

**NITRIFICATION/DENITRIFICATION  
IN A SUBMERGED ANAEROBIC  
MEMBRANE BIOREACTOR (SAMBR)  
FOR NITROGEN REMOVAL**

**By:**

**MOHD NAZRI MOHD NAWI**

**Department of Chemical Engineering**

**Faculty of Engineering**

**IMPERIAL COLLEGE LONDON**

**A thesis submitted in fulfilment for the degree of Doctor of  
Philosophy (PhD) and the Diploma of Imperial College (DIC)**

**FEBRUARY 2020**

## **DECLARATION OF ORIGINALITY**

It is hereby declared that the work presented in this thesis was candidate's own. All other information derived from the published and unpublished work of others have appropriately been referenced and acknowledged.

**MOHD NAZRI MOHD NAWI**

Imperial College London

February 2020

*'The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are licensed under a Creative Commons Attribution-Non Commercial 4.0 International Licence (CC BY-NC). Under this licence, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose. When reusing or sharing this work, ensure you make the licence terms clear to others by naming the licence and linking to the licence text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes. Please seek permission from the copyright holder for uses of this work that are not included in this licence or permitted under UK Copyright Law.'*

## ABSTRACT

Due to serious drawbacks in the conventional nitrification-denitrification process for nitrogen removal from wastewaters, such as high energy and carbon inputs and significantly increased sludge production, the more efficient and cost-effective Anammox process is starting to be used more widely. Anammox is an anaerobic biological process where around equimolar amounts of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  are oxidised/reduced, respectively, to produce dinitrogen gas without using a carbon donor. In the first part of this study, the start-up and performance of the Anammox process were evaluated in a 3-litre submerged anaerobic membrane bioreactor (SAMBR). The start-up using seed culture from an anaerobic digester (Anglian Water, UK) was relatively quick (60 and 70 d) for SAMBR 1 (HRT = 2d) and SAMBR 2 (HRT = 4d), respectively, compared to other reports in the literature. Both reactors showed quite high  $\text{NH}_4^+$  and  $\text{NO}_2^-$  removal efficiencies of over 80% and 65%, respectively, resulting in a molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption of 1:0.9, which was comparable to recently reported values in the literature which are lower than the originally cited ratio of 1:1.32. Despite different HRTs, there were no significant differences in performance between the reactors. The use of a flat sheet membrane panel (~0.4  $\mu\text{m}$  pore size-Kubota, UK) was shown to be capable of shortening the start-up period for Anammox compared to continuous flow through reactors such as conventional CSTRs.

The second major part of this work examined the novel process of partial pre-oxidation of  $\text{NH}_4^+$  using nanofiltration (NF) hollow fiber membrane modules, to provide a feasible alternative to conventional partial nitrification preceding the Anammox process. Prior to investigating the feasibility of this process, ammonium oxidising bacteria (AOB) were enriched from both activated sludge and full-scale SAMBR sludge, in batch reactors, in order to determine whether it was possible to use anaerobic sludge as a source of nitrifiers. The enriched AOB demonstrated stable and high nitrifying activity throughout the enrichment period of 200-300 days, with average  $\text{NH}_4^+$  removal efficiencies of over 90%. In the pre-oxidation process, AOB in the shell-side of the membrane unit was shown to be capable of oxidising the  $\text{NH}_4^+$  mainly to  $\text{NO}_2^-$  which then diffused back into the tube side, resulting in a mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the exit stream from the membrane unit. It was found that only flow rates of above 3.0 L/h were feasible, with a maximum  $\text{NH}_4^+$  flux in the range of 8 – 10  $\text{g/m}^2 \text{ h}$ . After 48 hours of operation, and at a flow rate of 5.0 L/h, an

approximately equimolar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  was observed in the exit stream, and this would meet the requirement for the Anammox process as suggested by previous reports.

This study has demonstrated the potential benefits of applying the Anammox process in a SAMBR for the treatment of nitrogen-containing wastewater as it could reduce the process start-up period, and the operation can be carried out at a short HRT. The application of a membrane process for the pre-oxidation of  $\text{NH}_4^+$  was found to be reasonably promising at a laboratory-scale, and practically viable at a scale similar to actual SHARON reactor (Whitlingham STC, UK) based on an estimation of the number of HF modules needed. However, a proper optimisation study of the process is strongly recommended so that its feasibility could be further examined at a larger scale linking both processes together.

## ACKNOWLEDGEMENTS

My foremost acknowledgement is directed towards my supervisor, Professor David Stuckey, for his continuous support, advice and constructive criticism throughout this study, all of which has broadened my perspectives towards academia and sharpened my inclination to engage in active research work. I am also grateful to him for his willingness to accept me as one of his students, and for believing in me until the end of this journey. The opportunity to learn from a distinguished and experienced academic like him has been most worthwhile.

Special recognition goes to my senior research colleagues, Dr. Rachel Fox and Dr. Hafiz Puteh. They were helping and teaching me on how to do things in the laboratory during my early years, despite their hectic schedule in finishing their own research work. In addition, thank you also to Fadzlie Wong, Keeran Ward and Bonahis Oko who were working together with me in the laboratory during this work; for always extending hands, ideas and support, as well as for having some interesting discussion either on academic or non-academic matters. I wish them the very best in their study and future career.

I would like to acknowledge the Ministry of Education, Malaysia, and my employer, IIUM, for providing me with financial support during the four years of this study, for which I have gone neither hungry nor homeless. It is equally important to acknowledge Dr. Hedley Skeleton and Tom Wakerly of Anglian Water UK; and Peter Pearce and Tom Gillespy of Thames Water UK, for allowing and helping me to collect wastewater sludge from their respective treatment plants, without which this research work would not be possible.

There are many other people, who I would like to express my gratitude to, without them this journey would have not been so meaningful. Unfortunately, I am not able to name them all here; it is, however, worth mentioning some of them, mostly those who have been close companions of mine throughout the good and bad times. To Dr. Hasbullah, Dr. Owan Saiful, Dr. Zulhafiz, Badrin, Azrudi, Ibtisham, Izzal, Erwan, Hazmil, Nasrul Hadi, Dr. Jeong, Dr. Mioa, Dr. Bhavish, Dr. Channarong, Dr. Hidayati, Dr. Ana, and Dr. Wen Chen, thanks for all the advice and help you have given me. I am also very thankful to my best friend and a working colleague at IIUM, Dr Firdaus, for helping me and sticking with me until the end.

Finally, I am very thankful for the patience and support from my parents and all my family members. "Thank you" is simply not good enough to express how grateful I am for their true inspiration, unconditional love, and some financial support they have provided me in completing the study.

# TABLE OF CONTENTS

<b>ABSTRACT</b>	.....	<b>3</b>
<b>ACKNOWLEDGEMENTS</b>	.....	<b>5</b>
<b>TABLE OF CONTENTS</b>	.....	<b>6</b>
<b>LIST OF FIGURES</b>	.....	<b>10</b>
<b>LIST OF TABLES</b>	.....	<b>13</b>
<b>NOTATION</b>	.....	.....
	.....	<b>14</b>
<b>CHAPTER 1 INTRODUCTION</b>	.....	<b>16</b>
1.1	Overview: The Need for Efficient Water Management.....	16
1.2	Anaerobic Biological Treatment of Wastewater .....	17
1.3	MBR Technology for Wastewater Treatment .....	18
1.4	Nitrogen Removal from Wastewater .....	20
1.5	Research Motivation .....	21
<b>CHAPTER 2 LITERATURE REVIEW</b>	.....	<b>23</b>
2.1	Anaerobic Digestion (AD) Process.....	23
2.2	Activated Sludge (AS) Process.....	24
2.3	Nitrogen-Containing Wastewater .....	26
2.4	Conventional Biological Nitrification/Denitrification.....	29
2.5	The SHARON Process.....	34
2.6	Partial Nitrification Process .....	36
2.6.1	Factors Affecting Partial Nitrification.....	37
2.7	The Anammox Bacteria .....	40
2.8	The Anammox Process .....	42
2.9	Partial Nitrification with the Anammox Process .....	46
2.10	Membrane Bioreactor for Wastewater Treatment .....	46
2.11	MBR Design and Configurations .....	51
2.11.1	Reactor Configurations: Side-Stream vs. Submerged.....	51
2.11.2	Membrane Configurations.....	54

2.12	The Anammox Process in an MBR .....	55
2.13	State of the Art: Integration of an Anammox Process into a Wastewater Treatment System .....	57
2.14	Pre-Oxidation of Ammonium Using Membrane Modules .....	59
2.15	Summary of the Literature .....	62
2.16	Problem Statement.....	65
2.17	Objectives of Study.....	66
2.17.1	General Research Aim.....	66
2.17.2	Specific Scientific Objectives .....	67
<b>CHAPTER 3 MATERIALS AND METHODS .....</b>		<b>69</b>
3.1	Submerged Anaerobic Membrane Bioreactor (SAMBR).....	69
3.2	Process Design and Configurations .....	72
3.3	Membrane Modules .....	72
3.3.1	Kubota Flat Membrane Panel (for SAMBR) .....	72
3.3.2	Membrane Access in SAMBR .....	73
3.3.3	Nano-Filtration Membrane Modules (Pre-Oxidation Process) .....	75
3.4	Strategy of Operations (Experimental Work).....	77
3.5	Origin of Biomass .....	77
3.6	Growth Media .....	78
3.6.1	Growth Media for the Anammox Bacteria.....	78
3.6.2	Growth Media for Nitrifying Bacteria (AOB) .....	79
3.7	Analytical Techniques .....	79
3.7.1	Ammonium, Nitrite and Nitrate Analyses.....	79
3.7.2	Hydrazine and Hydroxylamine Analyses.....	82
3.7.3	Chemical Oxygen Demand (COD) Analysis .....	84
3.7.4	Total and Volatile Suspended Solids (TSS/VSS) Analysis.....	84
3.7.5	pH Measurement .....	85
3.7.6	Biogas Production .....	86
3.7.7	Biogas Composition .....	86
3.7.8	Experimental Replication and Control .....	86

<b>CHAPTER 4 THE ANAMMOX PROCESS IN A SUBMERGED ANAEROBIC</b>	
	<b>MEMBRANE BIOREACTOR .....88</b>
4.1	INTRODUCTION .....88
4.2	MATERIALS AND METHODS.....89
4.2.1	Strategy of Operations .....90
4.2.2	Sampling and Analysis .....92
4.3	RESULTS AND DISCUSSION .....93
4.3.1	Characteristics of Seed Cultures.....93
4.3.2	Anammox Process Performance.....94
4.3.3	Analysis of COD .....104
4.3.4	Biogas Production .....105
4.4	SUMMARY .....107
<b>CHAPTER 5 ENRICHMENT OF AMMONIUM-OXIDISING BACTERIA (AOB)</b>	
	<b>FROM DIFFERENT SEED CULTURES IN BATCH REACTORS...111</b>
5.1	INTRODUCTION .....111
5.2	MATERIALS AND METHODS.....113
5.2.1	Strategy of Operations .....113
5.2.2	Sampling and Analyses .....115
5.3	RESULTS AND DISCUSSION .....115
5.3.1	Initial Characteristics of Seed Cultures .....116
5.3.2	Growth of Nitrifying Bacteria .....117
5.3.3	Reactor Performance .....125
5.4	SUMMARY .....128
<b>CHAPTER 6 PRE-OXIDATION OF AMMONIUM USING NANO-FILTRATION</b>	
	<b>MEMBRANE MODULES.....130</b>
6.1	INTRODUCTION .....130
6.2	MATERIALS AND METHODS.....132
6.2.1	Nano-Filtration (NF) Membrane Modules .....132
6.2.2	Strategy of Operation .....132
6.3	RESULTS AND DISCUSSION .....137



6.3.1	Membrane Permeability Study .....	137
6.3.2	Pre-Oxidation of Ammonium using NF Membranes .....	145
6.3.3	Feasibility of Using the NF Membrane Modules for Pre-Oxidation Process of Ammonium .....	151
6.4	SUMMARY .....	156
<b>CHAPTER 7 GENERAL DISCUSSION .....</b>		<b>158</b>
<b>CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS.....</b>		<b>163</b>
8.1	The Anammox Process in a SAMBR .....	163
8.2	Enrichment of AOB in Batch Reactors.....	164
8.3	Pre-Oxidation of Ammonium using NF Membrane System .....	165
8.4	Recommendations for Future Work .....	166
8.4.1	Use of molecular characterisation techniques to identify and characterise species of bacterial cells.....	166
8.4.2	Use of seed sludge with higher biomass concentrations and quality .....	167
8.4.3	Optimisation study of membrane process used in the pre-oxidation of ammonium .....	168
<b>REFERENCES .....</b>		<b>169</b>
<b>APPENDICES .....</b>		<b>179</b>
Appendix 1 Details of SAMBR Design .....		179
Appendix 2 Calibration Curves .....		180
Appendix 3 Regression Analysis on COD .....		183

## LIST OF FIGURES

<b>Fig. 1-1</b>	Schematic of (a) external re-circulation (side-stream), and (b) submerged MBR systems.....	19
<b>Fig. 1-2</b>	Flow diagram of partial nitrification in series with the Anammox process. ....	21
<b>Fig. 2-1</b>	Schematic of an activated sludge process.....	25
<b>Fig. 2-2</b>	Relative distribution of ammonia and ammonium ions with pH (Gerardi, 2005)... .....	26
<b>Fig. 2-3</b>	Classical N-cycle involving nitrification and denitrification processes (Ahn, 2006). .....	30
<b>Fig. 2-4</b>	The proposed SHARON process in a well-mixed CSTR (Hellings et al., 1998).	34
<b>Fig. 2-5</b>	Minimum residence time of ammonium and nitrite oxidisers as a function of temperature (Hellings et al., 1998). ....	38
<b>Fig. 2-6</b>	A schematic of the cell plan of the Anammox Planctomycetes (Fuerst and Sagulenko, 2011) .....	41
<b>Fig. 2-7</b>	Possible metabolic pathways for the Anammox process (van de Graaf et al., 1997). ....	44
<b>Fig. 2-8</b>	Regional share of the MBR market based on the number of plants in 2003 (Pearce, 2008). ....	48
<b>Fig. 2-9</b>	Comparison between conventional activated sludge and membrane bioreactor layouts (Le-Clech, 2010). ....	49
<b>Fig. 2-10</b>	Trend of reduction in membrane replacement cost per m <sup>2</sup> .....	50
<b>Fig. 2-11</b>	Side-stream membrane bioreactor configuration. ....	51
<b>Fig. 2-12</b>	Submerged membrane bioreactor configuration. ....	53
<b>Fig. 2-13</b>	Activated sludge process integrated with anaerobic digestion and the Anammox process (adapted from AMPC, 2016) .....	58
<b>Fig. 2-14</b>	Schematic of a vertical laboratory-scale MABR containing UF-HF membranes. The reactor length, ID and working volume were 1.47 m, 7 mm and 430 mL, respectively (Brindle et al., 1998).....	61
<b>Fig. 3-1</b>	The SAMBR flat panels, with a stainless steel diffuser. ....	70
<b>Fig. 3-2</b>	A complete SAMBR unit placed in a water bath. ....	70
<b>Fig. 3-3</b>	Schematic diagram of a SAMBR operations (Hu, 2004). ....	71
<b>Fig. 3-4</b>	Schematic of a partial nitrification reactor and SAMBR process flow diagrams.	72

<b>Fig. 3-5</b>	Kubota (type 203) flat panel membrane module used in the SAMBR.....	73
<b>Fig. 3-6</b>	The original design of how the membrane is encased inside the reactor unit. The membrane module could only be removed by disassembling the whole reactor unit. ....	74
<b>Fig. 3-7</b>	New design of how the membrane was inserted inside the reactor unit. The membrane module could be removed without the need to disassemble the whole reactor unit. ....	75
<b>Fig. 3-8</b>	The NF membrane module brought from NTU, Singapore. ....	76
<b>Fig. 4-1</b>	A schematic of the Anammox process in a SAMBR .....	91
<b>Fig. 4-2</b>	SAMBR in operation for the Anammox process. ....	92
<b>Fig. 4-3</b>	Profile of nitrogen compounds ( $\text{NH}_4^+$ , $\text{NO}_2^-$ , $\text{NO}_3^-$ ) and COD concentrations in the effluent of SAMBR 1.....	96
<b>Fig. 4-4</b>	Profile of nitrogen compound concentrations ( $\text{NH}_4^+$ , $\text{NO}_2^-$ , $\text{NO}_3^-$ ) and .....	101
<b>Fig. 4-5</b>	Biogas production in SAMBR1 and SAMBR 2.....	106
<b>Fig. 5-1</b>	Aerobic batch reactor used to grow nitrifying bacteria, placed in a constant temperature room of 35 °C.....	114
<b>Fig. 5-2</b>	$\text{NH}_4^+$ , $\text{NO}_2^-$ and $\text{NO}_3^-$ profiles in NR 1 during 275 days of operation. Other than at the start, fresh growth media was introduced into the reactor on day 80, 130, and 225, as indicated by the dotted vertical lines. ....	119
<b>Fig. 5-3</b>	$\text{NH}_4^+$ , $\text{NO}_2^-$ and $\text{NO}_3^-$ profiles in NR 2 during 300 days of operation. Other than at the start, fresh growth media was introduced into the reactor on day 75, 140, and 230, as indicated by the dotted vertical lines. ....	122
<b>Fig. 5-4</b>	Removal efficiency of $\text{NH}_4^+$ in NR 1 and NR 2 during the enrichment period of AOB in four different cycles (NR 1 = RAS, NR 2 = SAMBR). ....	125
<b>Fig. 5-5</b>	Relationship between $\text{NH}_4^+$ removal rates and COD/N ratio observed in NR 1 during the enrichment period. ....	127
<b>Fig. 5-6</b>	Relationship between $\text{NH}_4^+$ removal rates and COD/N ratio observed in NR 2 during the enrichment period. ....	127
<b>Fig. 6-1</b>	The tubular NF membrane module that consists of concentric hollow fibre tubes. ....	133
<b>Fig. 6-2</b>	Schematic of the proposed pre-oxidation of $\text{NH}_4^+$ using NF membrane modules. ....	135
<b>Fig. 6-3</b>	$\text{NH}_4^+$ mass fluxes of the NF membrane at different feed flow rates. ....	139

<b>Fig. 6-4</b>	$\text{NH}_4^+$ mass fluxes and membrane rejection of $\text{NH}_4^+$ at different initial feed concentrations. ....	143
<b>Fig. 6-5</b>	Glucose mass fluxes and membrane rejection at different flow rates with a COD concentration of 50 mg/L.....	145
<b>Fig. 6-6</b>	$\text{NH}_4^+$ mass fluxes at different flow rates, while a cell suspension of AOB circulated through the shell side of the membrane. ....	147
<b>Fig. 6-7</b>	Average membrane rejection of $\text{NH}_4^+$ at different flow rates, with and without cell circulation through the shell side of the module. ....	148
<b>Fig. 6-8</b>	Concentration profiles of $\text{NH}_4^+$ and $\text{NO}_2^-$ in the tube and shell sides of the membrane module at a flow rate of 3.0 L/h.....	149
<b>Fig. 6-9</b>	Concentration profiles of $\text{NH}_4^+$ and $\text{NO}_2^-$ in the tube and shell sides of the membrane module at a flow rate of 5.0 L/h.....	149
<b>Fig. 7-1</b>	Proposed schematic of the pre-oxidation of $\text{NH}_4^+$ using NF membrane module connected to the Anammox reactor. ....	162
<b>Fig. A-1</b>	Details of SAMBR design (Hu,2004).....	179
<b>Fig. A-2</b>	Calibration curve for $\text{NH}_4^+$ using $\text{NH}_4\text{Cl}$ as standard.....	180
<b>Fig. A-3</b>	Calibration curve for $\text{NO}_2^-$ using $\text{NaNO}_2$ as standard.....	180
<b>Fig. A-4</b>	Calibration curve for $\text{NO}_3^-$ using $\text{NaNO}_3$ as standard.....	181
<b>Fig. A-5</b>	Calibration curve for chemical oxygen demand (COD), using KHP as standard .....	181
<b>Fig. A-6</b>	Calibration curve for hydrazine ( $\text{N}_2\text{H}_4$ ), using $\text{N}_2\text{H}_4\cdot 2\text{HCl}$ as standard.....	182
<b>Fig. A-7</b>	Calibration curve for hydroxylamine ( $\text{NH}_2\text{-OH}$ ), using $\text{NH}_2\text{OH-HCl}$ as standard .....	182
<b>Fig. A-8</b>	Histogram of COD residuals in SAMBR 1.....	183
<b>Fig. A-9</b>	COD PP Plot in SAMBR 1.....	184
<b>Fig. A-10</b>	COD PP Plot in SAMBR 1.....	185
<b>Fig. A-11</b>	COD Standardized Residuals Plot in SAMBR .....	186
<b>Fig. A-12</b>	COD Distribution Plot in SAMBR 1.....	187

## LIST OF TABLES

<b>Table 2-1</b>	Industrial discharges of ammonium, nitrite and nitrate ions (Gerardi, 2005) ....	28
<b>Table 2-3</b>	Characteristics for a range of membrane modules (Fane and Chang, 2002). ....	54
<b>Table 2-4</b>	Some of the full-scale Anammox systems operated worldwide and their operational information (Ni and Zhang, 2013).....	59
<b>Table 3-1</b>	Characteristics of NF membrane modules (Wang, Personal communication). .	76
<b>Table 4-1</b>	Synthetic wastewater composition (adapted from van de Graaf et al. (1996)). ...	91
<b>Table 4-2</b>	Initial characteristics of AD sludge used to start-up the Anammox process in a SAMBR. ....	94
<b>Table 4-6</b>	Comparison of parameters and findings between SAMBR 1 and SAMBR 2.	109
<b>Table 5-1</b>	cSynthetic media used for the enrichment of AOB in batch reactors. ....	113
<b>Table 5-2</b>	Initial characteristics of seed sludge used to grow AOB in batch reactors. ....	116
<b>Table 5-3</b>	A summary and comparison of the three nitrifying reactors performance.....	128
<b>Table 6-1</b>	Experimental runs to investigate the effects of different parameters on membrane permeability and selectivity towards $\text{NH}_4^+$ and glucose.....	136
<b>Table 6-2</b>	Experimental runs to investigate the potential of using the NF membrane process for the pre-oxidation of $\text{NH}_4^+$ at different flow rates, HRT and NLR. The $\text{NH}_4^+$ concentration was set at 100 mg/L, and the biomass used was from activated sludge.....	137
<b>Table 6-3</b>	Average $\text{NH}_4^+$ rejection by NF membrane at different flow rates .....	141
<b>Table 6-4</b>	Amount of $\text{NH}_4^+$ and $\text{NO}_2^-$ in retentate (tube side) after 24 h and 48 h at different flow rates.....	154

## NOTATION

<b>AD</b>	Anaerobic digestion
<b>Anammox</b>	Anaerobic ammonium oxidising
<b>AnMBR</b>	Anaerobic membrane bioreactor
<b>ANOVA</b>	Analysis of variance
<b>AOB</b>	Ammonium-oxidising bacteria
<b>APHA</b>	American Public Health Association
<b>AS</b>	Activated sludge
<b>CANON</b>	Complete autotrophic nitrogen removal over NO <sub>2</sub> -
<b>CAS</b>	Conventional activated sludge
<b>COD</b>	Chemical oxygen demand
<b>CSTR</b>	Continuous stirred tank reactor
<b>DGGE</b>	Denaturing gel gradient electrophoresis
<b>EPS</b>	Extracellular polymeric substances
<b>Eq.</b>	Equation
<b>EU</b>	European Union
<b>Fig.</b>	Figure
<b>FISH</b>	Fluorescent in-situ hybridisation
<b>FP</b>	Flat plate
<b>GHG</b>	Greenhouse gases
<b>HF</b>	Hollow fibre
<b>HRT</b>	Hydraulic retention time
<b>ID</b>	Inner diameter
<b>KHP</b>	Potassium hydrogen phthalate
<b>LPH</b>	Litre per hour
<b>MABR</b>	Membrane aeration bioreactor
<b>MBR</b>	Membrane bioreactor
<b>MF</b>	Microfiltration
<b>MLVSS</b>	Mixed-liquor volatile suspended solids
<b>MSBR</b>	Membrane sequencing batch reactor
<b>MWCO</b>	Molecular weight cut-off

<b>NF</b>	Nanofiltration
<b>NLR</b>	Nitrogen loading rate
<b>NOB</b>	Nitrite-oxidising bacteria
<b>NR</b>	Nitrifying reactor
<b>OD</b>	Outer diameter
<b>OLAND</b>	Oxygen limited autotrophic nitrification and denitrification
<b>PE</b>	Population equivalent
<b>RAS</b>	Return activated sludge
<b>RO</b>	Reverse osmosis
<b>rpm</b>	Revolutions per minute
<b>SAA</b>	Specific Anammox activity
<b>SAMBR</b>	Submerged anaerobic membrane bioreactor
<b>SBR</b>	Sequencing batch reactor
<b>SHARON</b>	Single reactor system for high ammonium removal over nitrite
<b>SMP</b>	Soluble microbial products
<b>SNAD</b>	Simultaneous partial nitrification, Anammox and denitrification
<b>SNAP</b>	Single-stage nitrogen removal using Anammox and partial nitrification
<b>SRT</b>	Solid retention time
<b>TMP</b>	Transmembrane pressure
<b>TSS</b>	Total suspended solids
<b>UF</b>	Ultrafiltration
<b>UN</b>	United Nation
<b>VFA</b>	Volatile fatty acid
<b>VSS</b>	Volatile suspended solids
<b>WWAP</b>	World Water Assessment Programme
<b>WWDR</b>	World Water Development Report
<b>WWTP</b>	Wastewater treatment plant

# CHAPTER 1

## INTRODUCTION

### 1.1 Overview: The Need for Efficient Water Management

It is commonly known that over 70% of the earth surface is covered by water, and of all this water, about 97.5% is comprised of salty water in the oceans, while the remaining 2.5% is fresh water, comprised of lakes and frozen water locked up in glaciers and the polar ice caps, and this also includes ground water sources. About 2.5 billion people worldwide depend solely on ground water resources for their basic daily water needs (UN-WWDR., 2015). Without proper care of this already limited water resource, it is possible that one day the world would face a critical clean water scarcity for human consumption. The challenge is not only to preserve the existing water resources for the sustainability of the world, but to also make sure that proper and systematic waste management programmes are in place. As global water demand is largely influenced by population growth, it is undoubtedly true that one of the most challenging tasks faced by most countries is to properly ensure that clean water resources continue to exist, and be able to fulfill the increasing human consumption of water, while at the same time addressing the serious problems of water pollution.

Water pollution, which results from various sources, has always been a major environmental problem which has been faced for many decades. Other than residences, almost all types of industry produce waste, which is normally voluminous and varied in its composition. Based on the statistics provided by the UN World Water Assessment Programme (WWAP), about two million tons of sewage and industrial and agricultural waste are produced every day and discharged into the world's water (UN-WWAP., 2003). Untreated wastewater, or improper treatment of such wastewater prior to its release into the environment would result in serious environmental threats, and continuous depletion of fresh water resources; these consequently pose extreme health risks to human beings. Increasing environmental concerns, and the introduction of more stringent regulations imposed on environmental discharges have shifted the focus towards minimising the consumption of resources, along with water recovery, reuse, and recycling from wastewater. This would require an extensive improvement of the existing conventional wastewater



treatment technologies. In order to attain the required effluent quality, many plants have been gradually upgraded.

## **1.2 Anaerobic Biological Treatment of Wastewater**

Although a number of disposal options, which include both physicochemical and biological processes, can be used for the treatment of wastewater, no single process can be applied in the treatment of all types of wastewaters. For that reason, unique and improved wastewater treatment technologies with maximal process efficiency, reduced environmental impact of the plant, and economically viable costs must be continuously developed. Biological treatment of wastewaters, where bacteria and other microorganisms are used to remove various types of contaminant have long been the main focus of treatment technologies as it offers great advantages over conventional treatment systems, i.e. physical and chemical methods. Among the advantages are: low capital and operating costs; oxidation of a wide variety of organic compounds; removal of reduced inorganic compounds such as sulfides and ammonia; removal of nitrogen through denitrification; operational flexibility to handle a wide range of flows and wastewater characteristics, and; reduction in aquatic toxicity.

Biological treatment of wastewaters can generally be divided into two major categories, namely aerobic and anaerobic. In recent years, interest in anaerobic biological treatment of wastewaters has increased substantially due to its lower energy consumption, low sludge production and biogas generation (Huang et al., 2010). Anaerobic wastewater treatment differs from conventional aerobic treatment in that no aeration is applied. The absence of oxygen leads to the controlled anaerobic conversion of wastes into carbon dioxide, methane, water and a very small amount of biomass. In contrast, aerobic treatment uses aerobic bacteria to digest the organic wastes, which are then broken down with free oxygen to carbon dioxide, water and more biomass. The excess biomass produced in an aerobic reactor has to be treated prior to disposal, which adds considerably to the operating cost. For that reason, there has been an increasing interest in the application of anaerobic biological treatment due to its lower operating cost (no aeration required), lower energy consumption, low sludge production, and biogas generation.

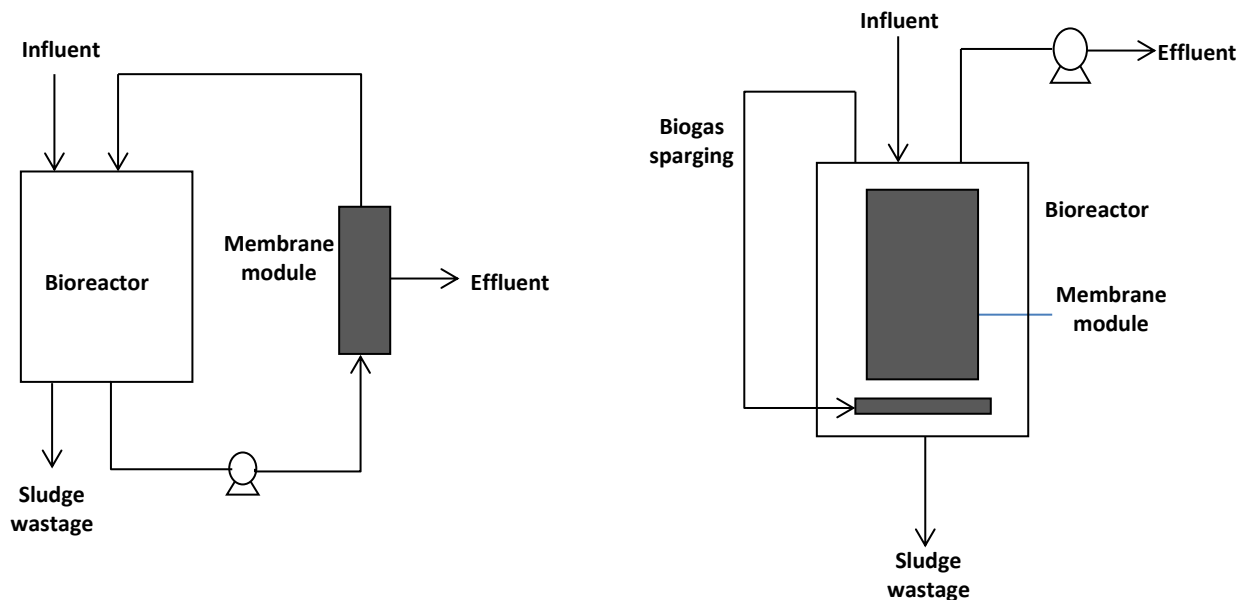
Nevertheless, the slow growth of anaerobic bacteria, especially in low-strength wastewaters, has been an issue of concern, which makes anaerobic treatment less attractive when compared with aerobic treatment. The difficulty in retaining these slow-growing bacteria with a short hydraulic retention time (HRT) is also a challenge that needs to be overcome. Therefore, efficient reactor designs that will enable a reactor to operate at short hydraulic retention times (HRT), independent from the solids retention time (SRT), is of high priority (Stuckey, 2012). One way this issue can be resolved is by applying membrane separation in anaerobic treatment processes as the membrane can effectively retain biomass, producing a solids-free effluent and preventing unintended sludge wash-out (Huang et al., 2010). This has led to the development of anaerobic membrane bioreactor (AnMBR) technology, which have evolved from the earlier versions of aerobic membrane bioreactors (AMBRs). AnMBRs offer various advantages over AMBRs because they have low energy use, produce low solids yields, and their emission of greenhouse gases (GHG), i.e. CO<sub>2</sub>, N<sub>2</sub>O (for nitrifying/denitrifying) is very low (Stuckey, 2012). The AnMBR was shown to be capable of allowing biomass growth to extremely high concentrations that consequently lead to high removal efficiencies of organic matter, i.e. > 90% (Fuchs et al., 2003).

### **1.3 MBR Technology for Wastewater Treatment**

The MBR is a relatively recent technology that integrates the biological treatment of waste products with membrane filtration, and has been shown to be effective in removing various types of contaminants from wastewaters (Yang et al., 2006, Judd, 2008, Le-Clech, 2010). It has received considerable attention worldwide as the current demand for more efficient and reliable processes for municipal and industrial wastewaters treatment rises. Since 2000, the global market for MBRs has grown significantly at between 11.6 – 12.7% per annum (Santos et al., 2011). The use of membranes in the biological treatment of wastewaters is promising as it can replace the conventional multiple treatment processes with a single membrane separation unit, hence reducing the overall reactor size and operational costs. The main advantage of using an MBR over other conventional bioreactors, particularly with slow-growing anaerobic bacteria, is the ability of the membrane to retain the biomass inside the reactor, and hence promoting the growth of the bacteria.

Apart from the ability of membranes to retain biomass and promote its growth, the MBR also allows the hydraulic retention time (HRT) to be controlled independently of the solids retention time (SRT), providing more flexibility in coping with flow rate and feed quality variations (Pearce, 2008). As membranes are impermeable to biomass, the SRT can theoretically be infinite, and it is possible to achieve high biomass concentrations in a bioreactor through the use of an MBR by coupling short HRTs with long SRTs. The effluent from an MBR also requires less post processing than a conventional treatment plant effluent as it has passed through a membrane filter and hence contains almost no solids or bacteria.

To date, commercial MBR systems would have two different types of membranes incorporated into the bioreactor design: in an external loop (side-stream) or integrated into the bioreactor (submerged), as illustrated in **Fig. 1-1**. The side-stream system was preferred in the past due to simple membrane cleaning and replacement without interfering with the reactor. Nevertheless, other than the smaller footprint required, Hu and Stuckey (2006) and Le-Clech (2010) later demonstrated that the use of submerged MBR systems seems potentially the most interesting and favorable in terms of lower operating costs, gentle mixing (no high shear rates through an external module), and very high volumetric efficiencies.



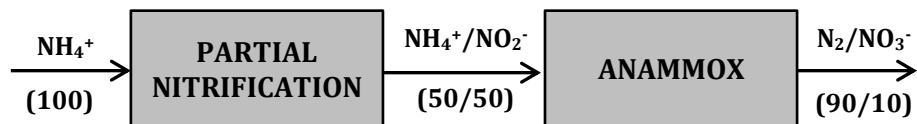
**Fig. 1-1** Schematic of (a) external re-circulation (side-stream), and (b) submerged MBR systems.

## 1.4 Nitrogen Removal from Wastewater

The presence of nitrogen in wastewater resulting from various industries, i.e. food processing, animal feedlots, has become a serious problem faced by many countries. Since excess nitrogen in wastewaters can pose adverse effects on humans and aquatic ecosystems, it has drawn increased attention from the relevant authorities, environmental experts and researchers in order to overcome this problem, not only by enforcing more stringent laws, but also by using better treatment technology for nitrogen removal. However, many treatment plants are still unable to fulfill the standard requirements of total nitrogen in the effluent before being released into the environment because of a lack of space to enable its removal, or an unfavorable wastewater composition (van Dongen et al., 2001a). A conventional treatment system that principally combines the two biological processes, namely aerobic nitrification and anaerobic denitrification, has long been used for the nitrogen removal from wastewaters. However, the process is expensive as it requires large amounts of oxygen for nitrification and the addition of external carbon source (electron donor) during denitrification.

Due to the drawbacks of the conventional treatment system, substantial efforts have been made to develop a more efficient and economical treatment technology. Accordingly, Ahn (2006) previously reported that several novel and cost-effective biological nitrogen removal processes have been developed in the past few years, which include partial nitrification, nitrifier denitrification, the Anammox process, and a combination of partial nitrification with the Anammox process. In 1994, a new and promising way to treat nitrogen-containing wastewater with a deficiency in chemical oxygen demand (COD) was first discovered, known as the anaerobic ammonium oxidising (Anammox) process (Mulder et al., 1995). This process uses  $\text{NO}_2^-$  as the electron acceptor, and was shown to be capable of removing  $\text{NH}_4^+$  and  $\text{NO}_3^-$  simultaneously from wastewater. The Anammox process offers great advantages over the conventional system for nitrogen removal, particularly in terms of its operational cost. The exclusion of an external carbon source and aeration during the Anammox process in a reactor, with low amounts of surplus sludge would allow for a significant reduction in costs compared with a conventional aerobic system. The Anammox process is also environmentally friendly as it reduces carbon dioxide emissions into the atmosphere.

In the past, the combination of partial nitrification followed by the Anammox process has been shown to be promising, and has become the new alternative for nitrogen removal technologies. In partial nitrification,  $\text{NH}_4^+$  is first partially oxidised (about 50 – 55%) to  $\text{NO}_2^-$  by ammonium-oxidising bacteria (AOB) under limited aerobic conditions. The mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  from the partial nitrification step would then be denitrified to nitrogen ( $\text{N}_2$ ) gas and a small amount of  $\text{NO}_3^-$  by the Anammox bacteria under anoxic conditions; no addition of an external carbon source is needed during the denitrification step. **Fig. 1-2** illustrates a simple combination of partial nitrification with the Anammox process in series.



**Fig. 1-2** Flow diagram of partial nitrification in series with the Anammox process.

However, the Anammox bacteria are slow-growing and normally require a long start-up period. Since the MBR has been shown capable of promoting the growth of slow-growing bacteria by retaining the biomass inside the reactor, the combination of MBR technology with the Anammox process should be able to reduce the lengthy start-up of the process. Various processes that made use of the concept of partial nitrification and/or the Anammox process, and with the use of MBR technology were developed thereafter. Different reactor designs and configurations, along with different operational strategies were used with the aim of further increasing the efficiency of the process as a whole.

## 1.5 Research Motivation

As the Anammox process has been shown to have promise in replacing the conventional nitrification and denitrification process for nitrogen removal, and with increasing interest in using MBR technology mainly for the slow anaerobic processes, the aim of this study was to further explore some other aspects with regards to these two fields, i.e. Anammox and membrane and MBRs. In terms of reactor choice, the submerged

anaerobic membrane bioreactor (SAMBR) has attracted considerable interest recently due to its potential advantages over aerobic processes, and the experiences gained from the successful application of aerobic submerged MBRs for wastewater treatment (Huang et al., 2010, Stuckey, 2012). The SAMBR provides high retention of biomass within the bioreactor, and better control of the microbial population (Vallero et al., 2005).

Back in 2004, a novel SAMBR with a working capacity of 3-liters was successfully designed and built at Imperial College London (Hu, 2004). It was later shown capable of removing various contaminants, such as chemical oxygen demand (COD) (Hu and Stuckey, 2006), saline organic waste (Vyrides and Stuckey, 2009), and bacteriophages (Fox and Stuckey, 2015) from wastewater through anaerobic process in our laboratory. However, the reactor has yet to be tested for the removal of nitrogen, particularly using the Anammox process. Therefore, this research mainly aims to explore the feasibility of carrying out the Anammox process in this 3-litre laboratory-scale SAMBR for the treatment of a nitrogen-containing wastewater.

The research also aims to make use of the partial nitrification concept prior to the Anammox process in a SAMBR. The challenge is, instead of using a typical stirred tank reactor to run the partial nitrification process, this work intends to replace the partial nitrification tank with a hollow fiber nano-filtration membrane module; the module was fabricated by membrane experts at Nanyang Technological University (NTU) in Singapore. In using such a membrane, species of nitrifying bacteria responsible for the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  (formerly enriched and grown in batch reactors) is grown on the shell side of the hollow fiber membrane tubes, while wastewater containing  $\text{NH}_4^+$  is fed through the tube side. However, due to limitations in time, and the complexity of the process since these membrane modules have never been tested for such an application previously, both the pre-oxidation step and the Anammox reaction in a SAMBR were not connected in series.

## **CHAPTER 2**

# **LITERATURE REVIEW**

This chapter presents the fundamentals and an overview of the field of nitrogen removal, and reviews the existing findings available in the literature which is necessary in order to define the specific objectives of the study. It begins with the basic principles of anaerobic digestion and activated sludge processes, as the seed cultures used in this work were collected from an anaerobic digestion and an activated sludge treatment plant in the UK. A large part of the review focuses on nitrogen-containing wastewater and its effects on human health and the ecosystem, followed by some previous and existing treatment technologies used to remove nitrogen from wastewater, mainly on the Anammox process and other related process, i.e. partial nitrification and SHARON, upon which this work is based. A review on the use of membrane bioreactors and their operational strategies, specifically with respect to the submerged anaerobic membrane bioreactors (SAMBR) is also carried out. Finally, based on the preceding review, specific research objectives are presented at the end of this chapter.

### **2.1 Anaerobic Digestion (AD) Process**

Neither the experimental work nor the thesis was involved directly with any anaerobic digestion process. However, a brief review on anaerobic digestion is made here considering the fact that the seed cultures used for the experimental work, mainly for the Anammox process in SAMBR, were collected from an anaerobic digestion treatment plant located in Cambridge (Anglian Water, UK). Basically, the seed culture collected was the product of a complete anaerobic digestion process carried out in the treatment plant. Typical CAMBI™ anaerobic digestion process is operated at Anglian Water Treatment Plant in Cambridge (Wakerley, 2014).

Anaerobic digestion is a series of biodegradation processes in which microorganisms break down large organic compounds into biogas, typically methane and carbon dioxide in the absence of oxygen. It involves quite a complex process involving many classes of bacteria and Archaea acting in a series of linked reactions with each other. There are four

main reactions involved in an anaerobic digestion process, namely: hydrolysis, acidogenesis (or fermentation), acetogenesis, and methanogenesis (Gujer and Zehnder, 1983, Zehnder, 1988).

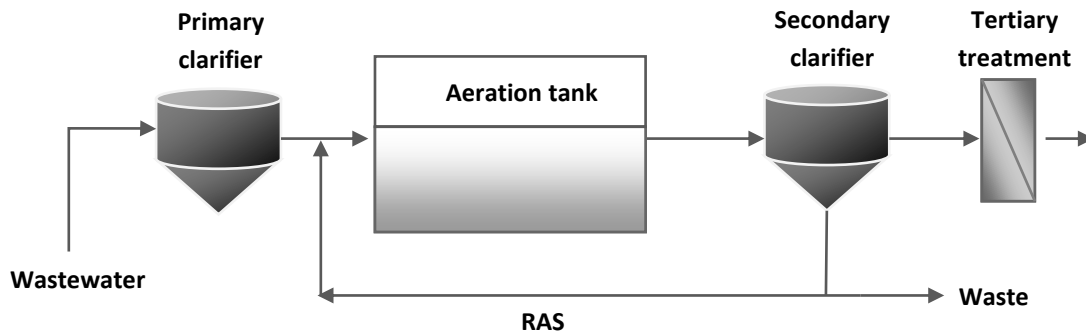
One of the important points to highlight is that since the seed cultures were collected from an anaerobic digestion plant, it is most likely that the seed contains quite a broad spectrum of bacteria and substrates/products, i.e. COD, acetate, and other soluble organic materials. This fact has to be considered as the interest of this study is about the nitrifying and Anammox bacteria. No pure culture of either nitrifying or Anammox bacteria was used in the experimental work.

## **2.2 Activated Sludge (AS) Process**

Other than anaerobic digestion sludge, some major parts of the experimental work in this study also involved the use of sludge collected from an activated sludge treatment plant operated by Thames Water UK, specifically located in Mogden, West London. For that reason, it is equally important to understand some of the basic principles of the conventional activated sludge process, mainly in terms of the bacterial community that might be present in the sludge.

The activated sludge process remains one of the most commonly used and predominant biological treatment processes for the treatment of industrial wastewater, although the selection of biological treatment system would depend on several factors, such as waste strength, space availability, effluent requirement, and also cost (Cheremisinoff, 1996). The process contains at least one aeration tank and one clarifier, as shown in **Fig. 2-1**. A primary clarifier or sedimentation tank is usually placed upstream of the activated sludge process (aeration tank). The purpose of a primary clarifier is to remove heavy solids that settle to the bottom of the clarifier, and also floating materials such as oils and greases (Gerardi, 2002). The activated sludge is usually followed by another clarifier (secondary clarifier). After secondary clarification, the treated wastes are subjected to further treatment (tertiary treatment) depending on the waste strength and the required effluent quality.





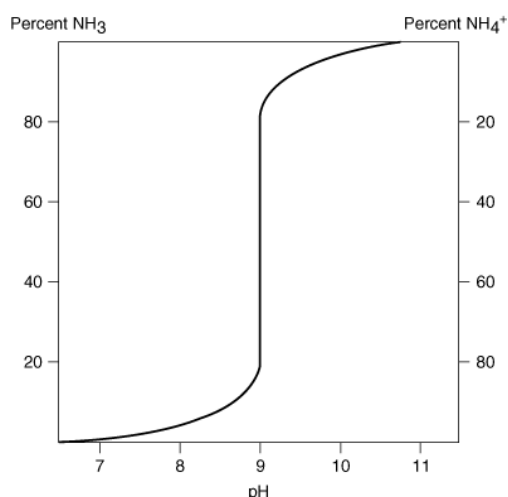
**Fig. 2-1** Schematic of an activated sludge process.

The aeration tank is a biological reactor where relatively large numbers of bacteria are aerated. Carbonaceous and nitrogenous wastes are introduced into the reactor and completely mixed with the bacterial suspension by means of a mechanical stirrer (or aeration) for a certain period of time. These wastes are degraded by the bacteria in the presence of dissolved oxygen, and in return this results in the growth of the bacterial population, hence producing more bacteria (Gerardi, 2002). Upon leaving the aeration tank and entering the secondary clarifier, the bacteria are typically in the form of floc particles. In the clarifier, the floc particles settle to the bottom of the tank and are returned to the aeration tank, known as return activated sludge (RAS), to continue the waste degradation process.

Cheremisinoff (1996) previously reported that bacteria present in activated sludge are capable of performing hydrolysis and oxidation reactions. Wastes oxidised in the aeration tank are converted to carbon dioxide, water, ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), sulphate ( $\text{SO}_4^{2-}$ ), phosphate ( $\text{PO}_4^{2-}$ ), and more bacterial cells (MLVSS) (Gerardi, 2002). It is apparent that the activated sludge process is also capable of oxidising some organic-nitrogen compounds such as proteins, into various inorganic products, including ammonium ions.  $\text{NH}_4^+$  are the substrate for the bacteria responsible for oxidising  $\text{NH}_4^+$  to  $\text{NO}_2^-$  ions, while  $\text{NO}_2^-$  are the substrate for the bacteria that oxidise  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . Both the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , followed by oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  is known as the nitrification process that occurs in series, but is carried out by different species of nitrifying bacteria. Therefore, activated sludge apparently contains diverse groups of bacteria, and this also includes nitrifying bacteria, which is of great interest in this study.

## 2.3 Nitrogen-Containing Wastewater

Nitrogen (N) is a chemical element that has the ability to exist in seven oxidation states, ranging from -3 to +5, and is therefore found in many compounds (Cheremisinoff, 1996, Jetten et al., 2009). In wastewaters, nitrogen is found in four dominant forms: organic nitrogen, ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2^- \text{-N}$ ), and nitrate nitrogen ( $\text{NO}_3^- \text{-N}$ ). Ammonia nitrogen may exist in aqueous solution as either ammonium ion ( $\text{NH}_4^+$ ) or unionized or free ammonia ( $\text{NH}_3$ ), and the relative concentrations of  $\text{NH}_4^+$  and  $\text{NH}_3$  are dependent on the pH and temperature of the water. As values of pH and temperature tend to increase, the concentration of undissociated  $\text{NH}_3$  also increases, while the concentration of  $\text{NH}_4^+$  decreases (Camargo and Alonso, 2006). The pH-dependent relationship between the two forms of ammonia nitrogen can be expressed using **Eq. 2-1**; at pHs less than 9.0, the reaction towards the right is more favourable, and hence  $\text{NH}_4^+$  is predominant. The relative distribution of free ammonia and ammonium ions based on pH is schematically shown in **Fig. 2-2**. The pKa value of ammonium is 9.25.



**Fig. 2-2** Relative distribution of ammonia and ammonium ions with pH (Gerardi, 2005).

Nitrite ions ( $\text{NO}_2^-$ ) are highly unstable since they usually do not accumulate in wastewater and are considered to be short-lived intermediate compounds during the oxidation of ammonia nitrogen to nitrate nitrogen when they would be quickly converted to

nitrate ions (Gerardi, 2005); if present in wastewater, their concentration is usually less than 1.0 mg/L (Cheremisinoff, 1996). Nitrate nitrogen is the most highly oxidised form of nitrogen, and although its discharge into receiving waters from wastewater treatment plants will not result in any oxygen demand in terms of nitrogenous oxygen demand (NOD),  $\text{NO}_3^-$  is, however, an important nutrient for algae growth, and can be a serious health issue in drinking water supplies.

The presence of nitrogen is a common problem in the wastewater from many different sources, primarily the industrial sector.  $\text{NH}_4^+$  and other nitrogenous compounds can be found in many industrial wastewaters that are normally discharged into activated sludge process. **Table 2-1** shows nitrogenous compounds discharged by various industrial processes, mainly in the forms of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ . Other than that, organic nitrogen made up of a variety of compounds including amino acids, sugars, urea and uric acid, and purines and pyrimidines (Kadlec and Knight, 1996) are also discharged from industrial wastewaters, which are finally converted to  $\text{NH}_4^+$  through hydrolysis and mineralisation (Paredes et al., 2007).

Several chemical processes are known to produce an effluent containing  $\text{NH}_4^+$  and organic substances. By considering the volume discharged and effluent compositions, the waste generated from chemical industries could be rated as one of the most polluting of all the industrial sectors. In addition, nitrogen-containing wastewater also arises from other different sources such as municipal solid waste landfills (500 – 3000 mg/L), domestic sewage (20–100 mg/L), swine wastewater (115–175 mg/L), sludge liquor (100–2000 mg/L), yeast effluent (180–450 mg/L), fertilizer manufacture, and agricultural activities (500–1000 mg/L) (Berge et al., 2005, Suneethi and Joseph, 2011b). It was reported that many wastewater treatment plants do not meet the current discharge standard of 10 mg N per liter since most existing wastewater treatment facilities were not properly designed for nitrogen removal (Jetten et al., 2002). However, the discharge standard may differ based on where the effluent is discharged to.

**Table 2-1** Industrial discharges of ammonium, nitrite and nitrate ions (Gerardi, 2005)

Industrial discharge	Nitrogenous Compound		
	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>
Automotive	/		
Chemical	/		
Coal	/		
Corrosion inhibitor		/	
Fertilizer	/		
Food	/		
Leachate		/	/
Leachate (pretreated)	/		
Livestock	/		
Meat	/		
Meat (flavouring)			/
Meat (preservative)		/	
Meat (pretreated)		/	/
Ordinance	/		
Petrochemical	/		
Pharmaceutical			/
Primary metal	/		
Refineries	/		
Steel	/	/	/
Tanneries	/		

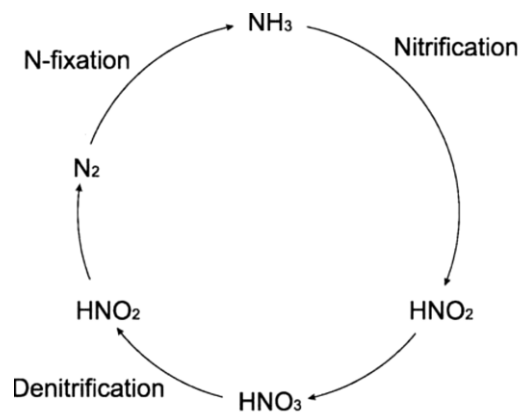
The adverse environmental effects of nitrogenous compounds on aquatic systems have long been recognised. The inappropriate discharge of nitrogenous compounds from wastewater into surface water bodies results in increased algal biomass (eutrophication), emissions of nitrous oxide to the atmosphere during the oxidation of ammonia, and toxicity to aquatic species (Cheremisinoff, 1996, Philips et al., 2002, Zhang et al., 2008). NO<sub>3</sub><sup>-</sup> was reported to be an important nutrient for algae and phytoplankton growth, which is responsible for promoting eutrophication in streams and lakes if present in excessive quantities. In the case of potable water supplies, the maximum allowable concentration of NO<sub>3</sub><sup>-</sup> set by the EU Nitrates Directive is 50 mg/L (NIEA, 2011) since high concentrations (90-104 mg NO<sub>3</sub><sup>-</sup>/L) have been shown to cause methemoglobinemia in infants under four months old (Cheremisinoff, 1996). In drinking water supplies, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> are toxic to humans causing the oxidation of haemoglobin, and in turn diminishing the oxygen transport

capacity of blood. The presence of ammonia in drinking water is therefore considered to be a potential health risk (van der Aa et al., 2002).

Free ammonia ( $\text{NH}_3$ ) is considered as one of the most significant pollutants in the aquatic environment because of its relatively highly toxic nature, and its ubiquity in surface water systems (Russo, 1985).  $\text{NH}_3$  is very toxic to aquatic life, particularly to fish, whereas  $\text{NH}_4^+$  are considerably less toxic (Constable et al., 2003), and Cheremisinoff (1996) and Kadlec and Knight (1996) reported that free ammonia in concentrations of above 0.2 mg/L has been shown to be fatal to many forms of aquatic life. Moreover, free ammonia can cause toxicity to *Nitrosomonas* and *Nitrobacter* bacteria, inhibiting the nitrification process, which can also result in an increased accumulation of  $\text{NH}_4^+$  (plus  $\text{NH}_3$ ) in the aquatic environment, intensifying the toxicity to bacteria and aquatic animals (Russo, 1985). Ammonia can also influence the oxygen balance of a river; wastewater effluent containing 20 mg/L of  $\text{NH}_4^+$  will have an oxygen demand of over 90 mg/L, about 4.5 times the ammonia concentration. Hence, to oxidise ammonia from wastewater is as important as to oxidise the carbonaceous demand (Kowalchuk and Stephen, 2001).

## 2.4 Conventional Biological Nitrification/Denitrification

The removal of nitrogen, which is present mainly in the form of  $\text{NH}_4^+$ , from wastewater can be carried out conventionally by various means that have been well developed such as breakpoint chlorination (Pressley et al., 1972), magnesium- $\text{NH}_4^+$ -phosphate (MAP) precipitation and air stripping (Kabdasli et al., 2000) and catalytic oxidation (Huang et al., 2001). Although these methods have been effective for clean water, there is no reported long term application for industrial wastewater. The combination of two biological processes – aerobic nitrification and anaerobic denitrification therefore remains the preferred and relatively cost effective method for the removal of nitrogen from both municipal and industrial wastewater (Fux et al., 2002, Ahn, 2006). Nitrification is the microbial conversion of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , and subsequently  $\text{NO}_3^-$ , whereas denitrification is the reduction of  $\text{NO}_3^-$  to nitrogen gas (Gerardi, 2005), as illustrated in **Fig. 2-3**. This conventional biological nitrogen removal process proceeds slowly due to low microbial activity and yield, and is generally performed on wastewater containing low nitrogen concentrations (Ahn, 2006).



**Fig. 2-3** Classical N-cycle involving nitrification and denitrification processes (Ahn, 2006).

The nitrification process is a chemolithoautotrophic oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  under strict aerobic conditions, and is sequentially conducted in two stages:  $\text{NH}_4^+$  oxidation and  $\text{NO}_2^-$  oxidation. Each stage is performed by different bacterial genera which are all aerobes and predominantly autotrophic (Ward, 2008). They use  $\text{NH}_4^+$  or  $\text{NO}_2^-$  as an energy source and molecular oxygen as an electron acceptor, while  $\text{CO}_2$  is used as a carbon source (Ahn, 2006). The most commonly recognised genus of bacteria that carries out  $\text{NH}_4^+$  oxidation is *Nitrosomonas*, while the most well-known nitrite oxidiser genus is *Nitrobacter* (Cheremisinoff, 1996, Ahn, 2006). However, *Nitrosococcus*, *Nitrospira*, *Nitrosovibrio*, and *Nitrosolobus* are also able to oxidise  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , whereas *Nitrospira*, *Nitrospina*, *Nitrococcus*, and *Nitrocystis* are known to be involved in the  $\text{NO}_2^-$  oxidation stage (Ahn, 2006). Both *Nitrosomonas* and *Nitrobacter* are considered to be autotrophs since they derive energy for growth and synthesis from the oxidation of inorganic nitrogen and carbon compounds. *Nitrosomonas* is coccus shaped with a diameter of 0.5-1.5  $\mu\text{m}$ , while *Nitrobacter* is bacillus (elongated) shaped with a diameter of 0.5-1.0  $\mu\text{m}$ . *Nitrosomonas* is mobile and reproduces by binary fission. In contrast, *Nitrobacter* is non mobile and reproduces by budding (Gerardi, 2005). Both of these groups of bacteria are Gram-negative, and have rather specific environmental requirements in terms of pH, temperature, and dissolved oxygen (Cheremisinoff, 1996).

Nevertheless, nitrification is not just limited to chemolithoautotrophic bacteria. Under aerobic conditions, a number of heterotrophic microorganisms among algae, fungi and bacteria were also found to be capable of oxidising a variety of nitrogenous compounds. This process is known as heterotrophic nitrification (Castignetti and Hollocher, 1984, Sakai

et al., 1996, Schmidt et al., 2003). Some of the main genera that have demonstrated heterotrophic nitrification include *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Pseudomonas* and *Bacillus* (Sakai et al., 1996, Stevens et al., 2002, Kim et al., 2007). Compared with those of autotrophs, the nitrification rates of heterotrophic nitrifiers are relatively slower by one or two orders of magnitude, hence limiting their application (Stevens et al., 2002). Therefore, heterotrophic nitrification was thought to preferentially take place under conditions that are not favourable for autotrophic nitrification, i.e. in acidic environments (Schmidt et al., 2003).

In the nitrification process,  $\text{NH}_4^+$  is firstly oxidised to  $\text{NO}_2^-$  (ammonium oxidation) by ammonium-oxidising bacteria (AOB) (**Eq. 2-2**). The  $\text{NO}_2^-$  is then sequentially oxidised to  $\text{NO}_3^-$  (nitrite oxidation) by nitrite-oxidising bacteria (NOB) (**Eq. 2-3**). A complete nitrification process requires two moles of oxygen since ammonium oxidisers use 1.5 moles and nitrite oxidisers use 0.5 mole of oxygen per mole of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , respectively (**Eqs. 2-2 and 2-3**). The complete nitrification process is represented by **Eq. 2-4**:



The  $\text{NO}_3^-$  produced from the nitrification process (**Eq. 2-4**) could then be reduced to nitrogen gas ( $\text{N}_2$ ) by means of denitrifying bacteria. The process is known as denitrification, a reduction process by which denitrifying bacteria reduce  $\text{NO}_3^-$  or  $\text{NO}_2^-$  to gaseous end-products of dinitrogen ( $\text{N}_2$ ), nitric oxide (NO) or nitrous oxide ( $\text{N}_2\text{O}$ ) under anoxic conditions (Skiba, 2008), which is then released to the atmosphere. This anoxic denitrification process could be accomplished with a variety of electron donors, i.e. methanol, which involves the reactions represented by **Eqs. 2-5 and 2-6** (Khin and Annachatre, 2004). Dinitrogen gas was reported to be the main end product of denitrification, while the other nitrogenous gases (NO and  $\text{N}_2\text{O}$ ) occur as intermediates in low concentrations, which may themselves be further reduced to  $\text{N}_2$  (Knowles, 1982).



Unlike the autotrophic nitrifying bacteria, the denitrifying bacteria are composed of ubiquitous, heterotrophic organisms, which use  $\text{NO}_2^-$  or  $\text{NO}_3^-$ , instead of oxygen as electron acceptors, while oxidising organic matter for carbon and as an energy source (Schmidt et al., 2003, Ahn, 2006). Some of the most common denitrifying bacteria are *Bacillus denitrificans*, *Micrococcus denitrificans*, *Pseudomonas stutzeri*, and *Achromobacter* sp. (Cheremisinoff, 1996). As denitrification is a heterotrophic process, a source of organic carbon as an electron donor, i.e. methanol, ethanol, acetic acid, acetate and lactic acid, is therefore required (Akunna et al., 1993, Schmidt et al., 2003, Khin and Annachhatre, 2004, Trigo et al., 2006, Jin et al., 2008). In many applications, methanol has gained acceptance as a cheap and reliable carbon source for denitrification compared with other carbon sources (Ahn, 2006, Jetten et al., 2009).

However, the paradigm that the only way to biologically remove nitrogen from wastewater necessitates the complete oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  followed by heterotrophic denitrification, has become obsolete (Schmidt et al., 2003). Since nitrifying bacteria are strict aerobes, the nitrification process consumes a large amount of oxygen, and its reaction rate is influenced by the DO concentration in the aeration tank. High requirements for oxygen certainly contribute towards the high cost of the process. It was reported that nitrification can only efficiently take place within the DO range of 2 – 3 mg/L (Gerardi, 2005).

Furthermore, this method is only suitable for the treatment of nitrogenous wastewater rich in biodegradable carbon. Enough carbon must be available in order to completely denitrify the  $\text{NO}_3^-$  formed during nitrification, which means that the COD/N ratio coming into the plant needs to be sufficiently high. For example, 4.2 g COD/g N was required for total nitrogen removal, including assimilation, when glucose was the carbon source (Henze, 1991). However, less than a decade later, it was further found that heterotrophic nitrification may only be of relevance when the wastewater contains a COD/N ratio of more than 10 (van Loosdrecht and Jetten, 1998). In many wastewaters, the low level



of biodegradable carbon is often not sufficient for complete nitrification, and hence it requires an additional source of external organic matter as a carbon source. In this case, higher costs will be involved for the treatment of wastewater with low biodegradable carbon and high nitrogen content such as effluents from the anaerobic digestion of sludge from wastewater treatment plants due to the necessity for external carbon sources during the denitrification stage (van Dongen et al., 2001b, Trigo et al., 2006).

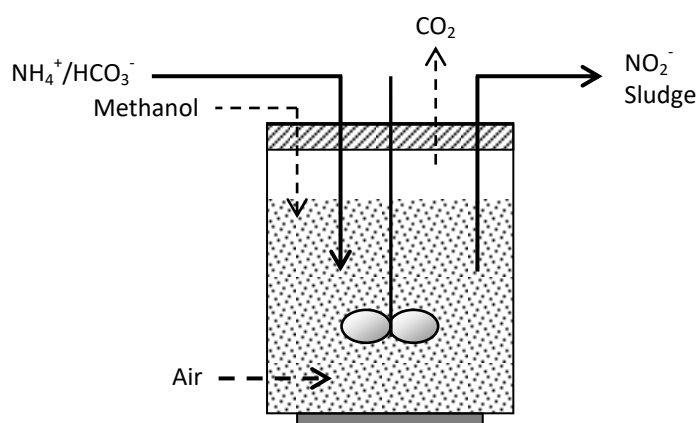
McCarty et al. (1969) investigated the use of five commercially available organic compounds as carbon sources during denitrification: methanol, acetic acid, ethanol, acetone and sugar. Of the five carbon sources, methanol was selected as the most preferable since it costs less on an equivalent basis, is a highly pure liquid and can be added easily and accurately to the wastewater. In 1969, McCarty et al. computed that the concentration of methanol to be 2.47 times the  $\text{NO}_3^-$  concentration, 1.53 times the  $\text{NO}_2^-$  concentration and 0.87 times the DO concentration (McCarty et al., 1969.). The following stoichiometric relationship between methanol concentration ( $C_m$ , in mg/L) with  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and DO concentrations ( $\text{N-NO}_3^-$ ,  $\text{N-NO}_2^-$ , and DO, respectively, in mg/L) was proposed (**Eq. 2-7**). Gerardi (2005) also recommended that 2.5 mg/L of methanol is used for every 1 mg/L of  $\text{NO}_3^-$ .

$$C_m = 2.47(\text{N-NO}_3^-) + 1.53(\text{N-NO}_2^-) + 0.87(\text{DO}) \quad (\text{Eq. 2-7})$$

The process is also unfavourable since it requires separate oxic and anoxic units for treatment, as nitrification and denitrification are carried out under different conditions and by different microorganisms. It results in high sludge production (1 kg VSS/kg N) with a substantial requirement for resources in terms of energy (2.8 kWh/kg N) and space (Ganigué et al., 2008), which add to the costs involved. Additional treatment of the surplus sludge will also certainly increase the operating cost (Gong et al., 2008). Moreover, the process is considered less environmentally-friendly due to the emissions of carbon dioxide and nitrogen oxide to the atmosphere (Jetten et al., 2009). Alternatively, these conventional processes can be replaced by more affordable and promising techniques. In 1998, a process known as SHARON was developed, where  $\text{NH}_4^+$  is oxidised to  $\text{NO}_2^-$  in a single reactor under aerobic conditions, and  $\text{NO}_2^-$  is then converted to nitrogen gas under anoxic conditions with the addition of a carbon source, e.g. methanol. (Hellings et al., 1998, van Dongen et al., 2001a). The SHARON process is further described in **Section 2.5**.

## 2.5 The SHARON Process

Several different research projects on nitrogen removal from wastewater have been carried out in the past. The SHARON process, an acronym for Single reactor High activity Ammonia Removal over Nitrite (Hellinga et al., 1998) was one of the processes, originally developed for the treatment of ammonium rich waste streams, i.e.  $> 500$  mg/L (van Dongen et al., 2001b), normally produced by the direct dewatering of warm digested sludge. The SHARON reactor could be a simple continuous stirred tank reactor (CSTR), as shown in **Fig. 2-4**, operated at unique operating conditions: an HRT of one day (for aerobic) and 0.5 day (for anoxic), no sludge retention (SRT equals to HRT), temperature of  $30 - 40$  °C, and the pH above 7.0 (Hellinga et al., 1998, Ahn, 2006). This process combines both autotrophic nitrification and heterotrophic denitrification in a single SHARON reactor system using intermittent aeration. During the denitrification step, the addition of a carbon source, e.g. methanol, is needed not only to reduce  $\text{NO}_2^-$  to nitrogen gas (Schmidt et al., 2003), but also for pH control and alkalinity production to compensate for the acidifying effect resulting from the previous nitrification phase (Ahn, 2006). Methanol is added periodically while the aeration is switched off.



**Fig. 2-4** The proposed SHARON process in a well-mixed CSTR (Hellinga et al., 1998).

In the SHARON process,  $\text{NH}_4^+$  is partially oxidised to a mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  under aerobic conditions, and then the  $\text{NO}_2^-$  is converted to nitrogen gas ( $\text{N}_2$ ) under anoxic condition with the addition of a carbon source, such as methanol (Hellinga et al., 1998). In such cases, the process needs less aeration, and the subsequent denitrification step consumes less COD, since only  $\text{NO}_2^-$  and not  $\text{NO}_3^-$  has to be reduced to nitrogen gas. The SHARON

process makes use of different growth rates between ammonium and nitrite oxidisers at sufficiently high temperatures, i.e. more than 26 °C (Hellings et al., 1998). Since the specific growth rate of ammonium oxidisers is reportedly much higher than that for nitrite oxidisers at an elevated operational temperatures, the ammonium oxidisers would outcompete nitrite oxidisers and hence preventing the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . The HRT of around one day was also selected as it is higher than the growth rate of nitrite oxidisers but lower than ammonium oxidisers (Schmidt et al., 2003). Furthermore, since there is no sludge retention in the SHARON process, nitrite oxidisers would be washed out in the effluent, thus  $\text{NO}_2^-$  becomes the stable end product of nitrification.

The SHARON process offers great advantages in the treatment of ammonium rich wastewater when compared with conventional nitrification and denitrification process as it reduces the cost for aeration and added carbon. It requires 25% less aeration because the oxidation is stopped at the  $\text{NO}_2^-$  stage, and 40% less added carbon source since only  $\text{NO}_2^-$  needs to be reduced to nitrogen gas. Khin and Annachhatre (2004), in a review on various microbial nitrogen removal processes, suggested that the SHARON process appears to be the most feasible to substantially remove  $\text{NH}_4^+$  from concentrated wastewater. Due to its simple reactor requirement, i.e. well-mixed CSTR without sludge retention (Hellings et al., 1998), the process does not need much initial investment (Khin and Annachhatre, 2004). A number of successful SHARON reactors were constructed and used to treat wastewater with a high concentration of  $\text{NH}_4^+$  has been previously reported in the literature. The first full-scale SHARON process was successfully scaled-up from 1.5 L laboratory-scale reactor and constructed at the Rotterdam Dokhaven wastewater treatment plant, used for the treatment of sludge liquor. The reactor with a capacity of 1800 m<sup>3</sup> was operated for two years, with 90% removal efficiency and managed to treat 830 kg N day<sup>-1</sup> (Mulder et al., 2001).

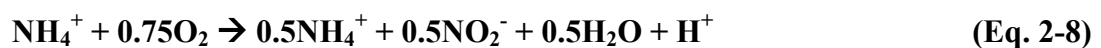
One of the drawbacks of the SHARON process is obviously the need for the addition of a carbon source during denitrification, and methanol is the most commonly used carbon source due to its low cost. Theoretically, the minimum stoichiometric demand for methanol as a carbon source would be 1.9 g/g  $\text{NO}_3\text{-N}$  or 1.14 g/g  $\text{NO}_2\text{-N}$  denitrified (Mulder et al., 2001). Considering the biomass yield, this demand could be much higher, i.e. expected to be 3.5 and 2.2 g/g N denitrified, respectively (Hellings et al., 1998), which would make full-scale denitrification quite expensive. The other limitations of the process are: it is a high temperature dependency process, thus not suitable for all wastewaters; and the process

is only ideal for wastewater containing high concentrations of ammonium (concentrated wastewater). Due to these limitations, the SHARON process is still subjected to some modifications, so that a much better treatment alternative for the removal of nitrogen from wastewater could be further developed.

The concept of the SHARON process, i.e. partial nitrification, was very much needed for the improvement of nitrogen removal from wastewater, particularly when combined with other processes like the Anammox. Prior to the Anammox process,  $\text{NH}_4^+$  in wastewater must be partially oxidised to  $\text{NO}_2^-$  (about 50 – 60%), but not to  $\text{NO}_3^-$ . Therefore, the Anammox process needs to be applied in a series of operations preceded by a partial nitrification, i.e. partial SHARON (without heterotrophic denitrification). The strategies used in the SHARON process to suppress the growth of nitrite oxidisers, thus preventing the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ , and other operating conditions should be strictly followed to provide the adequate influent conditions for the Anammox reaction. Thereafter, various processes that apply the concept of partial nitrification of  $\text{NH}_4^+$ , followed by denitrification of  $\text{NH}_4^+/\text{NO}_2^-$  to nitrogen gas by the Anammox reaction have been developed, aimed at overcoming the drawbacks of the conventional treatments (Sliemers et al., 2002, Windey et al., 2005, Lieu et al., 2006, Chen et al., 2009, Lan et al., 2011). In this method, partial nitrification of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  is carried out by fast growing ammonium oxidisers, while denitrification of  $\text{NO}_2^-$  to nitrogen gas is achieved using  $\text{NH}_4^+$  as the electron donor. (Schmidt et al., 2003, Ahn, 2006, Feng et al., 2007).

## 2.6 Partial Nitrification Process

Partial nitrification is the oxidation of wastewater  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , but not to  $\text{NO}_3^-$ , with fractions of  $\text{NH}_4^+$  remaining unconverted. The stoichiometry of the partial nitrification reaction is given by **Eq. 2-8** (Feng et al., 2007), where only approximately 50% of  $\text{NH}_4^+$  is oxidised to  $\text{NO}_2^-$ . Partial nitrification is often used as a shortcut to biological nitrogen removal, based on the fact that  $\text{NO}_2^-$  is an intermediary compound in both steps of nitrification and denitrification (Ciudad et al., 2005).



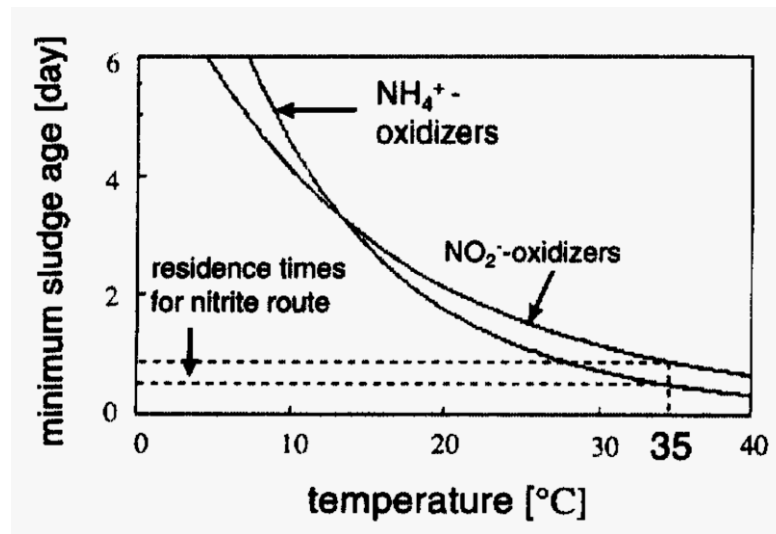
Operational costs of the conventional biological nitrogen removal process are to a great extent related to the oxygen and organic matter requirements for nitrification and denitrification, respectively. Unlike complete nitrification, partial nitrification uses only 0.75 mole of oxygen to oxidise one mole of  $\text{NH}_4^+$  (Eq. 2-8). Hence the process needs less aeration, and the subsequent denitrification consumes less COD since only  $\text{NO}_2^-$  and not  $\text{NO}_3^-$  has to be reduced to nitrogen gas (Schmidt et al., 2003). Therefore, it would be preferred to perform the partial nitrification prior to the denitrification stage, so that a significant reduction in operational costs in terms of aeration and carbon source can be made.

### 2.6.1 Factors Affecting Partial Nitrification

To achieve a stable partial nitrification, subsequent oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  should be prevented by controlling the operational variables such as temperature, pH, solids retention time, initial substrate concentration or alkalinity and the dissolved oxygen (DO) level (Ruiz et al., 2003, Lu et al., 2006, Feng et al., 2007, Zhang et al., 2008). Furthermore, other factors such as operation mode, aeration pattern, reactor configurations and operating costs are equally important and therefore should be considered comprehensively. Partial nitrification requires a reduction in the activity of nitrite oxidising bacteria (nitrite oxidisers), without affecting ammonium oxidising bacteria (ammonium oxidisers) (Ciudad et al., 2005), hence the conditions that favour  $\text{NH}_4^+$  oxidisers development must be established.

The two groups of bacteria are quite sensitive to temperature, in which elevated temperature facilitates ammonium oxidisers (Hellings et al., 1998). A stable nitrification process was observed at a temperature over 35 °C in the SHARON process as previously reported by Hellings et al (1998), shown in Fig. 2-5. In fact, it was reported that the maximum specific growth rate of ammonium oxidisers is double of that for nitrite oxidisers (1 and 0.5 day<sup>-1</sup>, respectively), at the operational temperature of 35 °C (Cheremisinoff, 1996). This finding was supported by Khin and Annachatre (2004) who proposed that the operation be performed at relatively high temperatures, i.e. above 35 °C, to enable the ammonium oxidisers to effectively outcompete the nitrite oxidisers. Recently, the nitrification process was also successfully started up and maintained between 15 and 30 °C (Yamamoto et al., 2006). However, system performance was observed to deteriorate

significantly below 15 °C, which is in agreement with the theoretical value found by Hellinga et al. (1998).



**Fig. 2-5** Minimum residence time of ammonium and nitrite oxidisers as a function of temperature (Hellinga et al., 1998).

At elevated temperatures, the doubling time of the ammonium oxidisers is shorter than that of the nitrite oxidisers. Hence the solids retention time (SRT) should be properly controlled in a limited range that enables the retention of ammonium oxidizers, but washes out the nitrite oxidizers (Zhang et al., 2008). Based on the full scale experience of a wastewater treatment plant by van Kempen et al. (2001), an SRT between 1 to 2.5 days was suggested. In fact, the ammonium oxidizers in a SBR can be selectively enriched and granulated resulting in wash out of the nitrite oxidizers by shortening the SRT (Kim and Seo, 2006). Nevertheless, it was later found that an SRT of up to 5 days in a sequential batch reactor (SBR) also created favourable conditions for ammonium oxidizers to outcompete nitrite oxidizers (Galí et al., 2007).

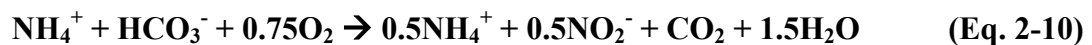
The oxidation of  $\text{NH}_4^+$  is also an acidifying process. It requires pH control prior to the Anammox process in order to prevent process inhibition (van Kempen et al., 2001). pH directly influences growth rates of the two groups of bacteria, but the nitrite oxidizers are more susceptible to a changing pH, where its activity is likely to be suppressed at elevated pH, i.e. a pH above 7 (Zhang et al., 2008). The relationship between the ammonium ion ( $\text{NH}_4^+$ ) and unionized free ammonia ( $\text{NH}_3$ ) is pH-dependent (Eq. 2-9), where the reaction is

displaced towards the left at a pH greater than 7, which promotes the growth of ammonium oxidizers (Khin and Annachatre, 2004).



Hellinga et al. (1998) also reported that wastewaters with a pH around 8 can create an environment containing more  $\text{NH}_3$ . Therefore, a high pH, i.e. pH 8, is preferred for obtaining an effluent that is high in  $\text{NH}_3$ , but low in  $\text{NH}_4^+$ . In contrast,  $\text{NH}_4^+$  oxidation will no longer take place when the pH drops below 6.5. At this low pH, the concentration of free ammonia becomes too low for sufficient growth of the ammonium oxidisers as the reaction is displaced towards the right, hence promoting the growth of nitrite oxidizers (Khin and Annachatre, 2004). Nonetheless, it was also found that the nitrification process would decline at pHs above 8 as too much  $\text{NH}_3$  is apparently toxic for the  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidisers. In fact, complete inhibition of nitrification take places at a pH lower than 6.45 and higher than 8.95 (Ruiz et al., 2003).

Feng et al. (2007) reported that there is a high correlation between  $\text{NH}_4^+$  oxidation and the initial alkalinity of the influent. Since  $\text{NH}_4^+$  oxidation is an alkaline-consuming (acidifying) reaction, one mole of alkali (bicarbonate) per mole of  $\text{NH}_4^+$  should be used to assure a proper ratio of  $\text{NH}_4^+ / \text{NO}_2^-$  (Eq. 2-10) (Zhang et al., 2008), hence a stable partial nitrification can be achieved. Both laboratory experiments and engineering practice have shown that an  $\text{NH}_4^+ / \text{NO}_2^-$  ratio around 1 is suitable for partial nitrification prior to the Anammox process (van Dongen et al., 2001b, Fux et al., 2002, Feng et al., 2007).



The addition of calcium bicarbonate to the wastewater would be needed only once at the beginning, serving as a carbon source, and alkalinity can be adjusted as necessary for partial nitrification (Feng et al., 2007). The sufficient alkalinity contributes to the maintenance of a favourable pH for ammonium oxidisers, and hence is essential for achieving partial nitrification.

The effect of dissolved oxygen (DO) concentration on the nitrification rate has also been extensively investigated by a number of researchers (Picioreanu et al., 1997,

Mosquera-Corral et al., 2005a, Canziani et al., 2006, Blackburne et al., 2007, Guo et al., 2009) using both pure and mixed cultures, and cultures found in wastewater treatment systems, in different types of reactors. DO strategy for the nitrification process control is based on different affinities of ammonium oxidisers and nitrite oxidisers. Picioreanu et al. (1997) observed that the nitrite oxidisers have a lower affinity for oxygen than the ammonium oxidisers, and therefore a limited DO level is certainly restrictive for the growth of nitrite oxidisers (Jianlong and Ning, 2004). Furthermore, by considering the fact that the oxygen affinity constants of ammonium and nitrite oxidisers are 0.3 and 1.1 mg/L, respectively (Wiesmann, 1994), operation at a low DO level in the range of 0.7 to 1.4 mg/L can also effectively suppress the activity of nitrite oxidisers (Ruiz et al., 2003, Ciudad et al., 2005). Of the operational variables previously described, the control of the DO level may be the most critical and is therefore a key factor for achieving partial nitrification (Shrestha et al., 2001, Canziani et al., 2006, Feng et al., 2007).

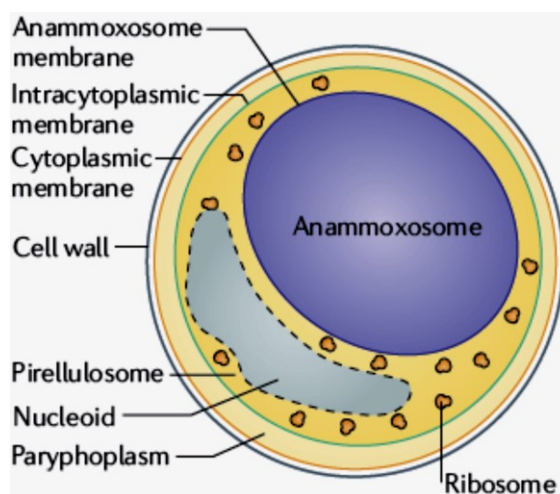
## 2.7 The Anammox Bacteria

Anaerobic ammonium-oxidising (Anammox) bacteria that belong to the order *Brocadiales* and are affiliated to the *Planctomycetes* were first discovered in wastewater sludge in the early 1990s (Kuenen, 2008, Jetten et al., 2009). This Gram-negative bacteria could be found widely either in wastewater treatment plants, or in freshwater and marine ecosystems (Schmid et al., 2005). Anammox bacteria have played significant roles in environmental and industrial microbiology, and derive their energy for growth from the conversion of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  into nitrogen gas ( $\text{N}_2$ ), with  $\text{NO}_2^-$  as electron acceptor in the complete absence of oxygen (Schmidt and Bock, 1997, Jetten et al., 2009). In fact, about 30% - 70% of gaseous nitrogen production is attributed to the Anammox process in the nitrogen cycle (Thamdrup and Dalsgaard, 2002). The remarkable advances in molecular biology techniques have revealed a great variety of information on the biodiversity of the Anammox bacteria (Schmid et al., 2001, Schmidt et al., 2003).

In 1999, the Anammox cells from a laboratory enrichment culture were physically purified, which marked the first description of Anammox bacterium (Strous et al., 1999a). The purified Anammox cells were shown to be capable of converting  $\text{NH}_4^+$  and  $\text{NO}_2^-$  into nitrogen gas in the absence of oxygen. The phylogeny of Anammox bacteria was



established using purified cells in a full molecular analysis based on the complete 16S rRNA gene sequence and fluorescence in situ hybridization (FISH) with specific oligonucleotide probes (Schmid et al., 2005). The cells display complex cell architecture with a central compartment, reminiscent of that of other members of the *Planctomycetes*, to which Anammox bacteria are phylogenetically related (Jetten et al., 2009). The *Planctomycetes* have been found to be morphologically and phylogenetically distinct from other Gram-negative bacteria. Their distinct phenotypic characteristics involve a red colour, budding production, crateriform structure on the cell surface, an intracellular compartment known as an anammoxosome, and intracytoplasmic membrane containing ladderane lipid (van de Graaf et al., 1996, Lindsay et al., 2001, Damste' et al., 2002). A schematic of an Anammox cell structure is shown in Fig. 2-6.



**Fig. 2-6** A schematic of the cell plan of the Anammox Planctomycetes (Fuerst and Sagulenko, 2011)

These bacteria have a highly unusual physiology, in that they live by consuming ammonia in the absence of oxygen (Ahn, 2006), while carbon dioxide is the main carbon source for their growth (van de Graaf et al., 1996). As concluded from electron microscopy observations, chemical analysis, genome sequencing and resistance to beta-lactam antibiotics and other cell wall-targeting antibiotics, *Planctomycetes* lack peptidoglycan, an almost universal polymer found within the domain Bacteria (Liesack et al., 1986, Fuerst, 2005). Furthermore, their cell wall is not surrounded by one membrane on the outer and one membrane on the inner side of the cell wall as is the case for other Gram-negative bacteria. Instead, there are two membranes on the inner side and no membrane on the outer side of

the cell wall, which consists mainly of proteins (Liesack et al., 1986, Jetten et al., 2009). The outermost of these two membranes, defined as the cytoplasmic membrane, is closely positioned to the cell wall, while the innermost membrane has been defined as an intracytoplasmic membrane as it is on the inside of the cytoplasmic membrane (Jetten et al., 2009).

The cytoplasm in Anammox bacteria is divided into three cytoplasmic compartments separated by single bilayer membranes: (1) the outer region, i.e. the paryphoplasm, occurs as an outer rim defined on its outer side by the cytoplasmic membrane and cell wall and on the inner side by the intracytoplasmic membrane; (2) the riboplasm, containing DNA, ribosomes and storage materials (glycogen granules); and (3) the inner ribosome-free compartment, the anammoxosome, bounded by the anammoxosome membrane and comprising 50–70% of the total cell volume (van Niftrik et al., 2008). The membrane bounding this compartment is often highly curved, possibly to increase the surface to volume ratio. Lindsay et al. (2001) further described the functions of the special organelle in the cell, anammoxosome. It was considered to have three functions: (1) providing a place for catabolism; (2) generating energy for ATP synthesis through a proton motive force across the anammoxosome membrane; (3) protecting the bacteria from the proton diffusion and intermediate toxicity due to their dense and impermeable membranes. Of the toxic intermediates that are produced and play significant roles in the Anammox process are hydrazine and hydroxylamine, which are further discussed in the next section.

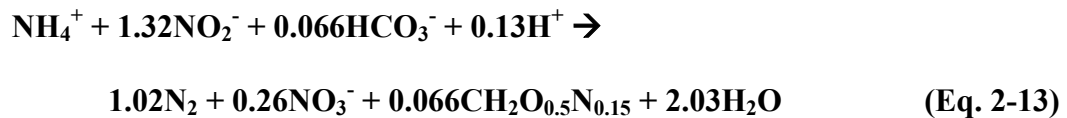
## 2.8 The Anammox Process

In recent years, great interest has been shown in investigating the potential of the Anammox process as a new and promising way to treat wastewater containing high concentrations of  $\text{NH}_4^+$ , with a deficiency in chemical oxygen demand (COD) content and organic matter (Trigo et al., 2006, Xiao et al., 2009). The process is based on energy conservation from anaerobic  $\text{NH}_4^+$  oxidation with  $\text{NO}_2^-$  as the electron acceptor without the addition of an external carbon source (Jetten et al., 1999). The Anammox process, which was first discovered in a laboratory-scale anaerobic fluidized bed reactor in 1994 (Mulder et al., 1995), was shown to be capable of removing  $\text{NH}_4^+$  and  $\text{NO}_3^-$  simultaneously (Eq. 2-11). Later, it was found that  $\text{NO}_2^-$  is the preferred electron acceptor for the process (Eq. 2-12)

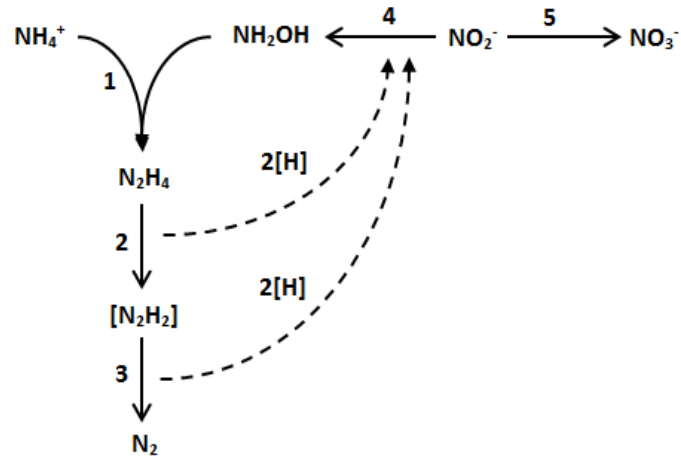
(Bock et al., 1995, van de Graaf et al., 1995). Since then, a lot of effort has been put into investigating the mechanism of microorganisms responsible for the Anammox process, together with its application in wastewater treatment.



From several basic studies, the stoichiometry of the Anammox reaction based on a mass balance over Anammox enrichment cultures is represented by **Eq. 2-13** (Strous et al., 1998). Although the main product of the Anammox reaction is nitrogen gas ( $\text{N}_2$ ), about 10% of the fed nitrogen ( $\text{NH}_4^+ / \text{NO}_2^-$ ) is also converted to  $\text{NO}_3^-$  (van de Graaf et al., 1996).



As previously described, nitrification and denitrification reactions, including the Anammox process, are often represented by rather simple equations, and mainly involve nitrogen transformations and cell growth (for Anammox). Nonetheless, it is recognised that the reactions may be more complex than they appear to be, and involve the formation of various intermediates. For example, highly toxic and reactive hydrazine ( $\text{N}_2\text{H}_4$ ) and hydroxylamine ( $\text{NH}_2\text{OH}$ ), are known to be amongst some intermediates of the process (van de Graaf et al., 1997, Jetten et al., 1999). The possible metabolic pathways for anaerobic  $\text{NH}_4^+$  oxidation are shown in **Fig. 2-7**.



**Fig. 2-7** Possible metabolic pathways for the Anammox process (van de Graaf et al., 1997).

In Step 1,  $\text{NH}_4^+$  is biologically oxidized using hydroxylamine as the electron acceptor to form hydrazine. Then,  $\text{N}_2\text{H}_2$ , reducing equivalents derived from hydrazine (Step 2) reduce  $\text{NO}_2^-$  to form nitrogen gas (Step 3) and even more hydroxylamine (Step 4). The presence of these two compounds could demonstrate the occurrence of the Anammox process. For example, hydrazine in particular, is a distinct compound and rarely found as an intermediate in microbial nitrogen conversions other than Anammox (Ahn, 2006).

The Anammox process offers great advantages over the conventional system of nitrogen removal from wastewater, particularly in terms of its operational cost. The exclusion of the external carbon source and aeration during the Anammox process in a reactor, with low amounts of surplus sludge would allow for a significant reduction in costs compared with the conventional nitrification-denitrification system (Xiao et al., 2009). It would save up to 90% of operational costs as compared with the conventional treatment systems for nitrogen removal (Jetten et al., 2001), and in terms of environmental concern, the Anammox process would also reduce carbon dioxide emissions to the atmosphere (Trigo et al., 2006). However, extensive experiments have shown that high levels of oxygen and low organic-carbon can completely inhibit Anammox activity when it is exposed to an enrichment culture (Waki et al., 2007). It was reported that Anammox bacteria are obligate anaerobes, and their metabolism is reversibly inhibited above  $2 \mu\text{M}$  oxygen (Strous et al., 1997a). Hence, the Anammox process should be carried out under strictly anaerobic conditions, and without the necessity for an additional organic carbon source.

A number of researchers reported that the application of the Anammox process requires a long start-up time due to their slow growing characteristics (Trigo et al., 2006, Jetten et al., 2009, Xiao et al., 2009). This slow growing characteristic of the bacteria, with a doubling time of 11-20 days (Jetten et al., 2009) is caused by their low substrate conversion rate (Strous et al., 1998). However, a doubling time of 1.8 days was later estimated from experiments in an anaerobic biological filter inoculated with a pre-culture (Isaka et al., 2005). The start-up of the Anammox process has been one of the most critical points in its application (Xiao et al., 2009), and hence considerable research effort has been dedicated to the more applied aspects of the process, particularly in the development and choice of reactors using Anammox. Various reactors, which include the fluidized bed reactor (van de Graaf et al., 1996), sequencing batch reactor (Strous et al., 1998), rotating biological contactor (Egli et al., 2001) and gas-lift reactor (Sliemers et al., 2003), were applied and optimised to start-up the Anammox process. Other successful systems were a reactor containing non-woven media for biomass immobilisation (Furukawa et al., 2003), an up-flow system seeded with anaerobic granular sludge (Imajo et al., 2004), and a non-woven rotating biological contactor (Chen et al., 2009).

However, a fraction of the generated biomass is inevitably washed out with the effluent in all these treatment systems, especially during unstable periods due to overloads, which provoke biomass flotation (Trigo et al., 2006). This situation requires further investigation since biomass retention inside the reactor is vital, especially in the case where Anammox activity of the inoculum is relatively low. In addition, the loss of a fraction of the sludge washed out with the effluent has also caused problems to the system (Trigo et al., 2006). A system with better efficiency and operation strategy is therefore required to avoid biomass being washed out with the effluent. Knowledge concerning the behavior of Anammox in a reactor during start-up, the interactive effects of various factors on the performance of the reactor, the types of aggregates formed by Anammox biomass, and the capacity of the systems to recover after  $\text{NO}_2^-$  build-up are still limited. The use of a membrane biological reactor for the treatment of wastewater would enable full biomass retention in an Anammox system, mitigating the issues previously described.

## 2.9 Partial Nitrification with the Anammox Process

Considering the drawbacks of the conventional nitrification and denitrification process in the treatment of nitrogen-containing wastewater, a process capable of biologically removing nitrogen but with less energy consumption in terms of aeration and without the need for external carbon sources is a very attractive option to make the whole process more sustainable. As such, the Anammox process has the potential to replace the conventional denitrification step if it is preceded by partial nitrification of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  in an initial aerobic reactor (Jetten et al., 1997, Fux et al., 2002, Schmidt et al., 2003, Op den Camp et al., 2006, Feng et al., 2007). This initial partial nitrification step produces  $\text{NH}_4^+/\text{NO}_2^-$  mixtures, which are subsequently reduced to nitrogen gas by the Anammox process, under strictly anoxic conditions. Prior to the Anammox process, a stable partial nitrification step must be performed by observing all the significant factors affecting the partial nitrification process, as previously discussed. The most essential aspects in the nitrification process are to continuously suppress the nitrite oxidisers, and to ensure that the molar ratio of  $\text{NH}_4^+/\text{NO}_2^-$  is about 1:1.3, as proposed by Strous et al. (1998) (Eq. 2-13).

The combination of partial nitrification and the Anammox process in the treatment of nitrogen-containing wastewater has gained significant attention from researchers. A number of processes such as SHARON (single reactor high activity ammonia removal over nitrite) (Mulder et al., 2001, van Kempen et al., 2001), CANON (complete autotrophic nitrogen removal over nitrite) (Sliekers et al., 2002), OLAND (oxygen limited autotrophic nitrification and denitrification) (Windey et al., 2005), SNAP (single-stage nitrogen removal using Anammox and partial nitrification) (Lieu et al., 2006), and SNAD (simultaneous partial nitrification, Anammox and denitrification) (Chen et al., 2009, Lan et al., 2011) have been identified and developed, based on the concept of partial nitrification and/or the Anammox process, in order to improve the efficiency of nitrogen removal, as well as to overcome the drawbacks of conventional treatment systems.

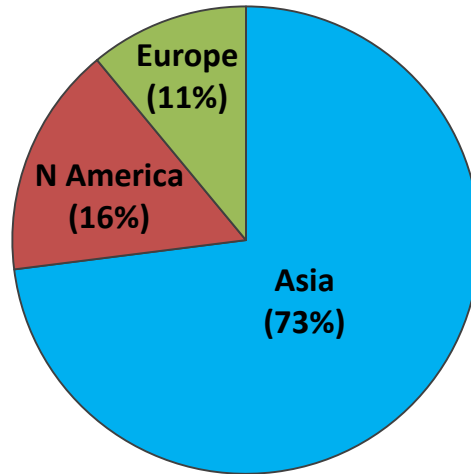
## 2.10 Membrane Bioreactor for Wastewater Treatment

A number of reactors have been developed over the last few decades for use in wastewater treatment aimed at achieving a high level of treatment with moderately low cost. Researchers had found that coupling membrane technology and biological reactors for the

treatment of wastewaters has led to the development of three generic membrane biological reactors (MBRs): for separation and retention of solids (water filtration); for bubble-less aeration (gas diffusion) within the bioreactor; and for the extraction of priority organic pollutants from industrial wastewaters (Stephenson et al., 2000, Visvanathan et al., 2000). The application of MBR technology for the separation of different types of contaminant from both municipal and industrial wastewaters has received considerable attention as they offer various advantages over the previously used technologies.

The MBR is generally described as a biological wastewater treatment process that uses membranes to replace the gravitational settling of the conventional activated sludge (CAS) process for the solid–liquid separation of sludge suspensions (Ng et al., 2006). This technology was first developed and commercially used 40 years ago. Among the earliest full-scale commercial MBR processes were those in North America in the late 1970s, and then in Japan in 1980s (Hu and Stuckey, 2006), followed by South Korea and China (Pearce, 2008). Historically, the market for MBRs has been dominated by activity in Asia. The regional share of the MBR market, based on the number of plants in 2003 is illustrated in **Fig. 2-8**.

In the UK, the first full-scale MBR plant for domestic wastewater treatment was installed in 1997 in Porlock, with a capacity of 1.9 megalitres per day involving a total membrane area of 2880 m<sup>2</sup> (Judd, 2002). Since then, there has been an increase in the number and diversity of applications for the technology. It was reported that by 2006, more than 100 municipal MBR plants with a capacity larger than 500 person equivalents were in operation in Europe. Currently, several thousand MBRs have been commissioned worldwide, and some are designed to treat up to 100 megalitres per day, i.e. in the Taihu Lake region, China (Le-Clech, 2010). Given the dramatic increase in both the number of operational MBR plants and their scale, confidence in this technology keeps increasing, making MBRs a technology of choice for wastewater treatment and reuse. In wastewater treatment, MBR technology can now be considered as an established system that competes directly with conventional processes such as activated sludge.



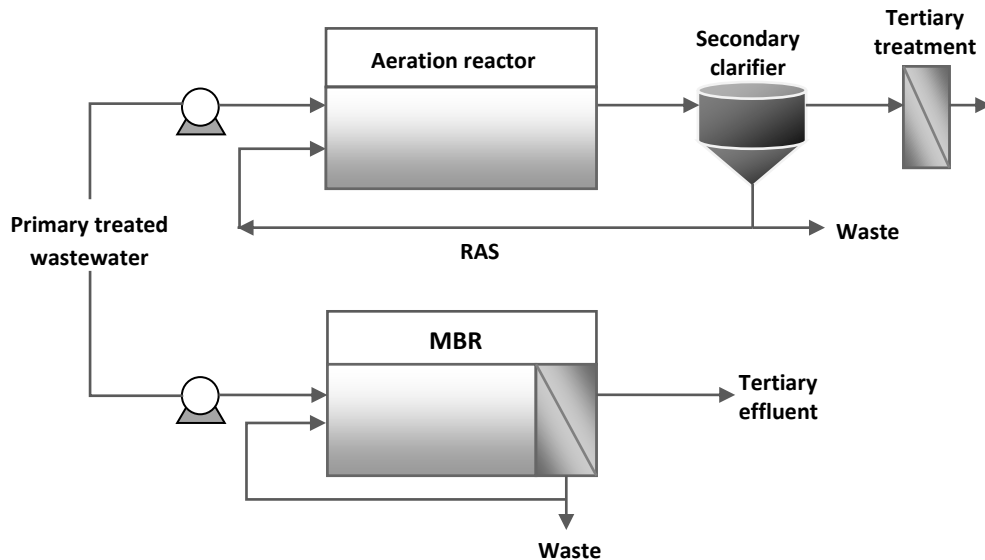
**Fig. 2-8** Regional share of the MBR market based on the number of plants in 2003 (Pearce, 2008).

The primary role of the membrane in an MBR is to provide a barrier against suspended solids. However, as mixed liquor from a bioreactor is often a complex mixture, the removal or partial removal of other species will largely depend on the choice of membranes. The most commonly used membranes in MBRs are either microfiltration (MF) or ultrafiltration (UF) membranes, while nanofiltration (NF) membranes are rarely applied due to their high hydraulic resistance, but may be of interest in niche applications (Fane and Chang, 2002). The application of the MF and UF membranes has led to significant improvements and advantages when MBRs are compared with conventional activated sludge processes. Certain physical and chemical properties of membranes that favour their use in MBRs are hydrophilicity, robustness, modest cost and ease of fabrication (Fane and Chang, 2002).

Among the advantages of MBRs is the large footprint reduction for the overall treatment system as the secondary clarifiers in conventional activated sludge processes are replaced by more compact membrane modules, as illustrated in **Fig. 2-9** (Le-Clech, 2010). Conventional activated sludge is normally followed by large gravity clarifier, or a membrane-based (MF/UF) filtration system (tertiary treatment) in a separate unit operation. However, MBRs provide an alternative to the CAS-MF/UF system by combining biological oxidation with the MF/UF membrane separation in a single unit operation. Furthermore, since biomass retention in an MBR is not based on gravitational settling, it allows for a



significantly smaller tank to be used for biological treatment. Unlike conventional activated sludge, a larger tank would be required to ensure good removal of low-density bio-solids that settle relatively slow (Pearce, 2008).



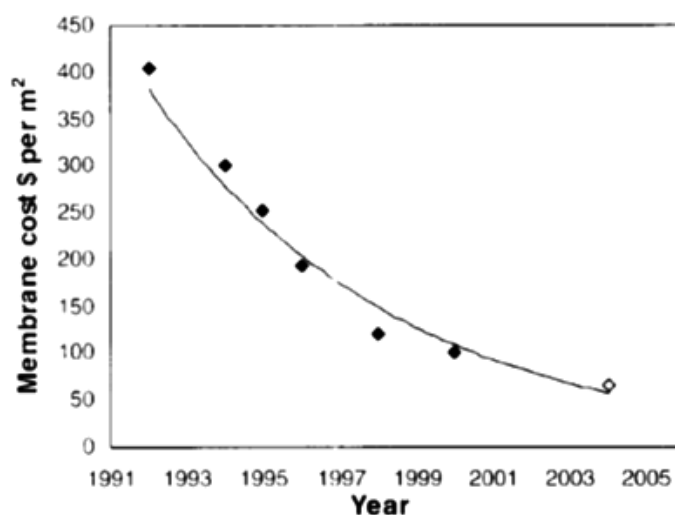
**Fig. 2-9** Comparison between conventional activated sludge and membrane bioreactor layouts (Le-Clech, 2010).

Apart from this consideration, the use of membrane filtration as a separation process also improves the quality of the produced effluent. It offers good disinfection capabilities by allowing the complete physical retention of bacterial flocs and most of the suspended solids (Pearce, 2008, Le-Clech, 2010). Due to its high-quality, MBR effluent can not only be directly discharged into the environment, but can also be reused for non-potable applications, i.e. irrigation and industrial application (Le-Clech, 2010). The more stringent regulations imposed for environmental discharge worldwide has also driven the recent development of MBR technology. Most industrial wastewater feeds that are difficult to treat commonly require the use of membrane technology to meet discharge standards. Food, pharmaceutical, paper and pulp, landfill, textile and meat industries are some of the examples for which the MBR has been successfully applied to treat high-strength wastewaters (Yang et al., 2006).

MBRs consist of compact reactors that may operate with high biomass concentrations and an absolute control of solids and hydraulic retention times (Trigo et al., 2006). The concentration of biomass as high as 35 g/L in an MBR system was previously

reported in the literature (Chang et al., 2006). High concentrations of the biomass can increase the efficiency of the treatment process, thereby improving the removal of dissolved components and reducing sludge production (Judd, 2006, Pearce, 2008), while the control of solids and hydraulic retention time can provide more flexibility in dealing with the variation of flow rates and feed quality (Pearce, 2008). Le-Clech (2010) further stated that operation at high solids retention times not only improves the retention of slow growing microorganisms, but it could also lead to low-sludge yields, resulting in sludge minimisation.

Although the use of an MBR for the treatment of wastewaters would enable full biomass retention in the system (Trigo et al., 2006, Pearce, 2008, Wyffels et al., 2004), attention should also be given to some of the limitations associated with MBR processes, such as the cost of membranes and operating cost due to fouling (Le-Clech, 2010). The MBR process also requires higher energy inputs compared with traditional wastewater treatment plants (Trigo et al., 2006). Pearce (2008) reported that the equipment and energy cost of an MBR are higher than conventional treatment, but the total water costs can be competitive due to the lower footprint and installation costs. As MBR technology has become accepted, while the scale of installation has increased, there has been a steady downward trend in membrane prices since the early 1990s, as depicted in **Fig. 2-10** (Churchouse and Wildgoose, 2000).



**Fig. 2-10** Trend of reduction in membrane replacement cost per m<sup>2</sup> (Churchouse and Wildgoose, 2000)

## 2.11 MBR Design and Configurations

There have been a number of different MBR designs (side-stream, submerged) and membrane configurations (flat plate, hollow fibre, spiral wound, tubular) reported in the literature, which are further discussed below.

### 2.11.1 Reactor Configurations: Side-Stream vs. Submerged

There are two main configurations for the design of MBRs: side-stream and submerged. The early generation of MBR systems used in the 1980s was based mainly on the side-stream (also known as cross-flow) configuration, in which the membrane is located outside the bioreactor as a separate unit, and the reactor liquor is circulated at high cross-flow velocity (around 2 - 4 m/s) via a recirculation pump (Fig. 2-11). In addition to being operated at high cross-flow velocity, the side-stream MBRs tend to operate at high transmembrane pressures (TMP, 2 – 7 bars) and permeate flux rates (70 – 100 L/m<sup>2</sup> h) compared with submerged membrane systems (Stephenson et al., 2000, Bérubé et al., 2006). Bérubé et al (2006) stated that both TMP and cross-flow velocity are the most crucial operating parameters that affect permeate flux rates in side-stream MBR system.

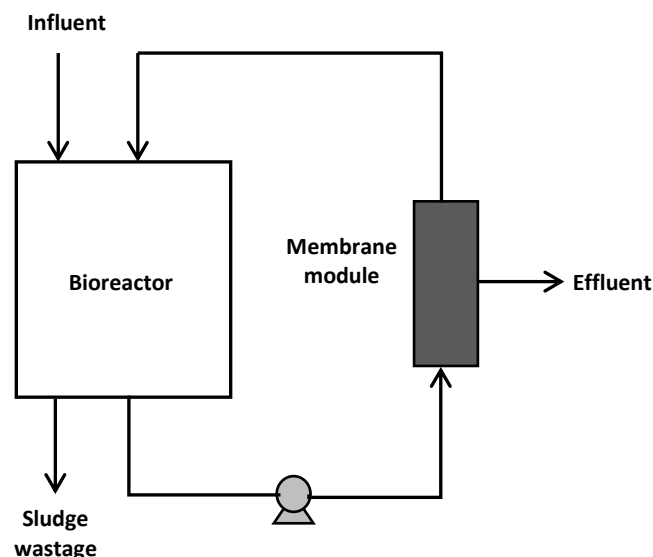
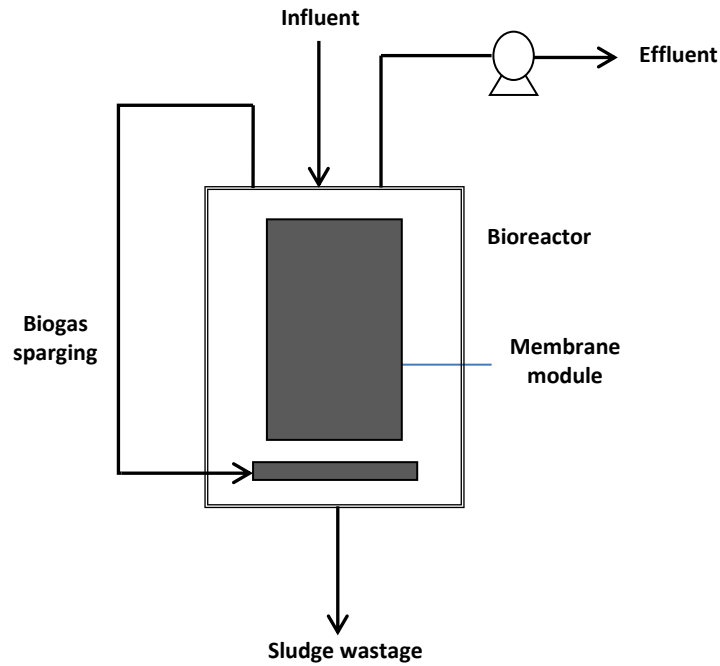


Fig. 2-11 Side-stream membrane bioreactor configuration.

This side-stream configuration has in the past been favoured over the submerged setup, since membrane removal, cleaning and replacement can be achieved without interfering with the reactor (Huang et al., 2008). Although featuring high permeate flux and relative simplicity, the system has rarely been developed on a large scale mainly due to the very fast fouling development (He et al., 2005) and high energy consumption of the recirculation pump (Le-Clech, 2010). Furthermore, high hydraulic shear from high mixed liquor recirculation not only reduces the microbial activity inside the reactors and affects biogas production (Kim et al., 2001), but also reduces the size of the biosolids in the mixed liquor and increases the release of soluble microbial products (SMP) (Bérubé et al., 2006). Given a number of factors in terms of energy consumption, fast membrane fouling, and shear damage that might cause a possible drop in the performance of side-stream MBRs, this option is no longer considered as the most promising.

Alternatively, the use of submerged bioreactor systems seems potentially the most interesting and favorable in terms of low operating costs, gentle mixing, and very high volumetric efficiency (Hu and Stuckey, 2006). Since the introduction of the submerged MBR systems in 1989, significant reductions in the capital and operating costs have been achieved, resulting in the development of a second generation of MBRs (Le-Clech, 2010). With the membrane directly submerged inside the bioreactor (**Fig. 2-12**), there is no pumping system required to pass the reactor liquor through the membrane unit, and the hydraulic shear force on the biomass is much lower. In this case, the slight negative pressure imposed on the permeate side is responsible for the driving force, allowing the clean water to permeate through the membrane (Le-Clech, 2010).



**Fig. 2-12** Submerged membrane bioreactor configuration.

The use of gas bubbling underneath the membrane has also been applied in this configuration as a method of membrane fouling control (Le-Clech et al., 2005) by producing a turbulent two-phase flow velocity (around 0.2 – 0.4 m/s) on the membrane surface, hence keeping the biomass in suspension (Le-Clech et al., 2003). Hu and Stuckey (2006) evaluated the effect of biogas sparging underneath both membranes in submerged anaerobic membrane bioreactors (SAMBR), and concluded that this method is as effective as aerobic reactors in maintaining reasonable fluxes in the reactors. Submerged reactor systems normally operate at lower TMPs (200 – 1000 mbar) and cross-flow velocity (less than 0.6 m/s) (Bérubé et al., 2006), resulting in lower permeate flux than that in the external side-stream systems, which consequently requires larger membrane areas. The TMPs, sparging intensity, and hydraulic retention time (HRT) have been identified as the most significant operating parameters that affect permeate flux rates in a submerged membrane system (Bérubé et al., 2006).

### 2.11.2 Membrane Configurations

Membrane configurations, i.e. the way they are shaped or housed to produce modules, are crucial in determining overall process performance. These configurations specifically determine characteristics such as relative energy demand, the ability to handle suspended solids, ease of cleaning and replacement, and the packing density (ratio of membrane area to module bulk volume) (Stephenson et al., 2000, Fane and Chang, 2002). **Table 2-2** summarises these characteristics for a range of membrane modules. The selection of suitable membrane modules for a particular application is as important as the selection of proper membrane materials. The standard MBR (direct membrane filtration of mixed liquor) requires a module that can handle suspended solids, has a relatively low energy demand and can accommodate reasonably high membrane packing densities. These requirements tend to suggest the use of contained flat sheet systems or submerged membranes (Fane and Chang, 2002).

**Table 2-2** Characteristics for a range of membrane modules (Fane and Chang, 2002).

<b>Characteristics</b>	<b>Flat Plate (FP)</b>	<b>Hollow Fibre (HF)</b>	<b>Spiral-Wound (SW)</b>	<b>Tubular</b>
Packing density	Moderate	High	High	Low
Energy	Low-Moderate	Low	Moderate	High
Solids handling	Moderate	Moderate/Poor	Poor	Good
Cleaning	Moderate	Backflushing possible	Difficult	Good-physical cleaning possible
Replacement	Sheet/cartridge	Element	Element	Tubes/element

In submerged MBR processes, membranes can be configured as either vertical or horizontal hollow fibres or vertical flat plates (flat sheet). These membranes are generally mounted in modules or cassettes, which include aeration ports, permeate flow connections, and supporting frames. Flat sheet membranes are the simplest as these are simply a supported sheet of the membrane material, while hollow fibre membranes are membranes with a small (<1mm) diameter. Hollow fibre membranes are generally cheaper to

manufacture, allow high membrane packing density and can tolerate vigorous backwashing (Le-Clech, 2010). However, fluid dynamics and distribution may be easier to control for flat plate and tubular membranes, where the membrane channel width is well defined (Cui et al., 2003). As a result, hollow fibres may be more susceptible to fouling and clogging, hence require more frequent and demanding cleaning protocol.

Amongst the numerous membrane manufacturers, Kubota (flat plate configuration), GE-Zenon, Mitsubishi Rayon and Siemens Water Technologies-Memcor (hollow-fibre configuration) are the main current membrane suppliers for MBR systems. Hu and Stuckey (2006) compared the performance of a SAMBR for the treatment of dilute wastewater using both Mitsubishi Rayon hollow fibre and Kubota flat sheet membranes, and concluded that both configurations had comparable COD removal rates. However, for the desired HRT, the flat sheet membrane required a lower TMP and was therefore slightly better. Huang et al. (2010), who studied the effects of HRT and SRT on the performance of SAMBR with flat plate module in the treatment of low-strength wastewater, highlighted that anaerobic MBR process are advantageous due to low production of biological waste, low nutrition requirements, ability to treat high organic loadings, and the formation of biogas as a useful end product (Huang et al., 2010).

## **2.12 The Anammox Process in an MBR**

The fact that Anammox are slow-growing bacteria that require efficient retention of biomass has driven research towards developing different types of MBRs. In the last 20 years, MBR technology has been utilised to promote biomass retention instead of secondary clarifiers in wastewater treatment plants (Trigo et al., 2006). In MBRs, the effluent is withdrawn via a membrane that is impermeable for microbial cells, which promotes cultivation of slow-growing bacteria with full biomass retention inside the reactor. Le-Clech (2010) reported that the complete retention of biomass within the MBR is also responsible for the development of slow growth nitrifying bacteria. Concurrently, biomass retention could minimise unintended wash-out of these slowly-growing bacteria in the effluent. MBRs have also appeared to be a promising alternative treatment technology, especially in the cases where spaces and water resources are limited, while high quality of water in the effluent is required (Cema et al., 2004).

MBRs can also enable the Anammox bacteria to grow as freely suspended cells in the reactor with a stirrer or gas bubbles, and thus a more homogeneous distribution of substrates and biomass can be achieved, and thus a high growth rate of the Anammox bacteria (van der Star et al., 2008). Cui et al. (2003) previously reported that gas bubbling or gas sparging was preferred in MBR operation, instead of mechanical stirrer, in a way that gas bubbles pose less risk to the membrane and to the cells, as well as are easily separated from the process stream. Other than to keep cells in suspension, gas bubbling could serve another purpose; in an aerobic reactor, it is used for oxygen supply, while in an anaerobic reactor, it is used for scouring the membrane to keep it relatively unfouled.

Due to the various advantages of using MBRs in wastewater treatment, and the efficiency of the Anammox process for the removal of nitrogen, the application of the Anammox process in MBRs have been widely studied (Cema et al., 2004, Trigo et al., 2006, van der Star et al., 2008, Suneethi and Joseph, 2011a, Wang et al., 2012). Wang et al. (2009) who had successfully started-up the Anammox process in a laboratory-scale MBR, proposed that MBRs could be developed as a brand-new alternative to starting-up the Anammox process as it is capable of overcoming some limitations faced by sequencing batch reactors (SBR) and other biofilm based reactors, and significantly reduce the start-up period of the Anammox process.

Further experiments were carried out to compare the start-up of the Anammox process in MBRs and SBRs, demonstrating that the Anammox start-up period in MBR (59 days) was much shorter than in a SBR (101 days); furthermore, the removal efficiency in the MBR was also higher than that of the SBR (Wang et al., 2012). Hence the MBR was proven to be a very efficient and powerful tool to cultivate slow-growing Anammox bacteria in a way that it does not only promote biomass retention, but also produce high purity cells inside the reactor (van der Star et al., 2008). The application of the Anammox process in an MBR has been proven to be a “perfect” and powerful combination that could be used to enhance the efficiency of the previous and existing nitrogen removal processes. However, other nitrogen removal processes and reactors must not be abandoned as they might be useful in some other ways. For example, van der Star et al. (2008) reported that the SBR has previously been widely used for the enrichment of the Anammox bacteria prior to the Anammox process in an MBR.



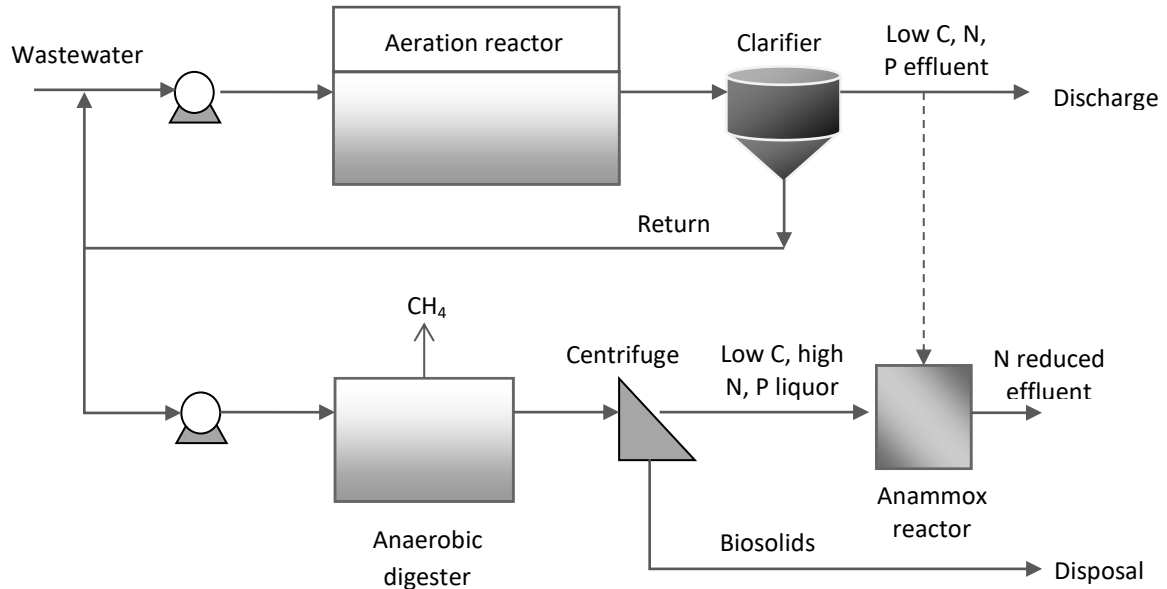
## 2.13 State of the Art: Integration of an Anammox Process into a Wastewater Treatment System

Although the activated sludge process remains one of the most commonly used treatment processes throughout the World for both industrial and municipal wastewater, the application of MBRs in wastewater treatment has also gained considerable attention due their advantages, as previously described in **Section 2.10**. The process layouts for both activated sludge and MBR systems were also compared and illustrated in **Fig. 2-10**. However, both activated sludge and MBR systems still require a tertiary treatment unit to further remove other types of contaminants, which includes nitrogen, depending on the waste strength and the required effluent quality. This tertiary treatment unit could either be the conventional nitrification-denitrification system, or the proposed Anammox process coupled with a partial nitrification reactor.

Apart from that, it was also reported that an aerobic SBR was integrated with anaerobic digestion and the Anammox processes for red meat processing wastewater in Australia (AMPC., 2016). The SBR process was used for the removal of carbon and nutrients, while the anaerobic digestion process was for sludge destabilisation and biomethane production, and any residual nitrogen mainly in the sludge dewatering liquor generated from anaerobic digester was eliminated using the subsequent Anammox process. The process flow diagram combining the three processes is as shown in **Fig. 2-13**. It was found that after the Anammox process, the sludge dewatering liquor containing acceptable concentration of nitrogen can be effectively discharged.

Based on many successful studies of the Anammox process at laboratory scale, the first full-scale granular Anammox reactor was built and operated in Rotterdam, the Netherlands with a size of 70 m<sup>3</sup>, designed to treat 500 kg- N/d (van der Star et al., 2007). The reactor was operated for about 3.5 years. Initially, it was inoculated with nitrifying sludge obtained from a wastewater treatment plant, followed by settled biomass from an Anammox enrichment reactor. The long start-up period was needed mainly due to a lack of Anammox sludge, particularly in the first two years. Although several other issues occurred, i.e. incidental nitrite toxicity, biomass washout and unexpected operational problems, the

operator still managed to treat 750 kg- N/d, higher than the original design load, proving the capability of Anammox granular technology on a full-scale.



**Fig. 2-13** Activated sludge process integrated with anaerobic digestion and the Anammox process (adapted from AMPC, 2016)

Besides the first application of the Anammox process in the Netherlands, many other full-scale partial nitrification-Anammox systems have been constructed over the past decade. Lackner et al. (2014) reported that by 2014 there were about 100 such facilities in operation worldwide which mostly focused on treating wastewater containing high ammonium concentrations, particularly reject water. It was also revealed that SBR technology was the most commonly used reactor, i.e. more than 50% of all partial nitrification-Anammox systems, followed by granular systems and MBBRs (Lackner et al., 2014). **Table 2-3** provides a summary of the operational information for the Anammox systems worldwide. As of 2013, more than 30 full-scale Anammox plants were in operation around the world (Ni and Zhang, 2013), indicating that the Anammox process has started to be widely used as a commercial technique, and is becoming the preferred choice for the treatment of nitrogen-containing wastewater.

**Table 2-3** Some of the full-scale Anammox systems operated worldwide and their operational information (Ni and Zhang, 2013)

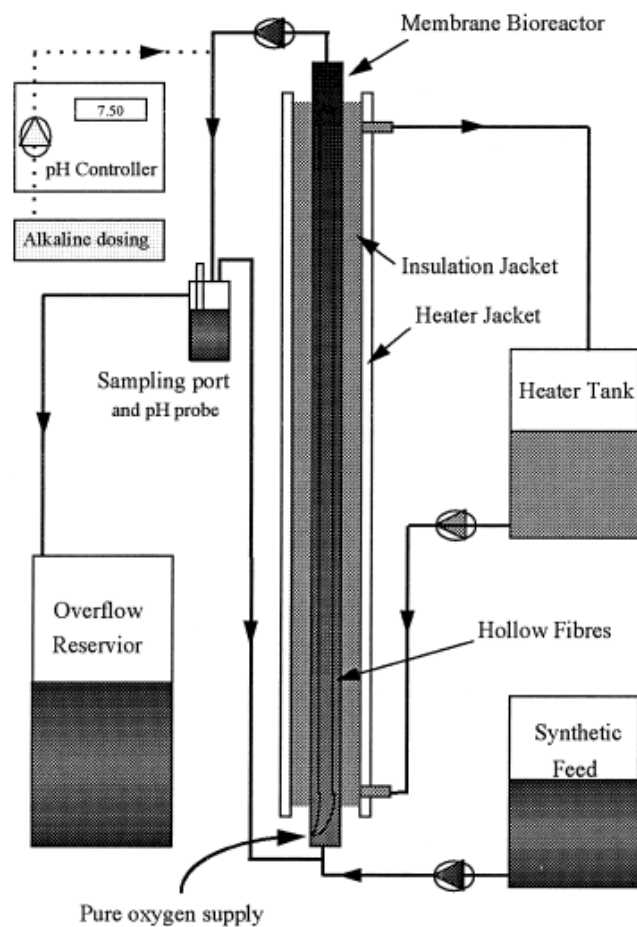
Process	Location	Influent	Reactor volume (m <sup>3</sup> )	Designed load (kg N/d)	Year
SHARON-Anammox	Rotterdam, NL	Reject water	72	500	2002
Nitrification-Anammox	Lichtenvoorde, NL	Tannery	100	325	2004
Anammox	Olburgen, NL	Potato processing	600	1200	2006
Nitrification-Anammox	Mie prefecture, JP	Semiconductor	50	220	2006
Anammox	Niederglatt, Switzerland	Reject water	180	60	2008
Anammox	Tongliao, China	Monosodium glutamate	6600	11000	2009
Anammox	Yichang, China	Yeast production	500	1000	2009
Anammox	The Netherlands	Reject water	425	600	2010
Anammox	Poland	Distillery	900	1460	2011
Anammox	Wuxi, China	Sweetener	1600	2180	2011
Anammox	Coventry, UK	Reject water	1760	4000	2011

## 2.14 Pre-Oxidation of Ammonium Using Membrane Modules

As previously highlighted, the combination of partial nitrification and the Anammox process for the removal of nitrogen from nitrogenous wastewater has been extensively reported in the literature. Partial nitrification is often carried out in a CSTR (Hellinga et al., 1998, Mosquera-Corral et al., 2005b, Chen et al., 2010), i.e. SHARON reactor without heterotrophic denitrification, or in an activated sludge unit coupled with an external settler (Campos et al., 1999, Ruiz et al., 2003), where all the operational conditions favourable to the growth of AOB are carefully controlled and maintained. However, the major drawback of using these reactors for partial nitrification are the large space requirements, and this could be quite challenging when the reactor needs to be scaled up to a much larger capacity to accommodate larger production requirement. For example, a full scale SHARON reactor (CSTR) with a working capacity of 1800 m<sup>3</sup> was needed in order to treat 830 kg N/day (Mulder et al., 2001) as operated at the Dokhaven wastewater treatment plant in Rotterdam.

Many other reports, however, suggested that oxidation of ammonium, including partial nitrification can also be carried out in a membrane bioreactor, which is also called a membrane aerated biofilm reactor (MABR) (Brindle et al., 1998, Casey et al., 1999, Feng et al., 2007, Dvorak et al., 2013). The use of a membrane that is usually incorporated into a bioreactor would allow the biomass to attach to it forming a biofilm layer, although it may also be suspended in liquid phase (cell suspension) (Reij et al., 1998). Gas-permeable membranes, e.g. hollow fibres, have been widely used in the MABR as they allow higher oxygen transfer efficiency due to their large surface area, whilst occupying a relatively small volume within the reactor (Brindle et al., 1998). This system offers various advantages, as it provides a very large surface area for biofilm attachment, protects microorganisms against unfavorable surroundings, and enables high sludge retention times (Feng et al., 2007). High sludge retention times are important to prevent washout of the nitrifying bacteria, thus maintaining a high biomass concentration in the system.

Brindle et al (1998) previously showed that a vertical laboratory-scale tubular reactor containing porous polyethylene hollow fibers with a pore size range of 0.04 - 0.1  $\mu\text{m}$  (UF) where a nitrifying biofilm attached to the membrane surfaces was capable of oxidising  $\text{NH}_4^+$  to  $\text{NO}_2^-$ . Pure oxygen (or air) was supplied through the tube side (lumen) of the module, which passed through the membrane walls and was utilised by the bacteria in the biofilm, while synthetic wastewater containing  $\text{NH}_4^+$  was supplied through the bulk phase (shown in **Fig. 2-14**). This concept, i.e. growing a nitrifying biofilm on gas permeable membranes, has later been widely applied mainly for the removal of nitrogen through nitrification (Semmens et al., 2003, Satoh et al., 2004, Feng et al., 2007, Feng et al., 2008), although different reactor configurations, types and characteristics of membranes, operational strategies, and sources of biomass were used.



**Fig. 2-14** Schematic of a vertical laboratory-scale MABR containing UF-HF membranes. The reactor length, ID and working volume were 1.47 m, 7 mm and 430 mL, respectively (Brindle et al., 1998).

These previous reports have shown that the use of membrane modules to carry out  $\text{NH}_4^+$  pre-oxidation, i.e. partial nitrification, as a pre-treatment for the Anammox process is very feasible at a laboratory scale. However, the selection of appropriate membrane filtration systems to be used in a module (bioreactor) has to be carefully made, and this usually depends on several factors, i.e. membrane materials, pore size, selectivity, and permeability. Membranes that are normally used in MABRs can be categorized into three types: microporous membranes (polyethylene), dense membranes (silicone), and composite membranes (Casey et al., 1999). Microporous membranes are generally preferred due to their higher permeability compared with dense membranes, although the latter can be more advantageous due to its higher specific selectivity and lower susceptibility to membrane clogging (Reij et al., 1998), although they cannot be used for ionic species due to their charge. Composite membrane possesses both the characteristics of microporous and dense

membranes since a thin layer of dense material is used to coat a microporous membrane, making it more suitable to be used in MABRs to achieve high-rate nitrification (Casey et al., 1999).

Nanofiltration (NF) membranes have recently become an advanced technology and are of the most appropriate methods for water treatment, i.e. water softening, removal of colorants and organic matter (Szoke et al., 2003, Lin et al., 2007, Izadpanah and Javidnia, 2012). They are preferred to MF, UF and RO membranes due to their high retention of multivalent anion salts, and organics with molecular sizes of above 300, and can be operated at low operating pressure and are more cost effective than RO membranes in certain applications (Ali et al., 2010). With a pore size smaller than UF and larger than RO, NF membranes display separation characteristics in the intermediate range between the UF and RO, which can widely separate solvent, monovalent salts and small organics from divalent ions and larger species (Mulder, 1996, Ali et al., 2010). The application of a composite NF membrane module not only is suitable for the oxidation of  $\text{NH}_4^+$  using the nitrifying biofilm concept, but at the same time it can also be used to separate other unwanted large species from wastewater. In such cases, one of the important characteristics of the membrane module that makes it suitable for this application is to have high  $\text{NH}_4^+$  permeability (high flux) and high rejection towards unwanted solutes, e.g. organic solutes, and monovalent ions.

## 2.15 Summary of the Literature

The overall picture obtained from reviewing the literature is as follows:

1. It has been widely reported that the presence of excess nitrogen, mainly in the forms of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and free  $\text{NH}_3$  in wastewaters that mainly result from various industries can pose adverse effects both on human and aquatic ecosystems (methemoglobinemia in infants, eutrophication, toxicity), thus triggering the relevant authorities and environmental experts to enforce more stringent laws and develop better treatment technology for nitrogen removal.
2. Conventional biological nitrification-denitrification has long been the most commonly used process for the removal of nitrogen from both municipal and

industrial wastewater. In this process,  $\text{NH}_4^+$  is first aerobically oxidised to  $\text{NO}_2^-$  through the action of AOB, i.e. *Nitrosomonas*, and  $\text{NO}_2^-$  is subsequently oxidised to  $\text{NO}_3^-$  by NOB, i.e. *Nitrobacter*; this is followed by anoxic denitrification of  $\text{NO}_3^-$  to nitrogen gas by means of denitrifying bacteria. However, the process is inefficient and expensive as it requires large amounts of oxygen (2 moles of  $\text{O}_2$  is consumed for each mole of  $\text{NH}_4^+$  oxidised to  $\text{NO}_3^-$ ) for nitrification, and the addition of an external carbon source (methanol) during the denitrification stage, particularly when wastewaters do not contain sufficiently high biodegradable carbon.

3. The Anammox (Anaerobic Ammonium Oxidising) process was later discovered in 1994 and its use has since been gradually growing for the treatment of nitrogen-containing wastewaters. This process uses  $\text{NO}_2^-$  as the electron acceptor and was shown to be capable of removing  $\text{NH}_4^+$  and  $\text{NO}_2^-$  (in a molar ratio of 1:1 to 1:1.3) simultaneously from wastewater in the complete absence of oxygen, thus eliminating the need for aeration and the addition of an external carbon source during denitrification. The Anammox process was reported to be able to save up to 90% of the operational costs compared with conventional treatment systems. However, the Anammox bacteria (obligate anaerobes, found in the early 1990s) are a very slow-growing microorganism with a doubling time of 11-20 days, hence requiring a long start-up time.
  
4. In 1998, a process known as SHARON (Single reactor High activity Ammonia Removal Over Nitrite) was successfully developed for the treatment of ammonium rich wastewaters, i.e.  $> 500 \text{ mg/L}$ . In this process, both aerobic nitrification and anoxic denitrification were combined in a single CSTR system using intermittent aeration where  $\text{NH}_4^+$  is partially oxidised to a mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  under aerobic conditions, and then  $\text{NO}_2^-$  is reduced to nitrogen gas under anoxic condition with the addition of a carbon source (methanol). The SHARON process was advantageous since it requires 25% less aeration (energy) because oxidation is stopped at the  $\text{NO}_2^-$  stage, and it consumes 40% less added carbon since only  $\text{NO}_2^-$  needs to be reduced to nitrogen gas. However, due to the need for the addition of an external carbon source during denitrification stage that contributes towards high operating costs, the SHARON process was still subjected to some further modifications.

5. The concept of the SHARON process, i.e. partial nitrification of  $\text{NH}_4^+$ , however, was very useful when it is combined with the Anammox process in series, and has been shown to be capable of significantly improving the efficiency and reducing the cost for nitrogen removal from wastewater. The mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  produced from the partial nitrification process is subsequently fed to an Anammox reactor, where both components are converted to nitrogen gas by the Anammox bacteria under anoxic conditions. This process combination offers great advantages over the conventional system of nitrogen removal, particularly in terms of operational cost, not only because of the exclusion of the external carbon sources and aeration, but also due to low amounts of surplus sludge production.
  
6. Since the discovery of the Anammox process, its application in various types of bioreactor has been widely reported in the literature. Different experimental designs and reactor configurations, as well as operational strategies have been applied with the aim of improving process performance and achieving maximum efficiency of nitrogen removal from wastewaters. Apart from this, different types of bacterial culture have been used to enrich and grow the Anammox bacteria, and start-up the process in a bioreactor. Of all types of bioreactors, there has been a strong trend towards the use of submerged MBRs, not only because of the lower operational costs and space, but also because of their better treatment efficiency as it can reduce the process start-up time due to the presence of membrane inside the reactor that can promote biomass growth and prevent wash-out. The use of submerged MBR systems is necessary considering the slow-growing characteristics of the Anammox bacteria.
  
7. Preceding the Anammox reactor, partial nitrification is often carried out in a single CSTR system, i.e. SHARON reactor, an activated sludge unit coupled with external settler, or in an MABR. The concept of using a nitrifying biofilm on a membrane tubes (mostly UF-HF membranes) incorporated in a module/bioreactor has recently been preferred for nitrification as it allows for higher oxygen transfer efficiency due to its high surface area, protects microorganisms from unfavourable conditions, and achieves high sludge retention time. The use of composite NF-HF membranes, however, is more promising as NF displays better removal efficiency than UF and more cost effective than RO.



## 2.16 Problem Statement

Based on the summary of the literature above, the main drawback of the conventional biological nitrification-denitrification process for the treatment of nitrogen-containing wastewater is its large requirement for oxygen, while the addition of an external carbon source (methanol) during the denitrification stage further adds to the operating cost. As the Anammox process was shown capable of simultaneously removing  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , it has been gradually used to replace the conventional nitrification-denitrification process. However, the Anammox process requires a long start-up time due to the slow-growing nature of the bacteria. Researchers have proposed using different techniques, types and reactor configurations to shorten the Anammox start-up period, including the use of a submerged membrane bioreactor.

Back in 2004, a novel 3-liter laboratory-scale submerged anaerobic membrane bioreactor (SAMBR) was designed by Hu (2004) and built at Imperial College London to be used for the removal of various contaminants from wastewater through an anaerobic process. Although the reactor's capability in removing COD (Hu, 2004), saline organic waste (Vyrides, 2009), and bacteriophages (Fox, 2012) from wastewater was previously proven, no work has been carried out to investigate the feasibility of using the reactor for the removal of nitrogen, particularly by incorporating the Anammox process into the reactor. Stuckey (2012) in a review on recent developments in anaerobic membrane reactors, suggested that more work is needed in order to better understand the complexity of the process, and one of them was to investigate whether nitrogen removal can be carried out in anaerobic membrane bioreactors.

As such, this work focused on carrying out the Anammox process in this SAMBR, examining the feasibility of the process, and investigating the reactor performance with respect to some controllable operational parameters. The findings of the study would be able to answer whether the Anammox process in the reactor is feasible, and whether the process start-up time can be reduced, comparable to some recently reported values available in the literature. The process performance, often represented by the removal efficiency of nitrogen, and the duration of process start-up are highly dependent on the types of biomass used, reactor types and configurations, and operating conditions. In this study, wastewater

sludge collected from an anaerobic digester in the UK was used to enrich and grow the Anammox bacteria.

Preceding the Anammox process,  $\text{NH}_4^+$  must be partially oxidised to a mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , but not  $\text{NO}_3^-$ . This partial nitrification process is often carried out using a CSTR system, activated sludge unit or MABR with a strict control of operating conditions to ensure that only ammonium oxidisers grow, while the growth of nitrite oxidisers must be continuously suppressed. Apart from the strict requirements for operating conditions, the conventional partial nitrification reactor requires a large footprint, and hence has a high cost too. Most of the previous reports on the use of MABRs for nitrification showed that the UF-HF membranes incorporated into a bioreactor or module act as a supporting material for the nitrifying bacteria to grow on and form a biofilm layer. This study intends to use this concept, i.e. nitrifying biofilm on membrane surfaces, with slight modifications, where  $\text{NH}_4^+$  is fed through the tube side while nitrifying bacteria (enriched from wastewater sludge) are grown on the outer surface of the membranes by continuously circulating the cell suspension through the shell side. This system is believed to be able to improve the removal efficiency, and reduce the operating costs compared with the existing treatment technology.

## **2.17 Objectives of Study**

The previous findings on nitrogen removal in membrane bioreactors can be used as an important basis on which to carry out further research in order to improve the process, but they are limited by certain conditions, i.e. reactor configurations and sizes, operational strategies, membrane types and configurations, and source of biomass. There was no single process that could represent the whole scenarios related to this field, instead a combination of findings from different researchers would be able to provide better view on how the process works and can be further improved.

### **2.17.1 General Research Aim**

In view of the above observations, this study aims to develop an innovative, efficient and economical biological process for the removal of nitrogen from wastewaters using Anammox bacteria in a 3-litre laboratory-scale submerged anaerobic membrane bioreactor

(SAMBR). Prior to the Anammox process in the SAMBR, wastewater containing  $\text{NH}_4^+$  was pre-oxidised to  $\text{NO}_2^-$  by ammonium oxidisers using NF membrane modules. This pre-oxidation process using NF membrane filtration system could become a promising alternative for the conventional partial nitrification reactor. It would be expected that this study could provide some useful information on the feasibility and efficiency of the Anammox process in a SAMBR, alongside the pre-oxidation process using the selected NF membrane system.

### 2.17.2 Specific Scientific Objectives

1. To start-up and investigate the performance of the Anammox process in a 3-litre laboratory-scale submerged anaerobic membrane bioreactor (SAMBR). The Anammox bacteria were enriched from anaerobic digestion sludge, with two different HRTs applied, i.e. two and four days. The aim of this Anammox process was to subsequently reduce the effluent from the preceding pre-oxidation step containing a  $\text{NH}_4^+/\text{NO}_2^-$  mixture to nitrogen gas ( $\text{N}_2$ ). However, both the pre-oxidation step and the Anammox reactor were not connected in series. The reactor performance and process efficiency were monitored based on the  $\text{NH}_4^+$  and  $\text{NO}_2^-$  removal efficiency, biogas production, COD removal and other characteristics of SAMBR effluent, i.e. biomass development.
2. To grow, enrich and study the characteristics of nitrifying bacteria, specifically the ammonium-oxidising bacteria (AOB) in synthetic wastewater containing  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and other mineral salts in 2-litre laboratory-scale batch reactors. The bacteria were enriched from two different sources of sludge; (1) activated sludge treatment plant in London, and full-scale SAMBR in Cambridge. The successfully enriched nitrifying bacteria would be subsequently used in the pre-oxidation of  $\text{NH}_4^+$  prior to the Anammox process. The next step was highly dependent on the successful enrichment of the bacteria since there was no pure culture of nitrifiers used in this study.
3. To perform the pre-oxidation process of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  using a NF membrane module consisting of concentric hollow fibre membrane tubes. The aim of the process was to propose a feasible alternative for the conventional partial nitrification

process that is normally in combination with the Anammox process. The nitrifying bacteria enriched in the previous step were grown on the shell side of hollow fiber membrane tubes, and thus the bacteria would oxidise the  $\text{NH}_4^+$  diffusing through from the tube side (feed). The feasibility and process performance were accessed based on the membrane permeability and rejections of  $\text{NH}_4^+$  and glucose, as well as the final molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  produced in the retentate (1:1 to 1:1.3) at the end of the process.

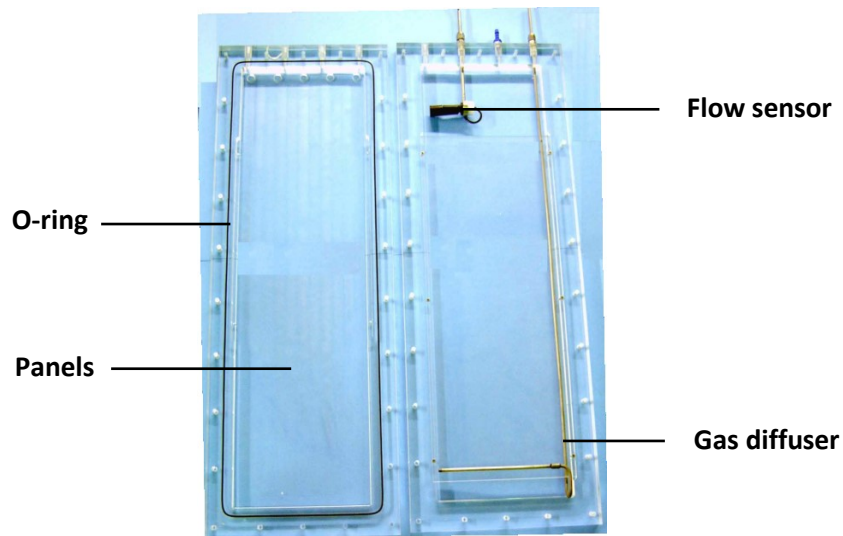
## **CHAPTER 3**

# **MATERIALS AND METHODS**

This chapter contains a description of the materials and methods used in this study. This includes the detailed design of the Submerged Anaerobic Membrane Bioreactor (SAMBR) system, the process design and operational parameters for the treatment of nitrogen-containing wastewater, as well as information on the biomass and their growth media. However, details of some specific process designs or operational strategies were made available at the beginning of each chapter in which they were relevant. Finally, the analytical techniques used for monitoring the process performance are also documented.

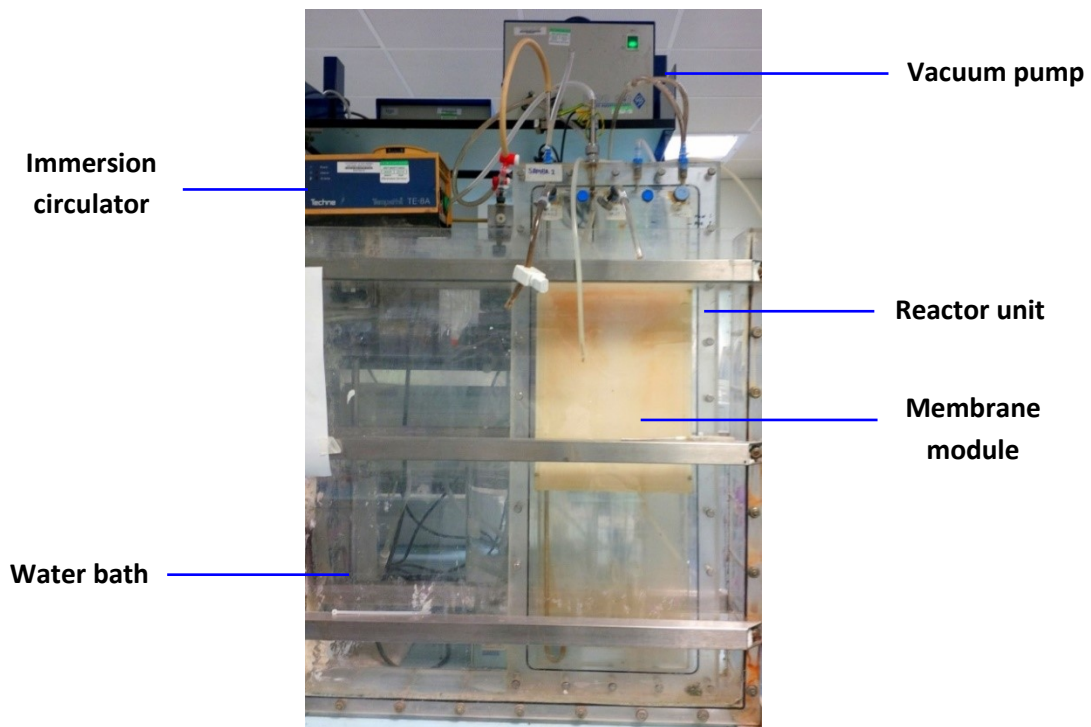
### **3.1 Submerged Anaerobic Membrane Bioreactor (SAMBR)**

The SAMBR used in this study was designed and fabricated in the Chemical Engineering departmental workshop (Hu, 2004). The reactor had an optimum working volume of three litres of sludge with head space for gas to be collected. It consisted of two transparent flat panels of cast acrylic plastic screwed together with an O-ring seal. A flat plate membrane module was submerged inside the main reactor unit and the effluent was pumped out through it. Biogas collected at the top of the reactor was pumped around the system and into a long stainless steel diffuser at the bottom of the reactor. The reactor transparent flat panels with a stainless steel diffuser are shown in **Fig. 3-1**. The coarse bubbles from the diffuser rise up and are forced along both sides of the membrane due to the baffle in the unit to provide scouring. This scouring action was intended to minimise the build-up of foulants on the membrane, while providing good mixing for the system.



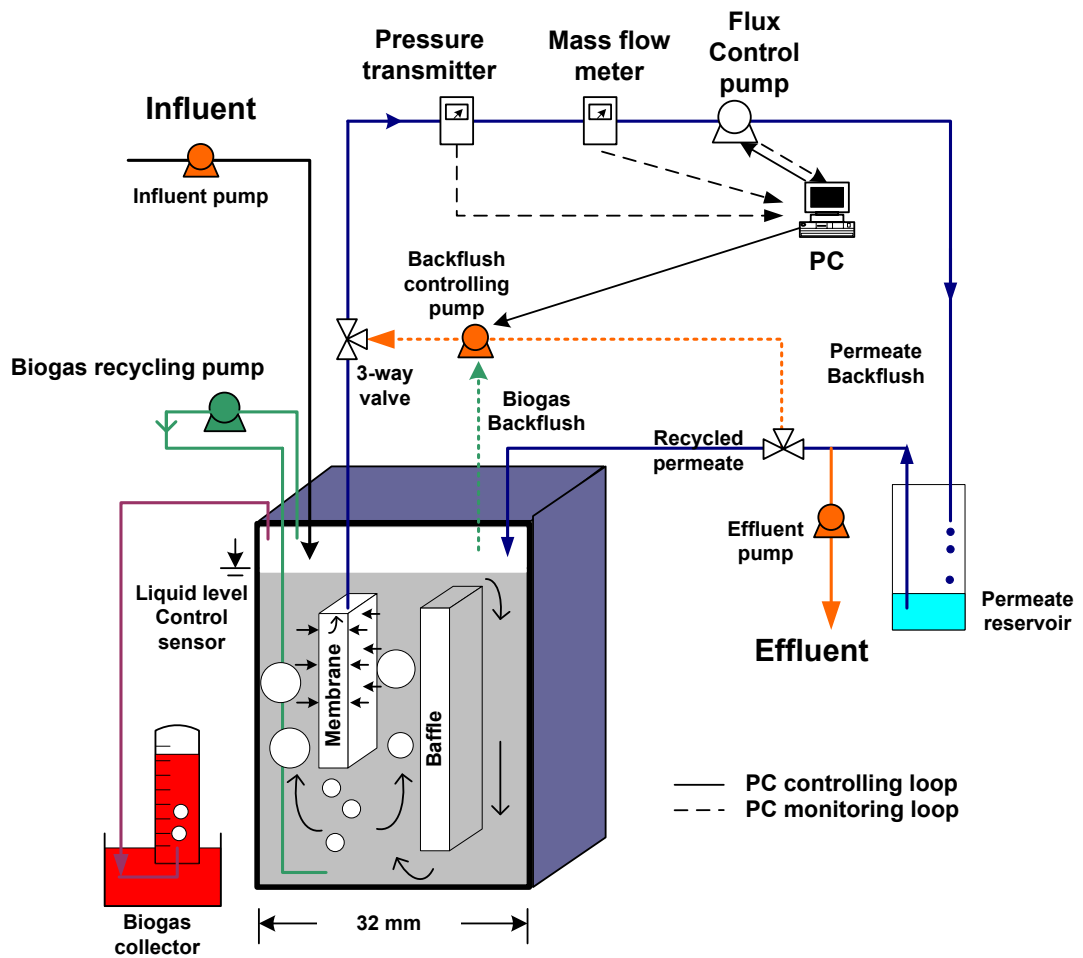
**Fig. 3-1** The SAMBR flat panels, with a stainless steel diffuser.

Once the reactor was assembled, it was placed in a water bath, where a portable immersion circulator (Techne, TE 8A) was used to maintain water temperature inside the water bath at about 35 °C. **Fig. 3-2** shows a complete SAMBR unit placed in a water bath. The water bath was later fully insulated with polystyrene and covered with aluminium foil to minimise the heat loss and prevent light from enhancing the growth of photosynthetic bacteria/algae inside the reactor.



**Fig. 3-2** A complete SAMBR unit placed in a water bath.

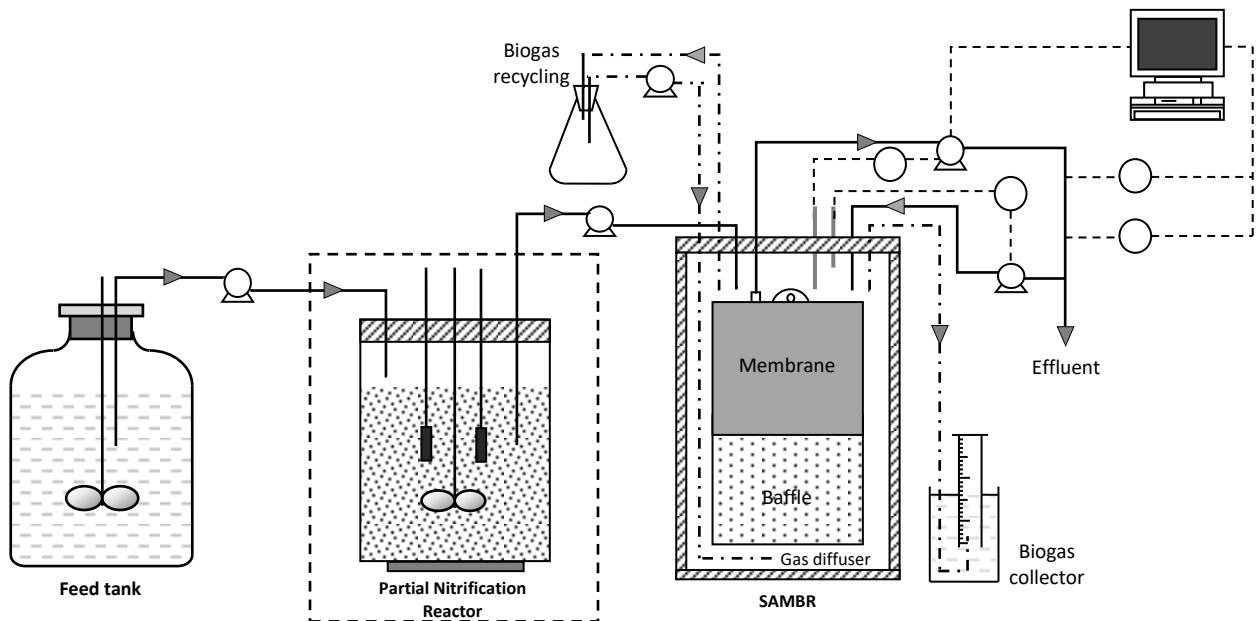
The whole SAMBR system can be divided into a few major parts; the main reactor, liquid level control system, biogas recycling system, biogas production collecting system, and process control unit. The feed and effluent were pumped by variable-speed peristaltic pumps (Watson-Marlow, 101U), while the gas line used a vacuum pump (Charles Austin, B100 SEC) drawing from the gas space of the reactor to create the scouring bubbles in the reactor, thus keeping the biomass and substrate in suspension at all times. The original schematic diagram illustrating a complete system of SAMBR operations was first drawn by Hu (2004), as shown in **Fig. 3-3**. Detailed designs and drawings of the SAMBR panels, with their exact dimensions, are shown in **Appendix 1**.



**Fig. 3-3** Schematic diagram of a SAMBR operation (Hu, 2004).

## 3.2 Process Design and Configurations

A schematic of the process flow diagram of an initial partial nitrification reactor followed by a SAMBR is shown in **Fig. 3-4**. However, as previously proposed and described in the objectives of study (**Section 2.15**), the initial partial nitrification reactor would be replaced by a pre-oxidation process of  $\text{NH}_4^+$  using nano-filtration (NF) membrane modules.



**Fig. 3-4** Schematic of a partial nitrification reactor and SAMBR process flow diagrams.

## 3.3 Membrane Modules

This study involved two different types of membranes modules for two different purposes. They were Kubota flat membrane panel used in the SAMBR; and nano-filtration membrane modules used for the pre-oxidation process of  $\text{NH}_4^+$  into  $\text{NO}_2^-$  prior to the Anammox process in a SAMBR. Details and characteristics of both membranes are further described below.

### 3.3.1 Kubota Flat Membrane Panel (for SAMBR)

The membrane used in this project was a Kubota type 203 module, which was donated by Kubota UK. The membrane module consisted of a solid acrylonitrile butadiene



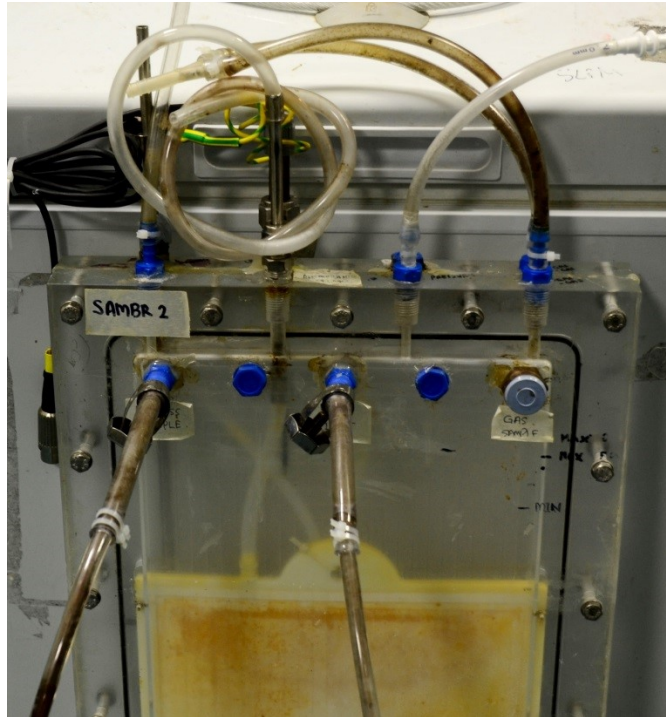
styrene support plate (5mm thick) with a spacer layer between it, welded to the polyethylene flat sheet membrane. The pore size was 0.4  $\mu\text{m}$  on a total membrane surface area of 0.11  $\text{m}^2$ . The membrane module is shown in **Fig. 3-5**.



**Fig. 3-5** Kubota (type 203) flat panel membrane module used in the SAMBR.

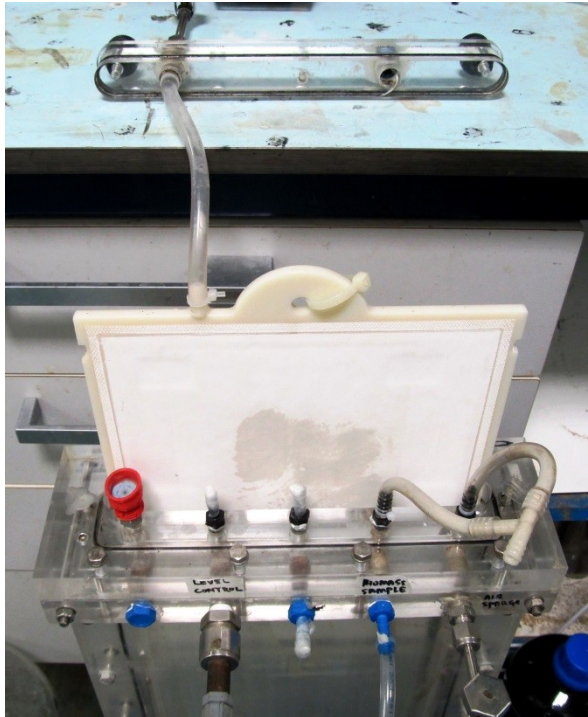
### **3.3.2 Membrane Access in SAMBR**

There were two slightly different reactor designs used in this study, the original reactor design was the one completed by Hu (2004) as illustrated in **Fig. 3-6**. Based on this original design, the membrane was encased inside the reactor where the two flat panels are sealed and screwed together using 26 screws and nuts. The drawback of this design was that the only way to take the membrane module out of the reactor for the purpose of analysis or cleaning is by unscrewing all the 26 screws and nuts in order to disassemble the whole reactor unit. It would take a lot of time not only to disassemble the reactor, but to reassemble it once the membrane module was cleaned.



**Fig. 3-6** The original design of how the membrane is encased inside the reactor unit. The membrane module could only be removed by disassembling the whole reactor unit.

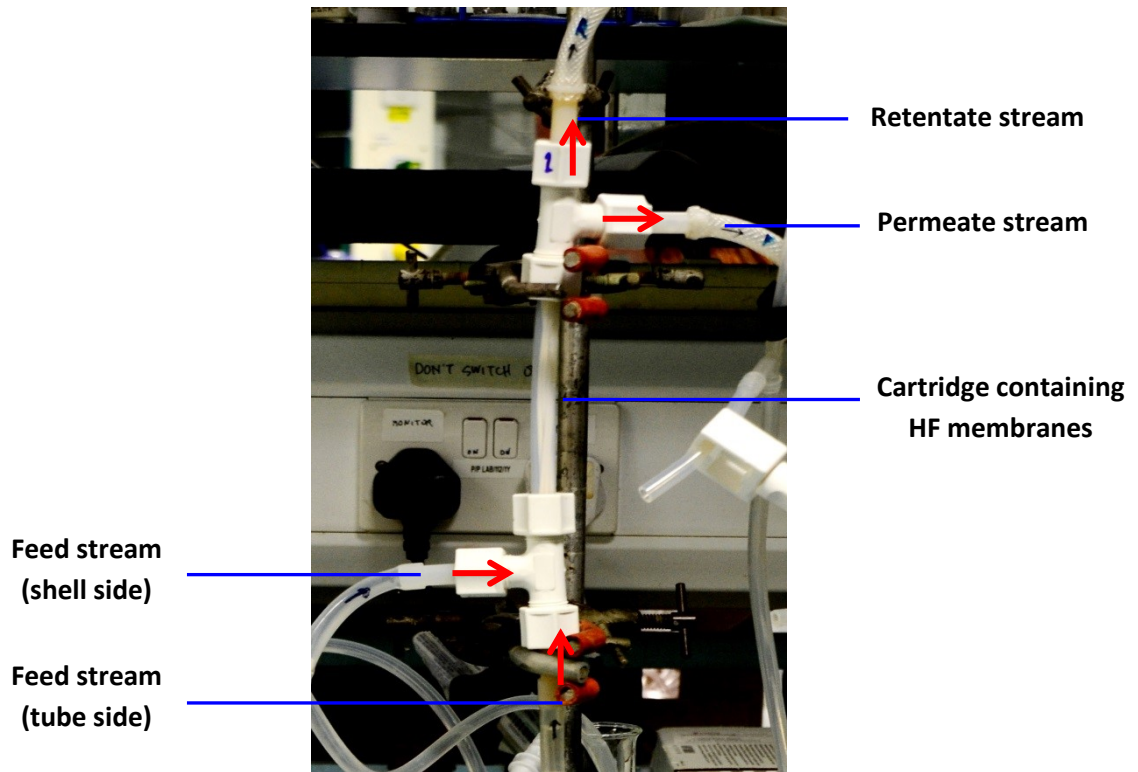
In order to allow for easier access to the membrane, a second reactor was built in 2008. This newly designed SAMBR did not require a complete disassembly of the reactor unit to enable the membrane module to be taken out. A new top section was designed such that a segment could be removed from the top of the reactor which was wide enough for the membrane to be pulled out, as shown **Fig. 3-7**. This would save a significant amount of operational time when compared with the original design. The segment was sealed into the reactor with an O-ring, and weighted down during operation to prevent any high pressure inside the reactor causing a gas leak. To access the membrane the sparging pump was first switched off, and a mixture of 70% N<sub>2</sub> and 30% CO<sub>2</sub> was bubbled into the reactor through the sample port to maintain anoxic conditions in the reactor when the membrane was lifted out.



**Fig. 3-7** New design of how the membrane was inserted inside the reactor unit. The membrane module could be removed without the need to disassemble the whole reactor unit.

### **3.3.3 Nano-Filtration Membrane Modules (Pre-Oxidation Process)**

In this study, there were two identical composite nanofiltration (NF) hollow fibre (HF) membrane modules used for the pre-oxidation process of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , aimed at replacing the initial partial nitrification process prior to the Anammox process in a SAMBR. These membrane modules were prepared by research colleagues in Nanyang Technological University (NTU) in Singapore. **Fig. 3-8** shows how the module was built; with six hollow fibre membrane tubes housed in the cartridge, while containing two inlet streams and two outlet streams. Meanwhile, the characteristics of the membrane modules are listed in **Table 3-1**:



**Fig. 3-8** The NF membrane module brought from NTU, Singapore.

**Table 3-1** Characteristics of NF membrane modules (Wang, Personal communication).

Characteristics	Values
Pore size	1.29 nm
Membrane surface area	0.0025 m <sup>2</sup>
MWCO	500 Da
No. of HF membranes inside each cartridge	6
HF ID	1.05 mm
HF OD	1.38 mm
Cartridge length	22 cm
Cartridge diameter	2 cm
Cartridge volume	69 mL

This NF membrane module was used for the pre-oxidation process of  $\text{NH}_4^+$  into  $\text{NO}_2^-$  prior to the subsequent process in a SAMBR. The aim was to grow a nitrifying bacterial culture on the shell side of the HF membrane tubes by continuously circulating the

cell suspension through the side stream of the module. Meanwhile, solution containing  $\text{NH}_4^+$ /glucose at certain concentration was fed through the tube side, and due to its low molecular weight compared to most of the organics in solution, e.g. glucose, it was expected that more  $\text{NH}_4^+$  would diffuse through the membrane than the organics. The AOB growing on the shell side of the membrane would oxidise the  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , which in turn would diffuse back into the tube side and collected in the retentate stream. The process was run continuously for 24 – 48 hours, and samples were taken at pre-determined intervals for further analyses.

### **3.4 Strategy of Operations (Experimental Work)**

There were three different parts of experimental work carried out over the duration of the study. The first part focused on the application of the Anammox bacteria in a SAMBR for nitrification and denitrification of wastewater containing  $\text{NH}_4^+$  and  $\text{NO}_2^-$ . The second part was about the enrichment and growth of AOB in batch reactors, where the initial seed culture was collected from different wastewater treatment plants in the UK. The final part focused on pre-oxidation of  $\text{NH}_4^+$  by AOB using the NF membrane modules. Details of the operational strategies were described in the beginning of each chapter, i.e. Chapter 4, Chapter 5, and Chapter 6.

### **3.5 Origin of Biomass**

The study aimed to grow and make use of two different types of bacteria, namely nitrifying bacteria and Anammox bacteria. Seed of these bacteria were mainly collected as sludge from several different sources in treatment plants, which were the anaerobic digestion (AD) plant and full scale of submerged anaerobic membrane bioreactor (SAMBR) at Anglian Water in Cambridge, and; return activated sludge (RAS) from the Mogden Sewage Treatment Works in London. The sludge was kept at 4 °C until further use. Prior to the use of the sludge, they were analysed for their nitrogen, TSS/VSS, COD, and hydrazine/hydroxylamine content.

## 3.6 Growth Media

Two different types of growth and enrichment media were used in this study. The first was specific for the Anammox bacteria, while the other one was for nitrifying bacteria (AOB). All chemicals used to prepare the media were of analytical grade and obtained from major retailers.

### 3.6.1 Growth Media for the Anammox Bacteria

The synthetic wastewater feed was the one described by van de Graaf et al. (1996), which is at the moment, the most used synthetic medium to grow the Anammox bacteria (Strous et al., 1997b, Dapena-Mora et al., 2004, Imajo et al., 2004, Trigo et al., 2006). The medium contained (per litre of deionised water): 400 mg  $(\text{NH}_4)_2\text{SO}_4$ ; 420 mg  $\text{NaNO}_2$ ; 500 mg  $\text{KHCO}_3$ ; 27.5 mg  $\text{KH}_2\text{PO}_4$ ; 200 mg  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 180 mg  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; 7 mg/L EDTA, 12 mg/L  $\text{FeSO}_4$ ; and 1.25 mL trace element solutions I and II. Trace element solution I contained (per litre deionised water): 5 g EDTA; 5 g  $\text{FeSO}_4$ ; and trace element solution II contained (per litre deionised water): 15 g EDTA; 0.43 g  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.24 g  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ; 0.99 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ; 0.25 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.22 g  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ ; 0.19 g  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ; 0.21 g  $\text{NaSeO}_4 \cdot 10\text{H}_2\text{O}$ ; 0.014 g  $\text{H}_3\text{BO}_4$ . The mineral medium was autoclaved at 121 °C for 20 minutes.

Solutions of trace elements was sterilised separately at 121 °C and added aseptically to the autoclaved medium. After cooling, the medium was flushed with nitrogen gas for at least 30 min to achieve anaerobic conditions. The pH of the feeding medium was adjusted to around 8.0 by means of 1M sulphuric acid solution (van de Graaf et al., 1996). The above-mentioned growth media was prepared in a 10-L bottle and continuously fed into the reactor using a peristaltic pump. Its concentration, however, was increased by doubling the concentrations of each of the elements mentioned above whenever the  $\text{NH}_4^+$  concentration was found to be at a minimum level, i.e. no decrease in  $\text{NH}_4^+$  concentration as the reactor runs.

### 3.6.2 Growth Media for Nitrifying Bacteria (AOB)

To grow nitrifying bacteria (AOB), the media used was adapted and modified from Bhaskar and Charyulu (2005). The medium contained (per litre of deionized water): 468 mg  $(\text{NH}_4)_2\text{SO}_4$ ; 400 mg  $\text{KH}_2\text{PO}_4$ ; 80 mg  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; 80 mg  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 5.5 mg  $\text{FeSO}_4$ ; 10 mg EDTA; and 10 mg phenol red. The pH of the medium was adjusted to 8.0, and autoclaved at 121 °C for 20 minutes.

## 3.7 Analytical Techniques

The performance of the SAMBR and the Anammox process were evaluated by employing several analytical techniques. These include the analyses for  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ , hydrazine, hydroxylamine, total suspended solids (TSS) and volatile suspended solids (VSS), pH, biogas composition, and chemical oxygen demand (COD). Sampling was performed at regular intervals to monitor the effluent quality. All analyses were carried out in triplicate, while all chemicals used to prepare reagents were of analytical grade and obtained from major retailers.

### 3.7.1 Ammonium, Nitrite and Nitrate Analyses

The concentration of  $\text{NH}_4^+$  was determined using the Phenate Method as described in Section 4500-NH<sub>3</sub>-F, while  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were determined using the Colorimetric Method as described in Section 4500-NO<sub>2</sub><sup>-</sup>-B and 4500-NO<sub>3</sub><sup>-</sup>-B, respectively, of the Standard Methods for the Examination of Water and Wastewater, section (APHA, 1999).

#### 3.7.1.1 Phenate Method – Analysis for Ammonium ( $\text{NH}_4^+$ )

The measurement of  $\text{NH}_4^+$  was based on the Phenate method. All the reagents required were prepared prior to the analysis; they were phenol solution, 0.5% (w/v) sodium nitroprusside solution, and oxidising solution. Phenol solution was first prepared by mixing 11.1 mL of liquefied phenol with 95% (v/v) ethanol to a final volume of 100 mL. Phenol solution was prepared on a weekly basis, and extra caution has to be taken while preparing the solution, i.e. wearing proper PPE and having good ventilation, as phenol is a toxic volatile substance. The 0.5% (w/v) sodium nitroprusside solution was prepared by dissolving 0.5 g of sodium nitroprusside in 100 mL of deionised water. This solution was

prepared monthly, and kept in an amber bottle. Prior to preparing oxidising solution, alkaline citrate solution and sodium hypochlorite (commercial solution of 5% concentration) were needed. Alkaline citrate was prepared dissolving 200 g trisodium citrate and 10 g sodium hydroxide in deionised water, and diluting to a final volume of 1000 mL. Oxidising solution was prepared daily, only when it was needed for the analysis, by mixing 100 mL of alkaline citrate with 25 mL of sodium hypochlorite.

For the analysis, 25 mL of diluted sample (centrifuged at 5000 rpm for 5 minutes, and filtered through 0.22  $\mu\text{m}$  filter) was added to a 50 mL Erlenmeyer flask and mixed with 1 mL of phenol solution, 1 mL of sodium nitroprusside solution, and 2.5 mL of oxidising solution. The flask was covered with paraffin wrapper and left at room temperature in subdued light for at least one hour to let the color develop. The color was stable for 24 hours. After one hour, the absorbance of the solution was measured at 640 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1800). Concentrations of  $\text{NH}_4^+$  (mg/L) contained in the samples were calculated using a calibration curve over the appropriate range of concentrations, which was prepared prior to the analysis.  $\text{NH}_4\text{Cl}$  was used to prepare the  $\text{NH}_4^+$  standard solutions. The coefficient of variance for five identical samples was  $\pm 4\%$ .

### **3.7.1.2 Colorimetric Method – Analysis for Nitrite ( $\text{NO}_2^-$ )**

The determination of  $\text{NO}_2^-$  was based on a Colorimetric method. Color reagent was first prepared by adding 85% phosphoric acid, 10 g sulfanilamide, and 1 g of N-(1-naphthyl)-ethylenediamine dihydrochloride (after 10 g sulfanilamide was completely dissolved) in 800 mL of nitrite-free water, and then diluting it to a final volume of 1000 mL. In this case, the nitrite-free water used was the double deionised water (ultra-pure water) obtained from the Analytical Laboratory. The solution was stored in a dark bottle at 4  $^\circ\text{C}$ .

For the analysis, 50 mL of diluted sample (centrifuged at 5000 rpm for 5 minutes, and filtered through 0.22  $\mu\text{m}$  filter) was thoroughly mixed with 2 mL of color reagent in a 100 mL flask. After 10 minutes, the absorbance of the solution was measured at 543 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1800). Concentrations of  $\text{NO}_2^-$  (mg/L) contained in the samples were calculated using a calibration curve over the appropriate range of concentrations, which was prepared prior to the analysis.  $\text{NaNO}_2$  was used to



prepare the  $\text{NO}_2^-$  standard solutions, and the coefficient of variance for five identical samples was  $\pm 2\%$ .

### 3.7.1.3 UV Technique – Analysis for Nitrate ( $\text{NO}_3^-$ )

Both the measurement of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  require the use of ultra-pure water (nitrite- and nitrate-free water) to prepare all the reagents and standards. Dry  $\text{KNO}_3$  (dried at  $105^\circ\text{C}$  for 24 hour) was used to prepare the standard solutions needed to construct a calibration curve. For the analysis, 50 mL of clear sample (centrifuged at 5000 rpm for 5 minutes, and filtered through  $0.22\ \mu\text{m}$  filter) was mixed with 1 mL of hydrochloride,  $\text{HCl}$  (1 N). The absorbances of the sample at 220 nm and at 275 nm were obtained using a UV-Vis spectrophotometer (Shimadzu, UV-1800). To obtain the final absorbance of the sample needed to calculate  $\text{NO}_3^-$  concentration (mg/L), two times the absorbance reading at 275 nm was subtracted from the reading at 220 nm. Absorbance reading at 275 nm represents the interference that might exist in the sample due to dissolved organic matter. The coefficient of variance for five identical samples was  $\pm 4\%$ .

This rapid determination of  $\text{NO}_3^-$  concentration in wastewater was widely used by many researchers (Dapena-Mora et al., 2004, Trigo et al., 2006, Xiao et al., 2009, Suneethi and Joseph, 2011a, Wang et al., 2011). The dual wavelengths correction scheme method was commonly adopted to estimate  $\text{NO}_3^-$  concentration when organic matter is also present in the sample (Shaw et al., 2014, Causse et al., 2017). Since organic matter may also absorb at 220 nm, but  $\text{NO}_3^-$  does not absorb at 275 nm, the absorbance at 275 nm multiplied by two is used to correct the  $\text{NO}_3^-$  value. The newly adjusted 220-nm absorbance is then used with the  $\text{NO}_3^-$  calibration curve (Appendix, **Fig. A-4**) to estimate the  $\text{NO}_3^-$  concentration.

However, although the method used to measure  $\text{NO}_3^-$  in this study is only suitable for the screening of uncontaminated water (low in organic matter), since most of the organic matter and other contaminants in our initial sample would have been removed as the sample was centrifuged and filtered through a  $0.22\ \mu\text{m}$  filter prior to the analysis, we feel the method can still be used. In addition, since the wastewater sample taken from the reactor was also diluted up to 250 times prior to analysis, in such cases the interference of COD on the  $\text{NO}_3^-$  measurement will be minimised. The interference of phenol red (pH indicator) on

$\text{NO}_3^-$  was also assumed to be negligible as the amount added to the media was only 10 mg per liter, and it would be washed out quickly in the early stages of the process.

### **3.7.2 Hydrazine and Hydroxylamine Analyses**

Both hydrazine ( $\text{N}_2\text{H}_4$ ) and hydroxylamine ( $\text{NH}_2\text{OH}$ ) were reported to be intermediates produced in the Anammox process (van de Graaf et al., 1997, Jetten et al., 1999). Analyses for hydrazine and hydroxylamine were carried out according to the methods previously described in the literature (Watt and Chrisp, 1952, Frear and Burrell, 1955, ASTM., 2007)

#### **3.7.2.1 Analysis for Hydrazine ( $\text{N}_2\text{H}_4$ )**

The measurement of hydrazine in the samples was adapted from the methods described by Watt and Chrisp (1952) and ASTM (2007). The reagents required for the analysis were hydrazine solution, hydrochloric acid, and p-Dimethylaminobenzaldehyde solution. Hydrazine dihydrochloride ( $\text{N}_2\text{H}_4 \cdot 2\text{HCl}$ ) was used to prepare the standard hydrazine solutions needed to construct a calibration curve. The stock solution of hydrazine was first prepared by dissolving 0.328 g of hydrazine dihydrochloride in 100 mL of deionised water and 10 mL of HCl. It was subsequently diluted and mixed with deionized water to 1000 mL in a volumetric flask. The concentration of stock solution was 100  $\mu\text{g}/\text{mL}$ . A series of standard hydrazine solutions with concentrations of 5 – 200  $\mu\text{g}/\text{L}$  were prepared from the stock solution to construct the calibration curve. Every 100 mL of standard solution should be acidified with 1 mL of concentrated HCl. p-Dimethylaminobenzaldehyde solution was prepared by dissolving 4 g of the chemical in 200 mL of methanol, and added with 20 mL of HCl. The solution was stored in a dark bottle to keep out of direct sunlight.

Prior to the analysis, 50 mL of sample was acidified with 1 mL of concentrated HCl as soon as they were taken from their sources. The ratio of sample to HCl volume was scaled down accordingly, depending on sample availability. Sample dilution was made if necessary by adding deionized water. 5 mL of the sample was then pipetted into a suitable flask or test tube, added and mixed with 1 mL of p-Dimethylaminobenzaldehyde solution. After a minimum of 10 minutes, but no longer than 100 minutes, the absorbance of each

solution was measured at 458 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1800). The concentrations of hydrazine (mg/L) were determined by referring the absorbance obtained for the sample to the calibration curve prepared earlier. All samples were analysed in triplicate, with coefficient of variance for five identical samples being  $\pm 3\%$ .

### 3.7.2.2 Analysis for Hydroxylamine

Determination of hydroxylamine in the samples was performed based on a color test described by Frear and Burrell (1955). Reagents required for the analysis were hydroxylamine stock solution (0.0695 g of dry hydroxylamine hydrochloride was dissolved in deionised water and diluted to 1000 mL volume, resulting in 0.001 M of concentration), 8-Quinolinol solution (1 g of 8-Quinolinol was dissolved in 100 mL of absolute ethanol), sodium carbonate solution (1 M), trichloroacetic acid solution (12% (w/v)), manganese chloride solution (0.001 M), and phosphate buffer solution, pH 6.8.

To construct a calibration curve for hydroxylamine, a series of hydroxylamine standard solutions with concentrations of 0.05 – 0.25  $\mu\text{mole/mL}$  were prepared from its stock solution. One mL of the standard solution was pipetted into a test tube, with 1 mL of phosphate buffer (pH 6.8) and deionised water added to bring its volume to 2.8 mL. Then, 0.2 mL of trichloroacetic acid solution and 1 mL of 8-Quinolinol solution were added to the test tube; after a gentle mixing, 1 mL of sodium carbonate solution (1 M) was finally added to the tube. The tube was closed and vigorously swirled, before being placed in a boiling water bath for one minute to develop the green colour. On removal from the water bath, the solution was cooled for 15 minutes. After that, the absorbance of each solution was measured at 705 nm in a UV-Vis spectrophotometer (Shimadzu, UV-1800). A graph of standard hydroxylamine concentrations was plotted against their absorbance readings at 705 nm.

Similar steps were carried out to analyse for hydroxylamine in each wastewater sample, except that the standard solutions were replaced with sample. Sample dilution was made if necessary. The concentrations of hydroxylamine (mg/L) were then determined by referring the absorbance obtained for the sample to the calibration curve prepared earlier. Samples were analysed in triplicate, with the coefficient of variance for five identical samples being  $\pm 5\%$ .

### 3.7.3 Chemical Oxygen Demand (COD) Analysis

The measurement of COD concentration was based on the Standard Closed Reflux Colorimetric Method described in Section 5220-D of Standard Methods (APHA, 1999). Digestion solution was first prepared by adding the following materials in 500 mL of deionised water: 10.216 g of  $K_2Cr_2O_7$ , previously dried for two hours at 103 °C; 167 mL of concentrated  $H_2SO_4$ ; and 33.3 g of  $HgSO_4$ . The mixture was then left to cool at room temperature before diluting to 1000 mL.

A sample of 1 mL was added to a Hach reflux tube followed by 0.6 mL of digestion solution. Then 1.4 mL of sulphuric acid reagent (2.5% w/w silver sulphate in sulphuric acid) was carefully run down the inside of the tube so that an acid layer was formed under the sample/digestion solution layer. The tubes were then tightly sealed and inverted three times to mix properly, and then refluxed in a COD reflux reactor (Hach, Model 45600) at 150 °C for two hours. After cooling (preferably overnight), the absorbance of the sample was measured at 600 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1800). The concentrations of the samples (g/L) were determined with the aid of a calibration curve shown in **Appendix 2**. To construct the calibration curve, potassium hydrogen phthalate (KHP) with a theoretical COD value of 1.176 g  $O_2$  per g KHP was used to prepare standard solutions. Each sample was analysed in triplicate, and an average value was taken, with the coefficient of variance for five identical samples being  $\pm 2\%$ . As  $NO_2^-$  has a theoretical oxygen demand of 1.1 mg COD per mg  $NO_2^-$ , its interference on COD concentrations was taken into consideration. All COD data presented in the thesis were normalized by subtracting them with their corresponding  $NO_2^-$  concentration.

### 3.7.4 Total and Volatile Suspended Solids (TSS/VSS) Analysis

The measurements of TSS and VSS, which represent the biomass concentrations and sludge characteristics, were performed according to the Standard Methods for the Examination of Water and Wastewater, section 2540-D and 2540-E, respectively (APHA, 1999), and all analyses were carried out in triplicate, with the coefficient of variance for five identical samples being  $\pm 7\%$ . Firstly, glass microfiber filters with a pore size of 1.5  $\mu m$  and

a diameter of 47 mm (GE Healthcare, Whatman™ 934-AH™) were washed by filtering 20 mL of deionised water for three times. The filters were placed on aluminium trays and put in a furnace (Carbolite, ELF 10/6) at 550 °C for 30 minutes. This was necessary to remove and evaporate any trace of vapour from the filter papers. After being cooled down in a desiccator, the initial weight of the filter paper was measured using an analytical balance (Sartorius, M-Power 210g x 0.1mg), and then they were placed in a desiccator until needed.

To measure TSS, a known volume of a well-mixed sludge, i.e. 5 mL, was filtered through the filter paper under vacuum, and the sludge residue left on the filter paper was dried in an oven at 103-105 °C for two hours. The filters were then allowed to cool down in a desiccator. The resulting weight was measured and recorded for the measurement of TSS. For the measurement of VSS, the tray was then placed in a furnace (Carbolite, ELF 10/6) at 550 °C for one hour to ignite the residue. After cooling down, the final weight of the filter paper was recorded. Calculations of TSS and VSS were then performed according to the Standard Methods (APHA, 1999), as follows:

$$TSS \left( \frac{g}{L} \right) = \frac{\text{Weight of filter and dried residue} - \text{Initial weight of filter, in } g}{\text{Volume of sludge sample, in } L}$$

$$VSS \left( \frac{g}{L} \right) = \frac{\text{Weight before ignition} - \text{Weight after ignition, in } g}{\text{Volume of sludge sample, in } L}$$

### 3.7.5 pH Measurement

The pH of any solution was measured using a pH meter (Hanna Instruments, Model 213), calibrated with buffer solutions of pH 4, pH 7 and pH 10 at room temperature. Values obtained were accurate to within  $\pm 0.02$  units. Adjustment of pH of solution, if needed, was made by the addition of 1 M sulphuric acid (H<sub>2</sub>SO<sub>4</sub>)/hydrochloric acid (HCl) or 1 M sodium hydroxide (NaOH)/sodium carbonate (NaCO<sub>3</sub>).

### 3.7.6 Biogas Production

The amount and rate of biogas production was determined using the water displacement method. Water in the collection chamber was slightly acidified using sulphuric acid to prevent carbon dioxide from dissolving in the water and giving a false composition reading. Measurements of the volume of gas produced (based on the volume of water displaced) were taken at regular intervals to determine the biogas production rate.

### 3.7.7 Biogas Composition

The composition of biogas produced in the reactor was analysed using a gas chromatography GC-TCD (Shimadzu, GC 14A) fitted with a Porapak N column (1500 × 6.35 mm). The carrier gas was helium with a flow rate of 16 mL/min, while the column temperature, detector temperature and injector temperature were set at 28 °C, 38 °C, and 128 °C, respectively. Peak areas and the percentage of gas composition were calculated and printed out on a Shimadzu Chromatopac C-R6A integrator. About 1 mL of biogas sample was collected from reactors using 1 mL plastic syringe (Terumo), and injected into the GC. The GC was capable of detecting three different gases: nitrogen (N<sub>2</sub>), methane (CH<sub>4</sub>), and carbon dioxide (CO<sub>2</sub>). The coefficient of variance for five identical samples was ±3%.

### 3.7.8 Experimental Replication and Control

All analyses were carried out in triplicate as to ensure the precision of the measurements, and would be repeated should the coefficient of variance of the measurements be higher than 10%. In addition, the linear calibration curves for various compounds (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, COD, hydrazine, hydroxylamine) fitted with a high correlation coefficient, i.e.  $R^2 > 0.99$  (attached in the Appendices). Analysis of variance (ANOVA) was conducted to determine which parameters significantly influenced the Anammox reaction. Regression analysis on COD was conducted to determine whether its concentrations change significantly over time during the Anammox reaction. The data collected was initially assessed for linearity using a scatterplot of COD versus time. Then, the residuals of the data were checked for homoscedasticity and normality of residuals. This was done using the Durbin-Watson test, Kurtosis and Skewness analyses. Once all the checks were passed, a linear regression model was developed, and any coefficient with a

probability of less than 0.05 was considered to have a statistically significant effect over time.

Handling of the sludge containing microorganisms was made on a clean bench in the laboratory, but not under laminar flow safety cabinet, at room temperature as the laboratory was only specifically used for research work related to sludge and wastewater treatment. Handlings of toxic and corrosive chemicals were all made inside the biosafety cabinet.

## CHAPTER 4

# THE ANAMMOX PROCESS IN A SUBMERGED ANAEROBIC MEMBRANE BIOREACTOR

### 4.1 INTRODUCTION

The Anammox process, discovered by Mulder et al. (1995) seems to have gained considerable attention as a new and economical alternative method for the removal of nitrogen from wastewater. Nevertheless, its application is quite limited due to a long start-up period, which prompted researchers to put more effort into improving its start-up process. Over the last two decades, studies on the start-up of the Anammox process and evaluation of its performance have been carried out in different types of reactors, and with different configurations and operational strategies. Due to the slow growth of the Anammox bacteria, i.e. 11 days of doubling time (Strous et al., 1998), a reactor that can assure efficient retention of biomass inside the system, particularly during the start-up, should be highly desirable. Prior to that, the reported doubling time for the Anammox bacteria grown in a fluidised-bed reactor was 30 days (van de Graaf et al., 1996). Strous et al. (1998) reported that insufficient biomass build-up, i.e. continuous loss of small amounts of biomass via the effluent, could impede the start-up, hence leading to significantly longer doubling times.

The cultivation of this slow-growing microorganism also relies mostly on the ability of the microbial population to form biofilms or aggregates such as flocs or granules (van der Star et al., 2008). Previously, this could be achieved by using sequential batch reactors (SBR) (Strous et al., 1998, Fux et al., 2002) or biofilm based reactors, i.e. fixed-bed reactor, fluidised-bed reactor, gas-lift reactor (Sliekers et al., 2003, Dapena-Mora et al., 2004). In a fluidised-bed reactor, the microbial community grew as biofilms on sand particles. However, its cultivation was not satisfactory due to the difficulties involved in operating the laboratory-scale reactor, and at times the retention of biomass inside the reactor was not sufficient to maintain its activity (Strous et al., 1998).



Apart from the conventional SBR and biofilm based reactors, Trigo et al. (2006) started-up the Anammox process using a membrane sequencing batch reactor (MSBR) with a hollow fibre membrane module submerged inside the reactor. The membrane module was mainly used to avoid biomass from being washed-out from the system. These findings have showed that the use of a membrane inside a reactor was suitable for nitrogen removal by the Anammox process, and could improve its start-up as well by shortening the start-up period. This study was then followed by a number of other established researchers in order to investigate the feasibility and efficiency of the Anammox process in membrane bioreactors (MBRs). In an MBR, the effluent is withdrawn via a membrane which is impermeable for microbial cells, which enables full biomass retention inside the system. It was reported by many researchers that MBRs were capable of starting-up Anammox process, with an increase in the efficiency of the process and moderately low cost when compared with conventional batch reactors (Cema et al., 2004, Trigo et al., 2006, van der Star et al., 2008, Xiao et al., 2009, Wang et al., 2012).

This study aims to enrich and grow the Anammox bacteria from anaerobic digestion sludge, and simultaneously start-up the Anammox process in a 3-litre laboratory-scale submerged anaerobic membrane bioreactor (SAMBR). A flat-plate hollow fibre membrane module was submerged inside the reactor, not only to facilitate the treatment of nitrogen-containing wastewater, but also to make use of the membrane to retain the biomass from being washed out into the effluent. Start-up time and performances of the Anammox process in a SAMBR for the removal of nitrogen were studied. This study would provide some useful information on the feasibility and efficiency of the Anammox process in a SAMBR.

## **4.2 MATERIALS AND METHODS**

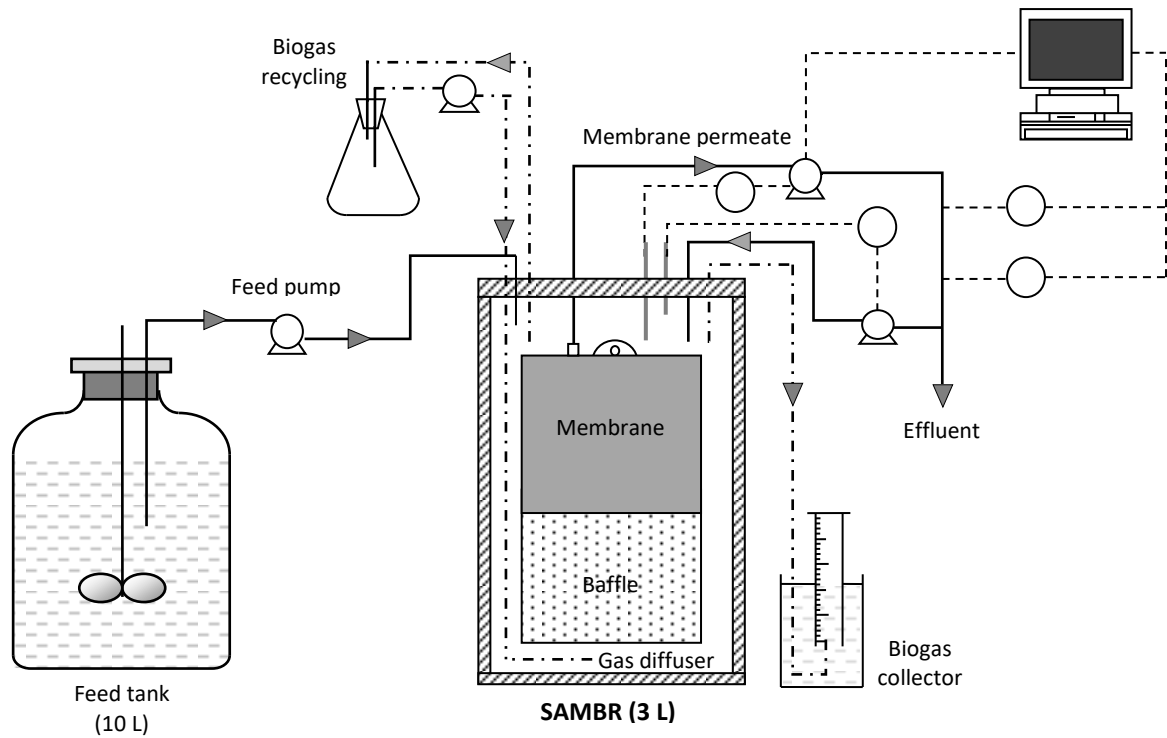
This section explains how the experiments were conducted, and then focuses mainly on the strategy of operations. Details of specific methods, i.e. analytical techniques, used in the experiments were previously described in Chapter 3.

### 4.2.1 Strategy of Operations

This study was initially carried out in a number of 3-litre submerged anaerobic membrane bioreactor (SAMBR) containing ultrafiltration HF membrane module (Kubota type 203, UK), as shown previously in Chapter 3 (**Fig. 3-5**). However, only operations in two reactors (out of five reactors) could be considered successful, and their findings are presented in this thesis. The membrane pore size of 0.4  $\mu\text{m}$  was designed for operation in a membrane bioreactor for wastewater treatment, and is impermeable to microbial cells. Seed culture from an anaerobic digester in the Anglian Water Treatment Plant in Cambridge was inoculated into the reactor to start-up the process. Some of initial characteristics of the seed culture are presented in **Section 4.3 (Table 4-2)**.

For the start, sludge and substrate (synthetic wastewater) as described by Van de Graaf et al. (1996) were mixed in 1:1 ratio inside the reactor. The reactor was then sparged with 70% nitrogen-30% carbon dioxide gas for about 20 minutes to remove traces of oxygen gas and create anoxic conditions. The experiment was then carried out in a system where the reactor was continuously fed with synthetic wastewater using a peristaltic pump (Watson-Marlow, 101U) at a rate of 1.5 L/d, the same way permeate was pumped out via the membrane panel, resulting in an HRT of two days (for SAMBR 1). A schematic of the process flow diagram of the SAMBR operation without pre-oxidation process is shown in **Fig. 4-1**.

A molarity ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  should be maintained in the range of 1:1 to 1:1.3. This synthetic wastewater (**Table 4-1**) was prepared in a 10-litre bottle every few days. Although  $\text{NO}_3^-$  is not needed for the growth of Anammox bacteria, about 10 mg/L of  $\text{NO}_3^-$  was added along with the medium during the start-up period to favour the elimination of denitrifiers and to prevent the generation of hydrogen sulphide ( $\text{H}_2\text{S}$ ) by sulphur reducing bacteria (Xiao et al., 2009). Biomass and substrate were mixed and kept in suspension inside the bioreactor by mean of biogas recycling via the stainless steel diffuser using a vacuum pump (Charles Austin, B100 SEC).



**Fig. 4-1** A schematic of the Anammox process in a SAMBR

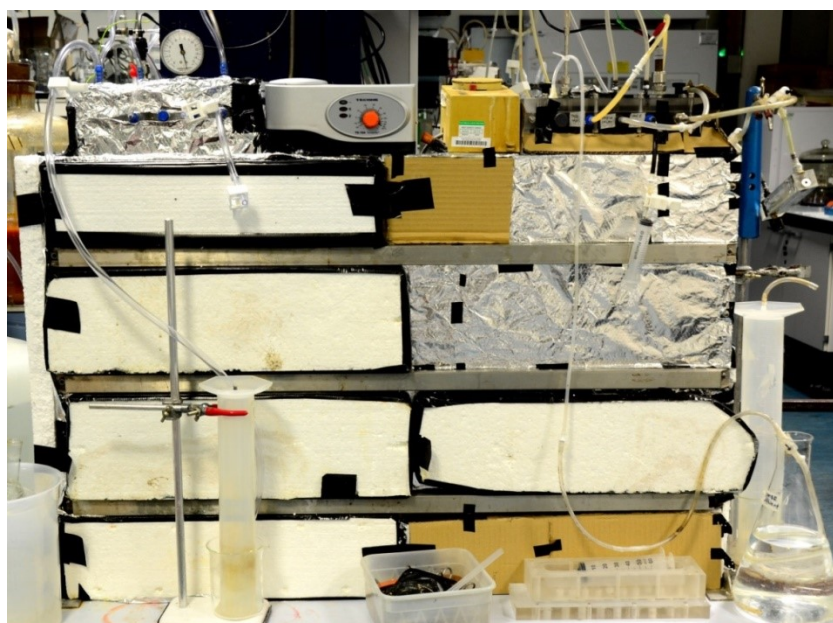
**Table 4-1** Synthetic wastewater composition (adapted from van de Graaf et al. (1996)).

Compound	Concentration (mg/L)
$(\text{NH}_4)_2\text{SO}_4$	400
$\text{NaNO}_2$	420
$\text{KHCO}_3$	500
$\text{KH}_2\text{PO}_4$	27.5
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	180
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	200
EDTA	7
$\text{FeSO}_4$	12
Trace elements	1.25 mL/L

The two SAMBRs used in this experiment were only operated at different HRTs, while the other operating conditions, i.e. temperature, pH and media composition, were similar for both reactors. The details of operating conditions were as follows: HRTs of two days and four days, for SAMBR 1 and SAMBR 2, respectively, temperature of 35 °C, and

pH between 7.5 and 8.0. The reactor units were placed in a water bath, where a portable immersion circulator (Techne, TE 8A) was used to maintain water temperature inside the water bath at about 35 °C. The reactor was tightly sealed to maintain anoxic conditions, while the water bath in which the reactor was placed in was covered with aluminium foil or/and polystyrene to protect the Anammox bacteria from light and algal growth. The use of polystyrene to cover the water batch could also minimise the heat loss to the surroundings. The SAMBRs during their operation are as shown in **Fig. 4-2**.

One of the most critical factors, especially during the enrichment process, was to ensure that the reactor operates in fully anoxic conditions as the Anammox bacteria could be inhibited even by low DO concentrations (Strous et al., 1997b). Sodium bicarbonate solution (1 M) was added to the system to maintain pH at the desired values. Nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_2^-$ ) loading rate was increased by increasing the concentrations of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NaNO}_2$  in the feed vessel or by shortening the HRT.



**Fig. 4-2** SAMBR in operation for the Anammox process.

#### **4.2.2 Sampling and Analysis**

At predetermined time intervals, about 10 mL of wastewater sample was withdrawn from the reactor for regular analysis using a syringe. Samples were centrifuged at 5000 rpm for about 5 minutes, and filtered through 0.45  $\mu\text{m}$  syringe filter prior to the analysis.

Nitrogen transformations inside the bioreactor were studied from the analysis of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and biogas compositions. Apart from that, the biomass development was also studied based on COD, TSS and VSS content, all of which was performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1999).

Removal efficiencies ( $R_e$ ) of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  were calculated as the following equation:

$$R_e (\%) = \left(1 - \frac{C_e}{C_i}\right) \times 100$$

where  $R_e$  was the removal efficiency;  $C_i$  was the initial concentration of nitrogen compounds;  $C_e$  was the concentration of nitrogen compounds in the treated effluent.

Since hydrazine and hydroxylamine are known to be intermediates of the Anammox process, as previously reported in the literature (van de Graaf et al., 1997, Jetten et al., 1999), they were analysed according to the methods previously described in Chapter 3.

## 4.3 RESULTS AND DISCUSSION

Results of the experimental work are presented and discussed in different sub-topics, namely: (1) Process performances, which covered growth characteristics, and nitrogen mass balances between the influent and effluent of the reactor; (2) Feasibility study of the Anammox process in SAMBR; The discussion of results was made based on the Anammox process operated in two different SAMBRs, namely SAMBR 1 and SAMBR 2. Both reactors were inoculated with the same anaerobic digestion sludge and operated at similar operating conditions, except that SAMBR 1 was operated at an HRT of two days, while SAMBR 2 was operated at an HRT of four days.

### 4.3.1 Characteristics of Seed Cultures

The initial characteristics of AD sludge used to start-up the Anammox process in a SAMBR are presented in **Table 4-2**. The sludge was moderately thick, viscous and black in colour (11.7 g/L TSS and 5.8 g/L VSS). It initially contained very high amount of  $\text{NH}_4^+$  (245 mg/L), and organic content (11.7 g/L COD), with a pH in the range of 7 – 8. The  $\text{NH}_4^+$  content was about 18 times higher than the  $\text{NO}_2^-$  and  $\text{NO}_3^-$  concentrations in total, and traces of hydrazine and hydroxylamine were also detected in the sludge.

**Table 4-2** Initial characteristics of AD sludge used to start-up the Anammox process in a SAMBR.

Parameters	Values (mg/L, except pH)
pH	7.5
TSS	11,700
VSS	5,800
NH <sub>4</sub> <sup>+</sup>	245
NO <sub>2</sub> <sup>-</sup>	0.40
NO <sub>3</sub> <sup>-</sup>	13.1
CODs	11,735
Hydrazine	0.150
Hydroxylamine	1x10 <sup>-4</sup>

#### 4.3.2 Anammox Process Performance

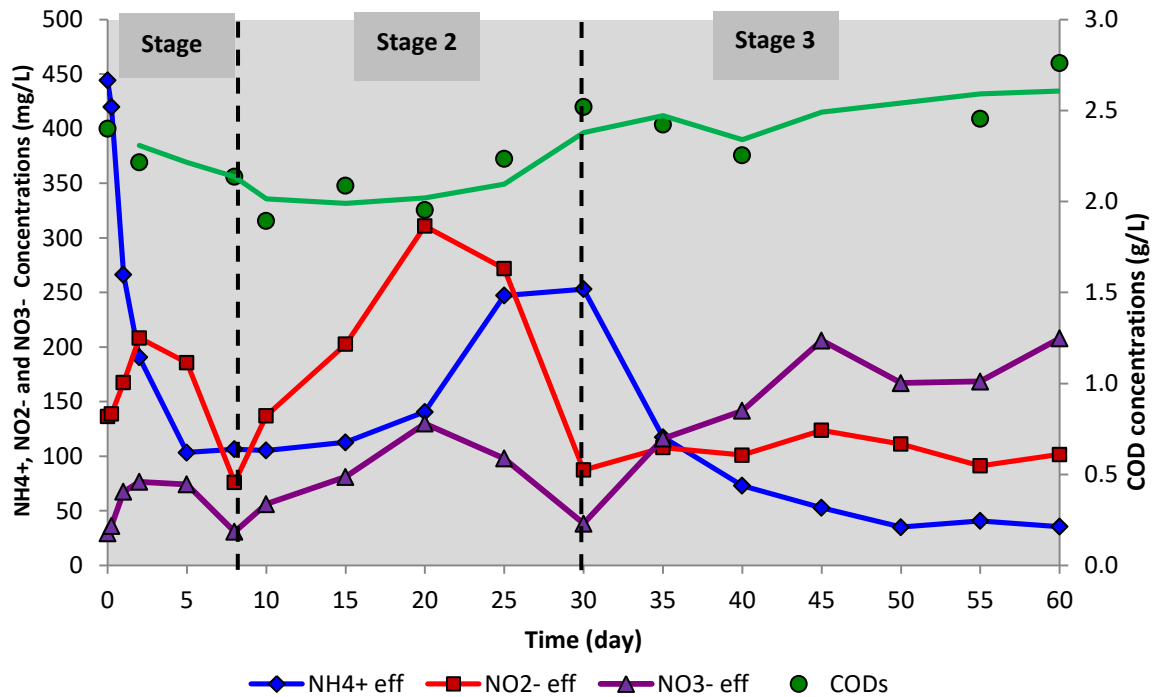
Evaluation of the Anammox process performance in the SAMBRs was mainly made based on a number of criteria: their growth characteristics, and mass balances of the nitrogen species in the influent and effluent. Then, based on these two criteria, the feasibility of using the SAMBR to grow and start-up the Anammox process could be assessed. The SAMBR used in these experiments also served as enrichment reactors, aimed at studying if the origin of seed sludge plays a role in nitrogen removal performance. The results shown in this chapter were part of the many batches of experimental work that were carried out, some of which did not produce satisfactorily enough results to be presented in the thesis. Since there were two SAMBRs successfully run in this study, the results and discussion on their process efficiency and microbial growth characteristics are made separately. However, findings for both reactors are at times compared and discussed together.

### **4.3.2.1 The Anammox Process in SAMBR 1**

#### **4.3.2.1(a) Bacterial Growth**

The experiments were carried out continuously in a SAMBR for the duration of 60 days, and **Fig. 4-3** represents the profile of effluent nitrogen concentrations in the forms of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  over the experimental period. As illustrated in the figure, the experimental period of 60 days could be divided into three distinct stages: **Stage 1** (day 1 – 8); **Stage 2** (day 8 – 30); and **Stage 3** (day 30 – 60), based on a number of reports available in the literature that dealt with the enrichment and start-up of the Anammox process, using different types of reactors (Trigo et al., 2006, Wang et al., 2009, Suneethi and Joseph, 2011b, Wang et al., 2012), though the duration of each stage selected was quite subjective. In the early stage, i.e. first 8 days, constant concentrations of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  were maintained in the influent, i.e. 109 mg/L and 280 mg/L, respectively. The initial concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  inside the reactor measured just after the inoculation of seed culture (before the media was continuously fed) were 180 mg/L, 145 mg/L, and 11 mg/L, respectively.

In Stage 1, as the growth media started to be continuously fed to the reactor, it was found that the initial concentrations of  $\text{NH}_4^+$  in the effluent were much higher than that in the influent, i.e. over 400 mg/L, while the concentration of  $\text{NO}_2^-$  was initially about 140 mg/L, and this increased in the first two days due to the high feed concentration (280 mg/L). This phenomenon was previously reported by other researchers (Dapena-Mora et al., 2004, Third et al., 2005, Chamchoi and Nitorisavut, 2007, Wang et al., 2009), where initial concentrations of  $\text{NH}_4^+$  in the reactor were much higher than that in the influent. During this early stage, Wang et al. (2009) explained that denitrifying activity was the favoured process in the absence of oxygen, and in presence of  $\text{NO}_2^-$ , but no apparent Anammox activity appeared. This phenomenon would usually occur during the transition period of a process.



**Fig. 4-3** Profile of nitrogen compounds ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ) and COD concentrations in the effluent of SAMBR 1.

Prior to the inoculation into the SAMBR, the seed culture was kept in a 4 °C room, which slowed the bacterial growth rate. Thus, the first few days of operation could be considered as adaptation period to a new environment. In the initial stage, after the seed sludge was inoculated into the SAMBR, it may lyse due to the change in environment, i.e. growth media composition, pH and temperature. Aerobic bacteria, which were not able to adapt to the given environment and conditions, would lyse and the organic nitrogen would breakdown to ammonia (Chamchoi and Nitorisavut, 2007), which resulted in a significant increase in  $\text{NH}_4^+$  concentrations inside the SAMBR, i.e. over 400 mg/L on day 1. This could explain why the concentrations of  $\text{NH}_4^+$  were apparently much higher than that in the influent (about 100 mg/L).

Thereafter, a rapid decrease in the concentration of  $\text{NH}_4^+$  in the effluent was observed from day 1 to day 5, reaching its lowest value of about 100 mg/L, almost identical to its initial concentration in the influent media. This could be due to the activity of nitrifying bacteria inside the reactor that started to use up  $\text{NH}_4^+$  for growth and synthesis. In



these first five days, taking into account that the initial  $\text{NH}_4^+$  concentration in the reactor was 180 mg/L, about 45% of  $\text{NH}_4^+$  was consumed by the nitrifying bacteria, while the other 55% was probably washed-out of the reactor. In contrast, the concentrations of  $\text{NO}_2^-$  increased slightly during the first two days due to high feed concentration (280 mg/L), and started to be consumed from day 2 until it reached its lowest value of about 75 mg/L on day 8. The initial concentration of  $\text{NO}_2^-$  in the reactor was about 145 mg/L, which means that approximately 50% of the  $\text{NO}_2^-$  removal was achieved in the first 8 days.

The slight loss of biomass concentration during this early stage (from 5.8 g/L to 5.5 g/L VSS) in the reactor without any sludge discharge indicates that some bacteria died most likely aerobic bacteria that were not able to adapt to the change in environment. This could also support the occurrence of increasing  $\text{NH}_4^+$  concentrations in the effluent. At this stage, microbial activity inside the reactor could also be disrupted by a variety of events, hence decreasing the efficiency of nitrogen removal. One of the events could be salt precipitation (calcium phosphate), which was observed by Trigo et al. (2006) who had successfully started-up the Anammox process in a membrane sequencing batch reactor (MBSR). Despite the fact that the analysis for salt precipitation was not carried out in this study, interference with microbial activity inside the reactor could not be ruled out.

In **Stage 2**, the  $\text{NH}_4^+$  and  $\text{NO}_2^-$  concentrations in the effluent increased as the nitrogen loading was increased as  $\text{NH}_4^+$  and  $\text{NO}_2^-$  concentrations were lowest, i.e. 105 mg/L and 75 mg/L, respectively, at the end of **Stage 1**. This increase in  $\text{NH}_4^+$  and  $\text{NO}_2^-$  concentrations also indicated that the activity of denitrifying bacteria, which was predominant in **Stage 1**, had decreased. Wang et al. (2009) suggested that at this moment, most of the soluble organic substrate resulting from the breakdown of the seed sludge in **Stage 1** had been completely consumed, which subsequently decreased the activity of denitrifying bacteria. The rate of  $\text{NH}_4^+$  increase in the reactor was, however, slightly slower than that of  $\text{NO}_2^-$ , i.e.  $\text{NH}_4^+$  peaked on day 30, while  $\text{NO}_2^-$  on day 20. In this case, it is apparent that there was little  $\text{NO}_2^-$  reduction as the peak of  $\text{NO}_2^-$  increased to around the feed of concentration, while  $\text{NH}_4^+$  increased more slowly because of cell lysis and very little Anammox activity.

It is clear from **Fig 4-3** that the Anammox activity had started to appear in the reactor in the last 10 days of **Stage 2** indicated by the significant decrease in  $\text{NO}_2^-$

concentration. In this stage, the Anammox bacteria were presumably more dominant than the other bacterial species, considering that the substrates provided and conditions favoured them. Wang et al. (2009) and Xiao et al. (2009) hypothesised that  $\text{NO}_2^-$  was most likely removed through the combine function of both denitrifying and Anammox bacteria. Apart from this,  $\text{NH}_4^+$  and  $\text{NO}_2^-$  consumption rates seemed to exhibit a fairly good correlation, and at some points their concentrations fluctuated with each other, which demonstrates that the Anammox bacteria might be the dominant species in the reactor, particularly at the end of **Stage 2**.

At the beginning of **Stage 3**,  $\text{NH}_4^+$  concentration was at its maximum, i.e. 250 mg/L, while both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  concentrations were at their minimum, i.e. 90 mg/L and 40 mg/L, respectively. As the  $\text{NH}_4^+$  concentrations started to decrease quite significantly thereafter through the action of the Anammox bacteria, it can be observed that  $\text{NO}_3^-$  concentrations in the effluent increased accordingly, while  $\text{NO}_2^-$  concentrations were quite constant towards the end, most probably due to its constant concentrations in the feed that limited the Anammox activity. The steady decrease in  $\text{NH}_4^+$  concentrations from day 30 until the end of Stage 3, i.e. day 60, exhibited that the Anammox process had achieved stability. This was also supported by the occurrence of synchronised removal of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the reactor. On day 60, the  $\text{NH}_4^+$  concentration in the effluent was about 35 mg/L.

The removal efficiencies of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in **Stage 2 – 3** of SAMBR 1, were found to be 86% and 67%, respectively. At the end of **Stage 3**, the final concentrations of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the effluent of the reactor were about 35 mg/L and 100 mg/L, respectively. Furthermore, traces of hydrazine and hydroxylamine were detected and were relatively constant during the whole experimental period, ranging from 0.2 – 0.45  $\mu\text{g/L}$ , and 0.0003 – 0.08  $\mu\text{g/L}$ , respectively. The presence of these intermediates appears to indicate that the Anammox bacteria were active in the system since they both play significant roles in the Anammox reaction, and make Anammox a distinct process as compared to other nitrogen removal processes (Ahn, 2006).

The average molar ratio of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  consumption by the Anammox bacteria in the final stage was 1:0.9, and this was relatively lower compared with the previously reported ratios of 1:1.32 (Strous et al., 1998), 1:1.22 (Trigo et al., 2006), and 1:1.15 (Wang et al., 2009), but similar to that reported by Wyffels et al. (2004) of 1:0.95, while Suneethi

and Joseph (2011a) reported a much lower ratio of 1:0.84. In such cases, more  $\text{NH}_4^+$  was consumed compared with  $\text{NO}_2^-$ , and this could be due to the activities of other bacterial species that might co-exist in the reactor, i.e. AOB-like microorganisms. Apart from this, the stoichiometric differences could also occur due to the differences in reactor composition at a given time, and this is strongly related to the types of wastewater sludge used to start-up the process (Wyffels et al., 2004).

Apart from analysing the nitrogen consumption ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ) and production ( $\text{NO}_3^-$  and  $\text{N}_2$ ) in the reactor based on the concentration profiles, the other method to explain the reactions that took place in the reactor, and hence verifying the process was by using the stoichiometric equation of the Anammox. For example, based on the stoichiometric equation of the Anammox proposed by van de Graaf et al. (1996) (Eq. 2-13), one mole of  $\text{NH}_4^+$  and 1.32 mole of  $\text{NO}_2^-$  will produce 0.26 mole of  $\text{NO}_3^-$ . It was found that from Stage 1 until the end of Stage 2, an average of 0.39 mole of  $\text{NO}_3^-$  was produced per 1.32 mole of  $\text{NO}_2^-$ . However, in **Stage 3**, the average amount of  $\text{NO}_3^-$  produced per 1.32 mole of  $\text{NO}_2^-$  increased to 1.56 mole.

In this study, the Anammox organism was enriched from an AD sludge, which potentially contained different kinds of microorganisms that might have interfered with the process, unlike the use of pure Anammox bacterial strains. Apart from this, the elemental composition and the amount of biomass present in the system might not have been similar to the one proposed earlier by Strous et al. (1998) in the stoichiometry of the Anammox reaction, i.e.  $0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15}$ . However, analysis for elemental composition of the biomass was not carried out in this study.

As can also be seen in **Fig. 4-3**, an average of 100 mg/L of  $\text{NO}_3^-$  was produced in the reactor over the whole experimental period. The initial growth medium contained about 10 mg/L of  $\text{NO}_3^-$ , as it was necessary to prevent the generation of hydrogen sulphide ( $\text{H}_2\text{S}$ ) by sulphur reducing bacteria (Xiao et al., 2009). Its concentrations fluctuated with the concentrations of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , and seemed to exhibit a similar trend to that of  $\text{NO}_2^-$  over the 60 day period. van de Graaf et al. (1997) previously reported that  $\text{NO}_3^-$  was produced from the oxidation of  $\text{NO}_2^-$  as the Anammox bacteria grew and propagated. This  $\text{NO}_3^-$  could be further reduced to nitrogen gas by small percentage of denitrifying bacteria that

might still exist in the reactor, and gas chromatography analysis had revealed that over 95% of biogas produced was composed of nitrogen gas.

Analysis of variance (ANOVA) was conducted to determine whether the effect of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  on the Anammox reaction is statistically significant. From this analysis,  $\text{NH}_4^+$  was found to be statistically significantly decreased over time in the Anammox reaction with  $p < 0.0001$  ( $F(4,10) = 3608.12$ ). Similarly,  $\text{NO}_2^-$  was found to be statistically significantly decreased over time, ( $F(4,10) = 42083.73$ ,  $p < 0.001$ ), while  $\text{NO}_3^-$  was significantly increased with  $p < 0.0001$   $F(4,10) = 3307.59$ ).

#### ***4.3.2.1(b) Analysis of TSS/VSS***

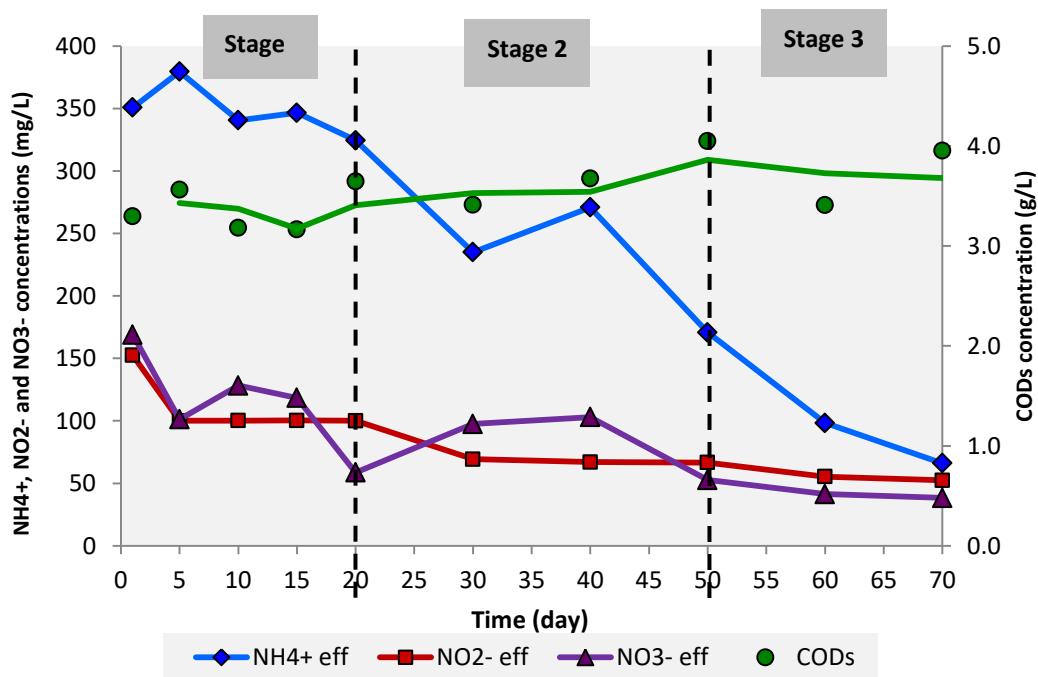
Despite the scouring effects created by the biogas being recycled through the diffuser from the bottom of the reactor (the vacuum/gas pump was initially set at 5 litres per hour-LPH), the distribution of biomass and substrate through the reactor was not completely homogenous. Since a membrane flat panel was submerged inside the reactor, which provided good retention of biomass, thus preventing the washout of biomass, the TSS/VSS could either theoretically increase, or stay constant, or decrease due to cell lysis throughout the process. However, there were moments when the TSS/VSS was found to slightly decrease (from 5.8 g/L to 5.5 g/L VSS). This may have been due to the fact that biomass was attached to the surface of the membrane panel, and not fully mixed in the suspension. For that reason, analysis of TSS/VSS in the reactor at certain times might not represent the concentrations of the whole microbial community.

In addition, some other bacteria, i.e. aerobic bacteria, could also die due to the lack of nutrients, or be unable to adapt to the change in environment, particularly during the early stages of the experiment and this could have contributed towards the decrease in biomass concentration. The final concentration of the enriched biomass after 60 days was about 5.0 g/L VSS, which was slightly higher than that found by Dapena-Mora et al. (2004b) (3.5 g/L VSS) who grew and enriched the Anammox biomass in an SBR for about 6 months. The initial biomass concentration inside the reactor was about 5.8 g/L VSS, indicating that there was a slight loss of total biomass, i.e. about 20%, during the whole experiment, when in fact cell growth was expected. This could be a result of the biomass

attaching to the surface of the membrane inside the reactor, and not being fully mixed in the suspension.

#### 4.3.2.2 The Anammox Process in SAMBR 2

SAMBR 2 was run with an HRT of four days, but the other conditions were kept identical to those of the first SAMBR, i.e. pH, temperature, and feed composition. This second SAMBR (newly designed reactor as previously shown in **Fig. 3-7**) was run continuously for a total of 70 days. However, extra precautions had to be taken as this newly designed reactor was more susceptible to oxygen leakage through its top segment used to pull the membrane out of the reactor unit. The profile of nitrogen concentrations in the effluent in the forms of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  over the experimental period are shown in **Fig. 4-4**. As illustrated in the figure, the experimental period was divided into three different stages, similar to what had been done for the analysis of SAMBR 1, but with slightly different durations for each stage: **Stage 1** (day 1 – 20); **Stage 2** (day 20 – 50); and **Stage 3** (day 50 – 70). This could be due to the longer HRT applied in the process.



**Fig. 4-4** Profile of nitrogen compound concentrations ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ) and CODs concentration in the effluent of SAMBR 2

As previously mentioned, the first few days of operation could be considered as a transition period to a new environment. For SAMBR 2, this transition period could have been longer due to the longer HRT applied for its operation (four days). One of the indications that the reactor was in a transition period in the initial stage was the higher concentrations of  $\text{NH}_4^+$  (about 350 mg/L) found in the effluent when compared with its initial concentration after the seed sludge was inoculated into the reactor (175 mg/L). In fact, its concentration increased to about 380 mg/L in the first five days. A few factors could have caused this, and this includes the breakdown of the biomass, i.e. aerobic bacteria, due to the change in environment. Cell lysis of aerobic bacteria would cause the organic nitrogen to be broken down to  $\text{NH}_4^+$ , which would increase  $\text{NH}_4^+$  concentrations inside the reactor (Chamchoi and Nitorisavut, 2007). This increase in  $\text{NH}_4^+$  concentration was in agreement with the previously reported phenomenon of having higher concentrations of  $\text{NH}_4^+$  detected in the effluent than its concentrations in the influent (Chamchoi and Nitorisavut, 2007, Wang et al., 2009) demonstrating that denitrifying bacteria were predominant in the early stage of inoculation.

Unlike in SAMBR 1, there was no sudden decrease in the concentrations of  $\text{NH}_4^+$  in the effluent, measured during the first 20 days in SAMBR 2, although its overall concentrations decreased slightly to about 325 mg/L from an earlier concentration of 350 mg/L. The  $\text{NH}_4^+$  started to be slowly consumed from day 5, most likely through the action of the Anammox bacteria that started to grow in the reactor. This could be further supported by the occurrence of  $\text{NO}_2^-$  removal, i.e. about 50% of the influent  $\text{NO}_2^-$ , in the first 20 days. During that stage too, quite significant concentrations of  $\text{NO}_3^-$  were detected in the effluent.

The occurrence of a simultaneous removal of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  from the reactor demonstrated clear and stable Anammox activity in **Stage 2** and **Stage 3**. From day 20, the concentrations of  $\text{NH}_4^+$  in the effluent decreased quite rapidly (325 to 170 mg/L), resulting in about 50% of  $\text{NH}_4^+$  removal. During that period,  $\text{NO}_2^-$  level continued to deplete to as low as 65 mg/L, representing over 75% removal efficiency, while  $\text{NO}_3^-$  was found to be in the range of 50 - 100 mg/L. Furthermore, considering the substrates provided and that the conditions were favourable for Anammox bacteria, they were expected to be more dominant than other bacterial species during **Stage 2**. It was previously hypothesised that all  $\text{NH}_4^+$  was removed by the Anammox bacteria, while  $\text{NO}_2^-$  was removed through the combined function of both denitrifying and Anammox bacteria (Wang et al., 2009, Xiao et al., 2009).

Quite significant levels of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were still maintained in the reactor, and this could be due to  $\text{NH}_4^+$  being oxidised by both the Anammox and AOB-like bacteria.

**Stage 3** demonstrated the stability of the Anammox process as both  $\text{NH}_4^+$  and  $\text{NO}_2^-$  were continuously consumed. At this stage, the Anammox bacteria could be the most dominant species available inside the reactor. On day 70, which marked the end of the experiment, about 65 mg/L of  $\text{NH}_4^+$  was observed in the effluent of the reactor, which represents about 81% of  $\text{NH}_4^+$  removal efficiency from its initial concentration. Likewise, about 66% of the  $\text{NO}_2^-$  was successfully removed at the end of the experiment, where the final  $\text{NO}_2^-$  concentration was about 50 mg/L. The average molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption in the reactor in the final stage was also 1:0.9, identical to that achieved in SAMBR 1.

On the other hand, about 38 mg/L of  $\text{NO}_3^-$  was also detected at the end of the experiment. Considering the whole period of the experiment, an average of 90 mg/L of  $\text{NO}_3^-$  was produced in the reactor, which was quite similar to the average amount of  $\text{NO}_3^-$  produced in SAMBR 1 after two months of operation, i.e. 100 mg/L. Based on the Anammox stoichiometric equation, SAMBR 2 produced much higher  $\text{NO}_3^-$  in the first two stages compared to that of SAMBR 1, i.e. an average of one mole  $\text{NO}_3^-$  produced per mole of  $\text{NO}_2^-$  consumed. However, the production of  $\text{NO}_3^-$  reduced to 0.55 mole per mole  $\text{NO}_2^-$ , suggesting that the Anammox activity in the reactor was higher than in the earlier stages.  $\text{NO}_3^-$  could be produced in the reactor from the oxidation of  $\text{NO}_2^-$  as the Anammox bacteria grew and propagated (van de Graaf et al., 1997). About 10% of the fed nitrogen, i.e.  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , is also converted to  $\text{NO}_3^-$  (van de Graaf et al., 1996).

Analysis of variance (ANOVA) was conducted to determine whether the effect of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  on the Anammox reaction in SAMBR 2 is statistically significant. From this analysis,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were found to be statistically significantly changed over time in the Anammox reaction with  $p < 0.0001$  ( $F(4,10) = 784.64$ ),  $p < 0.0001$  ( $F(4,10) = 2213.8$ ), and  $p < 0.0001$  ( $F(4,10) = 215.79$ ), respectively.

### 4.3.3 Analysis of COD

The seed sludge collected from the AD treatment plant initially contained significantly high concentrations of soluble COD, i.e. about 12 g/L. However, it would have degraded slightly after being kept in the 4 °C room for some times, and was diluted with the addition of the synthetic wastewater that did not contain any COD matter during the start-up of the process in the SAMBR. The presence of COD could have some effects on the growth of bacterial species, specifically denitrifying bacteria, inside the reactor. This was explained by Wang et al. (2009), where denitrifying bacteria used COD as a carbon source and electron donor, with the influent  $\text{NO}_2^-$  as an electron acceptor. For that reason, denitrifying bacteria might predominate in the first stage since anaerobic heterotrophic denitrifying bacteria grow much faster than the autotrophic Anammox bacteria.

However, the COD concentrations in both SAMBR 1 and SAMBR 2 were found to be almost consistent about its mean throughout the experimental period, i.e. about 2.5 g/L and 3.5 g/L in SAMBR 1 and SAMBR 2, respectively, showing that the presence of COD had minimal interference with the process. This is also supported by the statistical analysis (ANOVA) on the COD concentrations over time, which were found to be not significant ( $F(4,10) = 0.29$ ,  $p = 0.877891$ ). Based on the consistent COD concentrations and the results of statistical analyses, it could be concluded that the growth of denitrifying bacteria in the reactor was minimal. Since the sludge was collected after an anaerobic digestion process, most of the available carbon sources have been converted into methane and carbon dioxide (Akunna et al., 1992). Without organic carbon matter in the sludge nor additional supply of carbon electron donor, it is likely that denitrification did not take place in the reactor.

It has also been previously reported that residual COD found in the effluent of anaerobic treatment process is usually relatively high (~2% of the influent COD), and is mostly soluble microbial products (SMP) produced by the cells in the reactor itself (Aquino and Stuckey, 2002, Jianga et al., 2008). For instances, SMPs contain extracellular polymeric substances (EPS) that are detached from the cells (Janga et al., 2007). Therefore, SMPs represent a mixture of polymeric organic substances released by microorganisms, produced from cell lysis and adsorbed organic matter from wastewater, such as polysaccharides, proteins, humic substances and nucleic acids (Sheng et al., 2010). These solutes are known

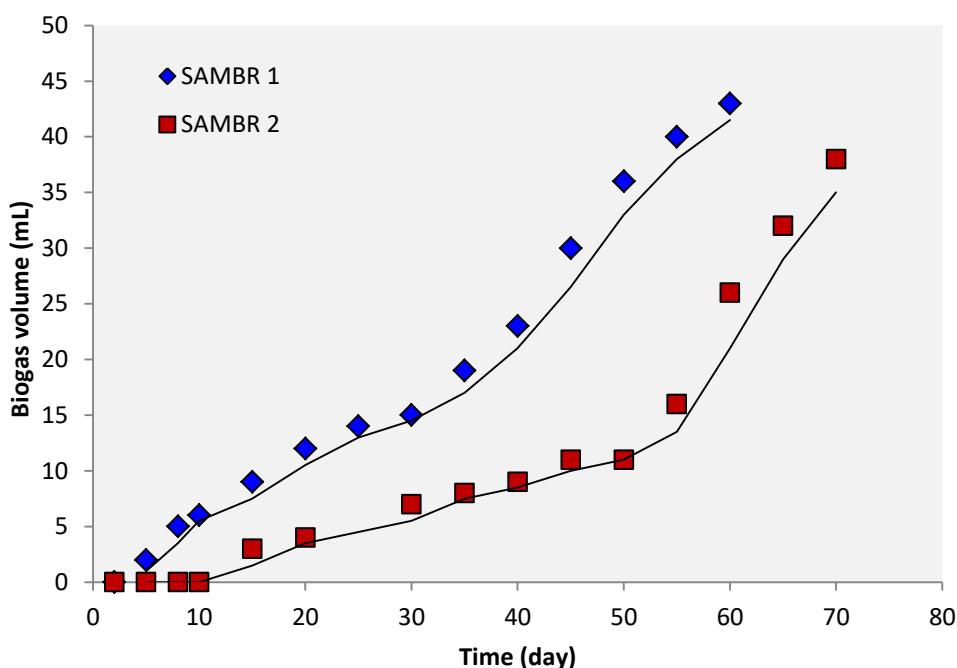


to be resistant to anaerobic degradation (hence their presence in the effluent), and hence do not support the growth of heterotrophic denitrifying bacteria.

In addition, based on some reports in the literature, denitrification was found to decrease when the COD/N ratio is above 15, depending on the type of carbon sources, i.e. acetate, methanol, or glucose, used for the process (Akunna et al., 1992, Adav et al., 2010, Ge et al., 2012). However, identification of the type of carbon sources contained in our COD is beyond the scope of this study. While the possibility of denitrification occurring could not be completely ruled out, the consistent level of COD concentrations observed in this study suggests that the effect of denitrification was negligible within the time period of the recorded Anammox process.

#### **4.3.4 Biogas Production**

One of the criteria that could be used to validate the occurrence of the Anammox reaction is the production of nitrogen gas, as stoichiometrically shown by **Eq. 2-13**, i.e. one mole of  $N_2$  gas is produced for every mole of  $NH_4^+$  and 1.32 mole of  $NO_2^-$  consumed. The biogas production from both reactors was monitored and observed using the water displacement method. The measurement of biogas production was made every five days, and its production trend is as illustrated in **Fig. 4-5**. Based on GC analysis, it was found that the biogas was mainly composed of dinitrogen ( $N_2$ ) gas, i.e. more than 95%, while the remaining gas was composed of methane ( $CH_4$ ) and carbon dioxide ( $CO_2$ ), which could be considered as negligible. However, due to the limitations of the GC equipment, other nitrogenous gases, i.e.  $N_2O$ ,  $NO$ ,  $NO_2$ , were not analysed, although Strous et al. (1998) reported that traces of those gases were also produced apart from  $N_2$  gas. In addition, other intermediates that may be present in the reactions, such as hydrazine and hydroxylamine, were also neglected in the mass balance analysis due to their low concentrations, i.e. the highest concentrations of hydrazine and hydroxylamine recorded were 0.81 mg/L and 0.085  $\mu$ g/L.



**Fig. 4-5** Biogas production in SAMBR1 and SAMBR 2

The volumes of biogas collected, presumed to be pure nitrogen, were converted into moles, and was compared to the number of moles of the Anammox reactants ( $\text{NH}_4^+$  and  $\text{NO}_2^-$ ) to check whether their molar ratios follow the theoretical ratios of the Anammox reaction. Theoretically, the molar ratio of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  consumption to  $\text{N}_2$  production for the Anammox reaction is 1:1.3: 1. It was found that biogas production at the end of the process was very close to the theoretical values, i.e. about 1.1 and 0.98 moles of  $\text{N}_2$  were produced for every mole of  $\text{NO}_2^-$  consumed, in SAMBR 1 and SAMBR 2, respectively.

The ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption in the reactor is the determining indicator of the Anammox reaction, while the  $\text{N}_2$  production could be used to support its occurrence. The gas displacement method used to detect biogas production, while it is a common method, may not reflect the actual volume, and thus moles of biogas produced at a certain time. This method was used to detect biogas production, while its composition was determined by GC. The sampling of the biogas was neither automated nor continuous, and hence a slight error in measurement is expected. The qualitative sampling was done to show that  $\text{N}_2$  gas was produced at about an equimolar consumption of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ .

## 4.4 SUMMARY

The Anammox process was successfully started up from anaerobic digestion sludge in two units of a laboratory-scale SAMBR in about two months (60 days for SAMBR 1 and 70 days for SAMBR 2). Both reactors were operated under similar operating conditions, i.e. pH 7.5-8.0, temperature 35°C, except that the HRT used for SAMBR 1 was two days, while SAMBR 2 operated at an HRT of four days. Both start-up periods (60 and 70 days) were much shorter than the previously reported values. Strous et al. (1997b) enriched a high-purity Anammox culture with the specific Anammox activity (SAA) of 0.08 g NH<sub>4</sub><sup>+</sup>/g VSS d in a fluidised-bed reactor within 150 days, while Dapena-Mora et al. (2004) required 200 days to start-up the Anammox process in an SBR also from an already-enriched culture, but achieved much higher SAA of 0.44 g NH<sub>4</sub><sup>+</sup>/g VSS d. A shorter enrichment period of 100 days was recorded by Third et al. (2005) who also started-up the process in an SBR, but from an activated sludge with SAA of 0.1 g NH<sub>4</sub><sup>+</sup>/g VSS d.

Trigo et al. (2006) later investigated the start-up of the Anammox process in a membrane sequencing batch reactor (MSBR) for a period of 375 days. Due to the use of a membrane module (submerged HF membrane) to retain the biomass inside the reactor, they concluded that it was possible to achieve high nitrogen removal rates, i.e. > 0.7 g NH<sub>4</sub><sup>+</sup>/L d, with low concentrations of biomass, i.e. < 2 g VSS/L, compared to other systems that started with higher biomass concentrations. The reported SAA of the biomass was 0.35-0.45 g NH<sub>4</sub><sup>+</sup>/g VSS d (Trigo et al., 2006). These findings were quite similar to those of Wyffels et al. (2004) and Suneethi and Joseph (2011a) who also suggested that the use of MBR system is more advantageous than the previously reported Anammox systems, achieving NH<sub>4</sub><sup>+</sup> removal rate of as high as 1.1 g NH<sub>4</sub><sup>+</sup>/L d with removal efficiency of over 80%. However, no SAA value was reported.

The two months start-up period recorded in this study was, however, quite comparable to some recently available reports. Wang et al. (2012) studied the Anammox start-up performances (enriched from conventional activated sludge mixed with nitrifying activated sludge) in both the MBR and SBR, and they found that the MBR had a notably shorter start-up period of only 59 days compared to 101 days in an SBR, with an NH<sub>4</sub><sup>+</sup> removal rate of 0.16 g NH<sub>4</sub><sup>+</sup>/L d and over 90% removal efficiency in the MBR. The final

SAA of the biomass was previously reported to be 0.35 g NH<sub>4</sub><sup>+</sup>/g VSS d (Wang et al., 2009).

The shorter start-up periods were most likely due to the use of a membrane impermeable to microbial cells, which enabled the biomass to be fully retained inside the reactor. In fact, this complete retention of biomass inside the system is necessary considering the fact that the Anammox organism is a very slow-growing bacteria, and tends to grow in aggregates (Trigo et al., 2006). The phenomenon of simultaneous nitrification and denitrification (SND) in the inoculation seed as previously proposed by Wang et al. (2009) could also contribute towards the shorter start-up time for the Anammox process. In this case, some of the Anammox species might have already accumulated in the sludge where SND occurred.

It is important to highlight that the operations of the previously reported Anammox systems were carried out with higher concentrations of biomass that already showed significant Anammox activity enriched in other systems prior to that. However, in this study, the enrichment of the Anammox culture was made purely from raw sludge of an anaerobic digester, resulting in much lower NH<sub>4</sub><sup>+</sup> removal rates of 27 mg NH<sub>4</sub><sup>+</sup>/L d and 4.0 mg NH<sub>4</sub><sup>+</sup>/L d, with SAA of 4.7 mg NH<sub>4</sub><sup>+</sup>/g VSS d and 0.7 mg NH<sub>4</sub><sup>+</sup>/g VSS d in SAMBR 1 and SAMBR 2, respectively. Suneethi and Joseph (2011b), who enriched the Anammox population from both anaerobic and aerobic sludge in batch reactors (operated in fed-batch mode), however, reported that anaerobic seed showed better NH<sub>4</sub><sup>+</sup> removal efficiency (98%) and stabilisation than aerobic seed (94%) in 70 days of enrichment period, with an SAA of 5.1 mg NH<sub>4</sub><sup>+</sup>/g VSS d, comparable to the SAA recorded in SAMBR 1. These low NH<sub>4</sub><sup>+</sup> removal rates and SAA in this study could also suggest that AD sludge might not be capable of enriching high activity of the Anammox culture, although the initial biomass concentrations used to inoculate the reactor were relatively high, i.e. > 5 g VSS/L.

Despite the low NH<sub>4</sub><sup>+</sup> removal rates and SAA shown by the Anammox culture, both reactors managed to record quite high NH<sub>4</sub><sup>+</sup> removal efficiencies, with 86% and 81% in SAMBR 1 and SAMBR 2, respectively, after about two months of operation. However, NO<sub>2</sub><sup>-</sup> removal efficiency for both reactors was fairly similar, i.e. 67% and 66%, respectively. **Table 4-3** summarises and compares some important parameters and findings

for SAMBR 1 and SAMBR 2. In terms of the average molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption, both reactors resulted in a similar ratio of 1:0.9, demonstrating that more  $\text{NH}_4^+$  was consumed compared with  $\text{NO}_2^-$  during the experiments. The ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption in various Anammox reactors also depends on the substrate, operating conditions, and reactor configurations, and can be in the range of 0.25 – 2 (Strous et al., 1999b). The findings also show that there were no significant differences in terms of reactor performance between HRTs of two and four days.

**Table 4-3** Comparison of parameters and findings between SAMBR 1 and SAMBR 2.

Parameters	SAMBR 1	SAMBR 2
HRT (days)	2	4
Start-up period (days)	60	70
$\text{NH}_4^+$ removal (%)	86	81
$\text{NO}_2^-$ removal (%)	67	66
$\text{NH}_4^+$ removal rate (mg $\text{NH}_4^+$ /L d)	27	4
SAA (mg $\text{NH}_4^+$ /g VSS d)	4.7	0.7
Ratio of $\text{NH}_4^+$ / $\text{NO}_2^-$ consumption	1:0.9	1:0.9
Ratio of $\text{NH}_4^+$ / $\text{NO}_2^-$ consumption to $\text{N}_2$ production	1:0.9:1	1:0.9:0.88

The submerged membrane type is also preferred to the side-stream membrane mainly because of its simple design, and it does not require much space for operation. The only drawback to using a membrane inside the reactor was membrane fouling. It was found that after two months of continuous operation, the membrane started to clog due to biomass accumulation and attachment on the surface of the membrane. In such a case, the reactor was stopped, emptied, and disassembled (in the case for SAMBR 1), and the membrane was taken out for cleaning. In this study, a cleaning protocol for Kubota membranes previously proposed by Le-Clech et al. (2006) was employed, and the process resumed as soon as the cleaning was completed.

Apart from membrane fouling, another issue that was commonly faced during the operation was internal blocking of the gas diffuser by biomass. In less severe cases, this blockage could be removed by increasing the sparging gas flow rate, i.e. from 5 to 10 LPH; otherwise the whole reactor had to be disassembled for manual cleaning. The new design of the SAMBR has been proven to be more favourable than the old design, and it could save a lot of time as it was much easier to access the membrane without the need to disassemble the whole reactor unit every time membrane cleaning had to be carried out. However, extra care had to be taken when using the newly designed SAMBR due to its susceptibility to oxygen leakage around the top segment of the reactor.

In conclusion, the SAMBR used in this study was found to be a promising and feasible alternative to start up the Anammox process, mainly because of the ability of the membrane to minimise the loss of microbial activity by avoiding the biomass from being washed out. A short start-up period (about two months) and considerably high removal efficiency of  $\text{NH}_4^+$  were achieved (over 80%) in both reactors. However, the removal efficiency of  $\text{NO}_2^-$  was relatively lower (< 70%), and this has resulted in a relatively lower molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption when compared with previously reported values. Considerable effort would have to be made in order to further improve the process performances, particularly in terms of removal efficiency and process stability. For example, the reactor could be operated for a longer duration and in a larger scale to investigate the stability of the Anammox process. Previous investigations on various types of nitrogen converters, including the Anammox bacteria, in larger scale MBRs for longer experimental periods has demonstrated better process stability (Fux et al., 2002, Ozdemir et al., 2011).

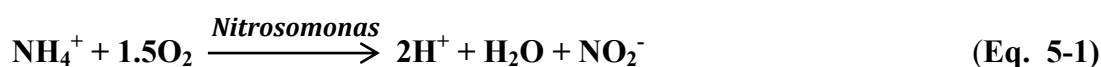
## CHAPTER 5

# ENRICHMENT OF AMMONIUM-OXIDISING BACTERIA (AOB) FROM DIFFERENT SEED CULTURES IN BATCH REACTORS

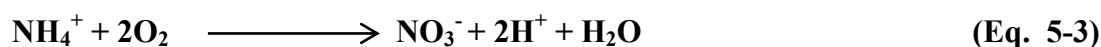
### 5.1 INTRODUCTION

Nitrification is a two-step process by which  $\text{NH}_4^+$  is firstly oxidised to  $\text{NO}_2^-$  by ammonium-oxidising bacteria (AOB), followed by the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  by  $\text{NO}_2^-$ -oxidising bacteria (NOB). Both of these AOB and NOB, generally known as nitrifying bacteria, are considered to be autotrophs as they derive energy for growth and synthesis from the oxidation of inorganic compounds ( $\text{NH}_3$ ) and carbon dioxide, respectively. The most commonly recognised genus of AOB is *Nitrosomonas*, while the most well-known NOB genus is *Nitrobacter* (Cheremisinoff, 1996). Apart from these organisms, other nitrifying bacteria that include *Nitrosococcus*, *Nitrosopira*, *Nitrosovibrio*, and *Nitrosolobus* are also able to oxidise  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , whereas *Nitrospira*, *Nitrospina*, *Nitrococcus*, and *Nitrocystis* are known to be involved in the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (Ahn, 2006). Nitrification by organisms other than nitrifying bacteria, however, occurs at relatively much slower rates, i.e. 1000 – 10,000 times slower than the rate of nitrification by nitrifying bacteria (Gerardi, 2005).

The two-step nitrification process by AOB (*Nitrosomonas*) and NOB (*Nitrobacter*) is shown by Eq. 5-1 and 5-2, respectively. The reactions are generally coupled and proceed rapidly to the  $\text{NO}_3^-$  form, thus  $\text{NO}_2^-$  level at any given time is relatively low. The conversion of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  alone (Eq. 5-1) is also referred as nitritation (formation of  $\text{NO}_2^-$ ), which in some applications is often termed as partial nitrification.



The overall nitrification reaction may be represented by combining Eq. 5-1 and 5-2, as shown in Eq. 5-3:



Nitrification occurs in nature and in activated sludge processes, although the population of nitrifying bacteria is relatively low compared with other bacterial species. For instance, activated sludge that is used for nitrification normally contains only about 3 – 10% of nitrifying bacteria (Gerardi, 2005). In contrast, it is less reported that nitrification can also naturally occur in anaerobic digestion processes. Prior to the discovery and development of the Anammox process, nitrification coupled with subsequent denitrification process has been the preferred and commonly used method for the removal of nitrogen from municipal and industrial wastewaters (Huang et al., 2001, Fux et al., 2002, Ahn, 2006). With the discovery of the Anammox process (Mulder et al., 1995), new possibilities and alternatives have opened up. A more economical method for nitrogen removal was later developed, which combined partial nitrification (an adaptation of the SHARON process) with the Anammox process in series (Jetten et al., 1999, Fux et al., 2002, Schmidt et al., 2003, Feng et al., 2007).

One of the objectives of this study was to specifically enrich and grow the AOB in batch reactors from two different sources: full-scale SAMBR sludge, and return activated sludge. To do this, operating conditions that favour and could promote the growth of AOB must be applied, while at the same time suppressing the growth of NOB inside the reactor. The strategies to promote only the growth of AOB, while suppressing NOB, have been widely applied in partial nitrification processes in which  $\text{NH}_4^+$  is only needed to be oxidised to  $\text{NO}_2^-$ , but not to  $\text{NO}_3^-$ . Suppressing the growth of NOB would prevent further oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . High concentrations of  $\text{NO}_2^-$  and low concentrations of  $\text{NO}_3^-$  found inside the reactor could demonstrate that AOB are the predominant species, and thus the biomass at that specific time could be harvested and used for the pre-oxidation process in the NF membrane modules.



## 5.2 MATERIALS AND METHODS

This section describes the operational strategies used in the experiment, i.e. process design, and operating conditions. Details of specific methods, i.e. analytical techniques, used in the experiments were previously described in Chapter 3.

### 5.2.1 Strategy of Operations

Seed sludge used in this study was collected from full-scale SAMBR reactor at Anglian Water in Cambridge, and return activated sludge (RAS) from Mogden Sewage Treatment Works in London. The sludge was inoculated into 2-litre glass batch reactors, and mixed with synthetic wastewater adapted and modified from the previously reported literature (Bhaskar and Charyulu, 2005) as shown in **Table 5-1**. The reactors were named nitrifying reactor 1 (NR 1), and nitrifying reactor 2 (NR 2). The growth media contained about 128 mg/L of  $\text{NH}_4^+$ , but did not contain any  $\text{NO}_2^-$  as this was necessary to prevent the growth of NOB.

**Table 5-1** Synthetic media used for the enrichment of AOB in batch reactors.

Compound	Concentration (mg/L)
$(\text{NH}_4)_2\text{SO}_4$	468
$\text{KH}_2\text{PO}_4$	400
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	80
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	80
$\text{FeSO}_4$	5.5
EDTA	10
Phenol red	10

Since nitrifying bacteria need to grow under slightly aerobic condition, with dissolved oxygen (DO) of above 0.5 mg/L, one of the feed vessels to the reactors was left open. The DO level was checked and measured using a DO probe. The pH of the reactor contents was monitored regularly as the nitrifying reaction is an acidifying process that could cause the pH to drop significantly, hence inhibiting nitrification. The addition of sodium carbonate solution (1 M) was made accordingly to maintain the pH at around 7.8 –

8.0. The reactors were placed on orbital shakers in a constant temperature room at 35 °C. The speed of the orbital shakers was set at 150 rpm to mix the biomass and substrate, and hence keeping the biomass suspended as free cells. The duration of operation was slightly different, ranging from four months to a year, mainly depending on the reactor performance, i.e. rate of  $\text{NH}_4^+$  removal and conversion to  $\text{NO}_2^-$ . The nitrifying batch reactor is as shown in Fig. 5-1.



**Fig. 5-1** Aerobic batch reactor used to grow nitrifying bacteria, placed in a constant temperature room of 35 °C.

Whenever the added  $\text{NH}_4^+$  was found to have been consumed in the batch reactors, and had reached a low level, i.e. < 50 mg/L, biomass was left to settle for about an hour, while the supernatant synthetic wastewater, i.e. growth media, was pumped out of the reactors using a peristaltic pump (Watson-Marlow, 101U). Fresh growth media was then introduced into each reactor, and the process was restarted. The introduction of fresh growth media was also needed whenever the concentrations of  $\text{NO}_3^-$  substantially increased, while the concentrations of  $\text{NO}_2^-$  depleted. This step was essential to maintain the growth of the intended bacterial species, and to avoid changes in reactors composition.

## 5.2.2 Sampling and Analyses

Sampling of wastewater was done every few days over a varying interval, i.e. 5-15 days, to monitor the effluent quality, and the frequency of sampling depended mainly on the rate of  $\text{NH}_4^+$  removal from the reactor. Prior to sampling, biomass was left to settle at the bottom of reactor for about one hour by turning off the orbital shaker. About 10 mL of the supernatant sample was carefully withdrawn from the reactor for regular analysis using a syringe. A similar amount, i.e. 10 mL, of fresh growth media was added to the reactor every time a supernatant sample was withdrawn. Samples were centrifuged at 5000 rpm for 5 minutes, and then filtered through a 0.45  $\mu\text{m}$  syringe filter prior to analyses to remove any biomass. Samples were also diluted if needed, then analysed for nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ ), and COD content. Biomass was also withdrawn from the reactor to be analysed for TSS and VSS content, but the sampling was less frequent than that for nitrogen analysis.

Nitrogen transformations inside the bioreactor were studied from the analysis of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ . Apart from that, the biomass development was also studied based on other parameters such as COD, TSS and VSS, all of which was performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1999). Removal efficiencies ( $R_e$ ) of  $\text{NH}_4^+$  from the reactor were calculated using the following equation:

$$R_e (\%) = \left(1 - \frac{C_e}{C_i}\right) \times 100$$

where  $R_e$  is the removal efficiency;  $C_i$  is the initial concentration of  $\text{NH}_4^+$ ; and  $C_e$  is the concentration of  $\text{NH}_4^+$  in the effluent. In addition, nitrogen mass balances between the influent and effluent were also performed at certain times interval to monitor the validity of nitrogen transformation inside the reactors.

## 5.3 RESULTS AND DISCUSSION

Experimental results on the enrichment of nitrifying bacteria in batch reactors are presented and discussed in terms of the reactor performances and bacterial growth, mainly based on nitrogen profiles inside the reactor.

### 5.3.1 Initial Characteristics of Seed Cultures

The seed culture were first analysed for their initial characteristics, and are presented in **Table 5-2**. It is important to carefully investigate the initial characteristics of the sludge, particularly their nitrogen content as it could be related to the presence of bacterial species intended for the experiments. The nature and purpose of the process where the sludge was collected also provide some significant insights into the availability of the different types of bacterial culture in the sludge.

**Table 5-2** Initial characteristics of seed sludge used to grow AOB in batch reactors.

Parameters (mg/L, except pH)	Sources of seed cultures		
	Return Activated Sludge (NR 1)	Pilot-scale SAMBR (NR 2)	Anaerobic digester (for comparison)
pH	7.70	7.20	7.50
TSS	6700	460	11700
VSS	3045	247	8800
NH <sub>4</sub> <sup>+</sup>	3.70	190	245
NO <sub>2</sub> <sup>-</sup>	0.38	0.65	0.40
NO <sub>3</sub> <sup>-</sup>	32.50	17.30	13.10
CODs	7100	400	11735
CODt	14000	743	19600

Initially, there were two types of wastewater sludge collected from Anglian Water Treatment Plant: anaerobic digestion (AD) sludge, and full-scale SAMBR sludge. The AD sludge, however, was not used to grow AOB in this study due to some existing reports and information stated that it had less potential to start-up nitrification process. At Anglian Water in Cambridge, the treatment processes were primarily operated for the removal of organic content, and not for nitrogen removal, thus the sludge might not contain many or any species of nitrifying bacteria (Skelton, 2012). The significantly high amount of NH<sub>4</sub><sup>+</sup> (245 mg/L) found in the AD sludge could also indicate that there was no nitrification occurred in the plant. The sludge was moderately thick, viscous and black in color with a high organic content (11.7 g/L CODs). In contrast, the seed culture from the full-scale SAMBR was much more dilute and less viscous, containing about 25 times less suspended

solids content compared with the AD sludge. Its organic content was only 0.4 g/L CODs, about 30 times less than that of the AD sludge.

As for the activated sludge obtained from Mogden Sewage Treatment Works in London, its initial characteristics seemed to fall in between the AD and SAMBR sludge, particularly in terms of its organic and suspended solids content. Both TSS and VSS content were about half of that in the AD sludge. The low concentration of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  present in the sludge, i.e. 3.7 mg/L and 0.38 mg/L, respectively, demonstrates that the treatment plant had experienced nitrification process, in which most of the  $\text{NH}_4^+$  and  $\text{NO}_2^-$  had been oxidised to  $\text{NO}_3^-$ , or reduced to nitrogen gas by denitrifying bacteria. Based on the information provided by Thames Water Innovation Centre, the sludge collected from the Mogden Plant was supposed to have a “good” and robust population of nitrifying bacteria, and should be able to nitrify quickly once all of their growth requirements are met (Pearce, 2013).

The availability of a nitrifying bacterial community in activated sludge processes could be further verified by some information in the literature, which said that operators of activated sludge processes may promote nitrification even though many of the plants are not required to satisfy an ammonia or total nitrogen discharge limit. In fact, the presence of nitrogenous compounds are not only of concern to operators of activated sludge reactors, but also to regulatory agencies due to their undesired impacts on activated sludge processes and receiving body of water, respectively. For example, Gerardi (2005) reported that operators of activated sludge reactors may use the  $\text{NO}_3^-$  ions produced during anoxic nitrification periods to obtain improved floc particle structure, and decreased operational costs. It was further reported that, of the total population, about 3 – 10% of a nitrifying bacterial culture were found in activated sludge used for nitrification (Gerardi, 2005).

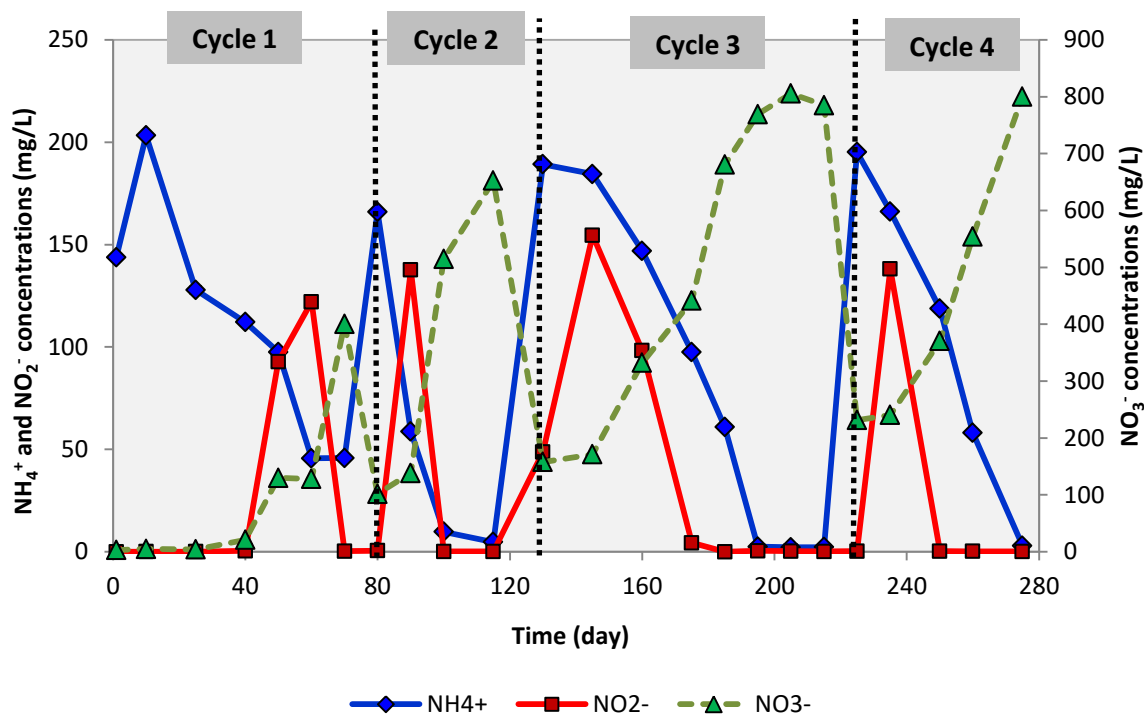
### **5.3.2 Growth of Nitrifying Bacteria**

During the whole experiment, a suspension of free cells was maintained inside each reactor. Discussion on bacterial growth characteristics and reactor performance are made based on different nitrifying reactor, named as NR 1 and NR 2.

### **5.3.2.1 Nitrifying Batch Reactor 1 (NR 1): Activated Sludge**

The reactor (NR 1) was operated for a total of 275 days, in which fresh growth media was introduced into the reactor on three occasions, i.e. day 80, 130 and 225, other than at the start. Biomass was harvested from the reactor when  $\text{NO}_2^-$  concentrations were very high, indicating that AOB were the predominant species at that moment. High concentrations of  $\text{NO}_2^-$  were usually observed in the reactor 10 – 20 days after the introduction of fresh growth media, before  $\text{NO}_3^-$  started to accumulate, and NOB outcompete AOB species. For simplicity of the discussion, the experimental period could be divided into four different cycles; **Cycles 1 – 4**, each was separated based on the day the new growth media was re-introduced into the reactor (indicated by the dotted lines), as shown in **Fig. 5-2**. The reactor was also bubbled with air every time new growth media was introduced to ensure that the DO level was around 1 - 2 mg/L.

At the beginning of the experiment, as soon as the sludge was mixed with growth media containing about 128 mg/L of  $\text{NH}_4^+$ , samples of the media was withdrawn for analyses. On day 1, the concentration of  $\text{NH}_4^+$  was apparently much higher than the theoretical concentration of  $\text{NH}_4^+$  in the growth media. This was quite common as the sludge also contained a certain amount of either  $\text{NH}_4^+$  or free ammonia. Apart from that, the effect of the “adjustment period” could have also taken place, in which bacterial species living inside the sludge tried to adapt to the change in environment, i.e. temperature, pH and growth media. As this was an aerobic process, any obligate anaerobes would not be able to survive. Consequently, dead and lysed biomass could also have contributed towards the increase in free ammonia concentrations.



**Fig. 5-2** NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> profiles in NR 1 during 275 days of operation. Other than at the start, fresh growth media was introduced into the reactor on day 80, 130, and 225, as indicated by the dotted vertical lines.

In addition, it is quite common that a process known as “ammonification” naturally precedes nitrification and denitrification. Ammonification is a process in which organic nitrogen is converted to ammonia and NH<sub>4</sub><sup>+</sup> through hydrolysis. The ratio of ammonia and NH<sub>4</sub><sup>+</sup> created from ammonification largely depends on the pH of the solution and temperature, although in many cases more NH<sub>4</sub><sup>+</sup> could be found than ammonia. At a pH less than 9.0, NH<sub>4</sub><sup>+</sup> are more predominant than free ammonia (Gerardi, 2005) since the pK<sub>a</sub> of ammonia is 9.25. In view of this, the concentrations of NH<sub>4</sub><sup>+</sup> in the reactor were found to be apparently much higher than that initially supplied in the growth media. After 10 days of enrichment, the concentration of NH<sub>4</sub><sup>+</sup> in the reactor was about 200 mg/L, almost double of that initially supplied in the growth media.

From day 10 onwards, however, it seemed that AOB had started to oxidise NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup>. As the concentration of NH<sub>4</sub><sup>+</sup> decreased, the NO<sub>2</sub><sup>-</sup> concentrations slowly increased. After two months of operation, NH<sub>4</sub><sup>+</sup> concentration reached its lowest (45mg/L), while the NO<sub>2</sub><sup>-</sup> concentration was significantly higher (122 mg/L), resulting in about 68% NH<sub>4</sub><sup>+</sup>

removal at a rate of 1.64 mg  $\text{NH}_4^+\text{-N}$  / L day. In spite of having fairly good removal rates of  $\text{NH}_4^+$  and the formation of  $\text{NO}_2^-$ , it was observed that  $\text{NO}_3^-$  had also started to accumulate alongside  $\text{NO}_2^-$ , indicating that some of the  $\text{NO}_2^-$  was being oxidised to  $\text{NO}_3^-$ . Presuming that AOB were the predominant species due to the high concentration of  $\text{NO}_2^-$  detected in the reactor, the first batch of the bacteria was harvested on day 60 to be used for the pre-oxidation of  $\text{NH}_4^+$  using the NF membrane modules in the next part of the study. Details of how the cells were harvested are described in the next chapter (**Chapter 6**).

At the end of **Cycle 1**, although 45 mg/L of remaining  $\text{NH}_4^+$  concentration was considered quite low, the reactor was left to operate for another 10 days to observe if more  $\text{NH}_4^+$  was consumed. However, on day 70, the concentration of  $\text{NH}_4^+$  remained at about 45 mg/L, indicating that no more  $\text{NH}_4^+$  oxidation was taking place. In contrast, the concentration of  $\text{NO}_2^-$  dropped dramatically to as low as 0.2 mg/L from 122 mg/L, while the concentration of  $\text{NO}_3^-$  rose significantly from 127 mg/L to 400 mg/L. After certain times, as the  $\text{NO}_2^-$  concentration was found to decrease indicating that  $\text{NO}_2^-$  had been completely oxidised to  $\text{NO}_3^-$ , while the significant presence of  $\text{NO}_3^-$  could promote the growth of NOBs inside the reactor.

As  $\text{NO}_3^-$  started to accumulate in the reactor, while  $\text{NO}_2^-$  depleted significantly, the existing growth media was pumped out of the reactor and replaced with new growth media on day 80. It was not possible, however, to completely drain the growth media from the reactor as this would also cause some loss of biomass. As a result of the media replacement, the immediate observable  $\text{NH}_4^+$  concentration was 165 mg/L, while the concentration of  $\text{NO}_3^-$  was found to be in the region of 100 mg/L, and  $\text{NO}_2^-$  was maintained in the range of 1 – 2 mg/L. The DO level was also checked accordingly, and the sludge would be aerated if needed, i.e. if the DO level was less than 0.5 mg/L.

Twenty days after the introduction of new growth media, about 94% of the  $\text{NH}_4^+$  was found consumed, clearly oxidised to  $\text{NO}_2^-$  as the concentration of  $\text{NO}_2^-$  drastically increased from less than 0.5 mg/L to almost 140 mg/L during that period. Correspondingly, the  $\text{NO}_3^-$  concentration slightly increased from 100 mg/L to 138 mg/L, indicating that a small percentage of the  $\text{NO}_2^-$  was also oxidised to  $\text{NO}_3^-$  by NOB. The slow accumulation of  $\text{NO}_3^-$  that took place in the reactor after  $\text{NH}_4^+$  had been oxidised to  $\text{NO}_2^-$  also means that it was quite difficult to practically suppress the growth of other bacterial species, mainly NOB



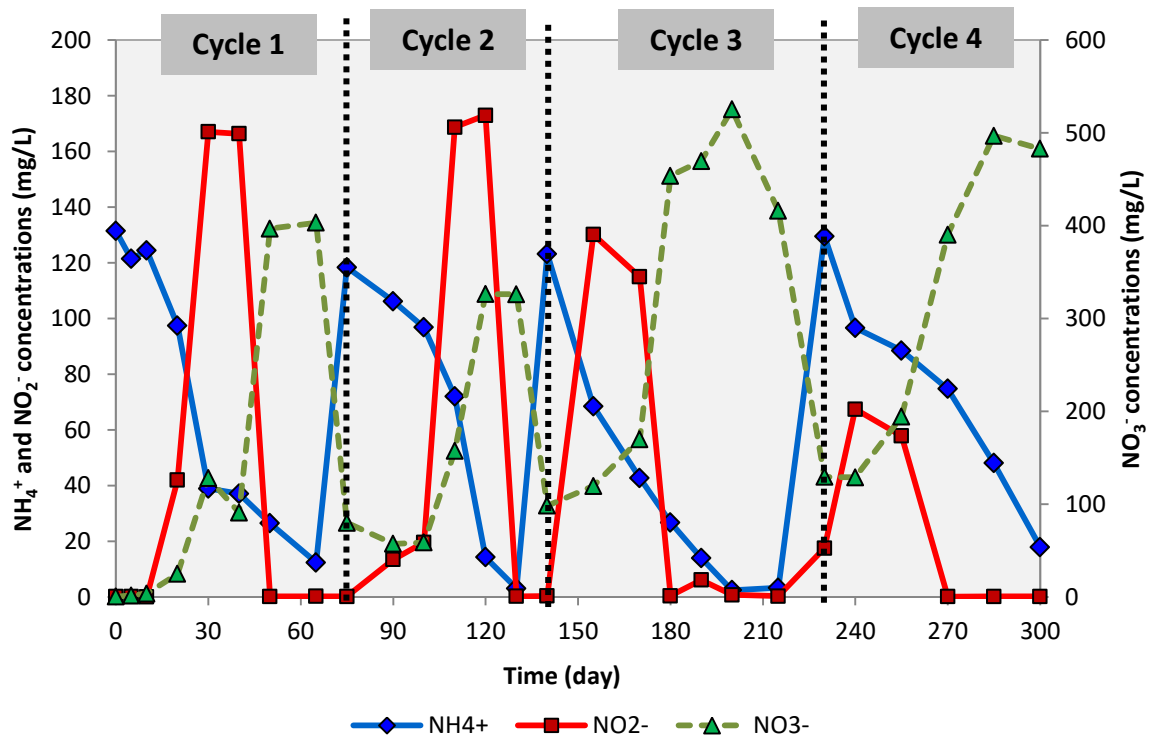
to prevent the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . Consequently, the maximum concentration of  $\text{NO}_3^-$ , i.e. 650 mg/L, was observed on day 115, while  $\text{NH}_4^+$  and  $\text{NO}_2^-$  decreased to their lowest concentrations of 4.5 mg/L and 0.1 mg/L, respectively. In such a case, it was time to restart the process by replacing the growth media, similar to what was done at the end of **Cycle 1**.

It was clearly observed that the presence of  $\text{NO}_2^-$  in the reactor in each cycle was apparently temporary as it was subsequently replaced by  $\text{NO}_3^-$ . This short period of  $\text{NO}_2^-$  in the reactor was, however, justifiable since  $\text{NO}_2^-$  is not the end product of nitrification and can easily be oxidised to  $\text{NO}_3^-$ .  $\text{NO}_2^-$  is unstable and merely exists as an intermediate compound during the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  (complete nitrification) (Cheremisinoff, 1996). On the other hand,  $\text{NO}_3^-$  is the most highly oxidised form of nitrogen and can only be reduced to nitrogen gas ( $\text{N}_2$ ) by heterotrophic denitrifying bacteria under anoxic conditions with the DO ideally below 0.2 mg/L (denitrification process). However, denitrification presumably did not take place as the reactor was aerobically operated. In addition, there was no apparent reduction in  $\text{NO}_3^-$  was observed in the reactor except when the growth media was replaced.

Ideally, the growth media should have been replaced as soon as  $\text{NH}_4^+$  and  $\text{NO}_2^-$  decreased to their lowest concentrations, otherwise, the  $\text{NO}_3^-$  level would keep increasing as a result of the oxidation of  $\text{NO}_2^-$  until there was insufficient nutrient left for microbial cells inside the reactor to grow. Although the  $\text{NO}_2^-$  concentration found on day 175 was only about 4 mg/L, the replacement of growth media was purposely delayed at the end of **Cycle 3** since the concentrations of  $\text{NH}_4^+$  was still quite high, i.e. about 100 mg/L. During the nitrite-lacking period, i.e. day 175 - 215, it could clearly be observed that the  $\text{NO}_3^-$  kept increasing and reached a concentration as high as 800 mg/L on day 205. This was due to the fact that the rate of  $\text{NH}_4^+$  oxidation and  $\text{NO}_2^-$  oxidation were balanced, and thus no  $\text{NO}_2^-$  (intermediate species) build-up was observed. The reactor was left to operate for another cycle (**Cycle 4**), while the steps of monitoring the growth and replacing the media were carried out as usual, in order to ensure that AOB was well grown and maintained inside the reactor.

### 5.3.2.2 Nitrifying Reactor 2 (NR 2): SAMBR Sludge

The reactor (NR 2) was operated for a period of 300 days in a fed-batch mode. For NR 2, growth media (containing 128 mg/L  $\text{NH}_4^+$ ) was freshly re-introduced on day 75, 140, and 230. It would also be appropriate to divide the whole experimental duration into a few different Cycles based on the introduction of the fresh media. The nitrogen profiles over the experimental duration with four different Cycles (Cycle 1 – 4) that can be used to understand the growth characteristics of the available nitrifying bacteria in NR 2 are shown in Fig. 5-3. Divisions of the four different cycles were as follows: Cycle 1 (day 0 – 75), Cycle 2 (day 75 – 140), Cycle 3 (day 140 – 230), and Cycle 4 (day 230 – 300).



**Fig. 5-3**  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  profiles in NR 2 during 300 days of operation. Other than at the start, fresh growth media was introduced into the reactor on day 75, 140, and 230, as indicated by the dotted vertical lines.

The phenomenon of having marginally higher concentrations of  $\text{NH}_4^+$  in the reactor than that supplied in the growth media was quite common in the early cycle of the enrichment period in all the reactors tried out. About 131 mg/L of  $\text{NH}_4^+$  was observed on day 1, just slightly more than that contained in the growth media, although this was much

lower than the  $\text{NH}_4^+$  observed in the early cycle of NR 1 operation. This lower concentration of  $\text{NH}_4^+$  found in NR 2 when compared with NR 1 was probably because of its lower initial suspended solids (0.254 g VSS/L and 1.02 g VSS/L in NR 2 and NR 1, respectively).

In **Cycle 1**, the reactor had started to exhibit nitrifying activity from day 10; in 20 days, about 70%  $\text{NH}_4^+$  removal was observed, yielding about 166 mg/L of  $\text{NO}_2^-$  in the reactor. The overall rate of nitrification, i.e. oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , in **Cycle 1** was found to be satisfactory, but quite slow as it took about two months to achieve 90% of  $\text{NH}_4^+$  removal, with maximum removal rate of 2.36 mg  $\text{NH}_4^+$ -N/L day. However, the presence of  $\text{NO}_3^-$  in the reactor was also detected as early as 20 days after the start of the enrichment, although its concentrations remained less than 100 mg/L up to day 40. It was then sharply increased to almost 400 mg/L in the next 10 days at the expense of  $\text{NO}_2^-$ . As the  $\text{NH}_4^+$  reached its minimum concentration of 12 mg/L after two months, and NOB started to outcompete AOB based on the rapid accumulation of  $\text{NO}_3^-$  and significant decrease in  $\text{NO}_2^-$ , new growth media was then introduced into the reactor on day 75 to mark the start of a new cycle.

The reactor was also left to operate for a total of four cycles, similar to NR 1. The bacterial growth was closely monitored, and growth media was replaced prior to the start of a new cycle. In each cycle, similar phenomena were observed where  $\text{NH}_4^+$  was oxidised to  $\text{NO}_2^-$  a few days after the introduction of new growth media to the reactor. This conclusion could be drawn based on the depletion of  $\text{NH}_4^+$  concentrations with time, while  $\text{NO}_2^-$  concentration rapidly increased to the maximum, indicating that AOB were active. As soon as  $\text{NO}_2^-$  reached a maximum,  $\text{NO}_3^-$  accumulation started to take place due the action of NOB that oxidised  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . The highest  $\text{NO}_3^-$  concentration of 525 mg/L was observed in **Cycle 3** (day 200) as the reactor was left to operate with the existing growth media, although  $\text{NO}_2^-$  had depleted to the lowest concentration on day 180.

As the nitrite-lacking period was prolonged until day 215, a 20% decrease in  $\text{NO}_3^-$  concentration was observed. A similar occurrence was also observed in NR 1 during the prolonged nitrite-lacking period, where the  $\text{NO}_3^-$  concentration decreased prior to the introduction of new growth media, although the magnitude of the decrease was not as significant as in NR 1. This could happen either because NOB had been inactive due to the

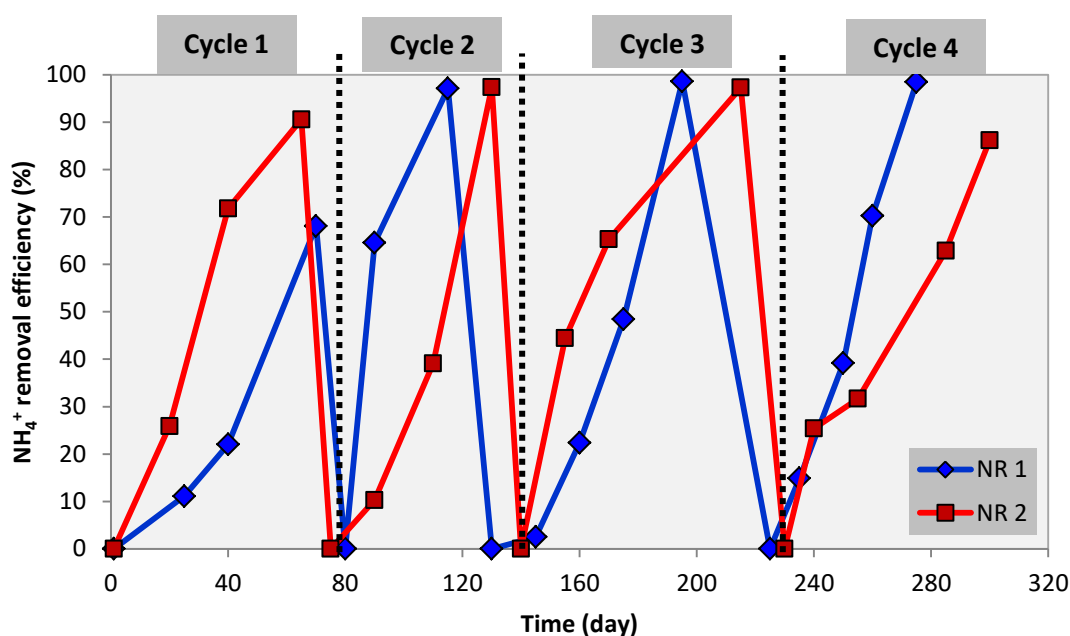
complete absence of  $\text{NO}_2^-$ , or/and some other bacterial species, i.e. denitrifying bacteria, had started to grow, most likely due to the depletion of DO levels in the reactor (less than 0.2 mg/L), hence reducing the  $\text{NO}_3^-$  to nitrogen gas. However, no further analysis was carried out to specifically identify the bacterial species responsible for this reduction in  $\text{NO}_3^-$  as the growth media was pumped out of the reactor and replaced with new media soon after that.

As nitrification is also an acidifying process that significantly consumes alkalinity, the pH of the reactor content was regularly checked, i.e. every 2-3 days. The pH was found to constantly drop with time, showing that  $\text{NH}_4^+$  was actively oxidised to  $\text{NO}_2^-/\text{NO}_3^-$ . At some points a pH of as low as 4.0 was recorded in NR 2, and in that case, nitrification was completely inhibited. Whenever the pH inside the reactor was found to be below 7.0, calcium bicarbonate solution (1 M) was immediately added to the reactor to compensate for the loss of alkalinity. Regular addition of calcium bicarbonate solution was necessary to ideally maintain the pH in the range of 7.5 – 8.0 at most of the times, hence assuring the continuity of the nitrification process. The reactor was stopped on day 300, which also marked the end of the final cycle, having achieved about 86% of  $\text{NH}_4^+$  removal in that cycle. For a comparison, it was quite noticeable that the trends of nitrogen transformations in NR 1 and NR 2 were fairly synchronised (**Fig. 5-2** and **5-3**), except that NR 2 required about 25 days more to be able to completely remove  $\text{NH}_4^+$ , mostly due to its relatively slower average removal rate of  $\text{NH}_4^+$  than that of NR 1.

Since the main purpose of the experiment was to enrich AOB needed for the next experiments, and AOB were harvested from the reactors on a few occasions, both reactors were stopped after 275 days (NR 1) and 300 days (NR 2). Based on the concentration profiles of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  after towards the end of the enrichment period (**Fig. 5-2** and **5-3**), it could be said that NOB was the dominant bacterial species in the reactors after 275 days and 300 days, although it was also possible that fractions of AOB species still remained. No confirmation of specific bacterial species present in the reactors could be made since characterisation analyses were not carried out.

### 5.3.3 Reactor Performance

In terms of reactor performance, both reactors had demonstrated significant nitrifying activities during the whole experimental period, hence indicating that AOB was successfully enriched and grown in the reactors. The removal efficiency of  $\text{NH}_4^+$ , along with  $\text{NH}_4^+$  removal rates throughout the experiments were analysed and compared. **Fig. 5-4** represents the efficiencies of  $\text{NH}_4^+$  removal recorded in both reactors in four different cycles, respectively.



**Fig. 5-4** Removal efficiency of  $\text{NH}_4^+$  in NR 1 and NR 2 during the enrichment period of AOB in four different cycles (NR 1 = RAS, NR 2 = SAMBR).

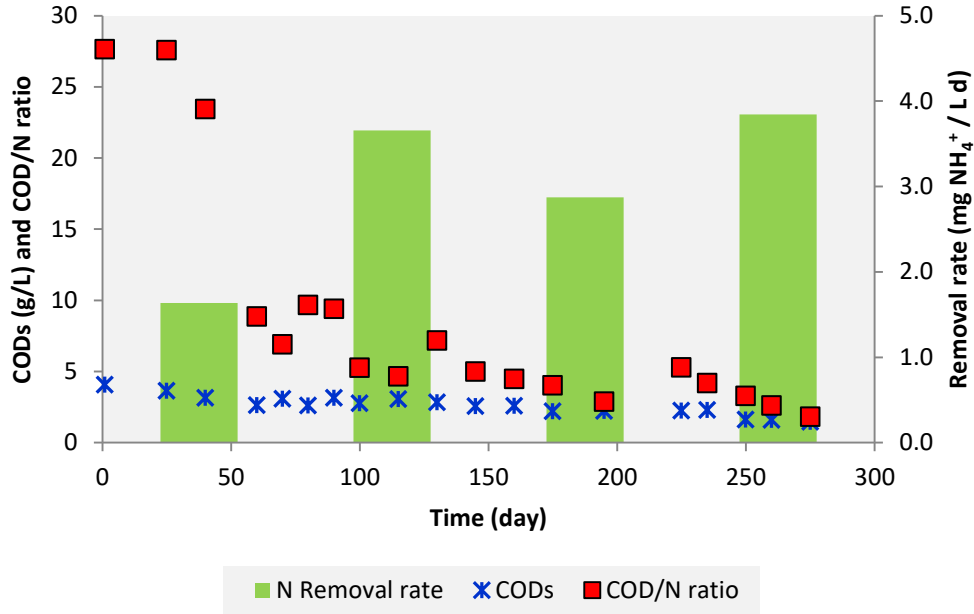
Similar to the nitrogen profiles observed in NR 1 and NR 2, the removal efficiencies of  $\text{NH}_4^+$  in both reactors were also in synchronisation with each other (**Fig. 5-4**). The only obvious difference in the removal efficiencies was observed at the end of **Cycle 1**, in which NR 2 achieved a maximum efficiency of 90%, while NR 1 only had a 68% removal despite having a “good” population of nitrifying bacteria. This low  $\text{NH}_4^+$  removal efficiency observed in NR 1 in the early cycle could be due to high organic content in the reactor (4.1 g/L CODs at the start of the cycle), which contributed to a high ratio of COD/N inside the reactor (the initial COD/N ratio was 27.6). It was previously reported that nitrification capacity is very sensitive to the COD/N ratio in the influent, where nitrogen removal rate,

i.e. nitrification rate, decreased at high COD/N ratio (Rostron et al., 2001, Wu et al., 2012). As the process continued and with CODs being gradually consumed, it can be seen that NR 2 recorded maximum efficiencies of 98% in the next three cycles, demonstrating that the sludge rightly contained “good” and robust population of nitrifying bacteria.

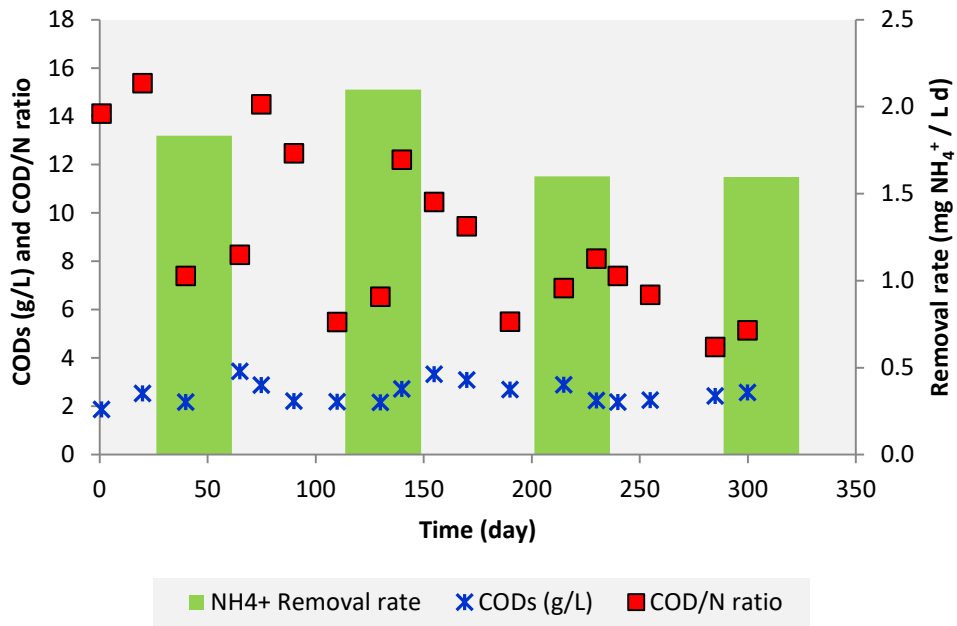
In contrast, NR 2 had higher removal efficiency as the sludge from SAMBR contained significantly lower organic content (about 1.9 g/L CODs at the start of the cycle), yielding a much lower COD/N ratio in the reactor, and hence achieving better rates of  $\text{NH}_4^+$  removal. In fact, NR 1 had maintained substantially high  $\text{NH}_4^+$  removal efficiency of more than 90% in the first three cycles, but only managed to keep it at about 86% at the end of **Cycle 4**. This was mostly caused by the slight loss of nitrifying activity of the bacterial community over time. In terms of averages, both reactors have recorded good and high efficiencies of  $\text{NH}_4^+$  removal of 91% and 93%, respectively, over the four cycles.

A comparison of the  $\text{NH}_4^+$  removal rates recorded in both NR 1 and NR 2, and their relationships with COD/N ratios in the reactors are represented in **Fig. 5-5** and **5-6**. It can be seen that lower  $\text{NH}_4^+$  removal rate in **Cycle 1** of NR 1 was caused by the significantly high COD/N ratio (higher than 20) during the early phase of the enrichment period. A high COD load was shown to have created competitive inhibition effect on nitrifying bacteria. According to previous report, heterotrophic bacteria would dominate the denitrifying bacteria at high COD/N ratio, and hence decreasing the  $\text{NH}_4^+$  removal efficiency (Okabe et al., 1996, Rostron et al., 2001, Carrera et al., 2004). Apart from that, a considerably longer start-up period will be needed to have a complete and stable nitrification as nitrifying bacteria compete for dissolved oxygen in the reactor (Okabe et al., 1996).

It can be observed that the  $\text{NH}_4^+$  removal rates in NR 1 in **Cycle 2 – 4** were significantly higher than that in Cycle 1 where it was previously depicted by a slightly lower  $\text{NH}_4^+$  removal efficiency due to a high COD/N ratio in the reactor. As the COD/N ratio gradually decreased, while a higher  $\text{NH}_4^+$  load was applied, the  $\text{NH}_4^+$  removal rate in NR 1 increased significantly in the next three cycles as it was easier for the nitrifying bacteria to compete for dissolved oxygen in the system with heterotrophic bacteria, yielding an average  $\text{NH}_4^+$  removal rate of 3.0 mg  $\text{NH}_4^+$ /L day.



**Fig. 5-5** Relationship between  $\text{NH}_4^+$  removal rates and COD/N ratio observed in NR 1 during the enrichment period.



**Fig. 5-6** Relationship between  $\text{NH}_4^+$  removal rates and COD/N ratio observed in NR 2 during the enrichment period.

## 5.4 SUMMARY

Both of the nitrifying reactors NR 1 and NR 2 used to enrich and grow the AOB, from different wastewater sludge showed good nitrifying activity in all four cycles. The performance of NR 1 and NR 2 were fairly comparable to each other, with both reactors recording over 90% of  $\text{NH}_4^+$  removal efficiency. **Table 5-3** summarises the performance of both nitrifying reactors, based mainly on their  $\text{NH}_4^+$  removal efficiencies and removal rates over their respective experimental periods. In terms of nitrifying activity, expressed as rates of  $\text{NH}_4^+$  removal per unit of reactor volume per unit time, it was observed that NR 1 recorded a slightly higher average of  $3.0 \text{ mg NH}_4^+ / \text{L day}$ , compared to  $1.78 \text{ mg NH}_4^+ / \text{L day}$  in NR 2. However, the findings also indicate that the seed sludge collected from the full-scale SAMBR in Cambridge inoculated into NR 2 showed clear nitrifying activities, and its performance was comparable with that of NR 1. In fact, the specific  $\text{NH}_4^+$  removal rate of biomass in NR 2 ( $7.5 \text{ mg NH}_4^+ / \text{g VSS day}$ ) was more than double of that in NR 1 ( $3.30 \text{ mg NH}_4^+ / \text{g VSS day}$ ).

**Table 5-3** A summary and comparison of the three nitrifying reactors performance.

Parameters for comparison	NR 1	NR 2
Source of seed culture	Activated sludge	SAMBR
Duration of experiment	275 days	300 days
Number of cycles completed	4	4
Average $\text{NH}_4^+$ removal efficiency	91%	93%
Maximum $\text{NH}_4^+$ removal rate ( $\text{mg NH}_4^+ / \text{L day}$ )	3.85	2.10
Specific $\text{NH}_4^+$ removal rate of biomass ( $\text{mg NH}_4^+ / \text{g VSS day}$ )	3.30	7.50

Other than operating conditions, i.e. pH, DO level, and media composition, the nature of the seed sludge used to start the enrichment may also have had a significant impact on the subsequent ability of the bacteria in the reactors. Since both reactors were operated with similar operating conditions, one main reason that could have contributed towards the high nitrification rates recorded in NR 1 was the nature and initial characteristics of the seed sludge. The activated sludge inoculated into NR 1 clearly



demonstrated that not only it contained a good population of nitrifying bacteria, but it was also robust against various environmental changes that might have happened in the reactor. These factors have enabled them to last longer in the reactor while maintaining their relatively high nitrifying activity, even after four complete cycles.

The significant and continuous decrease in pH observed in both reactors also proved that they had experienced clear nitrifying activities, indicating that AOB were successfully grown and enriched. Nitrification is naturally an acidifying process, and thus requires sufficient alkalinity to proceed. Theoretically, about 7.14 mg/L of alkalinity as  $\text{CaCO}_3$  is consumed for each mg/L of ammonia-nitrogen oxidised (Cheremisinoff, 1996). The release of  $\text{H}^+$  from the process would cause the pH to drop significantly, as observed in both reactors. Throughout the experiments, the pH of the reactor content were closely monitored and maintained in the range that favoured nitrification (pH 7 – 8), as it was known that nitrification would be completely inhibited at pH below 6.5 (Hellinga et al., 1998).

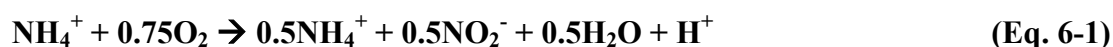
As the main objective of this work was to enrich and prepare AOBs at high concentration to be used for the subsequent partial nitrification process using an NF membrane, the key strategy here was to ensure that the operating conditions only favoured the AOB, while suppressing NOB. However, due to the prolonged process and complexity of the sludge, different bacterial species could grow and co-exist within the same period of time in the reactors, and possibly interfere with the nitrification process and cause the nitrification rate to decrease, although this has yet to be proven. Despite specific operational strategies being used to favour the growth of AOB which is responsible for the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  only, we could not assume that other bacterial species would not grow, mainly NOB. Although it was not practically possible to completely suppress the growth of NOB in the reactor, and hence inhibit the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ , the objective of this work could still be achieved as AOB were only harvested from the reactors at a point when  $\text{NO}_2^-$  started to decrease significantly, indicating that AOB activity was at its maximum, but before any significant growth of NOB.

## CHAPTER 6

# PRE-OXIDATION OF AMMONIUM USING NANO-FILTRATION MEMBRANE MODULES

### 6.1 INTRODUCTION

As previously discussed in Chapter 2 (**Section 2.9**), a combination of partial nitrification and the Anammox process have apparently brought significant improvements in the treatment of nitrogen-containing wastewaters (Jetten et al., 1999, Fux et al., 2002, Schmidt et al., 2003, Op den Camp et al., 2006, Feng et al., 2007). The partial nitrification process was normally carried out in a single reactor consisting of a nitrifying bacterial culture (AOB) that would partially oxidise  $\text{NH}_4^+$  to a mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  (Feng et al., 2007), as represented by **Eq. 6-1**.



Since only half of the  $\text{NH}_4^+$  is oxidised to  $\text{NO}_2^-$ , partial nitrification evidently requires half of oxygen needed for a complete nitrification. The subsequent Anammox process that replaces the conventional anoxic denitrification step could also eliminate the need for an external carbon source. Therefore, it is more convenient to combine partial nitrification with the Anammox process, so that a significant reduction in operational costs in terms of aeration and external carbon source can be made.

On the other hand, one of the drawbacks of using a partial nitrification reactor is that optimum conditions for the AOB must be strictly maintained, i.e. growth media, pH, temperature, DO. At a practical level, it is not easy to ensure that only the AOB are grown in the reactor, while NOB are suppressed in order to inhibit the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . As observed and discussed in Chapter 5, it was not possible to completely suppress the growth of NOB in the reactor for a long period due to the complexity of sludge that contained a broad range of microbial communities, unless a pure culture of AOB is used, rather than enriching it from wastewater sludge. The prolonged presence of  $\text{NO}_2^-$  in the

reactor could naturally promote the growth of NOB species. To avoid this, other than strictly maintaining the conditions that only favour the growth of AOB species, the process must be immediately followed by the Anammox process, so that the  $\text{NO}_2^-$  and  $\text{NH}_4^+$  could be subsequently converted to nitrogen gas.

In the previous chapters, it was highlighted that the use of membrane filtration combined with bioreactor technology, often termed membrane bioreactors (MBR), specifically for the treatment of wastewater has been expanding rapidly. Prior to this development, membrane filtration systems alone have been widely used for various wastewater treatment processes such as the desalination of salt water, and filtration of surface or ground water to remove dissolved contaminants. In these applications, membrane filtration is used for the removal of particles, ranging from large particulate materials to dissolved compounds, bacteria, viruses, and ions. The size and chemical characteristics of the membrane and the material being filtered determine which material will pass through the membrane. There are many different types of membrane filters available in a wide range of pore sizes and configurations (flat and tubular). Plate-and-frame and spiral wound modules involve flat membranes, whereas tubular, capillary and HF modules are based on tubular membrane configurations. The characteristics of some membrane modules were previously summarised in Chapter 2 (**Table 2-3**). HF membrane modules used in this study were in a tubular configuration.

Although the basic application of a membrane filter is for the removal of various particles, its application when combined with MBRs could be further extended as the membrane also acts as a platform for the biomass, i.e. bacteria cells, to attach and grow on, hence improving the efficiency of the treatment process. Being inspired by this concept, i.e. a membrane filter acting as a platform for bacterial cells to grow on, it was proposed that species of AOB be attached and grown on the shell side of the HF membranes and in the suspension, obtaining  $\text{NH}_4^+$  from the wastewater through the membrane (tube side), and oxidising it to  $\text{NO}_2^-$  which then diffuses back into the wastewater. The AOB culture was previously enriched from different wastewater sludge in batch reactors (discussed in Chapter 5).

In this study, we proposed using a NF membrane module to replace the conventional CSTR commonly used for partial nitrification preceding the Anammox process. However,

instead of using the term “partial nitrification”, it is more appropriate to refer the process as a pre-oxidation of  $\text{NH}_4^+$ , and it is meant to be performed prior to the Anammox process in a SAMBR. The membrane modules, consisting of concentric HF tubes that were brought from Singapore have never been tested for this process. The feasibility of using the membrane process to replace a conventional partial nitrification reactor was investigated in terms of whether the AOB grown on the shell side of the HF membrane were capable of oxidising the  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , and producing an acceptable ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  in the retentate prior to the Anammox process.

## **6.2 MATERIALS AND METHODS**

### **6.2.1 Nano-Filtration (NF) Membrane Modules**

Two NF membrane modules prepared in Nanyang Technological University (NTU), Singapore, were used to investigate the possibility of oxidising  $\text{NH}_4^+$  to  $\text{NO}_2^-$  prior to nitrification/denitrification process in SAMBR by the Anammox bacteria. Each module was 22 cm in length and a diameter of 2 cm (cartridge volume was 69 mL), housing six composite HF membrane tubes with MWCO of around 500 Da and an effective pore size and surface area of 1.29 nm and 25 cm<sup>2</sup>, respectively. The NF membrane modules were also tested with 1 g/L of  $\text{MgCl}_2$ , resulting in an average permeation rate and salt rejection of 20.5 L/m<sup>2</sup> h and 94%, respectively (Wang, Personal communication). The schematic of the membrane module is as shown in **Fig. 6-1**.

### **6.2.2 Strategy of Operation**

#### **6.2.2.1 Permeability Study for NF Membrane ( $\text{NH}_4^+$ and Glucose)**

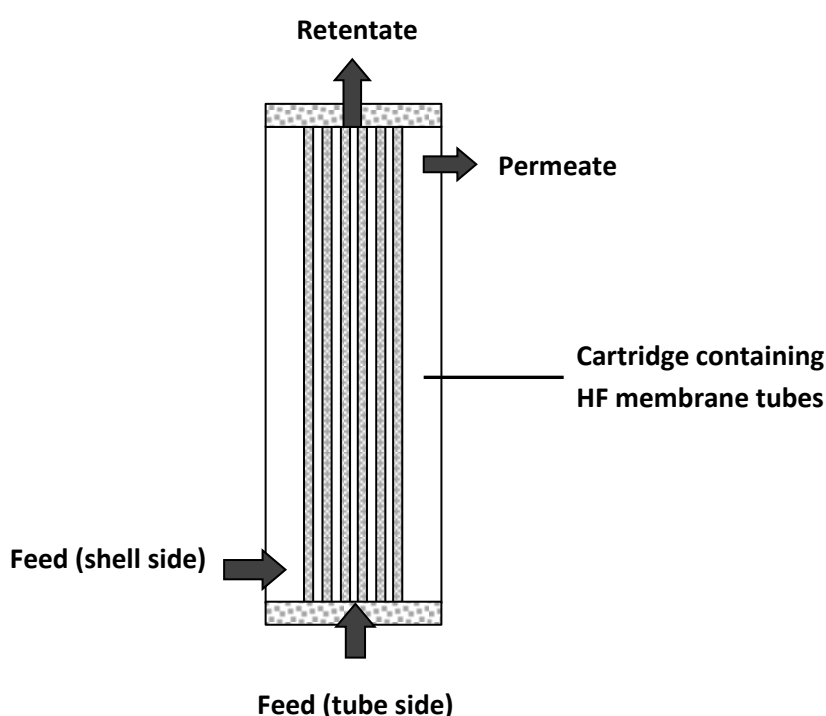
Prior to running the experiments specifically for the pre-oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , the membrane modules were first tested for their  $\text{NH}_4^+$  and glucose permeability. In this experiment, solutions containing  $\text{NH}_4^+$  and glucose at certain concentrations were pumped through the inner side of the HF tubes (“tube side”) at certain flow rates for predetermined periods of time. Samples of the solution in both the permeate and retentate streams were taken at predetermined intervals for further analysis. Concentrations of nitrogen species

(NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>), were analysed and determined using the Standard Method (APHA, 1999). The mass flux of a solute across a membrane was calculated as follows:

$$J = \frac{Q}{A \cdot \Delta t}$$

(Eq. 6-2)

where  $J$  is the mass flux across the membrane (g/m<sup>2</sup>.h),  $Q$  is the quantity of permeate crossing the membrane (g),  $A$  is effective membrane surface area (m<sup>2</sup>), and  $\Delta t$  is the sampling time (h). Mass flux is defined as the rate of mass flow per unit area per unit of time, and thus its unit is g/m<sup>2</sup> h.



**Fig. 6-1** The tubular NF membrane module that consists of concentric hollow fibre tubes.

Other than flux, another parameter that is often used to determine the efficiency of a membrane is its selectivity. As this study involved separation of solutes (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, glucose) from a solvent (water), the membrane selectivity is expressed as rejection (denoted as  $R$ ), and was calculated as follows:

$$R = \left( 1 - \frac{C_p}{C_f} \right) \times 100$$

(Eq. 6-3)

where  $C_f$  is the solute concentration in the feed, and  $C_p$  is the solute concentration in the permeate.  $R$  is dimensionless and can be expressed in terms of percentage, i.e. an  $R$  value of 100% represents a complete rejection of the solute, while 0% indicates that both solute and solvent freely pass through the membrane (Mulder, 1996).

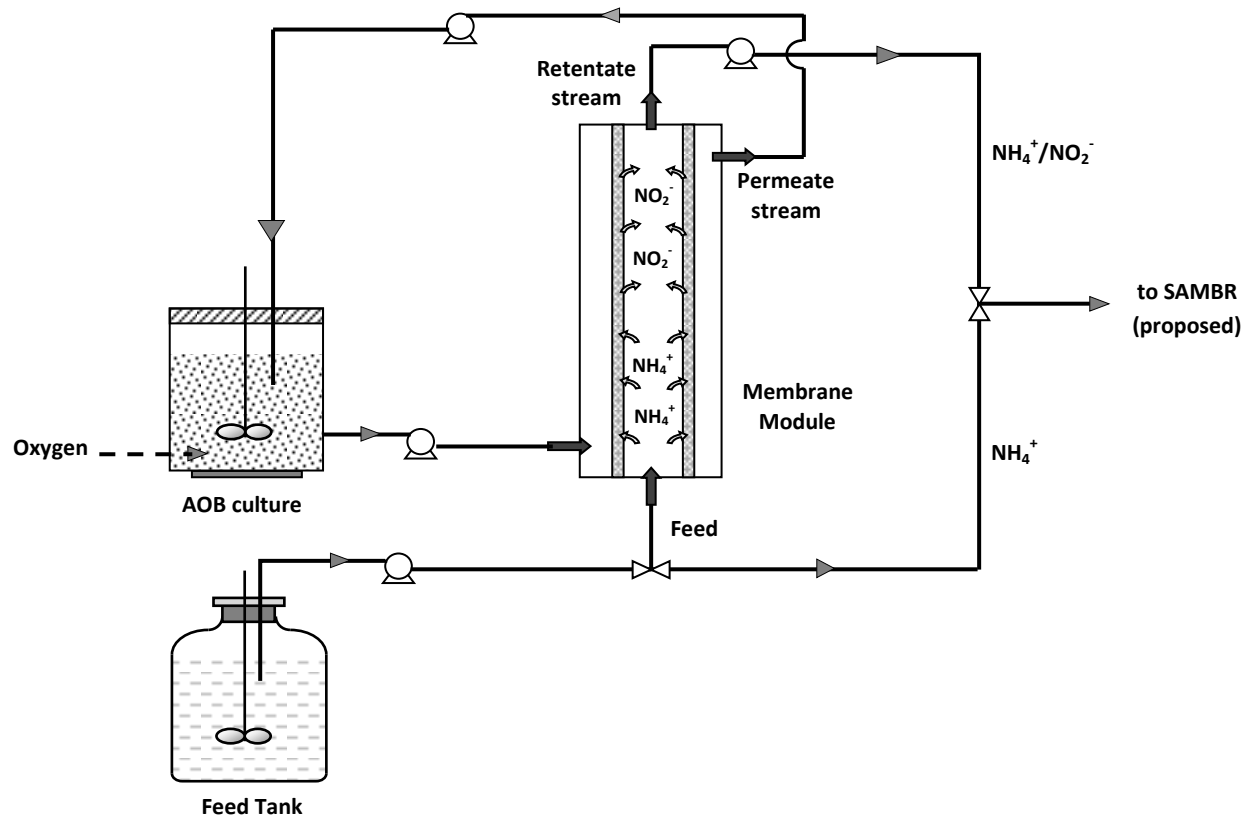
### **6.2.2.2 Preparation of a Cell Suspension**

In this experiment, the NF membrane modules were set-up to oxidise  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , with the aid of AOB, which were previously enriched and grown in batch reactors. In previous experiments, AOB culture that is responsible for the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , were harvested from batch reactors in which they were enriched and grown. The biomass was harvested at a time when the observable  $\text{NO}_2^-$  concentrations were maximal, and 200 mL of completely mixed sludge was removed from the batch reactors using a syringe. The sludge was then centrifuged at 7500 rpm for five minutes, and the cells (pellets) were re-suspended in 200 mL of a similar growth media used to enrich AOB, but lacking in nitrogen compounds ( $\text{NH}_4^+$  and  $\text{NO}_2^-$ ). The cells were thoroughly mixed with the growth media so that they could exist as a free suspension of cells, instead of in flocs or aggregates. The cells suspension was then ready for the pre-oxidation process.

### **6.2.2.3 Experimental Setup**

The proposed pre-oxidation process using the NF membrane modules is illustrated in **Fig. 6-2**. A cell suspension containing AOB culture was continuously circulated through the shell side of the membrane module using a gear pump at room temperature. After exiting the membrane module, the nitrifying culture was recycled back to a reactor. Continuous circulation of the cell suspension through the shell side of the module should allow the cells to attach and grow on the shell side of HF membranes inside the module.

A solution containing different concentrations of  $\text{NH}_4^+$  in a feed tank was pumped through the tube side at several different flow rates. At predetermined intervals, concentrations of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the permeate and retentate streams were analysed based on the Standard Methods (APHA, 1999). Each experiment was run for 24 – 48 hours.



**Fig. 6-2** Schematic of the proposed pre-oxidation of  $\text{NH}_4^+$  using NF membrane modules.

The process was initially operated for up to 72 hours, but later reduced to 24 hours (without nitrifying bacteria) and 48 hours (with nitrifying bacteria) since it was found that the volume of the solution in the feed tank (containing  $\text{NH}_4^+/\text{glucose}$ ) started to decrease significantly as the process approaching 48 hours. In this case, most of the solution/water from the feed tank has permeated through the membrane, causing the volume of the solution in the other tank (either containing water or AOB culture) to increase accordingly. Apart from that, based on flux analyses, it was observed that almost no  $\text{NH}_4^+$  could permeate through the membrane after 24 hours of operation (without nitrifying bacteria).

#### 6.2.2.4 Experimental Runs

The experimental runs in this study were divided into two different scopes: (a) membrane permeability study using  $\text{NH}_4^+$  and glucose, and (b) the pre-oxidation process of  $\text{NH}_4^+$  by AOB using the NF membrane modules.

#### 6.2.2.4 (a) Membrane Permeability Study (without Nitrifying Bacteria)

Although a number of parameters are known to have a significant influence on membrane performance, only the effects of flow rates (representing fluid velocity), and initial solute concentrations in the feed on membrane permeability were studied in this experiment. To do this, a simple design of experiments was used, where each parameter was set at three different levels, i.e. low, medium, and high. However, only a fixed concentration of COD was used in this study. The experimental parameters and their proposed values are summarised in **Table 6-1**.

**Table 6-1** Experimental runs to investigate the effects of different parameters on membrane permeability and selectivity towards  $\text{NH}_4^+$  and glucose.

Parameters	Levels		
	Low	Medium	High
Feed flow rates (L/h)	1.2	3.0	5.0
Initial $\text{NH}_4^+$ concentrations (mg/L)	50	100	150
Initial COD concentration (mg/L)		50	

#### 6.2.2.4 (b) Pre-Oxidation Process of Ammonium (with Nitrifying Bacteria)

The pre-oxidation of  $\text{NH}_4^+$  using the NF membrane module was carried out at different feed flow rates. In terms of biomass, two types of nitrifying cultures were considered for the study; cultures from activated sludge (previously enriched in NR 1), and from a SAMBR (previously enriched in NR 2). A fixed initial  $\text{NH}_4^+$  concentration of 100 mg/L in the feed tank was selected and used in this pre-oxidation study, and only flow rates were varied. Bacterial cultures harvested from NR 1 (activated sludge) were used for this process due to its robustness and high nitrifying activity shown in batch reactors. However, bacterial cultures from NR 2 (SAMBR) could still be used either as a back-up, or for future studies. The proposed experimental runs are summarised as follows (**Table 6-2**).



**Table 6-2** Experimental runs to investigate the potential of using the NF membrane process for the pre-oxidation of  $\text{NH}_4^+$  at different flow rates, HRT and NLR. The  $\text{NH}_4^+$  concentration was set at 100 mg/L, and the biomass used was from activated sludge.

	<b>Flowrates (L/h)</b>	<b>HRT (h)</b>	<b>NLR (g <math>\text{NH}_4^+</math>/L h)</b>
Run 1	1.2	0.058	1.736
Run 2	3.0	0.023	4.339
Run 3	5.0	0.014	7.231

## 6.3 RESULTS AND DISCUSSION

Discussion of the experimental findings are mainly centered on the effects of two experimental parameters, namely the feed flow rates and initial  $\text{NH}_4^+$  concentrations in the feed, on the membrane permeability, as well as the efficiency of  $\text{NH}_4^+$  oxidation by the AOB. The objective of the study was to find out whether the use of the NF membrane module to replace a conventional partial nitrification reactor prior to the Anammox process is feasible, and whether the process is practically viable on a larger scale.

### 6.3.1 Membrane Permeability Study

The performance or efficiency of a given membrane is determined by two parameters; its selectivity and permeability. The permeation rate is normally influenced by several factors, such as cross-flow velocity (flow rates), feed concentrations, temperature and trans-membrane pressure (TMP). Both of these parameters (selectivity and permeability) were used to investigate the performance and efficiency of the NF membrane, calculated using **Eq. 6-2** and **6-3** as previously shown. For this study, flow rates and initial feed concentrations were varied to investigate their effects on the membrane permeability and selectivity.

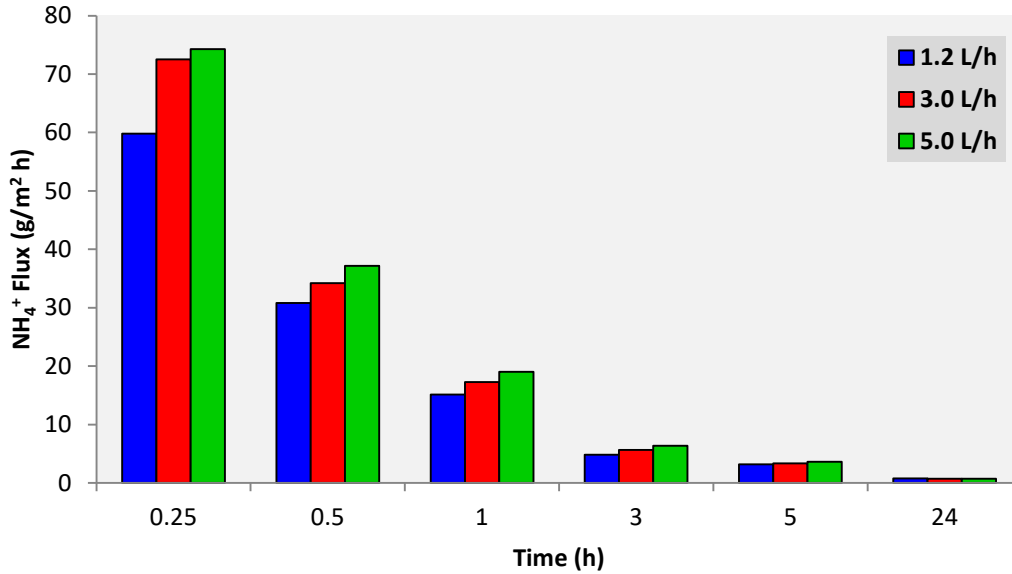
### **6.3.1.1 Ammonium Permeability**

The NF membrane process performance was measured in terms of its permeability and selectivity towards  $\text{NH}_4^+$  ions at three different flow rates and initial  $\text{NH}_4^+$  concentrations in the feed. In such cases, the experiments were carried out in two batches: (a) the effects of flow rates, and (b) the effects of initial  $\text{NH}_4^+$  concentrations.

#### **6.4.1.1 (a) The Effects of Flow Rates**

A solution containing  $\text{NH}_4^+$  at 100 mg/L, was pumped through the tube side of the membrane modules at three different flow rates: 1.2 L/h, 3.0 L/h, and 5.0 L/h, with the corresponding Reynolds numbers inside the tubes of 403, 1008, and 1680, respectively, implying that all flows were laminar. Samples were taken from both permeate (shell side) and retentate (tube side) streams at pre-determined intervals for 24 hours. In the first hour, samples in the tube side and shell side were made on three occasions, i.e. at 15, 30 and 60 minutes. Thereafter, the sampling frequency was reduced to every 2 hours up to 5 hours, before the final sample was taken after 24 hours. **Fig. 6-3** shows the mass fluxes profiles for  $\text{NH}_4^+$  across the membrane observed at specific time intervals at which samples were taken, with three different flow rates applied at the feed.

From **Fig. 6-3**, it can be observed that the  $\text{NH}_4^+$  fluxes across the NF membrane increased with increasing flow rates. The highest flux of  $74.3 \text{ g/m}^2 \text{ h}$  was observed at a flow rate of 5.0 L/h at minute 15, compared to  $72.5 \text{ g/m}^2 \text{ h}$  and  $59.8 \text{ g/m}^2 \text{ h}$  observed at flow rates of 1.2 L/h and 3.0 L/h, respectively. The NF membrane process in this study was built and operated in the cross-flow filtration mode, where the feed solution flows tangentially to the membrane surface. The permeating components would then move perpendicularly to the membrane surface because of the concentration gradient across the membrane. In this kind of process, the feed flow rates applied to the process have a direct influence on the permeation flux, i.e. fluid velocity is flow rate divided by cross sectional area of the membrane; a higher fluid velocity can reduce the stagnant boundary layer, and hence the diffusional resistance, and this increases the flux.



**Fig. 6-3**  $\text{NH}_4^+$  mass fluxes of the NF membrane at different feed flow rates.

This trend, i.e. increasing fluxes with increasing flow rates, could be seen for up to three hours of operation, although the difference between fluxes at different flow rates were quite minimal, i.e. less than 10%, after 30 minutes. However, after three hours, the  $\text{NH}_4^+$  fluxes were almost similar for all flow rates, indicating that the process had reached a steady-state condition. Over time the  $\text{NH}_4^+$  concentration in the bulk phase (tube side) decreases, and hence with a lower concentration driving force between the tube and the shell side, the flux decreased (assuming a constant mass transfer coefficient). The higher flow rates applied not only resulted in a higher initial flux across the membrane, but also caused a rapid decrease in flux over time as it induced additional resistances to the membrane surface, i.e. membrane fouling through the formation of a concentration polarisation layer, and hence this rapidly decreasing the flux.

Although flux increased with increasing flow rates, it apparently decreased with time, and so did the membrane performance, regardless of flow rates applied. This phenomenon is known as flux-time behavior, which states that flux through a membrane decreases over time. This was mainly caused by the decrease in  $\text{NH}_4^+$  concentration in the tube side over time. As seen from the graph (**Fig. 6-3**), there were initial rapid decreases in fluxes at all flow rates applied, i.e. first three hours, followed by a long and gradual decline. For example, the highest flux (about  $74.3 \text{ g/m}^2 \text{ h}$ ) was observed at minute 15 and flow rate of  $5.0 \text{ L/h}$ , and in the next 15 minutes, it decreased rapidly by 50%. After 3 hours of

operation, it could be seen that the rate of flux decrease at their respective flow rates had declined significantly, i.e. the decrease in flux became less apparent. Over time the  $\text{NH}_4^+$  was removed by diffusion, and hence the concentration driving force decreased, resulting in flux decline. When steady-state conditions have been reached, no further decrease in flux would be observed, i.e. flux will become constant with time when the  $\text{NH}_4^+$  concentrations on both sides of the membrane were very similar.

Some of other factors known to be responsible for the flux decline include concentration polarisation and membrane fouling, gel layer formation, and pore-blocking, for which they induce additional resistances on the feed side to transport solutes across the membrane. These factors, however, might qualitatively differ from process to process and from application to application. Although these factors were not discussed in detail in this study, concentration polarisation and membrane fouling are the most common phenomena observed during membrane process, particularly in pressure driven process. In short, concentration polarisation is a phenomenon where the retained solutes accumulate at the membrane surfaces, thus gradually increasing their concentrations that create additional resistance to the permeate flow towards the membrane.

It is known that the concentration polarisation phenomenon is reversible during operation, usually by increasing the feed velocity. Although the crossflow velocity for a single run in this study was not increased, it was clear that at the start of the experiment higher velocity led to higher fluxes as the boundary layer was thinner and hence flux was higher. However, in practice, a continuous decrease in flux could still be observed with time. The continuous flux decrease, however, could be the result of membrane fouling. Membrane fouling refers to the gradual deposition of retained particles on or in the membrane, which include adsorption, pore blocking, precipitation and cake formation (Mulder, 1996). The fouling layer can only be removed using specific treatment and cleaning methods, which include hydraulic, chemical, or mechanical cleaning. In this study, the membrane module was cleaned using both hydraulic and chemical methods, i.e. back-flushing with deionised water and 0.5% (v/v) sodium hypochlorite solution. Membrane cleaning was carried out immediately after every run.

Apart from having a reasonably high  $\text{NH}_4^+$  flux, another criterion that defines the feasibility of using the membrane module for the pre-oxidation of  $\text{NH}_4^+$  is to have low

membrane selectivity towards  $\text{NH}_4^+$ . Throughout the experimental period, the membrane rejection of  $\text{NH}_4^+$  slightly varied with the applied flow rates, and its averages after three and 24 hours operation are shown in **Table 6-3**. The lowest membrane rejection, with an average of 4% for the first three hours was observed at flowrate of 5.0 L/h. Only after three hours the membrane rejection started to increase slightly as the amount of  $\text{NH}_4^+$  ions that could diffuse through the membrane decreased due to the decrease in concentration difference between the two sides (tube and shell sides).

The results indicate that the NF membrane had a very low selectivity towards  $\text{NH}_4^+$ , with more than 90% of the  $\text{NH}_4^+$  ions diffusing through the membrane (at flow rates of 3.0 L/h and 5.0 L/h) caused by the concentration difference between the bulk phase and the shell side, due to its low molecular weight with respect to the membrane MWCO of 500 Da. At a flow rate of 1.2 L/h, the average membrane rejection was slightly higher, however, it was still below 25%, and this is in agreement with a report by Mulder (1996) that the typical rejection of monovalent ions by NF membranes should be less than 50%. Apart from this, two commercially available flat sheet NF membrane modules by Celgard that were previously applied for the removal of  $\text{NH}_4^+$  from potable water had resulted in membrane rejections of 27% and 12% after 24 hours of operation (Kurama et al., 2002).

**Table 6-3** Average  $\text{NH}_4^+$  rejection by NF membrane at different flow rates

Flow rates	Average $\text{NH}_4^+$ rejection	
	After 3 h	After 24 h
1.2 L/h	21.2%	25.1%
3.0 L/h	11.6%	12.1%
5.0 L/h	4.02%	5.75%

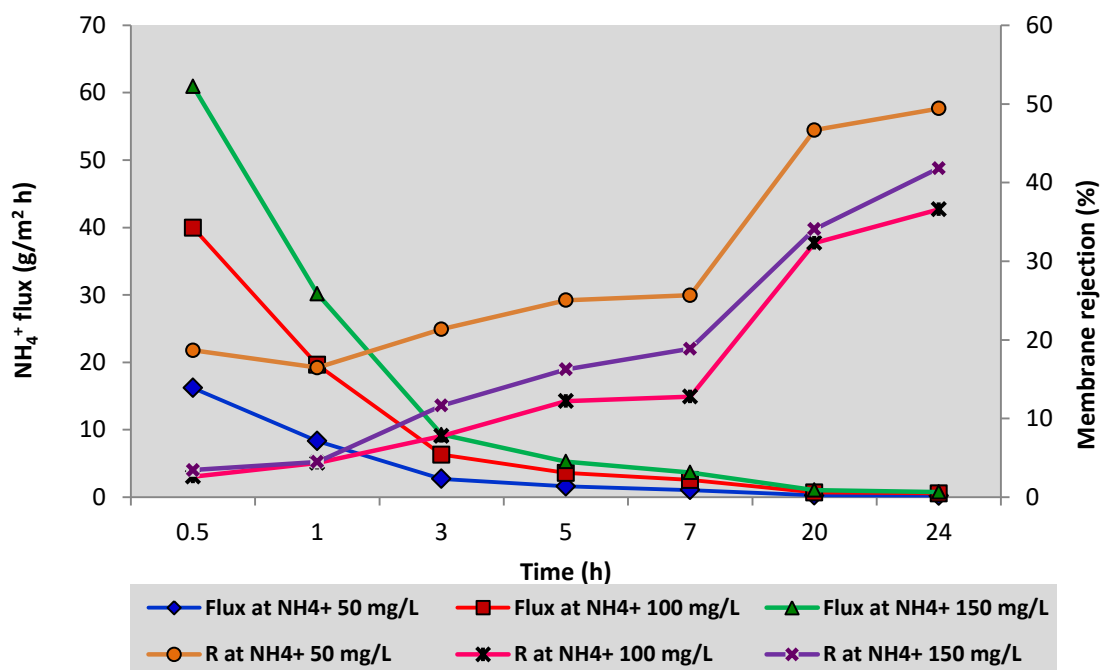
### ***Nitrogen Mass Balance Analysis***

Theoretically, at steady-state, the total mass entering a system should always be equal to the total mass leaving the system. As such, the total mass of nitrogen fed into the NF membrane module should be equal to the total mass of nitrogen leaving the module, both through the permeate and retentate. Based on the mass balance analysis of nitrogen in the feed, permeate and retentate, it could be shown that the  $\text{NH}_4^+$  fed into the tube side of the membrane module either passed through the membrane and was collected in the permeate, or was retained and collected in the retentate stream with only slight errors, i.e. 5-10%. The errors, represented by a loss of nitrogen from the feed, were most likely due to handling of chemicals and reagents during preparation and analysis, and analytical errors.

#### ***6.4.1.1 (b) Effects of Initial Ammonium Concentrations***

The membrane permeability was also studied based on different initial  $\text{NH}_4^+$  concentrations in the feed. A solution containing  $\text{NH}_4^+$  at concentrations of 50 mg/L, 100 mg/L, and 150 mg/L was pumped through the feed at a fixed flow rate of 3.0 L/h. At this flow rate, the hydraulic retention time was 0.023 hour (1.38 minutes), i.e. volume of the membrane cartridge divided by the flow rate, while the nitrogen loading rate (NLR) corresponding to the three  $\text{NH}_4^+$  concentrations were 2.1, 4.3 and 6.5 g  $\text{NH}_4^+$  / L h, respectively. **Fig. 6-4** shows the mass flux profiles for  $\text{NH}_4^+$  across the membrane, and the membrane rejection observed at specific time intervals at which samples were taken, with three different initial  $\text{NH}_4^+$  concentrations fed through the feed.

**Fig. 6-4** shows that the  $\text{NH}_4^+$  fluxes across the NF membrane increased with increasing initial  $\text{NH}_4^+$  concentrations in the feed. This is because a higher concentration gradient existed between the bulk phase and the shell side when a higher concentration of solute was fed through the tube side, hence increasing the driving force and causing more  $\text{NH}_4^+$  to diffuse through the membrane (Seyoum et al., 2012). As the concentration driving force decreased with time (due to the diffusion of  $\text{NH}_4^+$  to the shell side), as well as an increased resistance at the membrane surface due to concentration polarisation, the fluxes decreased with time, and were very similar for all initial  $\text{NH}_4^+$  concentrations after seven hours.



**Fig. 6-4**  $\text{NH}_4^+$  mass fluxes and membrane rejection of  $\text{NH}_4^+$  at different initial feed concentrations.

In terms of membrane rejection, it can be observed that the lowest membrane rejection of  $\text{NH}_4^+$  was at initial concentrations of 100 mg/L and 150 mg/L, i.e. 2.6% and 3.5%, respectively, while at 50 mg/L, the rejection was about 18%, all of which indicated that the membrane had a very low selectivity towards  $\text{NH}_4^+$ , and hence making it suitable for the proposed pre-oxidation process. After 24 hours, the average membrane rejection of  $\text{NH}_4^+$  was 29%, 16%, and 18% for initial  $\text{NH}_4^+$  concentrations of 50 mg/L, 100 mg/L, and 150 mg/L, respectively. The differences between membrane rejections at different initial  $\text{NH}_4^+$  concentrations were less significant as compared with the differences of the rejection observed at different flow rates, indicating that initial feed concentration had less impact on membrane selectivity compared with flow rates.

Although the findings showed that the  $\text{NH}_4^+$  flux increased as the initial  $\text{NH}_4^+$  concentration in the feed increased, this may only be relevant to cases in which the feed concentration contains up to 150 mg/L of  $\text{NH}_4^+$ . There is a possibility that the flux could decrease when the feed concentration exceeds certain limits, i.e. above 150 mg/L, which would require further experiments in order to investigate what is the critical concentration, alongside other factors that can cause the flux to decrease. Previous findings had shown that

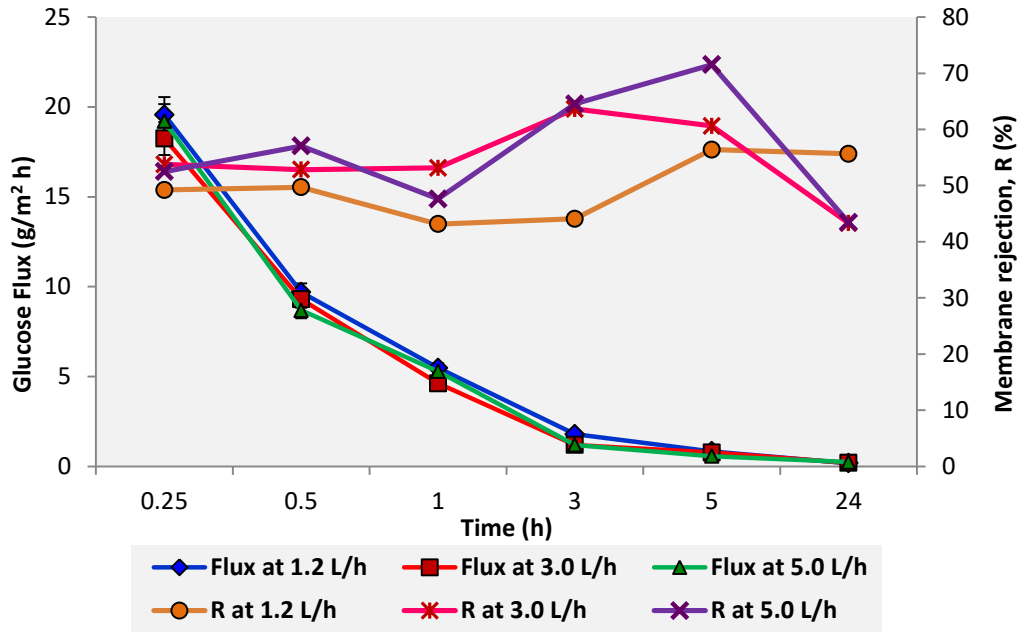
flux decreased with increasing feed concentrations due to an increase in boundary layer resistance, and a rapid accumulation of foulant material on the membrane surface when a higher concentration of solute was used. This presumption was, however, only consistent with a certain model of concentration polarisation, i.e. gel polarisation theory (DiGiano et al., 1995). Therefore, the question of whether flux increases or decreases with increasing solute concentration could also depend on the type and size of solutes that undergo the filtration process, i.e. suspended solids, organic matter, divalent ions, monovalent ions.

### **6.3.1.2 Glucose Permeability**

Other than  $\text{NH}_4^+$ , the membrane performance in terms of its permeability and selectivity towards glucose was also studied because this is important in terms of soluble COD permeating the membrane and being oxidised on the shell side thereby reducing  $\text{NH}_4^+$  oxidation, and increasing the oxygen demand. To do this, a solution containing glucose (expressed in terms of COD of 50 mg/L) was pumped through the tube side of the membrane module at three different flow rates. Similar to that of  $\text{NH}_4^+$ , both fluxes and membrane selectivity towards glucose (rejection) were calculated and studied. The mass fluxes of glucose and membrane rejection at different flow rates are shown in **Fig. 6-5**.

In contrast to  $\text{NH}_4^+$  fluxes, there were no significant differences between the glucose fluxes at different flow rates applied. In addition, the glucose fluxes were significantly lower than the  $\text{NH}_4^+$  fluxes observed at the respective flow rates. For example, at minute 15, the fluxes observed at all flow rates were about  $19 \text{ g/m}^2 \text{ h}$ , which were about four times less than the  $\text{NH}_4^+$  fluxes observed at a similar time. The lower glucose fluxes, and higher membrane rejection, indicate that most of the glucose was not able to diffuse through the membrane. This was mainly due to their higher molecular weight, i.e. 180 Da, as compared with that of  $\text{NH}_4^+$ . This means that with initial partial  $\text{NH}_4^+$  oxidation, with a bulk of the COD being rejected, the concept of using a membrane to pre-oxidise  $\text{NH}_4^+$  appears to be viable.





**Fig. 6-5** Glucose mass fluxes and membrane rejection at different flow rates with a COD concentration of 50 mg/L.

Lower glucose fluxes across the membrane could be further attributed to the high membrane rejection of glucose, where an average of 55% rejection was recorded over the 24 hours of operation at flow rates of 3.0 L/h and 5.0 L/h, while an average of 50% was observed at a flow rate of 1.2 L/h. The membrane rejection analysis basically shows that about half of the glucose in the feed was retained inside the tube side, while the other half could pass through the membrane. Mulder (1996) stated that NF membranes would have a greater than 50% rejection of microsolute with a molecular weight of over 100 Da, and hence in this case it could be said that the membrane was suitable for the rejection of glucose (or COD) in wastewater pre-treatment.

### 6.3.2 Pre-Oxidation of Ammonium using NF Membranes

The previous experiments (discussed in **Section 6.3.1**) were carried out to investigate membrane process efficiency based on the permeation rate and selectivity towards  $\text{NH}_4^+$  ions and glucose at three different flow rates and initial  $\text{NH}_4^+$  concentrations. In this section, similar experiments were carried out (with three different flow rates and a feed  $\text{NH}_4^+$  concentration fixed at 100 mg/L, but without glucose), except that a cell suspension containing AOB culture enriched from activated sludge was pumped through the

shell side of the membrane module. In addition, the experiments were extended to 48 hours, instead of 24 hours, in order to let the process achieve steady state. The aim was not only to study membrane performance, i.e. permeation flux and selectivity, but also to investigate the efficiency of  $\text{NH}_4^+$  oxidation, termed “pre-oxidation”, by the AOB culture grown on the shell side of the membrane module.

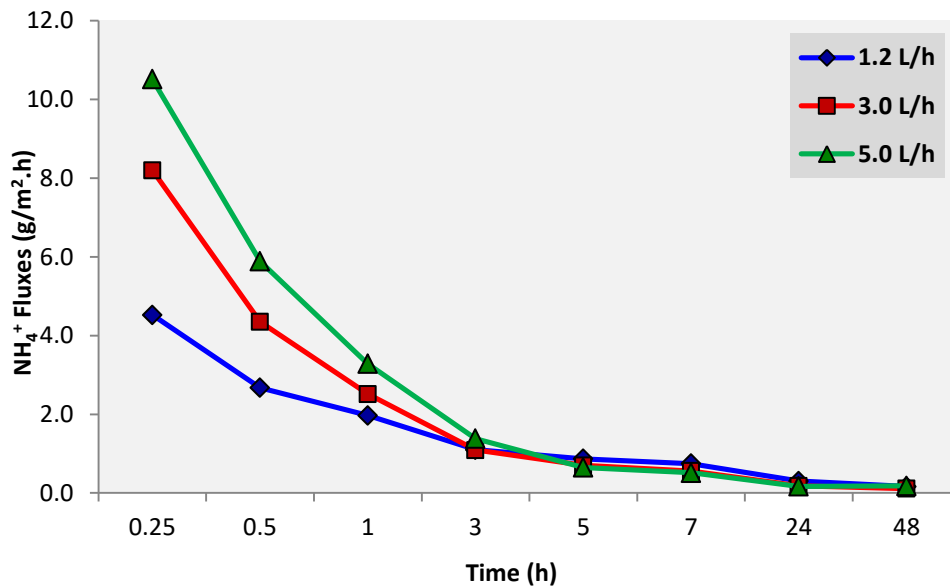
The attachment of AOB on the shell side of the HF tubes could be visually confirmed based on the presence of biomass attached to the fibres, and as freely suspended cells in the bulk phase of the shell side. The HF tubes that were originally white changed to slight brown yellowish indicating that the biomass had attached to their surface. Furthermore, as the solution containing only  $\text{NH}_4^+$  was fed through the tube side, the presence of  $\text{NO}_2^-$  (or/and  $\text{NO}_3^-$ ) either in the permeate or retentate streams also demonstrated that  $\text{NH}_4^+$  was oxidised by the bacterial cells attached to the shell side of the HF tubes. Samples from both permeate and retentate were collected and analysed for  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ .

### ***6.3.2.1 Membrane Process Performance: Ammonium Permeability and Membrane Rejection***

In this study, the performance of the pre-oxidation process was assessed based on the permeability and selectivity towards  $\text{NH}_4^+$ , as well as the rate of  $\text{NO}_2^-$  formation at different flow rates. **Fig. 6-6** shows the  $\text{NH}_4^+$  mass fluxes across the membrane at three different feed flow rates, i.e. 1.2 L/h, 3.0 L/h, and 5.0 L/h, with the cell suspension of AOB circulated through the shell side of the membrane module.

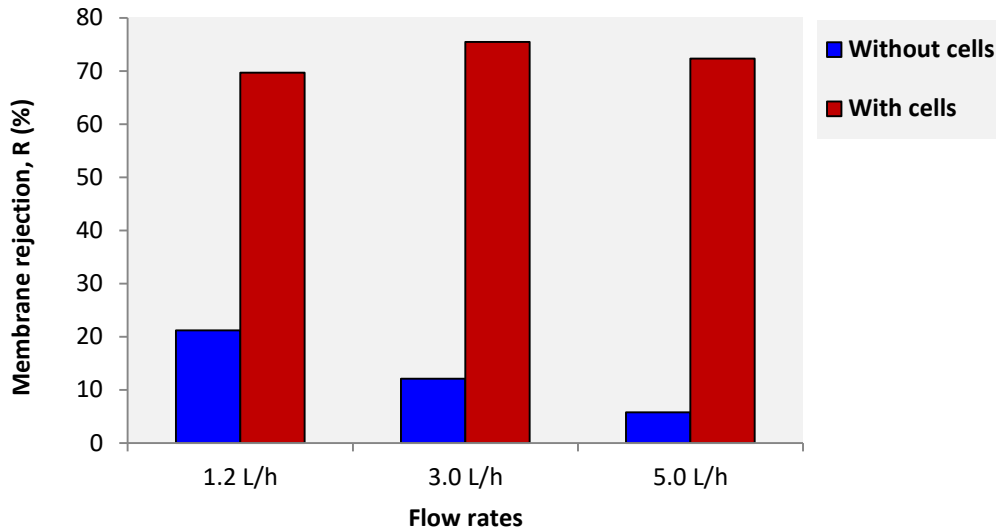
The fluxes inevitably decreased with time due to various factors that have previously been discussed, i.e. decreasing mass transfer driving force, concentration polarisation and membrane fouling. When comparing the flux observed at different flow rates, it is apparent that the higher the flow rates applied, the higher the fluxes were. This was due to the reducing boundary layer and hence increased diffusivity as the fluid velocity increased. The rates of flux decrease were substantially higher in the first hour of operation, i.e. over 40% at higher flow rates, i.e. 3.0 L/h and 5.0 L/h, but a much slower and gradual flux decrease was observed at a lower flow rate, i.e. 1.2 L/h, from the early stage of the operation. High

initial flux (due to high fluid velocity) tends to cause membrane fouling much earlier than at lower flow rates due to the quick build-up of solutes on the membrane surface.



**Fig. 6-6**  $\text{NH}_4^+$  mass fluxes at different flow rates, while a cell suspension of AOB circulated through the shell side of the membrane.

Since  $\text{NH}_4^+$  was fed through the tube side of the membrane module, the efficiency of the membrane process was also assessed based on its rejection of  $\text{NH}_4^+$ . **Fig. 6-7** compares the average membrane rejection of  $\text{NH}_4^+$  with and without cell circulation through the shell side at three different flow rates, and it appeared that more  $\text{NH}_4^+$  was retained inside the tube side, i.e. about 70%, when the process involved cell circulation through the shell side of the module. In contrast, the average membrane rejection of  $\text{NH}_4^+$  for the three flow rates was only 13% when there was no cell circulation involved. This is because the presence of bacterial cells on the tube walls and in the suspension would decrease the concentration difference that existed between the tube and the shell side. It was also quite possible that some of the bacterial cells could have caused membrane clogging, and hence decreasing the driving force that caused more  $\text{NH}_4^+$  to be retained inside the tube side.



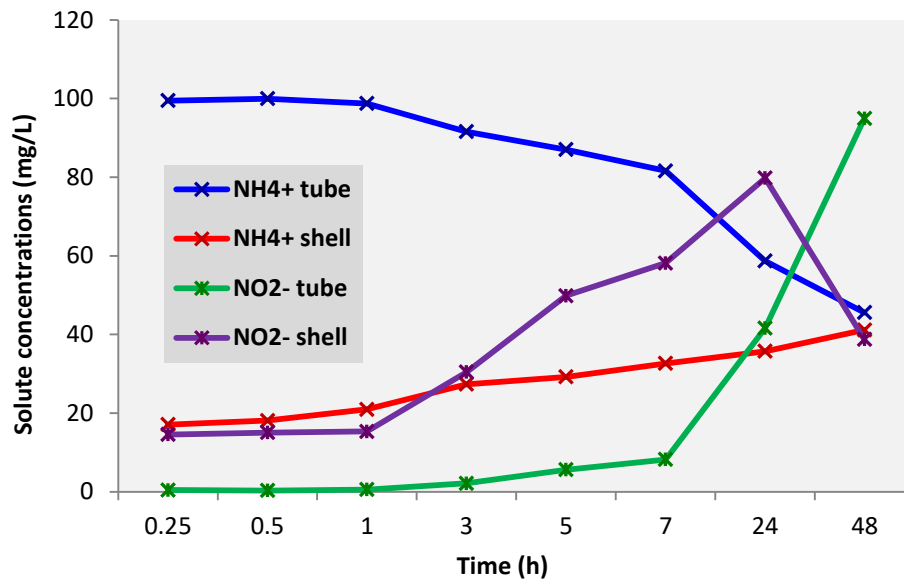
**Fig. 6-7** Average membrane rejection of  $\text{NH}_4^+$  at different flow rates, with and without cell circulation through the shell side of the module.

In addition, the average  $\text{NH}_4^+$  fluxes across the membrane decreased by more than 85% when bacterial cells were circulated through the shell side of the membrane. The  $\text{NH}_4^+$  flux, however, was expected to increase (decrease in membrane rejection) in the case involving bacterial cell circulation since  $\text{NH}_4^+$  concentrations on the shell side decreases due to the activity of AOB. The continuous recirculation of the bacterial cells through the shell side could have established a thin layer of biofilm on the outer surface of the HF tubes, and this could reduce the permeation of  $\text{NH}_4^+$  through the membrane. However, the real reasons for this observation are still not clear and might need further investigations.

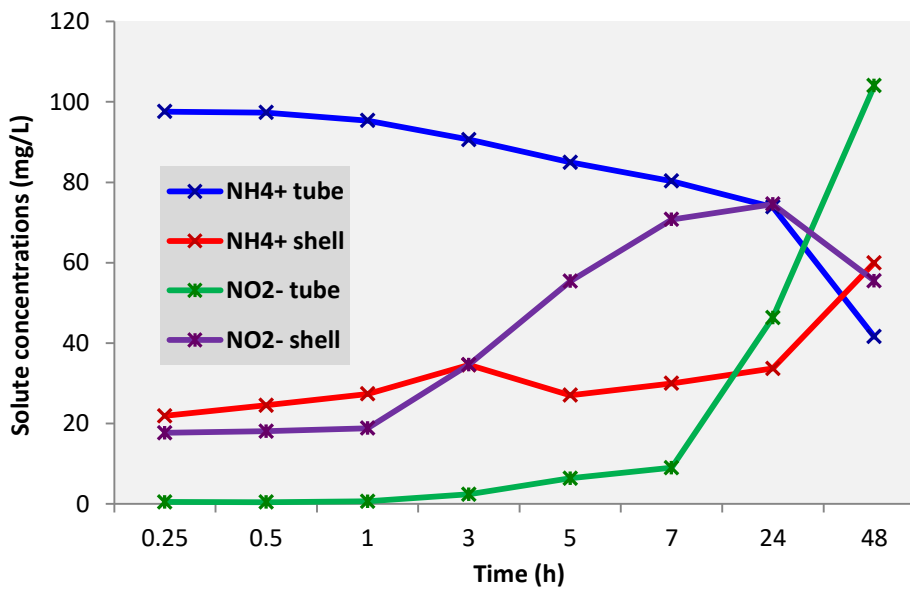
### **6.3.2.2 Formation of Nitrite ( $\text{NO}_2^-$ ) from Ammonium Oxidation**

In the case involving the circulation of AOB suspension through the shell side, the  $\text{NH}_4^+$  that diffused through the membrane were either oxidised to  $\text{NO}_2^-$ , or simply passed into the permeate side. The  $\text{NO}_2^-$ , as a result of  $\text{NH}_4^+$  oxidation on the shell side of the HF and in the suspension, would also either diffuse back into the tube side due to the concentration gradient, or remain in the shell side. Therefore, it was likely that both the permeate (shell side) and retentate (tube side) contained a mixture of both  $\text{NH}_4^+$  and  $\text{NO}_2^-$ . This was confirmed based on the presence of both components in the tube and shell sides,

as shown in **Fig. 6-8** and **6-9**; observations made at flow rates of 3.0 L/h and 5.0 L/h, respectively.



**Fig. 6-8** Concentration profiles of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the tube and shell sides of the membrane module at a flow rate of 3.0 L/h.



**Fig. 6-9** Concentration profiles of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the tube and shell sides of the membrane module at a flow rate of 5.0 L/h.

The concentration profiles of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  as illustrated in both figures proved that  $\text{NH}_4^+$  and  $\text{NO}_2^-$  were present in the tube and shell side of the membrane module, although  $\text{NO}_2^-$  had only started to appear at measurable concentrations in the tube side after one hour at both flow rates (3.0 L/h and 5.0 L/h).  $\text{NO}_2^-$ , in this case, was produced from the oxidation of  $\text{NH}_4^+$  by AOB. The rate of  $\text{NH}_4^+$  oxidation depended on a number of factors, and one of the most important ones is the quality and quantity of bacterial cells grown on the HF tubes, and in the shell side as a freely suspended cells. The presence of  $\text{NO}_2^-$  in both the permeate and retentate streams verified that the AOB culture was capable of oxidising  $\text{NH}_4^+$  to  $\text{NO}_2^-$ .

Initially, 100 mg/L of  $\text{NH}_4^+$  was fed to the membrane module through the tube side, and its concentration in the tube side gradually decreased as  $\text{NH}_4^+$  diffused into the shell side, and was subsequently oxidised by the AOB on the membrane shell side and in suspension. As a result of this,  $\text{NO}_2^-$  gradually increased both in the tube and shell side of the membrane module, and after 24 hours over 75 mg/L of  $\text{NO}_2^-$  was detected in the shell side of the module. After 48 hours, it can be observed that the  $\text{NO}_2^-$  concentrations in the shell side decreased by more than 50% at a flow rate of 3.0 L/h, while at a flow rate of 5.0 L/h, the  $\text{NO}_2^-$  had decreased by 25%. Concurrently, the  $\text{NO}_2^-$  concentration in the tube side increased significantly at both flow rates, indicating that the  $\text{NO}_2^-$  had diffused back into the tube side driven by a concentration gradient. After 48 hours, final  $\text{NO}_2^-$  concentrations of about 95 mg/L and 104 mg/L were found in the tube side at flow rates of 3.0 L/h and 5.0 L/h, respectively.

However, at a flow rate of 1.2 L/h, no  $\text{NO}_2^-$  was produced in the first 30 minutes, and only after one hour traces of  $\text{NO}_2^-$  were detected in the permeate.  $\text{NO}_2^-$  concentrations of only 2.4 mg/L and 3.1 mg/L were found in the shell side and tube side of the membrane, respectively, after 24 hours, which was considerably lower compared with the amount of  $\text{NO}_2^-$  produced at higher flow rates. Based on this low concentration of  $\text{NO}_2^-$ , it appears that a flow rate of 1.2 L/h was not sufficient to enable  $\text{NH}_4^+$  to diffuse through the membrane, and be oxidised by the AOB to produce  $\text{NO}_2^-$ .

### **6.3.2.3 Presence and Formation of Nitrate ( $\text{NO}_3^-$ )**

Other than  $\text{NO}_2^-$ , small amounts (2 – 10 mg/L) of  $\text{NO}_3^-$  were also found in both permeate and retentate streams; the presence of  $\text{NO}_3^-$  in the membrane process was from the oxidation of  $\text{NO}_2^-$  during the operation.  $\text{NO}_2^-$  is an intermediate compound during a complete nitrification (oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ ), and thus it can easily be oxidised to  $\text{NO}_3^-$ . Although samples were taken at a point immediately after exiting from membrane module (both retentate and permeate), some  $\text{NO}_2^-$  produced in the module could have also been oxidised to  $\text{NO}_3^-$  by NOB. This  $\text{NO}_3^-$  either remained in the tube side or permeated into the shell side.

The cell suspension used in this study was harvested from the enrichment batch reactors the moment that the maximum concentration of  $\text{NO}_2^-$  was found in the reactor, indicating that AOB was the predominant species. However, at the start of the experiment it was not guaranteed that the cell suspension only contained the AOB species responsible for the oxidation of  $\text{NH}_4^+$  of  $\text{NO}_2^-$ ; hence it might also have contained NOB species responsible for the oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . The presence of low concentrations of  $\text{NO}_3^-$  during the process was inevitable, but it was believed that this would not influence membrane performance and process efficiency. A nitrogen mass balance carried out between the initial feed and the effluent of the membrane (permeate and retentate) after 24 hours at all flow rates applied showed that  $\text{NH}_4^+$  either remained as it was, or was converted to  $\text{NO}_2^-$  and  $\text{NO}_3^-$  with acceptable percentage errors within 5%.

### **6.3.3 Feasibility of Using the NF Membrane Modules for Pre-Oxidation**

#### **Process of Ammonium**

Both the membrane performance and process efficiency would determine the feasibility of the process. Since this membrane process was initially proposed to replace a conventional partial nitrification reactor prior to the Anammox process, there is another important parameter to be looked at; the molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  after the process in the retentate (tube side). Apart from this, it is also necessary to evaluate the viability of the membrane process by calculating the quantity of membrane, i.e. membrane surface area, and hence the operating cost required to complete the process at a larger scale.

### 6.3.3.1 Molar Ratio of Ammonium to Nitrite

In partial nitrification, about 50% of the  $\text{NH}_4^+$  is oxidised to  $\text{NO}_2^-$ , producing a mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in a 50/50 ratio, which means that  $\text{NH}_4^+$  and  $\text{NO}_2^-$  should be provided at an approximately equimolar ratio to the Anammox process. Although Strous et al. (1998) previously found that a molar ratio of  $\text{NH}_4^+/\text{NO}_2^-$  of 1:1.32 was the ideal feed for the Anammox process, several recent reports have shown that  $\text{NH}_4^+$  and  $\text{NO}_2^-$  were consumed in a ratio of 1:1.22 (Trigo et al., 2006) and 1:0.95 (Wyffels et al., 2004) in their respective Anammox reactors.

Therefore, the feasibility and potential of using the NF membrane processes for the pre-oxidation of  $\text{NH}_4^+$  was also studied based on the molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  found exiting the process. Hence, the ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  found in the retentate (tube side) was more important as its product would be subsequently pumped into the next reactor running the Anammox process, as proposed and shown in **Fig. 6-2** at the beginning of this chapter. Apart from that, only findings from membrane processes with flow rates of 3.0 L/h and 5.0 L/h were considered for the feasibility study, since the amount of  $\text{NO}_2^-$  produced at flow rate of 1.2 L/h was less significant, making the ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  extremely high and not feasible.

Up to 24 hours of operation, the molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  in the retentate at a flow rate of 3.0 L/h was 1:0.3. This ratio was obviously imbalanced and not suitable for the Anammox process, as it indicates that more  $\text{NH}_4^+$  was still retained in the tube side instead of permeating through the membrane and reacted with AOB to produce  $\text{NO}_2^-$ . On the other hand, the ratio between the two components found in the permeate (shell side) was 1:0.9, much closer to the ideal ratio required for the Anammox process, although in this case the permeate would be recycled back into the reactor containing AOB culture, as it still contained remaining bacterial cells that was not attached on the shell sides of HF tubes. Quite similar ratios of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  found in the shell side after 24 h also showed that most  $\text{NO}_2^-$  still remained in the shell side, and had not diffused back into the tube side.

Similarly, at flow a rate of 5.0 L/h, the ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  in the retentate indicates that  $\text{NH}_4^+$  was still the primary component found in the retentate; for every mole of  $\text{NH}_4^+$ , there was only 0.25 mole of  $\text{NO}_2^-$  available. This finding also demonstrates that



the rate of  $\text{NH}_4^+$  oxidation by the AOB was quite slow, most likely due to the low concentration of bacterial cells available on shell sides of HF tubes and in suspension, thus a longer time was required for the process to be completed. The small amount of  $\text{NO}_2^-$  available in the retentate would not be able to compensate for the higher amounts of  $\text{NH}_4^+$ , and hence would not provide a balanced feed to the Anammox process.

The molar ratios of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  both in the retentate and permeate sides were then investigated for the run up to 48 hours. Prior to that, at 24 hours it was noticed that the process had still not reached its steady-state conditions as  $\text{NH}_4^+$  and  $\text{NO}_2^-$  fluxes across the membrane were still not constant, i.e. decreasing with time. It also meant that solutes could still be able to pass through the membrane, even at much smaller fluxes. After 48 hours of operation, a mixture of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the retentate was found in a molar ratio of 1:0.82 and 1:0.99 at flow rates of 3.0 L/h and 5.0 L/h, respectively. In this case, the  $\text{NH}_4^+$  to  $\text{NO}_2^-$  ratio of 1:0.99 at a flow rate of 5.0 L/h was similar to that reported by Wyffels et al. (2004). **Table 6-4** summarises the detailed amount of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , expressed in terms of moles, present in the retentate of the membrane module after 24 and 48 hours, at two different flow rates.

As the process was run for 48 hours, more  $\text{NH}_4^+$  had diffused through the membrane and been oxidised to  $\text{NO}_2^-$ . Furthermore, as the membrane process approaching steady-state conditions, it was noticed that more  $\text{NO}_2^-$  permeated back into the tube side and collected as a retentate, instead of staying in the shell side because  $\text{NO}_2^-$  in the shell side had increased from more  $\text{NH}_4^+$  coming through. This can be seen occurring based on the decreasing concentration of  $\text{NO}_2^-$  found in the shell side between 24 to 48 hours. Given sufficient time, and with a good population of bacterial cells grown on the shell sides of HF tubes and in the suspension, it is possible that more  $\text{NO}_2^-$  would be produced from  $\text{NH}_4^+$  oxidation, thus resulting in a much better molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , and hence providing the ideal feed into the Anammox process.

**Table 6-4** Amount of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in retentate (tube side) after 24 h and 48 h at different flow rates.

Solutes	Number of moles in the retentate (tube side)			
	Flow rate 3.0 L/h		Flow rate 5.0 L/h	
	24 h	48 h	24 h	48 h
$\text{NH}_4^+$	1.53	1.19	1.23	0.69
$\text{NO}_2^-$	0.43	0.97	0.30	0.68
$\text{NH}_4^+:\text{NO}_2^-$	1:0.28	<b>1:0.82</b>	1:0.24	<b>1:0.99</b>

### 6.3.3.2 Scale-Up of the Membrane Process

In membrane separation process, one of the goals of conducting laboratory-scale experiments is to accurately estimate the total membrane surface area (quantity) and the processing time needed to complete the process during large-scale processing, hence giving an idea whether the process is practically viable or not. The quantity of membrane is directly related to overall capacity of the treatment plant, while the processing time is proportional to the flow rates. As the membrane surface area increases, a larger size of filtration system (module) is required and larger pumps are required to maintain the recirculation flux across the membrane. To do this, a full-scale SHARON reactor that has been in full operation was selected for comparison.

One of the earliest full-scale SHARON reactors was successfully constructed at Dokhaven wastewater treatment plant (WWTP) in Rotterdam in 1999 (Mulder et al., 2001). The treatment plant was originally designed for BOD and nitrogen removal (470,000 PE) using aeration tanks and clarifiers in a two-stage process, whilst the construction of the SHARON system was necessary to meet the new legislation for nitrogen removal when a study had shown that significant fraction of nitrogen were still present in the digested sludge (rejection water) produced from the previous treatment stages. The system has been shown to be capable of treating of 830 kg  $\text{NH}_4^+$ /day and significantly improving the overall WWTP nitrogen removal efficiency of over 90% (Mulder et al., 2001). Based on a report published by Grontmij (2008) on their website, there were currently two full-scale

SHARON reactors operated in the UK; in Manchester (MVPC Shell Green) and Norwich (Whitlingham STC) with a capacity of 1400 m<sup>3</sup>/d and 900 m<sup>3</sup>/d, respectively.

In this study, the SHARON reactor located at Whitlingham STC (built in 2009) was selected for comparison. With a capacity of 900 m<sup>3</sup>/d, the system was used for the treatment of nitrogen-rich wastewater (filtrate) produced during dewatering of digested sludge containing NH<sub>4</sub><sup>+</sup> in the range of 2000 – 3000 mg/L (Bilt, 2007). However, a typical NH<sub>4</sub><sup>+</sup> concentration of 45 mg/L (medium level) in raw municipal wastewater with minor contributions of industrial wastewater (Henze and Comeau, 2008) was used in the calculation. Based on the capacity of the reactor (900 m<sup>3</sup>/d) and the concentration of the NH<sub>4</sub><sup>+</sup> to be treated (45 mg/L), the reactor will be capable of treating the wastewater at a rate of 40,500 g NH<sub>4</sub><sup>+</sup>/d. If the highest NH<sub>4</sub><sup>+</sup> flux achieved by our NF membrane module at a flow rate of 5.0 L/h and NH<sub>4</sub><sup>+</sup> concentration of 100 mg/L were to be maintained (252 g NH<sub>4</sub><sup>+</sup>/m<sup>2</sup> d), a total membrane surface area of 161 m<sup>2</sup> is required, and this represents a total of 64,400 modules of a similar size to our NF membrane module ( $A$  is 0.0025 m<sup>2</sup>/module) to complete the process. Considering the fact that our NF membrane module is significantly smaller (22 cm long and 2 cm in diameter) than the actual full-scale module, the number of modules can be significantly reduced if larger modules are built to house the HF tubes.

An estimation of capital cost to operate the membrane separation process was solely made based on the number of HF membranes required for the process, while other capital and operational costs were not considered in this study. The HF membrane cost was assumed to be €50/m<sup>2</sup> (about £37/m<sup>2</sup> as in 2015) (Verrecht et al., 2010), hence the total cost required for the 161 m<sup>2</sup> membrane is about £6,000. Therefore, it is practically and economically viable to operate the pre-oxidation process of NH<sub>4</sub><sup>+</sup> using the membrane module at a larger scale. In terms of processing time, at a rate of 40,500 g NH<sub>4</sub><sup>+</sup>/d (46 L NH<sub>4</sub><sup>+</sup>/d), about 22 days will be required to treat 1000 L of wastewater containing 45 mg/L of NH<sub>4</sub><sup>+</sup>. Taking into account the low capital cost required for the HF membrane alone, it is quite possible that the total cost needed for the whole process could be reduced compared with the conventional partial nitrification process, i.e. the SHARON reactor. Some limitations of the SHARON process, i.e. high temperature dependent and only suitable for wastewater containing low COD/N ratio, would make the pre-oxidation using the NF membrane module more favourable, as it was also shown to be capable of retaining large

fraction of COD inside the tube, and hence reducing the COD/N ratio in the shell side of the module.

## 6.4 SUMMARY

This study was a continuation of the study described in the previous chapter, where ammonium-oxidising bacteria (AOB) were enriched and grown from activated sludge and a SAMBR. It is important to highlight here that the study of using membrane filtration modules (specifically NF for this study) was just a preliminary study in order to investigate the potential of pre-oxidising  $\text{NH}_4^+$  to  $\text{NO}_2^-$  prior to the Anammox process, and hence replacing the conventional initial partial nitrification reactor should it be feasible. This membrane process could offer various advantages over the conventional reactor process, such as the process being able to be carried out continuously, with low energy consumption, no additives required, and easy scaling up, to name a few.

Membrane performance was first studied based on  $\text{NH}_4^+$  and glucose permeability, and its selectivity towards these components, at several different flow rates (1.2 – 5.0 L/h) and initial  $\text{NH}_4^+$  concentrations in the feed (50 – 150 mg/L) for 24 hours. The findings showed that the membrane had a very low rejection of  $\text{NH}_4^+$ , i.e. membrane rejection of less than 25%, over 24 hours of continuous operation with 100 mg/L of  $\text{NH}_4^+$  in the feed. The highest  $\text{NH}_4^+$  flux across the membrane was found to be in the range of 60 – 74  $\text{g/m}^2 \text{ h}$ , depending on the flow rates applied. It was found that the  $\text{NH}_4^+$  flux increased with increasing feed flow rates as higher fluid velocity can reduce the stagnant boundary layer, and hence the diffusional resistance.

In addition, initial  $\text{NH}_4^+$  concentration in the feed also had some impact on the permeation rate of  $\text{NH}_4^+$  through the membrane. At a fixed flow rate of 3.0 L/h, it was observed that the  $\text{NH}_4^+$  flux had increased as the feed concentrations increased, with the highest flux of 61  $\text{g/m}^2 \text{ h}$  recorded at an initial  $\text{NH}_4^+$  concentration of 150 mg/L. The relatively high flux across the membrane, with low rejection of  $\text{NH}_4^+$ , indicated that the membrane module is suitable for the proposed pre-oxidation process. In contrast, membrane rejection of glucose was relatively high, i.e. an average of 55% up to 24 hours of continuous operation with 50 mg/L glucose in the feed, making the membrane suitable for the

exclusion of glucose, i.e. COD in wastewater pre-treatment application. Less COD passing through the membrane also means that there would be less oxygen required for COD oxidation, and hence less biomass cells generated in the shell side, and possibly more methane generated in the Anammox reactor.

The study was followed by the pre-oxidation process of  $\text{NH}_4^+$  using NF membranes. The aim of the process was to enable  $\text{NH}_4^+$  to be oxidised to  $\text{NO}_2^-$  through the action AOB growing on the HF membrane tubes and as a freely suspended cells in the shell side. A cell suspension containing AOB was continuously circulated through the shell side of the module, concurrently with feeding a solution containing  $\text{NH}_4^+$  through the tube side. The experiments were carried out for 48 hours at three different flow rates (1.2 L/h, 3.0 L/h, and 5.0 L/h), considering the fact that fluid velocity is one of the significant factors influencing the permeation rate of solutes across a membrane. Of all the three flow rates applied, the performance of the membrane process was optimal at 5.0 L/h, based mainly on the molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  of 1:0.99 (almost equimolar) produced in the retentate after 48 hours operation, which was about 25% out of the ideal ratio of 1:1.32 proposed by Strous et al. (1998), but similar to that reported by Wyffels et al. (2004). The stoichiometric differences could be due to the differences in reactor composition including biomass concentration, as well as the nature of wastewater sludge used to start-up the process (Wyffels et al., 2004).

The preliminary findings in this study had shown that the potential of using membrane process for the pre-oxidation of  $\text{NH}_4^+$  is reasonably promising. This study can be considered successful based on the fact that the nitrifying bacteria (AOB) was only grown on the shell side of HF tubes and in the suspension of the membrane filtration module (instead of in a reactor) for a relatively short time (48 hours), but managed to produce a biomass-free effluent (retentate) containing  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in a molar ratio of 1:0.99 (at flow rate of 5.0 L/h). However, there are still a lot of improvements to be made, particularly in optimising the experimental operating conditions so that the process would seem feasible to be combined with the Anammox process for the treatment of nitrogen-containing wastewater. Apart from this, the operation of the process at a larger scale, i.e. plant capacity of 900 m<sup>3</sup>/d, could be practically feasible, where about 161 m<sup>2</sup> of HF membranes is required to build the membrane module to treat a typical municipal wastewater containing about 45 mg/L  $\text{NH}_4^+$ . However, issues related to capital and operating costs of the whole process must be given further and thorough consideration.

## CHAPTER 7

### GENERAL DISCUSSION

This chapter analyses all the findings in this thesis that have been explained and discussed in previous chapters in an overall manner, and tries to tie different strands of the work carried out together.

The anaerobic ammonium oxidising (Anammox) process discovered in 1994 (Mulder et al., 1995), was shown to be capable of removing  $\text{NH}_4^+$  and  $\text{NO}_2^-$  simultaneously from wastewater, and hence preferred to the conventional nitrification-denitrification process. However, as the Anammox consumes about equimolar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  to proceed, a partial nitrification process preceding the Anammox reactor is necessary. Therefore, this work focused on the application of NF membrane module for the partial nitrification (termed “pre-oxidation”) of  $\text{NH}_4^+$  to provide the ideal feed for the Anammox process.

Prior to the application of NF membrane for pre-oxidation of  $\text{NH}_4^+$ , the feasibility of Anammox process in a 3-L laboratory-scale submerged anaerobic membrane bioreactor (SAMBR) was first studied. The SAMBR was designed and fabricated at Imperial College London in 2004 (Hu, 2004). The SAMBR, as it applies the concept of membrane bioreactor technology, was shown capable of providing high retention of biomass within the reactor and better control of microbial population (Vallero et al., 2005), and hence reducing the process start-up period. The process performance in terms of its start-up period and process efficiency in the removal of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  from wastewater was assessed, discussed, and compared with the existing processes previously published in the literature.

Two SAMBR were used to culture and start-up the process, operated at different HRTs, i.e. two and four days. Relatively short start-up periods (60 and 70 days) with over 80% and 65%  $\text{NH}_4^+$  and  $\text{NO}_2^-$  removal, respectively, were achieved for both reactors. In addition, almost equimolar ratios of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption by the bacteria were found in both reactors, comparable to a number of reports in the literature (Wyffels et al., 2004, Wang et al., 2009), and not very different from the original value proposed by Strous et al.

(1998) (Eq. 2-13). Furthermore, the production of  $N_2$  gas was also stoichiometrically in agreement with the ratio of  $NH_4^+$  and  $NO_2^-$  consumed, i.e. one mole of  $N_2$  gas produced per mole of  $NH_4^+$  and  $NO_2^-$  consumed, respectively (summarised in Table 4-3). These findings support the occurrence of the Anammox reaction, and suggest that the SAMBR is a good reactor to grow the slow-growing Anammox bacteria, subsequently leading to a relatively quick start-up for the process of removing nitrogenous species from wastewater.

The second part of the work was the most critical, and represents the novelty of this particular study. The researcher proposed using a composite NF membrane module to pre-oxidise  $NH_4^+$  to a mixture of both  $NH_4^+$  and  $NO_2^-$  (1:1 ratio) through the action of AOB, which grew on the wall of hollow fiber membrane tubes, and as freely-suspended cells in the shell side of the membrane module. Prior to the process, the AOB was enriched from wastewater sludge in a 2-L batch reactor. Although this part served as a preparation work for the pre-oxidation part, it is still critical towards establishing a successful partial nitrification process prior to Anammox. The successfully enriched AOB would be used in the proposed pre-oxidation step using the NF membrane module in the next part of the work. The key factor in enriching the AOB was to suppress the growth NOB, mainly by controlling the dissolved oxygen level and pH of the culture.

Although the study is quite fundamental, the pre-oxidation process of  $NH_4^+$  using the NF hollow fibre membrane module was carried out to assess its feasibility, and potentially to improve the existing partial nitrification process prior to the Anammox reactor. In this process, AOB species responsible for the oxidation of  $NH_4^+$  to  $NO_2^-$  (formerly enriched and grown in batch reactors) was grown on the shell side of the membrane tubes, while synthetic wastewater containing  $NH_4^+$  and glucose was fed through the tube side. This concept, i.e. a nitrifying biofilm attached to the membrane surface, was adapted from Brindle et al. (1998), who showed that a vertical laboratory-scale tubular reactor containing UF membranes was capable of oxidising  $NH_4^+$  to  $NO_2^-$ . It was later found to be widely applied mainly for the removal of nitrogen through nitrification (Semmens et al., 2003, Satoh et al., 2004, Feng et al., 2008)

The process performance was assessed in terms of solute fluxes across the membrane, membrane rejection towards specific solutes, and  $NO_2^-$  concentration found in the permeate (shell side) and retentate (tube side) streams. The idea behind the proposed

work was that  $\text{NH}_4^+$  would permeate through the membranes due to concentration differences, and hence be oxidised into  $\text{NO}_2^-$  by the AOB. The  $\text{NO}_2^-$  would then permeate back into the tube side and be collected in the retentate stream; in this situation the retentate stream would contain a mixture of both unoxidised  $\text{NH}_4^+$  and  $\text{NO}_2^-$  (in an equimolar ratio). At the end the process, almost equimolar ratios of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  were found in the retentate stream, making an ideal feed for the Anammox process.

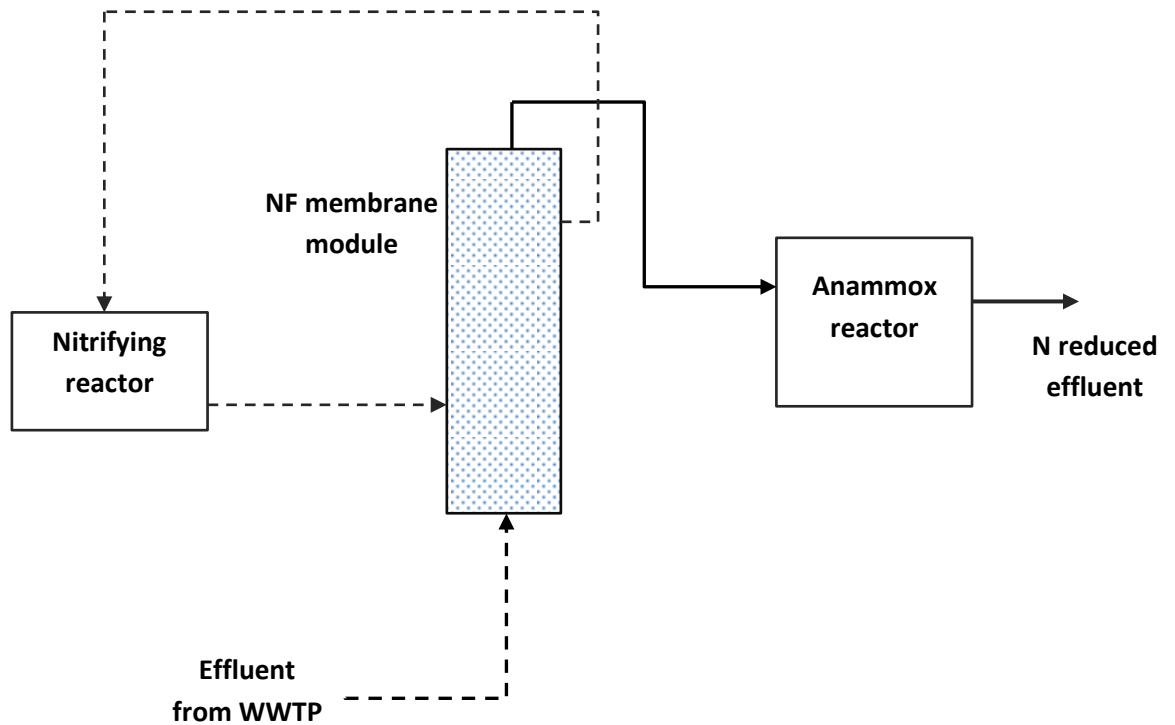
The evaluation of solute concentrations, flux, membrane selectivity and rejection were made at different points (time) and was not only based on a single point at 48 h. However, the final concentrations of solutes specifically at 24 hours and 48 hours were taken into consideration for comparison, and it was found that the process reached steady-state after 48 hours. It was previously explained in Chapter 6 (**Section 6.2.2.3**) that the preliminary process was ran for up to 72 hours, but as the process was extended, almost all of the solution in the feed tank permeated through the membrane and went to the other tank (supposedly to contain AOB culture). No further experiment was carried out to rectify the problem, as it was found that no  $\text{NH}_4^+$  permeated through the membrane after 24 h (without AOB culture). It is still possible to just maintain the AOB tank even after the system reaches steady state as not all AOB will attach to the shell side of the membrane, and hence be washed out into the permeate. Under ideal conditions, i.e. without any concentration polarization, membrane fouling, NOB presence and other negative factors, an equimolar ratio of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  could be found in the retentate stream. Likewise, similar ratio of both species could also be found in the permeate.

Apart from assessing the process feasibility in terms of solute fluxes and degrees of rejection, further analysis was carried out to study whether the proposed process was feasible to scale-up. This was carried out by taking an example of a real full-scale SHARON reactor located in Norwich, UK with a treatment capacity of  $900 \text{ m}^3/\text{d}$  (Bilt, 2007). The calculated cost of membrane was relatively low and economically feasible, i.e. about £6,000 for membrane with a total surface of  $161 \text{ m}^2$ , although the total operating cost to run the whole process has yet to be considered. However, significant reductions in the operating cost could possibly be achieved as the application of NF to partially oxidise  $\text{NH}_4^+$  requires neither intermittent aeration nor mechanical agitation, unlike in the SHARON process (Hellings et al., 1998, Ahn, 2006).



The findings of this pre-oxidation work suggest that it could be a feasible alternative to the conventional partial nitrification reactor operated prior to the Anammox process as it reduces reactor footprint, and hence its operating cost too. Not only is the process capable of producing a biomass-free effluent containing an equimolar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , but the findings also suggest that the membrane is suitable for wastewater pre-treatment, i.e. removal of organic matter, due to its high rejection of glucose (over 50%). It is important to remove the organics prior to the Anammox process as their presence could promote the growth of heterotrophic denitrifying bacteria, and hence affect the Anammox process. However, in our case, it was found that the presence of organics had a minimal impact on the Anammox process based on the consistent level of COD throughout the process, and this statement was supported by an ANOVA analysis (**Section 4.3.3**), indicating that a large portion of the COD was not consumed by the denitrifying bacteria. This is probably explained because the COD mainly comprised of SMPs as the sludge was collected from an anaerobic digestion treatment plant (Aquino and Stuckey, 2002, Jianga et al., 2008), which is hard to degrade anaerobically, and hence did not support the growth of denitrifying bacteria.

In practice, the pre-oxidation of  $\text{NH}_4^+$  using an NF membrane module could be connected in series with the Anammox reactor, while its feed is the effluent from wastewater treatment plant. AOB is grown in a separate nitrifying reactor and continuously fed through the shell side of the module, as schematically shown in Fig. 7-1. [Many full-scale Anammox systems have been operating worldwide for various applications \(Section 2.13\)](#), and one of the most successful applications includes integration with an aerobic SBR and anaerobic digestion for abattoir wastewater processing (AMPC., 2016).



**Fig. 7-1** Proposed schematic of the pre-oxidation of  $\text{NH}_4^+$  using NF membrane module connected to the Anammox reactor.

As previously highlighted, the application of the membrane filtration process for the pre-oxidation of  $\text{NH}_4^+$  was meant to serve as an alternative to the conventional partial nitrification process preceding Anammox. However, in this study, no further work was carried out to link the pre-oxidation process by NF membrane module with the Anammox process in a SAMBR. The linking of these two processes is the most important future work to be considered, apart from optimising the pre-oxidation process of  $\text{NH}_4^+$  by investigating several other factors further so that they could potentially contribute towards better process efficiency.

## CHAPTER 8

# CONCLUSIONS AND RECOMMENDATIONS

This chapter analyses to what extent the work completed in Chapters 4, 5 and 6 meet the objectives set out at the end of Chapter 2, and makes some recommendations for future work in order to improve the current findings.

### 8.1 The Anammox Process in a SAMBR

The first objective set out for this study was to start-up and investigate the performance of the Anammox process in 3-litre laboratory-scale submerged anaerobic membrane bioreactor (SAMBR) that was previously designed and fabricated in the department. Prior to this, the SAMBR had yet to be used and tested for the removal of  $\text{NH}_4^+$ , combined with the Anammox process, a very slow process with about 11 days bacterial doubling time. However, it was proven that the use of membrane bioreactor could significantly shorten the start-up period of the process when compared with other conventional reactor systems, i.e. sequencing batch reactors, and biofilm-based reactors. The Anammox process was successfully started-up from anaerobic digestion sludge, and operated in two SAMBRs, for 60 days and 70 days, respectively. The difference between the two reactors was only the HRT applied; two and four days.

SAMBR 1 (with an HRT of two days) demonstrated better performance, with 86%  $\text{NH}_4^+$  removal efficiency, compared to only 81% achieved in SAMBR 2 (with an HRT of four days).  $\text{NO}_2^-$  removal efficiencies were fairly similar in both reactors, with 67% and 66%, respectively. It was observed that more  $\text{NH}_4^+$  had been consumed compared to  $\text{NO}_2^-$  during the experiments, leaving the molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption of 1:0.9 in both reactors, which was quite comparable to the previously reported values in the literature. Strous et al. (1999b) reported that a ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  consumption in the range of 0.25 – 2 could still be considered as acceptable, as the values could vary depending on several factors such as substrate compositions, operating conditions and configuration of the Anammox process and reactor.

It was shown that not only was the process capable of removing nitrogen from wastewater, but the presence of a membrane inside the reactor also improved the growth of bacterial cells in a way that it provided complete retention of the cells from being washed-out in the effluent. The membrane also acted as a platform on which the bacterial cells could attach and grow in aggregates, hence increasing process efficiency. In conclusion, the combination of a SAMBR and the Anammox process for the removal of nitrogen from wastewater seemed to be very promising, and could be a feasible alternative to the existing treatment technologies in the future.

## 8.2 Enrichment of AOB in Batch Reactors

The second objective of this study was to enrich and grow nitrifying bacteria (AOB) responsible for the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  in 2-litre batch reactors (operated in fed-batch mode). The sources of the seed sludge were: return activated sludge (Mogden Sewage Treatment Works, London) and full-scale SAMBR sludge (Anglian Water Treatment Plant, Cambridge). Two batch reactors, marked as NR 1 and NR 2 used to enrich and grow AOB from activated sludge and SAMBR sludge, respectively, have both shown significant nitrifying activity throughout the experimental period, which also indicated that AOB was successfully enriched and grown inside the reactors.

NR 1 was operated for about 275 days while NR 2 lasted for 300 days over four cycles, with an average  $\text{NH}_4^+$  removal efficiency of 91% and 93%, respectively. In terms of  $\text{NH}_4^+$  removal rates, NR 1 (activated sludge) and NR 2 (SAMBR sludge) achieved maximum removal rate of 2.18 and 2.85 mg  $\text{NH}_4^+$ /day, with specific  $\text{NH}_4^+$  removal rate of 3.3 mg  $\text{NH}_4^+$ /g VSS d and 7.5 mg  $\text{NH}_4^+$ /g VSS d, respectively. NR 1 exhibited a higher and more stable removal rates and efficiency than that of NR 2, although the findings between the two reactors were fairly comparable. The findings have shown that both sludge from activated sludge processes and a SAMBR were capable of enriching AOB with satisfactorily high nitrifying activity. The enriched AOB culture would then be used for the pre-oxidation of  $\text{NH}_4^+$  combined with membrane process in the final part of the study.

### 8.3 Pre-Oxidation of Ammonium using NF Membrane System

The final objective set out for this study was to investigate the potential and feasibility of using composite NF membrane modules consisting of concentric HF tubes for the pre-oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ . The long term aim of the process was to provide an alternative to the conventional partial nitrification reactor prior to the Anammox process. This study was a continuity from the study described in Chapter 5, where the bacterial culture (AOB) previously enriched in batch reactors were made to grow on the shell side of the HF membrane tubes and as freely suspended cells in the shell side of the membrane module, so that  $\text{NH}_4^+$  would be oxidised to  $\text{NO}_2^-$ .

The membrane performance and process efficiency were first assessed based on  $\text{NH}_4^+$  and glucose permeability and rejection. The effects of feed flow rates (1.2 – 5.0 L/h) and initial feed concentrations (50 – 150 mg/L of  $\text{NH}_4^+$ ) on membrane permeability were investigated. After 24 hours of continuous operation, the average membrane rejection of  $\text{NH}_4^+$  was found to be in the range of 5 – 30%, with fluxes of 16 – 74  $\text{g/m}^2 \text{ h}$  depending on the flow rates applied or/and initial  $\text{NH}_4^+$  concentrations in the feed. High  $\text{NH}_4^+$  fluxes and low membrane rejection of  $\text{NH}_4^+$  ions showed that the NF membrane module was suitable to be used for the pre-oxidation process. In contrast, the membrane module had a relatively higher rejection of glucose (expressed in terms of COD) of 50 – 56%.

The final part of the study was to enable  $\text{NH}_4^+$  to be oxidised to  $\text{NO}_2^-$  through the action of AOB that was concurrently being circulated through the shell side of the membrane module. Similar to the earlier permeability study of the membrane, the operation was carried out at three different flow rates of 1.2 L/h, 3.0 L/h and 5.0 L/h. It can be concluded that AOB was successfully grown on the shell side of HF membrane tubes and in suspension, not only based on physical changes occurring to the membrane tubes, but more importantly, based on the formation of  $\text{NO}_2^-$  (and  $\text{NO}_3^-$ ) in both retentate and permeate streams throughout the process, as well as gradual decrease in  $\text{NH}_4^+$  concentration in the tube side due to the oxidation process carried out by AOB.

Operation at flow rates of 3.0 L/h and 5.0 L/h have shown some promising findings in terms of  $\text{NH}_4^+$  fluxes across the membrane, and the capability of the AOB to oxidise  $\text{NH}_4^+$

to  $\text{NO}_2^-$ . As the membrane process was proposed to provide an alternative to the conventional partial nitrification reactor prior to the Anammox process, it was also equally important to investigate the molar ratio of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  resulted after the process. In this study, approximately equimolar ratio (1:0.99) of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  in the retentate was recorded at 48 hours at a flow rate of 5.0 L/h, about 25% less than the ideal ratio of 1:1.32 previously reported for the Anammox process (Strous et al., 1998). This was probably due to insufficient time given for the oxidation to occur or/and low concentration of AOB cells on the shell side of HF tubes and in suspension.

In conclusion, the findings of the study have shown that the combination of membrane filtration system with nitrification process of  $\text{NH}_4^+$  by AOB prior to the Anammox process is practically feasible at laboratory-scale. Furthermore, an estimation of membrane area required to complete the process, followed by a costing analysis (based on the number of HF membranes required) has shown that the process is also practically and economically viable to be operated at a full-scale capacity (by comparing it with full-scale SHARON reactor with a capacity of 900  $\text{m}^3/\text{d}$ ). However, other capital and operational costs that might be needed for the actual full-scale process have yet to be considered. With proper optimisation study, followed by extensive experimental work, it is believed that this technique would be quite promising for a larger scale application in the future.

## **8.4 Recommendations for Future Work**

Considering the fact that the work done for this study was far from perfect, it is important for the researcher to provide some recommendations for future work, aimed at improving the findings or correcting mistakes, if any. The recommendations are as follows:

### **8.4.1 Use of molecular characterisation techniques to identify and characterise species of bacterial cells**

Part of the initial research proposal was to use specific molecular characterisation technique of denaturing gel gradient electrophoresis (DGGE) and fluorescence *in-situ* hybridisation (FISH) as identification tools to identify and characterise the specific types of bacteria involved in the study, as well as to further extend the understanding on the

behaviour of the bacterial communities. These techniques would allow investigation of the bacterial community structure, their diversity and phylogeny in almost all of the possible environments, quantitatively and qualitatively. FISH, for example will provide a clear insight into the interactions, concentrations and growth pattern of various bacterial groups available in microbial populations. DGGE, on the other hand, is a genetic fingerprinting technique that is normally employed to provide a profile representing the genetic diversity of a specific microbial species from a specific environment.

However, due to some inevitable technical issues and time limitation, no promising results were obtained particularly from FISH analysis, although enormous number of analyses was carried out. Both techniques require significantly a lot of time, and more importantly, precise experimental procedures.

#### **8.4.2 Use of seed sludge with higher biomass concentrations and quality**

In this study, the AOB was successfully enriched and grown from two different sources; activated sludge and full-scale SAMBR sludge. Although AOB culture enriched from the two aforementioned sources showed sufficient nitrifying activity in batch reactors, i.e. oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , their nitrifying ability inside the NF membrane module was still quite low since it took about two days of operation to achieve up to 60% of  $\text{NH}_4^+$  oxidised to  $\text{NO}_2^-$ . It was probably because of the insufficient concentration of bacterial cells attached and grown on the shell side of HF tubes and in the suspension. It is then proposed that biomass of higher concentration and purity be used particularly for the oxidation of  $\text{NH}_4^+$  inside the membrane module. To do this, optimum operating conditions should be ensured during the enrichment period of AOB in batch reactors, and bacterial cells should be harvested from the reactors at the right moment the AOB species is predominant over other species, followed by a more thorough and clean steps of a cell suspension preparation to minimise the risk of contamination.

Apart from that, commercially pure nitrifying bacterial culture could also be used in the membrane process, instead of enriching them from wastewater sludge, although this could add to the initial cost of the process. Higher concentration and purity of the bacterial cells would increase the rate of nitrifying activity, and hence reducing the time needed to

oxidise the  $\text{NH}_4^+$  to  $\text{NO}_2^-$ . Shorter time of operation is certainly needed to avoid serious loss of membrane performance in terms of flux decay due to various reasons.

### **8.4.3 Optimisation study of membrane process used in the pre-oxidation of ammonium**

The membrane performance and process efficiency should also be evaluated based on other significant experimental parameters such as the origin and concentrations of biomass (nitrifying culture), pH and temperature. In other words, a proper optimisation study of the membrane process used for the pre-oxidation of  $\text{NH}_4^+$  should be carried out. Optimisation study of a certain process would normally require a proper experimental design generated by suitable design software, such as Design Expert, Statistica, or Minitab to name a few. Comparison of outcomes between the actual (obtained from experimental work) and the theoretical values (generated by the software) would enable the software to precisely come out with the optimum operating conditions that consist of several experimental parameters in order to have an optimum process efficiency.



## REFERENCES

- Adav, S. S., Lee, D.-J. & Lai, J. Y. 2010. Enhanced biological denitrification of high concentration of nitrite with supplementary carbon source. *Applied Microbiology & Biotechnology*, 85, 773 - 778.
- Ahn, Y.-H. 2006. Sustainable nitrogen elimination biotechnologies: A review. *Process Biochemistry*, 41, 1709-1721.
- Akunna, J. C., Bizeau, C. & Moletta, R. 1992. Denitrification of anaerobic digesters: Possibilities and influences of wastewater COD/N-NO<sub>x</sub> ratio. *Environmental Technology*, 13, 825 - 836
- Akunna, J. C., Bizeau, C. & Moletta, R. 1993. Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: Glucose, glycerol, acetic acid, lactic acid and methanol. *Water Research*, 27, 1303–1312.
- Ali, N., Halim, N. S., Jusoh, A. & Endut, A. 2010. The formation and characterisation of an asymmetric nanofiltration membrane for ammonia-nitrogen removal: effect of shear rate. *Bioresource Technology*, 101, 1459-65.
- Ampc. 2016. Wastewater treatment with anaerobic digestion & anammox. Sydney: Australian Meat Processor Corporation Ltd (AMPC).
- Apha 1999. *Standard Methods for the Examination of Water and Waste Water*, Washington, American Public Health Association.
- Aquino, S. F. & Stuckey, D. C. 2002. Characterization of soluble microbial products (SMP) in effluents from anaerobic reactors. *Water Science and Technology*, 45, 127 - 132.
- Astm. 2007. Standard test method for hydrazine in water. West Conshohocken, PA: ASTM International.
- Berge, N., Reinhart, D. & Townsend, T. 2005. The fate of nitrogen in bioreactor landfills. *Critical Reviews in Environmental Science and Technology*, 35, 365-399.
- Bérubé, P. R., Hall, E. R. & Sutton, P. M. 2006. Parameters governing permeate flux in an anaerobic membrane bioreactor treating low-strength municipal wastewaters: A literature review. *Water Environment Research*, 78, 887-896.
- Bhaskar, K. V. & Charyulu, P. B. B. N. 2005. Effect of environmental factors on nitrifying bacteria isolated from the rhizosphere of *Setaria italica* (L.) Beauv. *African Journal of Biotechnology*, 4, 1145-1146.
- Bilt, D. 2007. SHARON N-removal over nitrite. Grontmij Nederland BV, Department of Water and Energy.
- Blackburne, R., Yuan, Z. & Keller, J. 2007. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. *Biodegradation*, 19, 303-312.
- Bock, E., Schmidt, I., Stüven, R. & Zart, D. 1995. Nitrogen loss caused by denitrifying *Nitrosomonas* cells using ammonia or hydrogen as electron donors and nitrite as electron acceptor. *Archives of Microbiology*, 163, 16-20.
- Brindle, K., Stephenson, T. & Semmens, M. J. 1998. Nitrification and oxygen utilisation in a membrane aeration bioreactor. *Journal of Membrane Science*, 144, 197-209.
- Camargo, J. A. & Alonso, Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, 32, 831–849.
- Campos, J. L., Garrido-Fernandez, J. M., Mendez, R. & Lema, J. M. 1999. Nitrification at high ammonia loading rates in an activated sludge unit. *Bioresource Technology*, 68, 141 - 148.
- Canziani, R., Emondi, V., Garavaglia, M., Malpei, F., Pasinetti, E. & Buttiglieri, G. 2006. Effect of oxygen concentration on biological nitrification and microbial kinetics in a cross-flow membrane bioreactor (MBR) and moving-bed biofilm reactor (MBBR) treating old landfill leachate. *Journal of Membrane Science*, 286, 202-212.

- Carrera, J., Vicent, T. & Lafuente, J. 2004. Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonium industrial wastewater. *Process Biochemistry*, 39, 2035–2041.
- Casey, E., Glennon, B. & Hamer, G. 1999. Review of membrane aerated biofilm reactors. *Resources, Conservation and Recycling*, 27, 203–215.
- Castignetti, D. & Hollocher, T. C. 1984. Heterotrophic nitrification among denitrifiers. *Applied and Environmental Microbiology*, 47, 620-623.
- Causse, J., Thomas, O., Jung, A.-V. & Thomas, M.-F. 2017. Direct DOC and nitrate determination in water using dual pathlength and second derivative UV spectrophotometry. *Water Research*, 108, 312 - 319.
- Cema, G., Surmacz-Górska, J. & Miksch, K. 2004. Implementation of the Anammox process in the membrane assisted bioreactor. In: SYSTEMS, I. A. O. O. U. S. (ed.) *Joint Polish-Swedish Seminars*. Stockholm, Sweden.
- Chamchoi, N. & Nitorisavut, S. 2007. Anammox enrichment from different conventional sludges. *Chemosphere*, 66, 2225-32.
- Chang, J. S., Chang, C. Y., Chen, A. C., Erdei, L. & S., V. 2006. Long-term operation of submerged membrane bioreactor for the treatment of high strength acrylonitrilebutadiene-styrene (ABS) wastewater: Effect of hydraulic retention time. *Desalination*, 191, 45-51.
- Chen, H., Liu, S., Yang, F., Xue, Y. & Wang, T. 2009. The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal. *Bioresource Technology*, 100, 1548-1554.
- Chen, J., Zheng, P., Yu, Y., Mahmood, Q. & Tang, C. 2010. Enrichment of high activity nitrifiers to enhance partial nitrification process. *Bioresource Technology*, 101, 7293-8.
- Cheremisinoff, N. P. 1996. Nitrification and denitrification in the activated sludge process. *Biotechnology for waste and wastewater treatment*. New Jersey: Noyes Publications.
- Churchouse, S. & Wildgoose, D. 2000. Membrane bioreactors progress from the laboratory to full-scale use. *Membrane Technology*, 111, 4-8.
- Ciudad, G., Rubilar, O., Muñoz, P., Ruiz, G., Chamy, R., Vergara, C. & Jeison, D. 2005. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. *Process Biochemistry*, 40, 1715-1719.
- Constable, M., Charlton, M., Jensen, F., McDonald, K., Craig, G. & Taylor, K. 2003. An ecological risk assessment of ammonia in the aquatic environment. *Human and Ecological Risk Assessment*, 9, 527-548.
- Cui, Z. F., Chang, S. & Fane, A. G. 2003. The use of gas bubbling to enhance membrane processes. *Journal of Membrane Science*, 221, 1-35.
- Damste, J. S. S., Strous, M., Rijpstra, W. I. C., Hopmans, E. C., Geenevasen, J. a. J., Duin, A. C. T. V., Niftrik, L. a. V. & Jettenk, M. S. M. 2002. Linearly concatenated cyclobutane lipids form a dense bacterial membrane. *Nature*, 419, 708-712.
- Dapena-Mora, A., Campos, J. L., Mosquera-Corral, A., Jetten, M. S. & Mendez, R. 2004. Stability of the Anammox process in a gas-lift reactor and a SBR. *Journal of Biotechnology*, 110, 159-70.
- Digiano, F. A., Braghetta, A., Utne, B. & Nilson, J. 1995. Hollow-fiber nanofiltration for treatment of a surface water high in natural organic matter. North Carolina: Water Resources Research Institute of North Carolina.
- Dvorak, L., Svojitka, J., Wanner, J. & Wintgens, T. 2013. Nitrification performance in a membrane bioreactor treating industrial wastewater. *Water Research*, 47, 4412-21.
- Egli, K., Fanger, U., Alvarez, P. J. J., Siegrist, H., Van Der Meer, J. R. & Zehnder, A. J. B. 2001. Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. *Archives of Microbiology*, 175, 198-207.
- Fane, A. & Chang, S. 2002. Membrane bioreactors: Design & operational options. *Filtration & Separation*, 36, 26-29.

- Feng, Y.-J., Tseng, S.-K., Hsia, T.-H., Ho, C.-M. & Chou, W.-P. 2008. Aerated membrane-attached biofilm reactor as an effective tool for partial nitrification in pretreatment of anaerobic ammonium oxidation (ANAMMOX) process. *Journal of Chemical Technology & Biotechnology*, 83, 6-11.
- Feng, Y. J., Tseng, S. K., Hsia, T. H., Ho, C. M. & Chou, W. P. 2007. Partial nitrification of ammonium-rich wastewater as pretreatment for anaerobic ammonium oxidation (Anammox) using membrane aeration bioreactor. *Journal of Bioscience and Bioengineering*, 104, 182-7.
- Fox, R. A. 2012. *Submerged anaerobic membrane bioreactors: Fouling, phage removal and flowsheet models*. PhD and Diploma of Imperial College (DIC), Imperial College London.
- Fox, R. A. & Stuckey, D. C. 2015. MS-2 and T4 phage removal in an anaerobic membrane bioreactor (AnMBR): effect of gas sparging rate. *Journal of Chemical Technology & Biotechnology*, 90, 384 - 390.
- Frear, D. S. & Burrell, R. C. 1955. Spectrophotometric method for determining hydroxylamine reductase activity in higher plants. *Analytical Chemistry*, 27, 1664-1665.
- Fuchs, W., Binder, H., Mavrias, G. & Braun, R. 2003. Anaerobic treatment of wastewater with high organic content using a stirred tank reactor coupled with a membrane filtration unit. *Water Research*, 37, 902-908.
- Fuerst, J. A. 2005. Intracellular compartmentation in Planctomycetes. *Annual Review of Microbiology*, 59, 299-328.
- Fuerst, J. A. & Sagulenko, E. 2011. Beyond the bacterium: planctomycetes challenge our concepts of microbial structure and function. *Nature Reviews Microbiology*, 9, 403-413.
- Furukawa, K., Rouse, J. D., Yoshida, N. & Hatanaka, H. 2003. Mass cultivation of anaerobic ammonium-oxidizing sludge using a novel nonwoven biomass carrier. *Journal of Chemical Engineering of Japan*, 36, 1163–1169.
- Fux, C., Boehler, M., Huber, P., Brunner, I. & Siegrist, H. 2002. Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant. *Journal of Biotechnology*, 99, 295-306.
- Galí, A., Dosta, J., Van Loosdrecht, M. C. M. & Mata-Alvarez, J. 2007. Two ways to achieve an anammox influent from real reject water treatment at lab-scale: Partial SBR nitrification and SHARON process. *Process Biochemistry*, 42, 715-720.
- Ganigué, R., López, H., Rusalleda, M., Balaguer, M. D. & Colprim, J. 2008. Operational strategy for a partial nitritation–sequencing batch reactor treating urban landfill leachate to achieve a stable influent for an anammox reactor. *Journal of Chemical Technology & Biotechnology*, 83, 365-371.
- Ge, S., Peng, Y., Wang, S., Lu, C., Cao, X. & Zhu, Y. 2012. Nitrite accumulation under constant temperature in anoxic denitrification process: The effects of carbon sources and COD/NO<sub>3</sub>-N. *Bioresource Technology*, 114, 137 - 143.
- Gerardi, M. H. 2002. *Nitrification and denitrification in the activated sludge process*, New York, John Wiley and Sons, Inc.
- Gerardi, M. H. 2005. Nitrification in the activated sludge process. In: LEHR, J. H. & KEELEY, J. (eds.) *Water Encyclopedia*. New York: John Wiley & Sons, Inc.
- Gong, Z., Liu, S., Yang, F., Bao, H. & Furukawa, K. 2008. Characterization of functional microbial community in a membrane-aerated biofilm reactor operated for completely autotrophic nitrogen removal. *Bioresource Technology*, 99, 2749-2756.
- Gujer, W. & Zehnder, A. J. B. 1983. Conversion processes in anaerobic digestion. *Water Science & Technology*, 15, 127–167.
- Guo, J., Peng, Y., Wang, S., Zheng, Y., Huang, H. & Wang, Z. 2009. Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. *Bioresource Technology*, 100, 2796-2802.
- He, Y., Xu, P., Li, C. & Bo, Z. 2005. High-concentration food wastewater treatment by an anaerobic membrane bioreactor. *Water Research*, 39, 4110–4118.

- Hellinga, C., Schellen, A. a. J. C., Mulder, J. W., Van Loosdrecht, M. C. M. & Heijnen, J. J. 1998. The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science & Technology*, 37, 135-142.
- Henze, M. 1991. Capabilities of biological nitrogen removal processes from wastewater. *Water Science & Technology*, 23, 669-679.
- Henze, M. & Comeau, Y. 2008. Wastewater characterization. In: HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment: Principles Modelling and Design*. London, UK: IWA Publishing.
- Hu, A. H. 2004. *Submerged anaerobic membrane bioreactor (SAMBR) for wastewater treatment*. PhD and Diploma of Imperial College (DIC), Imperial College London.
- Hu, A. Y. & Stuckey, D. C. 2006. Treatment of dilute wastewaters using a novel submerged anaerobic membrane bioreactor. *Journal of Environmental Engineering*, 132, 190-198.
- Huang, T.-L., Macinnes, J. M. & Cliffe, K. R. 2001. Nitrogen removal from wastewater by a catalytic oxidation method. *Water Research*, 35, 2113-2120.
- Huang, Z., Ong, S. L. & Ng, H. Y. 2008. Feasibility of submerged anaerobic membrane bioreactor (SAMBR) for treatment of low-strength wastewater. *Water Science & Technology*, 58, 1925-1931.
- Huang, Z., Ong, S. L. & Ng, H. Y. 2010. Submerged anaerobic membrane bioreactor for low-strength wastewater treatment: Effect of HRT and SRT on treatment performance and membrane fouling. *Water Research*, 45, 705-713.
- Imajo, U., Tokutomi, T. & Furukawa, K. 2004. Granulation of Anammox microorganisms in up-flow reactors. *Water Science & Technology*, 49, 155-163.
- Isaka, K., Date, Y., Sumino, T., Yoshie, S. & Tsuneda, S. 2005. Growth characteristic of anaerobic ammonium-oxidizing bacteria in an anaerobic biological filtrated reactor. *Applied Microbiology and Biotechnology*, 70, 47-52.
- Izadpanah, A. A. & Javidnia, A. 2012. The ability of a nanofiltration membrane to remove hardness and ions from diluted seawater. *Water*, 4, 283 - 294.
- Janga, N., Ren, X., Kim, G., Ahn, C., Cho, J. & Kim, I. S. 2007. Characteristics of soluble microbial products and extracellular polymeric substances in the membrane bioreactor for water reuse. *Desalination*, 202, 90 - 98.
- Jetten, M. M. S., Niftrik, L. V., Strous, M., Kartal, B., Keltjens, J. & Op Den Camp, H. J. M. 2009. Biochemistry and molecular biology of anammox bacteria. *Critical Reviews in Biochemistry and Molecular Biology*, 1-20.
- Jetten, M. S. M., Horn, S. J. & Van Loosdrecht, M. C. M. 1997. Towards a more sustainable municipal wastewater treatment system. *Water Science & Technology*, 35, 171-180.
- Jetten, M. S. M., Schmid, M., Schmidt, I., Wubben, M., Dongen, U. V., Abma, W., Sliemers, O., Revsbech, N. P., Beaumont, H. J. E., Ottosen, L., Volcke, E., Laanbroek, H. J., Campos-Gomez, J. L., Cole, J., Loosdrecht, M. V., Mulder, J. W., Fuerst, J., Richardson, D., Pas, K. V. D., Mendez-Pampin, R., Third, K., Cirpus, I., Spanning, R. V., Bollmann, A., Nielsen, L. P., Camp, H. O. D., Schultz, C., Gundersen, J., Vanrolleghem, P., Strous, M., Wagner, M. & Kuenen, J. G. 2002. Improved nitrogen removal by application of new nitrogen-cycle bacteria. *Reviews in Environmental Science and Biotechnology*, 1, 51-63.
- Jetten, M. S. M., Strous, M., Van De Pas-Schoonen, K. T., Schalk, J., Van Dongen, U. G. J. M., Van De Graaf, A. A., Logemann, S., Muyzer, G., Van Loosdrecht, M. C. M. & Kuenen, J. G. 1999. The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews*, 22, 421-437.
- Jetten, M. S. M., Wagner, M., Fuerst, J., Van Loosdrecht, M., Kuenen, J. G. & Strous, M. 2001. Microbiology and application of the anaerobic ammonium oxidation ('anammox') process. *Current Opinion in Biotechnology*, 12, 283-288.
- Jianga, T., Myngheer, S., De Pauw, D. J. W., Spanjers, H., Nopens, I., Kennedy, M. D., Amy, G. & Vanrolleghem, P. A. 2008. Modelling the production and degradation of soluble microbial products (SMP) in membrane bioreactors (MBR). *Water Research*, 42, 4955-4964.

- Jianlong, W. & Ning, Y. 2004. Partial nitrification under limited dissolved oxygen conditions. *Process Biochemistry*, 39, 1223-1229.
- Jin, R. C., Hu, B. L., Zheng, P., Qaisar, M., Hu, A. H. & Islam, E. 2008. Quantitative comparison of stability of ANAMMOX process in different reactor configurations. *Bioresource Technology*, 99, 1603-9.
- Judd, S. 2002. Submerged membrane bioreactors: Flat plate or hollow fibre? *Filtration & Separation*, 39, 30-31.
- Judd, S. 2006. *The MBR Book: Principles and applications of MBRs in water and wastewater treatment*, New York, Elsevier.
- Judd, S. 2008. The status of membrane bioreactor technology. *Trends in biotechnology*, 26, 109-16.
- Kabdasli, I., Öztürk, İ., Tünay, O., Yilmaz, S. & Arıkan, O. 2000. Ammonia removal from young landfill leachate by magnesium ammonium phosphate precipitation and air stripping. *Water Science & Technology*, 41, 237-240.
- Kadlec, R. H. & Knight, R. L. 1996. *Treatment Wetlands*, La Florida, Lewis Publishers, Boca Raton.
- Khin, T. & Annachatre, A. P. 2004. Novel microbial nitrogen removal processes. *Biotechnology Advances*, 22, 519-32.
- Kim, D. & Seo, D. 2006. Selective enrichment and granulation of ammonia oxidizers in a sequencing batch airlift reactor. *Process Biochemistry*, 41, 1055-1062.
- Kim, J.-S., Lee, C.-H. & Chang, I.-S. 2001. Effect of pump shear on the performance of a crossflow membrane bioreactor. *Water Research*, 35, 2137-2144
- Kim, Y. J., Rene, E. R. & Park, H. S. 2007. Effect of organic and metal ion concentration on the simultaneous biological removal of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> under anaerobic condition. *Bioresource Technology*, 98, 892-6.
- Knowles, R. 1982. Denitrification. *Microbiology and Molecular Biology Reviews*, 46, 43-70.
- Kowalchuk, G. A. & Stephen, J. R. 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annual Review of Microbiology*, 55, 485-529
- Kuenen, J. G. 2008. Anammox bacteria: from discovery to application. *Nature Reviews Microbiology*, 6, 320-326.
- Kurama, H., Poetzschke, J. & Haseneder, R. 2002. The application of membrane filtration for the removal of ammonium ions from potable water. *Water Research*, 36, 2905-2909.
- Lackner, S., Gilbert, E. M., Vlaeminck, S. E., Joss, A., Horn, H. & Van Loosdrecht, M. C. M. 2014. Full-scale partial nitrification/anammox experiences: An application survey. *Water Research*, 55, 292 - 303.
- Lan, C.-J., Kumar, M., Wang, C.-C. & Lin, J.-G. 2011. Development of simultaneous partial nitrification, anammox and denitrification (SNAD) process in a sequential batch reactor. *Bioresource Technology*, 102, 5514-5519.
- Le-Clech, P. 2010. Membrane bioreactors and their uses in wastewater treatments. *Applied Microbiology and Biotechnology*, 88, 1253-60.
- Le-Clech, P., Alvarez-Vazquez, H., Jefferson, B. & Judd, S. 2003. Fluid hydrodynamics in submerged and sidestream membrane bioreactors. *Water Science & Technology*, 48, 113-119.
- Le-Clech, P., Chen, V. & Fane, T. a. G. 2006. Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science*, 284, 17-53.
- Le-Clech, P., Jefferson, B. & Judd, S. J. 2005. A comparison of submerged and sidestream tubular membrane bioreactor configurations. *Desalination*, 173, 113-122
- Liesack, W., König, H., Schlesner, H. & Hirsch, P. 1986. Chemical composition of the peptidoglycan-free cell envelopes of budding bacteria of the *Pirella/Planctomyces* group. *Archives of Microbiology*, 145, 361-366.
- Lieu, P. K., Homan, H., Kurogl, A., Kawagoshi, Y., Fujii, T. & Furukawa, K. 2006. Characterization of sludge from single-stage nitrogen removal using anammox and partial nitrification (SNAP). *Japanese Journal of Water Treatment Biology*, 42, 53-64.

- Lin, S. W., Sicairos, S. P. & Navarro, R. M. F. 2007. Preparation, characterization and salt rejection of negatively charged polyamide nanofiltration membranes. *Journal of the Mexican Chemical Society*, 51, 129 - 135.
- Lindsay, M., Webb, R., Strous, M., Jetten, M., Butler, M., Forde, R. & Fuerst, J. 2001. Cell compartmentalisation in planctomycetes: novel types of structural organisation for the bacterial cell. *Archives of Microbiology*, 175, 413-429.
- Lu, G., Zheng, P., Jin, R.-C. & Qaisar, M. 2006. Control strategy of shortcut nitrification. *Journal of Environmental Sciences*, 18, 58-61.
- Mccarty, P. L., Beck, L. & Amant, P. S. Year. Biological denitrification of wastewaters by addition of organic materials. *In: 24th Industrial Waste Conference*, 1969. West Lafayette, IN, USA. 1271-1285.
- Mosquera-Corral, A., De Kreuk, M. K., Heijnen, J. J. & Van Loosdrecht, M. C. M. 2005a. Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor. *Water Research*, 39, 2676-2686.
- Mosquera-Corral, A., González, F., Campos, J. L. & Méndez, R. 2005b. Partial nitrification in a SHARON reactor in the presence of salts and organic carbon compounds. *Process Biochemistry*, 40, 3109-3118.
- Mulder, A., Van De Graaf, A. A., Robertson, L. A. & Kuenen, J. G. 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiology Ecology*, 16, 177-183.
- Mulder, J. W., Van Loosdrecht, M. C. M., Hellinga, C. & Van Kempen, R. 2001. Full-scale application of the SHARON process for treatment of rejection water of digested sludge dewatering. *Water Science & Technology*, 43, 127 - 134.
- Mulder, M. 1996. *Basic principles of membrane technology*, New Delhi, Kluwer Academic Publishers.
- Ng, H. Y., Tan, T. W. & Ong, S. L. 2006. Membrane fouling of submerged membrane bioreactors-impact of mean cell residence time and the contributing factors. *Environmental Science & Technology*, 40, 2706-2713.
- Ni, S.-Q. & Zhang, J. 2013. Anaerobic ammonium oxidation: From laboratory to full-scale application. *BioMed Research International*, 2013, 1 - 10.
- Niea 2011. European and national drinking water quality standards. Belfast: Department for Regional Development, Northern Ireland Environment Agency.
- Okabe, S., Oozawa, Y., Hirata, K. & Watanabe, Y. 1996. Relationship between population dynamics of nitrifiers in biofilms and reactor performance at various C:N ratios. *Water Research*, 30, 1563-1572.
- Op Den Camp, H. J. M., Kartal, B., Guven, D., Van Niftrik, L. a. M. P., Haaijer, S. C. M., Van Der Star, W. R. L., Van De Pas-Schoonen, K. T., A. Cabezas, A., Z. Ying, Z., Schmid, M. C., Kuypers, M. M. M., Van De Vossenberg, J., Harhangi, H. R., Picioreanu, C., Van Loosdrecht, M. C. M., Kuenen, J. G., Strous, M. & Jetten, M. S. M. 2006. Global impact and application of the anaerobic ammonium-oxidizing (anammox) bacteria. *Biochemical Society Transactions*, 34, 174-178.
- Ozdemir, B., Mertoglu, B., Yapsakli, K., Aliyazicioglu, C., Saatci, A. & Yenigun, O. 2011. Investigation of nitrogen converters in membrane bioreactor. *Journal of Environmental Science and Health. Part A, Toxic/hazardous substances & Environmental Engineering*, 46, 500-8.
- Paredes, D., Kuschik, P., Mbwette, T. S. A., Stange, F., Müller, R. A. & Köser, H. 2007. New aspects of microbial nitrogen transformations in the context of wastewater treatment – A review. *Engineering in Life Sciences*, 7, 13 - 25.
- Pearce, G. 2008. Introduction to membranes: An introduction to membrane bioreactors. *Filtration & Separation*, 45, 32-35.
- Pearce, P. 2013. *RE: Mogden nitrifying sludge*.

- Philips, S., Laanbroek, H. J. & Verstraete, W. 2002. Origin, causes and effects of increased nitrite concentrations in aquatic environment. *Reviews in Environmental Science & Biotechnology*, 1, 115–141.
- Picioreanu, C., Van Loosdrecht, M. C. M. & Heijnen, J. J. 1997. Modelling the effect of oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. *Water Science & Technology*, 36, 147–56.
- Pressley, T. A., Bishop, D. F. & Roan, S. G. 1972. Ammonia-nitrogen removal by breakpoint chlorination. *Environmental Science & Technology*, 6, 622–628.
- Reij, M. W., Keurentjes, J. T. F. & Hartmans, S. 1998. Membrane bioreactors for waste gas treatment. *Journal of Biotechnology*, 59, 155 - 167.
- Rostron, W. M., Stuckey, D. C. & Young, A. A. 2001. Nitrification of high strength ammonia wastewaters: Comparative study of immobilisation media. *Water Research*, 35, 1169-1178.
- Ruiz, G., Jeison, D. & Chamy, R. 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Research*, 37, 1371-1377.
- Russo, R. 1985. Ammonia, nitrite and nitrate. In: RAND, G. & PETROCELLI, S. (eds.) *Fundamentals of aquatic toxicology: Methods and applications*. Washington DC: Hemisphere Publishing Corporation.
- Sakai, K., Ikehata, Y., Ikenaga, Y., Wakayama, M. & Moriguchi, M. 1996. Nitrite oxidation by heterotrophic bacteria under various nutritional and aerobic conditions. *Journal of Fermentation and Bioengineering*, 82, 613-617.
- Santos, A., Ma, W. & Judd, S. J. 2011. Membrane bioreactors: Two decades of research and implementation. *Desalination*, 273, 148-154.
- Satoh, H., Ono, H., Rulin, B., Kamo, J., Okabe, S. & Fukushi, K. 2004. Macroscale and microscale analyses of nitrification and denitrification in biofilms attached on membrane aerated biofilm reactors. *Water Research*, 38, 1633 - 1641.
- Schmid, M., Schmitz-Esser, S., Jetten, M. & Wagner, M. 2001. 16S-23S rDNA intergenic spacer and 23S rDNA of anaerobic ammonium-oxidizing bacteria: implications for phylogeny and in situ detection. *Environmental Microbiology*, 3, 450-459.
- Schmid, M. C., Maas, B., Dapena, A., Van De Pas-Schoonen, K., Van De Vossenberg, J., Kartal, B., Van Niftrik, L., Schmidt, I., Cirpus, I., Kuenen, J. G., Wagner, M., Sinnighe Damste, J. S., Kuypers, M., Revsbech, N. P., Mendez, R., Jetten, M. S. M. & Strous, M. 2005. Biomarkers for in situ detection of anaerobic ammonium-oxidizing (Anammox) bacteria. *Applied and Environmental Microbiology*, 71, 1677-1684.
- Schmidt, I. & Bock, E. 1997. Anaerobic ammonia oxidation with nitrogen dioxide by *Nitrosomonas eutropha*. *Archives of Microbiology*, 167, 106-111.
- Schmidt, I., Sliemers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J. G., Jetten, M. S. M. & Strous, M. 2003. New concepts of microbial treatment processes for the nitrogen removal in wastewater. *FEMS Microbiology Reviews*, 27, 481-492.
- Semmens, M. J., Dahm, K., Shanahan, J. & Christianson, A. 2003. COD and nitrogen removal by biofilms growing on gas permeable membranes. *Water Research*, 37, 4343–4350.
- Seyoum, B., Mukhtar, H. & Lau, K. K. 2012. Removal of diethanolamine (DEA) from wastewater using membrane separation process. In: ISMAIL, A. F. & MATSUURA, T. (eds.) *Sustainable Membrane Technology for Energy, Water, and Environment*. New Jersey: John Wiley & Sons, Inc.
- Shaw, B. D., Wei, J. B., Tuli, A., Campbell, J., Parikh, S. J., Dabach, S., Buelow, M. & Hopmans, J. W. 2014. Analysis of ion and dissolved organic carbon interference on soil solution nitrate concentration measurements using ultraviolet absorption spectroscopy. *Vadose Zone Journal*, 13, 1 - 9.
- Sheng, G.-P., Yu, H.-Q. & Li, X.-Y. 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnology Advances*, 28, 882–894.

- Shrestha, N. K., Hadano, S., Kamachi, T. & Okura, I. 2001. Conversion of ammonia to dinitrogen in wastewater by *Nitrosomonas europaea*. *Applied Biochemistry and Biotechnology*, 90, 221-232.
- Skelton, H. 2012. RE: AW anaerobic digestion sludge.
- Skiba, U. 2008. Denitrification. In: JORGENSEN, S. E. & FATH, B. (eds.) *Encyclopedia of Ecology*.
- Sliemers, A. O., Derwort, N., Campos Gomez, J. L., Strous, M., Kuenen, J. G. & Jetten, M. S. M. 2002. Completely autotrophic nitrogen removal over nitrite in one single reactor. *Water Research*, 36, 2475–2482.
- Sliemers, A. O., Third, K. A., Abma, W., Kuenen, J. G. & Jetten, M. S. M. 2003. CANON and Anammox in a gas-lift reactor. *FEMS Microbiology Letters*, 218, 339-344.
- Stephenson, T., Judd, S., Jefferson, B. & Brindle, K. 2000. *Membrane bioreactors for wastewater treatment*, London, IWA Publishing.
- Stevens, W. E., Drysdale, G. D. & Bux, F. 2002. Evaluation of nitrification by heterotrophic bacteria in biological nutrient removal processes. *South African Journal of Science*, 98, 222-224.
- Strous, M., Fuerst, J. A., Kramer, E. H. M., Logemann, S., Muyzer, G., Van De Pas-Schoonen, K. T., Webb, R., Kuenen, J. G. & Jetten, M. S. M. 1999a. Missing lithotroph identified as new planctomycete. *Nature*, 400, 446-449.
- Strous, M., Heijnen, J. J., Kuenen, J. G. & Jetten, M. S. M. 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology*, 50, 589-596.
- Strous, M., Kuenen, J. G. & Jetten, M. S. M. 1999b. Key physiology of anaerobic ammonium oxidation. *Applied and Environmental Microbiology*, 65, 3248-3250.
- Strous, M., Van Gerven, E., Kuenen, J. G. & Jetten, M. 1997a. Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge. *Applied Environment and Microbiology*, 63, 2446–2448.
- Strous, M., Van Gerven, E., Zheng, P., Kuenen, J. G. & Jetten, M. S. M. 1997b. Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. *Water Research*, 31, 1955-1962.
- Stuckey, D. C. 2012. Recent developments in anaerobic membrane reactors. *Bioresource Technology*, 122, 137-48.
- Suneethi, S. & Joseph, K. 2011a. ANAMMOX process start up and stabilization with an anaerobic seed in Anaerobic Membrane Bioreactor (AnMBR). *Bioresource Technology*, 102, 8860-7.
- Suneethi, S. & Joseph, K. 2011b. Batch culture enrichment of ANAMMOX populations from anaerobic and aerobic seed cultures. *Bioresource Technology*, 102, 585-91.
- Szoke, S., Patzay, G. & Weiser, L. 2003. Characteristics of thin-film nanofiltration membranes at various pH-values. *Desalination*, 151, 123 - 129.
- Thamdrup, B. & Dalsgaard, T. 2002. Production of N<sub>2</sub> through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied and Environmental Microbiology*, 68, 1312-1318.
- Third, K. A., Paxman, J., Schmid, M., Strous, M., Jetten, M. S. M. & Cord-Ruwisch, R. 2005. Treatment of nitrogen-rich wastewater using partial nitrification and Anammox in the CANON process. *Water Science & Technology*, 52, 47–54.
- Trigo, C., Campos, J. L., Garrido, J. M. & Mendez, R. 2006. Start-up of the Anammox process in a membrane bioreactor. *Journal of Biotechnology*, 126, 475-87.
- Un-Wwap. 2003. The United Nations World Water Assessment Programme. *The world water development report 1: Water for people, water for life*. Paris: UNESCO.
- Un-Wwdr. 2015. The United Nations World Water Development Report. *Water for a sustainable world*. Paris: UNESCO.
- Vallero, M. V. G., Lettinga, G. & Lens, P. N. L. 2005. High rate sulfate reduction in a submerged anaerobic membrane bioreactor (SAMBaR) at high salinity. *Journal of Membrane Science*, 253, 217-232.



- Van De Graaf, A. A., De Bruijn, P., Robertson, L. A., Jetten, M. S. M. & Kuenen, J. G. 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology*, 142, 2187-2196.
- Van De Graaf, A. A., De Bruijn, P., Robertson, L. A., Jetten, M. S. M. & Kuenen, J. G. 1997. Metabolic pathway of anaerobic ammonium oxidation on the basis of <sup>15</sup>N studies in a fluidized bed reactor. *Microbiology*, 143, 2415-2421.
- Van De Graaf, A. A., Mulder, A., De Bruijn, P., Jetten, M. S. M., Robertson, L. A. & Kuenen, J. G. 1995. Anaerobic oxidation of ammonium is a biologically mediated process. *Applied and Environmental Microbiology*, 61, 1246–1251.
- Van Der Aa, L. T. J., Kors, L. J., Wind, A. P. M., Hofman, J. a. M. H. & L.C, R. 2002. Nitrification in rapid sand filter: phosphate limitation at low temperatures. *Water Supply* 2, 37–46.
- Van Der Star, W. R., Miclea, A. I., Van Dongen, U. G., Muyzer, G., Picioreanu, C. & Van Loosdrecht, M. C. 2008. The membrane bioreactor: A novel tool to grow anammox bacteria as free cells. *Biotechnology and Bioengineering*, 101, 286-94.
- Van Der Star, W. R. L., Abma, W. R., Blommers, D., Mulder, J.-W., Tokutomi, T., Marc Strous, Picioreanu, C. & Van Loosdrecht, M. C. M. 2007. Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research*, 41, 4149 – 4163.
- Van Dongen, L. G. J. M., Jetten, M. S. M. & Van Loosdrecht, M. C. M. 2001a. The combined Sharon/Anammox process: A sustainable method for N-removal from sludge water. *Water and Wastewater Practitioner Series: STOWA Report*. London.
- Van Dongen, U., Jetten, M. S. M. & Van Loosdrecht, M. C. M. 2001b. The SHARON®-Anammox® process for treatment of ammonium rich wastewater. *Water Science & Technology*, 44, 153-160.
- Van Kempen, R., Mulder, J. W., Uijterlinde, C. A. & Loosdrecht, M. C. M. 2001. Overview: full scale experience of the SHARON® process for treatment of rejection water of digested sludge dewatering. *Water Science & Technology*, 44, 145-152.
- Van Loosdrecht, M. C. M. & Jetten, M. S. M. 1998. Microbiological conversions in nitrogen removal. *Water Science & Technology*, 38, 1-7.
- Van Niftrik, L., Geerts, W. J. C., Van Donselaar, E. G., Humbel, B. M., Yakushevskaya, A., Verkleij, A. J., Jetten, M. S. M. & Strous, M. 2008. Combined structural and chemical analysis of the anammoxosome: A membrane-bounded intracytoplasmic compartment in anammox bacteria. *Journal of Structural Biology*, 161, 401-410.
- Verrecht, B., Maere, T., Nopens, I., Brepols, C. & Judd, S. 2010. The cost of a large-scale hollow fibre MBR. *Water Research*, 44, 5274 - 5283.
- Visvanathan, C., Aim, R. B. & Parameshwaran, K. 2000. Membrane separation bioreactors for wastewater treatment. *Critical Reviews in Environmental Science and Technology*, 30, 1-48.
- Vyrides, I. 2009. *Treatment of saline organic wastewater by a submerged anaerobic membrane bioreactor (SAMBR)*. PhD and Diploma of Imperial College (DIC), Imperial College London.
- Vyrides, I. & Stuckey, D. C. 2009. Saline sewage treatment using a submerged anaerobic membrane reactor (SAMBR): Effects of activated carbon addition and biogas-sparging time. *Water Research*, 43, 933–942.
- Wakerley, T. 2014. *RE: Process flow at Anglian Water*.
- Waki, M., Tokutomi, T., Yokoyama, H. & Tanaka, Y. 2007. Nitrogen removal from animal waste treatment water by anammox enrichment. *Bioresource Technology*, 98, 2775-80.
- Wang, R. 11 July 2012 Personal communication. *RE: Membrane characteristics*.
- Wang, T., Zhang, H., Gao, D., Yang, F., Yang, S., Jiang, T. & Zhang, G. 2011. Enrichment of Anammox bacteria in seed sludges from different wastewater treating processes and start-up of Anammox process. *Desalination*, 271, 193-198.

- Wang, T., Zhang, H., Gao, D., Yang, F. & Zhang, G. 2012. Comparison between MBR and SBR on Anammox start-up process from the conventional activated sludge. *Bioresource Technology*, 122, 78-82.
- Wang, T., Zhang, H., Yang, F., Liu, S., Fu, Z. & Chen, H. 2009. Start-up of the Anammox process from the conventional activated sludge in a membrane bioreactor. *Bioresource Technology*, 100, 2501-6.
- Ward, B. B. 2008. Nitrification. In: JORGENSEN, S. E. & FATH, B. (eds.) *Encyclopedia of Ecology*.
- Watt, G. W. & Crisp, J. D. 1952. A spectrophotometric method for the determination of hydrazine. *Analytical Chemistry*, 24, 2006-2008.
- Wiesmann, U. 1994. Biological nitrogen removal from wastewater. In: FIECHTER, A. (ed.) *Advances in Biochemical Engineering*. Berlin: Springer-Verlag.
- Windey, K., De Bo, I. & Verstraete, W. 2005. Oxygen-limited autotrophic nitrification-denitrification (OLAND) in a rotating biological contactor treating high-salinity wastewater. *Water Research*, 39, 4512-4520.
- Wu, L., Peng, C., Peng, Y., Li, L., Wang, S. & Ma, Y. 2012. Effect of wastewater COD/N ratio on aerobic nitrifying sludge granulation and microbial population shift. *Journal of Environmental Science (China)*, 24, 234 - 241.
- Wyffels, S., Boeckx, P., Pynaert, K., Zhang, D., Van Cleemput, O., Chen, G. & Verstraete, W. 2004. Nitrogen removal from sludge reject water by a two-stage oxygen-limited autotrophic nitrification denitrification. *Water Science & Technology*, 49, 57-64.
- Xiao, Y., Zeng, G. M., Yang, Z. H., Liu, Y., Ma, Y. H., Yang, L., Wang, R. J. & Xu Zh, Y. 2009. Coexistence of nitrifiers, denitrifiers and Anammox bacteria in a sequencing batch biofilm reactor as revealed by PCR-DGGE. *Journal of Applied Microbiology*, 106, 496-505.
- Yamamoto, T., Takaki, K., Koyama, T. & Furukawa, K. 2006. Novel partial nitrification treatment for anaerobic digestion liquor of swine wastewater using swim-bed technology. *Journal of Bioscience and Bioengineering*, 102, 497-503.
- Yang, W., Cicek, N. & Ilg, J. 2006. State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America. *Journal of Membrane Science*, 270, 201-211.
- Zehnder, A. J. B. 1988. *Biology of anaerobic microorganisms*, New York, John Wiley and Sons Inc.
- Zhang, L., Zheng, P., Tang, C. J. & Jin, R. C. 2008. Anaerobic ammonium oxidation for treatment of ammonium-rich wastewaters. *Journal of Zhejiang University Science B*, 9, 416-26.

# APPENDICES

## Appendix 1 Details of SAMBR Design

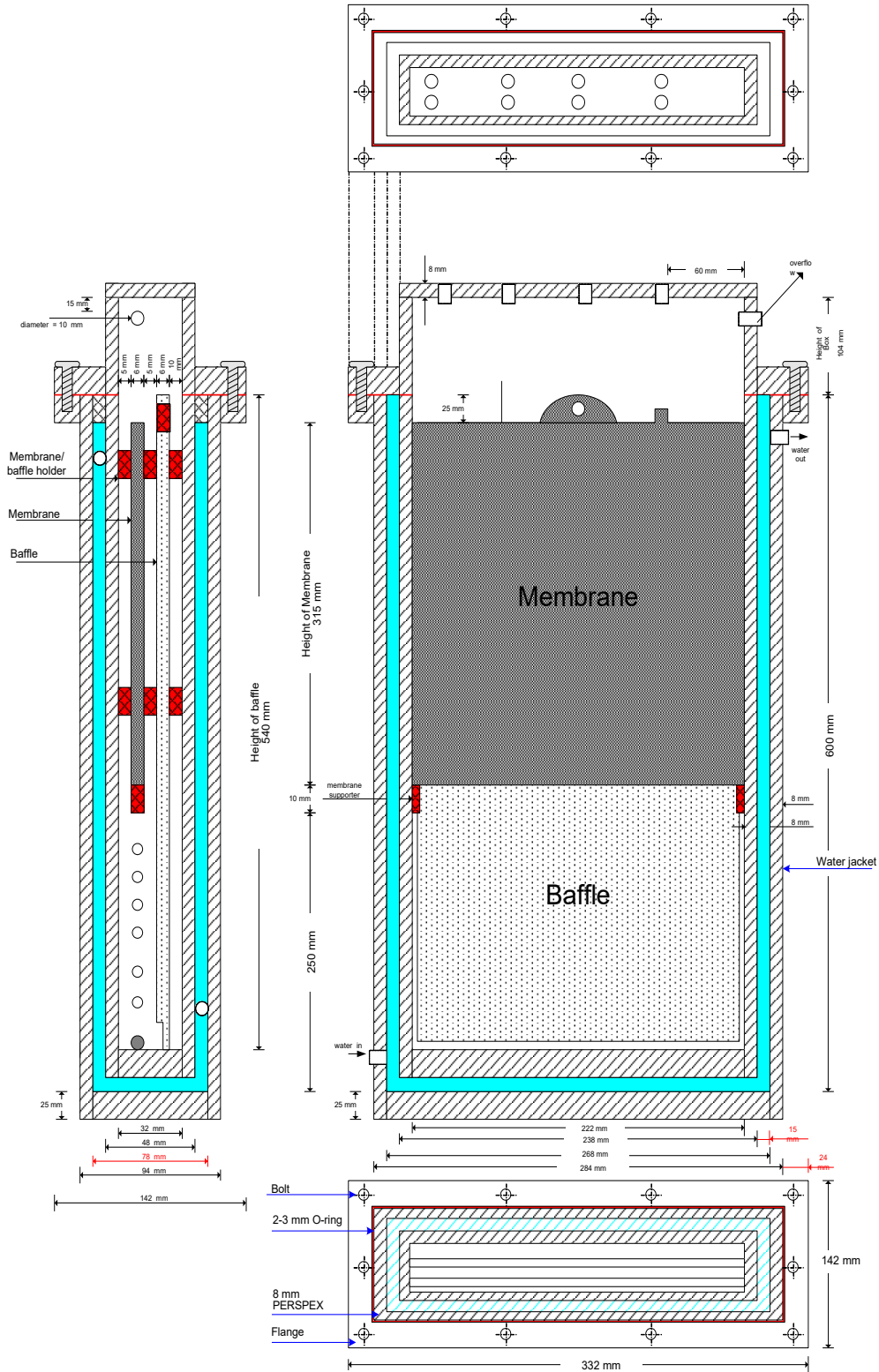


Fig. A-1 Details of SAMBR design (Hu, 2004)

## Appendix 2 Calibration Curves

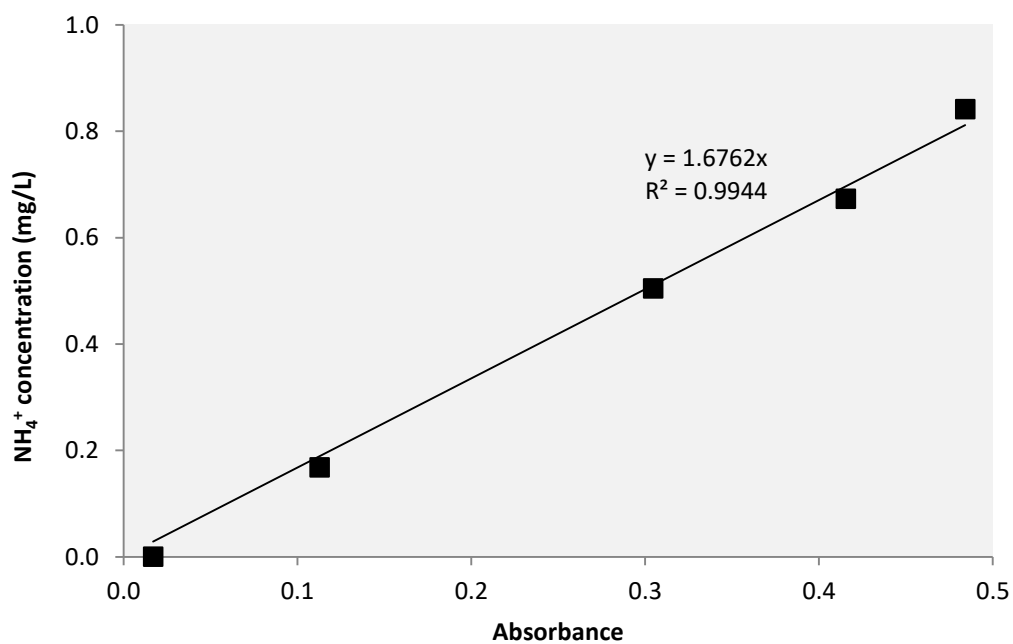


Fig. A-2 Calibration curve for  $\text{NH}_4^+$  using  $\text{NH}_4\text{Cl}$  as standard

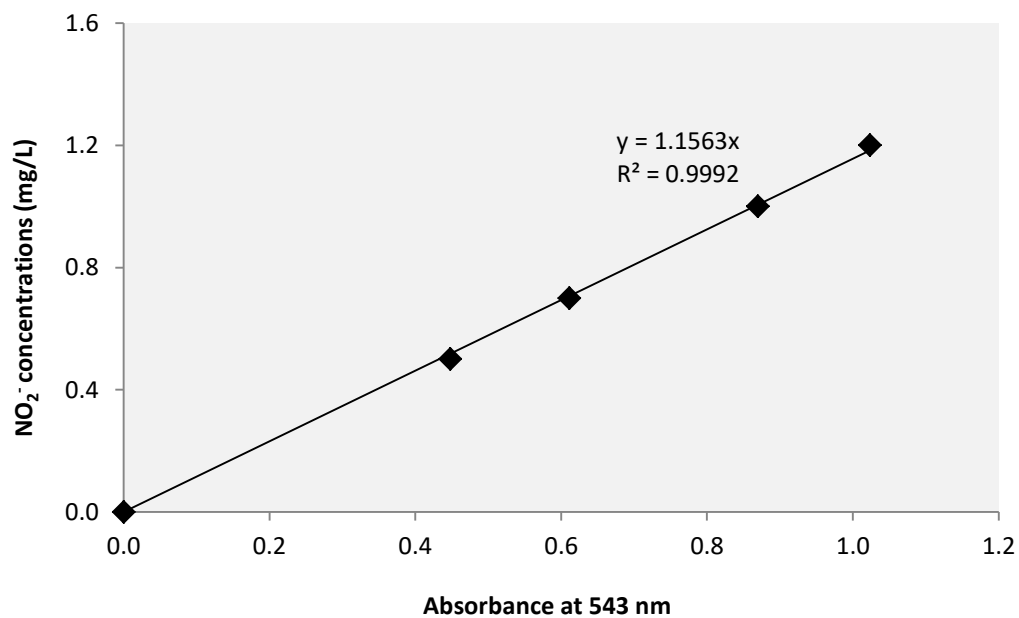
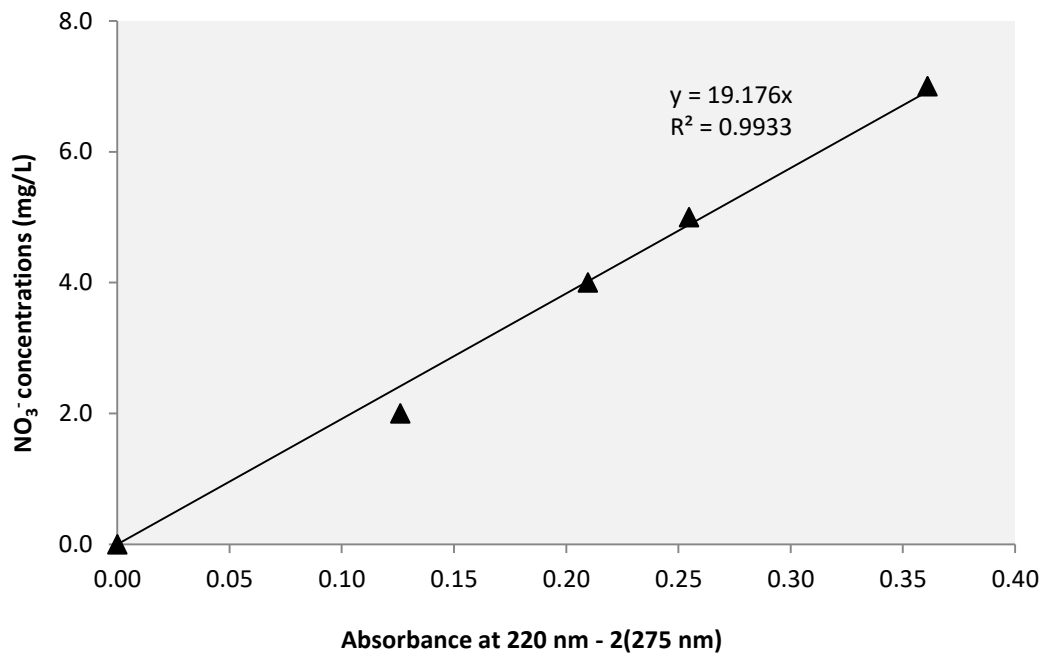
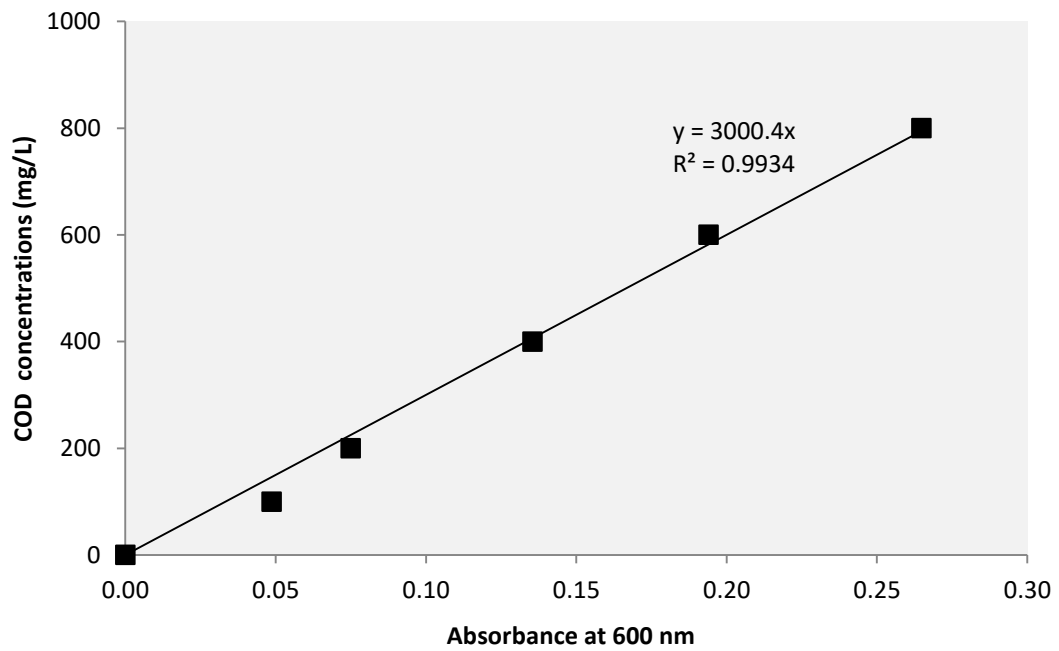


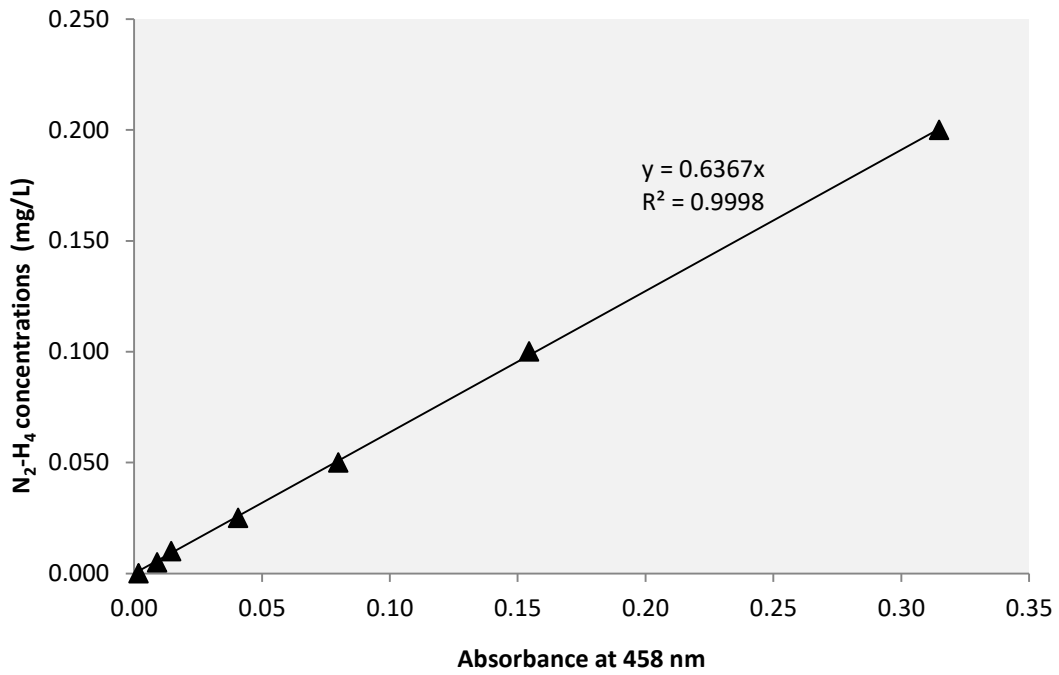
Fig. A-3 Calibration curve for  $\text{NO}_2^-$  using  $\text{NaNO}_2$  as standard



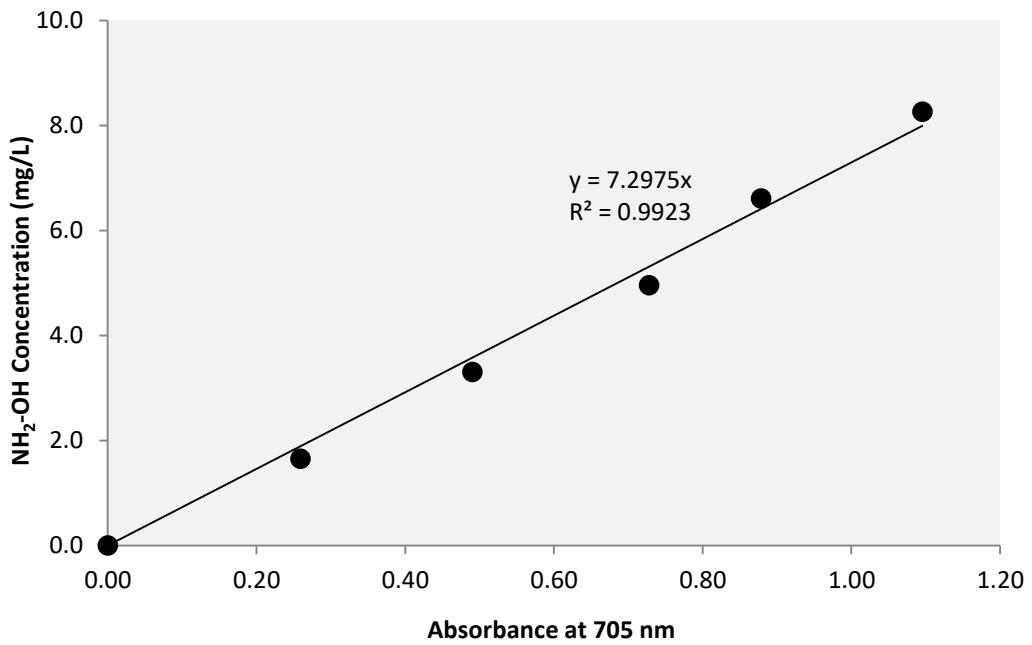
**Fig. A-4** Calibration curve for NO<sub>3</sub><sup>-</sup> using NaNO<sub>3</sub> as standard



**Fig. A-5** Calibration curve for chemical oxygen demand (COD), using KHP as standard

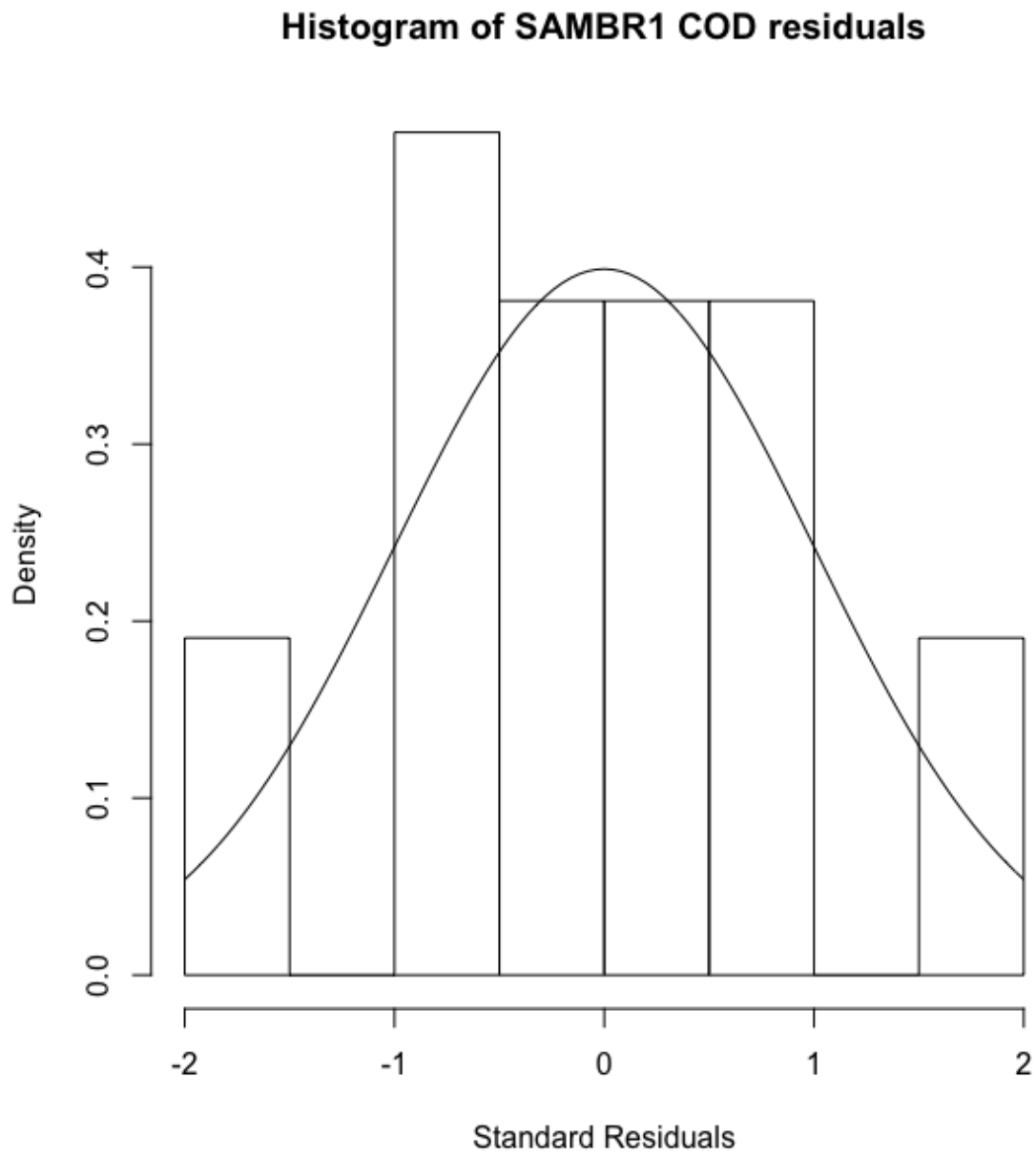


**Fig. A-6** Calibration curve for hydrazine (N<sub>2</sub>H<sub>4</sub>), using N<sub>2</sub>H<sub>4</sub>.2HCl as standard

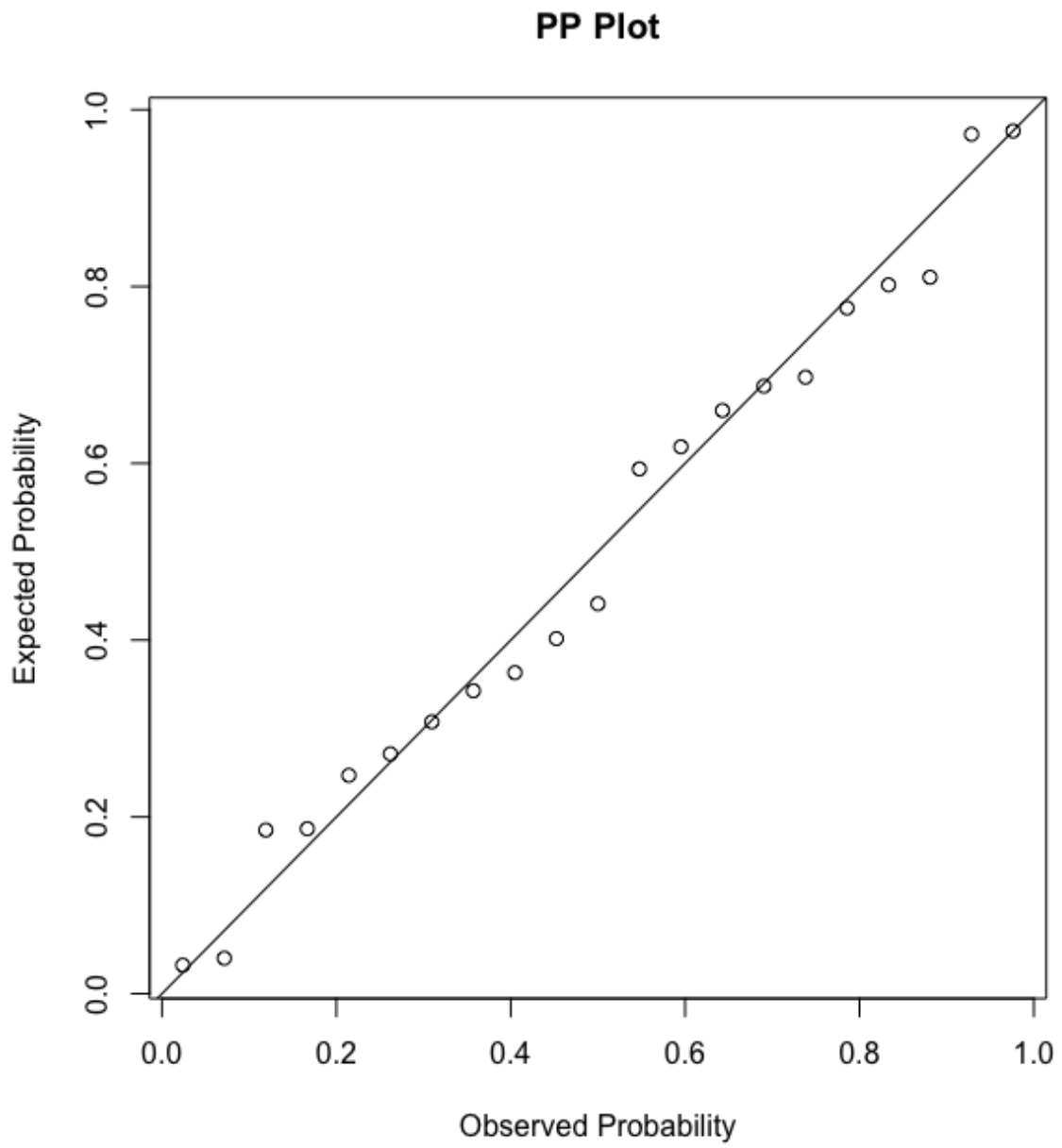


**Fig. A-7** Calibration curve for hydroxylamine (NH<sub>2</sub>-OH), using NH<sub>2</sub>OH-HCl as standard

### Appendix 3 Regression Analysis on COD

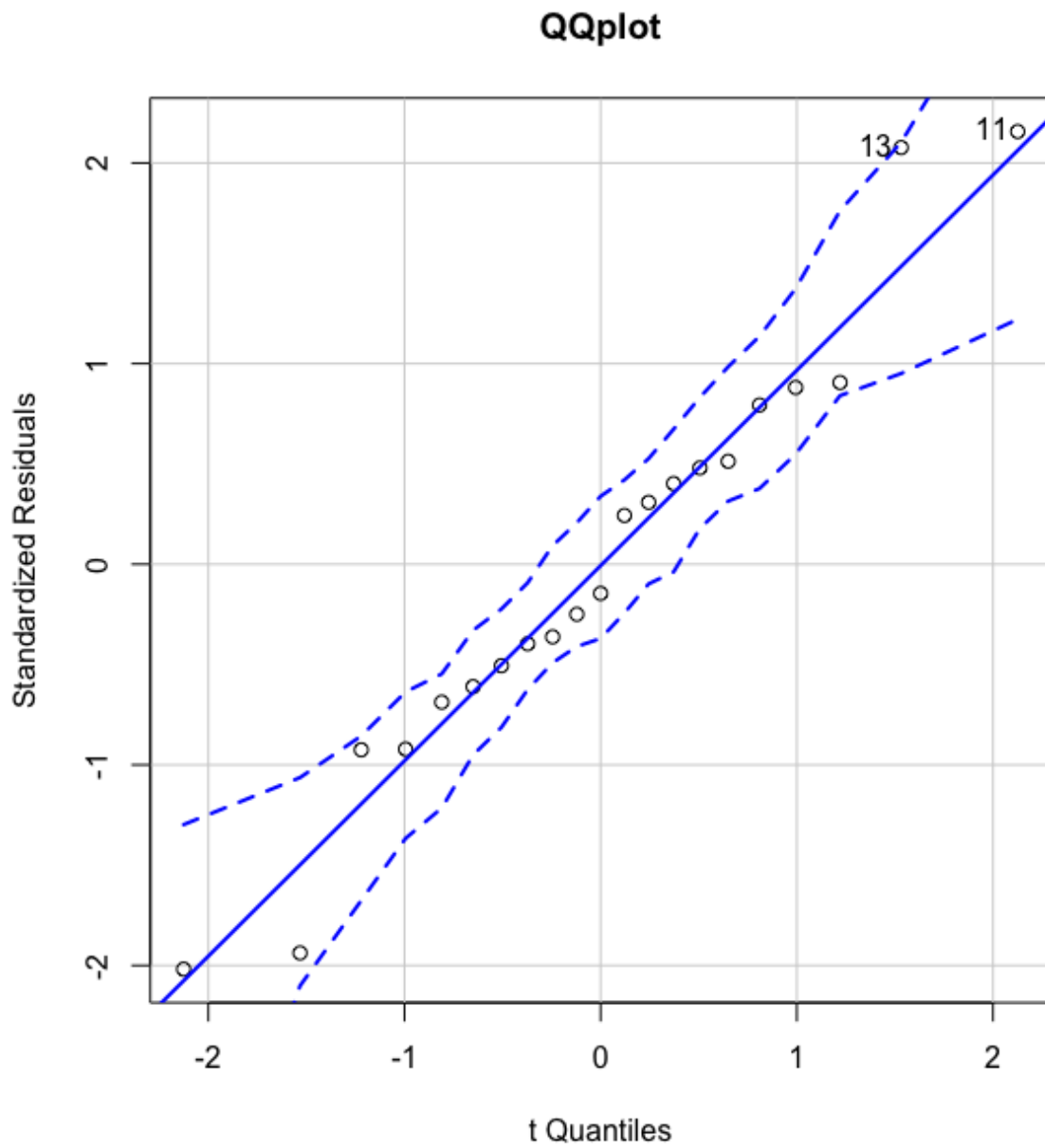


**Fig. A-8** Histogram of COD residuals in SAMBR 1

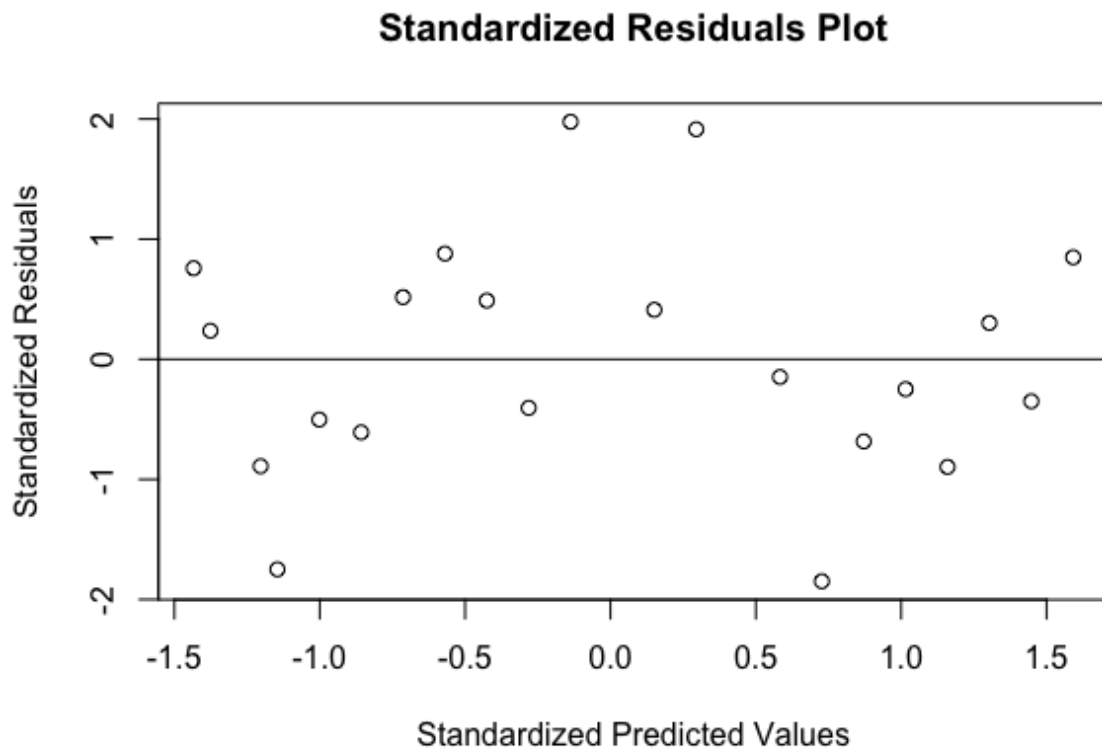


**Fig. A-9** COD PP Plot in SAMBR 1



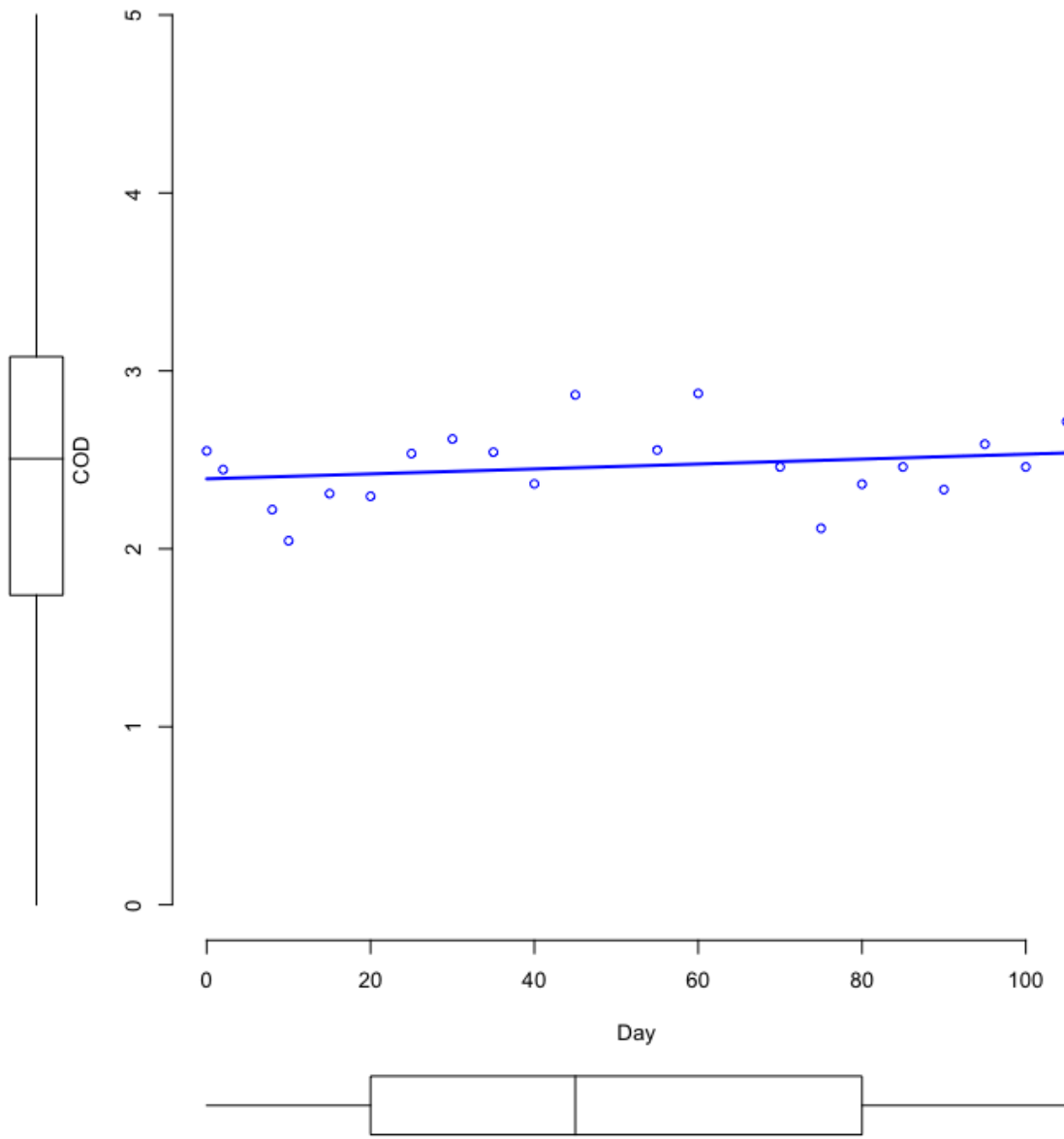


**Fig. A-10** COD PP Plot in SAMBR 1



**Fig. A-11** COD Standardized Residuals Plot in SAMBR 1

### SAMBR1 COD



**Fig. A-12** COD Distribution Plot in SAMBR 1