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MODELLING, SIMULATION AND OPTIMIZATION OF AUTOTROPHIC NITROGEN REMOVAL PROCESSES

MODELLEREN, SIMULEREN EN OPTIMALISEREN VAN AUTOTROFE STIKSTOFVERWIJDERINGSPROCESSEN

door

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Voor Annelies en Marion

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Contents

PART 1

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INTRODUCTION

Chapter 1

Introduction and problem statement

1 INTRODUCTION

Water is the most precious component on earth and more than 70 % of the blue planet's surface is covered by it, making a total volume of 1.36 10^9 km^3. Water is used as solvent for nutrients that are taken up by plants, animals and humans. It is used for basic hygiene, recreation and religious purposes. Electrical energy is created with the help of water in hydro-electric, thermic and nuclear power stations. However, only 0.6 % of all available water can be used for these purposes. Careful use, management and recycling of this water is therefore essential, especially in view of the ever growing world population.

This concern is already centuries old, but only the last 30-40 years attention has been given to it. In 1970, the United States of America (USA) established the Environmental Protection Agency (EPA). In the sixties and seventies several countries in Western Europe started issuing environmental laws. Further, in the seventies the European Union (EU) started issuing different regulations and directives with the aim of developing an environmental policy. These regulations are documents of general importance, binding and directly applicable in the whole European Union, while the directives are documents not directly applicable but needing an adequate, binding integration in every national body of laws. The most recent directive of the EU is the water framework directive (2000/60/EC) that takes an integrated approach to water management throughout the EU.

One of the elements of concern in wastewater is nitrogen, especially since the use of synthetic nitrogen fertiliser produced from atmospheric N_2 by the Haber-Bosch process has increased tenfold over the last 40 years. The human contribution to nitrogen pollution, for example in the form of urine, is ever increasing in view of the growing world population. Discharge of this nitrogen into the natural waters can lead to, amongst others, eutrophication, oxygen depletion and blue baby syndrome.

In most modern wastewater treatment plants (WWTP) nitrogen, which is generally in the form of ammonium or organic nitrogen, is removed by biological nitrification/denitrification. As a first step ammonium is converted to nitrate (nitrification). In a second step nitrate is converted to nitrogen gas (denitrification). Benefits of the process are the high potential removal efficiency,

high process stability and reliability, relatively easy process control, low area requirement and moderate cost (Metcalf and Eddy, 1991). A major stress factor on this nitrogen removal is reject water from sludge digesters, which is recycled back to the main WWTP. This reject water can represent up to 25 % of the total nitrogen load, but only 1 to 2 % of the volumetric load (Janus and van der Roest, 1997). Treating this return stream separately by nitrification/denitrification would become expensive and non sustainable as this treatment would require large oxygen consumption and the addition of a carbon source because of the high nitrogen concentrations (up to 2 $gN\,L^{-1}$) and the unfavourable carbon-to-nitrogen (C/N) ratio for denitrification (Pynaert, 2003).

The ANaerobic AMMonium OXidation (Anammox) process, which was discovered 10 years ago (Mulder, 1992) but already predicted to exist 30 years ago (Broda, 1977), could offer an alternative for the treatment of this return stream. Also, other streams with high nitrogen and low carbon content such as landfill leachates and evaporator condensates could be treated. In the Anammox process ammonium is oxidized under anoxic, i.e. oxygen depleted, conditions with nitrite as electron acceptor. Ammonium and nitrite are consumed on an almost equimolar basis. The Anammox process should always be combined with a partial nitritation process, such as the SHARON process (van Dongen *et al.*, 2001a&b), where half of the ammonium is oxidized to nitrite. Both autotrophic processes will increase the sustainability of wastewater treatment as the need for carbon addition (and concomitant increased sludge production) is omitted and oxygen consumption and the emission of nitrous oxide during oxidation of ammonia are largely reduced (Jetten *et al.*, 1997). As such, the combined process (partial nitritation and Anammox) was termed autotrophic nitrogen removal process.

The incorporation of such an autotrophic nitrogen removal process treating reject water from a sludge digester into the overall scheme of a waste water treatment plant is depicted in Figure 1.1.

Different research groups have studied the biochemistry, microbiology and physiology of the autotrophic nitrogen removal process. For this quite a number of lab and some pilot scale reactors were operated. Several questions still remain however towards the upgrade of the process to full scale, for example:

- How is the process operated optimally under different process conditions of influent concentration, pH and temperature?
- How long does it take to start-up such a system?
- What inoculum should be used for start-up?
- How necessary is control of the process?
- Should the process be engineered in 1 reactor, where both partial nitritation and Anammox are active, or in two reactors, thus separating nitritation and Anammox?

Some of these questions were tackled in this thesis. The core of the research has been the model-based analysis and simulation of the autotrophic nitrogen removal process, but considerable experimental work with lab-scale reactors has been performed as well.

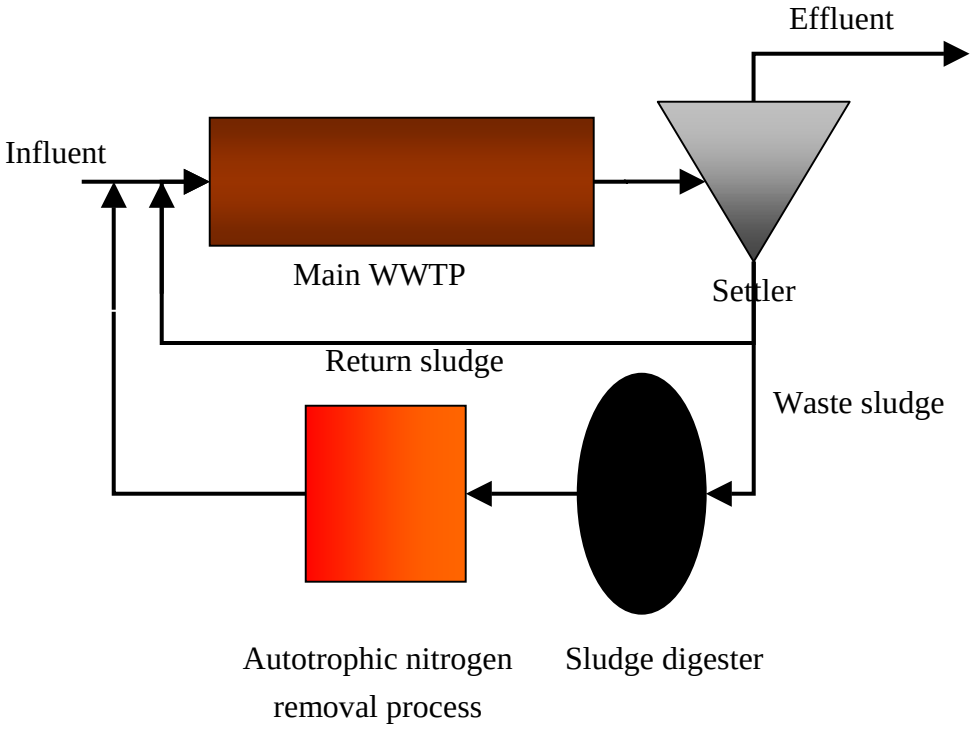

Figure 1.1. Autotrophic nitrogen removal process implemented in the return stream of a waste water treatment plant.

2 OUTLINE OF THE THESIS

The different chapters in this thesis can be grouped in six main parts. In the first part the outline of the thesis is introduced. The second part consists of a literature survey describing the different aspects and recent developments of autotrophic nitrogen removal. In a third part the model building, development and calibration process with the WEST® modelling and simulation software is discussed. The fourth part describes the results from lab-scale experiments with the process, with the focus on the first, partial nitritation, step. In the fifth part data from lab-scale reactors are compared with simulation results. Based on these results predictions on behaviour of the system are formulated. The performance of a one-reactor system is evaluated with the use of a model. Finally, in a sixth part the most important conclusions are drawn and perspectives towards further research are formulated.

PART 1: Introduction

In this part the outline of the thesis is introduced.

PART 2: Literature survey

Chapter 2 starts with the description of the origin and effects of nitrogen pollution. Further the different techniques for nitrogen elimination from wastewater are discussed. The rest of the chapter is focussing on the autotrophic nitrogen removal process. Different methods of obtaining an Anammox suited influent are given. The microbiology and physiology of Anammox are presented. Finally the practical implementation of the autotrophic nitrogen removal process is discussed

PART 3: Model building and development with WEST®

Chapter 3.1 presents the modelling and simulation environment WEST® (Vanhooren *et al.*, 2003) that was used throughout the thesis for model building, simulation, parameter estimation and sensitivity analysis. New developments such as the implementation of a stiff solver for calculations with the biofilm model are discussed.

Chapter 3.2 discusses the mathematical model for the autotrophic nitrogen removal process and competing processes, as well as the biofilm model developed for simulation in this thesis. Special emphasis is given to the fact that ammonia (NH_3) and nitrous acid (HNO_2) are considered as the actual substrates for nitrification. In this respect distinction is made throughout the thesis between the charged form of these components (ammonium (NH_4^+) and nitrite (NO_2^-)), the uncharged form (ammonia (NH_3) and nitrous acid (HNO_2)) and the sum of both (total ammonium nitrogen

(TAN) and total nitrite nitrogen (TNO_2)). The organisms responsible for the biological conversion are however still termed ammonium and nitrite oxidizers as generally done in literature. Further in this chapter the need for adequate calibration and validation of the model is stressed.

PART 4: Experimental study on autotrophic nitrogen removal

Chapter 4.1 shows the construction, start-up and operation of a partial nitritation SHARON reactor in view of coupling this reactor with an Anammox reactor. Different practical considerations are discussed. The reactor was operated at different influent concentrations investigating the effect of this on reactor performance. *The main part of this chapter was presented as an oral presentation at FAB2003, Ghent, Belgium, September, 18-19, 2003 (Van Hulle et al., 2003b).*

Chapter 4.2 verifies the assumption that ammonia is the actual substrate for nitrification by dedicated batch tests at different temperatures and pH levels. The inhibition of nitrous acid on nitrification is also investigated as well as direct temperature and pH effects. *The main part of this chapter was presented as an oral presentation at the IWA World Water Congress and Exhibition, Marrakech, Marocco, September, 19-24, 2004 (Van Hulle et al., 2004b).*

Chapter 4.3 investigates the use of an inexpensive and easy to automate titrimetric measurement technique for the monitoring of a SHARON-Anammox process and shows how this measurement can be used for controlling the process. *This chapter is in preparation for publication.*

PART 5: Simulation and optimization of autotrophic nitrogen removal

Chapter 5.1 describes the modelling of a partial nitritation membrane bioreactor (MBR). After comparing of experimental results with simulated data, the model is used for scenario analysis. The performance of the reactor at different temperatures, hydraulic and sludge residence times was investigated. *This chapter was published in Biotechnology & Bioengineering (Wyffels et al., 2004b).*

Chapter 5.2 describes the modelling of the start-up and operation of an Anammox sequencing batch reactor. Qualitative and quantitative experimental data are compared with modelling results. *This chapter was published in the Journal of Chemical Technology & Biotechnology (Dapena-Mora et al., 2004c).*

Chapter 5.3 estimates the necessary start-up times of an Anammox reactor under different operational conditions such as temperature, hydraulic residence time and initial Anammox biomass concentration. *This chapter is in preparation for publication.*

Chapter 5.4 describes how, based on simulation results with the biofilm model, more information concerning the parameter values of the Anammox biofilm process can be gained by measuring under certain experimental conditions. *This chapter was published in Water & Environmental Management Series: Young Researchers 2004 (Van Hulle et al., 2004a).*

Chapter 5.5 describes the influence of operational conditions on Anammox and competing processes in a biofilm. The effect of different hydrodynamic conditions, COD and TAN influent concentrations and nitrite inhibition was investigated. *Parts of this chapter were presented as a poster presentation at the IWA Biofilm symposium, Cape Town, South Africa, September, 14-18, 2003 (Van Hulle et al., 2003a).*

PART 6: Discussion and conclusions

Chapter 6 draws the most important conclusions and discusses future research work that is necessary for further improvement of autotrophic nitrogen removal systems.

PART 2

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LITERATURE REVIEW

Chapter 2

Literature review

ABSTRACT

In this chapter an overview of the origins and effects of nitrogen pollution is given. From this overview it becomes clear that a diverse range of nitrogen streams exists. For streams containing high nitrogen (100-5000 mgTAN L^{-1}) and low COD concentration, the autotrophic nitrogen removal process is proposed as a state-of-the-art and sustainable alternative to conventional nitrification/denitrification. The key process within this autotrophic nitrogen removal is the Anammox process. Hence, the (micro)biological, technical and engineering aspects of this Anammox process are presented. As this process requires a preceding partial nitrification step, the different aspects of (partial) nitrification are presented too, with a focus on the conditions that help producing an Anammox suited influent.

1 ORIGINS AND EFFECTS OF NITROGEN POLLUTION

Nitrogen is needed by all organisms for their synthesis of proteins, nucleic acids and other essential N containing compounds. Although it makes up 78 % of the earth's atmosphere as N_2 and is therefore abundantly present, this form of nitrogen cannot be used directly, except by a few specialised organisms (Pynaert, 2003). In many situations, fixed N is the limiting nutrient because its availability is usually much smaller than the potential uptake by, for instance, plants.

The supply of protein food for the global population by agriculture is therefore nowadays largely dependent on the use of synthetic nitrogen fertiliser produced from atmospheric N_2 by the Haber-Bosch process. In the last century the world's annual industrial output of nitrogenous fertiliser increased from 10 Mt N in 1960 to about 90 Mt N in 1998 (Mulder, 2003). The global estimate for biological nitrogen fixation is in the range of 200-240 Mt N, which shows that the anthropogenic mass flows for nitrogen have a major impact on the global nitrogen cycle (Gijzen and Mulder, 2001).

The consumption of protein will ultimately result in the discharge of the protein nitrogen in wastewater. In European countries approximately 18% of fertiliser nitrogen ends up in wastewater in the form of ammonium (TAN) or organic nitrogen (Mulder, 2003). Other polluting nitrogen compounds are nitrite (TNO_2) and nitrate. Nitrate is primarily used to make fertilizer, although it is also used to make glass and explosives and other chemical production and separation processes. TNO_2 is manufactured mainly for use as a food preservative, and both nitrate and TNO_2 are used extensively to enhance the colour and extend the shelf-life of processed meats (WHO, 2004). An overview of wastewaters containing nitrogen is presented in Table 2.1 and discussed in the next paragraph. It must be noted that these streams have one thing in common, a high nitrogen content, but differ considerably in other characteristics. Also some streams initially have a favourable carbon-to-nitrogen ratio, e.g. a sugar production wastewater (Austermaan-Haun *et al.*, 1999), but are treated first by anaerobic digestion. Hence, most of the COD that can be used for denitrification is removed by this step and a stream with a high nitrogen content, but unfavourable carbon-to-nitrogen ratio remains.

The discharge of these nitrogen compounds into the receiving waters would lead to several environmental and health risks. Ammonia is an essential plant nutrient and, after nitrification to nitrate, responsible for eutrophication, i.e. undesirable growth of aquatic plants and algae. The breakdown of these cells when they die can cause a depletion of oxygen in the water, which can take a heavy toll on fish. In addition, aquatic plants (including algae) influence the oxygen and pH of the surrounding water. The greater the growth of algae, the greater the fluctuations in levels of dissolved oxygen and pH. This can upset metabolic processes in organisms, which can result in disease or death. Also, blue-green algae can produce algal toxins, killing animals and

poisoning freshwater reservoir supplies. Ammonia itself is also toxic to aquatic organisms at concentrations as low as 0.03 mgNH_3-N L^{-1} (Solbe and Shurben, 1989). Nitrate pollution impedes the production of drinking water. Nitrites in drinking water can lead to oxygen shortage of newly borns ('blue baby syndrome') and, during chlorination of drinking water, carcinogenic nitrosamines may be formed by the interaction of nitrite with compounds containing organic nitrogen. Nitrogen compounds therefore needs to be removed from the wastewater. For this removal of nitrogen a wide variety of biological and chemical removal systems are available (Henze *et al.*, 1995) as discussed further.

2 OVERVIEW OF HIGH NITROGEN CONTAINING WASTEWATERS

2.1 Reject water

Waste sludge from a wastewater treatment plant (WWTP) can be treated by anaerobic digestion followed by dewatering. This results in dewatered sludge which can be burnt and reject water containing high concentrations of TAN. This reject water is normally recycled to the influent of the WWTP, but separate treatment becomes interesting in view of the more stringent discharge legislation. An analysis of the nitrogen balance of the WWTP Dokhaven in Rotterdam (The Netherlands) revealed that the reject water accounted for only a few percentages of the total flow, but 15 % of the nitrogen load (Mulder *et al.*, 2001; van Dongen *et al.*, 2001a&b). Hence, reducing the nitrogen load coming from the digesters can significantly help reduce the nitrogen concentration in the effluent of the main wastewater treatment plant. This way ever reducing discharge limits for nitrogen concentrations can be met.

2.2 Piggery manure

In certain regions an excess of piggery manure is produced. This means that manure can no longer be used completely as soil enhancer, but has to be disposed as waste. Raw manure is usually separated into a thick and a thin fraction. The thick fraction can be used as soil enhancer while the thin (liquid fraction) is treated. The composition of this thin fraction can vary and depends on the separation method and the composition of the animal feed (Feyaerts *et al.*, 2002). However, large amount of COD, nitrogen and phosphorus can be expected.

2.3 Landfill leachate

In Flanders domestic solid waste is normally burnt, but this is not everywhere the case. In Russia, for example, 96% of all domestic waste is put on landfills (Kalyuzhnyi and Gladchenko, 2003). The leachate of these landfills is heavily polluted and has to be captured and treated. Often this leachate is recycled, decreasing the COD content and increasing the nitrogen content because the

Table 2.1. COD, BOD, N and P concentrations (in mg L^{-1}) in waste streams with high nitrogen content (after Donckels, 2004)

Type wastewater	COD	BOD_5	Total nitrogen	Phosphorus	reference
Reject water	232–12587	81–750	260–958	33–207	Gil and Choi (2004)
	390–2720	n.m.	943–1513	n.m.	Jenicek *et al.* (2004)
	610	140	910	n.m.	Wyffels *et al.* (2003)
Thin fraction piggery manure	n.m.	2912	707	55	Chen *et al.* (2004)
	3969	1730	1700	147	Obaja *et al.* (2003)
	9000–13000	n.m.	3100–4300	20–40	Poo *et al.* (2004)
	6456	n.m.	695	91.8	Tilche *et al.* (1999)
Landfill leachate	2000–5000	1500–4000	500–1000	20–50	Chung *et al.* (2003)
	n.m.	45	310	n.m.	Ilies and Mavinic (2001)
	1300–1600	n.m.	160–270	n.m.	Jokela *et al.* (2002)
	9660–20560	n.m.	780-1080	20–51	Kalyuzhnyi and Gladchenko (2004)
Tannery waste water	300–1400	n.m.	50–200	n.m.	Carucci *et al.* (1999)
	1940–2700	n.m	123–185	n.m	Murat *et al.* (2003)
Slaughter house waste processing	1400–2400		170–200	35–55	Keller *et al.* (1997)(1)
Starch production	3000	990	1060	210	Abeling and Seyfried (1992)(2)
	5000–10000	2000–5000	800–1100	170–230	Abeling and Seyfried (1993)(2)
Pectine industry waste water	15000–22000	n.m	1280–2990	n.m	Austermann-Haun *et al.* (1999)
	8100	n.m.	1600	11	Deng Petersen *et al.* (2003)

n.m.: not mentioned

(1) After treatment in anaerobic lagune

(2) After treatment in anaerobic digester

landfill acts as an anaerobic bioreactor (Clabaugh, 2001). Similar to reject water this landfill leachate is characterized by a high TAN and a low COD content (Ilies and Mavinic, 2001).

2.4 Industrial wastewaters

Industrial processes can also generate streams with high nitrogen content. Also, industrial streams with a high COD content can lead to highly loaded nitrogen streams if they are first treated in an anaerobic digester. Examples can be found in the pharmaceutical industry (Carrera *et al.*, 2003), tanneries (Murat *et al.*, 2003), slaughterhouse waste processing (Keller *et al.*, 1997), potato processing industries, alcohol and starch production (Abeling and Seyfried, 1992) and formaldehyde production (Campos *et al.*, 2003; Garrido *et al.*, 2001). An additional problem is that these streams often contain recalcitrant and/or toxic components, resulting in a high effluent COD concentration. Despite the favourable C/N ratio there is still a need for carbon addition to allow the necessary denitrification. Carucci *et al.* (1999) report a minimum C/N ratio of 8 for tannery wastewater, which is much higher than the normally applied ratio of 4 to 6 (Mulder, 2003). This tannery wastewater also contains chromium, sulphide and chloride, all resulting in negative effects on the nitrification process (Murat *et al.*, 2003; Ros and Gantar, 1998). Wastewater of formaldehyde production, characterised by a high organic COD content, partially inhibits both nitrification and denitrification (Garrido *et al.*, 2001) and will thus lead to more difficult operation.

3 NITROGEN ELIMINATION FROM WASTEWATER

For a specific application the available alternatives need to be evaluated on cost aspects, chemical and energy requirements, operational experience, process reliability and environmental impact. The selection of the best alternative is generally based on cost-effectiveness. However, in practice the selection of either a biological or a physiochemical method is determined by the nitrogen concentration of the wastewater. Three concentration ranges can be distinguished (Mulder, 2003):

- Diluted wastewater with TAN concentration up to 100 mgTAN-N L^{-1}. In this range biological N-removal is the preferred process based on cost-effectiveness. Domestic wastewater is within this range.
- Concentrated wastewater with TAN concentrations in the range of 100-5000 mgTAN-N L^{-1}. A typical example is sludge reject water for which, after extensive investigations, biological treatment is to be preferred (Janus and Van der Roest, 1997). Although ammonia stripping and producing $MgNH_4PO_4$ were identified as interesting alternatives for resource recovery, these options are not cost-effective (Siegrist, 1996; Priestley *et al.*, 1997; Janus and Van der Roest, 1997). Recently developed biological nitrogen removal processes for these

concentrated streams are the SHARON-Anammox process (van Dongen *et al.*, 2001a&b), the OLAND process (Kuai and Verstraete, 1998) and the CANON process (Hao *et al.*, 2002a&b). All these processes will be discussed further in detail.

- Concentrated wastewater with TAN concentrations higher than 5000 mgTAN-N L^{-1}. In this range physicochemical methods are technically and economically feasible. A successful example is the steam stripping of a wastewater with high TAN concentration followed by TAN recovery which has been in operation on industrial scale since 1985 (Harmsen *et al.*, 1986)

3.1 Biological nitrogen removal systems

Since the nitrogen concentrations in the streams studied in this PhD are always below 5000 mgN L^{-1}, the focus will be on biological treatment systems. A short review redrafted after Mulder (2003) is presented here. The following systems are considered here and presented in Table 2.2.

- Activated sludge with conventional nitrification and denitrification. Around the world the activated sludge system with nitrification and denitrification is the most widely used system for nitrogen removal. It is available in many design variations (Henze *et al.*, 1995). In the nitrification process TAN is converted to nitrate via TNO_2. This nitrate is subsequently reduced to nitrogen gas. Per kg nitrogen removed about 4.57 kg O_2 and 3-6 kg COD are necessary.
- Activated sludge with nitrification/denitrification via TNO_2. In this process TAN is oxidised into TNO_2. This TNO_2 is subsequently reduced to nitrogen gas. Per kg nitrogen about 3.2 kg O_2 and 2-4 kg COD are necessary.
- Activated sludge with autotrophic nitrogen removal. Recently, the Anammox process in which TAN is oxidised under anoxic conditions with TNO_2 as electron acceptor was discovered (Mulder *et al.*, 1995). This Anammox process thus requires a partial oxidation of TAN. Per kg nitrogen removed only 1.71 kg O_2 and no COD are necessary, indicating the sustainability of this nitrogen removal process because much less energy and chemicals are necessary compared to other nitrogen removal processes.
- Constructed wetlands, algal and duckweed ponds. In constructed wetlands, algal and duckweed ponds ammonia is assimilated into algal biomass. The energy use in the algal pond is required for mixing and pumping.

Table 2.2. Typical values of specific operational parameters of biological nitrogen removal systems (after Mulder, 2003)

System	N-load [kgN ha^{-1} d^{-1}]	Energy consumption [kWh kgN^{-1}]	COD/N ratio	Sludge production [kgDW kgN^{-1}]	Removal efficiency	References
Activated sludge with conventional nitrification and denitrification	200-700	2.3	3-6	1-1.2	>75	Henze *et al.* (1995)
Activated sludge with nitrification denitrification via nitrite	200-700	1.7	2-4	0.8-0.9	>75	Abeling and Seyfried (1992)
Activated sludge with autotrophic nitrogen removal	>200-700	0.9	0	<0.1	>75	Mulder *et al.* (1995)
Algal ponds	15-30	0.1-1	6-7	10-15	23-28	Oswald (1995)
duckweed ponds	3-4	<0.1	28	20-26	74-77	Alaerts *et al.* (1996)
Constructed wetlands	3-26	<0.1	2-7	-	30-70	Haberl *et al.* (1995)

The last option is low in energy consumption compared to the first three. However, the removal capacity is also much lower. The most promising and sustainable option in terms of minimal energy consumption, absence of need for organic matter for denitrification, low sludge production, high nitrogen load, area requirement and N_2O emission is autotrophic nitrogen removal. This system will be discussed in detail.

3.2 Autotrophic nitrogen removal

Autotrophic nitrogen removal is the combination of partial nitrification and anaerobic ammonium oxidation (Anammox). Anammox oxidizes TAN with TNO_2 as electron acceptor. The molar TAN:TNO_2 consumption ratio of this process is about 1:1.32 (Strous *et al.*, 1998; Wyffels *et al.*, 2003). The Anammox process is therefore not a stand-alone process, but should always be combined with a partial nitrification process that produces this ratio. The updated nitrogen cycle with Anammox is depicted in Figure 2.1 (after Jetten *et al.*, 1999). In what follows both steps of the autotrophic nitrogen removal process will be discussed in detail.

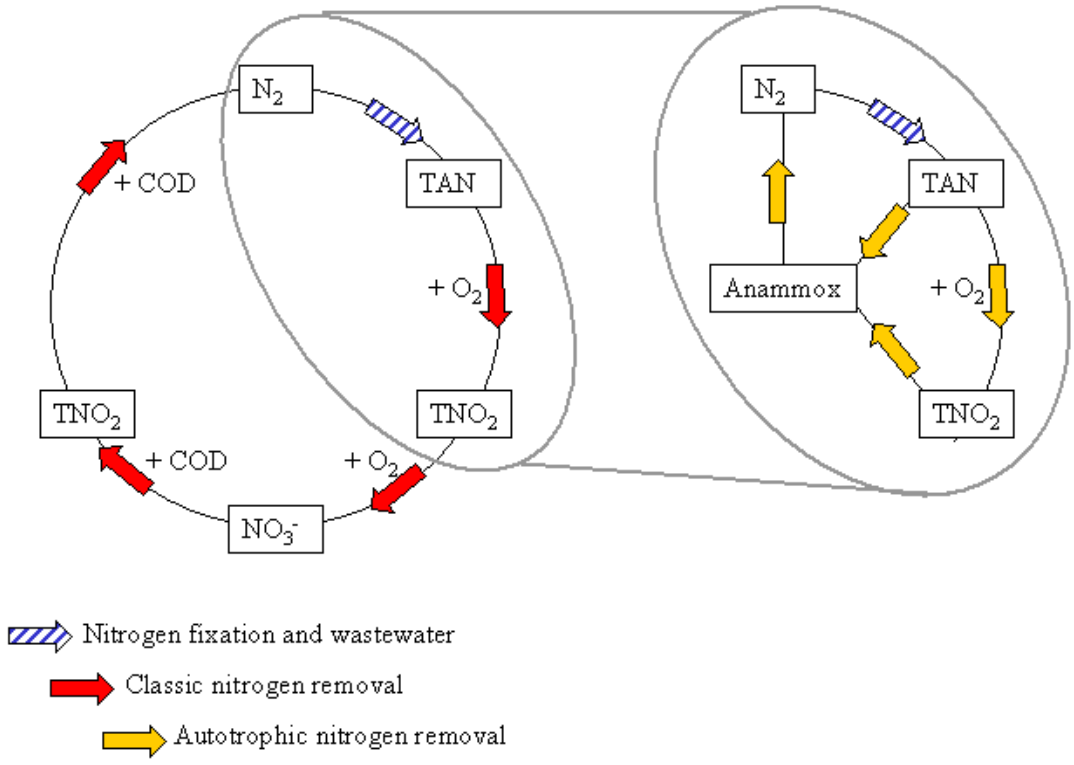

Figure 2.1. The updated nitrogen cycle with Anammox (after Jetten *et al.*, 1999)

3.2.1 (Partial) nitrification

3.2.1.1 General aspects

Nitrification is the aerobic oxidation of NH_3 to NO_3^-. It consists of two sequential steps carried out by two phylogenetically unrelated groups of aerobic chemolithoautotrophic bacteria. Some heterotrophic bacteria can also oxidize ammonium to nitrate, but this is only a very small contribution to the overall ammonia oxidation (Pynaert, 2003). First, NH_3 is oxidized to HNO_2 by the aerobic ammonia-oxidizing bacteria (X_{NH}). This step is also called nitritation. Approximately 2 moles of protons are produced for every mole of TAN oxidised. Ammonium oxidation is therefore an acidifying reaction. The second step where HNO_2 is oxidized to NO_3^- by the nitrite oxidizing bacteria (X_{NO}) is called nitratation. No single known autotrophic bacterium is capable of complete oxidation of NH_3 to NO_3^- in a single step (Abeliovich, 1992).
In Table 2.3 an overview of the main physiological and kinetic characteristics of both types of nitrifying organisms is given as summarised by Pynaert (2003). Values reported for all these parameters can vary significantly, depending on process conditions such as influent concentrations, temperature (T) and pH. It is therefore essential that in view of simulation studies the parameters are determined specifically for the system under study.

Table 2.3. Some important physiological parameters of the aerobic ammonia-oxidizing (X_{NH}) and nitrite oxidizing bacteria (X_{NO}) (Pynaert, 2003)

Parameter	Unit	X_{NH}	X_{NO}
Maximum specific growth rate (μ_{max})	d^{-1}	0.3-2.2	0.2-2.5
Biomass yield (Y)	$gVSS\ gN^{-1}$	0.04-0.13	0.02-0.08
Affinity constant for TAN (K_{TAN})	$mgTAN\ L^{-1}$	0.06-27.5	-
Affinity constant for TNO_2 (K_{TNO2})	$mgTNO_2\ L^{-1}$	-	0.1-15
Affinity constant for oxygen (K_{O2})	$mgO_2\ L^{-1}$	0.03-1.3	0.3-2.5
Temperature range	°C	4-42	4-46
pH range	-	4.5-8.5	4.5-9

The difference in values for maximum specific growth rate can be attributed by incorrect assessment of autotrophic decay rates, as this leads to errors in maximum specific growth rate estimation (Dold *et al.*, 2004).
The varying value for TAN and TNO_2 affinity constant can be explained by the fact that ammonia (NH_3) rather than ammonium (NH_4^+) and nitrous acid (HNO_2) rather than nitrite (NO_2^-) are the actual substrates for ammonium oxidizer and nitrite oxidizer growth, respectively (Suzuki

et al., 1974; Anthonisen *et al.*, 1976), although not everybody agrees with this statement (Groeneweg *et al.*, 1994).

It is generally accepted that nitrifiers grow optimally at slightly alkaline pH (7.2-8.2) and temperatures between 25 and 35 °C (Sharma and Ahlert, 1977). At a pH below 6.5, no growth of X_{NH} was observed, probably due to the limited NH_3 (substrate) availability at this low pH value (Burton and Prosser, 2001). The optimal dissolved oxygen (DO) level for ammonia and nitrite-oxidizers is 3-4 mg O_2 L^{-1} (Barnes and Bliss, 1983).

In view of autotrophic nitrogen removal of concentrated streams these boundaries for temperature, pH and dissolved oxygen concentration have been shifted to more extreme values. Operating temperatures for partial nitrification are between 30 and 40°C. Ammonium oxidisers were grown in a chemostat reactor for more than 1 year at a pH of about 6.8 (van Dongen *et al.*, 2001a&b; Van Hulle *et al.*, 2003b). Dissolved oxygen levels of 0.5 mgO_2 L^{-1} (Hanaki *et al.*, 1990) and even 0.1 mgO_2 L^{-1} (Wyffels *et al.*, 2003) and 0.05 mg L^{-1} (Abeliovich, 1987) supported significant rates of ammonia-oxidation.

Both groups of bacteria are chemolithoautotrophic and are considered obligatory aerobic. Autotrophic means they have to fix and reduce inorganic CO_2 for biosynthesis, which is an energy-expensive process explaining their low yield values compared to aerobic heterotrophs. The fact that they use a nitrogen electron donor even lowers their cell yield because of the lower energy release per electron equivalent compared to organic electron donors. As a consequence, nitrifiers are considered slow growers. Oxygen is used for respiration and as a direct reactant (aerobic bacteria).

Details about ecology and phylogeny are summarised by Kowalchuk and Stephen (2001).

3.2.1.2 Environmental conditions affecting (partial) nitrification

The most important environmental parameters influencing nitrification are the free ammonia (FA) and free nitrous acid (FNA) concentration, the temperature, pH and DO concentration. Other nitrogen components such as hydroxylamine also influence nitrification, but to a lower extent. Further, nitrification is partially inhibited by volatile fatty acids (Eilersen *et al.*, 1994) and other organic matter, phosphate and light (Philips *et al.*, 2002). The difference in sensitivity of ammonium and nitrite oxidisers towards these influences determines whether there will be TNO_2 accumulation in a nitrifying system. Indeed, generally nitrite oxidisers are more sensitive to detrimental environmental conditions than ammonium oxidisers.

In view of coupling a partial nitrification unit with an Anammox unit, nitrite oxidising activity should be suppressed and TAN should only be oxidised for about 50 % to TNO_2. The different influencing factors discussed hereafter can be used for this purpose.

3.2.1.2.1 Free ammonia and nitrous acid concentration

The uncharged nitrogen forms are considered to be the actual substrate/inhibitor for ammonium and nitrite oxidation. The amount of ammonia and nitrous acid present in the reactor can be calculated from the temperature and pH using the following equilibrium equations:

$$NH_4^+ \xleftrightarrow{K_e^{NH}} NH_3 + H^+ \quad (2.1)$$

$$HNO_2 \xleftrightarrow{K_e^{NO}} NO_2^- + H^+ \quad (2.2)$$

With TAN = NH_3 + NH_4^+ and $K_e^{NH} = \frac{NH_3 \cdot H^+}{NH_4^+}$ the fraction of total ammonium present in the form of uncharged ammonia (NH_3) is calculated as

$$C_{NH_3} = \frac{C_{TAN}}{1 + \frac{10^{-pH}}{K_e^{NH}}} \quad (2.3)$$

With TNO_2 = HNO_2 + NO_2^- and $K_e^{NO} = \frac{NO_2 \cdot H^+}{HNO_2}$ the fraction of total nitrite present in the form of uncharged nitrous acid (HNO_2) is calculated as

$$C_{HNO_2} = \frac{C_{TNO_2}}{1 + \frac{K_e^{NO}}{10^{-pH}}} \quad (2.4)$$

Anthonisen *et al.* (1976) proposed temperature (T in K) dependencies for the equilibrium constants: $K_e^{NH} = e^{\frac{-6344}{T+273}}$ and $K_e^{NO} = e^{\frac{-2300}{T+273}}$. Further, Helgeson (1967) proposed a temperature dependency for the ammonia/ammonium equilibrium: $K_e^{NH} = 10^{-\left(\frac{2835.8}{T+273} - 0.6322 + 0.00123(T+273)\right)}$. These equations can be used to compare equilibrium concentrations at different temperatures. Basically, a higher temperature and/or a higher pH results in a higher ammonia concentration and a lower nitrous acid concentration.

The concentrations of NH_3 and HNO_2 as function of total ammoniacal nitrogen (TAN=NH_4^+ + NH_3) and total nitrite concentrations (TNO_2=NO_2^- + HNO_2) and pH are presented in Figure 2.2. From these, boundary conditions of zones of nitrification inhibition were determined (Anthonisen *et al.*, 1976). A range of boundary conditions, depending on various

operating conditions, delimits each zone. Zone 1 (NH_3 > 10–150 mgNH_3–N L^{-1}) marks the inhibition of X_{NH} and X_{NO} by free ammonia, while in zone 2 (0.1–1.0 mgNH_3–N L^{-1} < NH_3 < 10–150 mgNH_3–N L^{-1}) NH_3 only inhibits X_{NO}. Complete nitrification is possible in zone 3 (NH_3 < 0.1– 1.0 mgNH_3–N L^{-1} and HNO_2 < 0.2–2.8 mgHNO_2-N L^{-1}). In zone 4 X_{NO} are inhibited by free nitrous acid (HNO_2 > 0.2–2.8 mgHNO_2-N L^{-1}). Because the concentrations of these two forms depend on the solution pH, NH_3 is the main inhibitor of nitrification at high pH (>8), whereas HNO_2 is the main inhibitor at low pH (<7.5). Prakasam and Loehr (1972) obtained 0.02 mgHNO_2-N L^{-1} as a threshold concentration of nitrite oxidation inhibition, which is lower than the threshold boundary range concentrations of 0.2–2.8 mgHNO_2-N L^{-1} or 0.06–0.83 mgHNO_2-N L^{-1} found by Anthonisen *et al.* (1976).

In any case, NH_3 and HNO_2 inhibition can be used to outcompete nitrite oxidisers and produce an Anammox suited effluent. However the potential of using only NH_3 and/or HNO_2 inhibition to obtain stable TNO_2 formation seems somewhat limited since adaptation of the nitrite oxidizing bacteria has been reported (Turk and Mavinic, 1989).

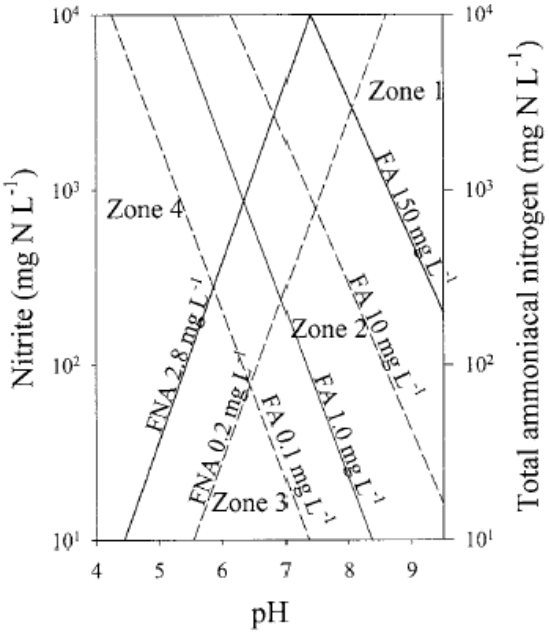

Figure 2.2. Relationship between concentrations of ammonia (FA) and nitrous acid (FNA) and inhibition to nitrifiers at ambient conditions. The dashed lines mark the lower limit and the solid lines mark the upper limit of the range of boundary conditions of zones of nitrification inhibition. (Anthonisen *et al.*, 1976).

3.2.1.2.2 Temperature

Temperature is a key parameter in the nitrification process, but the exact influence is hard to determine because of the interaction between mass transfer, chemical equilibria and growth rate dependency. A temperature rise creates two opposite effects: increased NH_3 inhibition as explained above and activation of the organisms according to the Arrhenius principal. This increased activity only holds up to a certain critical temperature above which biological activity decreases again.

Experiments with pure cultures gave an optimal temperature of 35°C for X_{NH} and 38°C for X_{NO} (Grunditz and Dalhammar, 2001) as can be seen from Figure 2.3. Brouwer (1995) indicates an optimal temperature range of 35-42°C for sludge cultivated at 30°C, but only investigated short-term effects. Long term exposure to temperatures above 40 °C is expected to lead to deactivation.

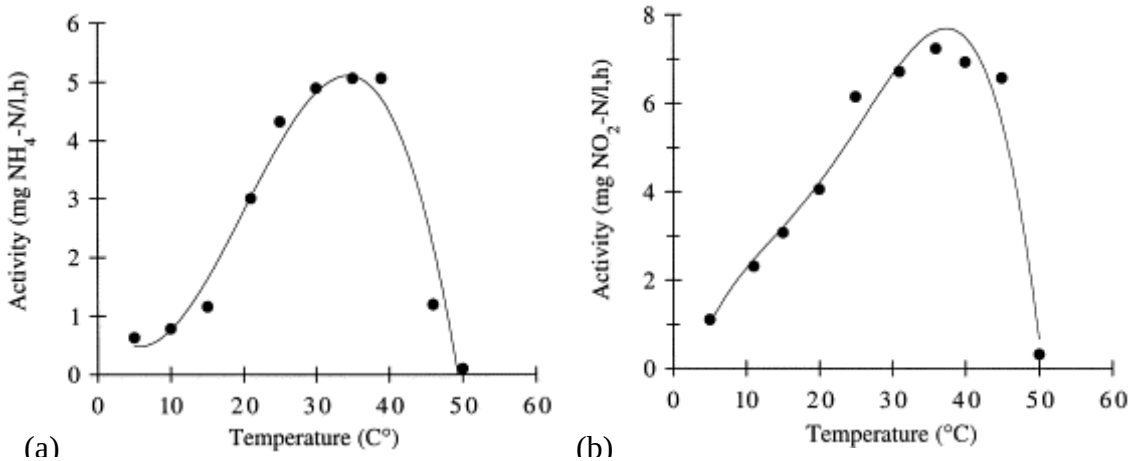

Figure 2.3. (a) Effect of temperature on the activity of ammonium oxidizers. (b) Effect of temperature on the activity of nitrite oxidizers (Grunditz and Dalhammar, 2001).

Literature values for activation energy of ammonium and nitrite oxidisers range from 72 to 60 kJ mol^{-1} and from 43 to 47 kJ mol^{-1} respectively (determined in studies from 7 to 30°C) (Jetten *et al.*, 1999; Helder and De Vries, 1983; Knowles *et al.*, 1965; Stratton and Mc Carty, 1967). This indicates that the activity of ammonium oxidisers will increase faster than the activity of nitrite oxidisers. The SHARON process (Single reactor High activity Ammonia Removal Over Nitrite) is based on this principle. In this process, nitritation of TAN to TNO_2 is established in a chemostat by working at high temperature (above 25°C) and maintaining an appropriate sludge retention time (SRT) of 1 to 1.5 days, so that ammonium oxidizers are maintained in the reactor, while nitrite oxidizers are washed out and further nitrification of TNO_2 to nitrate is prevented (Figure 2.4).

In order to produce an Anammox suitable influent only half of the TAN should be oxidized. In case the SHARON influent contains TAN and bicarbonate on an equimolar basis, the protons produced during conversion of half of the TAN are balanced 'exactly' via carbon dioxide stripping. For the high-concentrated streams to which the SHARON process is typically applied, the protons produced during TAN conversion above 50% would destroy the bicarbonate buffer system and cause a significant pH drop, preventing further nitrification.

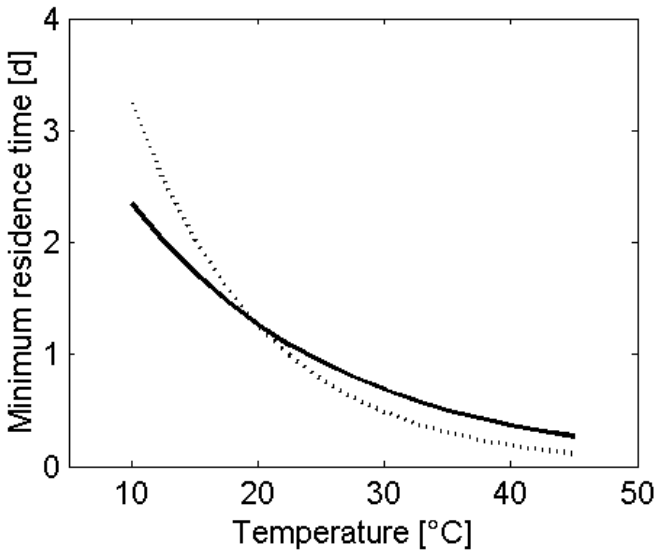

Figure 2.4. Effect of temperature on the minimal required cell residence time for ammonia and nitrite oxidation. Above 25°C it is possible to wash out the nitrite oxidizers (-) while maintaining the ammonium oxidizers (¨). Calculated with parameter values described in chapter 3.2.

3.2.1.2.3 pH

Despite a wide divergence of the reported effects of pH on nitrification, there seems to be a consensus that the optimum pH for both X_{NH} and X_{NO} lies between 7 and 8. As an example the results from Grunditz and Dalhammar (2001) are depicted in Figure 2.5.

An explanation for the preference of X_{NH} for slightly alkaline environments could be the fact that these organisms use NH_3 as substrate (Suzuki *et al.*, 1974). This optimum pH range is also linked with the pH dependent NH_4^+/NH_3 and HNO_2/NO_2^- equilibria, where NH_3 and HNO_2 can exhibit inhibitory effects starting from a certain pH, as explained above. Apart from the influence of pH on chemical equilibria, also pure pH effects exist. Below pH 7, the nitrification rate will decrease, although high nitrification rates at low pH were detected in a fluidized bed reactor with chalk as biofilm carrier (Tarre *et al.*, 2004). In this system the chalk probably acted as a local buffer system.

By working at pH between 7.5 and 8 growth of ammonium oxidisers is favoured over growth of nitrite oxidizers because of substrate availability and NH_3 inhibition. Baten *et al.* (1993) tested this strategy by applying a pH around 8 and a NH_3 concentration of 28 $mgNH_3$-N L^{-1} to outcompete the nitrite oxidizers in a nitrifying system treating ammonium rich waste water.

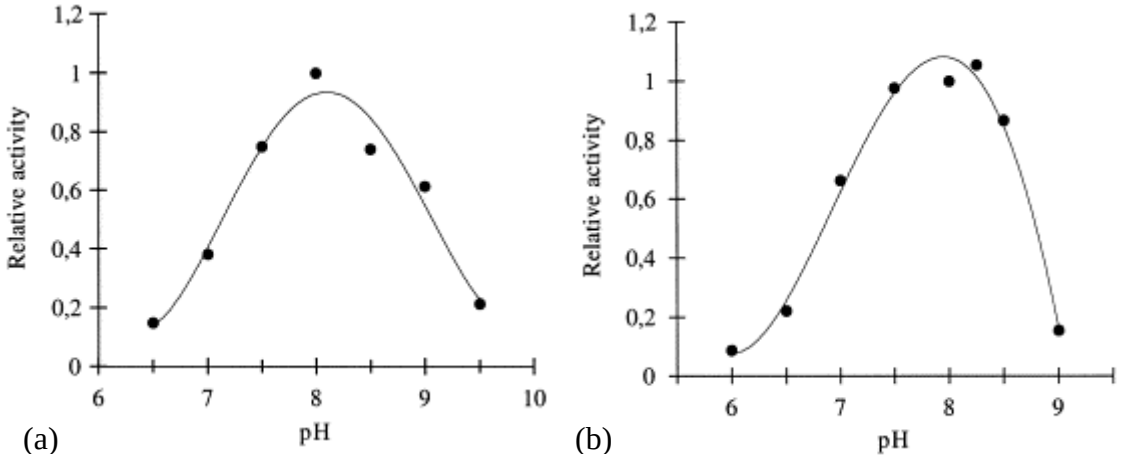

Figure 2.5. Effect of pH on the activity of ammonium oxidizers (a) and nitrite oxidizers (b), the activity at pH 8.0 was used as the reference activity (Grunditz and Dalhammar, 2001).

3.2.1.2.4 *DO*

When it comes to nitrification, the dissolved oxygen concentration is of utmost importance for both X_{NH} and X_{NO} (Philips *et al.*, 2002). X_{NH} seem to be more robust towards low DO than X_{NO}. Accumulation of TNO_2 at low DO is usually explained by the difference in oxygen half saturation constant DO (K_O) for X_{NH} and X_{NO} (Hanaki *et al.*, 1990). In other words, oxygen deficiency due to low DO influences the activity of X_{NO} more significantly than that of X_{NH} (Philips *et al.*, 2002).

According to Hunik *et al.* (1994) the half-saturation constant for O_2 is 0.16 mgO_2 L^{-1} and 0.54 for *Nitrosomonas europaea* and *Nitrobacter agilis* respectively. However, values for the half-saturation constant given in literature for activated sludge vary in the range of 0.25–0.5 mgO_2 L^{-1} and 0.34–2.5 mgO_2 L^{-1} respectively (Barnes and Bliss 1983). This variation is probably due to the fact that the oxygen concentration inside a sludge floc or biofilm not necessarily equals that of the water phase. The half saturation constant is therefore dependent on the biomass density, the floc size, the mixing intensity and the rate of diffusion of O_2 in the floc (Münch *et al.* 1996).

Imposing oxygen limiting conditions can thus be considered another way to outcompete nitrite oxidizers. However, it is also suggested that free hydroxylamine inhibition rather than a difference in affinity constants causes TNO_2 build-up in nitrifying systems at low DO (Dissolved Oxygen) concentration (Yang and Alleman, 1992).

The OLAND process (Oxygen Limited Autotrophic Nitrification Denitrification) (Kuai and Verstraete, 1998) and CANON (Completely Autotrophic Nitrogen removal Over Nitrite) (Sliekers *et al.*, 2002) processes are based on this oxygen limitation. In these systems the amount of oxidised TAN can be controlled by varying the amount of oxygen supplied to the system.

3.2.1.2.5 Lag time

Literature also mentions a lag time for nitrification (Peng *et al.*, 2004). If a reactor is operated with alternating anoxic and oxic phases, then TNO_2 peaks are noticed after the switch from the anoxic to the oxic phase. Hyungseok *et al.* (1999) report that nitrate formation can effectively be prevented by frequent switching between oxic and anoxic phases. Prolonging the aeration phases can lower the stress on nitrite oxidisers. This strategy was tested by Katsogiannis *et al.* (2003) in an SBR.

3.2.1.2.6 Other influencing factors

According to Hu (1990) hydroxylamine exhibited acute toxicity to *Nitrobacter* and this may also cause TNO_2 build-up in a nitrifying system. Hydroxylamine has been found to severely inhibit nitrite oxidizers (Castignetti and Gunner, 1982; Stüven *et al.*, 1992).

Formic, acetic, propionic and n-butyric acid all inhibited TNO_2 oxidation, but exhibited no significant effect on TAN oxidation (Eilersen *et al.*, 1994).

TNO_2 oxidation might also be affected by phosphorus deficiency (Nowak *et al.*, 1996). In a biological pre-treatment plant treating highly nitrogenous wastewaters ($T > 25°C$), TNO_2 oxidation was substantially reduced at phosphate levels below 0.2 $mgPO_4^{3-}$-P L^{-1}. Indeed, the phosphate half-saturation coefficient for X_{NO} is about one order of magnitude higher than for X_{NH} (0.2 $mgPO_4^{3-}$-P L^{-1} for X_{NO} and 0.03 $mgPO_4^{3-}$-P L^{-1} for X_{NH}) (Nowak *et al.*, 1996). Nitrite oxidizers are especially unable to oxidise TNO_2 to nitrate in the absence of phosphates, the so-called phosphate block.

Of a dozen compounds tested by Tomlinson *et al.* (1966), only chlorate, which is used to stop nitrite oxidation (Surmacz-Gorska *et al.*, 1996), cyanide, azide and hydrazine were more inhibitory to the oxidation of TNO_2 than TAN.

Light is inhibiting both X_{NH} and X_{NO}, through the oxidation of cytochrome c caused by light in the presence of O_2.

Other toxic components that influence TNO_2 oxidation are bromide and chloride (Peng *et al.*, 2004).

It can be concluded that an Anammox suited effluent can be produced by selection of the appropriate temperature, pH, substrate availability and NH_3 and HNO_2 inhibition level in order to

washout nitrite oxidisers from the system. In view of the Anammox stoichiometry, care should further be taken that only half of the TAN is oxidised.

3.2.2 Anammox

3.2.2.1 General aspects

When Mulder *et al.* (1995) observed unexplainable nitrogen losses in denitrifying fluidized bed reactors the idea was put forward that this could be attributed to ANaerobic AMMonium Oxidation (Anammox). Twenty years before Broda (1977) predicted that this process was possible on the basis of thermodynamic calculations. van de Graaf *et al.* (1995) showed by inhibition experiments that Anammox is a microbially mediated process and not the chemical Van Slyke reaction (Van Slyke, 1912).
First, it was assumed that nitrate acted as electron acceptor. However, van de Graaf *et al.* (1997) showed with ^{15}N-labeling experiments that TNO_2 is the actual electron acceptor. The key reaction is therefore given by equation 2.5.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \tag{2.5}$$

Note that this equation implies that the name anaerobic ammonium oxidation should actually be anoxic ammonium oxidation since TNO_2 is present as electron acceptor.
Anammox micro-organisms were cultivated by van de Graaf *et al.* (1996) on a synthetic medium containing bicarbonate with TNO_2 and TAN as only electron acceptor and –donor. Addition of organic carbon source was detrimental for the Anammox organisms because under such circumstances these anaerobic chemo-litho-autotrophs could not compete with heterotrophs.
It was found that TNO_2 was not only used for the oxidation of TAN, but it was also oxidised to nitrate. This oxidation was believed to generate the reducing equivalents necessary for carbon fixation (van de Graaf *et al.*, 1996; van de Graaf *et al.*, 1997). Consumption and production of TNO_2, TAN and nitrate have the relation (van de Graaf *et al.*, 1996) given by equation 2.6.

$$-\frac{\Delta[TAN]}{[1]} = -\frac{\Delta[TNO_2]}{[1.31 \pm 0.06]} = \frac{\Delta[NO_3^-]}{[0.22 \pm 0.02]} \tag{2.6}$$

The low growth rate and the difficulty in obtaining axenic cultures strongly hindered Anammox research. This low growth rate is attributed to the low TAN consumption, possibly because of the ‘kinetic difficulty’ of the metabolic strategy of Anammox (Strous *et al.*, 1998).
Strous *et al.* (1998) concluded that a sequencing batch reactor (SBR) is most suitable for the enrichment and cultivation of Anammox organisms. By making mass balances over the reactor a

number of kinetic parameters (Table 2.4) and the global stoichiometric equation using carbon fixation were determined as expressed in equation 2.7.

$$NH_4^+ + 1{,}32NO_2^- + 0{,}066HCO_3^- + 0{,}13H^+ \rightarrow 1{,}02N_2 + 0{,}26NO_3^- + 0{,}066CH_2O_{0,5}N_{0,15} + 2{,}03H_2O \quad (2.7)$$

Table 2.4. Parameters of anaerobic ammonium oxidation (after Jetten *et al.*, 2001)

Parameter	Unit	Anammox
ΔG	kJ/mol	-357
E_a	kJ/mol	70
μ_{max}	h^{-1}	0,003
Doubling time	d	10,6
Aerobic rate	mgN/g protein/min	0
Anaerobic rate	mgN/g protrein/min	0,7
K_{TNO2}	µM	<0.07
K_{TAN}	µM	0.07

In the mean time, Anammox activity has been discovered in different installations treating wastewater with high nitrogen load at low DO concentrations (Hippen *et al.*, 1997; Helmer and Kunst, 1998; Siegrist *et al.*, 1998; Helmer *et al.*, 2001; Toh *et al.*, 2002). Dalsgaard and Thamdrup (2002), Dalsgaard *et al.* (2003) and Kuypers *et al.* (2003) showed that Anammox contributes significantly to the nitrogen cycle as it was found in Skagerrak, Golfo Dulce (Costa Rica) and the Black Sea. Depending on the organic load up, to 70 % of the N_2 production in marine sediments can be attributed to Anammox (Dalsgaard and Thamdrup, 2002; Thamdrup and Dalsgaard, 2002). Further, Anammox activity was also found in sediments along the Thames estuary (Trimmer *et al.*, 2003), Arctic marine sediments (Rysgaard *et al.*, 2004) and an African freshwater wetland (Jetten *et al.*, 2003). This indicates that Anammox is present in different natural environments.

Anammox biomass has a brown-reddish colour, which is probably due to the high cytochrome contents (Jetten *et al.*, 1999).

3.2.2.2 Phylogeny

Strous *et al.* (1999a) showed that the bacteria responsible for the Anammox process are new members of the order of the Planctomycetes. Schmid *et al.* (2003) further placed the Anammox genera (Figure 2.6). In this Figure the phylogenetic tree reflecting the relationships of the Anammox organisms, other Planctomycetes and other reference organisms is represented (Schmid *et al.*, 2003). The triangles indicate phylogenetic groups. Phylogenetic analyses for this

Figure were performed with maximum likelihood, neighbour joining and maximum parsimony methods with 50% sequence conservation filters for Bacteria as well as Plantomycetes. Since no differences between all calculated trees in terms of branching order could be observed the tree based on maximum likelihood analysis with the 50% conservation filter for *Bacteria* is presented here. Filled circles indicate parsimony bootstrap values higher than 75%. Empty circles refer to values between 50 and 75%. The bar represents 10% estimated sequence divergence.

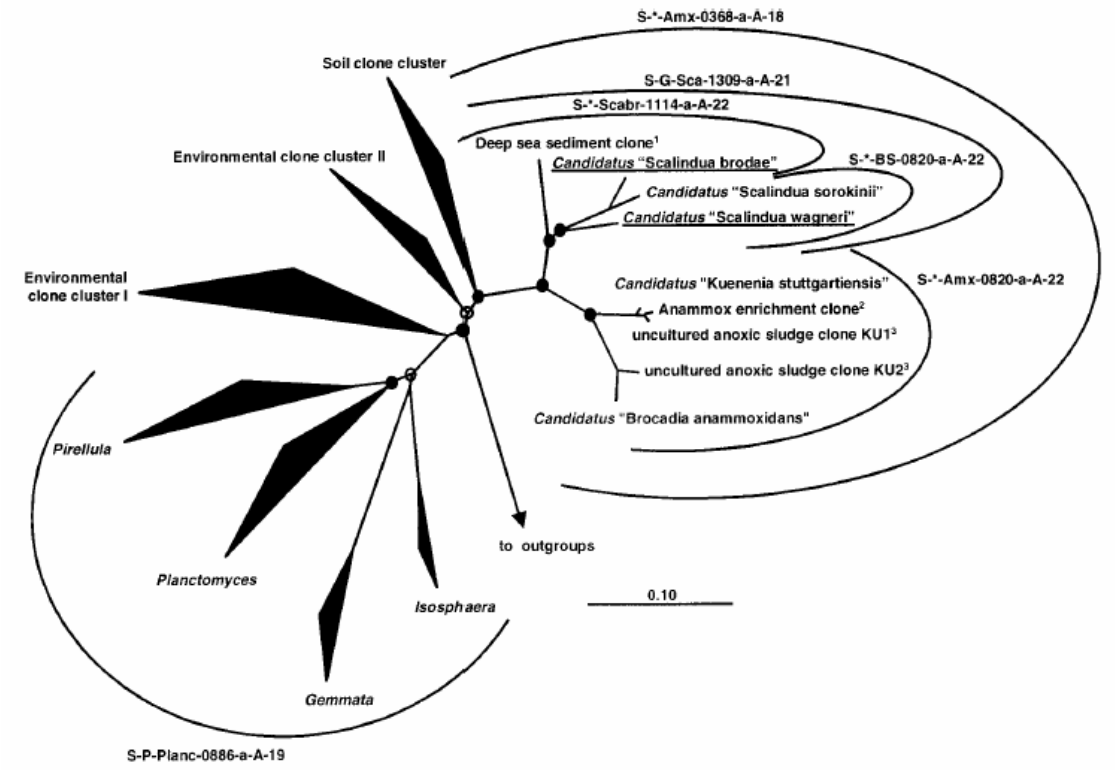

Figure 2.6. Situating the Anammox bacteria (after Schmid *et al.*, 2003)

The G-negative Anammox bacteria (van de Graaf *et al.*, 1996) were called *Candidatus Brocadia anammoxidans* (Strous *et al.*, 1999a). After analyses of 16 rDNA of 'Anammox micro-organisms' found in nitrifying rotating biological contractors (RBC) (Siegrist *et al.*, 1998) a new genus was discovered. This genus was named *Candidatus Kuenenia stuttgartiensis* (Schmid *et al.*, 2000). A third genus was detected both in Rotating Biological Contractors in a WWTP in Pitsea (UK) (Schmid *et al.*, 2003) and in the Black Sea (Kuypers *et al.*, 2003). The two members of the genus discovered in the UK were called *Candidatus Scalindua brodae* and *Candidatus Scalindua wagneri.* The Black Sea member was called *Candidatus Scalindua sorokinii.* With its detection in two very different habitats, "Scalindua" is possibly the most widespread Anammox genus identified so far. Since Anammox bacteria of different genera rarely occur in the same

WWTP or enrichment culture, it seems that they all occupy their own niche and environmental conditions select for only one of the different genera (Schmid *et al.*, 2003).

3.2.2.3 Compartmentalization in Anammox bacteria: the anammoxosome

Anammox, as most Planctomycetes, lack peptidoglycan and have a proteinaceous cell wall instead (König *et al.*, 1984) and have a complex compartmentalization. This compartmentalization involves a single intracytoplasmic membrane defining a major cell compartment (van Niftrik *et al.*, 2004). 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. One of these membranes is closely positioned to the proteinaceous cell wall. This membrane has been defined as the cytoplasmic membrane based on the finding of RNA in the 'paryphoplasm' compartment bounding its inner side. The other, innermost, membrane has been defined as an intracytoplasmic membrane as it is within the cytoplasm defined as any region of the cell containing RNA. A compartment – the paryphoplasm – is thus formed bounded by the cytoplasmic membrane on one side and the intracytoplasmic membrane on the other (Lindsay *et al.*, 2001). In Anammox bacteria, the compartment bounded by the intracytoplasmic membrane contains yet a second membrane bounded compartment (Figure 2.7), that is bounded by a single bilayer membrane. It is the site where catabolism takes place and is called the anammoxosome (van Niftrik *et al.*, 2004). This anammoxosome makes up for 29 to 61 % of the cell volume (Strous, 2000). The cytoplasm in Anammox bacteria is thus divided into three 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 the nucleoid and (3) the inner ribosome-free compartment, the anammoxosome, bounded by the anammoxosome membrane.

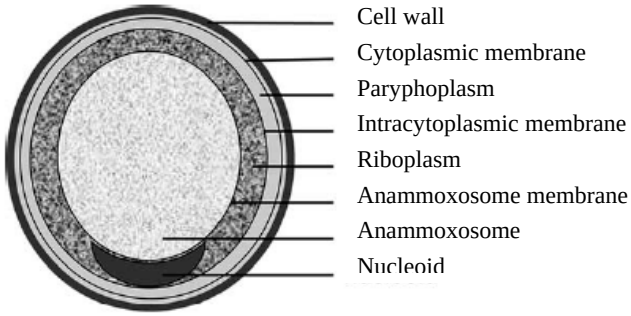

Figure 2.7. Cellular compartmentalization in Anammox bacteria (van Niftrik *et al.*, 2004)

Anammox bacteria contain a variety of abundant unconventional membrane lipids (Sinninghe Damsté *et al.*, 2002). The lipids occur in a wide variety of types and derivatives. Among these, unique structures have been found. They contain one, two or both of two different ring-systems, X and Y (Figure 2.8). Ring-system X is composed of three cyclobutane moieties and one cyclohexane moiety substituted with an octyl chain, which is ether-bound at its ultimate carbon atom to the glycerol unit. Ring-system Y is composed of five linearly concatenated cyclobutane rings substituted with a heptyl chain, which contains a methyl ester moiety at its ultimate carbon atom. All rings in ring-systems X and Y are fused by cis-ring junctions, resulting in a staircase-like arrangement of the fused rings, defined as ladderane. Lipids containing ladderane moieties X and Y are abundant membrane lipids in anammox bacteria. They represent 34% of total lipids in *Candidatus Brocadia anammoxidans*. The structure of the ladderane membrane lipids is unique in nature. Ladderane membrane lipids have so far been found only in Anammox bacteria. This raises the question of the functional significance of the ladderane lipids (van Niftrik *et al.*, 2004). Possibly these lipids help to form a very tight membrane to protect the cytoplasm from toxic intermediates produced in the anammoxosome (Jetten, personal communication).

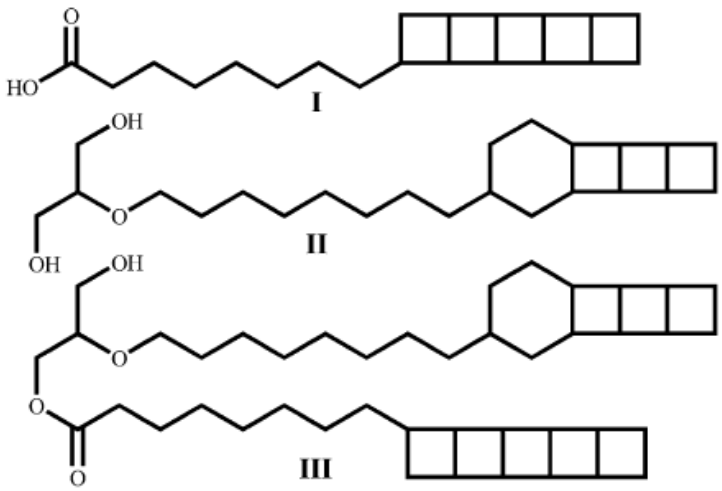

Figure 2.8. Structures of three characteristic ladderane lipids: I ladderane fatty acid-containing ring-system Y. II ladderane monoalkyl glycerol ether-containing ring-system X. III ladderane glycerol ether/ester containing both ring-systems, X and Y (van Niftrik *et al.*, 2004)

3.2.2.4 Biochemistry

Two possible pathways were hypothesized by van de Graaf *et al.* (1997) for the Anammox-process:

- Oxidation of ammonium to hydroxylamine, that reacts with nitrite yielding N_2O-gas. N_2O is then further reduced to nitrogen gas. Hydroxylamine-formation from ammonium via the ammonium monooxygenase, however, seems unlikely because of the strong oxygen inhibition (van de Graaf *et al.*, 1996; Jetten *et al.*, 1999).
- Partial reduction of nitrite with the formation of hydroxylamine (NH_2OH), that reacts further with ammonium to form hydrazine (N_2H_4). This hydrazine is further converted to nitrogen gas. This oxidation would give the necessary reducing equivalents for the initial reduction of nitrite.

^{15}N-labeling experiments showed that this second possibility is the correct one (van de Graaf *et al.*,1997). The addition of labelled hydroxylamine led to the formation of labelled nitrogen gas, in contrast to the addition of $^{15}N_2O$. Sustained growth on hydroxylamine or hydrazine is however not possible (Schalk *et al.*, 1998). Strous *et al.* (1999b) did notice that the addition of at least 50 µM of these intermediates resulted in complete recovery of the Anammox activity after inactivation with TNO_2. Schalk *et al.* (2000) succeeded in purifying and characterizing the hydroxylamine oxidoreductase/hydrazine reductase (HAO/HZO) of an Anammox culture. The HAO/HZO was able to oxidize both hydroxylamine and hydrazine under anoxic conditions to respectively NO, N_2O en N_2. The HAO/HZO made up 9 % of the total soluble protein fraction of the Anammox species *Candidatus Brocadia anammoxidans*. Schalk *et al.* (2000) also found that hydrazine strongly inhibits the oxidation of hydroxylamine. Kuenen and Jetten (2001) therefore suggested the most plausible hypothesis for the mechanism. Nitrite reduction by a nitrite reducing enzyme leads to the formation of hydroxylamine. An unknown hydrazine hydrolase converts ammonia and hydroxylamine to hydrazine that is converted to nitrogen gas by HAO/HZO. This oxidation would give the necessary reducing equivalents for the initial reduction of nitrite (Figure 2.9).

In the biochemical model, the Anammox reaction establishes a proton gradient by the effective consumption of protons in the riboplasm and production of protons inside the anammoxosome, a mechanism known as separation of charges. This results in an electrochemical proton gradient directed from the anammoxosome to the riboplasm.

Based on isotopic carbon analysis Schouten *et al.* (2004) concluded that different Anammox bacteria, such as *Candidatus Scalindua sorokinii* and *Candidatus Brocadia anammoxidans* use identical carbon fixation pathways, which may be either the Calvin cycle or the acetyl coenzyme A pathway.

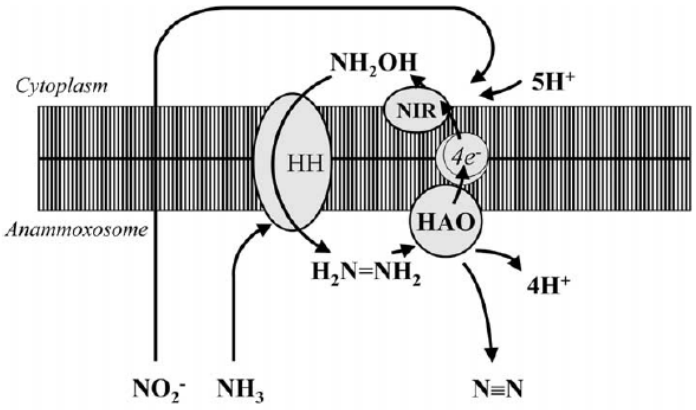

Figure 2.9. Biochemical pathway (Kuenen and Jetten, 2001)

3.2.2.5 Environmental conditions affecting Anammox

3.2.2.5.1 TAN, TNO_2 and NO_3^- inhibition

The Anammox process is not inhibited by TAN or by the by-product nitrate up to concentrations of at least 1 gN L^{-1}. In the presence of more than 100 mgTNO_2-N L^{-1}, Strous *et al.* (1999b) found that the Anammox process was completely inhibited. Fux (2003) showed in a long term experiment that maintaining a TNO_2 concentrations of 40 mgTNO_2-N L^{-1} over several days led to the inactivation of the Anammox organisms.

Remarkable is also the difference in tolerance for TNO_2 between the different Anammox genera. The inhibition experiments conducted by Strous *et al.* (1999b) were performed with *Candidatus Brocadia anammoxidans*. Experiments of Egli *et al.* (2001) with *Candidatus Kuenenia stuttgartiensis* showed that the Anammox process was only inhibited at concentrations higher than 182 mgTNO_2-N L^{-1}. Furthermore, experiments by Strous *et al.* (1999b) showed that increasing the TNO_2 concentration changed the stoichiometry of TAN and TNO_2 consumption from 1.3 gTNO_2-N/gTAN-N at 0.14 gTNO_2-N L^{-1} to almost 4 gTNO_2^--N/gTAN-N at 0.7 gTNO_2-N L^{-1}. From the distorted stoichiometry at high TNO_2 concentrations, it can be concluded that the micro-organisms under these conditions did not only use TAN as the electron donor but also must have generated an internal electron donor to reduce the TNO_2. This changing stoichiometry was also noticed at higher temperatures.

3.2.2.5.2 Oxygen

The anaerobic ammonium oxidation is strongly influenced by a number of physical and chemical factors. Oxygen completely inhibits the process at concentrations above 0.01 mgO_2 L^{-1} (van de

Graaf *et al.*, 1996) although Anammox has catalase and superoxide dismutase (Strous, 2000). It became clear from experiments with intermittent oxygen supply that this oxygen inhibition is reversible (Figure 2.10) (Strous *et al.*, 1997a). The horizontal bars below the graphs in this Figure indicate whether influent was supplied (shaded portion of bar) and whether air or argon was sparged through the reactor (shaded portions of bars). The dotted lines show the TAN and TNO_2 profiles that were expected when no Anammox activity occurred. Since the actual concentrations are lower than the expected concentrations it can thus be concluded that Anammox activity recovers from aerobic conditions.

This reversible inhibition makes partial nitrification and Anammox possible in one reactor (Strous *et al.*, 1997a). Both aerobic and anaerobic ammonium oxidisers can grow under oxygen limited conditions (Third *et al.*, 2001).

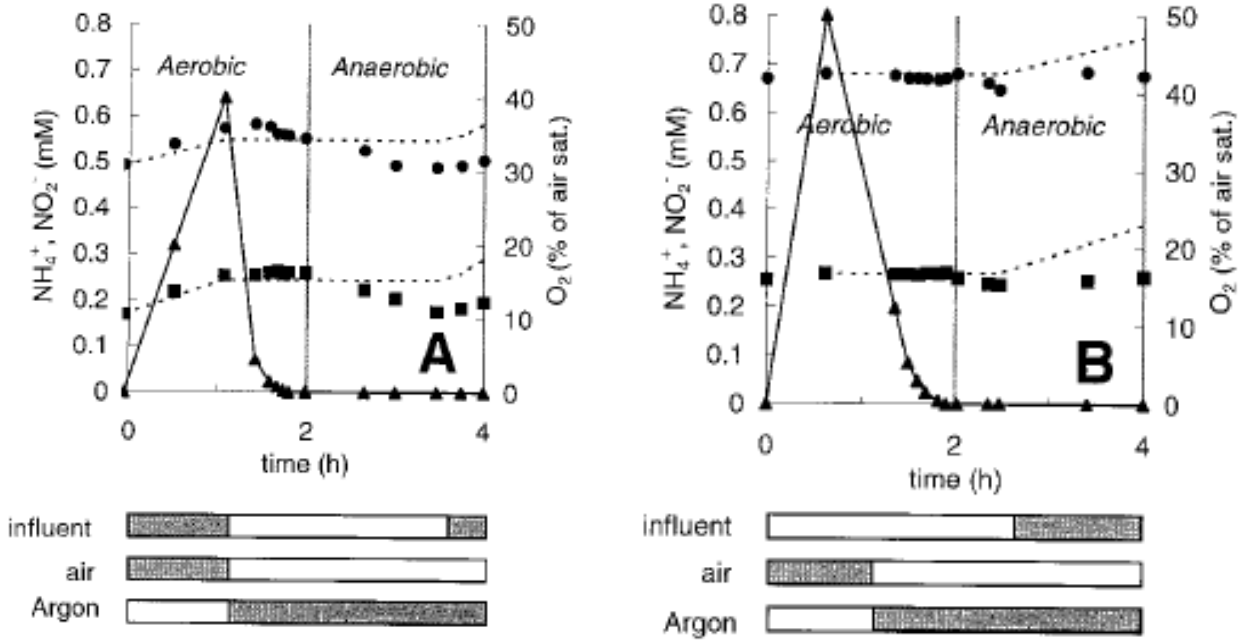

Figure 2.10. Anammox process under alternating aerobic and anaerobic conditions: TNO_2 (squares), TAN (circles), oxygen (triangles) and expected concentrations in absence of Anammox (dotted lines). Figures (A) and (B) represent different influent supplies (after Strous *et al.*, 1997a).

3.2.2.5.3 *Phosphate*

Similarly to TNO_2 inhibition a difference in tolerance for phosphate exists between *Candidatus Brocadia anammoxidans* and *Candidatus Kuenenia stuttgartiensis*. van de Graaf *et al.* (1996) experienced a loss of activity for *Candidatus Brocadia anammoxidans* at phosphate

concentrations above 155 $mgPO_4^{3-}$-P L^{-1}, while Egli *et al.* (2001) did not see any inhibitory effect of phosphate when a culture of *Candidatus Kuenenia stuttgartiensis* was supplied with up to 620 $mgPO_4^{3-}$-P L^{-1}. However, in batch tests using sludge from a highly loaded lab-scale rotating biological contactor containing *Candidatus Kuenenia stuttgartiensis*, phosphate was shown to partially inhibit the Anammox process (Pynaert *et al.*, 2003). Anammox activity decreased to 63% of the normal activity at 55 $mgPO_4^{3-}$-P L^{-1} and further to 20% at 110 $mgPO_4^{3-}$-P L^{-1}. At 285 $mgPO_4^{3-}$-P L^{-1} no further decrease was observed (80% inhibition).

3.2.2.5.4 Temperature and pH

According to Egli *et al.* (2001) *Candidatus Brocadua anammoxidans* prefers temperatures around 37°C. The *Candidatus Brocadia anammoxidans* has a minimum and maximum operating temperature of 10°C and 43°C respectively and an optimal operating temperature of 37°C (Strous *et al.*, 1999b). The optimal temperature of the Anammox micro-organisms studied by Dalsgaard and Thamdrup (2002) is however 15°C. Similarly, Rysgaard *et al.* (2004) found an Anammox optimal temperature of 12°C and Anammox activity in Arctic sediments at temperatures as low as –1.3°C. Apparently in an Arctic environment Anammox organisms are adapted to lower temperatures.

The optimal pH interval for Anammox organisms is 6.7 to 8.3 with an optimum of 8.0. Data concerning optimal temperature and pH intervals for *Scalindua* Anammox organisms are not available yet since these organisms were only discovered very recently.

3.2.2.5.5 Biomass concentration

The biomass concentration plays a crucial role for the Anammox activity. Strous *et al.* (1999a) found that Anammox is only active when cell concentrations are higher than 10^{10}-10^{11} cells ml^{-1}, even in purified cultures. This could be explained by the need for intercellular communication for activity (Hellingwerf *et al.*, 1998). Another potential explanation is that hydrazine diffuses relatively easy to the outside of the cell and a minimum concentration is necessary for Anammox activity. Sinninghe Damsté *et al.* (2002) however showed that the cellular membranes are less permeable than normal linear membrane lipids.

Maybe the presence of contaminating cells, 1 on 200-500, is necessary to sustain long term growth, because these cells can guarantee vitamin supply and the removal of toxic components (Kuenen and Jetten, 2001; Strous *et al.*, 1999a).

Pynaert *et al.* (2004) put forward the hypothesis that the presence of ammonium oxidizers is necessary for the re-activation of Anammox organisms after disturbance of the system. By the production or accumulation of NH_2OH by ammonium oxidizers, Anammox organisms can re-activate their metabolism. Once the process is re-established, the ammonium oxidizers are not

supposed to significantly participate in the Anammox process. This "sparking" was also described by Strous (2000) because it was found that the addition of the intermediates NH_2OH or N_2H_4 was necessary to restart Anammox activity after inhibition.

3.2.2.5.6 Other influencing factors

Experiments with continuous Anammox cultures by van de Graaf *et al.* (1996) showed that carbon sources such as acetate, glucose and pyruvate had a negative effect on Anammox activity. Anammox activity was also found to be sensitive to visible light. A decrease in activity of 30 to 50 % was observed by van de Graaf *et al.* (1996). As a result the equipment for further experiments by these researchers was covered with black plastic and paper to eliminate this light effect.

3.3 Practical implementation of autotrophic nitrogen removal

An Anammox reactor has to be preceded by a partial nitritation step. This can be accomplished in the same reactor (1-reactor system) or by using 2 separate reactors (2-reactor system). Typically the 1-reactor system is a biofilm reactor where the ammonium oxidizers are active in the outer layers of the biofilm, producing a suitable amount of TNO_2 for the Anammox organisms that are active in the inner layers. This way the Anammox organisms are protected from oxygen, which is consumed in the outer layers. However, examples of completely mixed 1-reactor systems were also described in literature (e.g. Sliekers *et al.*, 2003).

With a 2-reactor system nitritification and Anammox are separated in space. In a first reactor half of the TAN is converted to TNO_2, while in a second reactor Anammox is active. It is important that the influent of the Anammox reactor has a constant composition in view of the TNO_2 toxicity, independent of the strategy used to obtain this Anammox suited influent. With a 1-reactor system generally a higher volumetric nitrogen removal rate can be obtained for low loaded streams (Wyffels *et al.*, 2004a) and an important footprint reduction can be accomplished. A 2-reactor system is more flexible and will result in a more stable operation as both steps can be controlled separately (Verstraete and Phillips, 1998; Jetten *et al.*, 2002; Wyffels *et al.*, 2004a). Furthermore, with this system streams with higher nitrogen concentrations can be treated.

An overview of different reactors described in literature will be presented here. From this overview several things will become clear. First autotrophic nitrogen removal is mostly performed at temperatures above 30°C. Second, most studies concerning 2-reactor systems deal with either the first step or the second step. Few studies exist where both are coupled. Third, most of the experimental knowledge on autotrophic nitrogen removal is restricted to lab scale reactors, while full-scale expertise is very limited. Several questions on upgrading these promising processes are therefore not yet answered today.

3.3.1 1-reactor systems

Various names are used to decribe the 1-reactor systems (Fux, 2003): the OLAND-proces (oxygen limited autotrophic nitrification and denitrification) (Kuai and Verstraete, 1998), the CANON process (completely autotrophic nitrogen removal over nitrite) (Third *et al,* 2001) and aerobic/anoxic deammonification (Hippen *et al.*, 1997). The difference lies in the organisms that were originally assumed to be responsible for anaerobic ammonium oxidation. In both the OLAND-process and the aerobic/anoxic deammonification process nitrifiers were assumed to perform this ammonium oxidation under micro-aerobic conditions (Kuai and Verstraete, 1998; Helmer *et al.*, 1999). In the CANON process Anammox bacteria were assumed to be responsible. Recent studies (Pynaert *et al.*, 2003; Helmer-Madhok *et al.*, 2002) with FISH analyses confirmed that anaerobic ammonium oxidation in all reactors was performed by Anammox organisms, although Pynaert *et al.* (2003) did not exclude a specific role for the aerobic ammonium oxidizers.

Kuai and Verstraete (1998) first introduced the term OLAND describing lab-scale research with a 4 litre SBR reactor fed with synthetic influent containing 1 gTAN-N L^{-1} operated at 33°C and a pH of 7.2. About 50 mgTAN-N L^{-1} d^{-1} was removed from the reactor. However, for practical implementation this removal rate is far too low. Pynaert *et al.* (2002, 2003 and 2004) therefore constructed, operated and characterized an OLAND RBC system where high removal rates could be achieved. The reactor had a total water volume of 50 litres and was operated at a HRT of 1 day. Both synthetic and actual waste water were used as influent, with a TAN concentration of about 1 gTAN-N L^{-1}. The oxygen concentration was controlled at 0.8 mgO_2 L^{-1}. Within 100 days after inoculation a maximum TAN removal of 206 mgTAN-N L^{-1} d^{-1} was achieved.

The term aerobic/anoxic deammonification was first used when significant losses of inorganic nitrogen of up to 90 % were observed in the nitrification step of a rotating biological contractor (RBC) treating TAN-rich landfill leachate under low oxygen conditions (Hippen *et al*, 1997). Extended nitrogen loss was also observed in other RBC's in Switzerland and the UK (Siegriest *et al.*, 1998; Hippen *et al*, 2001). Influent nitrogen concentration to these RBC varied between 200 and 400 mgN L^{-1} (Hippen *et al*, 2001). None of the plants were built specifically for deammonification, but nitrogen elimination was established over time. In the Swiss RBC about 50 % of the bacteria population in the biofilm consisted of Anammox. Next to RBC's continuous flow moving-bed pilot plants were run as well. Optimal TAN elimination was achieved at a bulk oxygen concentration of 0.7 mgO_2 L^{-1}. The end product is always N_2, although Gaul *et al.* (2002) reported up to 12% N_2O production caused by incomplete heterotrophic denitrification under anoxic or oxygen-limited conditions.

The first full-scale application with deliberate deammonification in a moving bed reactor using Kaldnes® carriers was put into operation in April 2001 (Jardin *et al.*, 2001) at the WWTP of

Hattingen (Germany). Two identical reactors had a volume of 67 m^3 and an effective biofilm surface area of 13400 m^2. The oxygen concentration was kept below 1 $mgO_2\ L^{-1}$. First results are given in Cornelius and Rosewinkel (2002).
At the Delft University of Technology the 1-reactor system was termed CANON. Sliekers *et al.* (2002) and Sliekers *et al.* (2003) conducted experiments in lab-scale completely mixed reactors. Both reactors were started up by gradually introducing air in an Anammox reactor. In Sliekers *et al.* (2002) a SBR with a volume of 2 litre was operated at a HRT of 1 day, a temperature of 30°C and a pH of 7.8. Average influent and effluent concentrations were 131 and 56 $mgTAN\ L^{-1}$. In Sliekers *et al.* (2003) a gas lift reactor with a volume of 1.8 litre was operated at a HRT of 10 hours, a temperature of 30°C and a pH of 7.5. Average influent and effluent concentrations were 1545 and 899 $mgTAN\ L^{-1}$. This effluent concentration is still very high, although more then 600 $mgTAN\ L^{-1}$ was removed. In these completely mixed CANON reactors the oxygen concentration should always be below the detection limit in view of oxygen inhibition. With a simulation study Hao *et al.* (2002a) showed that the optimal bulk oxygen concentration for a CANON biofilm reactor is about 1 $mgO_2\ L^{-1}$, although this optimum depends on the biofilm thickness and density, boundary layer thickness, the COD content of the influent and the temperature. Oxygen control is therefore necessary.

3.3.2 Two reactor systems

3.3.2.1 Partial nitritation

The challenge for the first reactor is to obtain a stable, Anammox-suited effluent, i.e. with a molar TAN:TNO_2 ratio of 1:1.32 according to the stoichiometry proposed by Strous *et al.* (1998). In practice however this ratio will be closer to 1 in view of the desire to prevent TNO_2 inhibition, i.e. by providing an excess of TAN. Up to now three types of reactors were used: completely stirred tank reactors (CSTR), membrane bioreactors (MBR) and sequencing batch reactors (SBR).
The possibility to obtain an Anammox-suited effluent was tested by van Dongen *et al.* (2001a&b) in a 10 litre CSTR at a temperature of 30°C and a HRT of 1 day. The pH was not controlled and had an average value of 6.7. The average influent concentration was 1.18 $gTAN\text{-}N\ L^{-1}$. This TAN was for 53% oxidized to TNO_2 resulting in a TAN:TNO_2 ratio of 1.13. In the subsequent Anammox reactor TNO_2 was therefore the limiting component. Fux *et al.* (2002) also operated a CSTR to obtain a suited effluent. The 2.1 m^3 reactor was operated at a HRT of 1.1 days and a temperature of 30°C without pH control. Digester effluent from two different WWTPs was tested. The TIC:TAN ratio of both effluents was 1.2, while the TAN concentrations were about 625 $mgTAN\text{-}N\ L^{-1}$. At a pH between 6.6 and 7.2 an Anammox suited TAN:TNO_2 ratio of 1:1.32 was obtained. Udert *et al.* (2003) describe the partial nitritation of urine in a CSTR with a HRT of

4.8 d. Urine is characterized by a high TAN concentration (8200 mgTAN-N L^{-1}) and a high pH (9.2). A TAN:TNO_2 ratio of 1.0±0.05 was obtained. Egli *et al.* (2003) also produced an Anammox-suited influent in a 3 litre CSTR fed with synthetic influent at a concentration of 750 mgTAN-N L^{-1}. At 30°C and pH 7.5 the correct ratio was obtained at a HRT of 3.33 d. The reactor was however only operated for 1 or 2 weeks.

Wyffels *et al.* (2003) used a 1.5 l MBR as a first step of the autotrophic nitrogen removal process at low DO concentrations (<0.1 mgO_2 L^{-1}). The membrane had to be regularly cleaned to prevent clogging. The pH was controlled at 7.9 and the temperature was set to 35°C, although an experiment at room temperature was conducted too. Lowering the temperature had no significant effect on the obtained TNO_2:TAN ratio as can be seen from Table 2.5. Similarly, lowering the NH_3 concentration, and possibly lowering the NH_3 inhibition on nitrite oxidizers, had no significant effect on the obtained TNO_2:TAN ratio. This indicates that oxygen limitation is the most important operational factor.

Table 2.5. Influence of temperature, reactor NH_3 concentration and volumetric nitrogen load ($B_{v,N}$) on the TAN:TNO_2 ratio in an MBR reactor (Wyffels *et al.*, 2003)

$B_{v,N}$ [gTAN $L^{-1}d^{-1}$]	NH_3-N [mgNH_3-N L^{-1}]	Temperature [°C]	TNO_2:TAN:NO_3^- [-]
1	>20	35	0.81 / 1 /0.07
1	>20	22-24	0.91 / 1 / 0.03
0.5	>20	35	0.87 / 1 / 0.06
0.2	>7	35	0.89 / 1 / 0.08

Udert *et al.* (2003) used a 7.5 litre SBR to treat urine with a TAN concentration of 700-1300 mgTAN-N L^{-1}. The temperature was 24.5 °C and the oxygen concentration varied between 2 and 4.5 mgO_2 L^{-1}. The pH at the start of the reaction cycle was 8.8 and gradually decreased to a minimum of 6 as TAN conversion continued. At this pH TAN conversion stopped without apparent reason and a TAN:TNO_2 ratio of 1 was obtained.

Fux (2003) operated a 2.1 m^3 SBR at 30 °C. The oxygen concentration was controlled between 2 and 3 until the pH in the reactor dropped below 6.9. At that time aeration was put off. Influent TAN concentration of the digester effluent was on average 675 mgTAN-N L^{-1}, while the average TNO_2:TAN ratio was 1.47. This is somewhat high for safe operation of the Anammox reactor.

3.3.2.2 Anammox

Anammox reactors have start-up times up to 300 days because of the low growth rate and low cell yield of Anammox organisms. Hence, it is essential to use a reactor with high biomass retention. So far a large range of bioreactors have been evaluated for the enrichment of Anammox bacteria: trickling filters, packed-bed reactors, moving bed reactors, fluidised-bed-reactors, UASB-reactors, SBR, gas-lift reactors, MBR (Strous *et al.*, 2002; Wyffels *et al.*, 2004a). There are important differences between the first four and the last four bioreactors, as explained by Strous *et al.* (2002). In the former, the microbes grow as biofilms on a support material, while in the latter they form free-floating aggregates. These aggregates are maintained in the system via a settler, a membrane or, in a sequencing batch reactor by periodically allowing the aggregates to settle in the reactor itself before removing any effluent. The aggregates are well mixed and therefore the community composition is constant over all aggregates and the aggregates are spatially unorganised. Such reactors are the best option to study the black-box physiology of the aggregates. Biofilm reactors are not well mixed and the biofilm community differs in different parts of the reactor. Furthermore, biofilms are spatially organised and when they are disrupted, a loss of activity may occur, as is well documented for Anammox (Mulder *et al.* 1995; van de Graaf *et al.* 1995). Black box physiological study of such biofilms is impossible because the results are not reproducable according to Strous *et al.* (2002). However, these reactors are best for the initial enrichment of the desired microbes, because the conditions are different, both in the reactor as a whole and in the biofilm itself. It is more likely that somewhere in the system the conditions are sufficiently selective for the desired microbe.

A summary of the experimental studies described in literature is given in Table 2.6. From these studies the potential of the Anammox process can be seen as total nitrogen concentrations up to 2500 mgN L^{-1} are applied in the influent. The temperature and pH in the reactors are controlled between 30°C and 40°C and 7 and 8 to ensure optimal operational conditions. Almost always an excess of TAN is used and nitrate is present in the effluent. Also real wastewater is seldom used, probably because problems, such as inhibition, can be expected. Anammox reactors are purged with Ar or N_2 gas to ensure anoxic conditions. The fast start-up time of 14 days in an SBR reactor by Sliekers *et al.* (2002) was due to the inoculation of the reactor with fully active Anammox sludge. For the other reactors start-up time was significantly higher.

As most of the studies are still lab-scale applications, the challenge now lies in operating the Anammox process with full-scale reactors. The first full-scale Anammox reactor is currently being start-up in WWTP Dokhaven in Rotterdam (The Netherlands) Rotterdam, The Netherlands, as an addition to the SHARON reactor that is already in place. The reactor is estimated to have a return on investment of less than 7 years, because addition of methanol (currently used to sustain the denitrification) will no longer be required (Schmidt *et al.*, 2003).

Table 2.6. A summary of the experimental studies on Anammox

Type reactor	Start up time [d]	T [°C]	pH [-]	V [L]	HRT [d]	final influent concentration after start up [mgN L^{-1}]		final effluent concentrations after start up [mgN L^{-1}]			Type of waste water	Reference
						TAN	TNO_2	TAN	TNO_2	NO_3^-		
Fluidised bed reactor	83	30	7.0	2.5	0.18	420	490	70	0	70	Synthetic	van de Graaf (1996)
	80	36	8.0	2.3	0.83	840	840	80	0	ND	Synthetic	Strous *et al.* (1997b)
Fixed bed reactor	200	22	7.8	120.0	1.75	370	400	30	5	68	Synthetic	Fux *et al.* (2004)
SBR	120	32-33	7-8	15.0	0.93	517	502	103	0	105	Synthetic	Strous *et al.* (1998)
	14	30	7.8	2.0	1.00	257	203	103	0	45	Synthetic	Sliekers *et al.* (2002)
	130	31	7.5	2100	1.25	380	430	75	0	13	Effluent of SHARON reactor[b]	Fux *et al.* (2002)
	175	30-37	7-8	10.0	1.00	548	598	ND	0	ND	Effluent of SHARON reactor[b]	van Dongen *et al.* (2000)
	120	35	7.8-8	1.0	0.62	410	500	20	5	40	Synthetic	Dapena-Mora *et al.* (2004c)
Fixed bed	NA	36	8.0	1.4	0.84	840	840	65	0	ND	Synthetic	Strous *et al.* (1997b)
	60	37	7-8	1.0	1.67	90	90	5	5	ND	Synthetic	Toh *et al.* (2002)
gas lift reactor	100	30	7.5	1.8	0.28	1358	1370	275	4	ND	Synthetic	Sliekers *et al.* (2003)
	180	30	8.0	3.5	0.50	899	1100	20	5	ND	Synthetic	Dapena-Mora *et al.* (2004b)
	105	30	7-8	1.5	1	280	280	10	10	ND	Synthetic	Zheng *et al.*, 2004
CSTR[a]	200	30	7-8	10.0	5.00	644	784	30	0	100	Synthetic	Guven *et al.* (2004)
Uplflow reactor	350	30	7.5	200.0	0.38	868	1168	82	5	139	Effluent from partial nitrification reactor[b]	Imajo *et al.* (2004)
Non woven biomass carrier biofilm reactor	NA	25	ND	2.5	0.30	200	200	60	40	40	Synthetic	Furukawa *et al.* (2001)
MBR	NA	20-30	8.0	1.5	0.750	400	400	40	0	37.5	Effluent from partial nitrification MBR [b]	Wyffels *et al.* (2004a)

NA: not applicable/ ND: not determined

a: with outflow tube with perforated division (complete biomass retention)

b: fed with sludge digester effluent

PART 3

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MODEL BUILDING AND DEVELOPMENT WITH WEST®

Chapter 3.1

Model building and simulation with WEST®

ABSTRACT

The modelling and simulation environment WEST® provides the modeller a user-friendly platform to use existing models or to implement and test new models. Currently WEST® is mainly applied to the modelling and simulation of WWTP. Also in this thesis the simulator was used. WEST® consists of a modelling environment where the WWTP configuration is implemented and an experimentation environment where simulations, parameters estimations, scenario analysis, sensitivity analysis and optimal experimental design calculations can be performed. Both environments are linked by the modelbase where the models are implemented in the MSL-USER modelling language.

1 INTRODUCTION

Modelling, simulation and optimization is an efficient, cost reducing and elegant way to accomplish the well functioning of wastewater treatment plants (Coen *et al*., 1996). New techniques, such as autotrophic nitrogen removal systems, are being implemented and new installations are built every day. As a consequence the modelling exercise becomes more and more complex and challenging.

A first step in this exercise is the selection of an appropriate modelling and simulation environment. Criteria such as user-friendliness, reuse of previously developed models and versatility should be considered here.

Several packages for modelling and simulation of WWTP are available (Vanhooren *et al*., 2003, Copp, 2002). Typically, four types of simulators can be distinguished. First of all, it is possible to manually implement code in a programming language like Fortran or C++. Secondly, general-purpose simulators like Matlab/Simulink are available. In the third place, closed dedicated simulators like Biowin, EFOR and STOAT have been developed, which are specifically designed for modelling WWTPs and in which only predefined mathematical models can be used. Finally, open dedicated simulators like Aquasim, GPS-X, SIMBA and WEST® are also specifically designed for modelling WWTPs, but the user can write and use his own specific models if the models available do not fulfil his needs.

An overview of the different simulators used for WWTP modelling is presented in Table 3.1.1. In the Table results are presented from a questionnaire seeking information on the status of commercial WWTP simulators performed by Melcer *et al.* (2003) corrected with the most recent data. All these simulators can be used on a Microsoft Windows operating system.

Because of the in-house experience, direct application to WWTP and possibility to reuse previously developed models, the WEST® simulator was chosen as modelling and simulation environment for the work performed in this thesis. The information on WEST® presented in this chapter was derived from Vanhooren *et al.* (2003), Vanhooren (2001) and Meirlaen (2002), with the addition of the most recent developments. Further the different types of virtual experiments that can be performed with WEST® will be introduced shortly. Finally the numerical solvers to be used for simulations will be discussed.

Table 3.1.1. Summary of different dedicated WWTP simulators (modified from Melcer *et al.*, 2003)

Simulator	Aquasim	BioWin	EFOR	GPS-X	SIMBA	STOAT	WEST
Year introduced	1994	1990	1990	1991	1994	1994	1998
Company/institute	EAWAG	Envirosim	DHI	Hydromantis	IFAK systems	WRc	Hemmis/ BIOMATH
Country	Switzerland	Canada	Denmark	Canada	Germany	UK	Belgium
Current version	2.1	2.1	2003	4.1	4.0	4.2	3.7
Licenses sold/leased	>100	>300	100	>100	>170	>150	>150
User group (Percent of total)							
Consultants	20	66	60	40	24	50	40
Operators	0	12	30	20	23	48	10
Academic	80	20	10	30	30	2	45
Others	0	2	0	10	23	0	5

2 THE WEST® MODELLING AND SIMULATION SOFTWARE

2.1 General decription

The modelling and simulation package WEST® (World Wide Engine for Simulation, Training and Automation) provides the modeller with a user-friendly platform to use existing models or to implement and test new models (Vanhooren *et al*., 2003). WEST® is a modelling and simulation environment for any kind of process that can be described as a structured collection of Differential and Algebraic Equations (DAEs). Currently, however, WEST® is mainly applied to the modelling and simulation of wastewater treatment plants (Vangheluwe *et al.*,1998). Basically the WEST® modelling and simulation software consist of two environments: the modelling environment, which aims to enable reuse of model knowledge and the experimentation environment, which aims at maximising accuracy and performance. In the modelling environment the user can graphically implement the system under study as depicted in Figure 3.1.1. This can for example be a full scale WWTP, but also a lab-scale set-up. For each icon, representing a WWTP unit operation, that is put on the canvas of the modelling environment different models can be selected which are coded in the modelbase.
In the experimentation environment (Figure 3.1.2) the user can perform simulations, parameter estimations, sensitivity analysis, scenario analysis and optimal experimental design calculations. A strict distinction is made between these two environments.

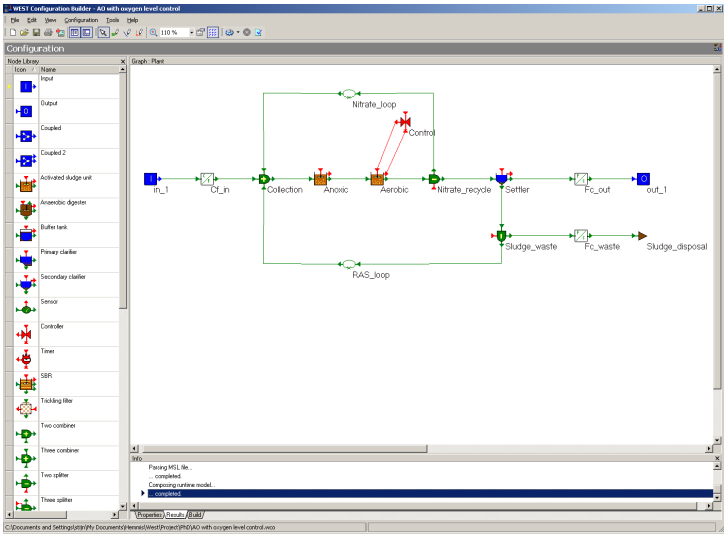

Figure 3.1.1. The graphical implementation of a WWTP in WEST®.

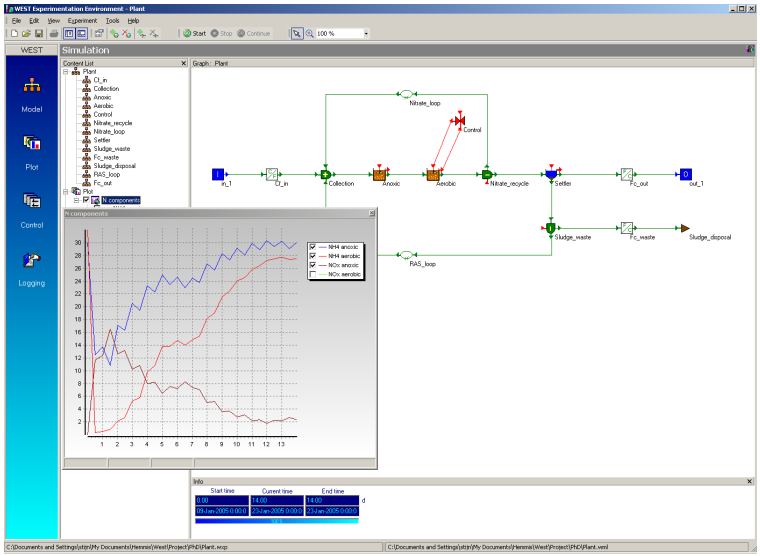

Figure 3.1.2. The experimentation environment in WEST®.

Next to these two user environments, the model base plays a central role in WEST®. In this model base, models are described in MSL-USER (MSL stands for model specification language), a high level object-oriented declarative language specifically developed to incorporate models, which allows for the declarative representation of the dynamics of systems. 'Declarative' means that the model is presented without specifying how to solve it.

The model base is aimed at maximal reuse of existing knowledge and is therefore structured hierarchically. All reusable knowledge – such as mass balances, physical units, default parameter values and applicable ranges – is thus defined centrally and can be reused by an expert user to build new models. This indicates that WEST® has an open structure and that the user is allowed to change existing models and define new ones as needed.

Both standard C as user-defined C++ functions can be used as external functions in the MSL-USER models. An example of such a user-defined function is the calculation of the pH in the activated sludge process.
To allow easy implementation of the models in WEST® a MSL editor was developed. This MSL editor offers a lot of functionality to create, edit and browse the MSL files. The editor provides the possibility to check the MSL files syntactically.

3 THE EXPERIMENTATION ENVIRONMENT: DIFFERENT EXPERIMENTS

As discussed above different experiments can be conducted in WEST®. Below, these different experiment types as implemented in the WEST® environment are presented.

3.1 Simulation experiment

The simulator calculates the trajectory of different variables over a certain time, by using the model, a set of parameter values, an input file, initial values of the state variables and a numerical integrator with associated settings. Output of the trajectory can be given in files, plots and other programs such as Excel.

3.2 Scenario analysis

It is possible to automatically perform a series of virtual experiments in WEST®. Output and integrator options can be controlled interactively. Most of the scenario-analysis performed in this work were performed this way.

3.3 Trajectory optimisation experiment

In this virtual experiment certain model parameters are varied by a number of search algorithms to minimise the distance between a simulated trajectory and a given (measured or desired) trajectory. This is mostly done for (constrained) parameter estimation, but it can also be used for controller tuning and process design optimisation. The distance measure is typically a sum of squares of differences between measured and simulated values though absolute values can also be used. The difference between measured and simulated values can be calculated at different points in time. In general, the differences can be weighed to account for measurement accuracy and possible differences in the order of magnitude of the different values in the objective function. Dochain and Vanrolleghem (2001) give an overview of optimisation methods that can be used. Two methods are implemented in WEST®. The method developed by Nelder and Mead (1964) (the simplex method) and the Praxis method (Brent, 1973) are implemented. Both are rather robust to local minima and quite efficient in terms of convergence. Genetic algorithms and the

Shuffled Complex Evolution (SCE) algorithm (Duan *et al.*, 1992) are currently under implementation.

3.4 End value optimisation experiment

Here the optimiser is used to vary some parameters (possibly constrained) to extremize a goal function that only evaluates variables at the final time of the experiment.

3.5 Sensitivity analysis experiment

The sensitivity of the model outputs with respect to model parameter variations can be investigated. A sensitivity function expresses how the sensitivity of an output variable to a parameter varies with time. The calculation of sensitivity functions is based on the finite difference method. This method calculates the difference between two simulation experiments, a reference experiment and a perturbation experiment. Dividing the difference in model outputs between these two simulation experiments by the parameter change results in the sensitivity function. The perturbation experiment is performed by perturbating a model parameter by a small factor (the perturbation factor). This perturbation factor can be positive or negative, depending on whether the user wants to use the forward or backward difference method for calculation of the sensitivity functions. Also an average value of both methods can be calculated, called the central difference method (De Pauw and Vanrolleghem, 2003).
Sensitivity functions cannot be easily compared as different sensitivity functions are expressed in different units. For easy comparison of sensitivities the relative sensitivity functions can be calculated. This is done by multiplying a sensitivity function with the corresponding parameter value and dividing it by the corresponding variable.

3.6 Optimal experimental design

Sensitivity functions form the basis of optimal experimental design because they indicate where the measurements are most sensitive to the parameters. Moreover, the Fisher Information Matrix (FIM), which is the cornerstone of experimental design, is calculated using sensitivity functions. This matrix is a measure for the information content of the simulated experiment. Under certain conditions (uncorrelated white measurement noise) the inverse of this FIM gives the lower bound of the parameter estimation covariance matrix according to the Cramer-Rao inequality (Ljung, 1999; Walter and Pronzato, 1997).
Measurement campaigns should conducted under conditions where the FIM is maximal. Maximizing this matrix can be done in different ways, which are all implemented in WEST® (De Pauw and Vanrolleghem, 2003b). The A- and D-optimal criterion aim at minimising the arithmetic and geometric mean of the identification errors respectively. The modified A-criterion

is similar to the A-criterion. The E-criterion aims at experimental designs that minimize the largest error. Finally the modified E-criterion tries to minimize the correlation between estimated parameters (Dochain and Vanrolleghem, 2001).

4 THE EXPERIMENTATION ENVIRONMENT: NUMERICAL SOLVERS IN WEST®

Different numerical solvers can be chosen interactively in the WEST® experimentation environment. Over ten different solvers are available, but only the ones used in this thesis will be discussed shortly. Basically two different types of simulations can be distinguished in this work: simulations with "simple" systems, i.e. systems consisting of 1 to 10 (completely mixed) process units and simulations with a biofilm system. For the first type of systems the WEST® default 4th order Runge-Kutta solver (Butcher, 1987) with variable time step size (*RK4ASC*) was used. During the development of the COST simulation benchmark for activated sludge systems, it was shown that this numerical integrator is preferred for simulating wastewater treatment systems (Copp, 2002). This method is a single step method because only the variables at the previous time step are used to calculate the variables of the next time step. This single step method, however, was not sufficiently strong to solve the complex and stiff biofilm model. A more powerful multistep method was used for solving this biofilm model which is implemented in the solvers LSODA (Petzold, 1983) and CVODE (Cohen and Hindmarsh, 1996). Because the LSODA C code was obsolete and could not be made thread-safe without a huge effort, only the CVODE solver was implemented in WEST®. In this solver the Adams method (orders 1-12 with functional iteration to solve the corrector equation) for non-stiff problems and backward differentation formulas (BDF) (orders 1-5 with Newton iteration) as the family of stiff methods is implemented. In contrast to LSODA no automatic switching between the solvers is implemented in CVODE. With this solver the user must therefore determine beforehand if the system under study is stiff or non-stiff and choose the correct solver (Cohen and Hindmarsh, 1996). Both methods proved to solve the biofilm model adequately, although the BDF solver is still considerably faster.

5 CONCLUSION

The WEST modelling and simulation software was selected for model building and model simulations as it offers a user-friendly platform to use existing models or to implement and test new models. The model base is written in MSL-USER in which standard and user defined C functions can be used. In the graphical modelling environment, the physical layout of the plant can be rebuilt, and each building block can be linked to a specific model from the model base. In the experimentation environment, the user can design different experiments like simulations, optimisations, scenario analysis and optimal experimental design.

Chapter 3.2

Modelling of autotrophic nitrogen removal

ABSTRACT

In WEST® a model for autotrophic nitrogen removal is implemented. The ASM1.e model is an extension of ASM1 with Anammox and two-step nitrification and denitrification. In the nitrification and denitrification model ammonia rather than ammonium, and nitrous acid rather than nitrite were used as actual substrates for ammonium oxidizer and nitrite oxidizer growth, respectively. In contrast to this, total ammonia nitrogen and total nitrite nitrogen instead of ammonia and nitrous acid were used to describe the Anammox growth kinetics, because it is not yet determined whether the uncharged form is the real substrate. For heterotrophic biomass kinetics from ASM1 were used. All kinetics were made dependent of temperature by applying Arrhenius type equations.

A method for calculating the pH, a very important variable in the autotrophic nitrification process, was implemented in WEST®. The method uses a charge balance for this pH-calculation.

A one-dimensional biofilm model was also implemented in WEST®. The model incorporates advective flow in the bulk phase, diffusive transport of solubles from the bulk phase to the biofilm, attachment and detachment of the biomass and reaction and diffusion processes in the biofilm.

Finally, the calibration and validation steps necessary in any modelling study are introduced and the need for new experimental techniques is stressed as standard experiments cannot be used straightforward for measuring autotrophic nitrogen removal activity.

Parts of this chapter were published in:

Wyffels, S., Van Hulle, S.W.H., Boeckx, P., Volcke, E.I.P., Van Cleemput, O., Vanrolleghem, P.A. & Verstraete, W. (2004b). Modelling and simulation of oxygen-limited partial nitritation in a membrane-assisted bioreactor (MBR). *Biotechnology & Bioengineering*, **86**, 531-542.

1 INTRODUCTION

In 1983 the 'International Association on Water Pollution Research and Control' (IAWPRC, now International Water Association (IWA)) formed a 'Task Group on Mathematical Modelling for Design and Operation of Activated Sludge Processes' (Henze *et al.*, 1987) to promote the development and facilitate the application of practical models for the design and operation of biological waste water treatment systems. This Task Group developed three consecutive models that described the most important wastewater treatment processes such as nitrification, denitrification and phosphorus removal (Henze *et al*., 2000).

The IWA task group also tried to create a uniform basis for compact representation of the activated sludge models. The task group chose the matrix format introduced by Petersen (1965) for the presentation of its models. The first step in setting up this matrix is to identify the components of relevance in the model. The second step in developing the matrix is to identify the biological processes occurring in the system, i.e. the conversions or transformations which affect the components listed. Before going into details on the components and processes of relevance concerning autotrophic nitrogen removal, the use of the Peterson matrix will be elaborated. Next to the model development, the calibration of the model parameters for autotrophic nitrogen removal will be commented upon in this chapter, as this calibration is strictly required prior to the application of the model.

2 THE PETERSEN MATRIX: A SIMPLE EXAMPLE

Consider the situation in which heterotrophic bacteria are growing in an aerobic environment by utilizing a soluble substrate for carbon and energy (Henze *et al.*, 2000; Vanhooren *et al.*, 2003). In one simple conceptualisation of this situation, two fundamental processes occur: the biomass increases by cell growth and decreases by decay. Other activities, such as oxygen utilization and substrate removal, also occur, but these are not considered to be fundamental because they are the result of biomass growth and decay and are coupled to them through the system stoichiometry. The simplest model of this situation must consider the concentrations of three components: biomass, substrate and dissolved oxygen. The matrix incorporating the fate of these three components in the two fundamental processes is shown in Table 3.2.1. In conformity with IWA nomenclature (Grau *et al*., 1982), particulate constituents are given the symbol X and the soluble components S. Subscripts are used to specify individual components: B for biomass, S for substrate and O for oxygen.

Only two processes are included in this example: aerobic growth of biomass and its loss by decay. These processes are listed in the leftmost column of the table. The kinetic expressions or

rate equations for each process are recorded in the rightmost column of the Table in the appropriate row. Process rates are denoted by r_j, where j corresponds to the process index.
If a simple Monod model is used for this situation, the rate expressions would be those in Table 3.2.1, although many other rate equations can be easily introduced in WEST®. The Monod equation, r_1, states that growth of biomass is proportional to biomass concentration in a first order manner and to substrate concentration in a mixed order manner. The expression r_2 states that biomass decay is first order with respect to biomass concentration.

Table 3.2.1. Process stoichiometry and kinetics for heterotrophic growth in an aerobic environment (after Henze *et al.*, 2000)

Process j	Component i			Process Rate r_j [$ML^{-3}T^{-1}$]
	1. Biomass X_B	2. Substrate S_S	3. Oxygen S_O	
1. Growth	1	$-\frac{1}{Y}$	$-\frac{1-Y}{Y}$	$\frac{\mu S_S}{K_S + S_S} \cdot X_B$
2. Decay	-1		-1	$b.X_B$
Stoichiometric Parameters: Growth yield Y	mgCOD L^{-1}	mgCOD L^{-1}	mgCOD L^{-1}	Kinetic Parameters: Maximum specific growth rate μ Half-velocity constant K_S Specific decay rate b

The elements within the Table comprise the stoichiometric coefficients, ν_{ij}, which set out the mass relationships between the components in the individual processes. For example, growth of biomass (*+1*) occurs at the expense of soluble substrate ($-\frac{1}{Y}$, Y is the yield parameter) and oxygen ($-\frac{1-Y}{Y}$). The coefficients ν_{ij} can easily be deduced by working in consistent units. In this case, all organic constituents have been expressed as equivalent amounts of chemical oxygen demand (COD); likewise, oxygen is expressed as negative oxygen demand. The sign convention used in the Table is negative for consumption and positive for production.
In matrix form, we obtain a stoichiometry matrix and a kinetics vector represented by equation 3.2.1 and 3.2.2.

$$\nu = \begin{pmatrix} 1 & -\frac{1}{Y} & -\frac{1-Y}{Y} \\ -1 & 0 & -1 \end{pmatrix} \tag{3.2.1}$$

$$r = \begin{pmatrix} \frac{\mu S_S}{K_S + S_S} \cdot X_B \\ b \cdot X_B \end{pmatrix} \tag{3.2.2}$$

Within a system, the concentration of a single component may be affected by a number of different processes (e.g. biomass). An important benefit of the matrix representation is that it allows rapid and easy recognition of the fate of each component, which helps in the preparation of mass balance equations. This Petersen matrix format is implemented in WEST® and a special editor for this matrix exists.

Another benefit of the Petersen matrix is that continuity (i.e. elemental mass balances hold) may be checked per process by horizontally moving across the matrix. This can only be done provided consistent units have been used, because then the sum of the stoichiometric coefficients must be zero. This can be demonstrated by considering the decay process. Recalling that oxygen is negative COD so that its coefficient must be multiplied by -1, all COD lost from the biomass through decay must be balanced by oxygen utilization. Similarly, for the growth process, the substrate COD lost from solution due to growth minus the amount converted into new cells must equal the oxygen used for cell synthesis.

3 THE AUTOTROPHIC NITROGEN REMOVAL MODEL

3.1 Overview

The autotrophic nitrogen removal model (ASM1.e) is based on ASM1 (Henze *et al*., 1987; Henze *et al*., 2000). This model was extended with Anammox and 2-step nitrification and denitrification (Hao *et al*., 2002a&b; Sin *et al.*, 2001; Liebig *et al.*, 2001). Heterotrophic activity in general and denitrification in particular and are strictly speaking not part of autotrophic nitrogen removal, but several studies showed that heterotrophs are present in autotrophic reactors (Wyffels *et al*., 2004b; van de Graaf *et al*., 1996) even when no external COD source is supplied (Kuenen and Gottschal, 1982). For example, Fux *et al*. (2002) noticed a strong decrease in nitrate concentration that was attributed to heterotrophs. Hence, heterotrophs were also incorporated in the ASM1.e model.

The relevant components in the autotrophic nitrogen removal model are:

- Readily biodegradable substrate (S_S)
- Oxygen (S_O)
- Total ammonium nitrogen (i.e. the sum of ammonium (NH_4^+) and ammonia (NH_3)) (S_{TAN})
- Ammonia (S_{NH3})
- Total nitrite nitrogen (i.e. the sum of nitrite (NO_2^-) and nitrous acid (HNO_2)) (S_{TNO2})
- Nitrous acid (S_{HNO2})
- Nitrate (S_{NO3})
- Heterotrophic biomass (X_H)
- Ammonium oxidizers (X_{NH})
- Nitrite oxidizers (X_{NO})
- Anammox biomass (X_{AN})
- Slowly biodegradable substrate (X_S)
- Inert biomass (X_I)

Bacterial growth and decay processes were modelled according to ASM1 (Henze *et al*., 1987) for the heterotrophic (X_H), autotrophic ammonium (X_{NH}) and nitrite oxidizing (X_{NO}) biomass and Anammox biomass (X_{AN}). Decay as described in ASM1 was preferred over endogenous respiration processes as described in ASM3 within the model because these are not yet clearly documented for ammonium and nitrite oxidizers and Anammox. This ASM1 death-regeneration concept is defined as the decay of biomass followed by growth (of heterotrophs) on secondary substrate arising from decay (van Loosdrecht and Henze, 1999). The different processes in the model are summarized schematically in Figure 3.2.1.

Readily degradable substrate (S_S) is used for heterotrophic growth. Readily degradable substrate can be available in the influent and is formed through hydrolysis of slowly degradable substrate (X_S). The slowly degradable substrate is formed during decay of biomass, along with inert particulate matter (X_I). Similar to ASM1 no nitrogen limitation was incorporated in the heterotrophic kinetics. Oxygen (S_O) is used as electron acceptor for heterotrophic growth. In addition, denitrifying heterotrophs use TNO_2 (S_{TNO2}) and nitrate (S_{NO3}) when oxygen becomes limiting.

The growth rate under anoxic conditions was assumed to be lower than the growth rate under oxic conditions, as expressed by the reduction factor η. The reduction factor for nitrate and TNO_2 is assumed to be the same, although discussion continues in literature that a preference for TNO_2 or nitrate exists (Nowak *et al*., 1995). In this respect also a switching function between TNO_2 and nitrate is introduced to prevent the growth rate under anoxic conditions to be higher than the

growth rate under oxic conditions if both TNO_2 and nitrate are present. Also the the anoxic yield was set lower than the aerobic yield according to Muller *et al.* (2003).

In the model presented here ammonia (S_{NH3}) rather than ammonium, and nitrous acid (S_{HNO2}) rather than nitrite were used as substrates since these are also the actual substrates for ammonium oxidizer and nitrite oxidizer growth, respectively (Suzuki, 1974; Anthonisen *et al.*, 1976). The stoichiometry of the reactions was however expressed in terms of corresponding concentrations of TAN (S_{TAN}) and TNO_2 (S_{TNO2}), as typically done (Volcke *et al.*, 2002a). Oxygen (S_O) was used as electron acceptor for nitrifier growth.

Total ammonium nitrogen (S_{TAN}) and total nitrite nitrogen (S_{TNO2}) instead of ammonia and nitrous acid were used to describe the dependency of the growth rate of Anammox on TAN and TNO_2. This is because it is not yet determined whether the uncharged form is the real substrate. It should also be noted that TNO_2 is not only a substrate, but is also inhibiting the Anammox process (Strous *et al.*, 1999b). Therefore, Haldane kinetics instead of Monod kinetics were used for the dependency of the growth rate on TNO_2.

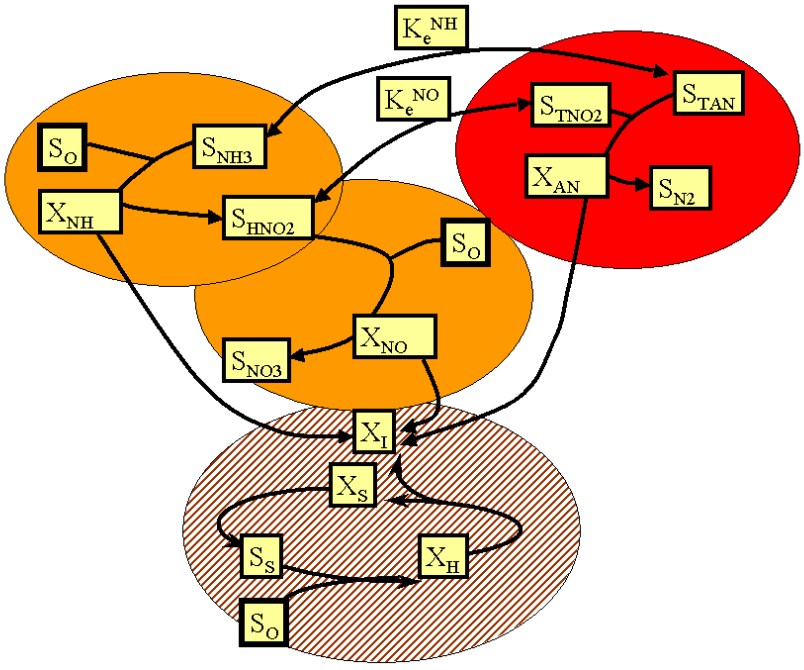

Figure 3.2.1. The extension of ASM1 with Anammox and 2-step nitrification-denitrification.

3.2 Kinetics and stoichiometry

The complete stoichiometric matrix in Petersen matrix format, together with the kinetic expressions are given in Tables 3.2.2 to 3.2.5. Parameter values in the tables are the so-called "default" parameters meaning that these values are used in the simulations, unless otherwise mentioned in the text. Most parameter values were derived from literature as mentioned in the

Tables. However some parameters concerning autotrophic nitrogen removal must be determined using dedicated tests, because adaptation of the organisms to different environmental conditions is reflected in varying parameter values. Especially for ammonium and nitrite oxidizers this was found to be true. Because of the highly concentrated TAN streams discussed in this thesis very different values for the affinity constants were found, up to 10 fold higher compared to values mentioned in literature for nitrifying sludge treating domestic wastewater (see chapters 4.2 and 5.1). Knowledge about Anammox stoichiometry and kinetics had to be gained only from Strous *et al*. (1998) and Strous *et al*. (1999b), indicating the still very limited knowledge on the model parameters of Anammox even though the Anammox organisms have now been studied for more than 10 years. In addition, so far no data exist on decay characteristics of Anammox. This is certainly a topic for future research.

Similar values for the parameter values for Anammox growth kinetics and stoichiometry were used by Hao *et al* (2002a&b), while Koch *et al.* (2000) used higher values for the growth rate (0.08 d^{-1}at 20°C) and affinity constants (21 mgTAN-N L^{-1} and 2 mgTNO_2-N L^{-1}). Hao *et al.* (2002b) stated that this value for the growth rate probably is to high, while the increased value for the affinity constants can be caused by diffusion limitation during experimental determination of the parameter.

Table 3.2.2. Kinetic equations of ASM1.e

	Process	Process rate equation
1	Hydrolysis of entrapped organics	$k_H \frac{X_S / X_H}{K_x + X_S / X_H} X_H$
2	Growth of X_H	$\mu_H^{\max} e^{\theta_H^{growth}(T-Tr)} \frac{S_O}{K_{O,H} + S_O} \frac{S_S}{K_{S,H} + S_S} X_H$
3	Decay of X_H	$b_H e^{\theta_H^{decay}(T-Tr)} X_H$
4	Growth of X_H on NO_3^-	$\mu_H^{\max} e^{\theta_H^{growth}(T-Tr)} \eta_{NO3} \frac{K_{O,H}}{K_{O,H} + S_O} \frac{S_{NO3}}{K_{NO3,H} + S_{NO3}} \frac{S_{NO3}}{S_{TNO2} + S_{NO3}} \frac{S_S}{K_{S,H} + S_S} X_H$
5	Growth of X_H on TNO_2	$\mu_H^{\max} e^{\theta_H^{growth}(T-Tr)} \eta_{NO2} \frac{K_{O,H}}{K_{O,H} + S_O} \frac{S_{TNO2}}{K_{TNO2,H} + S_{TNO2}} \frac{S_{NO2}}{S_{NO2} + S_{NO3}} \frac{S_S}{K_{S,H} + S_S} X_H$
6	Growth of X_{NH}	$\mu_{NH}^{\max} e^{\theta_{NH}(T-Tr)} \frac{S_O}{K_{O,NH} + S_O} \frac{S_{NH3}}{K_{NH3,NH} + S_{NH3}} \cdot X_{NH}$
7	Decay of X_{NH}	$b_{NH} e^{\theta_{NH}(T-Tr)} X_{NH}$
8	Growth of X_{NO}	$\mu_{NO}^{\max} e^{\theta_{NO}(T-Tr)} \frac{S_O}{K_{O,NO} + S_O} \frac{S_{HNO2}}{K_{HNO2,NO} + S_{HNO2}} \cdot X_{NO}$
9	Decay of X_{NO}	$b_{NO} e^{\theta_{NO}(T-Tr)} X_{NO}$
10	Growth of X_{AN}	$\mu_{AN}^{\max} e^{\theta_{AN}(T-Tr)} \frac{S_{TAN}}{K_{TAN,AN} + S_{TAN}} \frac{S_{TNO2}}{K_{TNO2,AN} + S_{TNO2}} \frac{K_{O,AN}}{K_{O,AN} + S_O} \cdot X_{AN}$
11	Decay of X_{AN}	$b_{AN} e^{\theta_{AN}(T-Tr)} X_{AN}$

Table 3.2.3. Stoichiometric matrix of soluble and particulate components of ASM1.e

	Component No	1	2	3	4	5	6	7	8	9	10	11	12
	Name	Oxygen	Readily biodegradable Substrate	TAN	TNO_2	Nitrate	Nitrogen gas	Heterotrophs	Ammonium oxidizers	Nitrite oxidizers	Anammox	Slowly degradable substrate	Inert particulates
	Symbol	S_o	S_s	S_{TAN}	S_{TNO2}	S_{NO3}	S_{N2}	X_H	X_{NH}	X_{NO}	X_{AN}	X_S	X_I
	Unit	$mgO_2\ L^{-1}$	$mgCOD\ L^{-1}$	$mgN\ L^{-1}$	$mgN\ L^{-1}$	$mgN\ L^{-1}$	$mgN\ L^{-1}$	$mgCOD\ L^{-1}$	$mgCOD\ L^{-1}$	$mgCOD\ L^{-1}$	$mgCOD\ L^{-1}$	$mgCOD\ L^{-1}$	$mgCOD\ L^{-1}$
	Process No												
1	Hydrolysis of entrapped organics		1									-1	
2	Growth of X_H	$-(1-Y_H)/Y_H$	$-1/Y_H$	$-i_{nbm}$				1					
3	Decay of X_H			$i_{nbm}-f_p i_{nxi}$				-1				$(1-f_i)$	f_i
4	Growth of X_H on NO_3^-		$-1/Y_{H,NO3}$	$-i_{nbm}$	$(1-Y_{H,NO3})/(1.14.Y_{H,NO3})$	$-(1-Y_{H,NO3})/(1.14.Y_{H,NO3})$		1					
5	Growth of X_H on TNO_2		$-1/Y_{H,NO2}$	$-i_{nbm}$	$-(1-Y_{H,NO2})/(1.71.Y_{H,NO2})$		$(1-Y_{H,NO2})/(1.71.Y_{H,NO2})$	1					
6	Growth of X_{NH}	$-(3.43-Y_{NH})/Y_{NH}$		$-1/Y_{NH}-i_{nbm}$	$1/Y_{NH}$				1				
7	Decay of X_{NH}			$i_{nbm}-f_p i_{nxi}$					-1			$(1-f_i)$	f_i
8	Growth of X_{NO}	$-(1.14-Y_{NH})/Y_{NH}$		$-i_{nbm}$	$-1/Y_{NO}$	$1/Y_{NO}$				1			
9	Decay of X_{NO}			$i_{nbm}-f_p i_{nxi}$						-1		$(1-f_i)$	f_i
10	Growth of X_{AN}			$-1/Y_{AN}-i_{nbm}$	$-1.52-1/Y_{AN}$	1.52	$2/Y_{NO}$				1		
11	Decay of X_{AN}			$i_{nbm}-f_p i_{nxi}$							-1	$(1-f_i)$	f_i

Table 3.2.4. Kinetic parameters of ASM1.e

Symbol	Definition	Value (at 20°C)	Unit	Reference
k_h	Maximum specific hydrolysis rate	3	gCOD $gCOD^{-1}d^{-1}$	Henze *et al.* (2000)
K_X	Saturation constant for slowly biodegradable substrate	0.03	gCOD $gCOD^{-1}$	Henze *et al.* (2000)
μ^{max}_H	Maximum growth rate of X_H	6	d^{-1}	Henze *et al.* (2000)
$K_{O,H}$	Saturation constant for S_O of X_H	0.2	$mgO_2\ L^{-1}$	Henze *et al.* (2000)
$K_{S,H}$	Saturation constant for S_S of X_H	20	mgCOD L^{-1}	Henze *et al.* (2000)
b_H	Decay rate of X_H	0.62	d^{-1}	Henze *et al.* (2000)
η_{NO3}	Anoxic reduction factor for NO_3^-	0.6	-	adapted from Henze *et al.* (2000)
η_{TNO2}	Anoxic reduction factor for TNO_2	0.6	-	adapted from Henze *et al.* (2000)
$K_{NO3,H}$	Saturation constant for S_{NO3} of X_H	1	$mgNO_3^-$-N L^{-1}	adapted from Henze *et al.* (2000)
$K_{TNO2,H}$	Saturation constant for S_{TNO2} of X_H	1	$mgTNO_2$-N L^{-1}	adapted from Henze *et al.* (2000)
μ^{max}_{NH}	Maximum growth rate of X_{NH}	0.8	d^{-1}	Wiesmann (1994)
$K_{O,NH}$	Saturation constant for S_O of X_{NH}	0.6	$mgO_2\ L^{-1}$	Wiesmann (1994)
$K_{NH3,NH}$	Saturation constant for S_{NH3} of X_{NH}	0.75	$mgNH_3$-N L^{-1}	Van Hulle *et al.* (2004)
b_{NH}	Decay rate of X_{NH}	0.05	d^{-1}	Wiesmann (1994)
μ^{max}_{NO}	Maximum growth rate of X_{NO}	0.79	d^{-1}	Wiesmann (1994)
$K_{O,NO}$	Saturation constant for S_O of X_{NO}	1.5	$mgO_2\ L^{-1}$	Wiesmann (1994)
$K_{HNO2,NO}$	Saturation constant for S_{HNO2} of X_{NO}	$8.723\ 10^{-4}$	$mgHNO_2$-N L^{-1}	Wiesmann (1994)
b_{NO}	Decay rate of X_{NO}	0.033	d^{-1}	adapted from Wiesmann (1994)
μ^{max}_{AN}	Maximum growth rate of X_{AN}	0.019	d^{-1}	Strous *et al.* (1998)
$K_{O,AN}$	Inhibition constant for S_O of X_{AN}	0.01	$mgO_2\ L^{-1}$	Strous *et al.* (1999b)
$K_{TNO2,AN}$	Saturation constant for S_{TNO2} of X_{AN}	0.05	$mgTNO_2$-N L^{-1}	Strous *et al.* (1999b)
$K_{TAN,AN}$	Saturation constant for S_{TAN} of X_{AN}	0.07	mgTAN-NL^{-1}	Strous *et al.* (1999b)
b_{AN}	Decay rate of X_{NO}	0.0025	d^{-1}	Dapena-Mora *et al.* (2004)

Table 3.2.5. Stoichiometric parameters of ASM1.e

Symbol	Definition	Value	Unit	Reference
$Y_{H,O}$	Heterotrophic yield on oxygen	0.67	gCOD $gCOD^{-1}$	Henze *et al*. (2000)
$Y_{H,NO3}$	Heterotrophic yield on NO_3^-	0.54	gCOD $gCOD^{-1}$	Muller *et al*. (2003)
$Y_{H,TNO2}$	Heterotrophic yield on TNO_2	0.54	gCOD $gCOD^{-1}$	Adapted from Muller *et al*. (2003)
$Y_{NH,O}$	Autotrophic yield of X_{NH}	0.15	gCOD gN^{-1}	Wiesmann (1994)
$Y_{NO,O}$	Autotrophic yield of X_{NO}	0.041	g COD gN^{-1}	Wiesmann (1994)
Y_{AN}	Autotrophic yield of X_{AN}	0.159	g COD gN^{-1}	Strous *et al.* (1998)
f_p	Production of X_I from decay	0.1	gCOD $gCOD^{-1}$	Henze *et al*. (2000)
i_{nxi}	N content of X_I	0.02	g N $gCOD^{-1}$	Henze *et al*. (2000)
i_{nbm}	N content of biomass	0.0583	g N $gCOD^{-1}$	Henze *et al*. (2000)

3.3 Temperature dependency

For all rate constants a temperature dependency was incorporated according to an Arrhenius type equation (Henze *et al*., 2000). This allows the investigation of the performance of the reactor at different temperatures as indicated by equation 3.2.3:

$$k(T) = k(T_r)e^{\theta(T-T_r)} \tag{3.2.3}$$

where k(T) is the kinetic parameter (maximum specific growth rate μ, decay coefficient b or hydrolysis constant k) at the actual temperature T, T_r is the reference temperature (20°C) and θ is the Arrhenius constant. This equation does not take the decrease of activity at higher temperatures (above 40°C) into account as discussed in chapter 4.2. However all simulation studies were performed at temperatures between 15 and 38°C, a range where the Arrhenius type equation appears valid.

The Arrhenius constant for autotrophs can be calculated with the activation energy (E_{act}) of the autotrophic biomass following equation 3.2.4 (Hao *et al*., 2002a).

$$\theta = \frac{E_{act}}{R\ 293\ (T+273)} \tag{3.2.4}$$

where R is the universal gas constant (8.31 J mol^{-1} K^{-1}). The activation energy of aerobic ammonium oxidizers ranges from 60 to 72 kJ mol^{-1} and the activation energy of nitrite oxidizers ranges from 43 to 47 kJ mol^{-1} (Jetten *et al*., 1999; Hunik *et al*., 1994; Hellinga *et al*., 1999;

Helder and De Vries, 1983; Knowles *et al.*, 1965, Stratton and Mc Carty, 1967). For the simulation studies presented in this thesis activation energies of 68 and 44 kJ mol^{-1} for ammonium and nitrite oxidizers respectively were assumed (Jetten *et al.*, 1999). This gives θ values of 0.094 and 0.061 for ammonium and nitrite oxidizers respectively. The activation energy of Anammox is 70 kJ mol^{-1} (Jetten *et al.*, 1999), so the θ value becomes 0.096. The same activation energy was used for autotrophic growth and decay, as no data is available in literature to determine two different activation energies.

For heterotrophs the θ values for maximum specific growth rate μ_H or decay coefficient b_H were assumed to be 0.069 and 0.11, respectively as proposed by Henze *et al.* (2000).

Also the oxygen transfer coefficient (K_La) and the oxygen saturation concentration (C_S) were temperature dependent as given in equation 3.2.5 and 3.2.6 (ASCE, 1996):

$$K_L a(T) = K_L a(T_r)\,\phi^{(T-T_r)} \tag{3.2.5}$$

$$C_s = 14.65 - 0.41\,T + 7.99\,10^{-3}\,T^2 - 7.78\,10^{-5}\,T^3 \tag{3.2.6}$$

where ϕ is the temperature correction factor (1.01-1.05).

3.4 pH determination

The use of ammonia and nitrous acid as substrate for X_{NH} and X_{NO} makes it necessary to have accurate knowledge concerning the pH. This can be accomplished by measuring the pH in the reactor used for simulation studies or by calculating the pH. In WEST® an external C++ function can be used for pH calculation. This implementation was based on the model developed by Hellinga *et al.* (1999). Details about the implementation in WEST® are given by Volcke *et al.* (2004). Here only a brief summary will be presented.

Basically the pH is calculated using a charge balance (Δ^+_-). For this three extra components are introduced in WEST®: S_{TIC}, S_{Z+} and S_{Z-}. The first component stands for the total inorganic carbon content, i.e. the sum of CO_2, HCO_3^- and CO_3^{2-}, the last two (virtual) components stand for all charged (respectively positive and negative) components that don't take part in any reaction and that are not influenced by the establishment of an equilibrium pH (e.g. Na^+ and Cl^-, …). In contrast to Hellinga *et al.* (1999) NO_3^- is explicitly considered in the charge balance and is not included in S_{Z-}, because NO_3^- can be consumed or produced. Also in contrast to Hellinga *et al.* (1999) two virtual charged components (S_{Z+} and S_{Z-}) instead of one are used because in WEST® concentrations cannot become negative.

It should also be noted that correct mass balances should be applied for all components. All processes that occur should be considered. These processes are generally transport, reaction and transfer to and from the gas phase. Especially the transfer to and from the gas phase is a process that is often forgotten in WWTP modelling, although for example stripping of CO_2 has an important influence on the pH (Wett and Rauch, 2002).

The calculation of the pH goes as follows. First a charge balance over the reactor is made. For a two step nitrification-denitrification activated sludge model this charge balance becomes (Volcke *et al*., 2002a):

$$\Delta^{+}_{-} = -C_{H^+} + C_{OH^-} - C_{NH_4^+} + C_{NO_2^-} + C_{NO_3^-} - C_{Z^+} + C_{Z^-} + C_{HCO_3^-} + 2C_{CO_3^{2-}} \tag{3.2.7}$$

In this charge balance the concentrations need to be expressed in mol L^{-1}, instead of the typically applied mg L^{-1}, to allow manipulations between different concentrations. Also, in the adapted approach only the lumped components (e.g. TAN stands for the sum of NH_4^+ and NH_3) are considered, since these are typically measured. With the help of the equilibrium constants of the chemical equilibria equation 3.2.7 can be expressed in terms of these lumped components (equation 3.2.8). In this charge balance the pH remains the only unknown, since $C_{H^+} = 10^{-pH}$, if the concentration of S_{Z^+} and S_{Z^-} is known.

$$\Delta^{+}_{-} = 10^{-pH} + \frac{K_W}{10^{-pH}} - \frac{C_{TAN}}{\left(1 + \frac{K_e^{NH}}{10^{-pH}}\right)} + \frac{C_{TNO_2}}{\left(1 + \frac{10^{-pH}}{K_e^{NO}}\right)} - C_{Z^+} + C_{Z^-} + C_{NO_3^-}$$
$$+ \frac{C_{TIC}}{\left(\frac{10^{-pH}}{K_e^{CO_2}} + 1 + \frac{K_e^{HCO_3}}{10^{-pH}}\right)} + 2\frac{C_{TIC}}{\left(\frac{10^{-2\,pH}}{K_e^{CO_2} K_e^{HCO_3}} + \frac{10^{-pH}}{K_e^{HCO_3}} + 1\right)} \tag{3.2.8}$$

This charge balance should be zero ($\Delta^{+}_{-}=0$). In WEST® a Newton-Raphson algorithm that is written in an external C-function is used to determine the nil point of the charge balance and, thus, calculate the pH.

The concentrations of S_{Z^+} and S_{Z^-} can be calculated from a mass balance for the two components. Since S_{Z^+} and S_{Z^-} do not take part in any reaction only transport is considered in this mass balance. Further, the influent concentration of S_{Z^+} and S_{Z^-} can be calculated from a charge balance for the influent and a measurement of the influent pH. Since the charge balance for the influent has to be zero, the following expression can be found for the difference of the concentration of S_{Z^+} and S_{Z^-}.

$$C_{Z^+}^{in} - C_{Z^-}^{in} = 10^{-pH^{in}} + \frac{K_W}{10^{-pH^{in}}} - \frac{C_{TAN}^{in}}{\left(1 + \frac{K_e^{NH}}{10^{-pH^{in}}}\right)} + \frac{C_{TNO_2}^{in}}{\left(1 + \frac{10^{-pH^{in}}}{K_e^{NO}}\right)} + C_{NO_3^-}^{in}$$
$$+ \frac{C_{TIC}^{in}}{\left(\frac{10^{-pH^{in}}}{K_e^{CO_2}} + 1 + \frac{K_e^{HCO_3}}{10^{-pH^{in}}}\right)} + 2\frac{C_{TIC}}{\left(\frac{10^{-2\,pH^{in}}}{K_e^{CO_2} K_e^{HCO_3}} + \frac{10^{-pH^{in}}}{K_e^{HCO_3}} + 1\right)} \qquad (3.2.9)$$

If this difference is positive, then the concentration of S_{Z+} can be set equal to this value and the concentration of S_{Z-} can be set to zero. If this difference is negative, then the concentration of S_{Z-} can be set equal to the absolute value of this value and the concentration of S_{Z+} can be set to zero.
For all equilibrium constants values are listed in literature (see for example Perry and Green, 1998 or Stumm and Morgan, 1996). However in view of simulations at different temperatures, a temperature dependency should be incorporated. As discussed in chapter 4.2 dependencies proposed by Stumm and Morgan (1996), Anthonisen *et al*. (1976) and Helgeson (1967) can be used.

4 THE BIOFILM MODEL

Because of the low growth rate of the Anammox organisms, biofilm systems are often used when applying autotrophic nitrogen removal. A one-dimensional biofilm model was therefore constructed in the WEST® software. It was opted not to use the model of Rauch *et al*. (1999) that was already implemented in WEST®. This model is based on substrate penetration depths and assumes a homogeneous distribution of the biomass throughout the biofilm. An essential property of autotrophic nitrogen removing biofilms is however the layered structure, where ammonium oxidizers are present at the top of the biofilm and Anammox biomass is active in the inner layers of the biofilm. A one-dimensional biofilm model was preferred over a two- or three-dimensional biofilm in view of the required calculation time and system complexity. Moreover, all these models predict approximately the same effluent concentrations (Morgenroth *et al*., 2004). Of course, for example, so-called hot spots of the toxic TNO_2 in the biofilm cannot be predicted by one-dimensional models (Picioreanu, 2003).
This one-dimensional biofilm reactor model presented here consists of a completely mixed bulk phase and a layered biofilm. The relevant processes are summarized in Figure 3.2.2.
Transport of solubles between the bulk phase and the biofilm is described through diffusion in a laminar boundary layer. The thickness of this boundary layer can be varied, depending on different process conditions. Particulate components can attach and detach to the biofilm. Different empirical equations exist for this attachment. However, in order not to complicate the

model, attachment was described as a first order process with respect to the concentration of the particulate component in the bulk phase. Following Horn and Hempel (1997) the rate of biomass detachment is formulated as being dependent on the velocity u_f by which the biofilm surface moves relative to the substratum. If the velocity is negative, then the rate of biomass detachment is zero. If this velocity is positive, then the rate of detachment of a particulate component is set equal to u_f C_I A, with C_i the concentration of a particulate component and A the total biofilm surface area. In the biofilm compartment, diffusion and biological conversion of soluble and particulate components takes place. The diffusion of particulates is of course much smaller than the diffusion of solubles. Diffusion coefficients for soluble nitrogen components considered in the ASM1.e model (TNO_2, NO_3^-, TAN and N_2) and oxygen were derived from Picioreanu *et al.* (1997), while the coefficient for the readily biodegradable substrate was derived from Henze *et al.* (1995) (see Table 3.2.6).

The biofilm diffusion coefficient for particulate components considered in the ASM1.e model was set equal to 10^{-10} m^2 d^{-1}. Biofilm porosity was set to 0.5. For the biofilm density, a typical value of 40 kgCOD m^{-3} was taken (Melcer *et al.*, 1995).

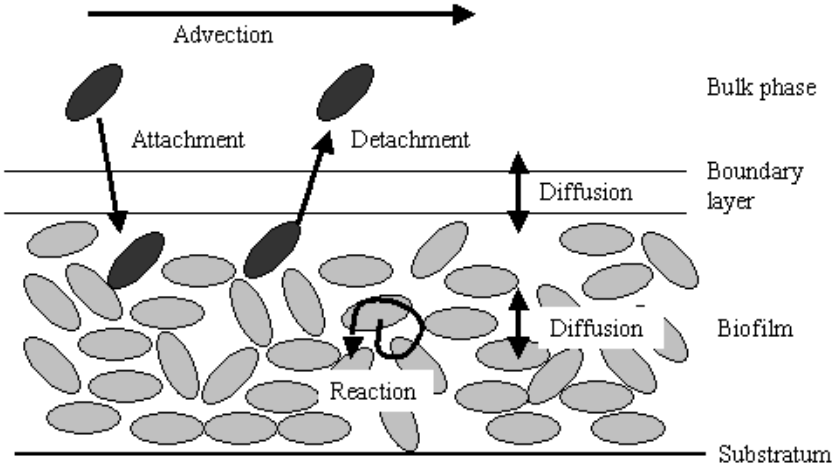

Figure 3.2.2. Relevant processes considered in the biofilm (after Wanner and Reichert, 1996).

Table 3.2.6. Diffusion coefficients for the soluble components considered in ASM1.e

Component	Diffusion coefficients [10^{-4} m^2 d^{-1}]
TNO_2	1.4
NO_3^-	1.4
TAN	1.5
N_2	2.2
O_2	2.2
S_S	0.58

The governing partial differential equation for both soluble and particulate components in the biofilm thus becomes equation 3.2.10 (Wanner, 2002).

$$\frac{\partial C_i}{\partial t} = D_i \frac{\partial^2 C_i}{\partial z^2} + r_i \qquad (3.2.10)$$

With C_i the concentration of a component, D_i the diffusion coefficient of a component, r_i the net conversion rate of a component and z the Cartesian co-ordinate perpendicular to the substratum. The partial differential equation is solved in two steps. First the partial differential equation is transformed to a set of ordinary differential equations by using the method of lines (Schiesser, 1991). In this thesis a 20 layer discretization is used. As a second step the resulting set of ordinary differential equations is integrated by the numerical integrator CVODE (Cohen and Hindmarsh, 1996).

5 MODEL CALIBRATION AND VALIDATION

Calibration and validation of activated sludge models is an inherent part of any type of model application. It consists of several steps, including dedicated lab-scale experiments and intensive measurement campaigns for the characterisation of the influent wastewater, the determination of the kinetics/stoichiometry of the biological processes and the hydraulic and settling behaviour of the system. In addition to the lab-scale experiments expert-knowledge and ad-hoc approaches can be used. In fact, calibration of a model without expert-knowledge was reported to be a very dangerous task, bound to lead to nonsense results (Andrews, 1991).

For even better model calibration, mathematical techniques can be used. One of the existing techniques for this purpose is optimal experimental design based on the Fisher Information Matrix (equation 3.2.10) (Dochain and Vanrolleghem, 2001).

$$FIM = \sum_{i=0}^{N} \left(\frac{\partial y}{\partial \theta} \right)_i^T Q_i^{-1} \left(\frac{\partial y}{\partial \theta} \right)_i \qquad (3.2.10)$$

where y is the vector of measured variables (e.g. consisting of TAN, TNO_2 and NO_3^-), θ the vector of parameters to be calibrated (e.g. consisting of μ^{max}_{AN}, $K_{TNO2,AN}$ and $K_{TAN,AN}$), Q the measurement error matrix and N the number of measurement points over time. This matrix represents the information content of a specific experiment. It is calculated based on two components: sensitivity functions and measurement error. Sensitivity functions express how sensitive certain measured variables are with respect to the model parameters. Measured variables which are sensitive to certain parameters contribute to the information content of the experiment in the sense that they, if measured, will provide useful data for calibration. Having sensitive measurement variables is not the only prerequisite for a well-designed experiment, correctly quantifying measurement error is equally important. Measurement errors express how much trust we can have in the measurements and to what extent they contribute to the information content of the experiment.

Optimal experimental design is based on model simulations and is therefore a very useful technique since it quantifies the information content of the data of a certain experiment before it is performed in practice. Using this technique, different experiments can be proposed based on the experimental degrees of freedom of the system under study: available measurements and experimental manipulations. The proposed experiments can be "virtually" simulated and their information content determined. Once the optimal experiment is found it can be performed in reality and the data collected. Based on this data the model can be (re)calibrated resulting in a model with more accurately estimated parameters.

For municipal WWTP numerous studies calibrating the biological, settling and hydraulical behaviour have been performed (Ekama *et al.*, 1986; Henze *et al.*, 1987; Sollfrank and Gujer 1991, Kappeler and Gujer, 1992; Coen *et al.*, 1996; Petersen *et al.*, 2002; among many others) and several protocols structurizing the calibration exercise were developed (Hulsbeek *et al.*, 2002, Vanrolleghem *et al.*, 2003; Langergraber *et al.*, 2004; Melcer *et al.*, 2003). A SWOT (strengths, weaknesses, opportunities and threats) analysis of these protocols was performed by Sin *et al.* (2004). From this SWOT analysis it became clear that the proposed methods for parameter calibration may not be applicable when applying a protocol to an industrial and non-domestic wastewater treatment plant. For example, the determination of the maximum specific growth rate of ammonium and nitrite oxidizers will not be as straightforward as described in WERF (2003) or Vanrolleghem *et al.* (2003), because nitrogen components can be present in such amounts that they will inhibit the ammonium and nitrite oxidation processes. Moreover, pH and salinity may also play an important role in the affinity and inhibition kinetics of these processes (Van Hulle *et al.*, 2004b; Moussa *et al.*, 2003). When modelling industrial WWTPs with high saline wastewater characteristics, Moussa *et al.* (2003) reported that the existing experimental methods could not help calibrate the model successfully. Instead, a new experimental methodology had to be

developed to study the effect of salinity on the activity of nitrifiers in several industrial WWTPs and determine (inhibition) kinetics of nitrifiers (Moussa *et al.*, 2003).
In this thesis dedicated experiments were developed and proposed for the calibration of the biological processes occurring during autotrophic nitrogen removal. As the autrotrophic nitrogen removal process in this thesis was only studied on lab-scale reactors the hydraulic and settling behaviour is fairly simple and was not studied in detail here. It should however be kept in mind that when the process is applied on full-scale this hydraulic and settling characterisation will be essential.

6 CONCLUSION

In the user-friendly modelling and simulation environment WEST® a model for autotrophic nitrogen removal was implemented. This model extends ASM1 with Anammox and 2-step nitrification and denitrification. In the model ammonia (S_{NH3}) rather than ammonium, and nitrous acid (S_{HNO2}) rather than nitrite were used as actual substrates for ammonium oxidizer and nitrite oxidizer growth, respectively (Anthonisen *et al.*, 1976). The stoichiometry of the reactions was however expressed in terms of corresponding concentrations of total ammonia nitrogen (S_{TAN}) and total nitrite nitrogen (S_{TNO2}), as typically done. Oxygen (S_O) was used as electron acceptor for autotrophic growth. Total ammonia nitrogen (S_{TAN}) and total nitrite nitrogen (S_{TNO2}) instead of ammonia and nitrous acid were used to describe the growth kinetics of Anammox. This is because it is not yet determined whether the uncharged form is the real substrate. To account for varying temperature and pH conditions, the influence of these parameters is incorporated in the model.
Decay as described in ASM1 was preferred over endogenous respiration processes as described in ASM3 within the model because these are not yet clearly documented for ammonium and nitrite oxidizers and Anammox. Further research on these subjects is certainly recommended.
Also a biofilm model was implemented in WEST® incorporating the above mentioned equations. A one-dimensional biofilm was developed as a compromise between process complexity and calculation time. The model incorporates advective flow in the bulk phase, diffusive transport of solubles from the bulk phase to the biofilm, attachment and detachment of the biomass and reaction and diffusion processes in the biofilm.
Calibration and validation of the proposed model is necessary before it will be used for model-based optimisation. Because of the special conditions occurring in autotrophic nitrogen removal, new experiments and protocols need to be developed. Several of these experiments will be performed and discussed in the next chapters.

PART 4

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EXPERIMENTAL STUDY ON AUTOTROPHIC NITROGEN REMOVAL

Chapter 4.1

Construction, start-up and operation of a continuously aerated lab-scale SHARON reactor in view of coupling with an Anammox reactor

ABSTRACT

In this study practical experiences during start-up and operation of a lab-scale SHARON reactor are discussed, along with the construction of the reactor. Special attention is given to the start-up in view of possible toxic effects of high nitrogen concentrations (up to 4000 mgN L^{-1}) on the nitrifier population and because the reactor was inoculated with sludge from a SBR reactor operated under completely different conditions. Because of these considerations, the reactor was first operated as an SBR to prevent biomass washout and to allow the selection of a strong nitrifying population. A month after the inoculation the reactor was switched to normal chemostat operation. As a result the nitrite oxidisers were washed out and only the ammonium oxidisers persisted in the reactor.

In this contribution also some practical considerations concerning the operation of a continuously aerated SHARON reactor, such as mixing, evaporation and wall growth, are discussed. These considerations are not trivial, since the reactor will be used for kinetic characterisation and modelling studies. Finally the performance of the SHARON reactor under different conditions is discussed in view of its coupling with an Anammox unit. Full nitrification was proven to be feasible for nitrogen loads up to 1.5 gTAN-N L^{-1} d^{-1}, indicating the possibility of the SHARON process to treat highly loaded nitrogen streams. Applying different influent concentrations led to different effluent characteristics indicating the need for proper control of the SHARON reactor.

The main part of this chapter was presented as oral presentation at FAB 2003:

Van Hulle, S.W.H., Van Den Broeck, S., Maertens, J., Villez, K., Schelstraete, G., Volcke, E.I.P & Vanrolleghem, P.A. (2003b). Practical experiences with start-up and operation of a continuously aerated lab-scale SHARON reactor. In: *Communications in Applied Biological Sciences* **68/2**(a), *Proceedings FAB Symposium.* Gent, Belgium, September 18-19, 2003, 77-84.

1 INTRODUCTION

Partial nitrification techniques, such as the continuously aerated SHARON process, have been denoted for quite a while as very promising for improved sustainability of wastewater treatment. (Abeling and Seyfried, 1992). Conventionally nitrogen removal in these wastewaters is achieved using nitrification/denitrification. In such systems, nitrifying bacteria oxidize TAN to nitrate under oxic conditions, and nitrate is subsequently or simultaneously reduced to dinitrogen gas, under anoxic conditions. Recently however, novel processes for nitrogen removal were developed, for example the combined SHARON-Anammox process (van Dongen *et al.*, 2001a&b).

In the SHARON (Single reactor High activity Ammonia Removal Over Nitrite) process, partial nitrification of TAN to TNO_2 is established by working at high temperature (above 25°C) and maintaining an appropriate sludge retention time (SRT) of 1 to 1.5 days, so that ammonium oxidizers are maintained in the reactor, while nitrite oxidizers are washed out and further nitrification of TNO_2 to nitrate is prevented as explained in detail below. In this way, significant aeration cost savings are realized in comparison with conventional nitrification to nitrate. The SHARON process is very suitable to reduce the load of streams with high TAN concentration (~1gTAN-N L^{-1}), rather than to meet strict effluent standards. It is typically applied for treating sludge digestion reject water in order to relieve the main wastewater treatment plant (WWTP) to which this stream is subsequently recycled. A full-scale SHARON process is operational since January 1999 at the Rotterdam Sluisjesdijk sludge treatment plant (Van Kempen *et al.*, 2001).

The TNO_2 produced in the SHARON process can be used as an electron acceptor for the oxidation of the remainder of the TAN by the recently discovered Anammox organisms (ANaerobic AMMonium OXidizers), that combine TAN and nitrogen to form nitrogen gas (Jetten *et al.*, 1999).

Benefits of the combined SHARON-Anammox process compared to the SHARON process with denitrification are the reduction by 50% of the aeration costs, since only half of the TAN is converted, the omission of the need for additional COD source, the virtual absence of sludge production and the possibility to obtain low nitrogen effluent concentrations through the subsequent autotrophic Anammox reaction. The latter has been an inspiring starting point for the development of more sustainable municipal wastewater treatment systems (Jetten *et al.*, 1997).

An experimental study on the treatment of TAN-rich wastewater by the combined SHARON-Anammox process performed by van Dongen *et al.* (2001a), showed that the combined SHARON-Anammox system can work stably over long periods and the process is ready for full-scale implementation.

2 THE SHARON PROCESS IN DETAIL

The continuously aerated SHARON process consists basically of a completely mixed reactor without sludge retention, operating at high temperature (above 25°C). At temperatures above 25°C the ammonium oxidizers grow faster than the nitrite oxidizers. The maximum specific growth rates at 20 °C of both populations are approximately the same (0.8 d^{-1} and 0.79 d^{-1} respectively) but the activation energies differ (68 and 44 kJ mol^{-1}) as discussed in chapter 3.2. Hence the maximum specific growth rate of the ammonium oxidizers will increase faster than the maximum specific growth rate of the nitrite oxidizers (Hellinga *et al.*, 1999). In Figure 4.1.1 the temperature dependency of the maximum growth rates is depicted. Nitrite oxidisers can thus be washed out by sufficiently lowering the hydraulic retention time, so that the dilution rate, which is the inverse of the HRT, becomes higher than the growth rate of nitrite oxidizers but lower than the growth rate of ammonium oxidizers. Nitrite oxidizers will then not be able to persist in the reactor.

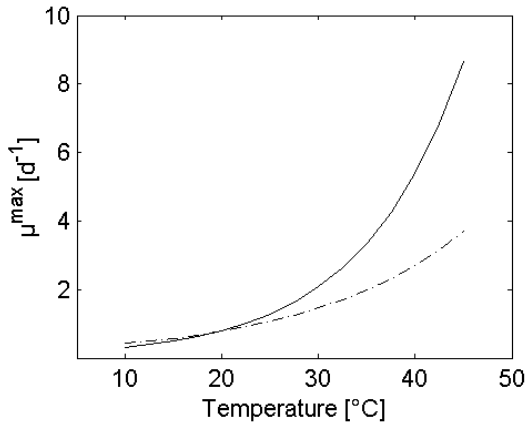

Figure 4.1.1. The temperature dependency of maximum specific growth rates of ammonium (-) and nitrite (- .) oxidizers. At temperatures above 25°C the growth rate of ammonium oxidizers is higher than the growth rate of nitrite oxidizers, indicating the possibility to outcompete nitrite oxidizers at higher temperatures.

Not only washout of the nitrite oxidizers has to be accomplished, but also only half of the TAN can be oxidized to TNO_2 in order to produce an Anammox-suited effluent. This is accomplished in the following way. Oxidation of 1 mole of ammonium to nitrite produces 2 moles of protons according to equation 4.1.1.

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad (4.1.1)$$

This production of protons leads to a significant pH-decrease and consequently a stop in nitrification especially with highly loaded nitrogen streams that are generally treated by the

SHARON reactor. However, normally SHARON influent contains bicarbonate next to TAN. This bicarbonate is stripped by the air in the form of CO_2:

$$H^+ + HCO_3^- \rightarrow CO_2 + H_2O \qquad (4.1.2)$$

This means that for every mole of TAN oxidized, 2 moles of bicarbonate are stripped. In case the SHARON influent has a molar TIC:TAN ratio of 1:1, the protons produced during conversion of half of the TAN are equal to the protons taken up via carbon dioxide stripping. Hence, TAN oxidation stops at 50 % conversion due to acidification and an Anammox suited effluent is produced.

However, the influent TIC:TAN ratio is not always 1:1. For example, data from Izzet *et al.* (1991) show that this ratio varies between 1.02 and 1.44 with an average of 1.2 for sludge digestion reject water (Figure 4.1.2). This type of wastewater is typically used as an example for SHARON influent.

So the question is what the result of this varying TAN:TIC ratio will be on the reactor performance. Also the influent TAN concentration will vary over time. This will also affect reactor performance. Process control seems therefore necessary since the Anammox reactor benefits from a constant influent composition.

In this chapter the experimental performance of the SHARON reactor under different influent conditions is discussed in view of its coupling with an Anammox unit.

Also practical experiences during start-up and operation of a lab-scale SHARON reactor are presented. The results of this study will be used in a simulation and control study. Therefore the results will also be discussed in view of this study.

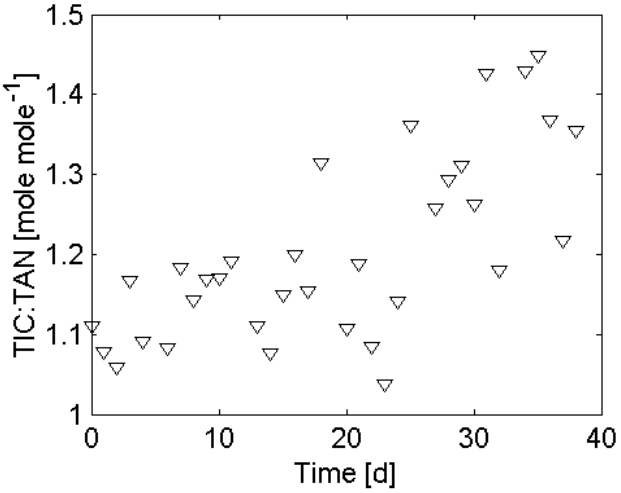

Figure 4.1.2. TIC:TAN ratio in the effluent of an anaerobic digester calculated from Izzet *et al.* (1991) showing that this ratio is not always 1:1 as commonly assumed.

3 MATERIALS AND METHODS

3.1 SHARON reactor

3.1.1 Hardware and software

The SHARON reactor is a 2 litre continuously stirred tank reactor (CSTR) without biomass retention. Its schematic and photo representation is shown in Figure 4.1.3.

The 2 litre reactor has a double borosilicate wall for heat exchange (Karlsruher Glastechnisches Werk 3.3 DIN/ISO 3585). The synthetic influent is pumped with a peristaltic pump (Gilson Minipuls 2) from the 5 litre influent vessel to the reactor. The pump flow rate of this influent pump determines both the hydraulic residence time and the sludge residence time (SRT), since both residence times are equal and defined as the ratio of the volume to the flow rate. The flow rate can vary between 0.2 to 6 litre per day, which gives the possibility of operating at HRTs between 10 and 0.33 days. The lower limit of the applied HRT is however determined by the growth rate of the ammonium oxidizers.

The effluent is pumped out of the reactor with a second peristaltic pump (Watson Marlow 101U/R) that operates at a higher flow rate than the first pump. The influent flow rate is however equal to the actual effluent flow rate since the withdrawal point is situated at the 2 litre mark.

The reactor is aerated through a pumice stone using air from a compressor (1 bar overpressure). The temperature of the reactor can be controlled between 20°C and 70°C with a Lauda thermostat A103, although the normal operational temperature is 35°C, as is usual for the SHARON process. In the reactor the dissolved oxygen and the pH are measured.

Data logging and control of the SHARON reactor are performed with the Labview® software (National Instruments, www.ni.com) installed on the computer. LabView® is a graphical programming language that uses icons instead of lines or text to create applications. In contrast to text-based programming languages, where instructions determine program execution, LabView® uses dataflow programming, where the flow of data determines execution.

The pH and DO are measured every 10 seconds using the software. The pH is controlled by addition of acid (HCl) and base ($NaHCO_3$ or NaOH) based on the measured pH. A setpoint is defined together with a pH boundary. If the pH exceeds this boundary a valve is briefly opened and acid or base is dosed. The developed software is easily extendable with additional control algoritms. More details concerning the hardware and software of the SHARON reactor, in particular the structure of the Labview® software can be found in Van Hulle *et al*. (2003c).

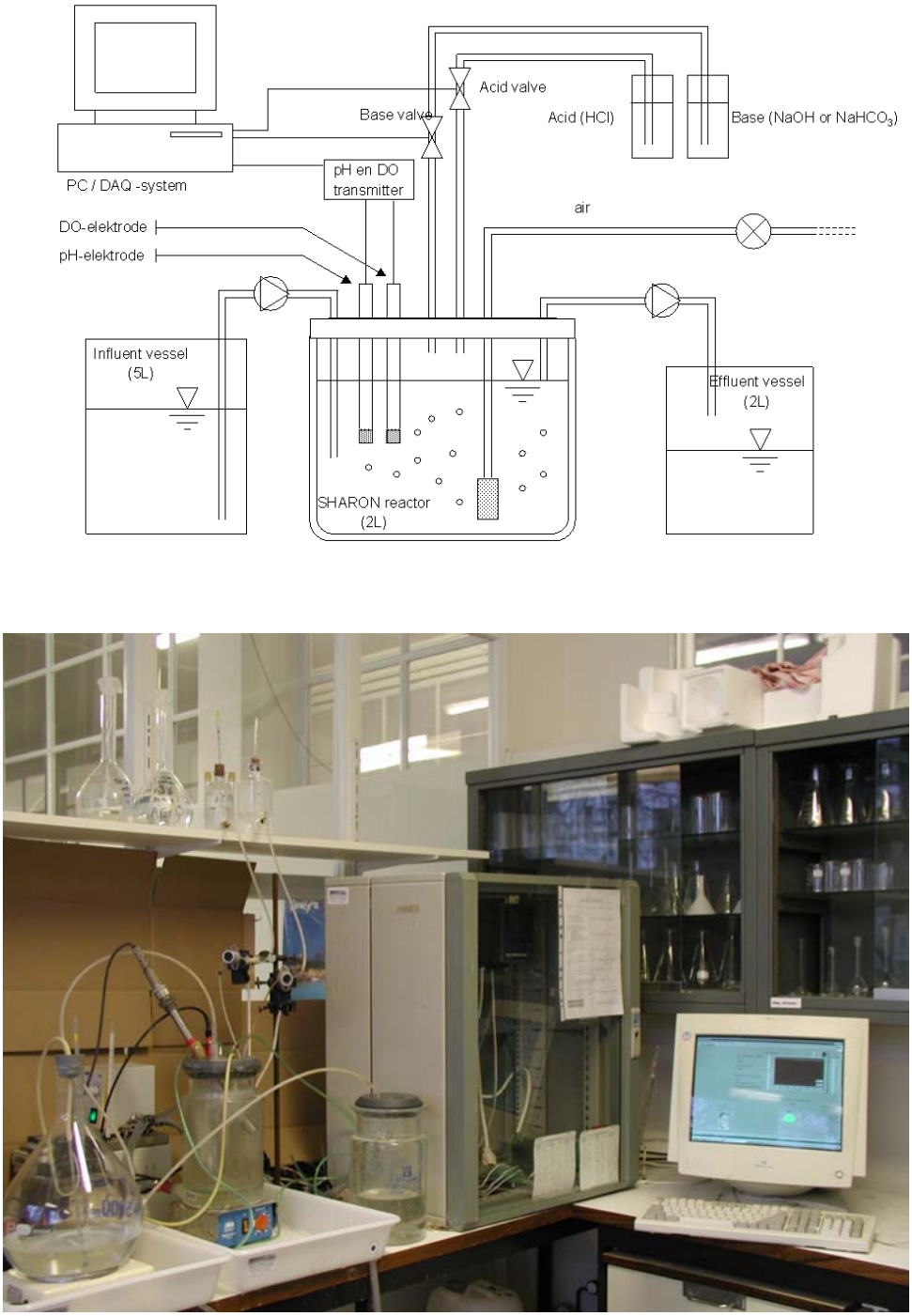

Figure 4.1.3. Schematic and photo representation of the lab-scale SHARON reactor used in this study (adopted from Van Den Broeck *et al.*, 2004 and Villez, 2003).

3.1.2 Inoculum

Two different inocula were tried as inoculum for the SHARON reactor. First, an inoculum from the SHARON reactor of the WWTP of Rotterdam (Mulder *et al*., 2001) was used. This SHARON reactor was operating under alternating oxic/anoxic conditions. These organisms are already adapted to the short residence times and high nitrogen concentrations typical for the SHARON process. Secondly, an inoculum from the sequencing batch reactor (SBR) in the BIOMATH lab (Lee and Vanrolleghem, 2003) was used. Since the organisms in the SBR are not adapted to high nitrogen concentrations, special attention was given to the start-up with this inoculum.

3.1.3 Influent

The reactor is fed with synthetic influent with composition given in Table 4.1.1. The TAN and TIC concentrations vary according to the type of experiment conducted. The influent concentrations used were 500, 1000, 2000 and 4000 mgTAN-N L^{-1}. The trace element composition is according to Visniac and Santer (1957) with the addition Pb^{2+}, Cr^{3+} and Ni^{2+} as adjusted by Capalozza (2001).

3.1.4 Microscopy

The microscopical observations were performed by using an optical microscope, Olympus CX40 (Olympus, Japan) equipped with a video camera Ikegami ICD-46E (Ikegami Electronics Inc., USA). A drop of mixed liquor was carefully deposited on a glass slide and covered with a cover slip before being observed through the microscope.

3.1.5 Chemical analyses

Concentrations of TAN, TNO_2 and NO_3 were analysed on a daily basis using spectrophotometric methods (Dr Lange GmbH, Germany). TSS (Total Suspended Solids) concentrations were determined according to Standard Methods (APHA, 1992). The dissolved oxygen was measured by an Ingold (Mettler Toledo) Clarck type oxygen electrode. The pH was measured with a glass electrode (Mettler Toledo HA 405-DXK-S8/120).

Table 4.1.1. Composition of the synthetic influent of the SHARON reactor used in this study

Main compounds	Concentrations [mg L^{-1}]
$(NH_4)_2SO4$	Depends on experiment; 1000 mgTAN-N L^{-1} = 4714 mg $(NH_4)_2SO_4$ L^{-1}
$NaHCO_3$	Depends on experiment; 1000 mgC L^{-1} = 6994 mg $NaHCO_3$
KH_2PO_4	1000
$MgSO_4.7H_2O$	600
Trace compounds	
$FeSO_4.7H_2O$	15
$PbCl_2$	3.4
$ZnCl_2$	7.2
$Cr(NO_3)_3.9H_2O$	8
$CuCl_2.2H_2O$	6
$MnSO_4.H_2O$	19
$NiSO_4.6H_2O$	2
$CoCl_2.6H_2O$	3.4
$(NH_4)_6Mo_7O_{24}.4H_2O$	4.8
$CaCl_2$	5
EDTA	150

4 RESULTS AND DISCUSSION

4.1 Start-up of the SHARON reactor: fast method versus slow method

4.1.1 Fast start-up method

Initially the reactor was inoculated with sludge from the SHARON reactor of Rotterdam. In order to start up in a fast way the reactor was set in CSTR mode with a HRT of 2.5 days after 24 hours of adaptation of the biomass to the reactor. The pH and temperature were fixed at 6.9 and 35°C respectively. Different influent concentrations, ranging from 300 to 800 mgTAN-N L^{-1}, were used, but all start-ups had the same outcome. As an example the results of a start-up with an influent concentration of 300 mgTAN-N L^{-1} is shown in Figure 4.1.4. The first 3 days all incoming TAN is oxidised to nitrate. After approximately 1 SRT TNO_2 starts to build up in the effluent, indicating the successful washout of the nitrite oxidisers. After 3 SRT however TAN builds up in the effluent, indicating the washout of ammonium oxidisers too. From Figure 4.1.4 it can thus be concluded that directly imposing short residence times on the nitrifying organisms coming from this full-scale SHARON reactor did not result in a stable operation of the SHARON process in contrast to the findings of van Dongen *et al*. (2001a&b). Toxic effects of ammonia and nitrous acid (Anthonisen *et al*., 1976) can be put forward as a possible explanation.

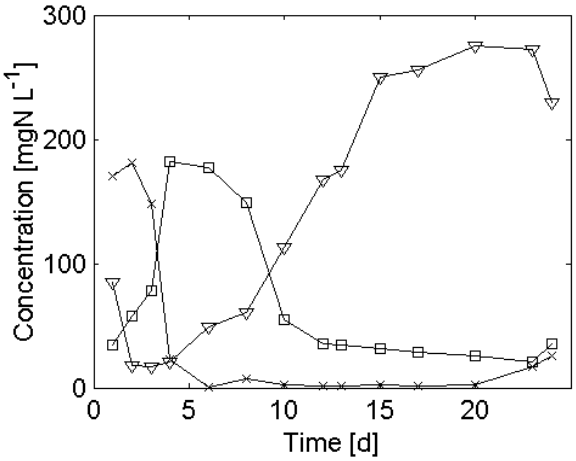

Figure 4.1.4. Fast start-up method for the SHARON reactor: evolution of TNO_2 (□), NO_3^- (x) and TAN (▽), indicating the washout of nitrifying organisms.

4.1.2 Slow start-up method

Since the fast start-up method had no success, a slow start-up method was tested. This time inoculum from a SBR reactor was used. Special attention was given to the start-up in view of possible toxic effects on the nitrifier population originating from a SBR reactor operated under completely different conditions (T=15°C, SRT = 10 d, TAN load 9 mgTAN-N L^{-1} d^{-1}). The ammonium oxidisers were therefore allowed to adapt slowly to the changed conditions (Van Den Broeck *et al.*, 2004).

The SHARON reactor was first operated as a SBR to prevent biomass washout, while the influent TAN load was stepwisely increased from 600 to 1480 mgTAN-N L^{-1} d^{-1}. The temperature too was step wisely increased from 23.4°C to 35°C. The pH was fixed at 7.1. Every 12 hours the sludge was allowed to settle and the effluent was withdrawn. A month after the inoculation of the reactor, a stable nitrifying population was established since all incoming TAN was oxidised to nitrate. The reactor was then switched to normal chemostat operation with a SRT of 2.7 days. This time the nitrite oxidisers were washed out since the incoming TAN was now oxidised to TNO_2 only and no nitrate was formed. After start-up the reactor was operated as discussed in the next paragraph.

Concerning the slow start-up method, it appears more appropriate to start-up with a general nitrifying sludge instead of dedicated SHARON sludge, since the former one is more readily available. However, even though the fast start-up method was unsuccessful, probably the slow start-up method would have worked also with the SHARON sludge.

4.2 Practical considerations concerning the SHARON reactor

Apart from the careful start-up, some other practical considerations can be pointed out when operating a continuously aerated SHARON reactor at high temperatures. Indeed, the conditions in the reactor have to be known as accurate as possible in order to compare experimental results with modelling results. For instance, evaporation and wall growth, among others, can hinder the interpretation of experimental results.

4.2.1 Low biomass concentration

The reactor is designed to operate at an effluent TAN:TNO_2 ratio of 1:1 and a HRT of 1.54 days, although the HRT at start-up was 2.7 days. For an influent TAN concentration of 2000 mgTAN-N L^{-1} the amount of TAN nitrified would then be 1000 mgTAN-N L^{-1} (N^{nitr}). According to Petersen *et al*. (2003) the concentration of ammonium oxidisers (X_{NH}) in the reactor can be calculated by equation 4.1.3.

$$X_{NH} = Y_{NH} \frac{SRT}{HRT} \frac{N^{nitr}}{1 + b_{NH} SRT} = 0.15 \frac{1.54}{1.54} \frac{1000}{1 + 0.1\ 1.55} \approx 130\ mgCOD\ \ L^{-1} \qquad (4.1.3)$$

with Y_{NH} the growth yield for ammonium oxidisers on TAN (mgCOD mgTAN-N^{-1}) and b_{NH} the decay rate for ammonium oxidisers (d^{-1}) (Wiesmann, 1994).

The combination of this low ammonium oxidiser concentration and the absence of other biomass in the reactor (since synthetic influent with only TAN and no carbon source is used) results in a reactor operation that is very sensitive to disturbances. Any disturbance can only be dealt with by the ammonium oxidisers and can lead to the malfunctioning of the reactor.

The low biomass concentration is also reflected in the low TSS concentrations. As an example the TSS values for a 1 month period in which the SHARON reactor was operated with an influent concentration of 2000 mgTAN-N L^{-1} is depicted in Figure 4.1.5.

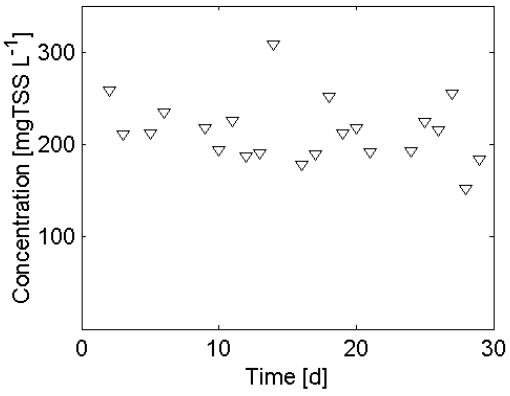

Figure 4.1.5. TSS concentrations for a 1 month period that the SHARON reactor was operated with an influent concentration of 2000 mgTAN-N L^{-1}, indicating the low biomass concentration.

4.2.2 Evaporation

Water evaporation is not negligible and can, depending on the air flow rate, amount to more than 20% of the influent flow when operating a 2 litre lab-scale reactor at 35°C. The effect of evaporation was detected because the nitrogen mass balance, assuming the influent and effluent flow rate to be the same, over the reactor did not close. In other words, the nitrogen concentration (in the form of TNO_2, nitrate and TAN and incorporated in the biomass) coming out the reactor was higher than the nitrogen concentration in the reactor as expressed by equation 4.1.4.

$$C_{TAN}^{in} \leq \left(C_{TAN}^{out} + C_{TNO_2}^{out} + C_{NO_3^-}^{out}\right) + i_{nbm} X^{out} \qquad (4.1.4)$$

with C^{in} the concentration of TAN in the influent, C^{out} the concentration of TAN, TNO_2 and nitrate in the effluent, i_{nbm} the nitrogen content of the biomass and X^{out} the biomass concentration in the effluent. This difference could only be explained by evaporation since numerous tests and dilution series were performed to exclude measurement errors.

Because of this evaporation the influent and effluent flow rates would differ. This evaporation was also noticed by Fux *et al*. (2002). It will also lead to an increase of the sludge age since the sludge age is determined by the outflow rate, as the sludge age is the ratio of the sludge mass to the outflow waste rate. If the sludge age is increased above the minimal sludge age for nitrite oxidizers, then these organisms can grow in and nitrate will be produced.

The amount of water evaporated can be calculated as follows. According to Perry and Green (1998) the vapour pressure of water at 35°C and 1 atm is 0.056 atm. From the ideal gas law it can be calculated that 1 m^3 of air contains 39.6 moles. Hence 1 m^3 of saturated air contains 2.3 moles

or 41.25 g or 41.25 ml of water, assuming a water density of 1 kg m^{-3}. If dry air enters the SHARON reactor and saturated air leaves the SHARON reactor, then for every m^3 of air that enters the reactor 41.25 ml of water is removed. Normally the air flow rate to the SHARON reactor is 3 to 8 l min^{-1} or 4.32 to 11.52 m^3 d^{-1}. So every day 178 to 475 ml of water is taken up by the air. Compared to a HRT of 1.54 d or an equivalent inflow rate of 1.3 l d^{-1} about 14 to 37 % of the flow evaporates! This was also checked experimentally. A batch reactor with a controlled temperature of 35°C was filled with 2 litres of water. After 24 hours of aeration the water volume reduction was measured. This experiment was repeated at different air flow rates and the resulting evaporation, expressed as a percentage of the initial water volume, are depicted in Figure 4.1.6.
In order to partially circumvent water evaporation, the air was subsequently saturated with water before entering the SHARON reactor.

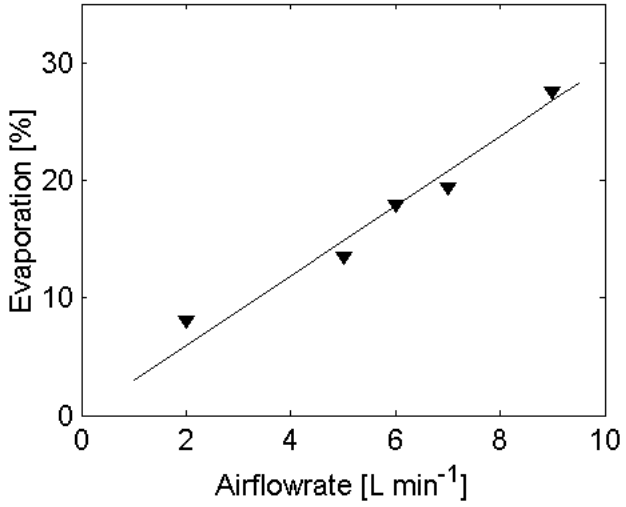

Figure 4.1.6. Theoretical (-) and experimental (▼) percentage water evaporation in the SHARON reactor.

4.2.3 Dilution by pH-control

The base addition for pH-control leads to a certain dilution. For example, the first 40 days of operation of the SHARON reactor about 200 ml d^{-1} of a 1 M $NaHCO_3$ solution was added.

4.2.4 Stripping of CO_2 from the influent

Due to CO_2 stripping from the influent vessel, the influent pH and TIC concentration vary over time. This has however no effect on the neutralising capacity of the influent since for every mole of CO_2 stripped one mole of OH^- ions is produced. The loss in buffering capacity is therefore converted to an equivalent pH increase. Generally batches of 5 litre influent are prepared, hence

after approximately 3 days the influent is finished, since the design HRT is 1.54 days. The pH evolution of 3 different influent batches when 12 g L^{-1} $NaHCO_3$ is added to the influent is depicted in Figure 4.1.7. It can be seen that the influent pH increases about 1 unit because of CO_2 stripping. Of course this pH-increase would stop if all TIC is stripped.

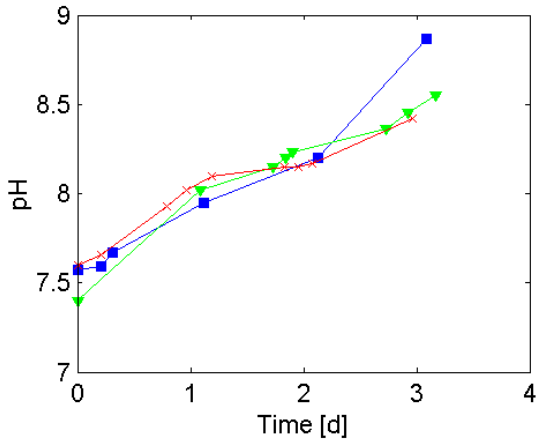

Figure 4.1.7. pH-evolution in 3 different influent batches, showing a pH increase because of stripping.

4.2.5 Mixing with air

Proper mixing of the SHARON reactor has to be ensured. However, during start-up and operation it was noticed that ammonium oxidisers are very sensitive to shear by mechanical stirring. Therefore, mixing of the SHARON reactor is performed by the air blown into the reactor.
The reduction of nitrifying activity by shear stress was also noticed by Ghyoot *et al.* (1999), among others, when operating a membrane bioreactor.

4.2.6 Wall growth

Measures had to be taken to prevent wall growth, since in a chemostat the sludge residence time has to equal the hydraulic residence time. Wall growth could increase the SRT and favour the growth of nitrite oxidizers. Wall growth could also induce anoxic conditions in the reactor and favour the growth of denitrifiers. Biomass of the reactor was therefore scraped off the walls every day. The oxygen electrode can also be covered by a biofilm. An example of the development of such a biofilm over the weekend is presented in Figure 4.1.8. The biofilm resulted in a decrease of the measured oxygen concentration because of the oxygen consumed in the biofilm on the electrode.

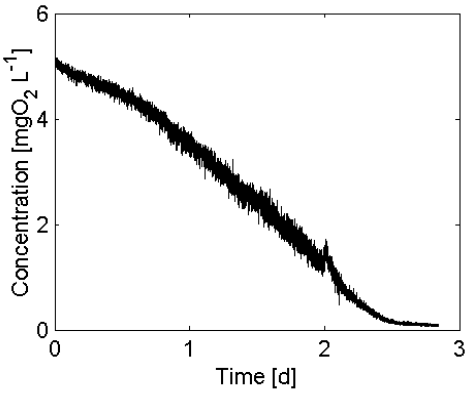

Figure 4.1.8. Biofilm development on the oxygen electrode and subsequent DO decrease over the weekend.

4.2.7 Ingrowth of nitrite oxidizers

The SHARON reactor was operated for more than 500 days with influent concentrations of 4000, 2000 and 1000 mgTAN-N L^{-1} at a HRT of 1.54 days. During this period malfunctioning of the reactor occurred due to biological instabilities, technical failures, software and computer crashes. At no point in time did nitrite oxidizers grow into the reactor resulting in nitrate build up. However, decreasing the influent concentration from 1000 mgTAN-N L^{-1} to 500 mgTAN-N L^{-1} led to the ingrowth of nitrite oxidizers even though the HRT was maintained at 1.54 days, indicating that next to temperature effects also inhibition of nitrous acid, ammonia and/or salinity may play a role in the competition between ammonium and nitrite oxidizers. Decreasing the HRT from 1.5 days to 1.2 and 1 day led to a decreasing nitrate concentration as indicated in Figure 4.1.9. However, nitrate persisted in the reactor indicating that faster growing nitrite oxidizers had grown in.

4.2.8 Protozoa

Protozoa can disturb the SHARON reactor, mainly if batches of real wastewater are used (van Dongen *et al.*, 2001a). A possible solution is to lower the reactor pH to 6 for 2 hours or to incorporate non-aerated periods. Non-aerated periods, however, clearly have a negative effect on the nitrogen conversion by nitrifiers. A pH-lowering in the SHARON reactor can be attained by reducing the influent flow under constant aeration. After one or two hours the pH will decline to approximately 6. This does not require large aeration intensity because conversion rates are relatively small. When anoxic periods have to be regularly provided to prevent protozoa growth, the SHARON reactor has to be 30 % larger to maintain good TNO_2 formation (van Dongen *et al.*, 2001a&b).

Protozoa can be observed via simple microscopic examination. This was done regularly during the operation of the SHARON reactor (Figure 4.1.10). Never protozoa were observed, possibly because synthetic influent was used. Concentrations are expressed in percentage of the total effluent nitrogen concentrations for easy comparison.

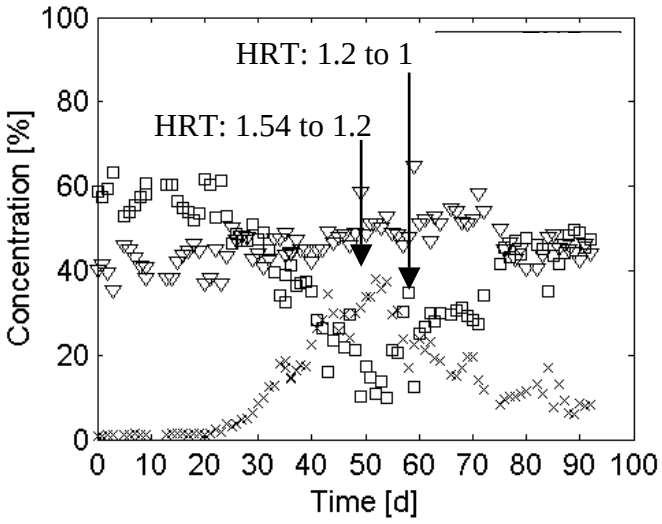

Figure 4.1.9. The evolution of TNO_2 (□), NO_3^- (x) and TAN (▽) showing the ingrowth of nitrite oxidizers after changing the influent concentration from 1000 mgTAN-N L^{-1} to 500 mgTAN-N L^{-1} on day 1.

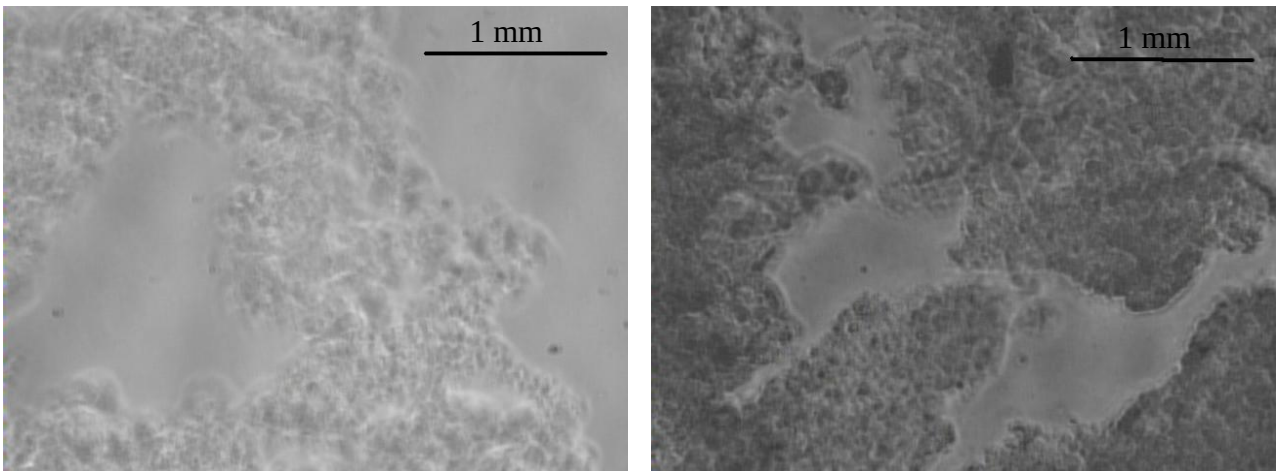

Figure 4.1.10. Microscopic images (40 x enlargement) of the SHARON sludge showing the absence of protozoa.

4.3 Experimental results

In the mean time the SHARON reactor ran for more than 2 years after successful start-up with the slow start-up method. Several instabilities occurred, but on the other hand several successful operational periods can be distinguished.

4.3.1 With pH control

During the first 45 days of operation the influent TAN concentration was 4000 mgTAN-N L^{-1} and HRT was set at 2.7 days resulting in an ammonium load of 1480 mgTAN-N L^{-1} d^{-1}. After 45 days the influent ammonium concentration and HRT were decreased to 2000 mgTAN-N L^{-1} and 1.54 days, resulting in an ammonium load of 1298 mgTAN-N L^{-1} d^{-1}. For both influent concentrations on average 80 % oxidation of TAN to TNO_2 was observed to be feasible at a pH controlled at 7.1 (results not shown), indicating the possibility of the SHARON process to treat highly concentrated nitrogen streams.
However, periods of reduced performance occurred and indicated that pH-control is not enough to produce a stable effluent. The concentrations of nitrate were always below 20 mgNO_3^--N L^{-1}, indicating the successful wash out of nitrite oxidisers.

4.3.2 Without pH control

The influence of the TIC:TAN-ratio on the behaviour of the SHARON reactor was also investigated. $NaHCO_3$ was added to the influent and the pH was only controlled to stay within the range 6-8. The SHARON reactor was operated at different TAN influent concentrations (2000, 1000, 500 mgTAN-N L^{-1}) and TIC:TAN ratio's (1:1, 0.5:1, 1.5:1). In all cases the HRT was 1.54 days. Influent concentrations and/or ratio's were only changed if a sufficiently long steady state was achieved. Typically this steady state had a duration of 15 to 30 days or 10 to 20 times the HRT. For an influent concentration of 500 mgTAN-N L^{-1} only results with a TIC:TAN ratio of 1:1 were obtained.
Average steady state values are summarised in Figure 4.1.11 together with calculated HNO_2 and NH_3 concentrations. These concentrations are calculated based on equations 2.3 and 2.4 presented in chapter 2. Effluent TAN and TNO_2 concentrations are expressed in percentage of the total effluent nitrogen concentrations for easy comparison between the different operation modes.
Transition from one operational mode to another one typically took about 8 days or 5 times the HRT as is usual in chemostat operation (Figure 4.1.12).

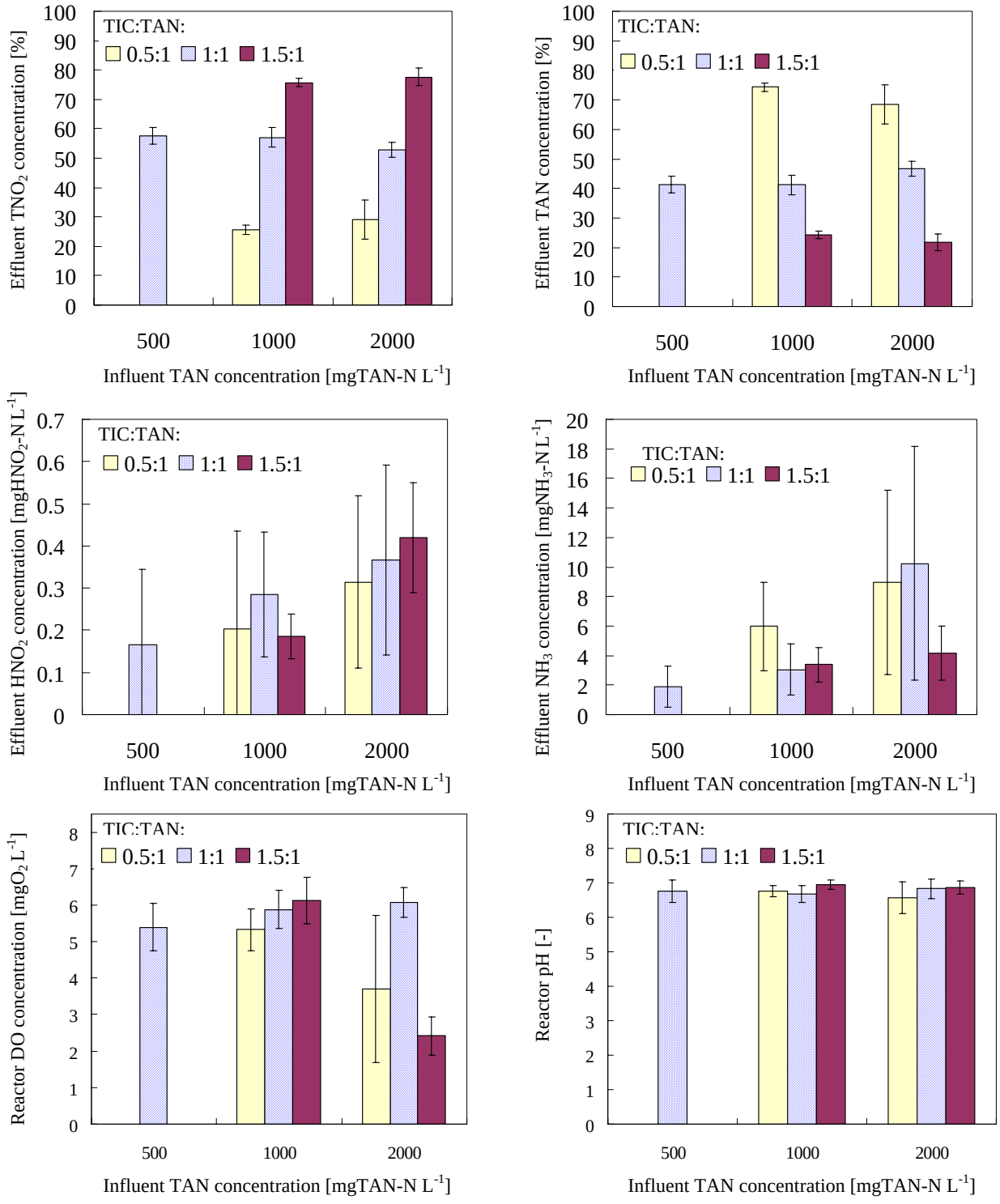

Figure 4.1.11. Average steady state results for different operating modes.

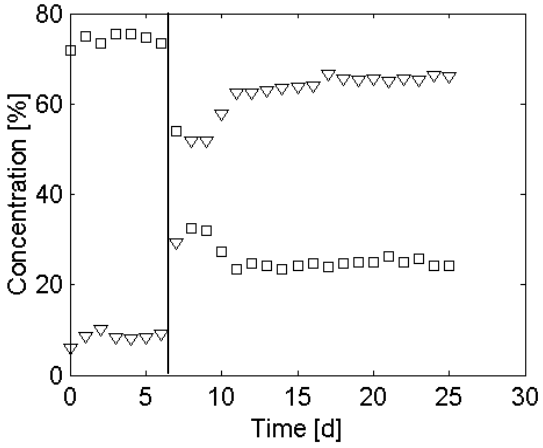

Figure 4.1.12. Transition of TNO_2 (□) and TAN (▽) concentration when switching to another operating mode on day 7.

For experiments with a TIC:TAN ratio of 0.5:1 and 1.5:1 and an influent TAN concentration of 1000 mgTAN-N L^{-1} the TNO_x instead of the TNO_2 concentration is presented because of the ingrowth of nitrite oxidizers. The experiments with a TIC:TAN ratio of 0.5:1 and 1.5:1 and an influent TAN concentration of 2000 mgTAN-N L^{-1} have a low DO concentration, possibly because a defective DO electrode was used for the measurements.

If a TIC:TAN ratio of 1:1 is applied, then a TNO_2:TAN ratio of approximately 1:1 is obtained. This effluent is an Anammox suited effluent. However, lower TAN influent concentrations but the same TIC:TAN ratio lead to slightly higher TIC:TAN effluent compositions. This becomes clear from Figure 4.1.13 where the relative concentration of TNO_2 and TAN are depicted over three experimental periods, two with an influent TAN concentration of 1000 mgTAN-N L^{-1} and one with an influent concentration of 2000 mgTAN-N L^{-1}.

This indicates that besides acidification also ammonia and nitrous acid inhibition play a role. Indeed, at lower influent concentrations more TAN can be converted to TNO_2 before the same nitrous acid concentration is attained. Another reason for the observations relates to the fact that the aeration rate is not changed during the different experimental runs. This means that relatively less CO_2 can be stripped at higher influent concentrations, as the CO_2 uptake capability of the gas phase remains unaltered.

With a TIC:TAN ratio of 0.5:1 about 25 % of the incoming TAN was oxidised, while a TIC:TAN ratio of 1.5:1 led to about 75 % of TAN oxidation. This confirms that a relationship exists between the influent TIC:TAN ratio and the effluent TNO_2:TAN ratio as was already predicted by a simulation study performed by Volcke *et al*. (2002b).

These results demonstrate the need for control of the SHARON reactor, since variations of the influent TAN concentration and the TIC:TAN ratio will occur in practice. These variations in the

influent will lead to variations in the effluent, which are undesirable in view of the sensitivity of the Anammox process towards process disturbances.

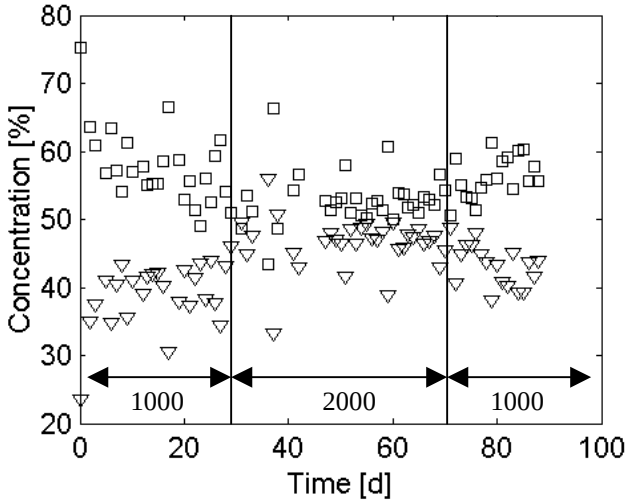

Figure 4.1.13. The evolution of TNO_2 (□) and TAN (▽) showing the effect of influent TAN concentration on effluent TAN and TNO_2 concentration. The numbers indicate the influent TAN concentration in mgTAN-N L^{-1}.

5 CONCLUSIONS

In view of future modelling, simulation and control studies a lab-scale SHARON reactor was constructed. Start-up of the reactor was feasible by slowly adapting sludge originating from a SBR to the conditions typical for the SHARON process. A dedicated start-up was indeed necessary as the conditions in the SBR are completely different from the conditions in the SHARON reactor. The sludge retention time, for example, was 10 days in the SBR reactor and only 1.54 days in the SHARON reactor. The temperature in the SBR was 15°C, while the SHARON reactor was operated at 35°C. Also toxic effects of ammonia and nitrous acid on the nitrifier population were expected in view of the high nitrogen concentrations typical for the SHARON reactor. The start-up phase began with the operation of the reactor as a SBR to prevent biomass wash out and to allow the selection of a strong nitrifying population. A month after the inoculation the reactor was switched to normal chemostat operation. As a result the nitrite oxidisers were washed out and only the ammonium oxidisers persisted in the reactor.

Once the reactor was started up the performance of the reactor could be assessed in view of its coupling with an Anammox unit. With pH controlled at 7.1, on average 80 % nitrification was

proven to be feasible for TAN loads up to 1.5 gTAN-N L^{-1} d^{-1}, indicating the possibility of the SHARON process to treat highly concentrated nitrogen streams.

Results of experiments with different TIC:TAN ratio's showed that both the influent TIC:TAN ratio and the influent TAN concentration influenced the resulting effluent concentrations, although generally it can be stated that the amount of TAN converted, or TNO_2 produced, is proportional to the influent TIC:TAN ratio. As such about 50 % of the influent TAN is converted to TNO_2 when the influent TIC:TAN ratio is 1:1. Process control of the SHARON reactor will be necessary as a constant influent for the Anammox reactor is prerequisite for the successful operation of the combined autotrophic nitrogen removal system. Indeed, varying influent TIC:TAN ratio's will result in varying and thus non-optimal TNO_2:TAN ratio's in the effluent of the SHARON reactor if no process control is applied.

When interpreting data of the lab-scale SHARON reactor for further modelling and control purposes some practical considerations, such as nitrate build-up, wall growth, water evaporation and CO_2 stripping from the influent should be considered. A list and quantification of these practical pitfalls was also presented in this contribution.

Chapter 4.2

Influence of temperature and pH on the kinetics of the SHARON nitritation process

ABSTRACT

The SHARON process is an innovative nitrogen removal process that improves the sustainability of wastewater treatment, especially when combined with an Anammox process. In order to further optimize this process by means of modelling and simulation, parameters of the biological processes have to be assessed. Batch tests with SHARON sludge clearly showed that ammonia rather than ammonium is the actual substrate and nitrous acid rather than nitrite is the actual inhibitor of the ammonium oxidation in the SHARON process. From these batch tests the ammonia affinity constant, the nitrous acid inhibition constant and the oxygen affinity constant were determined to be 0.75 mgNH_3-N L^{-1}, 2.04 mgHNO_2-N L^{-1} and 0.94 mgO_2 L^{-1}. The influence of pH and temperature on the oxygen uptake rate of SHARON biomass was determined, indicating the existence of a pH interval between 6.5 and 8 and a temperature interval from 35 to 45°C where the biomass activity is maximal.

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1 INTRODUCTION

With the discovery of the Anammox process almost 10 years ago (Mulder *et al*., 1995) a new path could be taken towards the sustainable removal of nitrogen from wastewater. In this Anammox process TAN and TNO_2 are combined on an equimolar basis to nitrogen gas, although also some nitrate is produced. The Anammox process requires a partial nitrification step in which half of the influent TAN concentration is oxidized to nitrite without further conversion to nitrate. As such a suitable influent for the Anammox reactor is produced. An example of such a partial nitritation process is the SHARON process (van Dongen *et al*., 2001a&b) in which stable nitrite formation at high temperature (35°C) and neutral pH is established by washing out the nitrite oxidizers, that grow slower than the ammonium oxidizers under these conditions. The combination of this Anammox process with a partial nitrification process has great potential since there is no longer need for external carbon addition, sludge production is very low, and oxygen input and aeration energy requirements are largely reduced (Jetten *et al*., 1997).

Very interesting and useful tools to further optimize the SHARON process are modelling and simulation environments such as WEST® (Vanhooren *et al*., 2003) or Matlab (The Mathworks Inc., www.mathworks.com). With such a simulation tool a large number of virtual experiments can be conducted in order to investigate the behaviour of the combined system under different operating conditions. In this way, time and money can be saved.

However, in order to have a correct representation of reality by these simulations, correct kinetic equations describing the biological processes have to be put forward. Furthermore the parameters in these equations have to be assessed.

Anthonisen *et al.* (1976) formulated the hypothesis that ammonia rather than ammonium is the actual substrate and that at higher concentrations ammonia becomes inhibiting. Nitrous acid inhibition, not discussed by Anthonisen *et al.* (1976), was also investigated in this study.

In this chapter the hypothesis of Anthonisen *et al.* (1976) was tested in separate batch experiments with SHARON sludge at two different temperatures (25 and 35°C) and three different pH's (6.5, 7 and 7.5). These batch experiments also allowed the determination of the ammonia affinity constant ($K_{NH3,NH}$) and the nitrous acid inhibition constant ($K_{IHNO2,\,NH}$).

The oxygen affinity constant (K_{O2}) was determined with a similar experiment. Further the maximum growth rate μ^{max}_{NH} and the influence of temperature and pH on the maximum oxygen uptake rate were determined.

2 MATERIALS AND METHODS

2.1 SHARON reactor

Sludge for the experiments was sampled from the SHARON reactor described in Chapter 4.1. The reactor is a 2 litre continuously stirred tank reactor (CSTR) without biomass retention. The synthetic influent is pumped with a peristaltic pump from the 5 litre influent vessel to the reactor. The reactor is aerated through a pumice stone using air from a compressor (1 bar overpressure). The temperature of the reactor is controlled to be 35°C. In the reactor the dissolved oxygen (DO) and pH are measured. The pH is controlled through the Labview® software (National Instruments, www.ni.com) by means of acid (HCl) or base (NaOH) addition. Data logging is also performed with the Labview® software.

2.2 Batch experiments

Respirometric batch experiments (Spanjers *et al.*, 1996) with the SHARON sludge were performed to asses the ammonia affinity constant, the nitrous acid inhibition constant, the oxygen affinity constant and the influence of pH and temperature on the maximum oxygen uptake rate. A schematic representation of the experimental set-up is shown in Figure 4.2.1.

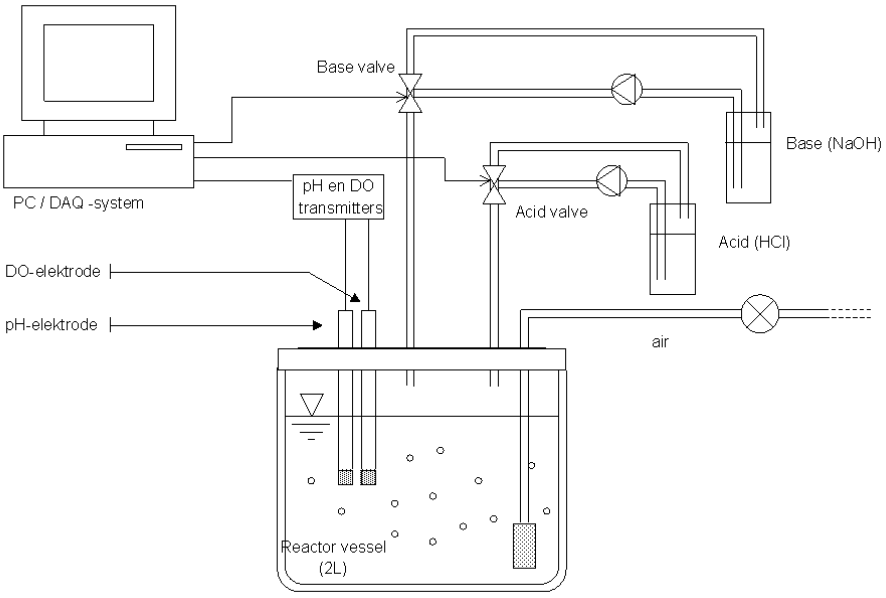

Figure 4.2.1. Schematic representation of the experimental set-up used for determining the influence of temperature and pH on the kinetics of the SHARON nitritation process.

Oxygen uptake rates were determined by turning off the aeration in the reactor and recording the drop in DO concentration. The slope of this DO concentration versus time plot equals the oxygen uptake rate (OUR = dDO/dt). For every experiment the OUR was measured twice under every condition. Linking this OUR to the conditions in the reactor (temperature, pH, TAN concentration, TNO_2, …) gives information on the kinetics of the SHARON process.
Aeration through the headspace was always smaller than 5% of the OUR. Hence, the error introduced by this aeration in the dissolved oxygen balance used for the oxygen uptake rate determination can be assumed negligible.
Before each experiment the sludge was washed with softened water to ensure that no TNO_2 or TAN was present at the beginning of the experiment. The same softened water was used for influent preparation of the SHARON reactor. Hence, a similar osmotic pressure during the experiments as during normal sludge conditions was ensured.
After each experiment it was verified qualitatively that no nitrate was formed, in order to link the oxygen uptake rate to ammonium oxidizer activity only.

2.2.1 Ammonia affinity and inhibition constant

Batch tests at two different temperatures (25 and 35°C) and three different pH's (6.5, 7 and 7.5) were performed for the determination of $K_{NH3,NH}$. In every batch test sequential additions of $(NH_4)_2SO_4$ were carried out and after each addition the OUR was determined. Before and after every oxygen drop a sample was taken for TAN (ammonium + ammonia) analysis. This way the OUR can be linked to the TAN concentration.
A similar experiment to determine ammonia inhibition was conducted at 35°C and pH 8. This last experiment was performed 3 months after the other experiments, but with sludge from the same reactor.

2.2.2 Nitrous acid inhibition constant

Similar batch tests as for the determination of $K_{NH3,NH}$ were conducted to determine the nitrous acid inhibition constant. Before the experiment, an excess of 1000 mgTAN-N L^{-1} was added to the reactor to exclude substrate limitation. In every batch test sequential additions of KNO_2 were carried out and after each addition the OUR was determined. Before and after every oxygen drop a sample was taken for subsequent TNO_2 (nitrous acid + nitrite) analysis. This way the OUR can be linked to the TNO_2 concentration.

2.2.3 Oxygen affinity constant

Again batch tests at two different temperatures (25 and 35°C) and three different pH's (6.5, 7 and 7.5) were performed for the determination of $K_{O2,NH}$. In every batch test an excess of

1000 mgTAN-N L^{-1} was added. Aeration was turned off. The drop in DO concentration was recorded until the concentration reached 0.1 mgO_2 L^{-1}. Plotting the time derivative of the DO concentration versus the concentration itself yields a Monod curve expressing oxygen limitation of the OUR.

2.2.4 Maximum oxygen uptake rate (OUR)

An excess of substrate to exclude substrate limitation was first added to the reactor. Starting from pH 7 the pH was varied between 5 and 9 in steps of 0.25 at a temperature of 25°C and 35°C. For every pH-value the maximum OUR was determined twice and was linked to pH.
A similar experiment was conducted for the temperature dependency. This time 6 different temperature setpoints (15, 20, 25, 30, 35 and 40°C) were applied, while keeping pH constant at 7. The temperatures in the experiment were applied in an increasing order because of practical considerations.
In Table 4.2.1 the cumulative substrate concentrations, pH and temperature profiles for the different experiments are summarized.

2.3 Analysis

Concentrations of TAN and TNO_2 were analysed after proper dilution using spectrophotometric methods (Dr Lange GmbH, Germany). Every sample was analysed twice. The absence of TAN and TNO_2 at the beginning and NO_3^- at the end of the experiment was checked semi-quantitatively with test strips (Merckoquant, www.vwr.com). The dissolved oxygen was measured by Ingold (Mettler Toledo) Clarck type oxygen electrode. The pH was measured with a glass electrode.

2.4 Parameter estimation

Parameter estimation was performed with the WEST® modelling and simulation software (Vanhooren *et al*., 2003).

Table 4.2.1. Cumulative substrate concentration, pH and temperature profiles for the different kinetic experiments performed in this chapter

Experiment	Ammonia affinity constant	Ammonia inhibition constant	Nitrous acid inhibition constant	Influence of pH	Influence of T
	Accumulated TAN concentration [mgTAN-N L^{-1}]	Accumulated TAN concentration [mgTAN-N L^{-1}]	Accumulated TNO2 concentration [mgTNO2-N L^{-1}]	pH values	T [°C]
	10	100	100	7	15
	25	250	300	6.75	20
	50	500	500	7.25	25
	75	1000	700	6.5	30
	100	2000	900	7.5	35
	200	3000	1100	6.25	40
	300	4000	1300	7.75	45
	500	5000	1500	6	50
	1000	6000	1700	8	
	2000	7000	1900	5.75	
		8000	2000	8.25	
		9000		5.5	
		10000		8.5	
		20000		5.25	
				8.75	
				5	
				9	

3 RESULTS AND DISCUSSION

3.1 SHARON kinetics

Biochemical experiments carried out over more than half a century on different cultures clearly indicated that kinetics are influenced by many physico-chemical and biological environmental factors among which the most important are: substrate concentration, product concentration, pH, temperature, dissolved oxygen and various inhibitors such as salts (Dochain and Vanrolleghem, 2001). The specific growth rate is then commonly expressed by the multiplication of individual terms, each of them referring to one of the influencing factors.

Ammonia rather than ammonium is the actual substrate in nitrification and at higher concentrations ammonia becomes inhibiting according to Anthonisen *et al.* (1976). Nitrous acid

inhibition was not discussed by Anthonisen *et al.* (1976), but in this study batch experiments were conducted to evaluate the effect of nitrous acid. In general Monod type expressions are used for the influence of ammonia and nitrous acid.

As discussed in chapter 3.2, the influence of temperature on biological activity is most often modelled by an Arrhenius-type of equation:

$$\mu(T) = \mu(T_r)\, e^{\theta(T-T_r)} \tag{4.2.1}$$

where μ(T) is the maximum specific growth rate μ at the actual temperature T, T_r is the reference temperature (often taken 20°C) and θ is the Arrhenius constant. The Arrhenius constant for autotrophs can be calculated with the activation energy (E_{act}) of the autotrophic biomass (Hao *et al*., 2002a) by equation 4.2.2.

$$\theta = \frac{E_{act}}{\left(R\ 293\ (T+273)\right)} \tag{4.2.2}$$

where R is the universal gas constant (8.31 J mol^{-1} K^{-1}). Since the activation energies of aerobic ammonium oxidation ranges in literature from 60 to 72 kJ mol^{-1} (Jetten *et al*., 1999; Helder and De Vries (1983). Knowles *et al*. (1965), Stratton and Mc Carty (1967)). The θ value lies in the range of 0.085 to 0.1. This equation however does not take the decrease of activity at temperatures above 40°C into account. Therefore equations such as the Hinshelwood model (equation 4.2.3) and the modified Rathowsky model (Zwietering *et al*., 1991) (equation 4.2.4) were put forward.

$$\mu = k_1\, e^{-\frac{E_1}{R(T+273)}} - k_2\, e^{-\frac{E_2}{R(T+273)}} \tag{4.2.3}$$

$$\mu = \left[b\left(T - T_{\min}\right)\right]^2 \left\{1 - e^{c\left(T-T_{\max}\right)}\right\} \tag{4.2.4}$$

The Hinshelwood model is based on the fundamental Arrhenius model, E_1 and E_2 are the activation energies of the reaction and the high-temperature denaturation respectively, but the parameters are strongly correlated and will therefore be very difficult to estimate. The modified Rathowsky model has no biological basis but was shown to be the most suitable to describe the specific growth rate as function of temperature in a study by Zwietering *et al*. (1991). The parameters T_{min} and T_{max} are the minimum and maximum temperature at which growth is observed. The parameters b and c are two parameters without biological basis.

The effect of pH on biological activity is normally less pronounced than the effect of temperature because the cell is reasonably well able to regulate its internal hydrogen ion concentration in the

face of adverse external concentrations, though the maintenance energy required to do this is obviously affected. In addition, the pH of the external medium has an important effect on the structure and permeability of the cell membrane (Sinclair, 1988). Up to now only a few good models for this pH-dependency have been suggested. One possibility is the bell-shaped function as given in equations 4.2.5 (Dochain and Vanrolleghem, 2001) and 4.2.6 (Henze *et al*., 1995).

$$\mu = \mu^{\max} \frac{1}{1+10^{pK_1 - pH} + 10^{pH - pK_2}} \tag{4.2.5}$$

$$\mu = \mu^{\max} \frac{K_{pH}}{K_{pH} - 1 + 10^{|pH_{opt} - pH|}} \tag{4.2.6}$$

In this contribution the function proposed by Henze *et al*. (1995) was chosen, because the obtained data showed a better fit to this equation. Based on the above elaborated considerations expression 4.2.7 for the growth rate of ammonium oxidizers is proposed:

$$\mu_{NH} = \mu_{NH}^{\max} \frac{S_{NH_3}}{S_{NH_3} + K_{NH_3,NH}} \frac{K_{I,NH_3,NH}}{S_{NH_3} + K_{NH_3,NH}} \frac{K_{IHNO_2,NH}}{S_{HNO_2} + K_{IHNO_2,NH}} \frac{S_{O_2}}{S_{O_2} + K_{O_2,NH}} \frac{K_{pH}}{K_{pH} - 1 + 10^{|pH_{opt} - pH|}} \left[b\left(T - T_{\min}\right)\right]^2 \left\{1 - e^{c\left(T - T_{\max}\right)}\right\} \tag{4.2.7}$$

The parameters for this equation will be determined for the SHARON process. Note that in all experiments the OUR and not the maximum specific growth rate is determined. However, if a constant biomass yield is assumed then both values are proportional:

$$OUR_{NH} = \frac{3.43 - Y_{NH,O}}{Y_{NH,O}} \mu_{NH} \; X_{NH} \tag{4.2.8}$$

The hypothesis of this constant biomass yield is not always valid, certainly when maintenance effects start playing a role. Further research on this maintenance is therefore necessary to allow the back calculation of the obtained OUR values to μ^{max}_{NH} values.

3.2 Ammonia affinity constant

Figure 4.2.2 summarizes the OUR values measured at different TAN concentration, for different pH values at 35°C. These three Monod curves are expressed in TAN concentration and %, with the highest OUR value for each experiment as reference. The Monod curves are expressed in % to enable comparison between the different experiments.

It can be seen from Figure 4.2.2 that for each experiment a different affinity constant would be obtained if the constant would be expressed in terms of the TAN concentration. A higher pH

results in a lower affinity constant for TAN: the TAN concentration at which the OUR reaches half of its maximum value is then lower.

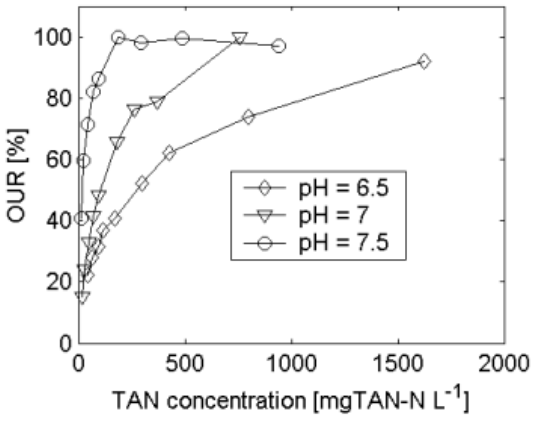

Figure 4.2.2. Process kinetics expressed in % of OUR_{max} and TAN concentration obtained at 35°C.

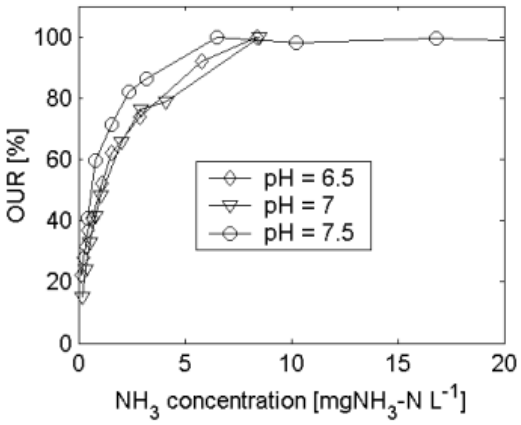

Figure 4.2.3. Process kinetics expressed in % of OUR_{max} and NH_3 concentration obtained at 35°C.

In order to test Anthonisen's hypothesis that the uncharged ammonia is the actual substrate for the ammonium oxidizers, the Monod curves of the three experiments were expressed in terms of NH_3 concentration in Figure 4.2.3. From equations 4.2.9 and 4.2.10 the fraction of total ammonium present in the form of uncharged ammonia (NH_3) is calculated by equation 4.2.11.

$$TAN = NH_3 + NH_4^+ \tag{4.2.9}$$

$$K_e^{NH} = \frac{NH_3 \cdot H^+}{NH_4^+} = 1.13\,10^{-9} \text{ at } 35°C \tag{4.2.10}$$

$$C_{NH_3} = \frac{C_{TAN}}{1 + \frac{10^{-pH}}{K_e^{NH}}} \tag{4.2.11}$$

Figure 4.2.3 shows that the Monod curves now coincide: the ammonia affinity constant, reflecting the concentration of uncharged ammonia at which the OUR reaches half of its maximum value, remains almost constant for varying pH.

The same experiment was conducted at 25°C. In order to compare experimental results at the two temperatures (25°C and 35°C) two temperature (T in K) dependencies for the equilibrium constant, proposed by Anthonisen *et al*. (1976) (equation 4.2.12) and Helgeson (1967) (equation 4.2.13) were used. Both dependencies yielded the same result.

$$K_e^{NH} = e^{\frac{-6344}{T+273}} \tag{4.2.12}$$

$$K_e^{NH} = 10^{-\left(\frac{2835.8}{T+273} - 0.6322 + 0.00123(T+273)\right)} \tag{4.2.13}$$

In Figure 4.2.4 all collected experimental data (of 2 temperatures) are given as function of the NH_3 concentration. This NH_3 concentration was calculated with the temperature dependent equilibrium constant and the measured TAN concentration using equation 4.2.10.

All data clearly overlap, indicating that NH_3 rather than NH_4^+ is the actual substrate. The affinity constant for ammonia can be considered as independent of pH and temperature and was determined to be 0.75 ± 0.052 mgNH_3-N L^{-1}. All experimental data except the experiment at 25°C and pH 6.5, because of experimental problems, were used for this parameter estimation.

In Table 4.2.2 the resulting affinity constants for the different experiments (T = 25 and 35°C, pH = 6.5, 7, 7.5) expressed in mgTAN-N L^{-1} are given. Large differences exist between the affinity constants. Independent of the experiment it can be seen that the ammonium affinity constant is high compared to values found in literature for normal nitrifying sludge (0.06-27.5 mgTAN L^{-1}, Pynaert, 2003; 0.034 mgNH_3–N L^{-1} at 20°C, Wiesmann, 1994), although Suzuki *et al.* (1974) found a fairly high affinity constant of 0.32 mgNH_3–N L^{-1} between 6.5 and 8.5 in cell-free extracts of *Nitrosomonas europaea*. A possible explanation is that the SHARON organisms are exposed to high ammonia concentrations and are as such not selected for their substrate affinity.

A similar high affinity constant was found by Hellinga *et al*. (1999) for their SHARON reactor (K_{NH3} = 0.47 mgNH_3–N L^{-1} at 35°C and pH 7) and Hunik *et al.* (1992) for a pure culture of *Nitrosomonas europea* (K_{NH3} = 0.3 mgNH_3–N L^{-1} at 35°C and pH 7). Wyffels *et al*. (2004b) used an ammonia affinity constant of 0.85 mgNH_3–N L^{-1} to simulate a partial nitritation OLAND reactor (see Chapter 5.1).

Table 4.2.2. Resulting TAN affinity constants expressed in mgTAN-N L^{-1}, indicating the wide range of values obtained at different pH and temperatures

pH T[°C]	6.5	7	7.5
25	420.1	133.4	42.7
35	210.8	67.2	21.7

The high ammonia concentration exposure might also explain why ammonia inhibition was only detected in an experiment at pH 8 at concentrations above 300 $mgNH_3$ –N L^{-1} as can be seen from Figure 4.2.5. This is in contrast with the findings of Groeneweg *et al.* (1994) who observed TAN inhibition at pH 8 at concentrations above 100 mgTAN–N L^{-1} or 10 $mgNH_3$–N L^{-1}.
Hellinga *et al*. (1999) performed a similar experiment at 40°C and pH 7 and found no inhibition until concentrations of 6000 $mgNH_4$–N L^{-1} or 93 $mgNH_3$–N L^{-1}. The decrease of OUR in this experiment can probably be attributed to salinity effects (Moussa *et al.*, 2003).
Inhibition of ammonia was therefore not considered further in this study that deals with treating digester effluent. The Monod term dealing with ammonia inhibition was therefore omitted from the kinetic expression.

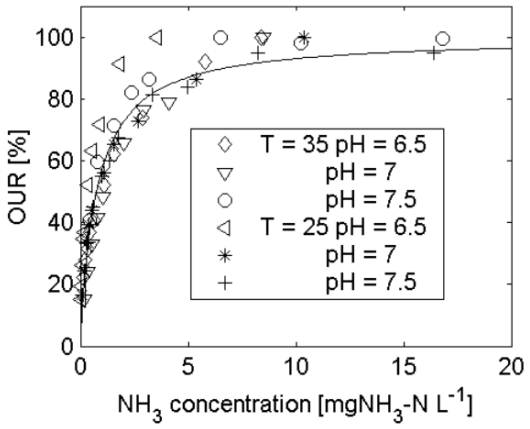

Figure 4.2.4. Process kinetics expressed in % of OUR_{max} and NH_3 concentration.

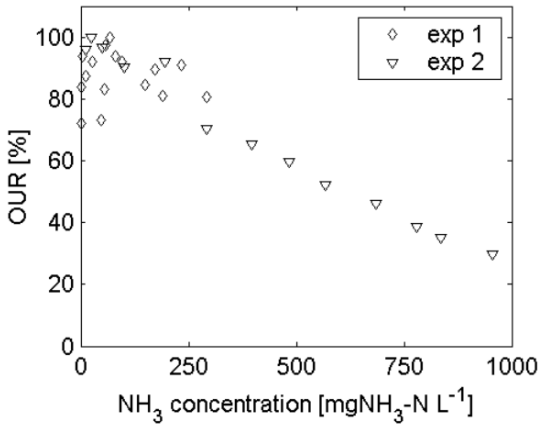

Figure 4.2.5. Process kinetics expressed in % of OUR_{max} and NH_3 concentration (35°C and pH=8).

3.3 Nitrous acid inhibition constant

The inhibition by TNO_2 at two different pH and two different temperatures is given in Figures 4.2.6a and b. The curves are again expressed in TNO_2 concentration and % relative to the highest OUR at the given temperature and pH. Clearly, the TNO_2 inhibition coefficient is different for the different cases but the temperature dependency is not significant.
Results for the six different experiments are again summarized in one Figure (Figure 4.2.7) by expressing the Monod curves in terms of HNO_2 by applying equation 4.2.14.

$$C_{HNO_2} = \frac{C_{TNO_2}}{1 + \frac{K_e^{NO}}{10^{-pH}}} \quad (4.2.14)$$

where K_e^{NO} is the acidity constant of the nitrite/nitrous acid equilibrium ($HNO_2 \leftrightarrow NO_2^- + H^+$). For this equilibrium constant a temperature (T in K) dependency was used as proposed by Anthonisen *et al*. (1976) and given by equation 4.2.15.

$$K_e^{NO} = e^{\frac{-2300}{T+273}} \quad (4.2.15)$$

From Figure 4.2.7 it is clear that HNO_2 is the real inhibitor since all curves now coincide, although less pronounced than for the affinity constant. This HNO_2 inhibition was not found by Anthonisen *et al*. (1976).

Note that the inhibition curve is only determined up to 60 % inhibition. This is because the experiments were stopped at 2000 mgTNO_2-N/l, which is in practice the upper level for TNO_2 concentrations in a SHARON reactor treating digester effluent. Also, from Figure 4.2.7 $K_{I,HNO2}$ could be determined to be 2.04 ± 0.017 mgHNO_2-N L^{-1}. This time all 6 experiments could be included for parameter estimation. The value is tenfold higher than the one determined by Hellinga *et al*. (1999) (0.203 mgHNO_2-N L^{-1} at pH 7 and T=35°C), indicating high nitrous acid resistance, possibly because the system has run at higher concentrations resulting in adaptation of the biomass.

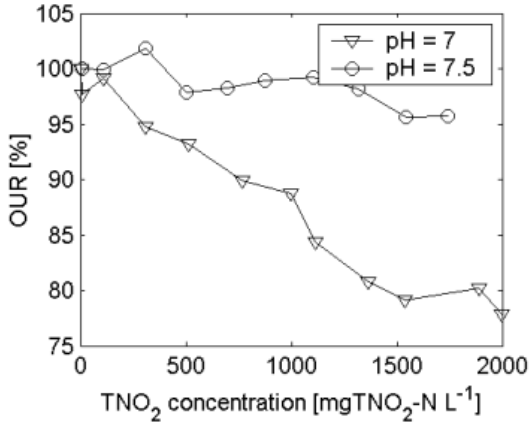

Figure 4.2.6a. Process kinetics expressed in % of OUR_{max} and TNO_2 concentration obtained at 35°C.

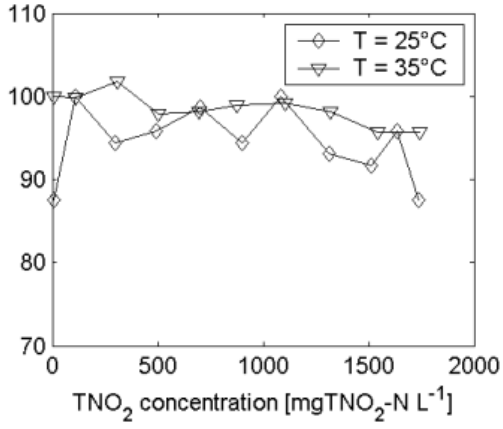

Figure 4.3.6b. Process kinetics expressed in % of OUR_{max} and TNO_2 concentration obtained at pH 7.5.

3.4 Oxygen affinity constant

No real influence of pH and/or temperature on K_{O2} was noticed in the different experiments in which the oxygen uptake rate evolution as function of a lowering oxygen concentration was observed. The average K_{O2} was determined to be 0.94 ± 0.091 $mgO_2\ L^{-1}$. This value is well in the range of values found in literature for activated sludge nitrifiers. As an example, the results from three experiments at pH 7 and 25°C are depicted in Figure 4.2.8.

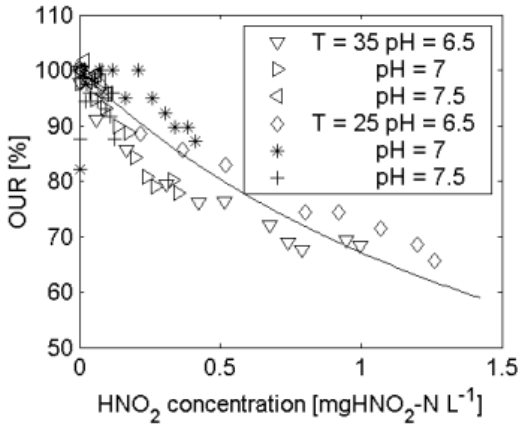

Figure 4.2.7. Process kinetics expressed in % and HNO_2 concentration.

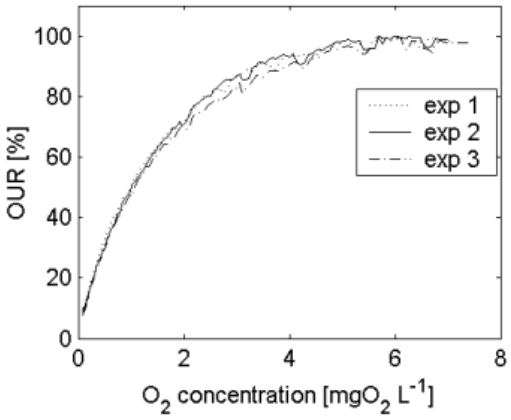

Figure 4.2.8. Process kinetics expressed in % and O_2 concentration for K_{O2} determination.

3.5 Maximum oxygen uptake rate-μ^{max}

The maximum specific growth rate (μ^{max}_{NH}) was determined from the parameters estimated above in dedicated batch experiments, as well as from steady state data of the continuous SHARON reactor over a 24 days period during which the hydraulic retention time (HRT) was 1.54 days, the influent TIC:TAN ratio was 1:1, the influent concentration was 2000 $mgTAN\text{-}N\ L^{-1}$ and the reactor temperature was 35°C (chapter 4.1). The average effluent NH_3, HNO_2 and DO concentrations in this period were 10.24 $mgNH_3\text{-}N\ L^{-1}$, 0.37 $mgHNO_2\text{-}N\ L^{-1}$ and 6.07 $mgO_2\ L^{-1}$ respectively. The average pH was 6.83.

Inserting the 24 daily measurements one by one in the well known chemostat equation 4.2.16 allowed the determination of a μ^{max}_{NH} of 1.0 ± 0.2 d^{-1}.

$$D = \frac{1}{HRT} = \mu_{NH} = \mu_{NH}^{\max} \frac{C_{NH_3}}{C_{NH_3} + K_{NH_3}} \frac{K_{I,HNO_2}}{C_{HNO_2} + K_{I,HNO_2}} \frac{C_{O_2}}{C_{O_2} + K_{O_2}} \qquad (4.2.16)$$

This is a value quite lower than normally found in literature (e.g. $1.5\,d^{-1}$ at 35°C and pH 7, Hellinga *et al.*, 1999), possibly because pH has a direct effect on the specific growth rate, which is not taken into account in the above equation.
In order to investigate the direct influence of pH at two different temperatures (25°C and 35°C) the oxygen uptake rate of the SHARON organisms at varying pH was measured (Figure 4.2.9a). Again curves are expressed in % relative to the highest OUR for the given temperature. This OUR was fitted to equation 4.2.17.

$$OUR[\%] = 100\frac{K_{pH}}{K_{pH} - 1 + 10^{|pH_{opt} - pH|}} \tag{4.2.17}$$

with K_{pH} and pH_{opt} estimated to be 8.21 ± 0.87 and 7.23 ± 0.027 respectively. According to this equation a pH of 6.8 would lead to approximately 20% reduction in oxygen uptake rate. So, at an optimal pH of 7.23 the μ^{max} would be 1.25 d^{-1} compared to 1.0 d^{-1} at pH 6.8. Figure 4.2.9a has an important engineering conclusion, i.e. there exists a narrow pH interval between 6.5 and 8 where the growth rate is optimal.
In Figure 4.2.9b the influence of temperature on the oxygen uptake rate of the SHARON organisms determined in two independent batch tests at pH 7 is depicted. This OUR was fitted to the modified Ratkowsky model (Zwietering *et al.*, 1991) (T in °C) given by equation 4.2.18.

$$OUR = 100\left[b\left(T - T_{\min}\right)\right]^2 \left\{1 - e^{c\left(T - T_{\max}\right)}\right\} \tag{4.2.18}$$

with b = 0.045 ± 0.002, c = 0.0459 ± 0.0068, T_{min} = 10.12 ± 0.76 and T_{max} = 56.06 ± 1.00. It is clear that temperatures between 35°C to 45°C are optimal for the SHARON process. However, only short-term temperature effects were investigated here. Long term exposure to a different temperature would lead to adaptation and temperatures above 40 °C would to lead to deactivation.

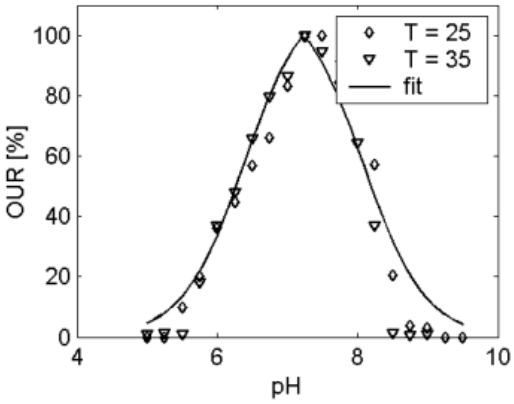

Figure 4.2.9a. Influence of pH at 25°C and 35°C on the oxygen uptake rate.

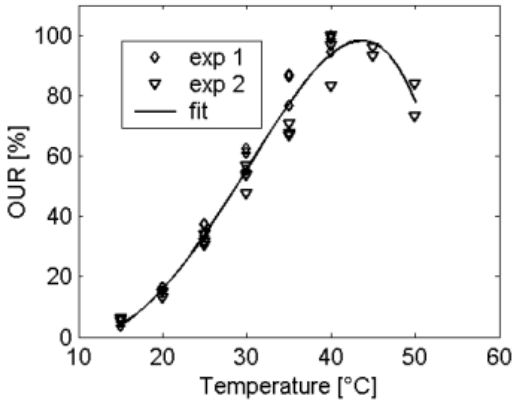

Figure 4.2.9b. Influence of temperature at pH 7 on the oxygen uptake rate.

4 CONCLUSIONS

Batch experiments at two different temperatures (25 and 35°C) and three different pH's (6.5, 7 and 7.5) with SHARON sludge clearly confirmed that ammonia rather than ammonium is the actual substrate for ammonium oxidizers. From these experiments the ammonia affinity constant could be determined to be 0.75 $mgNH_3$-N L^{-1}, and was found not to depend on pH nor temperature. In contrast with the findings of Anthonisen *et al.* (1976), ammonia inhibition of ammonium oxidisers was only detected at high concentrations. This can be attributed to adaptation of the SHARON process to high ammonia concentrations.

Further, similar inhibition-focussed experiments have shown that nitrous acid rather than nitrite is the actual inhibitor of the SHARON organisms. For the nitrous acid inhibition coefficient, a value of 2.04 $mgHNO_2$-N L^{-1} was found, also independent of pH and temperature.

The oxygen affinity constant was determined to be 0.94 mgO_2 L^{-1}, independent of pH and temperature.

The direct influence of pH and temperature on the maximum oxygen uptake rate of SHARON biomass was determined, indicating the existence of a narrow pH and temperature interval between 6.5 and 8 and 35 and 45°C respectively where the oxygen uptake rate is optimal.

The parameter values determined in this study can now be implemented in a simulation model for further optimization of the SHARON process.

Chapter 4.3

Titrimetric monitoring of a lab-scale SHARON-Anammox process

ABSTRACT

Fully autotrophic nitrogen removal processes, such as the combined SHARON-Anammox process, help to improve the sustainability of wastewater treatment. Successful operation of such a completely autotrophic system is among others based on the strict control of the SHARON reactor in order to produce an Anammox-suited influent with a 1:1 TAN:TNO_2 ratio. The high quality and high frequency measurements provided by a titrimetric set-up measuring the total ammonium (TAN) and total nitrite (TNO_2) concentrations facilitate this control considerably. In this study the use of a titrimetric set-up for monitoring the combined SHARON-Anammox process is investigated. The technique that interprets on-line collected titration curves was applied to a lab-scale system. Comparison with classic colorimetric results gave statistically indistinguishable results for two techniques for measurement of TAN and TNO_2 concentrations in the SHARON reactor. In the Anammox reactor only TAN could be determined by the investigated method due to the very low TNO_2 concentrations. Phosphate, a potential inhibitor of the Anammox process, was also shown feasible to be measured in the effluent of the SHARON reactor. Three measurements are thus combined in one single instrument. The proposed measuring technique holds different advantages over other TAN and TNO_2 measurement techniques such as on-site availability, easy automation, the absence of the need for high dilutions and cost reduction.

A reduced version of this chapter is accepted as oral presentation at ICA 2005 conference:
Van Hulle, S.W.H., Zaher, U., Schelstraete, G., & Vanrolleghem, P.A. (2005). Titrimetric monitoring of a completely autotrophic nitrogen removal process. In: *Proceedings 2nd IWA Conference on Instrumentation, Control and Automation for water and wastewater treatment and transport systems.* Busan, Korea, May 29-June 2, 2005 (in Press).

1 INTRODUCTION

1.1 The SHARON-Anammox process

Applying the SHARON-Anammox process (van Dongen *et al.*, 2001a&b) can significantly reduce the pressure on nitrogen removal in the main wastewater treatment plant (WWTP) by separately treating the sludge digester effluent (typically 1 gN L^{-1}) before this stream is recycled to the entrance of the WWTP. This highly loaded nitrogen stream is first partially oxidized in the SHARON reactor which works at a sludge retention time (SRT) of 1 to 1.5 days and a temperature between 30°C and 40°C. As such ammonium oxidizers are maintained in the reactor, while nitrite oxidizers are washed out and further nitrification of TNO_2 to nitrate is prevented. The SHARON reactor can produce an almost 1:1 total ammonium to total nitrite (TAN:TNO_2) ratio depending on the total ammonium to total inorganic carbon (TAN:TIC) ratio in the influent of the SHARON reactor (Van Hulle *et al.*, 2003b). The effluent of the SHARON reactor is then sent to the Anammox reactor where the remainder of the TAN is oxidized anoxically with TNO_2 as electron acceptor (Jetten *et al.*, 1999).

With the combined SHARON-Anammox process, low nitrogen effluent concentrations can be obtained, while aeration costs are significantly reduced, no additional carbon source is needed and sludge production is very low (Jetten *et al.*, 1997). Although the application of this completely autotrophic system is very promising, difficulties in operation are to be expected in view of the inhibition of the Anammox organisms by oxygen, phosphate and TNO_2 (Strous *et al.*, 1997a; Strous *et al.*, 1999b). Careful control of the preceding SHARON reactor is therefore essential for successful operation. A key factor in this control is the availability of high quality and high frequency measurements of TAN and TNO_2 as these are the principal components of the combined system. Up to now the methods used for the off-line measurement of these components (e.g. spectrophotometry and ion chromatography) are expensive and difficult to automate, especially in view of the concentration ranges typical for the SHARON reactor (1 gN L^{-1}). Indeed, large dilutions are required as will become clear from the following overview. In this contribution therefore the use of a titrimetric device for the monitoring and control of the combined SHARON-Anammox process is studied.

1.2 Overview of different methods for measuring TAN and TNO_2

The TAN and TNO_2 concentrations can be determined by different methods as summarized from Standard methods (1996) and the Dr Lange (Dr Lange GmbH, Germany) and Dionex (Dionex Corporation, USA) user manuals in Table 4.3.1. Selection of specific methods must be based upon the concentration level and the amount and types of interferences present.

The colorimetric method is used to measure unknown concentrations in a sample by measuring the sample's colour intensity. The colour of the sample after adding specific chemicals (reagents) is compared with colours of known concentrations. For TNO_2 the measuring range is 0.005 to 6 mg TNO_2-N L^{-1} and for TAN this range is 0.02 to 130 mgTAN-N L^{-1}. Usually the range of the TAN and TNO_2 concentration in the SHARON reactor is 350-750 mgN L^{-1}, whereas in the Anammox reactor this is 0-50 mgN L^{-1}. Due to this concentration range the colorimetric method requires dilution, which is a source of errors and additional manipulations. For the TNO_2 concentrations in the SHARON reactor this can amount up to 100 times. Moreover, the colorimetric method is an expensive method as 1 analysis costs up to 3 € (Dr LangeGmbH, Germany).

With the ion chromatography method for anion determination a sample is injected into a stream of bicarbonate eluent and passed through a series of ion exchangers. The anions of interest are separated on the basis of their relative affinities for a low capacity, strongly basic anion exchanger. For the determination of cations such as ammonium, the same principle of retention can be used, but an electrolytically generated methenesulfonic acid (MSA) eluent is used together with suppressed conductivity detection. With this method TAN and TNO_2 can be measured in the range of 0 to 40 mgN L^{-1}. Again dilution is necessary if the SHARON reactor is to be monitored.

The ammonia selective electrode uses a hydrophobic (water repelling) gas permeable membrane to separate a sample solution from the electrode internal solution of NH_4Cl. Dissolved ammonia ($NH_{3(aq)}$ and NH_4^+) is converted to $NH_{3(aq)}$ by rising the pH above 11 with a strong base. $NH_{3(aq)}$ diffused through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter. With this electrode no dilution is necessary for the SHARON process and also the TAN concentration in the Anammox process can be measured.

The TNO_2 selective biosensor contains bacteria that reduce NO_2^- (but not NO_3^-) to N_2O that is subsequently monitored by a built-in electrochemical sensor (Nielsen *et al*., 2002; Nielsen *et al*., 2004). This biosensor was made by substituting the *Agrobacterium radiobacter* used in the NO_x^- biosensor (Larsen *et al.,* 2000) with a strain that could only reduce NO_2^-, and not NO_3^-, to N_2O. This TNO_2 biosensor is suited for measuring TNO_2 in the Anammox reactor, but high dilution is again necessary for measuring TNO_2 in the SHARON reactor as the linear range of the sensor is limited from 0 to 30 mgTNO_2-N L^{-1} at 30°C. Also the lifetime of the sensor is limited (a few weeks at most) (Sin and Vanrolleghem, 2004).

With the titrimetric method acid or base is dosed on a drop by drop basis. Based on the obtained buffer curve a mathematical model calculates the concentration in the sample (a more exhaustive

description of this method can be found above). With this method both the TNO_2 as the TAN concentration in the SHARON as well as the Anammox reactor can be measured without dilution and at low costs. Also both ions can be measured in one low cost analysis, while for most other methods 2 separate analyses are necessary. Further, even additional ions such as phosphate can be determined with the same analysis if the concentrations are sufficiently high. The titrimetic method is often used for the analysis of component concentrations of anaerobic digesters (Vanrolleghem and Lee, 2003). Since the SHARON-Anammox process is typically used for the treatment of anaerobic digester effluent the titrimetric set-up will typically be already available on-site. This titrimetric method thus seems very promising for the monitoring of the SHARON-Anammox process.

Table 4.3.1. Comparison between different methods for TNO_2 and TAN measurement. (Table elaborated using information from the Standard Methods (1996) and the Dr Lange (Dr Lange GmbH, Germany) and Dionex (Dionex Corporation, USA) user manuals)

Component	Method	Range (mg N/L)	Interferences
TNO_2	Colorimetric	0,005 – 6	free chlorine, and nitrogen trichlorine (NCL_3)
	Ion Chromatography	0.01- 40	Any compound that has the same retention time as nitrite.
	Titrimetry	0,01-1000	Volatile alkaline
	TNO_2 selective electrode (biosensor)	At 8 ºC: 0-4,2 At 20ºC: 0-14 At 30ºC: 0-28	Temperature, Oxygen and Volatile alkalines.
TAN	Colorimetric	0.02 – 130	Volatile bases (i.e: hydrazine, amines)
	Titrimetry	> 5	Volatile alkaline
	Ammonia –selective electrode	0.03 – 1400	Amines, Hg^{2+}, Ag^{+} Volatile bases
	Ion Chromatography	0,05 - 40	Any compound that has the same retention time

2 MATERIALS AND METHODS

2.1 SHARON reactor

The lab-scale SHARON used in this study is a 2 litre continuously stirred tank reactor (CSTR) without biomass retention. The influent is pumped to the reactor at a rate of 1.3 litre per day. The pump flow rate of this influent pump determines both the hydraulic residence time (HRT) and the sludge residence time, since both residence times are equal and defined as the ratio of the volume to the flow rate.

The reactor is fed with synthetic influent. The influent TAN concentration is 1000 mg TAN-N L^{-1} and the TAN:TIC ratio is 1:1 as described in chapter 4.1. After 26 days of operation the amount of KH_2PO_4 was reduced 20 times (from 228 mgPO_4^{3-}-P L^{-1} to 11 mgPO_4^{3-}-P L^{-1}) to avoid toxic conditions for the Anammox organisms.

The reactor is aerated through a pumice stone using air from a compressor (1 bar). The normal operational temperature is 35°C, as is usual in practice for the SHARON process. The dissolved oxygen (DO) and pH in the reactor are logged with Labview® software. The pH was allowed to vary freely in a broad pH control range (pH 6-8), although the average pH during the experimental period was 6.9. The oxygen was always above 5 mgO_2 L^{-1}. Construction, start-up and operation of the SHARON reactor are described in detail in chapter 4.1.

2.2 Anammox reactor

The Anammox reactor is a 2 litre Sequencing Batch Reactor (SBR). The reactor is a Biostat B2 (BBI, Germany) and is equiped with sensors for pH, DO, redox and temperature. Operation and monitoring of the reactor is controlled via the BRAUN MFCS® software, as described by Wyffels (2004). The pH and temperature were controlled at 7.5 and 35°C respectively. When necessary N_2 gas was flushed through the reactor to ensure anaerobiosis. The reactor was fed with the effluent of the SHARON reactor that was diluted to obtain a TAN and TNO_2 concentration of 400 mgN L^{-1} each. When needed the TNO_2 or TAN influent concentrations were adjusted with $NaNO_2$ or $(NH_4)_2SO_4$. The biomass washed out from the SHARON reactor was not separated from the Anammox influent. The reactor was operated at an almost infinite SRT and a HRT of 4 days. Several times during operation the influent and effluent pumps were stopped in case TNO_2 concentrations became too high, as this would cause serious inhibition of the Anammox biomass.

2.3 Chemical analyses and standard solutions

Concentrations of TAN, TNO_2 and NO_3^- were analysed on a daily basis using colorimetric methods (Dr Lange GmbH, Germany).

Concentrations of TAN and TNO_2 were also analysed with a titrimetric set-up (Metrohm titrino 716, Metrohm, Switzerland) and the Buffer Capacity Software (BCS) developed by Zaher and Vanrolleghem (2004), of which a small summary is given in the following paragraph. Before titration the samples were acidified to pH 2 by the addition of a 37% HCl solution. As such the CO_2 in the sample was stripped. As such, the carbonate and bicarbonate buffer systems will not influence the titrimetric determination of TAN and TNO_2. The titrant (NaOH) was prepared by adding an appropriate amount of NaOH pellets to distilled water. Standard solutions were prepared by appropriate dilution from a 4 gN L^{-1} $NaNO_2$ and $(NH_4)_2SO_4$ stock solution.

2.4 Interpreting the titration curve with the BCS software

Titrimetric determination of buffers such as the monoprotic components TAN and TNO_2 (pKa values at 25°C respectively 9.2 and 3.4, Stumm and Morgan, 1996), the diprotic component bicarbonate (indicated as Total Inorganic Carbon (TIC) with pKa values at 25°C of 6.4 and 10.4, Stumm and Morgan, 1996) and the triprotic component phosphate (with pKa values at 25°C of 2.1, 7.2 and 11.9, Stumm and Morgan, 1996) is accomplished with the BCS software that performes a model-based interpretation of experimentally determined buffer capacity curves (Zaher and Vanrolleghem, 2004). From the measured buffer capacity curve, estimates of the different buffering components are computed using model selection and parameter estimation techniques. The methods used for model selection and parameter estimation are described elsewhere (Van De Steene *et al*., 2002).

The buffer capacity curve is calculated from the titration curve, which is obtained by measuring the pH as function of a stepwise addition of base. From this measured titration curve (typically around 30 to 50 points), the buffer capacity at each pH point is calculated as the derivative of the amount of base needed (meq l^{-1} pH^{-1}). This buffer capacity curve in fact consists of the sum of the buffer capacities of individual buffering components in the solution (Van Vooren *et al*., 2001), as demonstrated in Figure 4.3.1. In this Figure a typical example of a sample taken from a partial nitrifying reactor is depicted. The buffer capacity curve as measured titrimetrically is shown next to the contributions to the buffer capacity curve by TAN, phosphate and TNO_2, with a respective concentration of 10 mM (140 mgTAN-N L^{-1}), 5 mM (217 mgPO_4^{3-}-P L^{-1}) and 7 mM (98 mgTAN-N L^{-1}). These components are very critical for autotrophic nitrogen removal operation. Also, the contribution to the buffer capacity of the water buffer is given in the Figure. The area of each peak is related to the concentration of the component, while the position is depending on the pKa value(s) of the component. From the Figure it can be seen that possible interference of the water buffer with the TNO_2 buffer might impede the determination of the TNO_2 concentration, especially if the pKa value of TNO_2 shifts to a lower value because of the effect of salt contents, for instance.

The model based interpretation is performed as follows. The automatic model building of the BCS software starts with calibrating an initial model that is defined and initialised on the basis of available information concerning the expected buffer components in a certain system. The residuals (differences between model and data) are analysed and used to suggest a candidate model extension, i.e. adding an additional buffer with unknown pKa and concentration. Then another fitting cycle is performed with the extended model, the residuals are analysed, a new extension is defined, and so on, until one of the stop criteria is reached. The stop criteria are based on a set of model selection techniques, which determine the optimum buffer capacity model and then provide the estimates of the pKa's and concentrations of the significant buffers. Also, based on the fitting results, standard deviations are estimated for each of the pKa and concentration values.

Automation, with a measurement every 30 minutes, of this method is straightforward and is already available through the Anasense® titrimetric analyser (De Neve *et al.*, 2004) currently applied to titrimetric monitoring of anaerobic digesters (measuring VFA, bicarbonate, TAN, phenol and lactate).

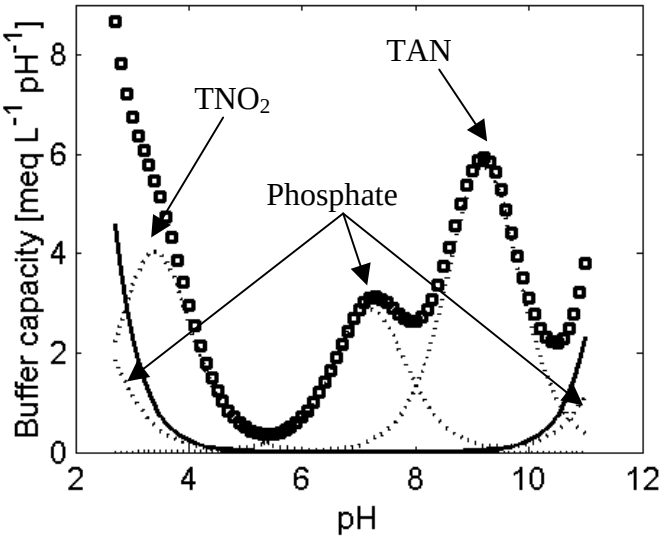

Figure 4.3.1. Contribution of individual components (…) (in casu TNO_2, phosphate and TAN) and the water buffer (-) to the buffer capacity curve (□).

3 RESULTS AND DISCUSSION

3.1 Titration with standard solutions

Before using the titrimetric method with the actual system, it was tested with standard solutions. Solutions of TAN and TNO_2 ranging from 10 to 4000 mgN L^{-1} were prepared from the stock solution and analysed with the titrimetric set-up in a 50 ml sample without applying any dilution. Titrant concentrations of 0.5, 0.2, 0.1 and 0.05 M were used in order to determine the optimal titrant concentration. Comparison between expected and measured concentrations with the different titrants revealed that only with the lowest concentration (0.05M) an acceptable agreement was obtained. With higher titrant concentrations the difference between expected and calculated concentration was increasing when low TAN and TNO_2 concentrations had to be measured. Especially for the TNO_2 concentration the difference is too large to be accurate. As an example of this discrepancy the expected and calculated concentrations measured with a 0.2 M titrant concentration are depicted in Figure 4.3.2, while in Figure 4.3.3 the relative error is presented. This error [%] was calculated as 100 times the ratio of the difference between the expected and the measured concentration to the expected concentration. The t-values calculated for a paired t-test (Weiss, 2002) for this example were 4.6 (n=3) and 3.7 (n=4) for TAN and TNO_2 respectively. As the tabulated t-values for the 95 % confidence interval are 4.3 ($t_{2,0.975}$) and 3.18 ($t_{3,0.975}$) the hypothesis that both methods are the same must be rejected. Clearly an underestimation of the TAN and TNO_2 concentrations occurs with this titrant. As a fairly linear relation still exists between the measured and expected concentrations a calibration line could be used. However such a calibration line would only hold under certain conditions and would undo the advantages of a model-based buffer curve interpretation.

For the 0.05 M titrant the concentrations of TAN and TNO_2 that were determined by the titrimetric method were much closer to the actual concentrations as depicted in Figures 4 and 5. The t-values calculated for a paired t-test were –0.887 and 0.245 (n=7) for TAN and TNO_2 respectively. As the tabulated t-value for the 95 % confidence interval is 2.45 ($t_{6,0.975}$) the hypothesis that both methods are the same can not be rejected. Further, an R^2-value of more than 0.99 was obtained for the correlation between the measured and actual values for both TNO_2 and TAN. Concentrations down to 10 mgN L^{-1} could be measured accurately.

In Figure 4.3.6 typical buffer capacity curves are presented for low (10 mgN L^{-1}) and high (500 mgN L^{-1}) TAN and TNO_2 concentrations using 0.05 M titrant. Corresponding fits calculated with the BCS software show good agreement with the measured curves.

Of course the application of such a lowly concentrated titrant limits the use of the titrimetric set-up to samples with a total nitrogen concentration below 1000 mgN L^{-1} since the physical

limitations of the titrimetric vessel do not allow higher concentrations, i.e. more titrant must be dosed than the volume of the test vessel.

Based on these preliminary measurements with stock solutions it was decided to use a 0.05 M titrant for the determination of the TAN and TNO_2 concentrations in the SHARON and Anammox reactors. These measurements will then be compared to measurements of TAN and TNO_2 with colorimetric methods (Dr Lange GmbH, Germany) as presented below.

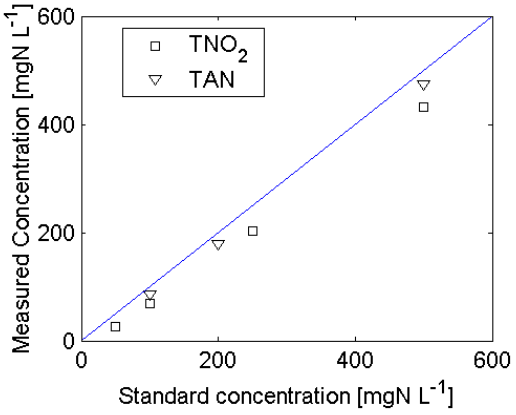

Figure 4.3.2. Comparison between expected and measured concentrations determined with a titrant of 0.2 M.

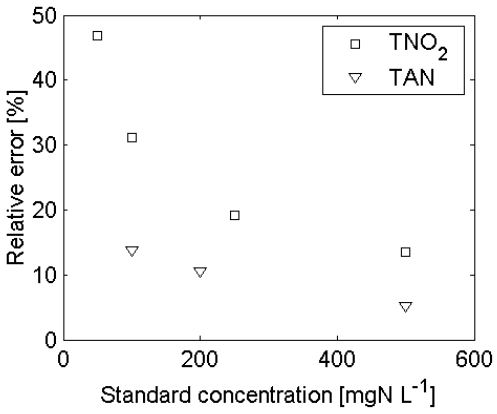

Figure 4.3.3. Relative error between expected and measured concentrations determined with a titrant of 0.2 M.

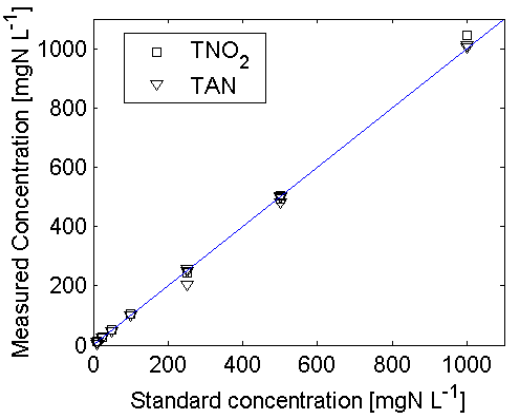

Figure 4.3.4. Comparison between expected and measured concentrations determined with a titrant of 0.05 M.

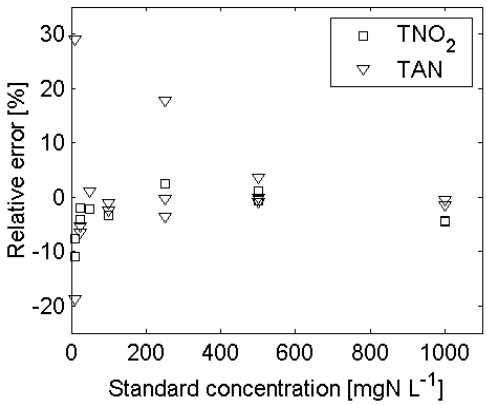

Figure 4.3.5. Relative error between expected and measured concentration determined with a titrant of 0.05M.

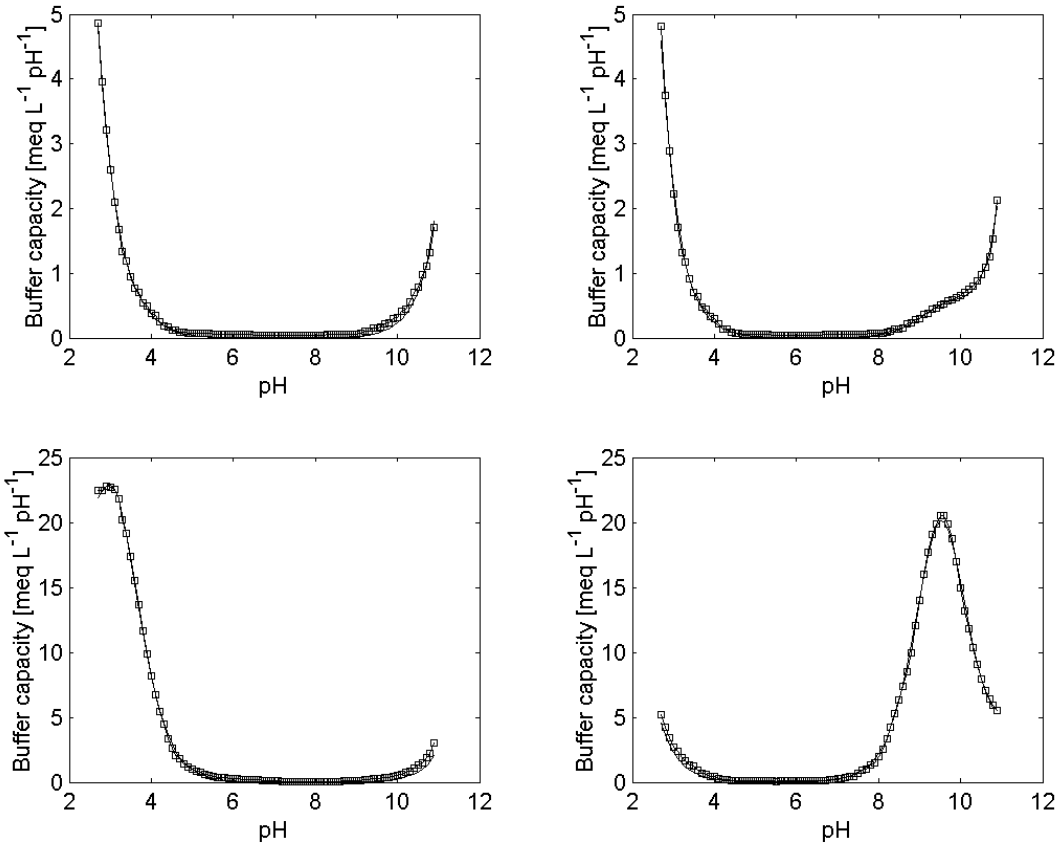

Figure 4.3.6. Typical buffer capacity curves for low (10 mgN L^{-1}, top) and high (500 mgN L^{-1}, bottom) TNO_2 (right) and TAN (left) concentrations using 0.05 M titrant (-: measured values, □: calculated values).

3.2 Monitoring the SHARON reactor

Samples from the SHARON reactor were taken daily and analysed once with the colorimetric method and twice with the titrimetric method. For practical reasons one of the two samples analysed with the titrimetric set-up was diluted twice and a 50 ml sample was used. For the other sample only a 35 ml volume was used.

Three times, around day 55, day 95 and day 110 (Figure 4.3.7), a disturbance occurred in the reactor causing the TAN concentration to increase and the TNO_2 concentration to decrease. With both methods this disturbance could be detected. The rest of the studied period the reactor operated stably even though the sampling for the titrimetric set-up caused the hydraulic residence time to fluctuate.

In Figure 4.3.7 (top) the comparison between colorimetric and titrimetric measurements is made for TAN and TNO_2 concentrations in the SHARON reactor. For TAN the concentrations

determined with both methods are very similar. The TNO_2 concentrations determined with both methods also follow the same trend, but here the colorimetric results show somewhat more scattering. This high variability may be due to the high dilution (100 times) that needs to be applied for determining the TNO_2 concentrations with the colorimetric method. As such, small errors during sample handling are amplified in the final result.

To statistically support the hypothesis that the titrimetric set-up offers an alternative to colorimetric methods for measuring the TAN and TNO_2 concentrations in a SHARON reactor 17 samples were analysed twice with each method. (Un)fortunately during the sampling period the reactor performance was not stable and TNO_2 and TAN concentrations fluctuated between 150 mgN L^{-1} and 1000 mgN L^{-1}. The t-values calculated for a paired t-test were 1.027 and 1.065 (n=17) for TAN and TNO_2 respectively. As the tabulated t-value for the 95 % confidence interval is 2.12 ($t_{16,0.975}$) the hypothesis that both methods are the same can not be rejected. In Figure 4.3.8 the TAN and TNO_2 concentrations measured with the colorimetric and titrimetric methods are compared in a q-q plot. The excellent agreement is obvious.

The average pKa values of TAN and TNO_2 determined with the BCS software for these 15 samples were 9.42 ± 0.05 and 2.85 ± 0.06 respectively. This indicates that a shift in pKa values occurred compared to the values mentioned by Stumm and Morgan (1996), possibly caused by temperature and salinity effects. The BCS software however easily deals with these shifts as it allows some freedom around the default values.

In addition to cost-effective TAN and TNO_2 measurements, the titrimetric measurement also offers the determination of phosphate, a component that was present in the influent of the SHARON reactor. However this only works on condition that the concentration is sufficiently high. From day 1 until day 26 the concentration of phosphate in the influent was 228 mgPO_4^{3-}-P L^{-1}, but after day 26 the phosphate concentration was reduced 20 times in view of the possible toxic effects of phosphate on the Anammox biomass (van de Graaf *et al.*, 1996). On average the phosphate concentration determined titrimetrically before day 26 was 244 ± 35 mgPO_4^{3-}-P L^{-1}. This concentration is somewhat higher than the one present in the influent, possibly because of the concentrating effect of evaporation that occurs in the SHARON reactor (Van Hulle *et al.*, 2003b). After day 26 phosphate could not longer be detected titrimetrically because the concentration was too low compared to the TNO_2 and the TAN concentrations.

Typical buffer capacity curves of the period before and after the phosphate reduction are depicted in Figure 4.3.9.

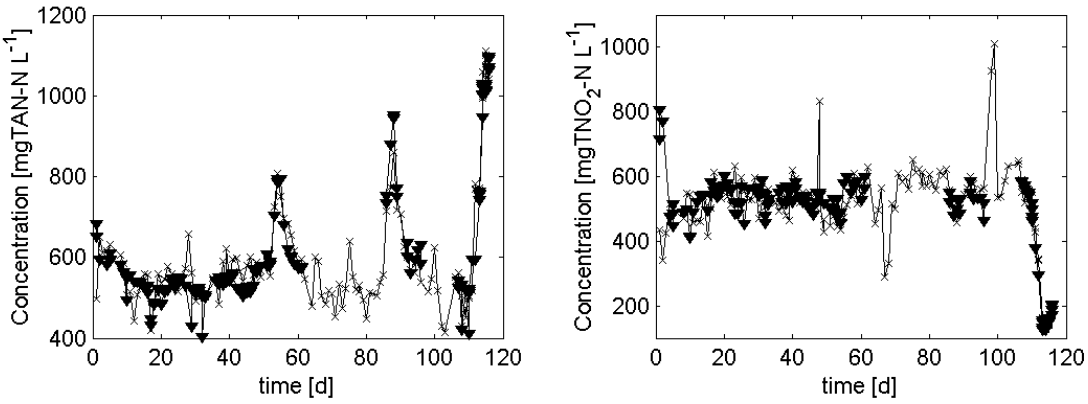

Figure 4.3.7. Comparison between colorimetric (x) and titrimetric measurements (▼) for TAN concentrations (left) and TNO_2 (right) in the SHARON reactor.

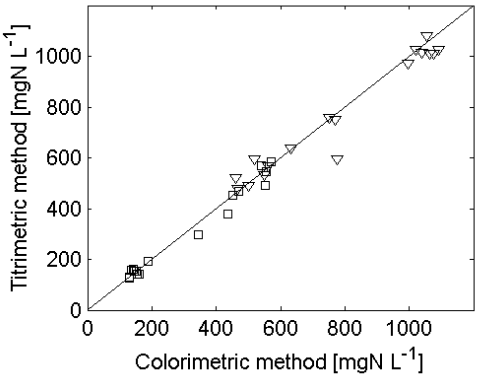

Figure 4.3.8. The q-q plot of TAN (▽) and TNO_2 (□) for comparing concentration measured in the 17 samples with the colorimetric and titrimetric method.

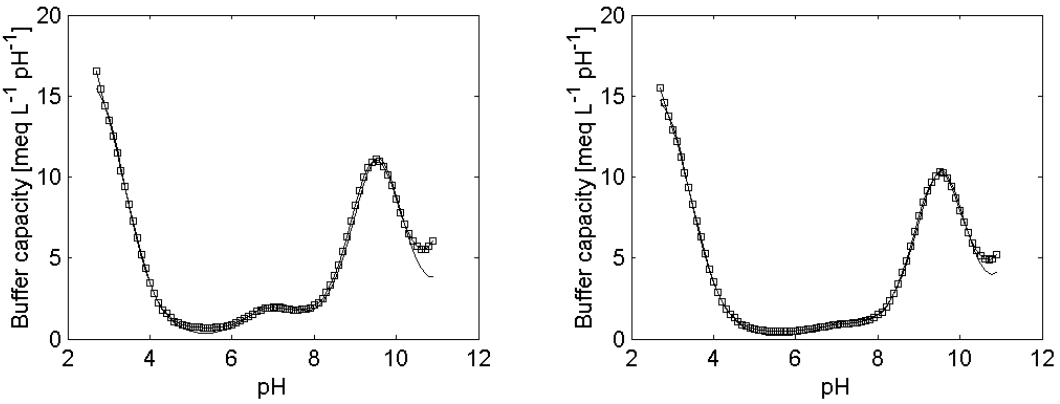

Figure 4.3.9. Typical buffer capacity curves for the period with 228 $mgPO_4^{3-}$-P L^{-1} phosphate in the influent (left) and the period with 11 $mgPO_4^{3-}$-P L^{-1} phosphate in the influent (right). Both samples were diluted twice and a 0.05 M titrant was used.

3.3 Monitoring the Anammox reactor

Samples from the Anammox reactor were also taken on a daily basis and analysed once with the colorimetric method and twice with the titrimetric method. A 50 ml sample without dilution was applied for the titrimetric method.

Unfortunately the Anammox reactor did not operate stably at the time of the study. Possible reasons for this are phosphate and TNO_2 inhibition. Also the presence of oxygen can inhibit the Anammox process, but the reactor was regularly purged with nitrogen gas and no oxygen was detected in the reactor.

Figure 4.3.10 compares between colorimetric and titrimetric measurements for TAN concentrations in the Anammox reactor. It can be seen that both analytical methods give approximately the same result.

The average pKa value of TAN determined with the BCS software was similar to the pKa value determined for the SHARON reactor (9.59 +/- 0.3), although the variation between the different samples is somewhat higher.

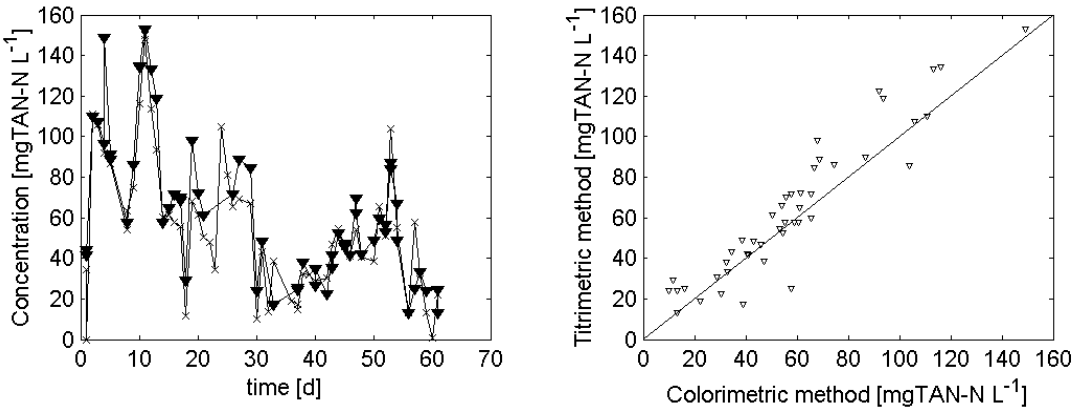

Figure 4.3.10. Comparison between colorimetric and titrimetric measurements for TAN concentrations in the SHARON reactor (left) and corresponding q-q plot (right).

Unfortunately, for the TNO_2 concentration no close agreement between both methods could be obtained. The TNO_2 concentrations determined with the titrimetric device were overestimated up to two times compared to the colorimetric method. Possibly interferences with other components present may have led to problems with the determination of the low TNO_2 concentration in the Anammox sample. This is disappointing, as specifically the TNO_2 concentration in the Anammox reactor should be monitored closely in view of the inhibition of Anammox at elevated TNO_2 concentrations. Further study is certainly warranted.

3.4 Control of autotrophic nitrogen removal by titrimetric data

As it was shown that the titrimetric technique can be employed for measuring TAN and TNO_2 in SHARON reactor and TAN in the Anammox reactor, these measurements can be used for efficient control of the autotrophic nitrogen removal system.

The measurement frequency of the components can however not be higher than once per hour as, due to the necessity to perform a slow titration to reach the required measuring accuracy (Zaher and Vanrolleghem, 2004), an analysis takes up to 45 minutes. However, as the residence time in both reactors is of the order of 1 day, this frequency is sufficient for the TAN and TNO_2 concentrations to be used as a measurement for the master controller in, for instance, a cascade controlled SHARON reactor as proposed by Volcke *et al.* (2003). In such a control structure the TAN:TNO_2 ratio is determined by using the titrimetric measurements. The difference between this ratio and the desired TAN:TNO_2 ratio necessary for the Anammox reactor (typically between 1 and 1.32) will determine the setpoint of, for example, pH or DO set by the master controller. The slave controller in the SHARON reactor will then adjust the pH or DO to the appropriate value.

How the controllers are tuned, what (other) variables should be measured and/or controlled is a subject for other (Volcke *et al.*, 2003) and further research.

4 CONCLUSIONS

In this study the possibility of using a titrimetric set-up for the determination of TAN and TNO_2 when monitoring the combined SHARON-Anammox process was investigated. First the optimal titrant concentration was determined to be 0.05 M based on measurements of standard solutions within the typical concentration range.

Then, measurements with both methods were compared for samples taken from a lab-scale SHARON reactor. For both nitrogen components the different methods gave similar results, although the TNO_2 concentrations determined with the colorimetric device varied considerably. A statistical test showed that both methods could not be distinguished from each other with 95% confidence.

For the Anammox reactor, only the TAN concentrations obtained with both methods agreed, while the TNO_2 concentrations were overestimated with the titrimetric set-up due to the low concentration of TNO_2 in the effluent and possible interferences by other components.

Basically, the titrimetric set-up gives the possibility to replace part of the analysis work by a cheaper and easy to automate method that requires no dilution and hence is less sensitive to manipulation errors. However, TNO_2 in the Anammox reactor can not be determined by the proposed method, so a combination of titrimetric and other measurements will still be necessary in the future.

Next to TAN and TNO_2, phosphate could also be detected as an additional measurement in the SHARON reactor, provided the concentration is sufficiently high (> 25 mgN L^{-1}).

The titrimetric method can be employed for control of the autotrophic nitrogen removal process, initially as a cost-effective and reliable off-line device, but in the future as an on-line titrimeter (Vanrolleghem and Lee, 2003).

PART 5

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SIMULATION AND OPTIMIZATION OF AUTOTROPHIC NITROGEN REMOVAL

Chapter 5.1

Modelling and simulation of oxygen-limited partial nitrification in a membrane-assisted bioreactor

ABSTRACT

The mathematical model for ammonium oxidation to nitrite and nitrite oxidation to nitrate described in Chapter 3.2 was calibrated and validated with data from a lab-scale membrane-assisted bioreactor (MBR) operated under oxygen-limited conditions. The reactor was fed with reject water from an anaerobic sludge digester and its main goal was to produce an Anammox-suited effluent, i.e. an effluent containing an almost equimolar amount of ammonium and nitrite. First, the mathematical model was calibrated with in situ batch tests determining the kinetic parameters for microbial growth. Second, the start-up and operational data of the oxygen-limited MBR were compared with model simulations. From these simulations it became clear that the model could accurately describe the experimental data. As such, a validated model was obtained. Third, the validated model was used for further process simulation and optimisation: steady state simulations were performed under different operational conditions including hydraulic retention time (HRT), K_La, temperature and sludge residence time (SRT). Simulation results indicated that stable nitrite production from sludge reject water was feasible with this process even at a relatively low temperature of 20°C with HRT down to a 0.25 days.

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1 INTRODUCTION

Removing nitrogen components from wastewater is an important issue nowadays in view of the adverse effects of these components on the environment and the increasingly stringent discharge standards. Especially the abatement of concentrated nitrogenous wastewater, such as sludge digestion reject water, becomes an important issue in today's treatment plants because these streams represent only a few percentages of the total flow, but up to 15 % of the nitrogen load (Mulder *et al.*, 2001). An innovative and sustainable process that can accomplish the treatment of highly loaded nitrogen streams is the combined partial nitrification-Anammox process. This combined completely autotrophic process has great potential since there is no longer need for external carbon addition, sludge production is very low, and oxygen input and aeration energy requirements are largely reduced (Jetten *et al*., 1997).

The application of a mathematical model, as described in chapter 3.2, to further improve and optimize the autotrophic nitrogen removal process is very useful, especially in view of the long start-up times required for this process. For example, with such a mathematical model several operational scenarios can be quickly simulated, while testing a large variation of conditions experimentally would require extremely long time (Hao and van Loosdrecht, 2004). Recently, some studies have been conducted dealing with simulation and optimization of nitrification and Anammox processes. Measured nitrification performance and nitrifier community composition in a membrane-assisted bioreactor (MBR) was compared with simulated data by Liebig *et al*. (2001). Behaviour of the SHARON process was simulated under different operational conditions (Hellinga *et al.*, 1999, Volcke *et al.*, 2002). Hao *et al.* (2002a&b) performed a thorough simulation study on the behaviour of a partial nitrification-Anammox system under different process conditions, such as varying temperature and dissolved oxygen concentration. However, in these studies, except for the study of Liebig *et al.* (2001), no verification with real experimental data was performed and no start-up dynamics were included.

In this chapter therefore start-up and operational data from a membrane-assisted bioreactor containing nitrifying biomass was used to calibrate and validate the first process (partial nitrification) involved in the autotrophic nitrogen removal process. In the subsequent chapter 5.2 data from an Anammox sequencing batch reactor (SBR) will be used for calibration and validation of the second process (Anammox) involved in the autotrophic nitrogen removal process. Further, in this chapter the partial nitrification MBR process will be evaluated in more detail through simulation. Based on these simulations optimal operating strategies can be suggested. The objectives of the present chapter are therefore twofold: (1) to accurately describe with a mathematical model the operation of the MBR during start-up and operation of the partial

nitrification process and (2) to assess the influence of operating parameters (K_La, temperature, HRT, SRT) on the steady-state process performance via simulations with the validated model.

2 MATERIALS AND METHODS

2.1 The membrane-assisted bioreactor (MBR)

The MBR used in this study was a 1.5 litre reactor operated as a Continuous Stirred Tank Reactor (CSTR) with a submerged microfiltration membrane module (with a total surface of 23 m^2 m^{-3} reactor) to allow continuous separation of biomass and reactor effluent (Wyffels *et al.*, 2003). All bacterial biomass was retained within the reactor as the hollow fiber membranes had a maximum pore size of 0.6 µm. Apart from sludge removal by sampling no sludge was wasted resulting in a virtual infinite sludge retention time (SRT) of 650 days.

The reactor was stirred at 200 rounds per minute (rpm). To maintain constant operating conditions, the reactor pH was controlled at 7.90±0.01 by adding 1M $NaHCO_3$, and the reactor temperature was controlled at 30.0±0.1°C. Strict temperature control at lower values was difficult during summer time due to the absence of cooling water in the control system

The reactor was fed with reject water from the anaerobic digestion of sewage sludge containing on average 931 ± 122 mgTAN L^{-1} and 605 ± 36 mgCOD L^{-1}. Sporadic BOD tests showed that about 30 % of this COD was biodegradable.

Further details on chemical analysis and operation of the MBR are described by Wyffels (2004).

2.2 Batch determination of oxygen transfer coefficient

The relationship between the measured airflow rate and the gas–liquid volumetric oxygen transfer coefficient (K_La) applied in the mathematical model was determined within the MBR in separate short-term batch experiments, taking 10–33 min depending on the imposed airflow rate. Experiments were conducted at the end of the reactor run under the same operating conditions as for the partial nitritation process, i.e., at a stirring rate of 200 rpm and a temperature of 30.0°C. After deoxygenation, the medium was re-aerated to oxygen saturation levels and the DO concentration was monitored at different imposed airflow rates, allowing calculation of the K_La using non-steady state methods (Cornel *et al.*, 2003). Airflow rates were monitored with rotameters and measured with a digital flow meter (ADM1000; J & W Scientific, Folsom, CA).

2.3 Modelling the MBR system

Modelling of the partial nitrification process was performed within the modelling and simulation environment WEST® (Vanhooren *et al.*, 2003) as described in chapters 3.1 and 3.2. The MBR configuration was implemented by placing a CSTR in series with an ideal point-settler with a

non-settleable fraction equal to zero, representing the complete retention of biomass. The recycle flow rate of the point-settler to the CSTR was chosen to be very small to prevent possible numerical stiffness of the system (Figure 5.1.1). The K_La was defined as an operational parameter to describe the effect of varying airflow rates and was controlled through an input file. Other operational and design parameters (temperature, pH, HRT, and influent TAN concentration) were also incorporated in the model via the input file.

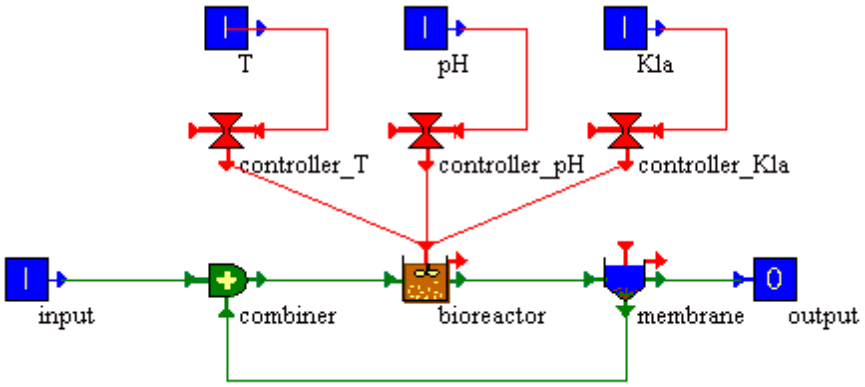

Figure 5.1.1. Implementation of the MBR configuration in WEST®. Operational and design parameters (T, pH, and KLa) are defined through an input file, as is the influent wastewater composition.

Activated Sludge Model 1 (Henze *et al.*, 2000) was chosen as the standard model and was extended with a two-step nitrification model. Bacterial growth and decay processes were modelled for the heterotrophic (X_H), and autotrophic ammonium (X_{NH}) and nitrite oxidizing (X_{NO}) biomass. Endogenous respiration processes were not incorporated within the model because these are not yet clearly documented for ammonium and nitrite oxidizers. The details of the model are discussed in Chapter 3.2.

Kinetic and stoichiometric parameters for heterotrophic growth were derived from literature. The substrate affinity constant ($K_{S,H}$) was assumed to be somewhat higher (50 mgCOD L^{-1}) than the reported value of 20 mgCOD L^{-1} (Henze *et al.*, 2000), because of the recalcitrant nature of the organic carbon present in the reject water. Similarly, the decay parameters for both heterotrophs and autotrophs were slightly increased (b_H=2.32 d^{-1}, b_{NH}=0.19 d^{-1}, b_{NO}=0.092 d^{-1} at 30°C) to account for the higher decay rates generally observed in nitrifying MBRs (Liebig *et al.*, 2001; Wyffels *et al.*, 2003).

An extensive range of parameters for ammonium and nitrite oxidizer growth is reported in literature, mainly determined by the microbial species involved and the operating process

conditions. To increase the accuracy of the kinetic model, a combination of experimental data derived specifically for the biomass from the partial nitritation process (operating at low DO and very high SRT) and literature values was used (Table 5.1.1). A temperature dependency was considered for the K_La and for the maximum growth rate (μ^{max}) and decay rate (b) as discussed in chapter 3.2.

Table 5.1.1. Kinetic parameters of ammonium and nitrite oxidizers

Symbol	Definition	Value (at 30°C and pH 7.9)	Unit	Reference
μ^{max}_{NH}	Maximum growth rate of X_{NH}	2.02	d^{-1}	This study
$K_{O,NH}$	Saturation constant for S_O of X_{NH}	0.235	$mgO_2\ L^{-1}$	This study
$K_{NH3,NH}$	Saturation constant for S_{NH3} of X_{NH}	0.85	$mgNH_3\text{-}N\ L^{-1}$	This study
b_{NH}	Decay rate of X_{NH}	0.19	d^{-1}	Wiesmann (1994)
μ^{max}_{NO}	Maximum growth rate of X_{NO}	1.36	d^{-1}	Wiesmann (1994)
$K_{O,NO}$	Saturation constant for S_O of X_{NO}	1.5	$mgO_2\ L^{-1}$	Wiesmann (1994)
$K_{HNO2,NO}$	Saturation constant for S_{HNO2} of X_{NO}	$8.723\ 10^{-4}$	$mgHNO_2\text{-}N\ L^{-1}$	Wiesmann (1994)
b_{NO}	Decay rate of X_{NO}	0.092	d^{-1}	adapted from Wiesmann (1994)

2.4 Batch determination of kinetic and stoichiometric parameters for microbial growth

Microbial growth parameters were assessed in respirometer batch experiments. Kinetic and stoichiometric parameters of the MBR biomass were determined at 30.0°C and pH 7.90 at stable operation of the oxygen-limited partial nitritation process after start-up. The MBR was switched to batch mode, the biomass was washed twice to remove background concentrations and pulses of NH_4Cl and KNO_2 were added to determine the kinetics of the ammonium oxidizing and nitrite oxidizing biomass, respectively. The DO concentration was monitored during the experiments and the calculated oxygen uptake rate (OUR) was used for parameter estimation.

3 RESULTS AND DISCUSSION

3.1 Operational strategy of the partial nitrification process

The main goal of a partial nitrification reactor such as the MBR under study is to provide an Anammox-suited effluent, i.e. with an almost equimolar TAN:TNO_2 ratio. Several practical strategies to accomplish this TAN:TNO_2 ratio are described in literature (chapter 2). Imposing oxygen-limited conditions to outcompete the nitrite oxidizers is one of them and this strategy was followed in the study presented here. In several other studies nitrite production is also linked to oxygen-limited conditions (Bernet *et al.*, 2001; Han *et al.*, 2001; Pollice *et al.*, 2002; Ruiz *et al.*,

2003). The phenomenon of TNO_2 accumulation at oxygen-limited conditions is attributed to the often-reported lower oxygen affinity (K_O) for nitrite oxidizers than for ammonium oxidizers, making the latter better competitors for limited amounts of oxygen. However, it is also suggested that free hydroxylamine inhibition rather than a difference in affinity constants causes nitrite build-up in nitrifying systems at low oxygen concentration (Yang and Alleman, 1992). Further, the pH in the MBR was controlled at pH 7.90, resulting in free NH_3 concentrations of 23–26 $mgNH_3\text{-}N\ L^{-1}$. Therefore, it is most likely that both low DO and high NH_3 concentrations contribute to a reduced nitrite oxidizer activity and a consequent stable nitrite production in the partial nitrification MBR (Wyffels *et al*., 2003). At such NH_3 concentrations no inhibition of ammonium oxidizing activity was observed in batch tests conducted with SHARON biomass (chapter 4.2), indicating the adaptation of ammonium oxidizers to high NH_3 concentrations.
By adjusting airflow rates and maintaining oxygen-limited conditions, ammonium conversion to nitrite can be controlled in the optimal range for further treatment with an Anammox-like process (Wyffels *et al*., 2003; Wyffels, 2004).

3.2 Start-up and performance of the oxygen-limited MBR

The MBR was started fresh using a nitrifying inoculum with complete ammonium oxidation to nitrate (Wyffels, 2004). Initially, the reject water was supplied at a high HRT of 5 days to allow biomass enrichment. The start-up consisted of a stepwise increase of the loading rate by decreasing the HRT. After 16 days of operation the HRT was reduced to 1 day and this HRT was maintained for the rest of the experimental period. The decrease of the HRT was followed by a gradual decrease of the airflow rate (final K_La values between 190–240 d^{-1}), thereby limiting the oxygen supply to the nitrifying biomass. This resulted in DO concentrations below 0.1 mg L^{-1} from day 33 onwards.
Increasing the loading rate was performed in a way to maintain complete ammonium oxidation to nitrate, while decreasing the airflow resulted in a concomitant decrease of nitrate concentration and an accumulation of TNO_2 and un-oxidized TAN. The evolution of HRT and K_La during start-up of the partial nitrification process, and the effluent concentration ratios of $TNO_2\text{-}N/NO_x^-\text{-}N$ and TNO_2-N/TAN are shown in Figure 5.1.2a and 2b, respectively. From day 36 onward nitrate is less than 8% of the total oxidized nitrogen species, and after fine-tuning the K_La on day 49 the MBR effluent contains TNO_2 and TAN at an approximately equimolar ratio. Stable operation of the partial nitrification process treating sludge reject water under oxygen-limited conditions was further maintained for another 3 months (Wyffels, 2004), making the MBR a suitable reactor system to precede an Anammox reactor within an integrated autotrophic nitrogen removal process.

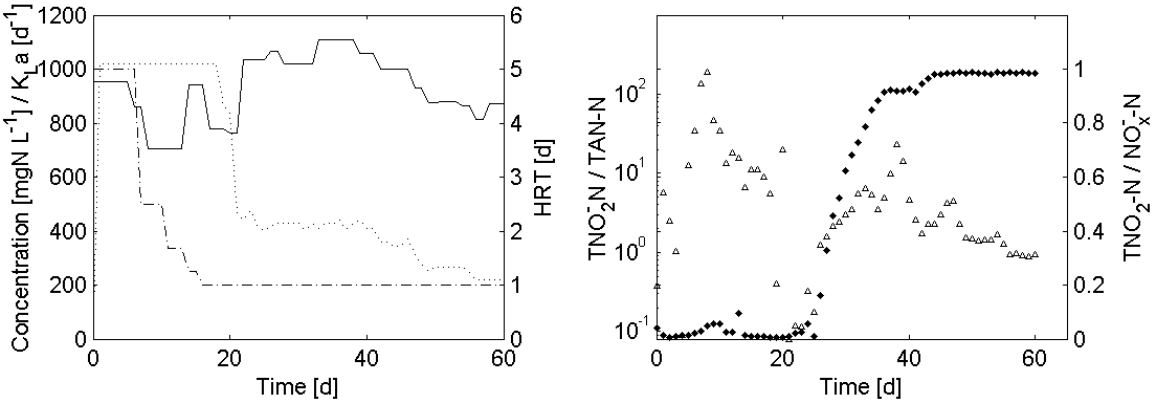

Figure 5.1.2. (a) Influent ammonium concentration (—), HRT (-——-) and K_La (---) profiles during start-up of the partial nitrification process. Gradually decreasing HRT and K_La generated oxygen-limited conditions after 21 days. (b) Evolution of the TNO_2-N/NO_x^--N (◆) and TNO_2-N/TAN-N (Δ) effluent ratios during start-up of the partial nitrification process.

3.3 K_La determination

Within WEST®, the applied aeration profiles with varying values for the gas–liquid oxygen transfer coefficient (K_La) are introduced via the input file. The K_La was influenced by operating temperature, stirring rate, and airflow rate. Reactor temperature (30.0°C) and stirring rate (200 rpm) were maintained constant during all experiments. The relationship between oxygen transfer coefficient (K_La, d^{-1}) and airflow rate (Q_A, L m^{-3} min^{-1}) was found to be $K_La = 0.55\ Q_A + 102$. Higher K_La values were found when the empty MBR was filled with tap water. Solids and dissolved salts tended to lower the gas–liquid oxygen transfer when aerating the MBR biomass feeding on the reject water. This is expressed within the α-value, which is defined as the ratio of the oxygen transfer coefficient for the MBR (K_La) to the oxygen transfer coefficient for tap water (K_La*). An α-value of 0.52 ± 0.04 was found for airflow rates between 47–520 L m^{-3} min^{-1} at a solids concentration of 10.3 gMLSS L^{-1}. A comparable α-value of 0.6 was determined for full-scale municipal MBRs with solids concentrations of 12 gMLSS L^{-1} (Cornel *et al*., 2003). Higher solids concentrations resulted in lower α-values. Similarly, at very high biomass concentrations in a biofilm airlift suspension reactor exceeding 17 gVSS L^{-1}, Garrido *et al*. (1997) showed that the K_La was negatively influenced by an ever-increasing biomass concentration due to a decreased oxygen hold-up.

Airflow rates were continuously monitored with rotameters allowing the calculation of the K_La at any point during the reactor run. To compensate for the lower solids concentration at the beginning of the run, the α-value was corrected with a factor 1.1–1.3 according to the observations of Cornel *et al*. (2003).

3.4 Model calibration

The kinetic conversion model was specifically calibrated for the oxygen-limited partial nitritation process in the MBR. Operational characteristics of this process are rather different from those encountered in most nitrification systems. It is expected that these differences are reflected in the composition of the nitrifying community, including some of their kinetic and stoichiometric parameters. For example, the low DO concentration puts a selective pressure upon the system for organisms coping well with limited oxygen availability, while high sludge ages allow the propagation of slow-growing organisms. A two-step nitrification model in WEST® (see chapter 3.2) accurately described the batch oxidation profiles of the respirometry experiments. Resulting parameter estimations (T = 30.0°C; pH = 7.90) determined experimentally for the ammonium oxidizing biomass were $\mu^{max}_{NH} = 2.02\ d^{-1}$, $K_{O2,NH} = 0.24\ mgO_2\ L^{-1}$, $K_{NH3,NH} = 0.85\ mgNH_3\text{-}N\ L^{-1}$. Compared to general literature data for the oxygen affinity constant ($K_{O2,NH} = 0.6\ mgO_2\ L^{-1}$ at 30°C; Wiesmann, 1994) a somewhat lower value was measured, which could be explained by the fact that the MBR (low DO, high SRT) selects for ammonium oxidizers having a higher oxygen affinity. The affinity constant for ammonia substrate is very high compared to literature values ($K_{NH3,NH} = 0.034\ mgNH_3\text{-}N\ L^{-1}$ at 30°C, Wiesmann, 1994), most likely due to the fact that the organisms were exposed to high ammonium concentrations and as such not selected for their substrate affinity.

With respect to this, a similar high affinity constant ($K_{NH3,NH} = 0.65\ mgNH_3\text{-}N\ L^{-1}$ at 30°C and pH 8) was found for ammonium oxidizer growth under SHARON conditions (Hellinga *et al*., 1999). Also the value for the ammonium affinity constant for SHARON biomass determined in chapter 4.2 ($0.75\ mgNH_3\text{–}N\ L^{-1}$) lies in this range. Parameter estimation for nitrite oxidation was, however, difficult because of too low oxygen uptake rates when dosing KNO_2. At steady-state operation of the process, the amount of nitrite oxidizers was simply too low, which can be explained by the fact that by maintaining low DO their growth rate was severely suppressed. This will also be illustrated by the simulation results in the following model validation section. Therefore, standard literature values were chosen for nitrite oxidizer kinetics (Wiesmann, 1994). Since nitrite oxidizers are also exposed to high substrate concentrations, a high substrate affinity constant for nitrous acid was assumed.

3.5 Model validation: modelling the start-up of the partial nitritation process

A comparison between modelled and measured data for the TAN, TNO_2, NO_3^- and DO concentrations is given in Figure 5.1.3. The modelled concentrations agree well with the measured concentrations. Low DO concentrations were measured during the first 4 days of the start-up, because the membrane of the oxygen sensor was fouled with biomass. The loading rate was set to zero (no addition of sludge reject water) on days 12, 19 and 25 resulting in lower

oxygen uptake rates and a temporary high bulk DO, which can be seen both from the experimental and modelled data. On day 25 the effect of no wastewater addition is also reflected in a decreased concentration of ammonium and nitrite, and an increased nitrate concentration.

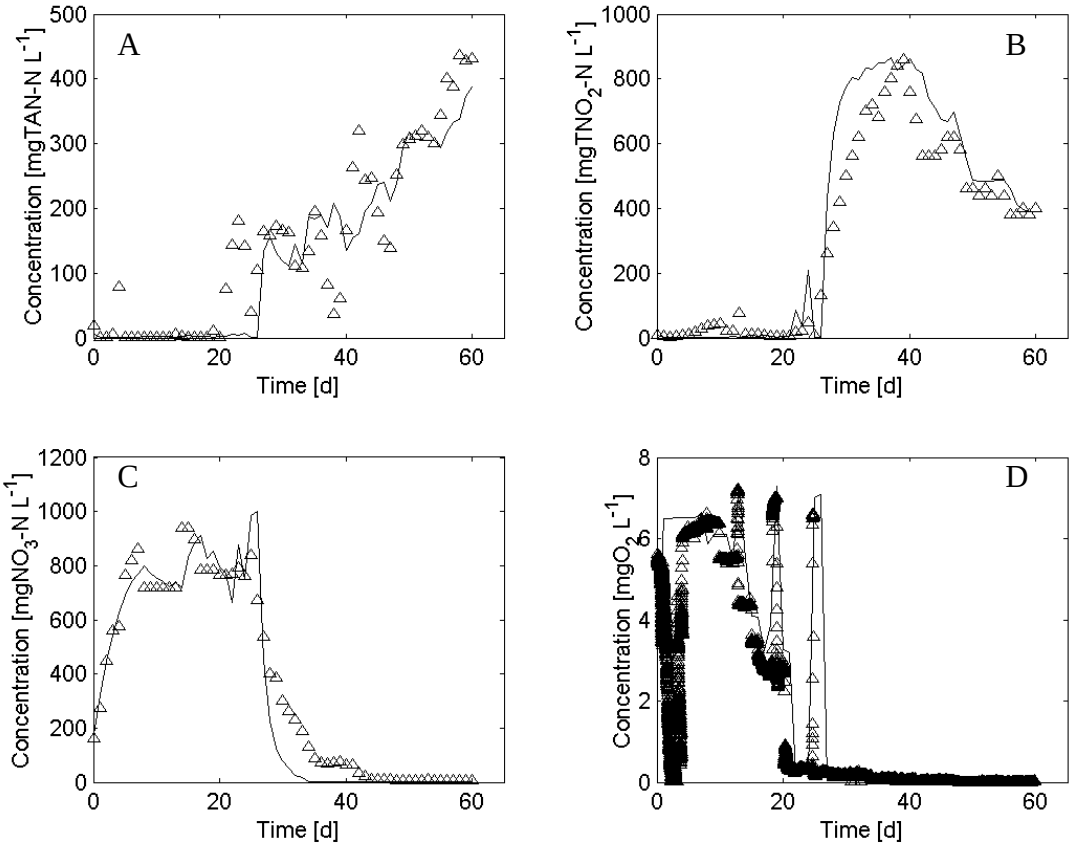

Figure 5.1.3. Measured (Δ) and modelled (—) MBR effluent concentrations of TAN (A), TNO_2-N (B), NO_3^--N (C) and DO (D) after decreasing HRT and K_La as outlined in Figure 5.1.2a.

The modelled concentration of particulate COD (particulate COD = inert particulate COD + biomass particulate COD) also agrees well with the measured values, although the measurement frequency was lower compared to the nitrogen compounds (Figure 5.1.4). As expected, operating the MBR without sludge waste (infinite SRT) resulted in the accumulation of inert particulate COD (X_I), which can be seen from the start-up simulation data in Figure 5.1.5. It was assumed that 15% of biomass decay products was inert particulate matter (Henze *et al*., 2000).

However, no significant accumulation of biomass VSS was measured (Figure 5.1.4) which also becomes obvious from the simulation data for concentrations of the ammonium oxidizers, nitrite oxidizers, and heterotrophs (Figure 5.1.5). The total biomass concentration was rather constant

through the start-up period with moderate fluctuations due to substrate availability. The amount of ammonium oxidizing biomass (X_{NH}) was negatively affected by the events of a zero loading rate on days 19 and 25. The concentration of nitrite oxidizers (X_{NO}) was steadily decreasing after reducing the airflow rate and thereby limiting the oxygen supply.

Estimated model-based calculations of the biomass concentration after 200 days of stable operation at constant reactor conditions (T = 30°C; pH = 7.9; $TAN_{influent}$ = 870 mgTAN-N L^{-1}; HRT = 1 d; K_La = 222 d^{-1}) revealed that X_{NH} was 0.41 gCOD L^{-1} while X_{NO} was nearly zero. Nitrite oxidizers were completely outcompeted at low DO concentrations (which resulted from a combination of high loading rate and a low airflow rate), but from a physiological point of view the additional effect of inhibiting high concentrations NH_3 could not be discriminated from the low DO effect. However, introduction of a NH_3 inhibition term in the kinetics of the ammonium and nitrite oxidizers had no influence on the model output of nitrogen compounds or biomass concentration. Therefore, the simplified model was used with Monod terms for microbial growth. As such, oxygen limitation and low DO were suggested as the main factors for maintaining nitrite accumulation. Heterotrophic bacteria were able to persist under the indicated reactor conditions and were only a fraction (15%) of the estimated active biomass concentration.

Based on the agreement of experimental and modelled data it was concluded that the conversion model was properly validated, allowing further predictions with model simulations.

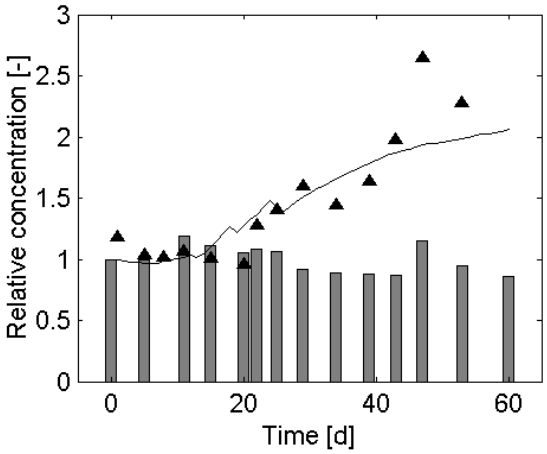

Figure 5.1.4. Measured (▲) and modelled (—) accumulation of particulate COD and evolution of VSS (grey bars). Concentrations of COD and VSS are shown relative to their initial concentration at the time of inoculation.

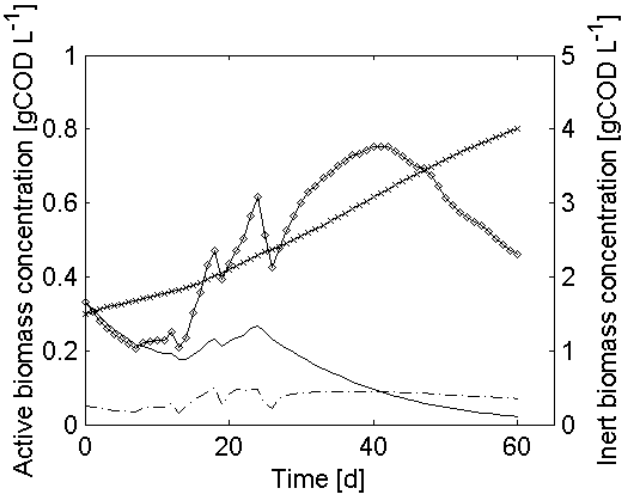

Figure 5.1.5. Simulation of inert particulate COD (×) accumulation and growth of ammonium oxidizing (◇), nitrite oxidizing (—) and heterotrophic (— -) biomass.

3.6 Simulation of the effect of temperature, K_La and HRT on TAN and TNO_2 conversion

Simulations with the validated kinetic model were performed to demonstrate the possibility of obtaining stable effluent concentrations, which are suited for subsequent autotrophic nitrogen removal via anaerobic ammonium oxidation. In a first series of simulations, the effect of operating reactor temperature on TAN and TNO_2 conversion was investigated. Simulations were carried out at 15, 20, 25, 30, and 35°C over a 200 days period with all other parameters kept constant (pH = 7.9; $TAN_{influent}$ = 870 mgTAN-N L^{-1}; HRT = 1 d). The airflow rate was also constant, resulting in constant K_La values (123, 150, 182, 222, and 270 d^{-1} for the five different temperatures, respectively). After 200 days of simulated operation, the effluent nitrogen concentrations and reactor biomass concentrations were in steady state. The results of this simulation are shown in Table 5.1.2. The absence of nitrite oxidizing activity under oxygen limitation was reflected in both the simulated nitrate concentrations and the simulated nitrite oxidizing biomass concentration, independent of reactor temperature. Nitrate concentrations and nitrite oxidizer biomass concentrations were always below 0.005 mgNO_3^--N L^{-1} and 2 mgCOD L^{-1}, respectively. As such, high temperatures are not a prerequisite for a stable process operation in terms of preventing nitrate production. The formation of nitrate during partial nitrification is disadvantageous since nitrate is not further removed in the anaerobic ammonium oxidation reactor. In chemostat systems without sludge retention such as the SHARON process, nitrate production is circumvented by washing out the nitrite oxidizers, which have a lower growth rate than ammonium oxidizers at high temperatures between 30–40°C (Hellinga *et al.*, 1998). In practice, a temperature of 35°C is needed to operate the SHARON process at its

maximum rate, which often requires reactor insulation or an external heating system (van Kempen *et al*., 2001; chapter 4.1). In the chemostat approach stable TNO_2 accumulation is achieved when the reactor temperature is at least 30°C (van Dongen *et al*., 2001b), although it has been stated that even at a temperature of 20°C TNO_2 accumulation occurred (van Loosdrecht, personal communication). At lower temperatures, nitrite oxidizer activity is likely to reappear and ammonium conversion rates are significantly decreased (Fux *et al*., 2002). Using the MBR for oxygen-limited partial nitrification, operating at a lower temperature is possible (no reactor heating and insulation required) while still maintaining a low HRT. More specifically, flexibility towards reactor temperature is desirable in these cases where the warm sludge reject water can't be treated directly from the digester drain point and is cooled down to ambient temperatures.
Biomass decay rates were found to be much higher at elevated temperature, resulting in lower concentrations of active biomass (Table 5.1.2). In the simulations at 30°C, the total amount of autotrophic and heterotrophic biomass was only 45% of its value at 20°C.

Table 5.1.2. Simulated effect of reactor temperature on the steady state concentrations of TAN, TNO_2, ammonium oxidizing (X_{NH}) and heterotrophic biomass (X_H) at K_La=150 d^{-1}.

Temperature [°C]	TAN [mgTAN-N L^{-1}]	TNO_2 [mgTNO_2-N L^{-1}]	X_{NH} [mgCOD L^{-1}]	X_H [mgCOD L^{-1}]
15	513	268	1254	395
20	477	302	860	224
25	434	345	594	127
30	385	395	412	71
35	332	452	285	38

Reactor temperature had a considerable influence on ammonium conversion, while no nitrite conversion to nitrate occurred (Table 5.1.2). At a temperature below 30°C, for example at 20°C, ammonium conversion to nitrite is only 0.3 kgTAN-N m^{-3} d^{-1} resulting in a sub-optimal TNO_2-N:TAN effluent ratio of 0.63. By simulating an increased HRT of 1.45 d, or an increased K_La of 195 d^{-1} at this temperature of 20°C, the TNO_2-N/TAN ratio in the effluent can be restored again to an optimal value of 1. Thus, working under continuous oxygen limitation and constant reactor pH, the rate of ammonium conversion at a given loading rate is mainly determined by temperature and K_La. This was also demonstrated in a series of process simulations at different values for K_La (obtained by varying the airflow rate) shown in Figure 5.1.6 for two different temperatures. The ammonium conversion rate and nitrite concentration increased with increasing K_La-values with a maximum nitrite concentration found at 325 d^{-1} and 400 d^{-1} at 20°C and 30°C, respectively. At higher K_La-values ammonium was limiting and oxygen became increasingly available for the nitrite oxidizing biomass resulting in the production of nitrate. Nitrite conversion was complete at K_La-values above 500 d^{-1} and 590 d^{-1} at 20°C and 30°C, respectively. The

oxygen availability for the nitrite oxidizers can be reduced again by increasing the loading rate and thus the oxygen uptake by the ammonium oxidizers.

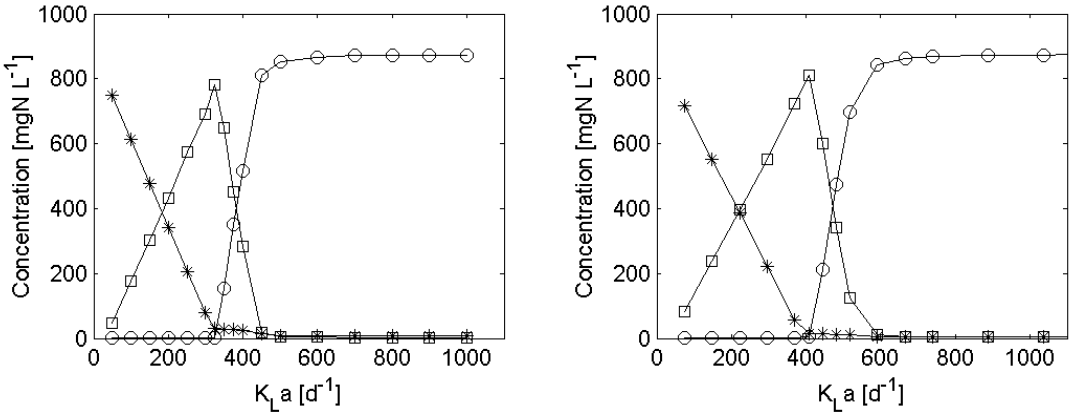

Figure 5.1.6. Simulated effect of the oxygen transfer coefficient on the steady state concentrations of TAN (∗), TNO_2-N (□) and NO_3^--N (O) at T=20°C (left) and 30°C (right).

In a second series of simulations, the dimensions of the partial nitrification process were designed by a combination of HRT and K_La. The required reactor volume and aeration capacity are determined by HRT and K_La, respectively. The key features of the process are: reactor operation under oxygen limitation, preventing nitrite oxidation and limiting ammonium conversion that results in the generation of effluents with a TNO_2:TAN ratio of 1:1, making them ideally suited for subsequent treatment via anaerobic ammonium oxidation. Shorter HRTs require higher K_La-values to obtain ideal effluent ratios (Figure 5.1.7). The effect of reactor temperature on the necessary K_La is more pronounced at HRTs below 1 day. From these simulations it was noted that a partial nitritation process with a HRT as short as 0.25 days should be possible, thereby achieving optimal ammonium conversion. Further shortening of the HRTs would require an unrealistically high aeration capacity. A short HRT or high volumetric loading rate means more costs for additional membrane surface since a higher effluent flux needs to be generated. However, these costs should be weighed against a strongly decreased reactor volume. With respect to partial nitrification processes in chemostat systems (e.g., SHARON process with HRT = SRT) the required reactor volume for generating an Anammox suited effluent can be largely decreased.

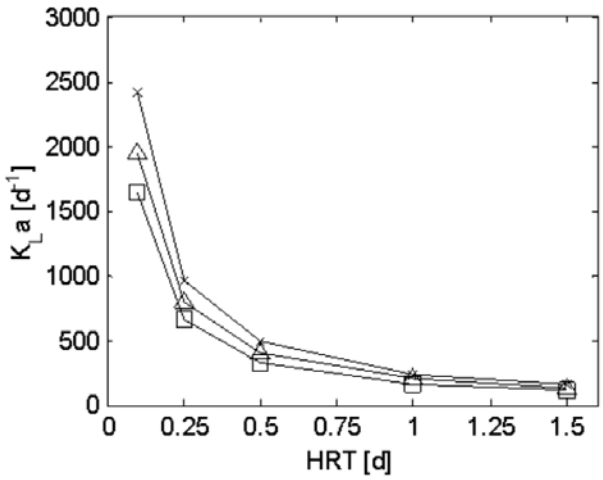

Figure 5.1.7. Simulated effect of the hydraulic residence time (HRT) on the volumetric oxygen transfer coefficient ($K_L a$) needed to produce a reactor effluent suitable for further anaerobic ammonium oxidation, at 15°C (□), 25°C (Δ), 35°C (×).

3.7 Simulation of the effect of SRT on the accumulation of particulate matter

In a third series of simulations the influence of SRT on the accumulation of particulate matter was investigated. So far, the SRT was always set infinite (complete sludge retention and no sludge waste) in both the experimental runs and in the simulations. An infinite SRT and subsequent increasing inert particulate COD concentration (Figure 5.1.4) is, however, not beneficial for the gas–liquid oxygen transfer, requiring more aeration energy to obtain the same oxygen input (or reach the same $K_L a$-value). Also an ever-increasing solids concentration could have a detrimental effect on the membrane filtration performance. Steady-state simulations were performed at SRT of 10, 25, 50, 75, and 100 days and constant reactor operating conditions (T = 30°C; pH = 7.9; $TAN_{influent}$ = 870 mgTAN-N L^{-1}; HRT = 1 d; $K_L a$ = 222 d^{-1}). The resulting total particulate COD, inert particulate COD and concentration of ammonium oxidizing biomass in the MBR as function of the SRT is shown in Figure 5.1.8. As expected, the total and inert particulate COD concentration increased with increasing SRT. The concentration of ammonium oxidizing biomass was nearly unaffected by the SRT since growth of the ammonium oxidizers was largely determined by the availability of substrate (in casu oxygen) and by the operating temperature. Lower SRTs showed only a small diluting effect on the nitrifying biomass. In addition, the effluent TNO_2:TAN ratio was not affected by increasing the sludge age, indicating the possibility of the MBR to be operated at different SRTs. In practice, operating the MBR at a SRT somewhere between 50 and 75 days should be appropriate in terms of minimized sludge waste production with still sufficient oxygen transfer.

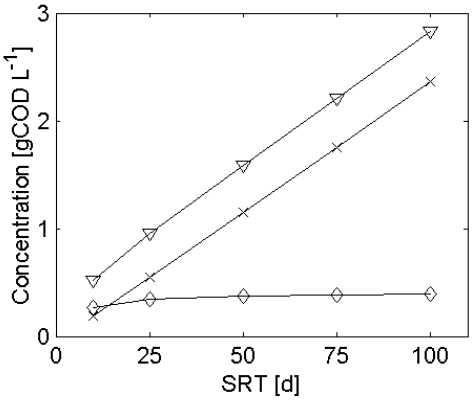

Figure 5.1.8. Total particulate COD (▽), inert particulate COD (×) and concentration of ammonium oxidizing biomass (◇) as function of the SRT.

3.8 Oxygen affinity of nitrite oxidizers

The assumption used in the above simulations, i.e. oxygen limitation and low DO are the main factors for maintaining nitrite accumulation, only holds if the oxygen affinity constant of nitrite oxidizers is higher than the oxygen affinity constant of ammonium oxidizers. In order to see the effect of a varying affinity constant a final simulation series was performed. Steady-state simulations were performed at constant reactor operating conditions (T = 30°C; pH = 7.9; $TAN_{influent}$ = 870 mgTAN-N L^{-1}; HRT = 1 d; K_La = 222 d^{-1}) and varying oxygen affinity constant for nitrite oxidizers. The oxygen affinity constant of ammonium oxidizers was kept constant at 0.235 mgO_2 L^{-1}. The resulting effluent ammonium, nitrite and nitrate concentrations are depicted in Figure 5.1.9a. For oxygen affinity constants higher than 0.5 mgO_2 L^{-1} nitrite accumulation is complete, i.e. no nitrate is produced. At lower affinity values a significant amount of nitrate is formed indicating the ingrowth of nitrite oxidizers as can be seen from Figure 5.1.9b. Baring in mind that reported values of the oxygen affinity constant of nitrite oxidizers lie between 0.5 and 3 mgO_2 L^{-1}, the assumption used above can be considered valid.

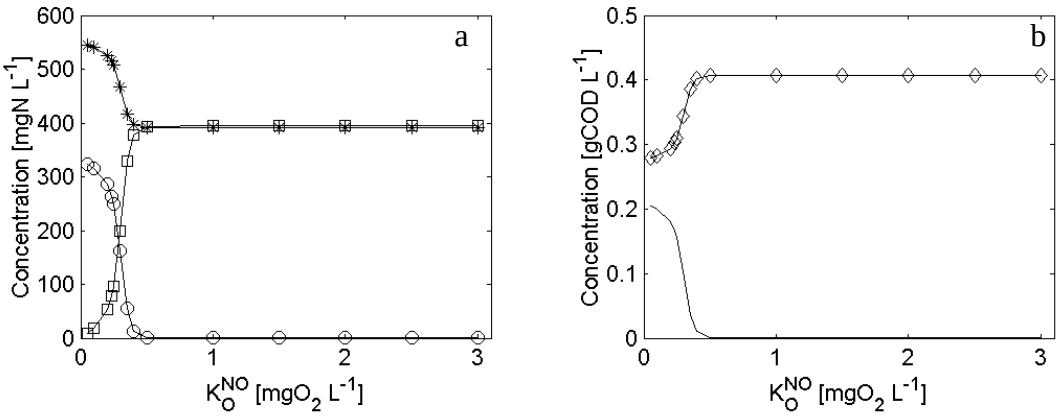

Figure 5.1.9. Simulated effect of oxygen affinity constant of nitrite oxidizers on the steady state concentrations of TAN (∗), TNO_2-N (□) and NO_3^--N (O) (a) and on the steady state concentrations of ammonium (◇) and nitrite (—) oxidizing biomass (b). Oxygen affinity constant of ammonium oxidizers: 0.236 mgO_2 L^{-1}.

4 CONCLUSIONS

The start-up and operational data of a nitrifying membrane-assisted bioreactor (MBR) operated under low DO concentrations were used for the calibration and validation of the mathematical model presented in chapter 3.2. This 2-step nitrification model describes the first step (partial nitrification) of the autotrophic nitrogen removal process. The model was first calibrated with dedicated batch experiments determining the kinetic constants of ammonium oxidation. For model validation the start-up phenomena as well as the steady state operational data were used. The calibrated model was able to describe these data accurately.

The mathematical model was then used for simulation of the oxygen-limited MBR under different operational conditions. This scenario analysis allowed postulating further suggestions for optimization of the oxygen-limited partial nitrification process. Based on the simulation results, the MBR for oxygen-limited partial nitrification is proposed as a suitable configuration to precede reactor systems for anaerobic ammonium oxidation (Anammox). The process features a biomass-free effluent, a high loading rate, and flexibility towards operating reactor temperature. It should even be possible to operate the process at ambient temperatures of 20°C and with a HRT down to 0.25 days.

Chapter 5.2

Enrichment of Anammox biomass from municipal activated sludge: Experimental and modelling results

ABSTRACT

Anaerobic Ammonia Oxidizing (Anammox) biomass was enriched from sludge collected at a municipal wastewater treatment plant (WWTP), employing a sequential batch reactor (SBR). After 60 days Anammox activity started to be detected, by consumption of stoichiometric amounts of TNO_2 and TAN in the system. FISH (Fluorescence In Situ Hybridization) analysis confirmed the increase of the Anammox bacteria concentration with time. A final concentration of enriched biomass of 3-3.5 gVSS $L^{-1}$was obtained, showing a Specific Anammox Activity (SAA) of 0.18 gTAN-N $gVSS^{-1}$ d^{-1}. The reactor was able to treat a nitrogen loading rate up to 1.4 kgN m^{-3} d^{-1} achieving a removal efficiency of 82%.

Start-up and operation of the Anammox SBR reactor were modelled with the Activated Sludge Model Nr. 1 extended for Anammox. The simulations predicted the experimental data related to the concentrations of nitrogenous compounds quite well and helped interpreting the experimental results. These simulations reveal that heterotrophs still remain in the system after the start up of the reactor and can protect the Anammox microorganisms from a negative effect by oxygen. These results demonstrate the feasibility to obtain Anammox biomass from a biological sludge, facilitating the implementation of this process in wastewater treatment plants.

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1 INTRODUCTION

Biological nitrogen removal from wastewaters having high nitrogen contents can be costly, in particular when the wastewater contains only small amounts of biologically degradable carbon compounds, e.g. effluents from anaerobic sludge digestion, food and agriculture wastewaters, fertilizers and leachate effluents (Seyfried *et al*., 2001).

Nitrogen in the wastewaters is usually in the form of ammonium nitrogen. A novel way to remove nitrogenous compounds of this type of wastewaters is the combination of:

1. A partial nitrification system of the type SHARON (Single High Ammonium Removal Over Nitrite), that oxidizes 50% of the TAN to TNO_2 by controlling the HRT, pH and T.
2. An Anammox system, where TAN is oxidised anaerobically, using the TNO_2 produced in the SHARON system as electron acceptor.

Application of this combined process would reduce the required oxygen input by 60% (compared to a conventional nitrification-denitrification process) and would alleviate the need for addition of methanol and concomitant increased sludge production (van Dongen *et al*., 2001a&b; Jetten *et al*., 1997).

The Anammox process was discovered by Mulder *et al*. (1995). The first identified Anammox organism branched in the *Planctomycetes* phylum and was named *Candidatus Brocadia anammoxidans* (Kuenen and Jetten, 2001). Nowadays different Anammox organisms have been detected by PCR, phylogenetic analysis or FISH in both wastewater treatment and natural systems where nitrogen losses occurred: *Candidatus Kuenenia stuttgartiensis, Candidatus Scalindua brodae, Candidatus Scalindua wagneri* and *Candidatus Scalindua sorokinii* (Schmid *et al*., 2003; Kuypers, *et al*., 2003).

The presence of Anammox organisms has been detected in several types of treatment units treating different wastewaters (Strous *et al*., 2002). So far, Anammox activity was observed in a rotating biological contactor treating TAN-rich leachate (Helmer *et al*., 2001), a trickling filter treating wastewater (Schmid *et al*., 2000), fixed and fluidised bed reactors and SBRs treating a synthetic medium (Strous *et al*., 1997b; Strous *et al*., 1998).

Application of the Anammox process is limited by the availability of Anammox biomass. The isolation and enrichment of Anammox biomass from a mixture of bacterial populations requires the optimisation of the conditions favouring the Anammox process while limiting the growth of any other kind of microbial population. In particular the exclusion of oxygen is essential, since the Anammox process is completely inhibited by it (Jetten *et al*., 1997). As the maximum specific growth rate of the Anammox bacteria is very low (0.003 h^{-1} at 32°C, Strous *et al*., 1998), it is important to use a system that minimises biomass washout, in order to maximise the biomass concentration in the system.

Strous *et al.* (1997b) performed the enrichment of Anammox biomass in a fluidised bed reactor, treating a sludge digester effluent, reaching a NLR (Nitrogen Loading Rate) of 1.5 kgN $m^{-3}d^{-1}$. Still, the retention capacity of the system was not good and the biofilm structure was not homogeneous through the reactor, causing difficult access of the biomass to the substrate. Strous *et al.* (1998) showed that the SBR is a suitable system to grow Anammox organisms (Strous *et al.*, 1999b). The strongly selective conditions achieved in this system permitted an enrichment of 74% of Anammox micro-organisms in the SBR. Using this type of reactor the authors could determine, for the first time, different stoichiometric and kinetic parameters of the Anammox process. These parameters can be used for modelling and subsequent optimisation of the Anammox process.

Hitherto, few studies have been conducted towards modelling start-up and dynamic behaviour of the Anammox process. Hao *et al.* (2002a&b) performed a thorough simulation study on the behaviour of a partial nitrification-Anammox system under different process conditions, such as varying temperature and dissolved oxygen concentration. However no verification with real experimental data was performed and no start-up dynamics were included in the study. Koch *et al.* (2000) performed simulations with a similar system, but also did not include any start-up or long-term dynamic effects. Furthermore it can be noted that in both studies total nitrogen concentration only amounted to about 150 mgN L^{-1}, while in a real Anammox reactor this concentration would be tenfold higher.

The purpose of this study is thus twofold. Firstly the possibility to isolate and produce Anammox biomass from sludge of a municipal WWTP by using a SBR system will be demonstrated. If successful, this would make the application of the Anammox process feasible in situations where this type of biomass may not be directly accessible. Second, the results of this enrichment will be interpreted with the Activated Sludge Model nr. 1, extended with submodels for 2 step nitrification-denitrification processes and the Anammox process as described in Chapter 3.2. Simulation results of both start-up and dynamic operation of the reactor will be compared to the measured values. Total nitrogen concentrations in the reactor will amount up to 900 mgN L^{-1} as will be the case in a real reactor. This simulation study will also quantify the kinetic properties of the enriched Anammox biomass.

2 MATERIALS AND METHODS

2.1 Description of the SBR

2.1.1 Inoculum

The reactor was inoculated with a mixture of sludges from the municipal WWTPs of Bertamiráns, Padrón and Negreira (A Coruña, Spain). All three WWTPs are operated very similarly in alternating conditions (oxic/anoxic) with a hydraulic retention time of 11 hours, a carbon loading rate of 0.86 kgCOD m^{-3} d^{-1} and a nitrogen loading rate of 0.065 kgN m^{-3} d^{-1}. About 90 % of the incoming COD and about 60 % of the incoming nitrogen are removed.

2.1.2 Mineral medium

The composition of the feeding mineral medium is similar to the medium used by van de Graaf *et al*. (1996) and Strous (2000) and is shown in Table 5.2.1. Concentrations of TAN, TNO_2 and NO_3^- were added as specified in the results section.

Table 5.2.1. Composition of the mineral medium

Compound	(g L^{-1})
$(NH_4)_2SO_4$	0.132 – 1.88
$NaNO_2$	0.069 – 2.46
$NaNO_3$	0 – 0.85
$KHCO_3$	1.25
NaH_2PO_4	0.05
$CaCl_2 \cdot 2H_2O$	0.30
$MgSO_4 \cdot 7H_2O$	0.20
$FeSO_4$	0.00625
EDTA	0.00625
H_2SO_4	0.5-1.25 mL/L
Trace elements solution*	1.25 mL/L

* van de Graaf *et al.* (1996)

2.1.3 Operation of the SBR

The process was carried out in a SBR with working volume of 1 litre (Figure 5.2.1). The system was maintained at a temperature of 35ºC and a pH between 7.8 and 8, maintained without specific control, although an emergency pH control was installed which could add H_2SO_4 in case the pH would rise above 8. The Hydraulic Retention Time (HRT) was fixed at 0.62 d. The medium was maintained homogeneous with a one blade mechanical stirrer operated at 50 rpm.

The stirrer had a diameter of one third of the internal diameter of the reactor. The reactor was flushed with a mixture of 95% Ar / 5 % CO_2 to maintain anaerobic conditions.

The operation strategy consisted of increasing the inlet concentrations of TAN and TNO_2 as the limiting substrate (TNO_2) was totally consumed.

The SBR worked in cycles of 6 hours, controlled by a PLC (CPU224, Siemens). Each cycle had three phases. In the first phase (5.5 hours) 400 ml of influent was fed continuously to the reactor. In the second phase (20 minutes), the stirrer and the influent supply were stopped and the biomass was allowed to settle. Finally, in a third phase (10 minutes), 400 ml of supernatant liquid was pumped out of the reactor. No idle phase was implemented as complete biomass retention is essential for the successful start-up of an Anammox reactor. The different phases in the SBR operation are summarized in Table 5.2.2.

Table 5.2.2. Summary of the SBR operation (total cycle time: 6 hours)

Phase	Duration
Fill and reaction	5 h 30 min
Settle	20 min
Draw	10 min
Idle	0 min

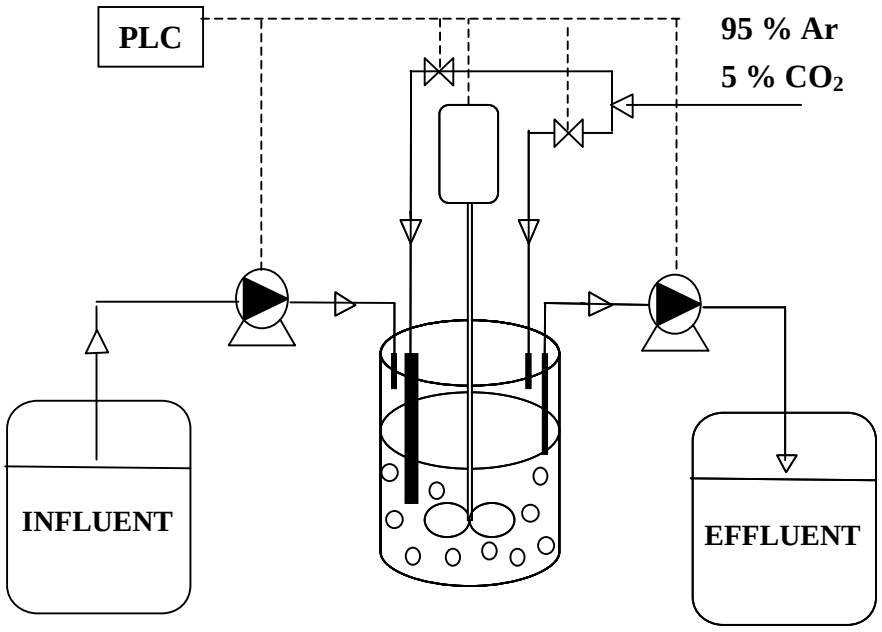

Figure 5.2.1. Experimental set-up of the sequential batch reactor used for enrichment of Anammox biomass.

2.1.4 Analytical methods

TAN was analysed by the phenol-hypochloride method (Wheatherburn, 1967). TNO_2 and nitrate were analysed by spectrophotometry (APHA, 1995). Biomass concentration was determined as gVSS/L (APHA, 1995). The bacterial population was monitored by Fluorescence In Situ Hybridation (FISH) analysis, according to Schmid *et al*. (2003). The applied oligonucleotide probes were: PLA46 [S-P-Planc-0046-a-A-18] (planctomycetes), EUB338 [S-D-Bact-0338-a-A-18] (eubacteria), AMX820 [S-*-Amx-0820-a-A-22] (Anammox of the type *Brocadia anammoxidans* and *Kuenenia stuttgartiensis*) and KST1273 (*Kuenenia stuttgartiensis*).

2.2 Modelling the Anammox SBR

2.2.1 Implementation of the Anammox SBR

All modelling was performed in the modelling and simulation environment WEST® (Vanhooren *et al.*, 2003). A standard SBR model in the WEST® modelbase was used to describe the SBR behaviour. In this model the five SBR-cycle phases were described (fill, reaction, settle, draw and idle). In view of the size of the experimental reactor (1 litre) and the presence of a stirrer system the SBR reactor was assumed to be ideally mixed. Although settling was ideal, the fraction of biomass withdrawn with the effluent was set to 0.5 %, to take the effect of sampling into account. The implementation of the SBR in the WEST® software is depicted in Figure 5.2.2.

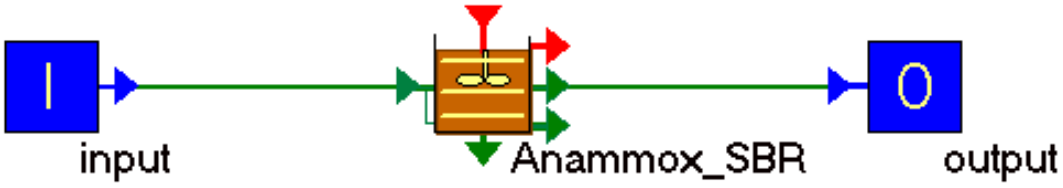

Figure 5.2.2. Implementation of the Anammox reactor in WEST®.

2.2.2 Extension of ASM1

For modelling purposes the Activated Sludge Model nr. 1 was extended with a 2-step nitrification-denitrification model and with the Anammox process as described in Chapter 3.2. In this model the death-regeneration concept (Henze *et al*., 2000) is preferred over the endogenous respiration concept as used in other simulation studies dealing with Anammox to describe decay (Koch *et al*., 2000; Hao *et al*., 2002a&b). This preference follows from the fact that Anammox biomass behaviour under substrate limiting conditions is not completely clear yet and from the observation that heterotrophs were shown to be active in the reactor. Only the application of this death-regeneration concept can explain this activity, since no biodegradable substrate (BOD) was

added with the influent. A value of 2 mgCOD L^{-1} biodegradable substrate in the influent was however assumed to account for any biodegradable substrate present in the water used for medium preparation, and biodegradable substrate originating from EDTA in the trace element solution.

The complete stoichiometric matrix in Peterson matrix format is already presented in chapter 3.2. Monod kinetics were used to describe the dependency of the growth rate of Anammox on TAN and TNO_2 concentrations. An additional Monod term was used to describe the inhibition of the Anammox organisms by oxygen as indicated by equation 5.2.1.

$$\rho_{growth} = \mu_{AN} \frac{K_{O2,AN}}{K_{O2,AN} + S_O} \frac{S_{TNO2}}{K_{TNO2,AN} + S_{TNO2}} \frac{S_{TAN}}{K_{TAN,AN} + S_{TAN}} X_{AN} \quad (5.2.1)$$

It should be noted, however, that TNO_2 is not only a substrate, but also inhibits the Anammox process (Strous *et al*., 1999b). Therefore, Haldane kinetics are perhaps more appropriate than the Monod kinetics applied. However, the measured TNO_2 concentrations in the reactor were never high enough to suggest an inhibitory effect, which prevented any calibration of the inhibition constant.

For the decay rate of Anammox expression 5.2.2 was used:

$$\rho_{decay} = b_{AN} X_{AN} \quad (5.2.2)$$

The maximum specific growth rate of the Anammox biomass (μ_{AN}) was derived from Strous *et al*. (1998) and set to a value of 0.08 d^{-1} at 35°C. The decay coefficient (b_{AN}) and the affinity constants were manually fitted to the available data. The decay coefficient was set to 0.0011 d^{-1} at 35°C, which is an order of magnitude below the maximum growth rate, while affinity constants for TAN ($K_{TAN,AN}$) and TNO_2 ($K_{TNO2,AN}$) were both set to 0.3 mgN L^{-1}. This value is higher than the one proposed by Strous *et al*. (1999b) indicating possible but minor substrate diffusion limitation in the SBR reactor.

The yield on TAN for Anammox organisms was set to 0.159 mgCOD $mgCOD^{-1}$ (Strous *et al*., 1998). The amount of TAN incorporated in the biomass (i_{nbm}) was set to 0.0583 mgN $mgCOD^{-1}$, while the fraction of inert biomass produced during cell decay was set to 0.08 mgCOD $mgCOD^{-1}$ (Henze *et al*., 2000).

3 RESULTS AND DISCUSSION

3.1 Start-up of the reactor

An SBR reactor was selected for the process because of its high biomass retention capacity (Strous *et al.*, 1998). The biomass washed out of the reactor was settled again and recycled, also with the purpose of increasing the retention of the system. The reactor was inoculated with a high concentration of VSS because during the first days there is an important decrease in the active biomass concentration into the system, due to decay of the original biomass.

Another important point to consider is the diffusion of oxygen into the system. Previous enrichment experiments with sludge collected from municipal WWTP have been made with the only resulting in a low nitrifying activity and a continuous decrease in the VSS concentration. Although the oxygen concentration was low in those previous attempts (around 0.1 $mgO_2\ L^{-1}$), its effect was more pronounced at low biomass concentration.

3.2 Operation of the reactor

An appreciable consumption of TAN and TNO_2 was observed in the system after two months of operation (Figure 5.2.3). As these compounds were consumed, their concentration in the feed was increased stepwisly. After 4 months of operation the influent composition was kept constant and the reactor was operated for another 2 months at an influent TAN and TNO_2 concentration of 410 $mgTAN\text{-}N\ L^{-1}$ and 500 $mgTNO_2\text{-}N\ L^{-1}$ respectively.

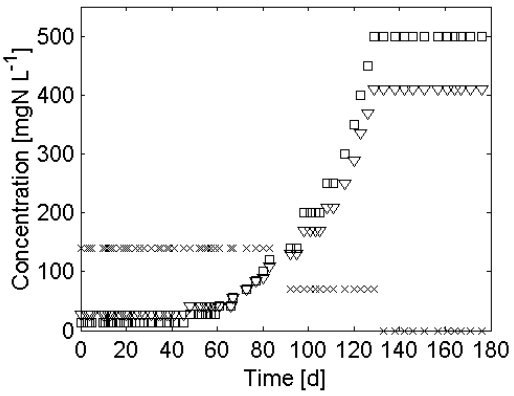

Figure 5.2.3. The influent TNO_2 (□), TAN (▽) and nitrate (x) concentrations to the Anammox reactor.

Initially, denitrifying activity was favoured (anaerobic atmosphere and presence of NO_3^-) to eliminate the organic matter present in the medium due to the lysis of aerobic bacteria. This fact was evidenced by the initial consumption of NO_3^- together with a significant decrease of the

biomass concentration during the first days. After the lysis of the aerobic bacteria, lysis of the denitrifying bacteria started due to a lack of organic substrate.

The biomass concentration in the system decreased during the first days, reaching a minimum of 1.7 gVSS L^{-1} at day 100. Starting from day 60 the specific Anammox activity (SAA) increased exponentially (Figure 5.2.4) and a concomitant increase of the biomass concentration was observed, as well as a gradual colour change, from brownish to reddish, a typical colour of Anammox biomass (van de Graaf *et al*., 1996).

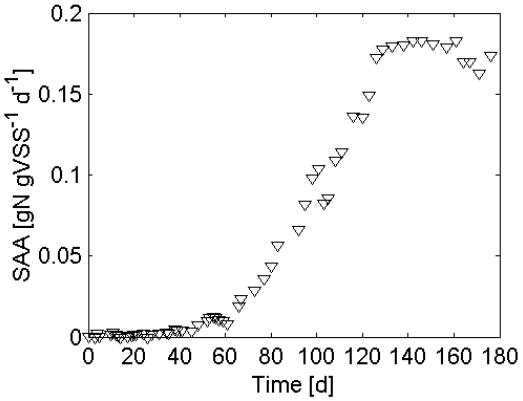

Figure 5.2.4. Measured specific Anammox activity in the Anammox reactor.

An average of 0.24 grams NO_3^--N were produced and 1.25 grams of TNO_2 were consumed for every gram of TAN-N consumed. These values approach the ones obtained by Strous *et al.* (1999b) for the Anammox process. The biomass showed an activity of 0.18 gTAN $gVSS^{-1}\cdot d^{-1}$ and the reactor was loaded at a NLR of 1.4 kgN $m^{-3}d^{-1}$ achieving a removal efficiency of 82 %. The maximum concentrations of TAN and TNO_2 in the influent were 410 mgTAN-N L^{-1} and 500 mgTNO_2-N L^{-1}, as would be the case with typical Anammox influent from sludge reject water.

FISH analyses were realized to follow the evolution of the bacterial populations in the reactor. The probe EUB338 was used to see the total quantity of active cells in the sample. Since Anammox organisms have many features in common with Planctomycetes, the probe PLA46 also hybridizes with Anammox bacteria and was used to detect their presence in the reactor. A more specific probe (AMX820), which hybridizes with *Candidatus Kuenenia stuttgartiensis* and *Candidatus Brocadia anammoxidans*, was also employed. Hybridization with PLA46 and AMX820 was not detectable at the beginning of the experiment but after two months an increasing positive signal with these two probes was observed. All biomass that hybridized with PLA46 also hybridized with AMX820, and the proportion of this biomass over eubacteria was increasing together with the Anammox activity in the reactor. Final FISH analysis of the enriched Anammox microorganisms showed that they were of the type "*Kuenenia stuttgartiensis*" (hybridized with KST1273).

3.3 Model simulations

The SBR was inoculated with 4 grams sludge. This corresponds to about 6 g COD sludge. No distinction was made between Anammox and other organisms. Therefore it was assumed that this 6 g COD biomass consisted of approximately 1.8 g COD heterotrophs, 50 mg COD ammonia oxidisers, 50 mg COD nitrite oxidisers, 4 g COD inert biomass and 10 mg COD Anammox biomass based on a steady state analysis of the municipal WWTP biomass as proposed by Petersen *et al.* (2003). These values were used as initial conditions for the simulations with the mathematical model.

The calculated values of the TNO_2, TAN and nitrate concentrations are compared with the measured ones in Figure 5.2.5. From these data the calculated values agree well with the measured ones. Also the effluent concentrations are low, indicating the possibility of the Anammox reactor to treat nitrogen rich streams.

In Figure 5.2.6, the time evolution of the Anammox organisms' concentration (X_{AN}), as well as the concentration of inert biomass (X_I) is depicted. The quantity of Anammox biomass is initially very low and starts to increase in an exponential way after 2 months. This is quantitatively in agreement with the results of the FISH analysis, i.e. after 2 months an increasing signal was observed. The simulations further show that Anammox become dominant after day 100. This explains the colour change from brownish to reddish, as mentioned above. This also shows the successful enrichment of Anammox organisms from wastewater sludge over the experimental period.

At first, most of the biomass is heterotrophic (X_H) and autotrophic (X_{NH} and X_{NO}), as the reactor was inoculated with sludge from a municipal WWTP, but these populations decrease because they do not have substrates. No or very little autotrophic (X_{NH} and X_{NO}) biomass could remain in the reactor after start-up. This can be seen from the strong decrease in autotrophic biomass concentration in Figure 5.2.7.

From Figure 5.2.8 it can be seen that the concentration of heterotrophic biomass also decreases markedly after start-up of the reactor. However, a detailed plot of the concentration of heterotrophic organisms from day 100 to the final day of the experiment (Figure 5.2.8 (right)) reveals that heterotrophs still remain in the system after this time and perform a "background" process. The presence of these heterotrophs was confirmed by sporadic respirometric batch tests. The persistence of the heterotrophs is probably because heterotrophic biomass is able to live on cell lysis products (cryptic growth) and from the biodegradable substrate in the influent even in the absence of oxygen, since these organisms can use nitrate and TNO_2 as electron acceptor. Autotrophic biomass is not able to do this and thus these organisms cannot survive in a strictly anoxic environment.

If, however, in a full-scale application oxygen would be present due to, for instance, leaks, both autotrophs and heterotrophs would use this oxygen for growth and thus would allow the Anammox bacteria to grow in an anoxic environment.

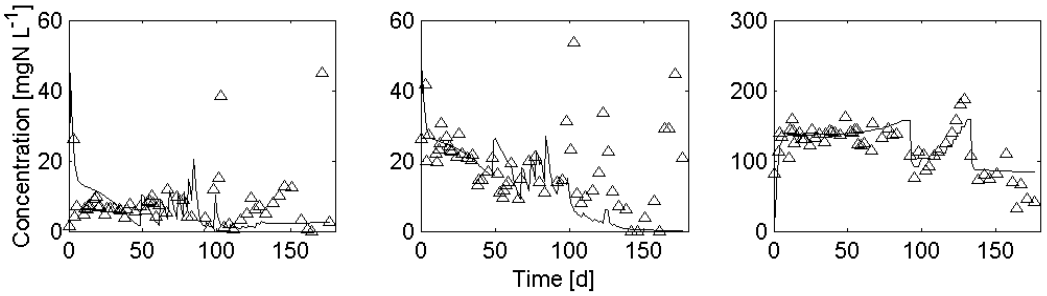

Figure 5.2.5. Comparison between the measured (△) and calculated (—) values of TNO_2 (left), TAN (middle) and nitrate (right) in the effluent of the reactor.

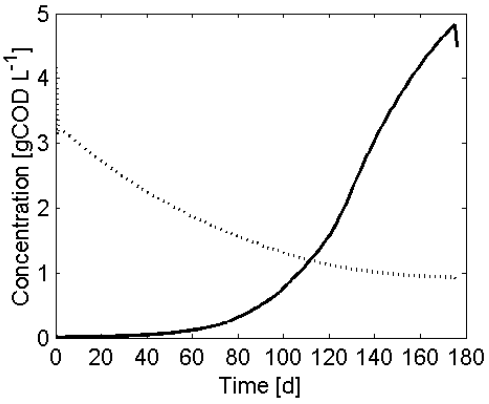

Figure 5.2.6. The calculated amounts of Anammox biomass X_{AN} (—) and inert biomass X_I (--) in the reactor.

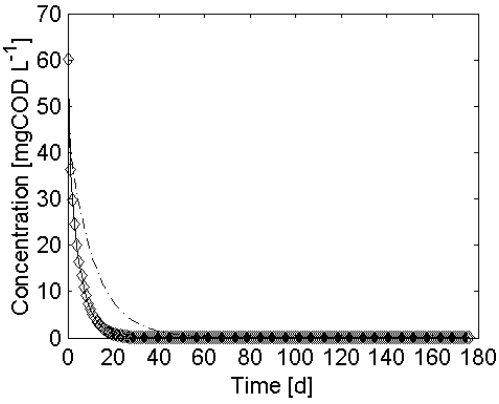

Figure 5.2.7. The calculated amounts of X_{NH} (◇) and X_{NO} (-.) over the total experimental period.

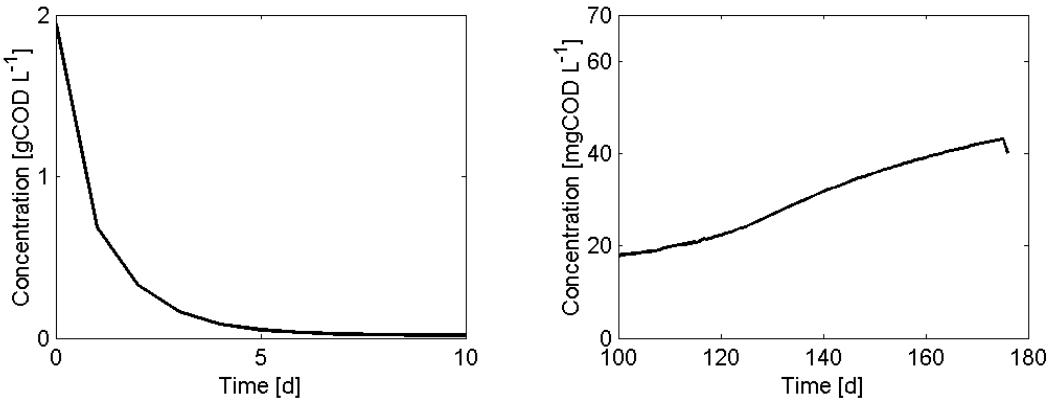

Figure 5.2.8. The calculated amounts of X_H during the first 10 days of the experimental period (left) and from day 100 onwards (right).

4 CONCLUSIONS

The isolation of Anammox biomass from WWTP sludge requires an anoxic atmosphere. The presence of oxygen, even in low quantities, can inhibit the process because of the low initial concentration of the Anammox biomass, in combination with their very low growth rate. A good choice to solve this problem could be the addition of nitrifiers during start-up, so that would consume the oxygen. This cooperation of aerobic and anaerobic ammonium oxidising bacteria allows the removal of ammonium in one single oxygen-limited step named CANON (Completely Autotrophic N-removal Over nitrite) (Sliekers *et al*., 2002).

The SBR is a very suitable system for the isolation of a microbial community with an extremely slow growth rate. An efficient retention of the biomass was achieved, the biomass concentration in the effluent being very low. The SBR permits a homogeneous distribution of substrates, products and biomass, preventing the formation of local accumulations of TNO_2 that could inhibit the process. The operation was stable and a high nitrogen removal efficiency was reached (82%).

The Anammox SBR reactor was modelled in the modelling and simulation environment WEST®. The Activated Sludge Model nr. 1 was extended with a 2 step nitrification-denitrification model and with the Anammox process to interpret the experimental results. Simulations were in good agreement with the measurement data and showed that both Anammox biomass and heterotrophs were active in the reactor. The presence of these heterotrophs is important, because they can consume any oxygen that might leak into the system, as could the nitrifying bacteria if they were present. This way the reactor can still stay anoxic, which favours the activity of the Anammox biomass.

Chapter 5.3

Model-based estimation of the minimum start-up time for Anammox bioreactors

ABSTRACT

The minimum start-up time of the Anammox process was studied for different reactor configurations and operational conditions. The start-up time increases with decreasing initial Anammox biomass concentration, decreasing temperature, decreasing separator efficiency, increasing influent concentration and decreasing HRT. The benefit of these model-based simulations is that design decisions can be taken based on these calculations. For example the choice between investing in a membrane for better separation and a longer and thus more expensive start-up can be weighted against each other. Further, operational conditions where start-up within a preset number of days is not possible can be identified.

1 INTRODUCTION

Ten year after its discovery in denitrifying fluidised bed reactors (Mulder *et al*., 1995) the Anammox process has been started up and studied in several lab-scale and pilot-scale reactor configurations such as sequencing batch reactors (Strous *et al*., 1998), rotating biological contractors (Helmer *et al*., 2001) and membrane bioreactors (Wyffels *et al*., 2004a). In the Anammox process TAN and TNO_2 are combined on an almost equimolar basis to nitrogen gas, while also a small amount of nitrate is formed.

Together with a preceding partial nitrification step such as the SHARON process (van Dongen *et al*., 2001a&b) this Anammox process combines to a completely autotrophic nitrogen removal process with great potential since there is no longer need for external carbon addition, sludge production is very low and oxygen input and aeration energy requirements are largely reduced (Jetten *et al.*, 1997).

A full-scale SHARON-Anammox process is constructed at the Rotterdam Sluisjesdijk sludge treatment plant (The Netherlands) and start-up is expected soon. A major difficulty of starting up such a (full-scale) Anammox reactor is the long start-up time as the doubling time of Anammox organisms is in the order of 11 to 20 days depending on the environmental conditions (Strous *et al*., 1998). Oxygen and TNO_2 inhibition might even delay start-up further. Other factors affecting the start-up time are the temperature, the retention capacity of the enrichment reactor and the initial amount of biomass. This last factor poses a problem for start-up of full-scale applications since the total amount of Anammox present in the world is currently still very limited and located at a few research institutes, not counting the Anammox present in the natural environment such as the Black Sea (Kuypers *et al*., 2003). Initial Anammox biomass for start-up will therefore be low and start-up could become lengthy or even impossible within a certain time frame.

With the help of model-based simulations the effect of initial biomass concentration, reactor configuration and temperature can be easily investigated. These simulations can help design decisions on the implementation of Anammox reactors.

2 MATERIALS AND METHODS

2.1 Extension of ASM1

For modelling purposes the Activated Sludge Model nr. 1 (Henze *et al.*, 1987) was extended with a 2-step nitrification-denitrification model and with the Anammox process as presented in chapter 3.2. Expression 5.3.1 for the Anammox growth rate was used.

$$\mu = \mu_{AN}^{\max} \frac{K_{O,AN}}{K_{O,AN} + S_O} \frac{S_{TNO2}}{K_{TNO2,AN} + S_{TNO2}} \frac{S_{TAN}}{K_{TAN,AN} + S_{TAN}} \quad (5.3.1)$$

The maximum specific growth rate of the Anammox biomass (μ_{an}^{max}) was derived from Strous *et al.* (1999b) and set to a value of 0.019 d^{-1} at 20°C. The affinity constants for TAN ($K_{TAN,AN}$) and TNO_2 ($K_{TNO2,AN}$) were both set to 0.3 mgN L^{-1}. The decay coefficient was set to 0.0025 d^{-1}, which is an order of magnitude below the maximum growth rate. An Arrhenius type temperature dependency given by equation 5.3.2 with 20°C as reference is incorporated in the kinetic expression.

$$k(T) = k(T_r) e^{\theta(T-T_r)} \quad (5.3.2)$$

where k(T) is the kinetic parameter (maximum specific growth rate μ or decay coefficient b) at the actual temperature T, T_r is the reference temperature (20°C) and θ is the Arrhenius constant. The Arrhenius constant for Anammox was set to 0.096 (see chapter 3.2). The temperature dependency proposed here can be used in the interval between 20 and 40°C.

2.2 The Anammox reactor

All modelling was performed in the modelling and simulation environment WEST® (Vanhooren *et al.*, 2003). The Anammox reactor was implemented as a completely mixed reactor followed by a pointsettler selected from the WEST® modelbase (Figure 5.3.1). Influent and effluent are supplied continuously. The separator efficiency of the Anammox settler is determined by f_{ns}, which is a parameter that indicates the fraction of biomass that is discharged. As such (1-f_{ns}) is the separator efficiency.

This reactor configuration was preferred over an SBR configuration as applied by Strous *et al.* (1998) and Dapena-Mora *et al.* (2004c) to have a continuous operation, to rule out discussion on optimal operation of the SBR and to keep the set-up as straightforward as possible.

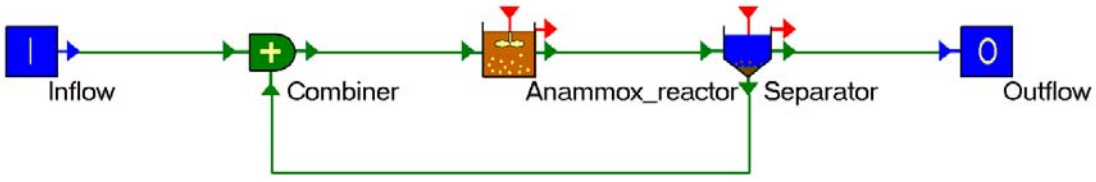

Figure 5.3.1. Implementation of the Anammox reactor in the WEST® software.

2.3 Simulation approach

Simulations were performed over a 500-day period. If after 500 days the reactor is still not started up the simulated start-up strategy cannot be considered appropriate since in reality the start-up time will even be longer as oxygen and TNO_2 inhibition and other disturbances that are not considered here will occur and will further slow down start-up.

In the simulations the effect was studied of 7 different separator efficiencies (100, 99.9, 99.5, 99, 97.5 and 95%), 5 different temperatures (20, 25, 30, 35 and 38°C), 3 different hydraulic residence times (HRT, 0.5, 1 and 2d) and 4 different initial Anammox biomass concentrations (10, 100, 250, 1000 mgCOD L^{-1}). It should be noted that the initial Anammox biomass concentration is different from the total initial biomass concentration in reality, as only part of the biomass will consist of Anammox organisms. This should be kept in mind when analysing the results of the simulations. The same remark holds for the separator efficiency, which is calculated without distinguishing between the different types of biomass. If for some reason the Anammox organisms grow in better retainable flocs than the "average" sludge floc, then the actual separator efficiency of the reactor will be larger if it is calculated on the basis of Anammox flocs alone.

Finally also 2 different TAN and TNO_2 influent concentrations were used: a typical Anammox influent of 500 mgTNO_2-N L^{-1} and 450 mgTAN-N L^{-1} and a low concentration stream of 100 mgTNO_2-N L^{-1} and 100 mgTAN-N L^{-1}.

The start-up time in a certain simulation was determined as the point were the TAN and TNO_2 concentrations were lower than 1% of the original concentrations and corresponds with the point where full activity was obtained. This is illustrated in Figure 5.3.2. In this example the initial biomass concentration was 10 mgCOD L^{-1}, the separator efficiency was 99.9 %, the HRT 1 day, the temperature 35°C and the influent TAN and TNO_2 concentration 450 and 500 mgN L^{-1} respectively. The point indicated by the arrow (68 days) was taken as the start-up time.

In this chapter the most important results will be highlighted.

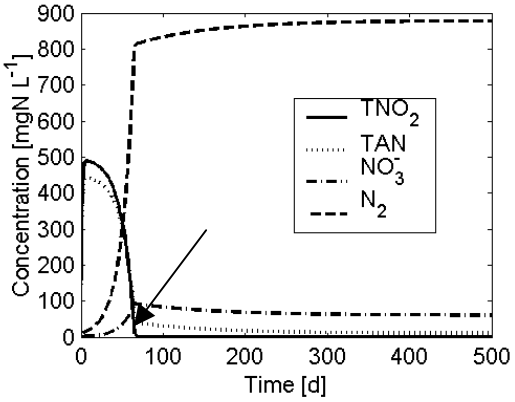

Figure 5.3.2. Determination of the start-up time of an Anammox reactor.

3 RESULTS AND DISCUSSION

3.1 Start-up time of a standard Anammox reactor

A literature survey showed that the average HRT of an Anammox reactor is 1 day and a typical influent concentration is 500 mgTNO_2-N L^{-1} and 450 mgTAN-N L^{-1} (based on reactors described by Dapena-Mora *et al*., 2004c; Strous *et al*., 1998; Fux *et al*., 2002 and Wyffels *et al*., 2004a among others). For these conditions the estimated minimal start-up time as function of temperature, initial Anammox biomass concentration and separator efficiency is presented in Figure 5.3.4.

Logically start-up time will increase with decreasing initial Anammox biomass concentration, decreasing temperature and decreasing separator efficiency. Based on this curve the design decision can be made between better separation efficiency (e.g. a membrane with an almost complete retention and longer (i.e. more expensive) start-up time.

From Figure 5.3.4 it also becomes clear that start-up of Anammox is also possible at temperatures below 25°C on the condition that the reactor efficiency is high enough since at 97.5 and 95% separator efficiency the minimal temperature for start-up within 500 days is 25°C and 30°C respectively.

Start-up time is therefore less dependent on the availability of initial Anammox biomass then on separator efficiency and temperature. Even with very low initial biomass concentration start-up is possible providing the reactor is operated at sufficiently high temperatures and separator efficiency. Also the importance of sludge breeding to allow the Anammox reactor to start-up with a higher biomass concentration becomes evident. This is very similar to sludge breeding for inoculation of anaerobic digesters. It is often performed in UASB reactors that degrade starch wastewater, whose waste sludge is used to inoculate.

3.2 Comparing different operational conditions

In Figure 5.3.5 the start-up times of two Anammox reactors with the same retention capacity (99.9 %) and influent concentration (500 mgTNO_2-N L^{-1} and 450 mgTAN-N L^{-1}) but different HRT (0.5 and 1 day) are compared. From Figure 5.3.5 it can be seen that decreasing the HRT increases the start-up time. Decreasing the HRT of the reactor can therefore lead to a more difficult start-up. Start-up at lower temperatures is however still possible at lower HRT.

In Figure 5.3.6 the start-up times of two Anammox reactors with the same retention capacity (99.9 %) and HRT (1d) but different influent concentration (500 mgTNO_2-N L^{-1} and 450 mgTAN-N L^{-1} and 100 mgTNO_2-N L^{-1} and 100 mgTAN-N L^{-1} respectively) are compared. From Figure 5.3.6 it can also be seen that decreasing the influent concentration decreases the

start-up time. Start-up will therefore be faster for lower loaded streams. Also the risk for TNO_2 inhibition will be lower for lower loaded streams, but this is not investigated here.

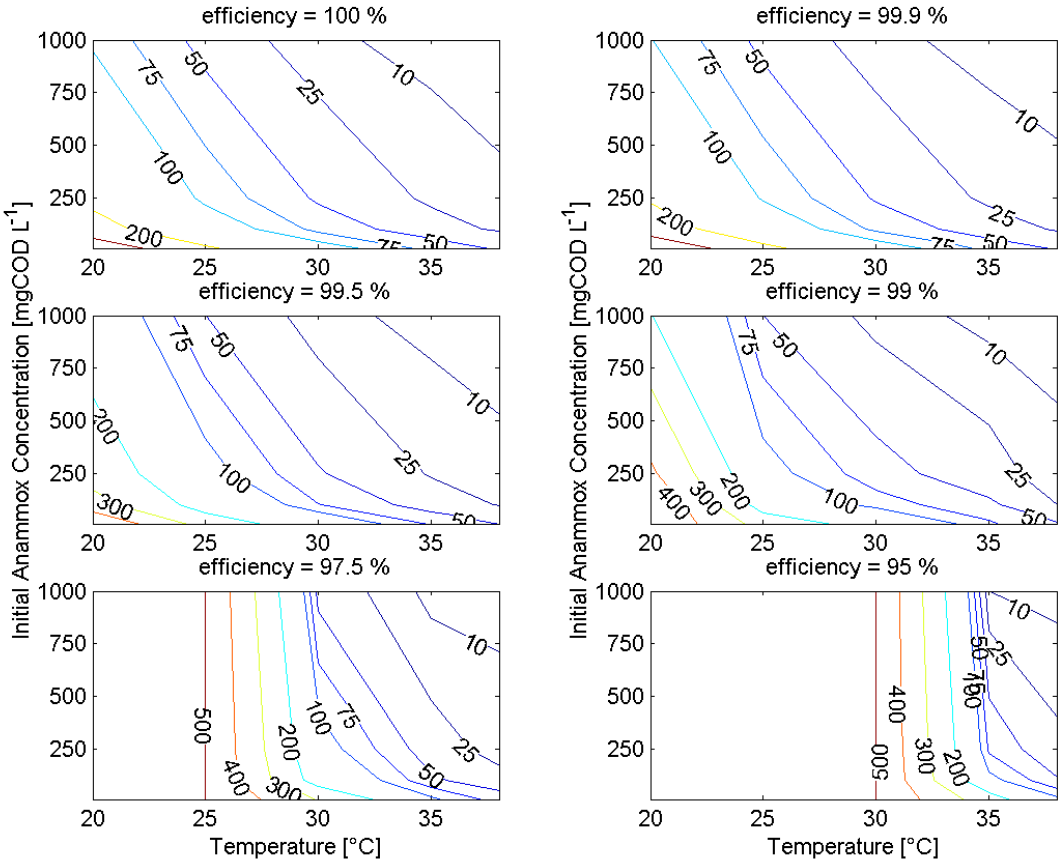

Figure 5.3.4. Estimated minimal start-up time (contourlines) of an Anammox reactor with a HRT of 1 day and an influent concentration of 500 mgTNO_2-N L^{-1} and 450 mgTAN-N L^{-1}.

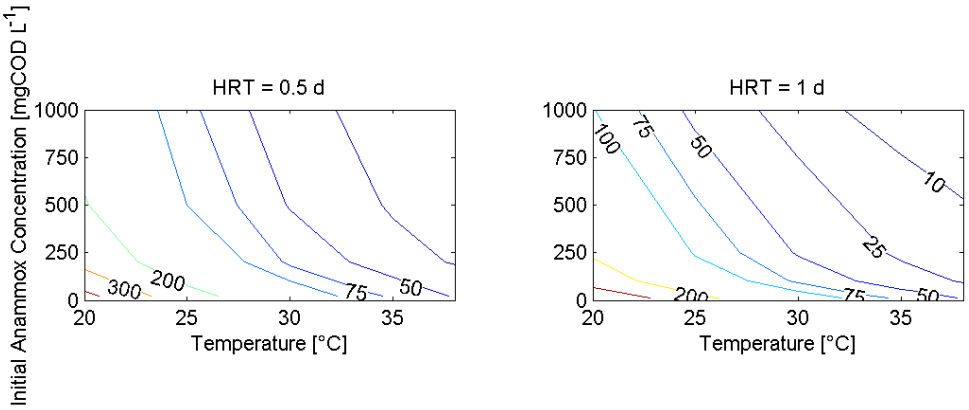

Figure 5.3.5. Start-up times of two Anammox reactors with different HRT (0.5 day (left) and 1 day (right)).

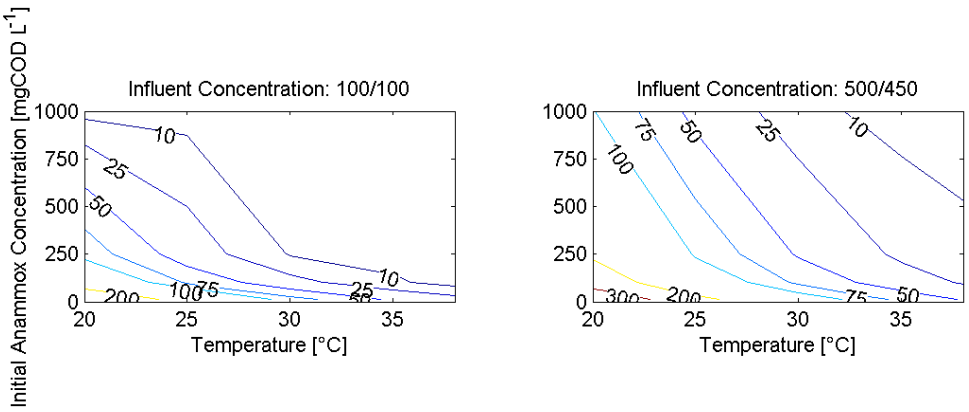

Figure 5.3.6. Start-up times of two Anammox reactors with different influent concentration (100 mgTNO_2-N L^{-1} and 100 mgTAN-N L^{-1} (left) and 500 mgTNO_2-N L^{-1} and 450 mgTAN-N L^{-1} (right)).

3.3 Effective influent concentration

Obviously the start-up strategy as discussed in the materials and methods section will not work as Anammox is inhibited by TNO_2. Exposure to TNO_2 concentrations of 100 mgTNO_2-N L^{-1} over a period of 12 hours or instantaneous TNO_2 concentrations higher than 140 mgTNO_2-N L^{-1} resulted in inhibition (Strous *et al*., 1999b). However, this inhibition is not accounted for in the model. Consequentially, in a real start-up the concentrations should be slowly increased as the Anammox organisms grow while all other conditions are kept constant, as demonstrated by Dapena-Mora *et al*. (2004c). For the example given in the materials and methods section the actual influent concentration is depicted in Figure 5.3.3. This profile was calculated by subtracting non-converted TNO_2 and TAN from the initial influent concentration (500 mgTNO_2-N L^{-1} and 450 mgTAN-N L^{-1}).

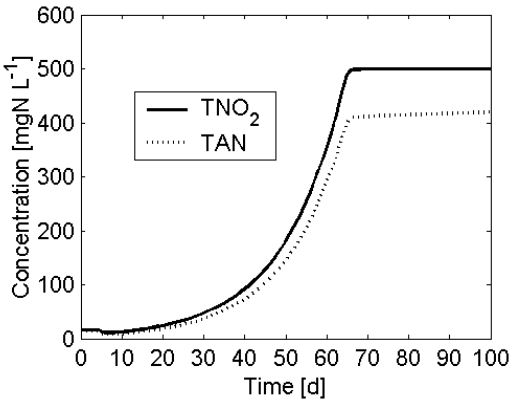

Figure 5.3.3. Effective influent TAN and TNO_2 concentration during start-up of an Anammox reactor to avoid inhibition by TNO_2.

4 CONCLUSIONS

Based on model simulations the minimum start-up time of the Anammox process for different reactor configurations and operational conditions was calculated. From these simulations it became clear that the start-up time increases with decreasing initial Anammox biomass concentration, decreasing temperature, decreasing separator efficiency, increasing influent concentration and decreasing HRT.

The real benefit of these simulations is that design decision can be taken based on the calculated curves. For example the choice between investing in a membrane for better separation and a longer and thus more expensive start-up can be weight against each other. Further, operational conditions where start-up within a preset number of days is not possible can be identified.

Chapter 5.4

Using parameter sensitivity analysis of the OLAND/CANON biofilm process: What to measure, where to measure and under which conditions?

ABSTRACT

Steady state sensitivity analysis revealed that among the nitrogen species measurements (TAN, TNO_2, NO_3^- and N_2) only the measurement of TAN and TNO_2 yields information concerning the maximum specific growth rate and the affinity constants of ammonium oxidizers and Anammox organisms in the OLAND/CANON process. However, almost no information concerning the Anammox TNO_2 inhibition constant could be derived using data obtained under steady state. Therefore, dynamic experiments are proposed where TNO_2 is injected to a continuously operated OLAND/CANON reactor. Based on experimental design calculations and practical considerations it was concluded that injecting a TNO_2 solution such that the mixed liquor concentration reached 30 mgTNO_2-N L^{-1} gave the most information concerning the TNO_2 inhibition constant of the Anammox process. The use of (bio)sensors to measure the TNO_2 and TAN concentrations during the dynamic experiments is advised because of the high quality and frequency of such data.

This chapter is published as:

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1 INTRODUCTION

Processes like the OLAND process (Kuai and Verstraete, 1998; Pynaert *et al.*, 2003) or the CANON system (Hao *et al.*, 2002a&b) offer an alternative to the SHARON-Anammox process for significantly improving nitrogen removal in case the influent TAN concentrations are of the order of 100 mgTAN L^{-1}. The process is based on the combination of partial nitritation, where TAN is partially oxidized to TNO_2, and anaerobic ammonium oxidation (Anammox), a process in which TAN and TNO_2 are combined to form nitrogen gas. As both 1-reactor processes are very similar the process studied here will be called the CANON/OLAND process.

This combined completely autotrophic process has great potential since there is no need for external carbon addition, sludge production is very low, and oxygen input and aeration energy requirements are largely reduced (Jetten *et al.*, 1997). Characteristics of the Anammox organisms are the low growth rate, low biomass yield and the inhibition by oxygen and TNO_2. Because of this low growth rate and biomass yield, start-up is lengthy and the use of a reactor with high retention capacity, such as a biofilm system, is essential.

A modelling and simulation environment such as WEST® (Vanhooren *et al.*, 2003) is a very suitable tool for further optimization of these completely autotrophic nitrogen removal process as experimental work is very time consuming. In literature, few studies towards modelling and simulation of the Anammox organisms are presented so far. Hao *et al.* (2002 a&b) performed a thorough simulation study on the behaviour of a CANON system under different process conditions, such as varying temperature and dissolved oxygen concentration. However, no verification with real data was performed and no start-up dynamics were included in the study. Koch *et al.* (2000) performed simulations with a similar system, but also did not include any start-up or long-term dynamic effects. In both studies different kinetic parameters for the Anammox process are used. In future simulation studies it is therefore considered important to calibrate the parameters for the system under study. Since this is a time-consuming task it is useful to follow a structured approach based on optimal experimental design for parameter estimation (OED-PE) (Dochain and Vanrolleghem, 2001).

With this OED-PE experiments can be designed that will produce high quality data required for an accurate model calibration. It is a solution to the complex problem of constrained choices resulting in an optimal experiment (De Pauw and Vanrolleghem, 2003b). The basis of OED-PE is the Fisher Information Matrix (FIM) that summarizes the information content of the data (to be) collected in a certain experiment. Essential to calculate this FIM are the sensitivity functions (De Pauw and Vanrolleghem, 2003b). These functions determine how much a parameter influences the variables of the process. Hence, if the sensitivity is large, the influence of the parameter on the variable is large and the measurement of the variable gives much information about the

parameter, so that the parameter can be estimated with high accuracy. Care should also be taken that parameters are not correlated. All this can be evaluated through the FIM (Dochain and Vanrolleghem, 2001).

2 MATERIALS AND METHODS

2.1 Extension of ASM1: ASM1.e

For modelling purposes the Activated Sludge Model nr. 1 (Henze *et al.*, 1987) was extended with the Anammox process and 2-step nitrification and denitrification: ASM1.e (see chapter 3.2).
The maximum specific growth rate (μ^{max}_{AN}, 0.019d^{-1} at 20°C) of the Anammox biomass was derived from Strous *et al*. (1999b). The decay coefficient was set to 0.0025 d^{-1}, which is an order of magnitude below the maximum growth rate. The affinity constants for TAN (K_{TAN}^{AN}) and TNO_2 (K_{TNO2}^{AN}) were both set to 0.3 mgN L^{-1}. Monod kinetics were used to describe the dependency of the growth rate of Anammox on TAN. TNO_2 is not only a substrate, but is also inhibiting the Anammox process (Strous *et al.*, 1999b). Therefore Haldane kinetics are more appropriate than Monod kinetics. An inhibition constant ($K_{I,TNO2}^{AN}$) of 20 mgTNO_2-N L^{-1} was selected based on the experiments performed by Strous *et al.* (1999b) and Wyffels (personal communication). The maximum specific growth rate (μ^{max}_{NH}) of the ammonium oxidizers was set to 0.8 d^{-1} at 20°C. The affinity constants expressed in terms of mgTAN-N L^{-1} ($K_{TAN,NH}$) and oxygen ($K_{O2,NH}$) were set to 2.4 mgTAN-N L^{-1} and 0.6 mgO_2 L^{-1} respectively. Kinetics of heterotrophs and nitrite oxidizers were derived from other studies (Hao *et al.*, 2002a; Dapena-Mora *et al.*, 2004c). Kinetics were made dependent of temperature by Arrhenius type equations.

2.2 Simulation, sensitivity analysis and OED-PE

Simulation, sensitivity analysis and OED-PE were performed in the modelling and simulation environment WEST® (Vanhooren *et al.*, 2003; De Pauw and Vanrolleghem, 2004). The biofilm model and the simulation approach elaborated in chapter 3.2 were used for simulations with the OLAND/CANON biofilm reactor. The sensitivity functions were calculated both in the bulk phase and at different locations along the biofilm depth. Influent TAN concentration was chosen to be 100 mgTAN-N L^{-1}. The hydraulic retention time and temperature were set to 6 h and 35°C respectively. The aeration coefficient (K_La) was set to 125 d^{-1}. This value results from the simple rule that the flux of TAN that should be oxidised is equal to the flux of oxygen to the reactor. The liquid boundary layer was set to 50 µm, while the initial biofilm thickness was set to 700 µm.
Sensitivity functions were calculated with the central difference method (De Pauw and Vanrolleghem, 2003a). The TAN, TNO_2, NO_3^-, N_2 and O_2 concentrations were used as variables for the calculation of the (relative) sensitivity functions. The growth parameters (maximum specific growth rate, affinity and inhibition constants) of the ammonium oxidizers and the

Anammox organisms were used as parameters for the calculation of the (relative) sensitivity functions.

For the OED-PE calculations the D-criterion (determinant of the FIM) was used as objective function of the OED-PE optimisation problem.

3 RESULTS AND DISCUSSION

3.1 Steady state concentration profiles

Table 5.4.1 presents steady state effluent concentrations of the OLAND/CANON system when the total influent nitrogen concentration is 100 mgTAN-N L^{-1}. As the sludge production was very low, the sludge nitrogen content was not accounted for in the Table. No stripping process for the nitrogen gas is incorporated in the model. Hence, all the nitrogen gas is recovered in the bulk phase.

Biomass concentration profiles in the biofilm are depicted in Figure 5.4.1.a. These concentration profiles are similar to the ones calculated by Hao *et al.* (2002a&b). In the OLAND/CANON biofilm system the top of the biofilm is dominated by ammonium oxidizers, while Anammox is predominant in the inner layers, although also inert and slowly biodegradable material is present. The oxygen concentration in the biofilm decreases rapidly because of its consumption by ammonium oxidizers as can be seen in Figure 5.4.1.b.

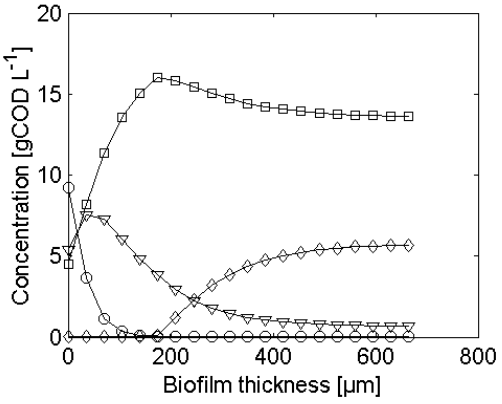

Figure 5.4.1.a. Biomass concentration profiles in an OLAND/CANON biofilm. Ammonium oxidizers (O), Anammox (□), inert (∇) and slowly biodegradable material (◊) are present.

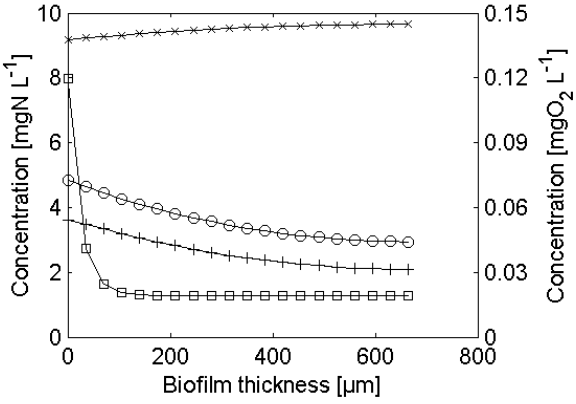

Figure 5.4.1.b. Concentration profiles in an OLAND/CANON biofilm of TAN (O), TNO_2 (+), NO_3^- (x) and oxygen (□).

Table 5.4.1. Steady state bulk concentrations of an OLAND/CANON biofilm system with a total influent nitrogen concentration of 100 mgTAN-N L^{-1}

	Concentration [mg L^{-1}]
TNO_2	3.5
NO_3^-	9
TAN	5.5
N_2	82
O_2	0.8

3.2 Steady state sensitivities of variables measured in the bulk phase

Calculation of the steady state relative sensitivities of variables measured in the bulk phase (Figure 5.4.2) revealed that the maximum specific growth rate influences the process variables most, while affinity constants are less influential, as was also observed by Hao *et al.* (2002b). In steady state operation information concerning the maximum specific growth rate and the affinity constants can be derived. However, almost no information concerning the TNO_2 inhibition constant can be derived using steady state sensitivity functions.

It can also be seen that measuring nitrogen gas or nitrate yields hardly any information concerning the parameters, while TNO_2 and TAN concentrations are most influenced by the parameters. The oxygen concentration is obviously most influenced by the kinetics of the ammonium oxidizers, but TNO_2 and TAN data are more sensitive. Further experimental efforts should therefore consider the measurement of TAN and TNO_2.

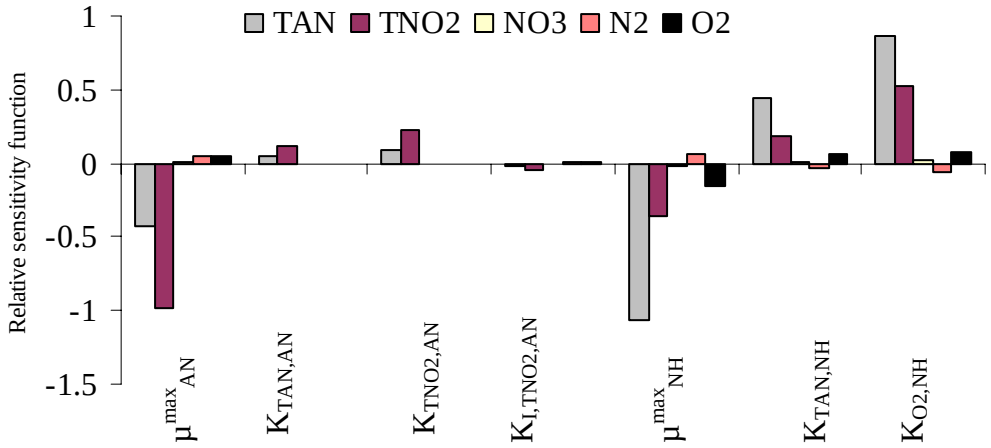

Figure 5.4.2. Steady state relative sensitivity functions in the bulk phase of the biofilm reactor.

3.3 Steady state sensitivities of variables measured in the biofilm

The sensitivity functions (e.g. of TNO_2 and TAN to the maximum specific growth rates of ammonium oxidizers and Anammox) tend to increase towards the inside of the biofilm (Figure 5.4.3). This means that in order to have the maximal amount of information, TNO_2 and TAN should be measured in the inner parts of the biofilm. This seems logical for the kinetic parameters of the Anammox process, since Anammox is active in the inner parts of the biofilm. Noteworthy is that the sensitivities related to ammonium oxidizers show the same trend.

Such experimental design has some obvious drawbacks, because the determination of concentrations inside the biofilm is a tedious or even impossible task, especially in full-scale installations.

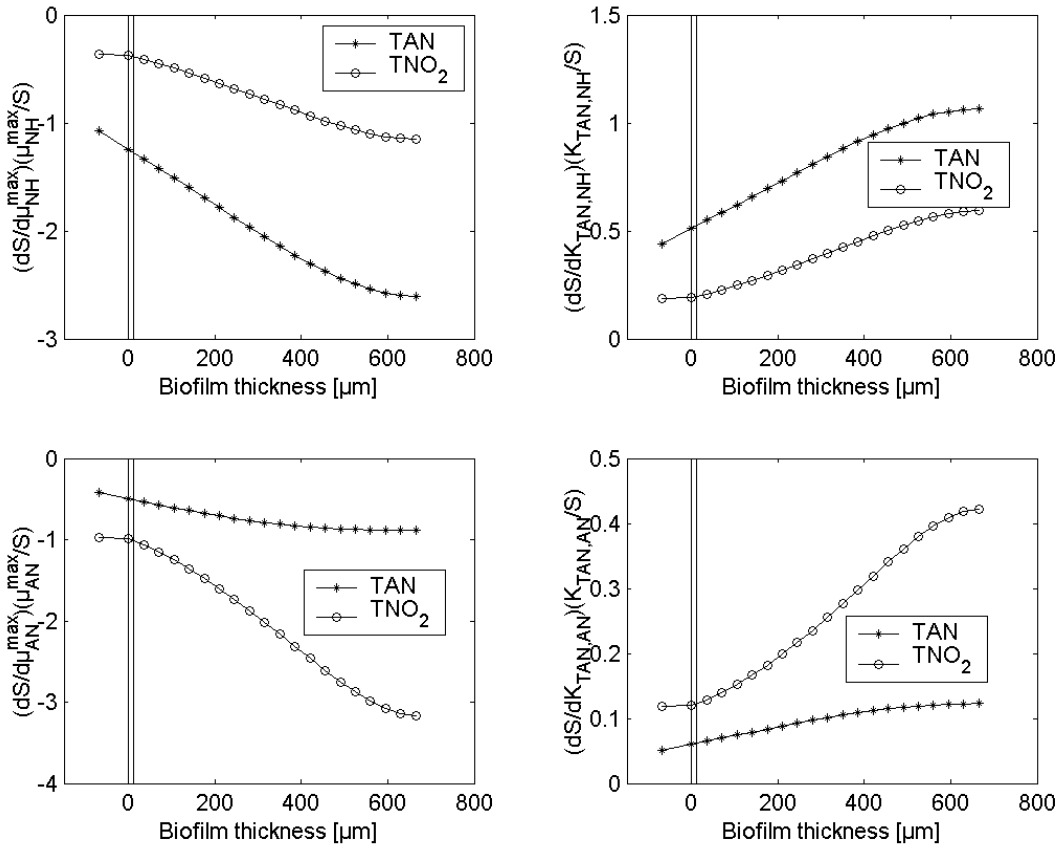

Figure 5.4.3. Steady state relative sensitivity functions of TAN and TNO_2 measured in the biofilm to the maximum specific growth rate and TAN affinity constant of ammonium oxidizers (top) and Anammox (bottom).

3.4 Sensitivities under dynamic conditions with square wave influent

Sensitivity analysis under dynamic conditions was also conducted. For example, in Figure 5.4.4 the average relative sensitivity functions related to the bulk TAN and TNO_2 concentrations are depicted when a square wave with 10 % variation with a twice a day frequency is added to the influent flow rate and concentrations. The values of the relative sensitivity functions are similar to the ones in Figure 5.4.2, except for the functions related to the TNO_2 inhibition constant, which are somewhat higher. Application of dynamic conditions will therefore lead to only somewhat more information concerning this inhibition constant. However, the Anammox process is very vulnerable to these dynamic conditions as TNO_2 accumulation or other inhibitions might lead to failure of the reactor.

Figure 5.4.4. Average relative sensitivity functions of measurements in the bulk phase of the biofilm reactor when a square wave with 10 % variation with a twice a day frequency is added to influent flow and concentrations.

3.5 Sensitivities under dynamic conditions with pulse substrate injection

The pulse injection of TNO_2 and/or TAN to the process could also lead to more information. This experiment is easy to implement, but the question is how much of what should be injected?

Two types of experiments can be conducted: an injection of TAN and an injection of TNO_2. Injection of, for example, 25 mgTAN-N L^{-1} TAN to the reactor would lead to less information on the TAN affinity constant of Anammox since the TAN concentration would increase above its value, 0.3 mgTAN-N L^{-1}. Similarly, the injection of TNO_2 would lead to less information on the TNO_2 affinity constant of Anammox. However, more information on the TNO_2 inhibition

constant would be obtained. This last experiment will be further discussed as up to now the information of the TNO_2 inhibition constant is very limited.

Injecting too much TNO_2 would seriously endanger the operation of the Anammox reactor. On the other hand, injection of small amounts of TNO_2 in the reactor would not lead to significant additional information. From preliminary simulations it became clear that injecting a TNO_2 solution such that the mixed liquor concentration reached more than 30 $mgTNO_2\text{-}N\ L^{-1}$ leads to operational failure of the reactor. Therefore, the optimal amount of TNO_2 injected would lie between 0 and 30 $mgTNO_2\text{-}N\ L^{-1}$. Since the information concerning maximum growth rate and affinity constant is already reasonable, the focus will be on the development of an optimal experiment for the determination of the TNO_2 inhibition constant.

The OED-PE algorithm as implemented in WEST® (De Pauw and Vanrolleghem, 2004) was used to determine the optimal TNO_2 injection, assuming a measurement frequency of once every 15 minutes and an 8 hours experiment duration. The D-criterion (determinant of the FIM) was used as objective function of the OED-PE optimisation problem. It focuses on an overall reduction of parameter uncertainty. For the determination of the optimal amount of TNO_2 the sensitivity functions of TAN and TNO_2 to the TNO_2 inhibition constant of the Anammox biomass were used in the FIM calculation.

From the OED-PE calculations it became clear that the maximum amount of TNO_2 injected gave most information concerning the TNO_2 inhibition constant. Hence, if the actual experiment would be performed with Anammox organisms with a higher tolerance towards TNO_2, then higher amounts of TNO_2 can be injected.

Corresponding relative sensitivity functions and mixed liquor TNO_2 and TAN concentration profiles are depicted in Figure 5.4.5. When determining the inhibition constant, a trade-off between process stability and information content becomes evident as the process is brought into more unstable conditions during the experiment. Also, the sensitivity function related to TAN has a higher (negative) maximum than the sensitivity function related to TNO_2. Hence, measuring TAN yields more information than measuring TNO_2, if the measurement errors for both components are assumed equal.

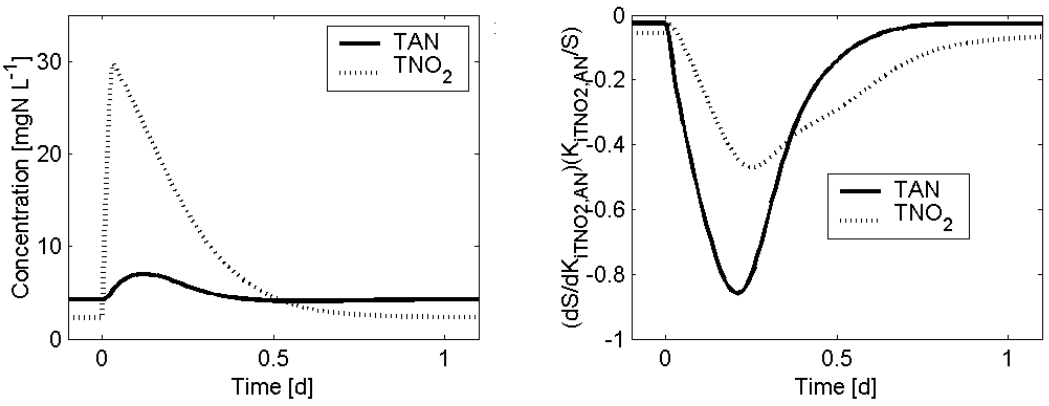

Figure 5.4.5. TAN and TNO_2 concentration profiles (left) and corresponding relative sensitivity functions (right) in the bulk phase of the biofilm reactor after injection of 30 mgTNO_2^--N L^{-1} at time 0.

3.6 Use of (bio)sensors

All the above-mentioned reactor operations and experiments and the corresponding sensitivity functions assume accurate measurements and sensors with high resolution. For TNO_2, a biosensor (Revsbech *et al.*, 2000, Sin *et al.*, 2004) is already described in literature and commercial application is expected soon. This TNO_2 biosensor contains bacteria that reduce TNO_2, but not nitrate, to N_2O that is subsequently monitored by a built-in electrochemical sensor. TNO_2 measurement will therefore not be interfered by the presence of nitrate. The biosensor has a linear calibration curve in a range of about 0-30 mgTNO_2-N L^{-1} at 35°C (Nielsen *et al.*, 2002; Sin *et al.*, 2004). It is in this range that the TNO_2 and TAN concentrations in the OLAND/CANON reactor will typically evolve. For TAN a sensor as described by Rieger *et al.* (2002) can be used. The operational principle is based on analyzing the potential difference between a reference electrode and a measuring electrode whose potential is sensitive to ammonium. The sensor has a linear response in a range of 0-30 mgTAN-N L^{-1}. With such sensors high quality and high frequency data can be collected.

The titrimetric measurement of the TAN and the TNO_2 concentrations will be difficult in view of the concentration range required here, as discussed in chapter 4.3.

4 CONCLUSION

Information concerning the maximum specific growth rate and the affinity constants of the OLAND/CANON process can be obtained by measuring the bulk TNO_2 and TAN concentrations in a biofilm reactor. Measuring nitrate or nitrogen gas hardly yields any information on the kinetic parameters.

In order to increase the information concerning the TNO_2 inhibition constant a dynamic experiment is proposed where TNO_2 is injected to the Anammox reactor and the TNO_2 and TAN evolutions are measured. Based on experimental design calculations and practical considerations it was concluded that injecting the maximum amount of 30 $mgTNO_2\text{-}N\ L^{-1}$ gave most information concerning the TNO_2 inhibition constant of the Anammox process.

The use of (bio)sensors to measure the TNO_2 and TAN concentrations during the dynamic experiments is advised because of the high quality and frequency of their data. In the future such experiments should be conducted to increase the knowledge of the kinetic parameters.

Chapter 5.5

Influence of operational conditions on Anammox and competing organisms in a biofilm

ABSTRACT

The performance of Anammox in a biofilm system under different operational conditions was investigated. In an OLAND/CANON system an optimal bulk oxygen concentration existed where nitrogen removal was optimal. Increasing the influent concentration led to the disappearance of this optimal removal because of TNO_2 inhibition. The performance of an Anammox biofilm system is only little affected by changing hydrodynamic conditions. An OLAND/CANON biofilm system on the other hand was very much affected by a change in hydrodynamic conditions. Nitrogen removal dropped drastically when the boundary layer increased, indicating the importance of the oxygen mass transfer resistance. This oxygen transfer resistance is an important limitation to the performance of an OLAND/CANON system and an important consideration in the design of these novel nitrogen removal processes. Competition of Anammox and heterotrophs will be important when high COD loads are applied. The amount of Anammox present decreases with COD load. Dynamic conditions also lead to lower Anammox activity because of inhibition by TNO_2 and competition of autotrophs and heterotrophs for electron donors and acceptors.

1 INTRODUCTION

Examples of new (autotrophic) nitrogen removal processes are the OLAND process (Kuai and Verstraete, 1998), the CANON (biofilm) process (Hao *et al.*, 2002a&b, Sliekers *et al.*, 2002; Sliekers *et al.*, 2003) and the combined partial nitritation-Anammox process (van Dongen *et al.*, 2001a&b, Wyffels *et al.*, 2004a). In both processes about half of the TAN is first converted to TNO_2 by ammonium oxidizers. This TNO_2 is then used as electron acceptor for the oxidation of the remainder of the TAN by the recently discovered Anammox organisms (van de Graaf *et al.*, 1995).

The difference between the two process configurations is that in the OLAND/CANON biofilm process both ammonium oxidisers and Anammox are active in one reactor (1-reactor system), while in the partial nitrification-Anammox process ammonium oxidisers and Anammox are active in two separate reactors (2-reactor system). Typically in the OLAND/CANON biofilm process the ammonium oxidizers are active in the outer layers of the biofilm, while Anammox is active in the inner layers. This way the Anammox organisms are protected from oxygen, which is consumed in the outer layers and which is inhibiting the Anammox activity (Figure 5.5.1).

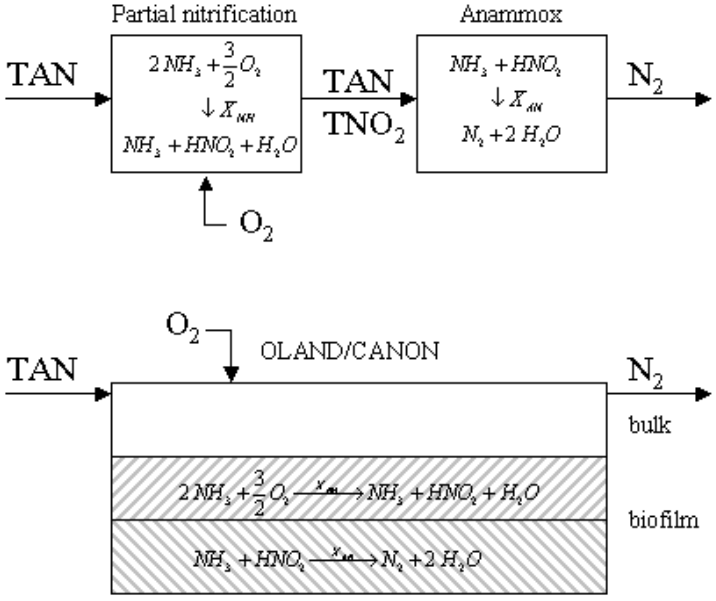

Figure 5.5.1. Schematic representation of a 2-reactor (top) and a 1-reactor (biofilm) (bottom) autotrophic nitrogen removal processes.

The question is whether the OLAND/CANON biofilm process is the favourite option or whether the partial nitritation and Anammox steps should be engineered separately (Jetten *et al.*, 2002)?

Single reactor systems require less footprint, but two reactor systems are easier to control and can handle higher nitrogen loads.

A key factor to answer this question is as in all nitrifying biofilm reactors (Zhu and Chen, 2001), the oxygen transfer to the OLAND/CANON reactor. This oxygen transfer is limited by two factors: the transfer from air to the bulk phase and the transfer from the bulk phase to the biofilm over a boundary layer. The type of aeration device and the reactor configuration determine the first limitation. In this chapter the effect of increasing oxygen transfer to the bulk phase and consequentially increasing bulk oxygen concentration will be demonstrated at different influent concentrations. The second limitation is determined by the thickness of the boundary layer which is mostly influenced by flow conditions. In literature, it has been stated that the maximum oxygen uptake of biofilms is on average 10 $gO_2\ m^{-2}d^{-1}$ (Logan, 1993; Hinton and Stensel, 1994). However, for lower oxygen bulk concentrations this oxygen uptake is lower. At an oxygen bulk concentration of 1 $mgO_2\ L^{-1}$, a typical value for an OLAND/CANON reactor, this oxygen uptake becomes 3 $gO_2\ m^{-2}d^{-1}$ (van Loosdrecht, personal communication). Hence, the maximum TAN load that the OLAND/CANON biofilm reactor can treat at an oxygen bulk concentration of 1 $mgO_2\ L^{-1}$ is about 350 mgTAN-N $L^{-1}d^{-1}$, assuming a biofilm surface area of 200 $m^2_{reactor}\ m^{-3}$, 50% TAN conversion, 3.43 mgO_2 needed per mgTAN-N converted and no COD in the influent. For a saturated oxygen bulk concentration this value becomes 1100 mgTAN-N $L^{-1}d^{-1}$. The maximum influent concentration that can be handled by an OLAND/CANON reactor is then a linear function of the hydraulic retention time (HRT) as depicted in Figure 5.5.2. However, the actually treated load will be lower than the maximum load. Hydrodynamic conditions will alter the boundary layer thickness and make oxygen transfer more difficult. This will be discussed in this chapter.

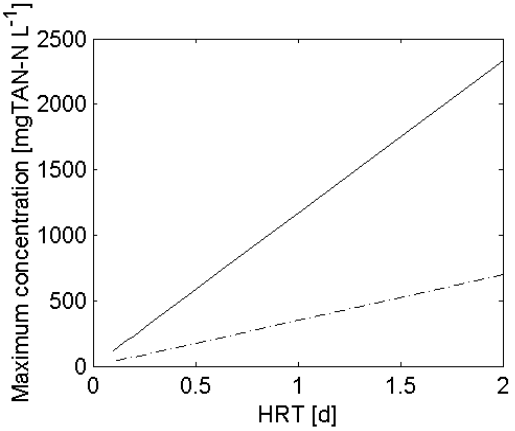

Figure 5.5.2. Maximum TAN influent concentration to the biofilm as function of the HRT for a bulk oxygen concentration of 1 $mgO_2\ L^{-1}$ (-.) and a saturated oxygen bulk concentration (-) assuming a biofilm surface area of 200 $m^2_{reactor}\ m^{-3}$, 50% TAN conversion and that 3.43 mgO_2 is needed per mgTAN-N converted.

This oxygen transfer limitation is a reason why a combined partial nitritation-Anammox system is to be preferred over an OLAND/CANON system at higher nitrogen loading rates. This study therefore researched the effect of oxygen transfer limitations in the application of the OLAND/CANON biofilm system.

Another influence on the performance of the Anammox process is the presence of biologically degradable carbon (COD). On the one hand the presence of this COD can enhance the performance of the Anammox process because the nitrate produced in the Anammox reaction can be denitrified by heterotrophs to TNO_2 rather than nitrogen gas. This TNO_2 may then serve again as electron acceptor for the Anammox process. On the other hand heterotrophs will also compete for TNO_2 with the Anammox organisms, as both organisms can use TNO_2 as electron acceptor.

The competition between heterotrophs and Anammox was already partially answered by Hao and van Loosdrecht (2004) who concluded that the presence of COD improved nitrogen removal and that heterotrophs and Anammox can coexist. However, no inhibition of TNO_2 nor dynamics were considered in this study. This chapter therefore tries to elaborate on these findings, linking the conclusions of Hao and van Loosdrecht (2004) with experimental data found in literature describing heterotrophs outcompeting Anammox organisms (van de Graaf *et al.*, 1996; Thamdrup and Dalsgaard, 2002).

Finally it is observed that TNO_2 itself is inhibiting the Anammox process. Because of this inhibition the performance of the Anammox process is significantly influenced by the dynamics inherent to wastewater treatment. Therefore, the influence of TNO_2 inhibition and process dynamics on the behaviour of Anammox was investigated.

2 MATERIALS AND METHODS

2.1 Extension of ASM1: ASM1.e

For modelling purposes the Activated Sludge Model nr. 1 (ASM1) was extended with a 2-step nitrification-denitrification model and with the Anammox process and will be denoted ASM1.e as discussed in chapter 3.2. Parameters characterising heterotrophic and autotrophic activity were taken from literature (Hao *et al*., 2002a; Henze *et al.*, 2000). The maximum specific growth rate of the Anammox biomass was derived from Strous *et al*. (1998) and set to 0.083 d^{-1} at 35°C. The decay coefficient was set to 0.01 d^{-1}, which is an order of magnitude below the maximum growth rate. The affinity constants for TAN and TNO_2 were both set to 0.3 mgN L^{-1}. Haldane kinetics were used to describe the dependency of the growth rate of Anammox on TNO_2, since TNO_2 is not only a substrate, but is also inhibiting the Anammox process. An inhibition constant of 20 mgN L^{-1} was selected as discussed in chapter 6.1. Note, however, that simulation results will be

strongly affected by the value of this inhibition constant and that research effort in the future should be directed to the more accurate determination of this constant.

2.2 Biofilm modelling

For the simulation study presented here, a one-dimensional multispecies biofilm reactor model was developed in the modelling and simulation environment WEST® (Vanhooren *et al.*, 2003) as discussed in chapter 3.2.

2.3 Process conditions and simulation approach

The reactor configuration and influent conditions in this study are very similar to studies performed earlier by other researchers (Hao *et al.*, 2002 a&b; Koch *et al.*, 2000). All simulations were performed at 35°C. Diffusion coefficients for soluble nitrogen components considered in the ASM1.e model (TNO_2, NO_3^-, TAN and N_2) and oxygen were derived from Picioreanu *et al.* (1997), while the coefficient for the readily biodegradable substrate was derived from Henze *et al.* (1995). The biofilm diffusion coefficient for particulate components considered in the ASM1.e model was set equal to 10^{-10} m² d^{-1}. Biofilm porosity was set to 0.5. For the biofilm density, a typical value of 40 kgCOD m^{-3} was taken (Melcer *et al.*, 1995). The initial biofilm thickness was set to 700 µm. Unless stated otherwise the boundary layer thickness was set to 50 µm.

3 RESULTS AND DISCUSSION

3.1 Performance of an Anammox and a OLAND/CANON system

In a first set of simulations the performance of an OLAND/CANON and an Anammox biofilm reactor were compared. Both reactors were operated at a HRT of 0.25 days. The influent concentration for the OLAND/CANON biofilm reactor was 100 mgTAN-N L^{-1}. The aeration coefficient (K_La) was set equal to 125 d^{-1}. This value results from the simple rule that the flux of TAN that can be oxidised is equal to the flux of oxygen to the reactor as given by equation 5.5.1. Oxygen transfer limitations are not considered in this expression:

$$K_l a = \frac{3.43\ f\ C_{TAN}^{in}}{HRT\ \Delta O_2} \approx 125\ d^{-1} \qquad (5.5.1)$$

with C^{in}_{TAN} the influent TAN concentration, f (≈ 0.55) the fraction of TAN to be oxidised and ΔO_2 (≈ 6 mgO_2 L^{-1} at 35°C) the driving force for air to liquid oxygen transfer. The influent concentrations for the Anammox biofilm reactor were 45 mgTAN-N L^{-1} and 55 mgTNO_2-N L^{-1}. The aeration coefficient was of course set to 0 d^{-1}, since Anammox organisms are inhibited by oxygen (Strous *et al.*, 1999b).

In Table 5.5.1 steady state bulk concentrations are presented of an OLAND/CANON and an Anammox reactor system. Again, as the sludge production was very low, the sludge nitrogen content was not accounted for in the Table. No stripping process for the nitrogen gas is incorporated in the model. Hence, all the nitrogen gas is recovered in the bulk phase.

Performances are very similar under steady state operation, although the Anammox reactor performs somewhat better.

Biomass concentration profiles in the biofilm for both systems are depicted in Figure 5.5.3. These concentration profiles are similar to the ones calculated by Hao *et al.* (2002a). In the Anammox reactor the Anammox organisms are present at the top of the biofilm, while the rest of the biofilm mainly consists of inert and slowly biodegradable biomass. In the OLAND/CANON biofilm system the top of the biofilm is dominated by ammonium oxidizers, while Anammox is predominant in the inner layers, although also inert and slowly biodegradable biomass is present. The oxygen concentration in the biofilm decreases rapidly because of the consumption by ammonium oxidizers.

Table 5.5.1. Steady state bulk concentrations in mg L^{-1} of an OLAND/CANON and an Anammox system with a total influent nitrogen concentration of 100 mgN L^{-1}

	OLAND/CANON reactor	Anammox reactor
TNO_2	3.5	2
NO_3^-	9	10
TAN	5.5	2
N_2	82	86
O_2	0.8	0

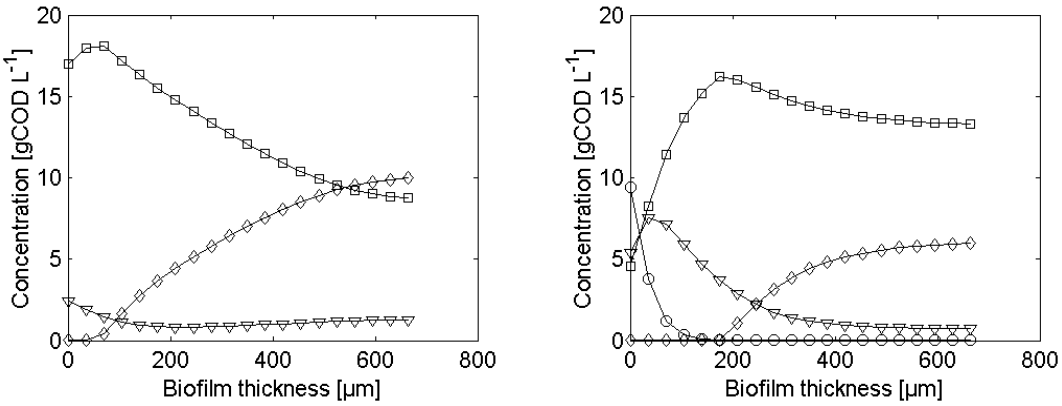

Figure 5.5.3. Biomass concentration profiles in an Anammox biofilm (left) and an OLAND/CANON biofilm (right). In both biofilms Anammox (□), inert (∇) and slowly slowly biodegradable matter (◊) are present, in the OLAND/CANON biofilm ammonium oxidizers (○) are present too.

3.2 Influence of aeration intensity on an OLAND/CANON system

The influence of increasing aeration intensity and resulting higher bulk oxygen concentration was investigated at different nitrogen loads. The reactor was operated at a HRT of 0.25 days and influent concentrations of 100, 200 and 400 mgTAN-N L^{-1} were used. Attention was paid to performance of the OLAND/CANON system at higher loads because this system was up to now only used for low loaded streams.

Aeration intensity plays an important role in the performance of the OLAND/CANON system, similar to the findings of Hao *et al.* (2002b). At an influent concentration of 100 mgTAN-N L^{-1} in total three areas can be distinguished (Figure 5.5.4).

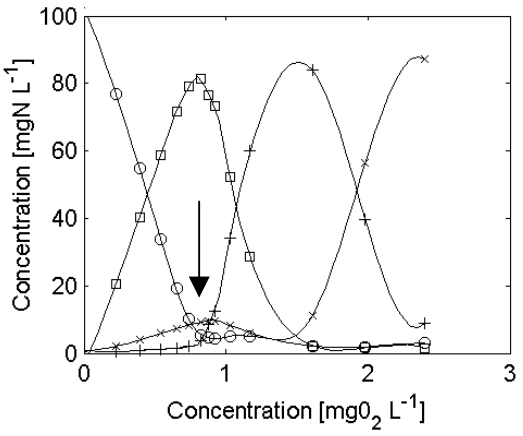

Figure 5.5.4. Concentration of TNO_2 (+), NO_3^- (x), TAN (o) and N_2 (□) in function of bulk oxygen concentration at an influent concentration of 100 mgTAN-N L^{-1}.

If the aeration is lower than the optimal aeration, then the conversion of TAN to TNO_2 is not complete. This leads to a surplus of TAN in the effluent of the reactor. Increasing the aeration will increase this conversion and eventually lead to an optimal nitrogen removal in the form of nitrogen gas. The optimal bulk oxygen concentration corresponding with this optimal removal is 0.8 mgO_2 L^{-1} for the conditions studied here. This is very similar to the value of 0.7 mgO_2 L^{-1} found by Hao *et al.* (2002b), but somewhat lower than the experimentally determined value of 2 mgO_2 L^{-1} by Koch *et al*. (2000). Further increasing the aeration beyond the optimal value will increase the oxygen concentration and the amount of ammonium oxidizers inside the biofilm and decrease Anammox perfomance. TNO_2 concentrations will therefore increase in the reactor, further decreasing Anammox activity. At even higher bulk oxygen concentrations above 1.5 mgO_2 L^{-1} nitrate will break through. Imposing low oxygen conditions is therefore essential for the operation of the OLAND/CANON reactor because otherwise nitrite oxidisers will be able to persist. So, the aeration intensity has to be high enough to ensure adequate oxidation of TAN, but

can also not be too high. Furthermore, in order to control the OLAND/CANON process frequent measurements of the nitrogen components are essential as a small shift in dissolved oxygen concentration (+/- 0.5 mgO_2 L^{-1}) can lead to large fluctuations in effluent conditions.

Increasing the influent TAN concentration should theoretically lead to a similar behaviour as described above, as long as the influent concentration is lower than the maximum influent concentration at the given HRT, as depicted in Figure 5.5.2. The optimal oxygen concentration can be expected to be somewhat higher compared to lower influent TAN concentrations. Also, inhibition of TNO_2 can play a more important role.

The effect of increasing influent concentration is first illustrated at an influent concentration of 200 mgTAN-N L^{-1} (Figure 5.5.5). At low aeration the increase of influent concentration gives similar results as above, although the bulk oxygen concentration were the nitrogen gas production is optimal had to be increased from 0.8 mgO_2 L^{-1} to 1.3 mgO_2 L^{-1}. At bulk oxygen concentrations above 1.3 mgO_2 L^{-1} the course of the nitrogen concentrations is however somewhat different. Anammox activity decreases rapidly because of increasing TNO_2 concentrations and consequentially increasing inhibition. As such control of the OLAND/CANON reactor at higher TAN influent concentrations will be more difficult. Increasing the oxygen concentration of the reactor to much will lead to a fast decrease of Anammox activity.

Also breakthrough of nitrate in the reactor is observed at higher dissolved oxygen concentrations (above 3.5 mgO_2 L^{-1}, results not shown). The rapid decrease of Anammox activity indicates that at higher influent concentrations a more stringent control will be necessary to have an optimal nitrogen removal, especially if the influent is highly dynamic. As a consequence, working with a small excess of TAN is advised.

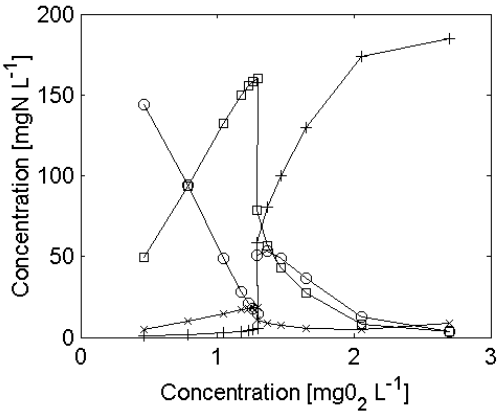

Figure 5.5.5. Concentration of TNO_2 (+), TAN (o) and N_2 (□) in function of bulk oxygen concentration at an influent concentration of 200 mgTAN-N L^{-1}.

Further increasing the influent concentration to 400 mgTAN-N L^{-1} leads to a completely different picture (Figure 5.5.6). No point exists anymore where nitrogen removal in the form of nitrogen gas is optimal. At an oxygen concentration of 1.4 mgO_2 L^{-1} TNO_2 breaks through and Anammox activity rapidly decreases. TAN is not limiting at the point of "maximum" Anammox activity, indicating the need for an excess of TAN as used in several studies (e.g. Sliekers *et al.*, 2003). From Figure 5.5.6 it can be seen that next to oxygen transfer limitation, the TNO_2 inhibition further limits the use of the OLAND/CANON process to treat higher TAN loads.

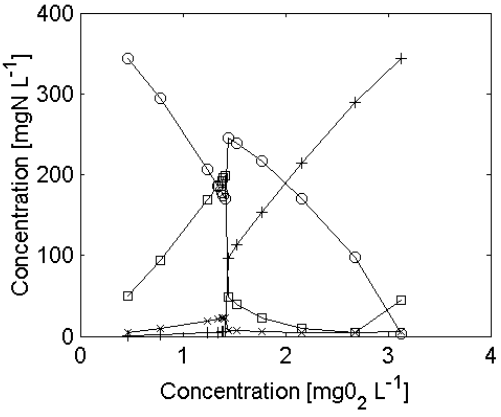

Figure 5.5.6. Concentration of TNO_2 (+), TAN (o) and N_2 (□) in function of bulk oxygen concentration at an influent concentration of 400 mgTAN-N L^{-1}.

3.3 Influence of hydrodynamic conditions on an Anammox and an OLAND/CANON system

To investigate the influence of the liquid boundary layer simulations were performed with boundary layer thicknesses of 50, 100, 200 and 400 µm, as 50-400 µm is the typical boundary layer thickness range (Horn and Hempel, 1998). The reactor was operated at a HRT of 0.25 days and an influent concentration of 100 mgTAN-N L^{-1}. Again the aeration coefficient (K_La) was set equal to 125 d^{-1}.

The increasing boundary layer thickness had only little influence on the behaviour of the biofilm system. In all cases about 85 mgN_2-N L^{-1} dinitrogen gas and 10 $mgNO_3^-$-N L^{-1} nitrate is formed. Only small amounts of TAN and TNO_2 were still present.

However, an increasing boundary layer thickness has an important influence on the performance of the OLAND/CANON system, in contrast to the Anammox biofilm system. Conversion of nitrogen dropped from 85 mgN L^{-1} to 50 mgN L^{-1} when the boundary layer thickness was increased from 50 µm to 400 µm (Figure 5.5.7). Bulk TAN concentration increased

simultaneously from 3 to 46 mgTAN-N L^{-1}. Also, the optimal oxygen concentration in the bulk phase increased from 1.0 to 3.5 mgO_2 L^{-1}.

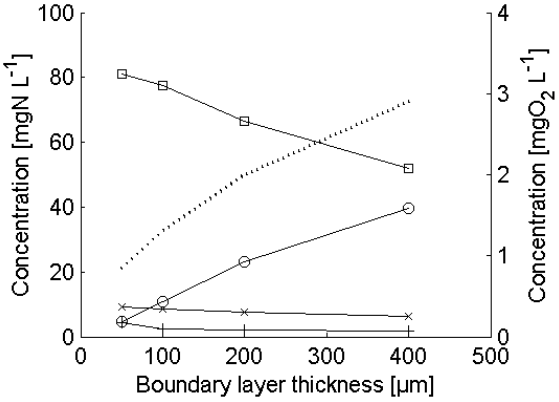

Figure 5.5.7. Bulk concentration profiles of TNO_2 (+), NO_3^- (x), TAN (o) and N_2 (□) and oxygen (--) as function of the boundary layer thickness.

From this Figure 5.5.7 it can be concluded that oxygen transfer resistance to the biofilm is a major factor influencing operation of the OLAND/CANON system. This should be taken into consideration in the design stage of such a process. Increasing the bulk oxygen concentration by increasing the aeration intensity to the reactor or biofilm surface area can of course circumvent the problem at hand, but this solution has a significant cost associated with it and might lead to other problems such as inhibition of TNO_2 as discussed above. Also, since both hydrodynamic conditions and reactor loading may vary considerably during dynamic operation, careful control of the aeration intensity will be necessary in order to obtain high nitrogen removal efficiency in a OLAND/CANON system. Control of an OLAND/CANON biofilm system can therefore be a difficult task and might complicate the application of the process. Obviously, this will be a point of future research.

3.4 Competition of heterotrophs and Anammox

To examine the competition between Anammox and heterotrophs in an OLAND/CANON system simulations were also performed with a reactor operated at a HRT of 0.25 days. The influent TAN concentration was set to 100 mgTAN-N L^{-1}. The influent S_S concentration was varied between 50 and 1000 mgCOD L^{-1} as is typical for this kind of biofilm reactors (Metcalf and Eddy, 1991). The aeration coefficient was adjusted according to the load to obtain full nitrogen and COD removal.

The influence of influent COD concentration on the competition between Anammox and heterotrophs in an Anammox system was not examined as it was assumed that most COD would have been consumed in the preceding partial nitrification reactor.

Addition of COD to the influent of an OLAND/CANON reactor increases nitrogen removal in comparison to a reactor with only Anammox organisms, since nitrate is denitrified to nitrogen gas by persisting heterotrophs. In Table 5.5.2 the steady state bulk concentrations of an OLAND/CANON system with 100 mgTAN-N L^{-1} and 100 mgCOD L^{-1} in the influent are compared with the effluent concentrations of an OLAND/CANON system with only 100 mgTAN-N L^{-1} in the influent (as also described in Table 5.5.1). The only difference between the two reactors is the aeration coefficient, since this coefficient is adjusted according to the load to the reactor. It can be seen that the nitrate concentration is reduced from 9 to 1 mgNO_3-N L^{-1}. No stripping process for the nitrogen gas is incorporated in the model. Hence, all the nitrogen gas is recovered in the bulk phase.

Table 5.5.2. Steady state bulk concentrations in mg L^{-1} of an OLAND/CANON system with a TAN influent concentration of 100 mgTAN-N L^{-1}, with (right) and without (left) the addition of 100 mgCOD L^{-1} to the influent.

	OLAND/CANON reactor without COD	OLAND/CANON reactor with COD
TNO_2	3.5	6
NO_3^-	9	1
TAN	5.5	6
N_2	82	87
O_2	0.8	1

Next to the improved nitrogen removal, a certain competition exists between Anammox and heterotrophs, especially if the COD load to the reactor increases. Nitrogen gas production by heterotrophs becomes higher if higher COD loads are applied. This is depicted in Figure 5.5.8, where the percentage of nitrogen gas produced by Anammox and heterotrophs in the reactor is depicted as function of the influent COD concentration. This percentage is higher than the one predicted by Hao and van Loosdrecht (2004). Also the ratio of Anammox to heterotrophs in the biofilm decreases with higher COD loads as depicted in Figure 5.5.9. The difference with the results of Hao and van Loosdrecht (2004) can be explained by the difference in biofilm density. In this contribution it is assumed that all biomass has the same density (40 kgCOD m^{-3}), while in Hao and van Loosdrecht (2004) it is assumed that autotrophes have a much larger density (70 kgCOD m^{-3}) than heterotrophs (25 kgCOD m^{-3}). Biofilm density is dependent of different factors

such as type of substrate (Villasenor *et al.*, 2000). Hence, both assumptions can be valid, and will depend on the conditions of the reactor under study.

Similar to Hao and van Loosdrecht (2004) an increasing COD load leads to an increasing required bulk DO concentration as can be seen in Figure 5.5.10.

These simulations thus link the findings of Hao and van Loosdrecht (2004), i.e. COD in the influent enhances nitrogen removal, and the findings of van de Graaf *et al.* (1996) and Thamdrup and Dalsgaard (2002), i.e. addition or presence of COD leads to less Anammox activity. It can be seen that the contribution of Anammox towards nitrogen removal lowers if COD is in the influent.

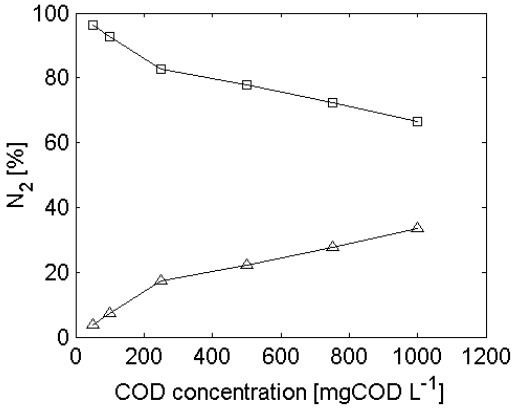

Figure 5.5.8. Percentage of nitrogen gas produced by Anammox (□) and heterotrophs (Δ) as function of influent COD concentration.

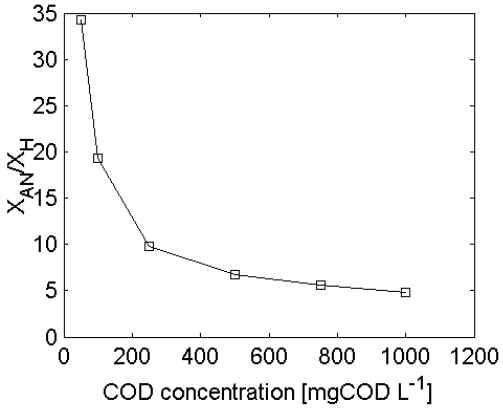

Figure 5.5.9. Ratio of Anammox to heterotrophs as function of influent COD concentration.

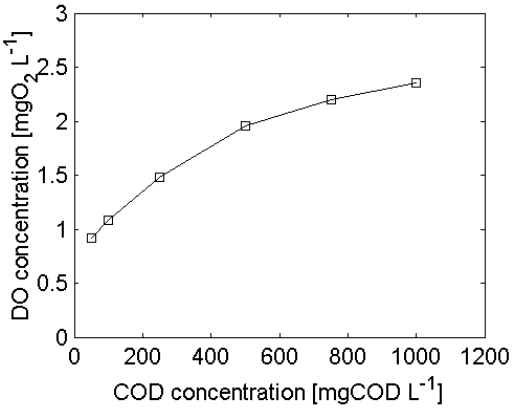

Figure 5.5.10. Bulk DO concentration as function of the influent COD concentration.

3.5 Influence of dynamic conditions of an OLAND/CANON system

The performance of an OLAND/CANON system was also investigated under dynamic conditions. Simulations were performed with a reactor operated at a HRT of 0.25 days. The influent TAN concentration was set to 100 mgTAN-N L^{-1}. The influent S_S concentration was varied between 50 and 1000 mgCOD L^{-1}. Square wave variations with a twice a day frequency were added to this influent flow rate and concentrations after the system had reached steady state operation. An initial steady state was necessary before applying dynamic conditions because directly applying dynamic conditions resulted in loss of all Anammox activity, indicating the need for a smooth start-up procedure.

Performance of the Anammox organisms in an OLAND/CANON biofilm system drops drastically when dynamic loading conditions are applied. In Figure 5.5.11 the increase or decrease in nitrogen species concentrations is depicted when applying a 5, 10 and 20 % blockwise variation with a frequency of twice a day at an average 500 mgCOD L^{-1} and 100 mgTAN-N L^{-1} influent concentration. The results are the average over a 1 year simulation period with dynamic conditions. It can be seen that because of the dynamic conditions nitrogen gas production decreases by up to 15% when applying a 20% variation on the influent conditions. Evidently lower variations will lead to a lower decrease in reactor performance. Too high variations will lead to process failure.

A first factor playing a role in the decrease of Anammox activity in the biofilm is the increased and varying TNO_2 concentration that inhibits the Anammox process. Second, the penetration of oxygen into the biofilm will also fluctuate, and this can also negatively affect the Anammox activity as Anammox is inhibited by oxygen (Strous et al., 1999b).

The decrease or increase in effluent nitrogen concentrations is very similar for lower influent COD concentrations (results not shown). However, with decreasing COD concentration less decrease in Anammox activity occurs. This indicates that at higher COD concentrations more competition for the different electron donors and acceptors occurs.

A similar decrease in activity can be expected in an Anammox reactor that is part of a 2-reactor system, although the partial nitrification step can already act as buffer, reducing the dynamics. Proper control of the first partial nitrification step (Volcke *et al.*, 2003) can further decrease dynamics and increase the Anammox reactor performance.

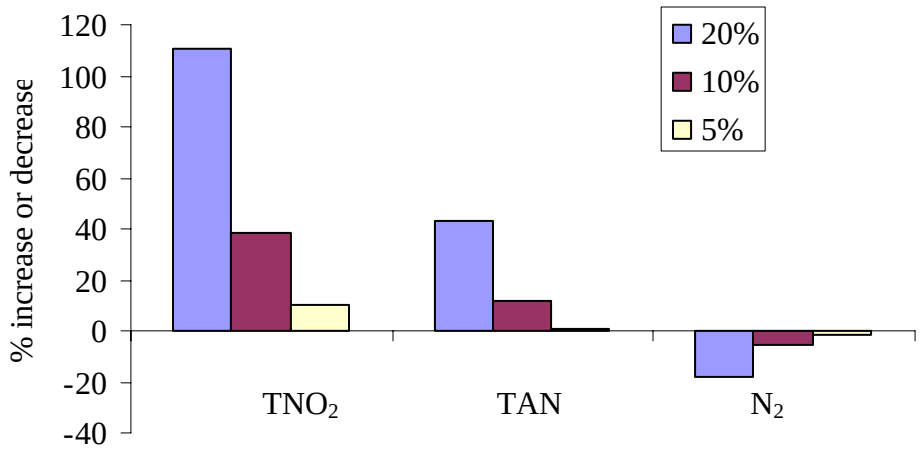

Figure 5.5.11. The increase or decrease in effluent concentration when applying 5, 10 and 20 % variation at 500 mgCOD $L^{-1}$and 100 mgTAN-N L^{-1} average influent concentration.

4 CONCLUSION

This study revealed that the most important factors affecting Anammox activity in a biofilm are the transfer of oxygen to the reactor and the biofilm, the influent TAN and COD concentration, TNO_2 inhibition and influent dynamics. Up to a certain influent concentration an optimal bulk oxygen concentration exists where the nitrogen removal is maximized. At higher influent concentrations inhibition of TNO_2 becomes important and makes it necessary to operate with a TAN excess.

This study showed that the performance of an Anammox biofilm system is only little affected by changing hydrodynamic conditions. At steady state, almost all incoming TAN and TNO_2 can be converted to dinitrogen gas. About 10% nitrate is formed. Hence, the combination of a partial nitritation and an Anammox reactor does not suffer from mass transfer limitations. The OLAND/CANON biofilm system on the other hand is very much affected by a change in hydrodynamic conditions. In the case of a small boundary layer (50 µm) 85% of the incoming TAN was converted to dinitrogen gas at steady state. When the boundary layer thickness was increased stepwisely to 400 µm, the TAN conversion dropped to 50%, indicating the importance of oxygen mass transfer resistance. This resistance is an important limitation to the performance of an OLAND/CANON system and a consideration in the design of these novel nitrogen removal processes. Increasing the aeration intensity could circumvent this decrease in nitrogen removal and careful control of the aeration of the OLAND/CANON system will therefore be essential.

Competition between Anammox and heterotrophs will be important when relatively high COD loads are applied. The amount of Anammox present decreases with COD load.

Dynamic influent conditions also lead to lower Anammox activity because of inhibition by TNO_2 and oxygen. Competition of Anammox with heterotrophs and other autotrophs for electron donors and acceptors will also lead to a lower Anammox activity. In a 2-reactor system the first (partial nitrification) reactor can act as a buffer for the Anammox reactor. Hence, the effect of dynamic conditions will be less detrimental in a 2-reactor configuration.

PART 6

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DISCUSSION AND CONCLUSIONS

Chapter 6

General discussion and conclusions

1 TOWARDS FULL-SCALE APPLICATIONS

Autotrophic nitrogen removal offers a useful alternative for treating highly loaded nitrogen streams with an unfavourable carbon to nitrogen (C/N) ratio. This autotrophic nitrogen removal consists of a partial nitritation step that produces a suitable influent for the second step, i.e. the Anammox process (chapter 2).

The production of this Anammox-suited effluent can be based on several principles. For the SHARON process (chapters 4.1, 4.2 and 4.3) the optimal total ammonium nitrogen:total nitrite nitrogen (TAN:TNO_2) ratio is obtained by two mechanisms. First the hydraulic and sludge residence times are reduced in such a way that nitrite oxidizers are washed out while ammonium oxidizers remain in the system. Second, in the absence of pH control the amount of total inorganic carbon (TIC) determines the amount of TAN that can be oxidized to TNO_2, although the TAN influent concentration plays a role in this as well (chapter 4.1). If the TIC:TAN ratio is 1:1 then normally a TAN:TNO_2 ratio of 1:1 will be produced in the SHARON reactor. This is a suited influent for the Anammox reactor.

In an oxygen-limited membrane bioreactor (chapter 5.1) the Anammox suited-effluent is obtained by reducing the oxygen concentration below 0.1 $mgO_2\ L^{-1}$. The nitrite oxidizers will not be active under these conditions as these organisms have a lower affinity for oxygen than the ammonium oxidizers. Moreover, by controlling the oxygen input to the reactor the amount of TAN oxidized to TNO_2 can be controlled.

Both processes (partial nitritation and Anammox) can also be engineered in a single reactor process. Both a completely mixed and a biofilm set-up have been shown to work (chapters 5.4 and 5.5).

All these systems have already been studied extensively on lab-scale and pilot-scale, both in this thesis and by other researchers and research groups. The real challenge for the future therefore is to move a step further and run the process on full-scale size. For the partial nitritation process this does not seem such as problem as for the Anammox process. So far, only two full-scale Anammox reactors have been constructed, both in The Netherlands. One of them was started up more than 2 years ago and is now at about 30% of its full removal capacity and activity is increasing daily (van Loosdrecht and Mulder, personal communication). The challenge for the future will be to have this and other full-scale Anammox reactors working in a stable and robust

way. Especially policy makers and water boards will have to be convinced that it is worth to wait for this process. Otherwise this fascinating process which is so promising on lab-scale will never be used world wide on full-scale.
The aim of this thesis was to help this upgrade by gaining more insight into the process with the help of mathematical models and dedicated lab-scale experiments. Unfortunately, no modelling exercise with full-scale data could be performed due to a lack of data of a stable full-scale system. In this final chapter the main results are discussed and future perspectives are given.

2 MODEL BUILDING AND DEVELOPMENT

All modelling, simulations, parameter estimation and sensitivity analysis was performed in the WEST® software (Vanhooren *et al*., 2003). This software allows the easy implementation of both new process models and reactor configurations (chapter 3.1). The biological model (chapter 3.2) for the autotrophic nitrogen removal process and other competing processes that was developed and used in this thesis was based on previous models (Hao *et al*., 2002a&b; Hellinga *et al*., 1999; Henze at al., 2000; Liebig *et al*., 2001 and Volcke *et al*., 2002a). The main features of the model are:

- Ammonia (NH_3) and nitrous acid (HNO_2) are considered to be the actual substrates for ammonium and nitrite oxidizers. Only the uncharged component is assumed to inhibit the process (Anthonisen, 1976)
- For the Anammox organisms TAN and TNO_2 are considered as to be actual substrates and TNO_2 inhibits the process at higher concentrations
- The death-regeneration concept is preferred over the endogenous respiration concept to describe decay (Henze *et al*., 2000)

Suzuki *et al*. (1974) and Anthonisen *et al*. (1976) proposed that NH_3 rather than NH_4^+ and HNO_2 rather than NO_2^- are the actual substrates for ammonium and nitrite oxidation. A long debate followed but at the end this conclusion has a consensus (Wood, 1986), although some researchers still disagree (Groeneweg *et al*., 1994). In this work (chapter 4.2) it was shown that indeed only the uncharged form of the nitrogen species acts as substrate or inhibitor for ammonium oxidizers active in the lab-scale SHARON reactor discussed in chapter 4.1. This assumption also means that temperature and pH are parameters that always have to be known or calculated when dealing with a nitrifying system because the temperature and pH will determine the equilibrium between the charged and uncharged component.
For Anammox the actual substrate is not yet determined and this is an interesting topic for future research. This could help in finding even more optimal conditions in terms of temperature and pH. Difficulties are however to be expected as the affinity constants of Anammox are very small

(in the order of 0 to 0.5 mgN L^{-1}) in contrast to the affinity constants of the partial nitritation step. This makes them difficult to estimate. Hence estimated values that differ at different temperatures and pH could easily be caused by experimental errors.

With respect to the determination of the Anammox parameters it is also essential that more experimental knowledge is gained towards the magnitude and form of the nitrite inhibition. In chapters 5.4 and 5.5 a Haldane kinetics is used and an inhibition constant was chosen based on qualitative discussions with Anammox research groups (in the TU Delft, the KU Nijmegen and Ghent University). In the future, dedicated experiments should be conducted to elucidate this inhibition. This could be based for example on the nitrogen gas production as proposed by Dapena *et al*. (2004a) and/or by injecting nitrite into a reactor as proposed in chapter 5.4. With this nitrite inhibition function more reliable model-based predictions of Anammox behaviour can be performed and process operation can become more robust.

In contrast to other simulation studies dealing with Anammox (Koch *et al*., 2000; Hao *et al*., 2002a&b) the death-regeneration concept as described in ASM1 (Henze *et al.*, 1987) is used. This ASM1 death-regeneration concept is defined as the decay of biomass followed by growth (of heterotrophs) on secondary substrate arising from decay (van Loosdrecht and Henze, 1999). The preference follows from the fact that Anammox biomass behaviour under substrate limiting conditions is not completely clear yet and from the observation that heterotrophs were shown to be active in some reactors (chapter 5.2). Only the application of this death-regeneration concept can explain this activity if no COD substrate is present in the influent of the reactor. As a result of this heterotrophic activity the observed TNO_2:TAN uptake ratio will be lower than theoretically predicted as nitrate will be reduced to TNO_2 by heterotrophs and used by Anammox organisms as an electron acceptor (van Dongen *et al.*, 2001a). As different other components influence the TNO_2:TAN uptake ratio, this hypothesis should be tested under defined lab conditions.

The decay concept of nitrifiers and Anammox, and especially the modelling of it, is definitely a topic for future research. As a start the ASM3 endogenous respiration approach defined as respiration with oxygen or nitrate using cell internal components (van Loosdrecht and Henze, 1999) can be further developed. First, it can be questioned whether endogenous respiration is also possible on unusual electron acceptors, in casu nitrate or TNO_2 for autotrophic ammonium and nitrite oxidizers and nitrate and oxygen for Anammox. It seems for example contradictory that Anammox organisms that are completely inhibited by oxygen would endogenously respire on oxygen. Of course the modelled endogenous respiration is more than the endogenous respiration sensu stricto, as for example oxygen consumption of protozoa can be considered (van Loosdrecht and Henze, 1999). Still, it seems better to go with the death-regeneration concept if no suitable electron acceptor is present. More concrete, this means that only heterotrophs would be able to use oxygen, nitrite and nitrate to respire endogenously. This anoxic endogenous respiration is

however smaller than its aerobic counterpart (Koike and Hattori, 1975) and a reduction factor should be applied. The aerobic autotrophs, ammonium and nitrite oxidizers, would not be able to use nitrite and nitrate as electron acceptor for endogenous respiration. Hence, endogenous respiration would only be possible under aerobic conditions. Hatziconstantinou and Andreakis (2002) and Siegrist *et al.* (1999) showed that anoxic incubation of nitrifying biomass results in a slower decay than in aerobic conditions. Anammox organisms would only be able to endogenously respire on TNO_2 (van de Graaf *et al.*, 1997). It remains an open question whether these assumptions are true and further research for this is necessary.

Another point for future research is the maintenance process in Anammox organisms. This maintenance is defined as the direct consumption of cell external or internal substrates for maintenance of the cell integrity (van Loosdrecht and Henze, 1999). Maintenance together with possible endogenous respiration of Anammox on TNO_2 may explain the varying TNO_2:TAN consumption ratio's of Anammox at varying TNO_2 concentrations as observed by Pynaert *et al*. (2003) and Strous (2000). These researchers performed batch experiments with Anammox. In these experiments TNO_2 and TAN were added in equimolar amounts and the consumption of the nitrogen species was monitored. It was observed that at higher initial TNO_2 concentration the TNO_2:TAN consumption ratio was also higher. For initial nitrogen concentrations over 100 mgN L^{-1} the TNO_2:TAN consumption ratio even increased above 1.32 (Pynaert *et al.*, 2003), which is the stoichiometric consumption ratio for Anammox growth (Strous *et al.*, 1998). Thus at higher TNO_2 levels, when Anammox is (partially) inhibited, the organisms consume more TNO_2 than stoichiometrically needed for growth. This extra TNO_2 consumption can possibly be linked to maintenance and endogenous respiration of Anammox on TNO_2.

The model presented in chapter 3.2 needs to be calibrated before use. Several examples of the calibration of the biological parameters are presented in subsequent chapters. These calibration studies are all done on lab-scale experimental set-ups where hydraulic and settling characteristics are fairly simple and easy to model. The reactors are assumed to be completely mixed and the settling behaviour of the biomass is assumed to be ideal.

Hence, the focus in these studies is on the biological part of the model. In case the Anammox process will be operated on full-scale, more comprehensive calibration will be necessary. For now, however, no clear calibration protocol exists for autotrophic nitrogen removal processes as these processes are fairly different from the classic municipal WWTP treating streams with nitrogen concentrations up to 50 mgN L^{-1}. Finding a general protocol will actually be very difficult as not all nitrifying sludge has the same behaviour. In some cases inhibition by ammonia and/or nitrous acid is noticed while in other cases no inhibition occurs. In the future, the Optimal Experimental Design (OED) methodology can help to minimize the experimental work for autotrophic nitrogen removal calibration. This OED methodology gives the mathematical basis

for the more accurate determination of the Anammox kinetic constants and in particular the TNO_2 inhibition constant. With the help of sensitivity functions and the Fisher Information Matrix (FIM), that summarizes the information content of an experiment (Dochain and Vanrolleghem, 2001), optimal experimental conditions can be found that generate information rich data concerning the Anammox parameters.

As an example of how experimental design can be used, a sensitivity analysis for an OLAND/CANON biofilm was conducted (chapter 5.4). This revealed that most information can be gained by measuring TNO_2 and TAN, but also that the sensitivities tend to increase towards the inside of the biofilm. Measuring nitrate and dinitrogen gas yields almost no information. By injecting nitrite into a continuously operated reactor considerably more information concerning the nitrite inhibition constant could be extracted as virtually no information concerning this constant is present during "normal" operation. Although the injection of nitrite is experimentally challenging, as this could affect the stability of the reactor, experiments such as the proposed one should be conducted for further information gathering concerning the kinetic constants.

Perhaps in the future even automated procedures as proposed by De Pauw and Vanrolleghem (2004) can be used for further parameter determination. With such an automated procedure all steps of model calibration are performed automatically. These steps include the finding of an optimal, information rich, experiment based on preliminary experiments, performing the experiment in practice and recalibrating the model (De Pauw and Vanrolleghem, 2004). The steps are repeated as long as the desired accuracy is not attained. It would be interesting to build such an automatic set-up next to a full-scale Anammox reactor that can provide enough active sludge.

3 EXPERIMENTAL STUDY ON AUTOTROPHIC NITROGEN REMOVAL

In chapter 4.1 the start-up and operation of a lab-scale SHARON reactor is discussed. Special attention was given to this start-up because inoculum sludge of a system treating synthetic wastewater (mimicking domestic wastewater) was used. For this sludge a fast start-up strategy directly applying the steady state SHARON reactor conditions in terms of hydraulic residence time and influent concentration did not succeed. Similar attempts with sludge originating from the SHARON reactor in Sluisjesdijk (The Netherlands) also failed. A slow start-up strategy did succeed and demonstrated the importance of a dedicated start-up procedure for the success of the process even at lab-scale. Especially towards full-scale applications attention will have to be paid towards start-up of the process. On the other hand it was demonstrated that a SHARON reactor can be started up with normal WWTP sludge. This facilitates the application of the process in places in the world where no actively nitrifying culture is present.

Some practical issues were also discussed. One of these concerns is the low sludge concentration in a continuously aerated lab-scale SHARON reactor. This makes the reactor vulnerable to

disturbances and reduces the robustness of the process. A second consideration worth mentioning here is the water evaporation due to aeration at high temperatures, amounting up to 20 % if the air is not humidified, a problem also noticed by Fux *et al.* (2002). As such this is not a problem because the TNO_2:TAN ratio will remain the same and after all this is the main purpose of the SHARON reactor. Data interpretation and optimisation based on these data can however become erroneous if these data are not corrected for evaporation. Further, evaporation can lead to an increased sludge age and hence alter the competition between ammonium and nitrite oxidizers. Another effect influencing this competition is the influent TAN concentration to the reactor. By reducing the influent concentration from 1000 mgTAN-N L^{-1} to 500 mgTAN-N L^{-1} the production of nitrate was found to increase exponentially. Both reduced nitrous acid and ammonia inhibition allowed the nitrite oxidizers to grow somewhat faster and accumulate in the reactor, causing this increase. Anyway, the SHARON reactor did no longer produce an Anammox-suited effluent. The persistence of these nitrite oxidizers in the SHARON reactor endanger the long-term stability and operation of the autotrophic nitrogen removal process and is a topic for future research.

Different influent TAN concentrations (500, 1000, 2000 mgTAN-N L^{-1}) and different TIC:TAN ratio's (3:2, 1:1, 1:2) were applied to see the effect of this on the produced TNO_2:TAN ratio. As expected theoretically a higher TIC:TAN ratio yielded a higher TNO_2:TAN ratio. Changing the influent concentration and keeping the same TIC:TAN ratio resulted in a slightly different effluent TNO_2:TAN ratio. The reason for this is a combination of nitrous acid inhibition, salt effects and CO_2 transfer variations. Because of these dependencies and the fact that variations in influent TAN concentrations and TIC:TAN ratio's will occur in practice, it will be necessary to apply control to the SHARON reactor. Which control strategy should be implemented, what variables should be measured and what variables should be manipulated is a subject for other (Volcke *et al*., 2003) and further research.

With respect to measuring the key components in the SHARON and Anammox reactor (TAN and TNO_2) a titrimetric measuring technique was evaluated in chapter 4.3. For the typical concentrations applied in the SHARON reactor (350-750 mgN L^{-1}) measurements performed with the titrimetric technique were in close agreement with the measurements performed with a colorimetric technique (Dr Lange GmbH, Germany). The same precision was obtained and with 1 low-cost analysis both components could be measured. For the Anammox reactor only TAN could be determined accurately with the technique, while TNO_2 could not be determined as its concentrations were too low. On the one hand this titrimetric measurement thus offers an inexpensive, easy to automate technique that requires no or little dilution for measuring the TAN and TNO_2 concentration in the SHARON reactor and TAN in the Anammox reactor. On the other hand the technique will need to be combined with other measuring techniques for measuring

TNO_2 in the Anammox reactor and nitrate in both reactors, since nitrate cannot be measured with this titrimetric technique.

Finally, in the experimental part the hypothesis that ammonia is the actual substrate and nitrous acid is the actual inhibitor of ammonium oxidation was verified in batch tests using sludge from the SHARON reactor (chapter 4.2). From these tests the affinity constants and inhibition constants of the SHARON biomass could be determined. The direct influence of temperature and pH on the maximum growth rate were determined. From the experiments it became clear that a narrow temperature and pH window exists where conditions are optimal for the SHARON biomass. Again the need for appropriate control becomes evident.

Testing such control strategies on lab-scale and full-scale systems should be the next step in experimental work. One of these strategies can be the partial bypassing of part of the influent of the SHARON reactor to the Anammox reactor. This way fast and flexible control of the combined SHARON-Anammox process can be achieved. By putting part of the influent directly to the Anammox reactor TNO_2 peaks can be dealt with efficiently. However some (possible) disadvantages and efficiencies of this bypass SHARON can be mentioned. First, only part of the buffering capacity of the TIC in the influent will be used in the SHARON reactor as part of the influent is bypassed. This means that extra pH correction in the SHARON reactor will be necessary. Van Kempen *et al*. (2001) reported however that TAN conversion of over 90 % is possible without pH correction, if the TAN concentration is below 0.6 gTAN-N L^{-1}.

On the other hand the pH in Anammox reactor could increase because of the TIC that is still present. Also sulfides originating from the (dissimilatory) sulphate reduction in the anaerobic digestor (Colleran *et al*., 1995) could negatively influence the performance of this bypass process. With a classic SHARON-Anammox system these sulfides are oxidized to sulphate in the SHARON-reactor. When applying this bypass part of the sulfide will go directly to the Anammox reactor without preceding oxidation. This could inhibit the Anammox process (Mulder *et al*., 1995). This inhibition is however pH dependent as the uncharged form, H_2S, is mainly responsible for it (Koster *et al*., 1986). Also, sulphide can be oxidized anoxically to sulphate, for example by *Thiobacillus denitrificans* (Sublette *et al*., 1998; van de Graaf *et al*., 1996). The conditions in the Anammox reactor seem to favour the proliferation of these chemo-autolithotrophs because of the lack of organic carbon source, the high temperatures (Chazal and Lens, 2000) and the nitrate produced by the Anammox organisms. Hence, the effect of sulphide inhibition is mainly determined by the sulphide load to the Anammox reactor and the nitrate available for sulphide oxidation. If necessary the sulphides can be removed by chemical precipitation.

Another control strategy that can be tested on lab-scale or on full-scale is the strategy proposed by Volcke *et al.* (2003) that was further developed since (Volcke, personal communication). In

this control strategy the effluent TAN:TNO_2 ratio necessary for successful operation of the Anammox reactor is maintained at a value (typically 1-1.32) by adjusting the reactor oxygen concentration. As discussed in chapter 4.3 the titrimetric measurement of TAN and TNO_2 can be used for this control strategy. Model-based evaluation of this strategy gave excellent results. Controlling the SHARON reactor in such a way doesn't only increase the conversion efficiency of the Anammox reactor, but is especially useful to avoid toxic TNO_2 concentrations, that inhibit the Anammox conversion (Volcke *et al.*, 2003).
Developing and implementing other (advanced) control strategies for the optimal operation of the combined SHARON-Anammox process or other autotrophic nitrogen removing processes is definitely a topic for further research.

4 SIMULATION AND OPTIMIZATION OF AUTOTROPHIC NITROGEN REMOVAL

The model for the autotrophic nitrogen removal processes was compared against lab-scale data in chapters 5.1 and 5.2. In chapter 5.1 the start-up and operational behaviour of a partial nitrifying membrane assisted bioreactor was simulated. By decreasing and fine-tuning the aeration intensity an Anammox-optimal influent composition could be obtained under oxygen-limited conditions.
Such an oxygen-limited membrane assisted bioreactor can be used as an alternative to the SHARON reactor studied in chapter 4.1. As biomass retention is complete by using a membrane, these reactors can be operated at an HRT (up to 6 hours) far lower the ones possible in the SHARON reactor and still produce an Anammox-suited effluent. In a SHARON reactor wash-out would occur at such low HRT as the dilution rate would be higher than the growth rate of the ammonium oxidizers in the SHARON chemostat. An important space limitation can thus be accomplished when applying membrane reactors. The governing factor for competition between ammonium and nitrite oxidizers in a membrane reactor is difference in oxygen affinity of both organisms. The governing factor for competition between ammonium and nitrite oxidizers in a SHARON chemostat is the difference in growth rate between both organisms. The governing factor in a membrane reactor is less influenced by temperature than the governing factor in a SHARON chemostat. Hence, application of an oxygen limited membrane reactor at lower temperatures (< 20°C) will be easier from an engineering viewpoint (Wyffels, 2004). However, with a membrane reactor more complex operation and problems such as membrane fouling are to be expected. Further as all the biomass is retained in the membrane reactor no continuous inoculation of biomass in the Anammox reactor will occur. This inflow of biomass can be beneficial for Anammox activity as the ammonium oxidizers can consume residual oxygen and provide "sparking" intermediates as such as NH_2OH discussed by Pynaert *et al.* (2004). Also, in oxygen limited reactors the airflow cannot be used anymore for CO_2 stripping control, for

example to limit the TNO_2 formation when streams with an excess buffer capacity are treated (Volcke *et al.*, 2004).

The influence on the composition of this influent by different operational strategies such as changing the HRT, SRT and temperature was investigated. This scenario analysis for example showed that it is possible to even obtain an Anammox-suited effluent at a temperature of 20°C. This opens the possibility to use autotrophic nitrogen removal systems in colder conditions as it was also shown in chapter 5.3 that start-up of an Anammox reactor is also possible at 20°C. Start-up time of the system will however be significant, because of the even lower growth rate of the Anammox organisms at lower temperatures.

In chapter 5.2 data from start-up and operation of a lab-scale Anammox SBR reactor were compared with model results. Quantitative data, i.e. TAN, TNO_2 and nitrate concentrations were in good agreement with model simulations. The simulations predicted a gradual increase of Anammox organisms and this was confirmed by qualitative data. The sludge colour changed over the experimental period from brownish to reddish, a typical colour of Anammox biomass. Also an increasingly positive signal with an Anammox specific FISH probe (AMX820) was observed. FISH analysis also showed that the Anammox population was of the type *Kuenenia stuttgartiensis* (hybridized with KST1273). Again, sludge from a municipal WWTP was used as inoculum, indicating the possibility to start up an autotrophic nitrogen removal system from municipal WWTP sludge.

Based on these findings and additional literature data an estimation of the minimal start-up time for an Anammox reactor as function of temperature, HRT, initial Anammox biomass concentration and reactor separator efficiency was performed (chapter 5.3). Based on these calculations different design decisions concerning HRT, temperature, … can be taken. It should be stressed however that the calculated start-up time is the very minimum time required for start-up as different disturbances and inhibitions will further increase the necessary time.

For further scenario analysis with full-scale Anammox reactors the hydraulic reactor model developed by Maertens (2003) can be used, although several experiments such as tracer tests will first be necessary to calibrate this model. In the future the full-scale Anammox reactor constructed at Sluisjesdijk (The Netherlands) can be used as test case, once the reactor will be started-up.

Finally, in chapter 5.5 the interaction of Anammox and competing processes was investigated in a biofilm reactor. Key factors affecting this competition are the oxygen transfer to the reactor and the biofilm, the influent TAN and COD concentration, TNO_2 inhibition and influent dynamics. Application of autotrophic nitrogen removal in a single biofilm reactor will be limited by the amount of oxygen that can be transferred to the biofilm. As such these systems will have to be operated at nitrite limiting conditions when higher influent nitrogen concentrations are applied.

Systems with two reactors (partial nitrification reactor and Anammox reactor) suffer less from this transfer limitation and therefore seem the better option for treating more concentrated streams. From the simulations it also became clear that the presence of COD is beneficial for the removal of TAN due to the simultaneous denitrification. Dynamic influent conditions also lead to lower Anammox activity because of inhibition by TNO_2 and oxygen. In a 2-reactor system the first (partial nitrification) reactor can act as a buffer for the Anammox reactor. Hence, the effect of dynamic conditions will be less detrimental in a 2-reactor configuration.

Results from the simulations with the biofilm reactor need to be tested in the future with experimental data to ensure the validity of the conclusions. No such data exist up to now.

5 CONCLUSIONS

The following conclusions can be made drawn from this work

- Start-up of autotrophic nitrogen removal processes is possible from “normal” WWTP sludge, although this start-up is slow and requires careful operation.
- The effluent of the SHARON reactor, which will be fed to the Anammox reactor, is not only dependent on the TAN:TIC influent ratio, but also on the TAN influent concentration. Hence control is necessary to obtain an Anammox suited effluent and to prevent nitrate build-up
- Ammonia and nitrous acid are the actual substrate and/or inhibitor of the nitritation process. Hence, temperature and pH are key parameters for the process as these parameters determine the concentrations of NH_3 and HNO_2.
- A titrimetric set-up can be used for the cost-effective determination of TAN and TNO_2 in the SHARON reactor and TAN in the Anammox reactor.
- Start-up of a partial nitritation reactor and an Anammox reactor can be described with the help of a mathematical model. With this model different scenarios were simulated to see the effect of changing operational conditions.
- Autotrophic nitrogen removal can start-up and operate at temperatures below 25°C
- The lack of knowledge on the TNO_2 inhibition constant limits further model-based evaluation of the Anammox process. Research efforts should be conducted towards the determination of this constant
- When treating higher loaded streams, a 2-reactor system is better controllable and easier to operate than a one-reactor system.

6 PERSPECTIVES

The following suggestions for further research can be formulated:

- The mathematical model for the autotrophic nitrogen removal process should be tested with full-scale data once this data is available. Next to the biological model, also a hydraulic model must then be developed to describe the flow in the full-scale installation.
- Further research on Anammox growth and decay kinetics is necessary. First it can be investigated whether the uncharged form is also the actual substrate/inhibitor for the Anammox biomass. Second the magnitude and form of the TNO_2 inhibition should be determined. Finally it should be investigated which concept is most appropriate to model decay of Anammox organisms.
- The development and use of model-based optimal experimental design is strongly advised for the study of Anammox kinetics.
- The effect of different dynamic and operational conditions, such as the operation of the SHARON reactor at different pH and oxygen set-points, needs to be further investigated both experimentally and with the aid of a mathematical model. As such better insight in the process can be obtained and this will help with the development of control strategies.
- Control strategies for the autotrophic nitrogen removal process in general and the SHARON process in particular should be further developed and tested on lab-scale reactors and full-scale installations.
- The successful start-up of a full-scale Anammox reactor is essential for further use, distribution and research of autotrophic nitrogen removal processes. As such research towards scale-up of the process should be conducted.

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List of abbreviations

ASM	Activated sludge model
ASM1	Activated sludge model number 1
ASM2	Activated sludge model number 2
ASM2d	Activated sludge model number 2d
ASM2dN	Activated sludge model number 2d extended with the hydrolysis of organic nitrogen module of the ASM1 model
ASM3	Activated sludge model number 3
ASM1.e	Extended activated sludge model number 1
b_{AN}	Decay rate of X_{NO} [d^{-1}]
b_H	Endogenous decay coefficient of heterotrophs [d^{-1}]
b_{NH}	Decay rate of X_{NH} [d^{-1}]
b_{NO}	Decay rate of X_{NO} [d^{-1}]
BOD	Biological oxygen demand [$mgO_2\ L^{-1}$]
C	Molar concentration [$mol\ L^{-1}$]
C/N	Initial COD to nitrogen ratio [$mgCOD\ mgN^{-1}$]
COD	Chemical oxygen demand [$mgCOD\ L^{-1}$]
CO_2	Dissolved carbon dioxide [$mgCO_2\ L^{-1}$]
DO	Dissolved oxygen concentration [$mgO_2\ L^{-1}$]
FIM	Fisher Information Matrix
f_p	Inert fraction of biomass [$mgCOD\ mgCOD^{-1}$]
H^+	Hydrogen [mole]
HRT	Hydraulic residence time [d]
i_{NBM}	Nitrogen content of biomass [$mgN\ mgCOD^{-1}$]
i_{NXI}	Nitrogen content of the inert fraction of biomass [$mgN\ mgCOD^{-1}$]
k_h	Maximum specific hydrolysis rate
$K_{HNO2,NO}$	Saturation constant for nitrous acid of nitrite oxidizers [$mgHNO_2\text{-}N\ L^{-1}$]
$K_{I,TNO2,AN}$	Inhibition constant for total nitrite nitrogen of of Anammox [$mgTNO_2\text{-}N\ L^{-1}$]
K_La	Volumetric mass transfer coefficient [d^{-1}]
$K_{NH3,NH}$	Saturation constant for ammonia of ammonium oxidizers [$mgNH_3\text{-}N\ L^{-1}$]
$K_{NO3,H}$	Saturation constant for nitrate of heterotrophs [$mgNO_3^-\text{-}N\ L^{-1}$]

$K_{O,AN}$	Inhibition for oxygen of Anammox [$mgO_2\ L^{-1}$]
$K_{O,H}$	Saturation constant for oxygen of heterotrophs [$mgO_2\ L^{-1}$]
$K_{O,NH}$	Saturation constant for oxygen of ammonium oxidizers [$mgO_2\ L^{-1}$]
$K_{O,NO}$	Saturation constant for oxygen of nitrite oxidizers [$mgO_2\ L^{-1}$]
$K_{S,H}$	Saturation constant for readily biodegradable substrate of heterotrophs [$mgCOD\ L^{-1}$]
$K_{TAN,AN}$	Saturation constant for total ammonium nitrogen of Anammox [$mgTAN\text{-}N\ L^{-1}$]
$K_{TNO2,AN}$	Saturation constant for total nitrite nitrogen of of Anammox [$mgTNO_2\text{-}N\ L^{-1}$]
$K_{TNO2,H}$	Saturation constant for S_{TNO2} of heterotrophs [$mgTNO_2\text{-}N\ L^{-1}$]
K_X	Saturation constant for slowly biodegradable substrate [$mgCOD\ L^{-1}$]
MBR	Mebrane bio-reactor
MLSS	Mixed liquor suspended solids [$mgSS\ L^{-1}$]
MLVSS	Mixed liquor volatile suspended solids [$mgSS\ L^{-1}$]
N	Nitrogen
N_2	Dinitrogen gas
NH_4^+	Ammonium
NH_3	Ammonia
NO_2^-	Nitrite
NO_3^-	Nitrate
OED	Optimal Experimental Design
OUR	Oxygen uptake rate [$mg\ O_2\ L^{-1}\ d^{-1}$]
pH	Negative logarithm of proton concentration
pK_A	Negative logarithm of dissociation constant
P	Phosphorous
PO_4^{3-}	Phosphate
SBR	Sequencing batch reactor
S	Concentration expressed in $mg\ L^{-1}$
SRT	Sludge residence time [d]
S_S	Soluble readily degradable COD [$mgCOD\ L^{-1}$]
S_{N2}	Nitrogen gas concentration [$mgN_2\text{-}N\ L^{-1}$]
S_{NH3}	Ammonia nitrogen concentration [$mgNH_3\text{-}N\ L^{-1}$]
S_{HNO2}	Nitrous acid nitrogen concentration [$mgHNO_2\text{-}N\ L^{-1}$]
S_{NO2}	Nitrate nitrogen concentration [$mgTNO_2\text{-}N\ L^{-1}$]
S_{O2}	Oxygen concentration [$mgO_2\ L^{-1}$]
S_{TAN}	Total ammonium nitrogen concentration [$mgTAN\text{-}N\ L^{-1}$]
S_{TNO2}	Total nitrite nitrogen concentration [$mgTNO_2\text{-}N\ L^{-1}$]
T	Temperature [°C]

t	Time [d]
TAN	Total ammonium nitrogen [mgTAN-N L^{-1}]
TNO_2	Total nitrite nitrogen [mgTNO_2-N L^{-1}]
WWTP	Wastewater treatment plant
X_{AN}	Anammox organisms [mgCOD L^{-1}]
X_I	Inert particulate COD [mgCOD L^{-1}]
X_H	Heterotrophic biomass [mgCOD L^{-1}]
X_{NH}	Ammonium oxidizers [mgCOD L^{-1}]
X_{NO}	Nitrite oxidizers [mgCOD L^{-1}]
X_S	Slowly degradable particulate COD [mgCOD L^{-1}]
$Y_{H,O}$	Heterotrophic yield on oxygen [mgCOD $mgCOD^{-1}$]
$Y_{H,NO3}$	Heterotrophic yield on nitrate [mgCOD mgN^{-1}]
$Y_{H,TNO2}$	Heterotrophic yield on total nitrite nitrogen [mgCOD mgN^{-1}]
$Y_{NH,O}$	Autotrophic yield of ammonium oxidizers [mgCOD mgN^{-1}]
$Y_{NO,O}$	Autotrophic yield of nitrite oxidizers [mgCOD mgN^{-1}]
Y_{AN}	Autotrophic yield of Anammox [mgCOD mgN^{-1}]

Greek symbols

α	Correction factor for the oxygen transfer in wastewater
η_{NO3}	Anoxic reduction factor for nitrate
η_{TNO2}	Anoxic reduction factor for total nitrite nitrogen
μ^{max}_{AN}	Maximum growth rate of Anammox [d^{-1}]
μ^{max}_{H}	Maximum growth rate of heterotrophs [d^{-1}]
μ^{max}_{NH}	Maximum growth rate of ammonium oxidizers [d^{-1}]
μ^{max}_{NO}	Maximum growth rate of nitrite oxidizers [d^{-1}]

Samenvatting

Voor de behandeling van ammoniumrijke stromen, zoals het rejectiewater van de anaërobe vergisting, kan het ANaerobe AMMonium OXidatie (Anammox)-proces gecombineerd met een partieel nitrificatieproces zoals het SHARON-proces een duurzaam alternatief bieden voor het conventionele nitrificatie-denitrificatieproces. Voor de verdere optimalisatie van dit zogenaamde autotrofe stikstofverwijderingsproces kunnen wiskundige modellen zeer waardevol zijn en het doel van dit onderzoek is dan ook een dergelijk model op te stellen. Het onderzoek werd hiervoor opgesplitst in 6 delen.

Na een kort inleidend eerste gedeelte wordt in een tweede deel (hoofdstuk 2) de state-of-the-art kennis betreffende het autotroof stikstofverwijderingsproces samengevat in een literatuurstudie. De invloed van verschillende factoren zoals temperatuur, pH en opgeloste zuurstof op de activiteit van nitrificeerders en Anammox-organismen werd besproken en vervolgens werd een overzicht gegeven van de verschillende reactorconfiguraties die reeds geëvalueerd werden.

In een derde deel (hoofdstukken 3.1 en 3.2) werd een model opgesteld voor het autotroof stikstofverwijderingssysteem en geïmplementeerd in de modelleer- en simulatieomgeving WEST® (Hemmis NV, België). Het model was gebaseerd op het ASM1-model en heeft als belangrijkste aandachtspunt dat de neutrale componenten ammoniak (NH_3) en salpeterig zuur (HNO_2) en niet de geladen componenten ammonium (NH_4^+) en nitriet (NO_2^-) worden beschouwd als substraat en inhibitor voor ammonium- en nitrietoxideerders. Deze aanpak werd in de experimentele studie (hoofdstuk 4.2) bevestigd.

In een vierde, experimenteel, deel werd eerst in hoofdstuk 4.1 de opstart en bedrijfsvoering besproken van een SHARON-reactor met het oog op de koppeling ervan met een Anammox-reactor. Enkele praktische bedenkingen werden ook behandeld. Eén daarvan was de verdamping van water in de reactor die kan oplopen tot 20% van het influentdebiet en kan zorgen voor een slibleeftijd die hoger is dan verwacht uit een standaard chemostat berekening. Als inoculum voor de SHARON reactor werd slib gebruikt komende van een SBR-systeem dat synthetisch huishoudelijk afvalwater behandelt. Dit slib was niet aangepast aan de condities in de SHARON-reactor. Een snelle opstartprocedure waarbij het slib direct onderworpen werd aan de “normale” operationele condities van de SHARON-reactor, zoals een totale ammonium (TAN) influentconcentratie van 1000 mgTAN-N L^{-1}, een temperatuur van 35°C en een hydraulische verblijftijd van 1 dag, resulteerde in de uitspoeling van de ammoniumoxideerders en bijgevolg falen van de reactor. Een trage adaptatie van het nitrificerend slib (in semi-SBR mode) aan de condities karakteristiek voor het SHARON-proces, bleek dan ook de beste methode voor de opstart. Een stabiele werking werd bekomen na 30 dagen.

Na de succesvolle opstart van de reactor werd de invloed van verschillende TAN influentconcentraties en verschillende totale anorganische koolstof:totale ammonium (TIC:TAN)-verhoudingen in het influent nagegaan. Zoals theoretisch verwacht resulteerde een hogere influent TIC:TAN verhouding in een hogere totale nitriet:totale ammonium (TNO_2:TAN)-verhouding in het effluent van de SHARON-reactor. Deze nitriet:ammonium verhouding is zeer belangrijk aangezien het Anammox proces een 1:1 verhouding nodig heeft voor een goede werking.

In hoofdstuk 4.2 werd de eerder vermelde aanname dat ammoniak (NH_3) het echte substraat en salpeterig zuur (HNO_2) de inhibitor is voor ammoniumoxideerders bevestigd aan de hand van batchexperimenten met slib uit de SHARON-reactor. Verder lieten batchexperimenten toe om de parameters die de kinetiek van de ammoniumoxidatie beschrijven, zoals de ammoniakaffiniteitsconstante, te bepalen. Tevens werd de rechtstreekse invloed van temperatuur en pH op de groeisnelheid van ammoniumoxideerders bepaald uit batch-testen.

Het experimentele deel werd afgesloten met hoofdstuk 4.3 waar een titrimetrische methode voor de simultane bepaling van TNO_2 en TAN ontwikkeld werd. Voor typische stikstofconcentraties in de SHARON-reactor (350-750 mgN L^{-1}) kwamen de metingen met deze titrimetrische methode goed overeen met metingen met een colorimetrische methode (Dr Lange GmbH, Duitsland). Bovendien hadden beide methodes een gelijkaardige nauwkeurigheid. Voor typische stikstofconcentraties in de Anammox-reactor (0-100 mgN L^{-1}) was er alleen voor de TAN-concentratie een goede overeenkomst tussen beide methodes, terwijl de TNO_2 concentratie niet met de gewenste nauwkeurigheid gemeten kon worden gezien de te lage concentraties.

In een vijfde deel, dat toegespitst was op de toepassing van het model, werd eerst in hoofdstuk 5.1 de opstart en operationele data van een OLAND membraan-bioreactor vergeleken met modelsimulaties. Een goede overeenkomst werd bekomen tussen de gemeten en gesimuleerde TAN-, TNO_2-, nitraat-, O_2- en particulaire COD-concentraties. Vervolgens werd de invloed van temperatuur, hydraulische verblijftijd en slibverblijftijd op de werking van de reactor nagegaan aan de hand van simulaties. Deze studie toonde onder andere aan dat onder zuurstofgelimiteerde omstandigheden een Anammox-geschikt effluent bekomen kan worden door het fijnregelen van de beluchting, zelfs bij lage temperaturen (vb. 20°C).

In hoofdstuk 5.2 werd de modelgebaseerde interpretatie van opstart en operationele data van een Anammox reactor besproken. Voor deze studie werd slib van een volle-schaal huishoudelijke afvalwaterzuiveringsinstallatie gebruikt als inoculum. Kwantitatieve data, i.e. TAN-, TNO_2- en nitraatconcentraties kwamen goed overeen met modelsimulaties. De simulaties voorspelden een geleidelijke toename van de Anammox organismen en dit werd bevestigd door kwalitatieve data. De kleur van het slib veranderde immers gedurende de experimentele periode van bruinachtig naar rood, de typische kleur van Anammox en er werd een steeds toenemend positief signaal van

de Anammox-specifieke FISH probe (AMX820) waargenomen. Verder werd aan de hand van FISH aangetoond dat de Anammox-organismen van het type "*Kuenenia stuttgartiensis*" waren.
In hoofdstuk 5.3 werd een schatting gemaakt van de tijd nodig voor het opstarten van een Anammox-reactor als functie van de influentconcentratie, temperatuur, hydraulische en slibverblijftijd, rekening houdend met het feit dat Anammoxinhibitie niet werd beschouwd.
De beperkte kennis van de kinetische parameters van het Anammox-proces wordt gezien als één van de belangrijkste knelpunten voor verdere simulatiestudies. Daarom werd in hoofdstuk 5.4 aan de hand van sensitiviteitsanalyse en optimaal experimenteel ontwerp een basis gelegd voor de verdere experimentele bepaling van deze parameters. Uit de studie bleek dat van de stikstofcomponenten alleen de meting van TNO_2 en TAN informatie verschaft over de parameters, terwijl de meting van nitraat en stikstofgas geen informatie gaf. Bovendien kon bij normale bedrijfsvoering van de reactor geen informatie verkregen worden over de TNO_2 inhibitieconstante. Daarom werd een experiment hierover om meer informatie te verkrijgen voorgesteld dat bestond uit het injecteren van een TNO_2 oplossing in de reactor zodat de bulk concentratie oploopt tot 30 mgTNO_2-N L^{-1}.
In hoofdstuk 5.5 werd de interactie tussen Anammox- en concurrerende processen zoals heterotrofe groei nagegaan in een biofilm reactor. De belangrijkste factoren die de Anammox-activiteit bepalen, bleken de zuurstofoverdracht naar de biofilm, de influent-TAN- en COD-concentratie, de TNO_2-inhibitie en een dynamisch influent te zijn. Uit de simulaties bleek dat toepassing van autotrofe stikstoverwijdering in 1 biofilmreactor gelimiteerd werd door de zuurstofoverdracht naar de biofilm. Systemen met 2 reactoren (1 reactor voor partiële nitrificatie en 1 reactor voor Anammox) hebben minder last van deze zuurstofoverdrachtlimitatie. Deze laatste lijken dan ook de betere optie voor de behandeling van zwaarder beladen stromen. Uit de simulaties werd het ook duidelijk dat de aanwezigheid van COD bevorderlijk is voor de verwijdering van TAN gezien het optreden van simultane denitrificatie. Tenslotte bleek dat dynamische influentcondities zorgen voor een lagere Anammox-activiteit door de inhibitie van TNO_2 en zuurstof. In een 2-reactorsysteem echter kan de eerste reactor als buffer dienen voor de Anammox-reactor en dus het effect van een dynamisch influent afzwakken.
In een zesde en laatste deel werden de belangrijkste besluiten van het werk algemeen besproken en perspectieven voor verder onderzoek geformuleerd. Een eerste interessant onderwerp voor toekomstig onderzoek is het verder nagaan van de Anammox groei- en afsterfkinetiek. In het bijzonder moet de grootte en vorm van de TNO_2-inhibitie worden bepaald. Ook zou moeten onderzocht worden welk concept (sterfte of onderhoud) het meest geschikte is om de reductie in biomassa te modelleren. Een tweede onderwerp voor verder onderzoek is het gebruik van het wiskundig model dat autotrofe stikstofverwijdering beschrijft voor simulatie en controle van volle-schaal installaties en het evalueren van het nut en de voordelen van aparte behandeling van slibrejectiewater voor de afvalwaterzuivering.

Summary

For the treatment of ammonia-rich streams, such as reject water of an anaerobic sludge digester, the combination of the ANaerobic AMMonium OXidation (Anammox) process with a process for partial nitrification such as the SHARON or OLAND process can offer a more sustainable alternative to a conventional nitrification-denitrification process. Mathematical models can help to optimize this so-called autotrophic nitrogen removal process. The goal of this research was therefore to develop such a model and with this aim in mind the research was divided into six parts.

After a short introduction part, the second part (chapter 2) summarizes the state-of-the-art knowledge concerning autotrophic nitrogen removal into a literature review. The influence of different factors such as temperature, pH and dissolved oxygen on the activity of nitrifying and Anammox organisms was discussed and an overview is presented of the different reactor configurations that have already been evaluated.

In a third part (chapters 3.1 and 3.2) a model was constructed for the autotrophic nitrogen removal process and implemented in the modelling and simulation environment WEST® (Hemmis NV, Kortrijk). The model was based on the ASM1 model and has as key feature that the uncharged components ammonia (NH_3) and nitrous acid (HNO_2) and not the charged components ammonium (NH_4^+) and nitrite (NO_2^-) are considered as the actual substrate and inhibitor of ammonium and nitrite oxidizers. This approach was confirmed in the experimental study in this thesis (chapter 4.2).

The fourth, experimental, part was started with chapter 4.1. In this chapter the start-up and operation of a lab-scale SHARON reactor in view of its coupling with an Anammox reactor is discussed. Also, several practical experiences with the SHARON reactor were presented. One concerns the evaporation of water from a lab-scale reactor. This evaporation can amount to 20% of the influent flow rate and in this way leads to a higher sludge age than expected from standard chemostat analysis. As inoculum for the SHARON reactor sludge from an SBR system treating synthetic domestic wastewater was used. This sludge was not adapted to the conditions prevailing in the SHARON reactor. A fast start-up procedure directly imposing the “normal” operating conditions of the SHARON reactor, such as an influent total ammonium concentration of 1000 mgTAN-N L^{-1}, a temperature of 35°C and a hydraulic and sludge residence time of 1 day, resulted in the wash-out of the ammonium oxidizers and thus failure of the process. A slow start-up procedure (in semi-SBR mode) allowing the nitrifying sludge to slowly adapt to the conditions prevailing in the SHARON reactor turned out to be the best method to start-up. Stable operation was achieved after 30 days.

After the successful start-up of the reactor, the influence of different TAN influent concentrations and different influent total inorganic carbon:total ammonium nitrogen (TIC:TAN) ratio's was tested. As theoretically predicted a higher TIC:TAN ratio in the influent resulted in a higher TNO_2:TAN ratio in the effluent of the SHARON reactor. This TNO_2:TAN ratio is very important as the Anammox process requires an almost 1:1 TNO_2:TAN ratio for proper operation.

In chapter 4.2 the assumption that ammonia (NH_3) is the actual substrate and nitrous acid (HNO_2) is the actual inhibitor for ammonium oxidation was confirmed by batch tests with sludge originating from the SHARON reactor. With these batch experiments it was furthermore possible to determine the kinetic parameters of ammonium oxidation, such as the ammonia affinity constant, as well as the direct influence of temperature and pH on the growth rate of ammonium oxidizers.

The experimental work was concluded with chapter 4.3, in which a robust titrimetric method for simultaneous determination of TNO_2 and TAN was developed. For nitrogen concentrations typical for the SHARON reactor (350-750 mgN L^{-1}) results from this titrimetric method and a colorimetric method (Dr Lange GmbH, Germany) were in close agreement and had similar measurement accuracies. For typical nitrogen concentrations in the Anammox reactor (0-100 mgN L^{-1}) only the TAN concentrations agreed. The TNO_2 concentration could not be measured with the desired accuracy, as the concentrations were too low.

The fifth part focussed on model applications and started with chapter 5.1 in which the start-up and operational data of an OLAND membrane bioreactor were compared with model simulations. A good agreement was obtained between measured and simulated TAN, TNO_2, nitrate, O_2 and particulate COD concentrations. With the calibrated model the influence of temperature, hydraulic and sludge residence time on the operation of the reactor was examined. The simulation study showed that under oxygen-limited conditions an Anammox-suited effluent could be obtained by fine-tuning the aeration, even at lower temperatures (e.g. 20°C).

In chapter 5.2 the model-based interpretation of start-up and operation of an Anammox reactor is discussed. For this study sludge from a full-scale domestic wastewater treatment plant was used as inoculum. Quantitative data, i.e. TAN, TNO_2 and nitrate concentrations were in good agreement with model simulations. The simulations predicted a gradual increase of Anammox organisms and this was confirmed by qualitative data. The sludge colour changed over the experimental period from brownish to reddish, a typical colour of Anammox biomass. Also an increasingly positive signal with an Anammox specific FISH probe (AMX820) was observed. Further FISH analysis showed that the Anammox organisms were of the type "Kuenenia stuttgartiensis".

In chapter 5.3 an estimate was made of the time necessary for start-up of an Anammox reactor as function of the influent concentration, temperature, hydraulic and sludge residence time, bearing in mind that no Anammox inhibition was considered.

The limited knowledge of the kinetic parameters of the Anammox process is seen as one of the most important bottlenecks for further model simulation studies. Therefore in chapter 5.4 a basis for the further experimental determination of the parameters was laid out based on sensitivity analysis and optimal experimental design. From this study it became clear that among the nitrogen species only the measurement of TNO_2 and TAN yields information on the parameters, while no information can be gained by measuring nitrate and nitrogen gas. Further, at normal operation of the reactor no information can be obtained concerning the TNO_2 inhibition constant and hence a dedicated experiment to gain more information was proposed. This experiment consisted of injecting a TNO_2 solution such that the mixed liquor concentration reaches 30 mgTNO_2-N L^{-1}.

In chapter 5.5 the interaction between Anammox and competing processes such as heterotrophic growth is studied in a biofilm reactor. The most important factors determining the Anammox activity in a biofilm are the oxygen transfer to the biofilm, the influent TAN and COD concentration, TNO_2 inhibition and influent dynamics. Application of autotrophic nitrogen removal in a single biofilm reactor was found to be limited by the amount of oxygen that can be transferred to the biofilm. Systems with two reactors (partial nitrification reactor and Anammox reactor) suffer less from this transfer limitation and therefore seem the better option for treating more concentrated streams. From the simulations it also became clear that the presence of COD is beneficial for the removal of TAN due to the occurrence of simultaneous denitrification. Dynamic influent conditions also lead to lower Anammox activity because of inhibition by TNO_2 and oxygen. In a 2-reactor system the first (partial nitrification) reactor can act as a buffer for the Anammox reactor. Hence, the effect of dynamic conditions will be less detrimental in a 2-reactor configuration.

In a sixth and final part the main conclusions of the work were generally discussed and perspectives for further work were formulated. A first interesting topic for future research is the further investigation of the Anammox growth and decay kinetics. Especially the magnitude and form of the TNO_2 inhibition should be determined. Also, it should be investigated which concept, decay or maintenance, is most appropriate to model decay of Anammox organisms. A second main topic for future research is the use of the mathematical model describing autotrophic nitrogen removal for simulation and control of full-scale systems and evaluate what the benefits of dedicated sludge reject water treatment are for the overall plant.

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