ASSESSMENT OF
A PARTIAL NITRITATION/ANAMMOX SYSTEM
FOR NITROGEN REMOVAL

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Assessment of a partial nitritation/Anammox system for nitrogen removal

"Att skapande är att befria det som redan finns."

Henning Mankell
"Berättelse på tidens strand"
Summary
Nitrogen removal from wastewater has been introduced in Sweden and in many other countries mainly by the implementation of a technology based on biological nitrification and denitrification processes. One vital factor negatively affecting the wastewater treatment in the biological nitrification/denitrification step is the recirculation of a nitrogen-rich stream originating from dewatering of digested sludge (supernatant). Separate treatment of the supernatant is often proposed to decrease the nitrogen load into the main stream. However, such type of wastewater contains small amounts of biologically degradable carbon compounds and the addition of an external carbon supply is necessary to perform treatment in the traditional nitrification/denitrification processes.

In the 1990s, a cost-effective deammonification process was proposed to separately treat ammonium-rich streams. In the first step of the deammonification process, equal amounts of ammonium and nitrite nitrogen are produced in the partial nitritation route to perform in the second stage the ANaerobic AMMonium OXidation (Anammox®) process. The latter step involves simultaneous biochemical removal of ammonium and nitrite by Anammox bacteria under oxygen-limited conditions, and results in the production of dinitrogen gas. The deammonification system, which is still under development, can be designed to perform this process in either one or two reactors. This novel wastewater treatment technology enables considerable savings through reduced aeration costs and elimination of the necessity for an external carbon source.

In Sweden, a technical-scale pilot plant continuously supplied with the supernatant was constructed and operated at the Himmerfjärden WWTP, Grödinge. A focus was given to perform the deammonification in two steps in a moving-bed™ biofilm partial nitritation/Anammox system®. As biofilm carriers, Kaldnes rings were used.

In this study, the successful establishment of the partial nitritation process was shown. The efficient nitrogen removal in the Anammox reactor was obtained under the two-year period. The Anammox reactor capacity was extended and the pH correction was excluded. The performance data were collected and evaluated in accordance with the system approach by means of univariate and multivariate data analyses.

As a result of this assessment, the interplay of the factors affecting both steps of the system (such as pH value, dissolved oxygen (DO) concentration, temperature, conductivity, nitrite concentration) was recognised and a control system has been proposed. The control strategy for the system consisted of adjusting the relevant factors (DO concentration, drop of the pH value) to obtain the nitrite-to-ammonium ratio (NAR) around 1.3 in the effluent from the partial nitritation reactor (R1). The effective nitrogen removal in the Anammox reactor (R2) was dependent on the performance of the preceding step and monitoring of the nitrite nitrogen concentration in the reactor. The dissolved oxygen concentration and nitrite nitrogen concentration increase were recognised as system bottlenecks. The influence of the influent supernatant characteristics on the process performance was evaluated as well. The study demonstrated that both aerobic and anaerobic oxidation of ammonium occurred in the R1 and R2 reactors, respectively, and could be monitored by conductivity measurements. An Oxygen Uptake Rate (OUR) test methodology for the nitrifying biofilm cultures has been developed. OUR tests regarding the nitrifying activity of the bacteria in both steps of the system were performed and evaluated. Batch tests enabled to estimate the reaction rates.

Assessment of the partial nitritation/Anammox system gave recommendations for future full-scale implementation. An array of process options has been proposed. Case-specific technological improvements of a two-step partial nitritation/Anammox system have been presented. A possibility of Simultaneous Partial Nitritation/Anammox (SPNA) system has been suggested for future investigations.
Assessment of a partial nitritation/Anammox system for nitrogen removal

Sammanfattning

Avlägsnande av kväve från avloppsvatten har införts i Sverige och i många andra länder främst med hjälp av en teknologi som baseras på de biologiska processerna nitrifikation och denitrifikation. En viktig faktor som inverkar negativt på avloppsseringen i det biologiska stege är recirkulationen av kväve av flöden som kommer från avvattnningen av slam (rejektvatten). Separat behandling av ammoniumrika rejektvatten har föreslagits för att minska kvävemängden till huvudflödet. Traditionella biologiska kväveavskiljningsprocesser är utformade för att renna avloppsvatten med hög ammoniumhalt kan bli mycket dyra, särskilt om avloppsvattnet innehåller små mängder av biologiskt nedbrytbara kolföreningar så att tillförelse av en extern kolkälla är nödvändig.

Under 1990-talet påbörjades utveckling av en kostnadseffektiv process för separat rening av ammoniumrika flöden med deammonifikationsprocessen som alternativ till det traditionella nitrifikations- och denitrifikationssystemet. I det första steget av deammonifikationsprocessen produceras approximativt lika stora mängder av ammonium och nitritkväve i nitritationsprocessen för att sedan fortsätta i ett andra steg med Anammox®. Det sista steget medför samtidig biokemisk avskiljning av ammonium och nitrit med hjälp av Anammoxbakterier under anaeroba förhållanden och resulterar i produktion av kvävgas. Deammonifikationssystemet, som fortfarande är under utveckling, kan utformas i antingen en eller två reaktorer. I denna studie ligger fokus på att utföra deammonifikationen som en två-stegs process med systemet partiella nitrifikation/Anammox®.


Till följd av utvärderingen har studerats faktors interaktion som påverkar båge steg av systemet (t.ex. pH-värde, syrehalt (DO), temperatur, konduktivitet, nitritkvävehalt) och kontrollsystem har föreslagits. Systemstrategin bestod i justering av relevanta faktorer (syrehalt, minskning av pH-värde) för att erhålla en kvot mellan ammonium och nitrit (NAR) på drygt 1,3 i avloppet från den partiella nitrificationprocessen (R1). En effektiv kväveborttagning i Anammox reaktorn (R2) berodde på det partiella nitrificationstegets utförande och övervakning av nitritkvävekoncentration i Anammoxreaktorn. DO koncentration och nitritkvävehaltens ökning var identifierade som processflaskhalsar. Inverkan av inkommande rejektvattens egenskaper i processen utvärderades även. Undersökningar visade att både aerob och anaerob ammoniumkväveoxidation i R1 respektive R2 kan övervakas med hjälp av konduktivitetsmätningar. Testmetodik för Oxygen Uptake Rate (OUR) (syreupptagningshastighet) för nitrifikationsbakterier i biofilm utvecklades. OUR tester angående bakteriernas aktivitet i Anammox steget i bäge steg av systemet utfördes och utvärderades. Diskontinuerliga tester möjliggjorde beräkning av reaktionshastighet.

Utvärdering av det partiella nitrification-Anammox systemet gav underlag för anvisningar för attutföra ett system i full skala i framtiden. Processutformningar har föreslagits och även tekniska förbättringarna av ett partiellt nitrification-Anammox system. Möjligheter att etablera ett samtidigt utnyttjande i enbart ett steg av partiell nitrification och Anammox har föreslagits för fortsatta studier.
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**Table of Content**

Summary .......................................................................................................................... v
Sammanfattning .................................................................................................................. vii
Acknowledgements ........................................................................................................... ix

Appended papers .............................................................................................................. xiii

Abstract ......................................................................................................................... 1

1. Biological nutrient removal – a sustainable approach .................................................. 1

2. Objectives of the thesis .............................................................................................. 3

3. New concepts in nitrogen removal from wastewater .................................................. 3
   3.1. Background ............................................................................................................... 3
   3.2. Ammonium-rich streams ......................................................................................... 4
   3.3. Overview of processes with nitrogen removal ......................................................... 9
   3.4. Applications of the Anammox process ................................................................ 18
   3.5. Modelling of the systems with biological wastewater treatment ......................... 21

4. Methodology .................................................................................................................. 22
   4.1. Pilot plant description ............................................................................................ 22
   4.2. System configurations and operational approach ............................................... 23
   4.3. Measurements and analytical procedures ............................................................. 24
   4.4. Batch tests .............................................................................................................. 25
   4.5. Oxygen Uptake Rate (OUR) tests ........................................................................ 25
   4.6. Modelling of the process data with the SIMCA-P software ................................ 26

5. Results and discussions .............................................................................................. 27
   5.1. Bacterial identification and activity ........................................................................ 27
       5.1.1. FISH tests ........................................................................................................... 27
       5.1.2. Application of OUR tests ................................................................................ 28
   5.2. Factors affecting system efficiency ...................................................................... 29
       5.2.1. Supernatant characteristics ............................................................................. 30
       5.2.2. Partial nitritation process ................................................................................. 31
       5.2.3. Anammox process .......................................................................................... 33
       5.2.4. Reaction rates .................................................................................................. 35

6. Implications for full-scale implementation .................................................................. 36
   6.1. Proposal for system configurations ....................................................................... 36
   6.2. System technology with partial nitritation/Anammox ........................................... 40
   6.3. Overall recommendations ...................................................................................... 40

7. Final conclusions ......................................................................................................... 43

8. Further research work ............................................................................................... 44

9. References .................................................................................................................... 45
APPENDED PAPERS

This thesis is based on the following papers, which are appended at the end of this thesis and referred to by their Roman numerals in the thesis text:


Assessment of a partial nitritation/Anammox system for nitrogen removal

**Abbreviations**

Anammox – anaerobic ammonium oxidation  
ASL – ammonium surface load  
BAF – bench-scale upflow biological aerated filter  
CANON – completely autotrophic nitrogen removal over nitrite  
DO – dissolved oxygen  
FBR – fixed-bed reactor  
FISH – fluorescent in situ hybridisation  
HRT – hydraulic retention time  
MBBR – moving-bed™ biofilm reactor  
MVDA – multivariate data analysis  
NAR – nitrite-to-ammonium ratio  
OLAND – oxygen-limited autotrophic nitrification-denitrification  
OUR – oxygen uptake rate  
p. e. – population equivalent  
PCA – principal component analysis  
PLS – partial least squares projections to latent structures  
SBR – sequencing batch reactor  
SHARON – single reactor system for high ammonium removal over nitrite  
SPNA – simultaneous partial nitritation/Anammox  
SRT – sludge retention time  
SS – suspended solids  
USAB – upflow anaerobic sludge blanket  
VSS – volatile suspended solids  
WWTP – wastewater treatment plant

**Chemical notations**

ATU – allylthiourea  
COD – chemical oxygen demand  
HNO₂ – nitrous acid  
NaClO₃ – sodium chlorate  
NH₃ – free ammonia  
NH₄-N – ammonium nitrogen  
NO₂-N – nitrite nitrogen  
NO₃-N – nitrate nitrogen  
N₂O – nitrous oxide  
NO – nitric oxide  
NO₂ – nitric dioxide  
NOₓ = N₂O, NO & NO₂
ABSTRACT

This thesis evaluates the performance of a deammonification system designed as a two-step technology consisting of an initial partial nitritation followed by an Anammox process. Operation of a technical-scale pilot plant at the Himmerfjärden Wastewater Treatment Plant (Grödinge, Sweden) has been assessed. Oxygen Uptake Rate (OUR) to evaluate the respiration activity of nitrifiers in the system and batch tests to assess reaction rates have also been applied in the study. It was found that the total inorganic nitrogen elimination strongly depended on the nitrite-to-ammonium ratio in the influent to the Anammox reactor, which was correlated with the performance of the partial nitritation phase. Therefore, a control strategy for oxidation of ammonium to nitrite has been proposed. Controlled oxygen supply to the partial nitritation reactor is obligatory to obtain a proper pH drop indicating oxidation of ammonia to nitrite at the adequate ratio. A very high nitrogen removal efficiency (an average of 84%) and stable operation of the system have been reached. Conductivity measurements were also used to monitor the system influent nitrogen load and the nitrogen removal in the Anammox reactor. The data gathered from the operation of the pilot plant enabled the use of multivariate data analysis to model the process behaviour and the assessment of the covariances between the process parameters. The options for full-scale implementation of the Anammox systems have been proposed as a result of the study.

Key words: Biofilm; Deammonification; Nitrogen removal; Oxygen Uptake Rate (OUR); Partial nitritation/Anammox system

1. BIOLOGICAL NUTRIENT REMOVAL – A SUSTAINABLE APPROACH

Currently, an increasing awareness of the need for sustainable water management results in an effort to reduce the load of nutrients imposed on receiving water bodies. A variety of factors are nowadays taken into account in order to decide on proper wastewater treatment systems. Population growth and more stringent effluent standards are amongst factors that play a vital role in choosing the most appropriate options for wastewater handling. An emphasis has been put on reducing the expenditure for aeration and chemical additions.

The European Union Water Framework Directive 91/271/EEC imperatively states to “protect the environment from any adverse affects due to discharge of (untreated) urban and industrial waters”. In this perspective the development of new technologies for finding solutions in water management is of highest concern for both stakeholders and citizens. The requirements for discharges from urban wastewater treatment plants to sensitive areas, which are subjected to eutrophication, as drawn up in the Directive 91/271/EEC, recently gave rise to an amending Directive 98/15/EC in February 1998. The total nitrogen discharge limit for plants with more than 100,000 p.e. is equal to 10 mg l⁻¹ with 70-80 minimum percentage of reduction whereas for total phosphorous 1 mg l⁻¹ (80 percent of minimum reduction).

At the end of the twentieth century, biological nutrient removal became a standard wastewater treatment option. Gradually, the traditional method of using nitrification/denitrification route in nitrogen removal has encountered difficulties in coping with the more stringent effluent standards imposed on existing wastewater treatment plants (WWTP). The influent load often increases and contributes to employ an upgrading procedure, which now is a common solution to increase the capacity of a WWTP. In many cases, however, the upgrading of a plant requires space that is not available. Hybrid systems have been proposed to improve the activated sludge system (Gebara, 1999; Ochoa et al., 2002). Carriers for biofilm growth have been used to enhance the existing processes and increase the capacity without expansion of the reactor footprint (Öde-

For further improvements, one has to identify the bottlenecks that are part of the existing systems. In the traditional nitrification/denitrification process, the generated sludge is digested and centrifuged at a WWTP and an ammonium-rich side stream is produced (digester supernatant). The supernatant contains as much as 2 kg N m\(^{-3}\) (Strous et al., 1997). Typically, it is recirculated to the inflow of a WWTP and contributes to the increase of the influent nitrogen load by 15-20% in comparison with the total influent nitrogen load (Plaza et al., 1989, 1990; Jansen et al., 1993; Jönsson et al., 2000). Separate collection and treatment of supernatant from digested sludge is now a promising alternative. In Sweden, more than 10 wastewater treatment plants have a system of full-scale separate supernatant treatment, mainly with activated sludge SBR-technology and nitrification/denitrification processes. Studies by Tendaj-Xavier (1985) and Mossakowska (1994) performed at KTH/Stockholm Water are examples of research works concerning the biological treatment of supernatant.

With the discovery of the Anammox bacteria (Mulder et al., 1995), new feasibility studies concerning implementation of the Anammox process into the existing infrastructure have been evaluated. It was shown that if the main component of the digester supernatant – ammonium nitrogen – was partially oxidised to nitrite in a preceding step, the Anammox bacteria could use nitrite as an electron acceptor and anaerobically convert ammonium and nitrite to nitrogen gas (Jetten et al., 1997). Slikkers et al. (2004) proposed a combination of aerobic nitrifying bacteria and anaerobic Anammox bacteria to treat urea in one single reactor.

Separate collection of urine is of highest interest nowadays (Jetten et al., 1997; Maurer et al., 2003; Wilsenach et al., 2003; van Loosdrecht et al., 2004). There is a new branch of research that focuses on treating urine, as it is the main source of nutrients in municipal wastewater. If successful, such sustainable handling of wastewater will result in the reduction of nitrogen and phosphorous loads in WWTPs. The residual part of the nutrients would therefore be used up completely for the generation of sludge. In this most probable case, all the nitrogen would be released as supernatant after sludge digestion and its treatment would be the most significant part of the treatment at a WWTP. Such a shift in wastewater management would put much more emphasis on establishing a reliable system for biological treatment of sludge liquors. Moreover, application of the Anammox process will prove to be important in the future perspective as it can actually be applied for treatment of supernatant, urine and other ammonium-rich streams like leachates.

In the field of environmental technology, the concept of treating many types of side streams currently receives a lot of attention. There is further potential for the implementation of the Anammox process to treat separately collected urine (Maurer et al., 2003; Slikkers et al., 2004), landfill leachate (Hippen, 2001; Hippen et al. 2001; Nikolić and Hultman, 2003), poultry and piggy waste thin fractions (Dong and Tollner, 2003; Ahn et al., 2004), and many industrial side streams. Among industrial wastewater there are examples of treating slaughterhouse wastewater (Keller et al., 1997), pharmaceutical streams (Carrera et al., 2003), tannery wastewater (Banas et al., 1999; Carruci et al., 1999), streams from the food and beverage industry (Austermann-Haun et al., 1999) and potato processing industries like alcohol and starch production (Abeling and Seyfried, 1992). Despite considerable concentrations of organic matter, usually expressed as COD (Chemical Oxygen Demand), these streams need to be treated with the external supply of easy biodegradable organic carbon to sustain the denitrification process.

The prospect of implementing a research idea in a full scale requires adequate questions to be answered successfully. A biological process has to be developed to give reliability in practice. Interdependence between conditions for proper bacterial growth and low-cost treatment might be an obstacle in reaching the expected treatment expenditures’
reduction. However, a biological system depended on autotrophic reactions may lead to savings on addition of chemicals. Additionally, the biofilm moving-bed systems have the advantage of compactness and low excess sludge production. Moreover, system reactions need to be scrutinized for side effects in accordance with characteristics of supernatant to be treated.

2. OBJECTIVES OF THE THESIS

This licentiate work focuses on biological nitrogen removal with the use of a two-step partial nitritation/Anammox process. The objectives are:

- To perform a literature study concerning different system designs for the most cost-effective nitrogen removal from ammonium-rich wastewater.
- To evaluate a two-step partial nitritation/Anammox system with the aim of establishing stable partial oxidation of ammonium to nitrite in the first step and effective removal of nitrogen in the second step.
- To assess the influence of a variable characteristic of supernatant from dewatering of digested sludge, as an ammonium-rich stream, on the system performance.
- To assess the presence of a nitrifying activity in the system in both a quantitative and qualitative manner.
- To prepare recommendations for an integrated and efficient biological system for the treatment of nitrogen-rich streams.

3. NEW CONCEPTS IN NITROGEN REMOVAL FROM WASTEWATER

3.1. Background

It was almost three decades ago that Brodda (1977) predicted the existence of chemolithoautotrophic bacteria using only thermodynamic calculations. It was demonstrated that the biological uptake of ammonium as an inorganic electron donor is nearly as energetically favourable as the aerobic nitrification process. It was only recently that this reaction was proven in a laboratory (Mulder et al., 1995; Strous et al., 1997; Helmer et al., 1999, 2001; Jetten et al., 1999; Seyfried et al., 2001). The research group from the Kluyver Laboratory for Biotechnology at the Delft University of Technology, the Netherlands, discovered anaerobic ammonium oxidizers (Anammox bacteria) in a fluidised bed reactor (Mulder et al., 1995). More comprehensive research concerning the Anammox started around the 1990s and publications concerning the process and its technology were released. Initially, the nomenclature was a little ambiguous and in the Anammox-related publications the term 'deammonification' was used to describe the novel process of nitrogen removal. A proposal for a more sustainable wastewater treatment system was made (Jetten et al., 1997) and consisted of treating wastewater in two steps. A partial nitritation reactor was designed to pre-treat wastewater with the aim of producing a proper feed to the Anammox reactor. The application of the SHARON (Single reactor system for High Ammonium Removal Over Nitrite) reactor in which the reaction is stopped at partial oxidation of the ammonia to nitrite ('partial' SHARON) was suitable for supplying the Anammox reactor. The digester supernatant was chosen to be the stream most adequate for applying the combination of the SHARON and Anammox processes. The processes for the treatment of ammonium-rich wastewater were patented (Mulder, 1992; van Loosdrecht and Jetten, 1997, 2003; Dijkman and Strous, 1999). A consultant company Paques (Paques home page), which specialises in the development and manufacture of biological water purification systems, developed the Anammox process for commercial purposes. The SHARON® process has been patented by “Grontmij Water and Waste Management” (Heijnen and van Loosdrecht, 1997, 1999). It was also proven that the Anammox bacteria largely contribute (up to 70%) to nitrogen cycle in the World’s oceans (Thamdrup and Dalsgaard, 2002; Dalsgaard et al., 2003, 2005; Devol, 2003; Kuypers et al., 2003).
Skagerrak, which is part of the Danish belt seaway, it was shown that Anammox reaction has a large importance in the N₂ production. At greater depths, where the sediment mineralisation rates are lower, the importance of Anammox in removing the nitrogen in the sediments seems to be highest (Dalsgaard et al., 2005). The natural occurrence of Anammox bacteria was also proven in marine sediments of the Thames estuary (Trimmer et al., 2003), in Golfo Dulce in Costa Rica (Dalsgaard et al., 2003), in freshwater wetland in Africa (Jetten et al., 2003) as well as in arctic sediments (Rysgaard et al., 2004).

Strous et al. (1999) reported that Planctomycetes could perform the Anammox process. Currently, three genera of Anammox bacteria have been discovered: Brocadia, Kuenenia and Scalindua. Genera of Brocadia and Kuenenia occur naturally in ammonium-rich environments and have been found in wastewater treatment systems. Candidatus Brocadia anammoxidans (Mulder et al., 1995; Jetten et al., 2001) and Candidatus Kuenenia stuttgartiensis (Egli et al., 2001) were identified by the FISH (Fluorescent In-Situ Hybridisation) method. The biodiversity of Anammox bacteria was extended by the discovery of a genus Scalindua at a WWTP treating landfill leachate in Pitsea, UK (Schmid et al., 2003). Two species were found: Candidatus Scalindua brodae and Scalindua wagneri. The genus of Scalindua has been also detected in the marine ecosystems of the Black Sea and the Candidatus was named Scalindua sorokinii (Kuypers et al., 2003). A brown-reddish colour is typical for all Anammox bacteria probably due to its high cytochrome content (Jetten et al., 1999).

Environmental and possibilities of economical advantages of these discoveries are substantial, and therefore give rise to large expectations in the future usage of naturally occurring Anammox bacteria in wastewater treatment technology. The first full-scale Anammox reactor at the Dokhaven WWTP, Rotterdam, the Netherlands was started in 2002 (Abma et al., 2005). At Hattingen WWTP, Germany a full-scale deammonification pilot plant with the Kaldnes moving-bed process is in operation (Jardin et al., 2001; Cornelius and Rosenwinkel, 2002; Rosenwinkel and Cornelius, 2005). Furthermore, at the Strass WWTP, Austria the deammonification single sludge SBR system was implemented on full scale.

Publications within this area of research are mainly from Europe with the leading centres being in the Netherlands and Germany. In Sweden, the most important research groups are in Stockholm (wastewater technology) and in Gothenburg (marine microbiology). At the Royal Institute of Technology, Stockholm, at the Department of Land and Water Resources Engineering there is an extensive research concerning technological aspects of the combined partial nitritation/Anammox system for digester supernatant treatment. It was initiated by SYVAB AB and PURAC AB in 2000. An overview of the research and commercial groups with a focus on the branch of research concerning the Anammox process is shown in Table 1.

### 3.2. Ammonium-rich streams

The data gathered in Table 2 shows the general characteristics of different ammonium-rich streams. It is mainly supernatant and landfill leachate that have been studied by different researchers. These streams differ from each other in the concentration of organic matter (expressed as COD). It is characteristic for supernatant to have a higher temperature compared to the raw wastewater at the inflow to a WWTP (Glixelli, 2003). The supernatant is a product of dewatering of the sludge that was earlier stabilised by the process of methane fermentation. Such sludge is usually characterised by a high percentage of mineral substances – products of fermentation. It is periodically disposed of the digestion chamber and dewatered in centrifuges or filter presses. The handling of supernatant causes a common problem in large wastewater treatment plants where anaerobic digestion of sludge is used. High concentrations of NH₄-N from the supernatant added at the inflow to the WWTP overload the biological nitrogen removal process. Despite the fact that the volumetric supernatant flow is 3-5% of the influent wastewater flow, the ammonium content in such a stream may be as high as 15-20% of
Table 1. Overview of the research and development of the Anammox process.

<table>
<thead>
<tr>
<th>Country/centre</th>
<th>Main topics</th>
<th>Examples of references</th>
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<tr>
<td>the Netherlands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delft University of Technology</td>
<td>Microbiology, application of the Anammox process, full-scale and pilot-plant</td>
<td>van Loosdrecht and Jetten (1998); Kuenen and Jetten (2001); Schmidt et al. (2003);</td>
</tr>
<tr>
<td></td>
<td>experiments; physiology of the Anammox bacteria, marine microbiology, biomarkers for</td>
<td>Siekers et al. (2003); Strous et al. (2002); Strous and Jetten (2004); IcoN project</td>
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<tr>
<td></td>
<td>detection of Anammox bacteria; the IcoN (Improved control and application of nitrogen</td>
<td>web page</td>
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<td></td>
<td>cycle bacteria for Nitrogen removal from wastewater) project</td>
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<tr>
<td>University of Nijmegen</td>
<td>Microbiology, application of the Anammox process, physiology of the Anammox</td>
<td>Jetten et al. (1997, 1999, 2002)</td>
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<tr>
<td></td>
<td>bacteria, marine microbiology</td>
<td></td>
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<td>Royal Netherlands Institute for Sea</td>
<td>Marine microbiology (the impact of Anammox on the past oceanic nitrogen cycle)</td>
<td>Sinninghe-Damsté et al. (2002)</td>
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<tr>
<td>University of Hannover</td>
<td>Deammonification biofilm moving-bed technology (full-scale and pilot-plant</td>
<td>Hippen et al. (1997); Helmer et al. (1999, 2001); Seyfried et al. (2001); Rosenwinkel</td>
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<td>application)</td>
<td>and Cornelius (2005); Rosenwinkel et al. (2005)</td>
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<td>Max Planck Institute For Marine</td>
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<td>(2005); IcoN project web page; BIOMATH web page</td>
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<td>University of Aarhus</td>
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<td>Ottosen et al. (2004)</td>
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<td>University of Birmingham</td>
<td></td>
<td>Mohan et al. (2004)</td>
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<td>Microbiology in estuarine sediments</td>
<td>Trimmer et al. (2003)</td>
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<td>Siegrist et al. (1998); Egli et al. (2001); Fux et al. (2002); Egli (2003); Fux (2003)</td>
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<tr>
<td>(EAWAG), Zurich</td>
<td></td>
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<td>Spain</td>
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<tr>
<td>University of Santiago de Compostela</td>
<td>Application of the Anammox process, inhibition studies, enrichment, modelling;</td>
<td>Dapena-Mora et al. (2004, 2005)</td>
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<td>the IcoN project</td>
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<tr>
<td>University of Cantabria</td>
<td>Model-based evaluation of the Anammox process</td>
<td>Domínguez et al. (2005)</td>
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<tr>
<td>Turkey</td>
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<tr>
<td>Istanbul Technical University</td>
<td>Stimulation of the Anammox activity; inhibition studies</td>
<td>Güven et al. (2004, 2005)</td>
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</table>
Table 1. Overview of the research and development of the Anammox process (contd).

<table>
<thead>
<tr>
<th>Country/centre</th>
<th>Main topics</th>
<th>Examples of references</th>
</tr>
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<tr>
<td><strong>France</strong></td>
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<tr>
<td>Genoscope, Evry, the French National Sequencing Center</td>
<td>Genetic information concerning Anammox bacteria</td>
<td>Genoscope web page</td>
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<tr>
<td><strong>Sweden</strong></td>
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<tr>
<td>Royal Institute of Technology, Stockholm</td>
<td>Deammonification moving-bed technology; technical-scale and lab-scale pilot plant studies; one-set and two-step technology; modelling studies</td>
<td>Plaza et al. (2002); Szatkowska (2004); Szatkowska et al. (2003a,b; 2004a,b); Trela et al. (2004a,b,c); Gut et al. (2005)</td>
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<td>Göteborg University</td>
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<td>Engström (2004)</td>
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<td><strong>Poland</strong></td>
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<td>Silesian University</td>
<td>Kinetics of the Anammox process; technological aspects of Anammox process, application of the Anammox process (laboratory-scale experiments)</td>
<td>Surmacz-Górśka et al. (1997); Cema et al. (2005a,b)</td>
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<td><strong>Australia</strong></td>
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<td>University of Queensland</td>
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<td>Murdoch University</td>
<td>Anammox process in the CANON system</td>
<td>Third et al. (2001); Third (2003)</td>
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<tr>
<td><strong>USA</strong></td>
<td></td>
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<tr>
<td>University of Georgia</td>
<td>Application of the Anammox process for poultry manure</td>
<td>Dong and Tollner (2001)</td>
</tr>
<tr>
<td><strong>USA/Brazil</strong></td>
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<td>Coastal Plains, Soil, Water and Plant Research Center, United States Department of Agriculture</td>
<td>Application of the Anammox process for livestock wastewater</td>
<td>United States Department of Agriculture web page</td>
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<td><strong>Japan</strong></td>
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<tr>
<td>Kumamoto University</td>
<td>Granulation of the Anammox bacteria, application of the Anammox process</td>
<td>Furukawa et al. (2001); Imajo et al. (2004)</td>
</tr>
<tr>
<td>Nagaoka University</td>
<td>Molecular Biological Analysis of Anammox, laboratory-scale experiments</td>
<td>Nagaoka University web page</td>
</tr>
<tr>
<td><strong>Korea</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korea University</td>
<td>Application of the Anammox process for piggery waste</td>
<td>Ahn et al. (2004); Hwang et al. (2004)</td>
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<tr>
<td><strong>China</strong></td>
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<tr>
<td>Beijing Institute of Civil Engineering and Architecture</td>
<td>Modelling of a partial nitritation-Anammox biofilm process; laboratory-scale experiments</td>
<td>Hao and van Loosdrecht (2003, 2004)</td>
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<tr>
<td>Tsinghua University</td>
<td>Granulation of the Anammox bacteria; laboratory-scale experiments</td>
<td>Jianlong and Jing (2005)</td>
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<td>Hunan University</td>
<td>Start-up of the deammonification process; laboratory-scale experiments</td>
<td>Li et al. (2004)</td>
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<td>Harbin Institute of Technology</td>
<td>Anammox process technology; laboratory-scale experiments</td>
<td>Wang et al. (2004)</td>
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<td>Ocean University of China</td>
<td>Enrichment and cultivation of Anammox microorganisms</td>
<td>Huang et al. (2004)</td>
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<td><strong>Commercialization of the technology</strong></td>
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<td>Paques BV, the Netherlands</td>
<td>Full-scale implementation of Anammox; the IcoN project</td>
<td>Paques home page</td>
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<tr>
<td>Grontmij Water and Reststoffen, the Netherlands</td>
<td>Coupling SHARON with the Anammox process</td>
<td>Grontmij Water and Reststoffen web page</td>
</tr>
<tr>
<td>PURAC AB, Sweden</td>
<td>Technical-scale pilot plant studies, deammonification studies</td>
<td>Johansson et al. (1998)</td>
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<tr>
<td>Unisense A/S, Denmark</td>
<td>Construction and use of micro and macro scale nitrogen-sensors for environmental analysis of the Anammox process</td>
<td>Unisense A/S web page</td>
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<tr>
<td>Kurita Water Industries Ltd., Japan</td>
<td>Commercial application of the Anammox process; pilot-scale experiments of Anammox process</td>
<td>Kurita Water Industries Ltd. Web page</td>
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</table>
Assessment of a partial nitritation/Anammox system for nitrogen removal

the raw wastewater load (Siegrist, 1996; Wett and Alex, 2003). Pre-treatment of such supernatant is necessary to lower the nitrogen load. Different deammonifying systems running with this medium as a substrate were investigated in many works (Table 2).

Recently the debate concerning the impact of waste landfills has put more interest in the second source of high ammonia waste streams – leachate. The leachate is concentrated and highly polluted water that soaked through the solid waste layer of landfill, transporting suspended solids and extracting soluble substances and other products of complex degradation processes in the landfill. Biochemical conditions, seasonal water regime of the landfill and changes in the solid waste composition affect both the quality and the quantity of this wastewater. Removal of ammonium is often not sufficient by treatment using a biological nitrification/denitrification method. Moreover, the nitrifying bacterial community is sensitive to toxic substances and high concentrations of ammonium. A seasonal decrease in the temperature can be a major drawback in the implementation of leachate treatment systems in the Northern European countries.

The flow variations at a WWTP and at a landfill site can cause changes in the quality of both media. The leachate water quality slowly changes with the landfill age. On the other hand, the supernatant’s quality is affected by operating problems with fermentation chambers or differences at the inflow to a WWTP (for instance the uneven flow of rainwater influences its operation). Yet, in the long run this is the medium with the most stable composition. Ammonium nitrogen concentration in leachate changes during a landfill’s life and it can exceed 2000 mg NH$_4$-N l$^{-1}$. Fluctuations of ammonium nitrogen concentration in supernatant can be high (from 400 to 1700 mg NH$_4$-N l$^{-1}$) and change in a matter of days or weeks. Because of this, it is necessary to control the treatment system in terms of changeable influent medium characteristics. Amounts of other inorganic nitrogen forms, like NO$_2$-N and NO$_3$-N, are very low in both types of waters. Minor concentrations of organic nitrogen forms are present in both supernatant and leachate as the ratio of NH$_4$-N/N$_{tot}$ usually falls just below 1 (Glixelli, 2003). The pH value is similar in both media. The amounts of COD are usually much higher in the leachate, although in some cases the supernatant can have a COD concentration larger than 1000 mg O$_2$ l$^{-1}$. The total phosphorus concentration in leachate is usually low and stable during the landfill’s existence (in the range 0.1-19.4 mg P$_{tot}$ l$^{-1}$). In the supernatant, its concentration changes to a higher extent (0.6-48.6 mg P$_{tot}$ l$^{-1}$). An additional advantage of the supernatant and the leachate is their high temperature, though the leachate’s temperature is more difficult to control and depends more on seasonal changes. Glixelli (2003) also reported the presence of other substances in leachate, like heavy metals, trace elements or toxic substances.

It is the location (standard of life, industry located in the municipal area) and the characteristics of waste treated at a municipal WWTP or a landfill (e.g. pre-treatment of solids waste) that affects the quality and quantity of both the supernatant and the leachate. A technology used in the wastewater treatment also determines the composition of the supernatant (i.e. supernatant from the chemical sludge is usually rich in metal salts used for precipitation). Moreover, it is of special importance to separate the supernatant stream from the other side streams generated at a WWTP, e.g. scrubber water and water from the cleaning of centrifuges (they may cause operational problems as well as being a source of toxic substances).

It was also recently proposed to independently treat urine collected in separating toilets (NoMix toilets) or waterless urinals (Jetten et al., 1997; Maurer et al., 2003). Urine is a major source of nitrogen, phosphorous and potassium in municipal wastewater and is a prime target to achieve a more sustainable treatment of nutrients today. During storage, the pH value of urine increases and therefore it is higher than the pH value of the supernatant. A high concentration of the total phosphorous differs urine from supernatant and leachate. Autotrophic processes were employed to treat urine, mainly traditional
Table 2. Literature overview of different studied ammonium-rich streams (nd - no data).

<table>
<thead>
<tr>
<th>Stream</th>
<th>NH$_4$-N (mg l$^{-1}$)</th>
<th>T ($^\circ$C)</th>
<th>pH (-)</th>
<th>COD (mg O$_2$ l$^{-1}$)</th>
<th>$P_{\text{tot}}$ (mg l$^{-1}$)</th>
<th>Comments</th>
<th>References</th>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Influent to Sharon reactor</td>
<td>Hellinga et al. (1998)</td>
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<tr>
<td>1000</td>
<td>30</td>
<td>8.1-8.4</td>
<td></td>
<td>810</td>
<td>27</td>
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<tr>
<td>1180</td>
<td>nd</td>
<td>6.7-6.8</td>
<td>nd</td>
<td>nd</td>
<td></td>
<td>Sharon-Anammox process</td>
<td>van Dongen et al. (2001b)</td>
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<tr>
<td>750</td>
<td>30</td>
<td>nd</td>
<td>277</td>
<td>nd</td>
<td></td>
<td>BABE reactor</td>
<td>Salem et al. (2001b)</td>
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<td>840</td>
<td>nd</td>
<td>nd</td>
<td>1044</td>
<td>nd</td>
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<td>SBR reactors</td>
<td>Fux et al. (2003)</td>
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<tr>
<td>657</td>
<td>nd</td>
<td>7.4-7.8</td>
<td>nd</td>
<td>0.6-7.3</td>
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<td>Fux et al. (2002)</td>
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<td>500-1500</td>
<td>30-37</td>
<td>7.0-8.5</td>
<td>nd</td>
<td>nd</td>
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<td>1200</td>
<td>30</td>
<td>7.2</td>
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<td>710</td>
<td>nd</td>
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<td>1250-1700</td>
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<td>11.9-12.8</td>
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<td>700-1000</td>
<td>nd</td>
<td>SBR reactor</td>
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<td>552-1004</td>
<td>nd</td>
<td>nd</td>
<td>384-711</td>
<td>1.2-33</td>
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<td>Partial nitration/Anammox system (Sweden)</td>
<td>Szatkowska (2004)</td>
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<td>436-797</td>
<td>nd</td>
<td>7.4-7.9</td>
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<td>262-650</td>
<td>19.4-48.6</td>
<td>Assessment of digester supernatant (Poland)</td>
<td>Musial (2000)</td>
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<td>1540-2310</td>
<td>23-27</td>
<td>7.9-9.8</td>
<td></td>
<td>1940-5704</td>
<td>11.8-19.4</td>
<td>Assessment of leachates (Taiwan)</td>
<td>Chen (1996)</td>
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<td>0.2-800</td>
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<td>5.2-8.7</td>
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<td>180-4700</td>
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<td>Assessment of leachates (Sweden)</td>
<td>Welander (1998)</td>
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<td>0.7-1520</td>
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<td></td>
<td>470-7200</td>
<td>0.1-13.6</td>
<td>Assessment of leachates (Poland)</td>
<td>Obrzut (1997)</td>
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<td>32-681</td>
<td>27-30</td>
<td>7.4-8.7</td>
<td></td>
<td>442-2900</td>
<td>nd</td>
<td>RBC systems: Mechernich, Germany; Kölliken, Switzerland; Pitsea, UK</td>
<td>Hippen et al. (2001)</td>
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<td>18-494</td>
<td>13-20</td>
<td>7.3</td>
<td></td>
<td>748-1593</td>
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<td>147-780</td>
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<td></td>
<td></td>
<td>nd</td>
<td></td>
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<tr>
<td>220-260</td>
<td>nd</td>
<td>7.0-7.2</td>
<td>nd</td>
<td>nd</td>
<td></td>
<td>RBC system</td>
<td>Siegrist et al. (1998)</td>
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<tr>
<td>8180</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>670</td>
<td>Energetic aspects of removal and recovery of nutrients</td>
<td>Maurer et al. (2003)</td>
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<td>1800-3800</td>
<td>nd</td>
<td>8.9-9.1</td>
<td>nd</td>
<td>80 (after precipitation)</td>
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<td>MMBR (nitrate production), CSTR, SBR (nitritation), Anammox batch reactor</td>
<td>Udert et al. (2003)</td>
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<td>Piggery manure</td>
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<td>8.4-8.6</td>
<td>56000</td>
<td>476-1260</td>
<td></td>
<td>Granular sludge UASB reactor</td>
<td>Ahn et al. (2004)</td>
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<td>Potato starch wastewater</td>
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<td>8.4-8.6</td>
<td>3000</td>
<td>210</td>
<td></td>
<td>Activated sludge nitrification/ denitrification via nitrite</td>
<td>Abeling and Seyfried (1992)</td>
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</tbody>
</table>
Assessment of a partial nitritation/Anammox system for nitrogen removal

Due to the enhanced production of piggery manure, the handling option has been proposed as an alternative of using it as the soil fertilizer. The thin fraction of the piggery manure can be treated and the application of the Anammox process has been successful (Ahn et al., 2004; Choi et al., 2004). The composition of the thin fraction of the piggery waste varies significantly and depends on the equipment used for separating thick and thin fractions of the sludge. High amounts of nitrogen, COD and total phosphorous are typical for this kind of wastewater.

Industrial processes also generate highly concentrated nitrogen streams and should be treated separately. Abeling and Seyfried (1992) name the following industry fields as producers of wastewaters with high inorganic nitrogen concentration: alcohol production, pectin industry, starch and potato processing industry, slaughterhouses, metallurgy and petrochemical industry. High COD concentration in these wastewaters is not always sufficient for denitrification. Moreover, industrial streams often contain toxic compounds that hinder the biological treatment processes.

3.3. Overview of processes with nitrogen removal

At a municipal WWTP, the influent ammonium is mainly the product of breaking down proteins. During the biological treatment a negligible part of the ammonium is transformed to ammonia in the gas phase. Moreover, ammonia is partly used by the activated sludge and biofilm bacteria and contributes to their organic biomass. That part of the ammonium nitrogen is only temporarily bounded due to the subsequent release of ammonium during the fermentation process (the sludge handling part of a WWTP) and results in the generation of a highly concentrated side stream of reject water. There are many different chemical and biochemical routes for the nitrogen transformation to nitrogen gas. Table 3 shows an overview of the most important processes in handling the nitrogen load imposed on WWTPs. The ultimate aim is to transform the ammonium to nitrogen gas with the least usage of resources and without formation of greenhouse gases like nitrous oxide (N\textsubscript{2}O). The paradigm that the only way to biologically convert the wastewater ammonium to nitrogen gas is through the aerobic conversion to nitrate followed by the heterotrophic denitrification is now obsolete. Discoveries of other metabolic paths of aerobic and anaerobic ammonia oxidizers are now used in the environmental biotechnology. A short outline of the processes follows (the reaction numbers refer to Table 3).

Traditional nitrification/denitrification

In Table 3 the traditional treatment system with the combination of nitrification and denitrification is illustrated by the reactions 4+5+6+7. At Swedish WWTPs, the nitrogen removal technology is consuming a considerable amount of resources: 4.57 g O\textsubscript{2} g\textsuperscript{-1} N and around 4 g COD g\textsuperscript{-1} N (Plaza, 2001; Trela, 2000). These values imply that there is a need to aerate the medium for nitrification and supply an external source of carbon for denitrification. It has to be taken into account that the internal content of easily biodegradable COD changes in different countries. The traditional biological treatment leads to a sizeable amount of produced sludge that must be treated in a proper manner. An efficient execution of the anoxic denitrification demands a variety of electron donors, such as acetate, methanol, ethanol, lactate or glucose (Henze et al., 2002). Dissimilar conditions for bacteria performing nitrification and denitrification result in designing separate reactors for both processes. This leads to high costs of construction, operation and maintenance of the biological part of a WWTP. Subsequent nitrification/denitrification is possible in the Sequential Batch Reactor (SBR) by alternating the conditions in a proper sequence of aerobic and anoxic phases.
Table 3. Reactions for biological conversions of nitrogen forms (modified after Plaza et al., 2003).

<table>
<thead>
<tr>
<th>No.</th>
<th>Reaction</th>
<th>Process</th>
<th>Microorganisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>$C_8H_7O_2N+4H_2O \rightarrow 2.5CH_4+1.5CO_2+HCO_3+NH_4^+$</td>
<td>Ammonification (anaerobic)</td>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>$C_8H_7O_2N+5O_2 \rightarrow 4CO_2+HCO_3^-+NH_4^++H_2O$</td>
<td>Ammonification (aerobic)</td>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>$NH_4^++OH^- \rightarrow NH_3+H_2O$</td>
<td>Ammonium/ammonia equilibrium</td>
<td>No (physical process)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>$4CO_2+HCO_3^-+NH_4^++H_2O \rightarrow C_8H_7O_2N+5O_2$</td>
<td>Assimilation</td>
<td>Bacteria, Algae (growth)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$NH_4^++1.5O_2+2HCO_3^- \rightarrow NO_2^-+2CO_2+3H_2O$</td>
<td>Nitritation</td>
<td>Nitrosomonas, e.g. N. eutropha, N. europaea; Nitrosospira</td>
<td>Rittmann and McCarty (2001); Henze et al. (2002)</td>
</tr>
<tr>
<td>5</td>
<td>$NO_2^-+0.5O_2 \rightarrow NO_3^-$</td>
<td>Nitratation</td>
<td>Nitrobacter, e.g. N. agilis, Nitrosospira, Nitrococcus, Nitrosocystis</td>
<td></td>
</tr>
<tr>
<td>4+5</td>
<td>$NH_4^++2O_2+2HCO_3^- \rightarrow NO_3^-+2CO_2+3H_2O$</td>
<td>Nitrification</td>
<td>Nitrifying bacteria</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>$C+2NO_3^- \rightarrow 2NO_2^-+CO_2$</td>
<td>Denitrification</td>
<td>Denitrifying heterotrophic bacteria</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$3C+2H_2O+CO_2+4NO_2^- \rightarrow 2N_2+4HCO_3^-$</td>
<td>Denitrification</td>
<td>Denitrifying heterotrophic bacteria</td>
<td></td>
</tr>
<tr>
<td>6+7</td>
<td>$5C+2H_2O+4NO_3^- \rightarrow 2N_2+4HCO_3^-+CO_2$</td>
<td>Denitrification</td>
<td>Heterotrophs: Pseudomonas, Bacillus, Alcaligenes, Paracoccus</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>$NH_4^++0.75O_2+HCO_3^-+0.5NH_4^++0.5NO_3^-+CO_2+1.5H_2O$</td>
<td>Partial nitritation</td>
<td>Ammonium-oxidizing bacteria</td>
<td></td>
</tr>
<tr>
<td>9a</td>
<td>$NH_4^++NO_2^- \rightarrow N_2+2H_2O$</td>
<td>Anammox (without cell synthesis)</td>
<td>Planctomycetales</td>
<td>van Dongen et al. (2001a)</td>
</tr>
<tr>
<td>9b</td>
<td>$NH_4^++1.32NO_2^-+0.066HCO_3^-+1.02N_2+0.26NO_3^-+0.066CH_2O_3+NO_2^-+2.03H_2O$</td>
<td>Anammox (with cell synthesis)</td>
<td>Planctomycetales</td>
<td></td>
</tr>
<tr>
<td>4+7</td>
<td>$4NH_4^++6O_2+3C+4HCO_3^- \rightarrow 2N_2+7CO_2+10H_2O$</td>
<td>Modified nitrogen removal</td>
<td>Bacteria</td>
<td>Rittmann and McCarty (2001); Henze et al. (2002)</td>
</tr>
<tr>
<td>4+5+6+7</td>
<td>$4NH_4^++8O_2+5C+4HCO_3^- \rightarrow 2N_2+9CO_2+10H_2O$</td>
<td>Traditional nitrogen removal</td>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>4+9</td>
<td>$NH_3+0.85O_2 \rightarrow 0.11NO_3^-+0.44N_2+0.14H^++1.43H_2O$</td>
<td>CANON</td>
<td>Nitrifying bacteria, Planctomycetales</td>
<td>Sliekers et al. (2002)</td>
</tr>
<tr>
<td>10</td>
<td>$NH_4^++0.75O_2 \rightarrow 0.5N_2+H^++1.5H_2O$</td>
<td>OLAND</td>
<td>Nitrosomonas</td>
<td>Verstraete and Philips (1998)</td>
</tr>
<tr>
<td>11</td>
<td>$3NH_4^++3O_2+3[H] \rightarrow 1.5N_2+3H^++6H_2O$</td>
<td>NO₃ process</td>
<td>Nitrosomonas</td>
<td>Schmidt et al. (2003)</td>
</tr>
</tbody>
</table>
Assessment of a partial nitritation/Anammox system for nitrogen removal

Modifications of traditional N-removal processes

The oxidation of ammonium to nitrite (reaction 4) followed by denitrification (reaction 7) has been the subject of extensive research (Turk and Mavinic, 1989; Surmacz-Górska et al., 1997; Jianlong and Ning, 2003; Ruiz et al., 2003; Wyffels et al., 2003; Ciudad et al., 2005). The minimisation of resources by partial nitrification and denitrification results in a more sustainable technology. Savings in the oxygen demand, reduction of the organic carbon requirement and the decrease in the surplus sludge are advantages of shortcutting the traditional nitrification/denitrification route. Nitrite accumulation is obtained by optimising the operational conditions by properly setting the parameters like the dissolved oxygen (DO), pH value and temperature (Hwang et al., 2000; Bae et al., 2002; Ruiz et al., 2005). The system set-up can consist of performing partial nitrification and partial denitrification in two steps (Ruiz et al., 2005) or using a one-stage activated sludge system (de Silva and Rittmann, 2001). Additionally, nitrite accumulation techniques were applied for low concentrated streams mainly (de Silva and Rittmann, 2001; Bae et al., 2002). However, high ammonium concentration wastewaters have also been treated (Wyffels et al., 2003; Yang et al., 2003; Ciudad et al., 2005; Ruiz et al., 2005).

SHARON process

The SHARON (Single reactor system for High Ammonium Removal Over Nitrite) process (reaction 8) was designed to reduce the load of streams with high ammonium concentration (ca. 1 g NH$_4$-N l$^{-1}$) rather than meet effluent standards. Conditions set in the SHARON reactor favour ammonium oxidizers by washing out nitrite oxidizers due to the short retention time (approximately 1 day) and a temperature over 30°C (van Dongen et al., 2001a). A full-scale SHARON reactor operates at the Dokhaven WWTP, Rotterdam, the Netherlands (van Dongen et al., 2001b; van Kempen et al., 2001). Initially, the process concept was aimed at exploiting the specific temperature of supernatant from the digested sludge and its composition. A full-scale application is operated with intermittent aeration in one reactor, which allows for longer aerobic and shorter anoxic phases (Hellenga et al., 1998). During the anoxic phase, methanol is added and the denitrification proceeds. Compared to the traditional processes of nitrification/denitrification, the oxygen demand is decreased by 25% and amounts to 3.43 g O$_2$/g N. To compare the usage of the organic materials, it is decreased by 40%, which equals 2.4 g COD/g N (Mulder et al., 2001; Hellenga et al., 1998). The sludge production is also lower and a simple well-mixed reactor can be used. Six full-scale SHARON units have been constructed at WWTPs in Rotterdam, Utrecht, Zwolle, Beverwijk, Garmerwolde and Den Haag, the Netherlands (total capacity 2,740,000 p.e.) and a plant is under construction in New York, USA (3,000,000 p.e.) (van Loosdrecht and Salem, 2005).

It appeared that characteristics of the recycled reject water streams are especially suitable to partially oxidize ammonium to nitrite in the 'partial' SHARON as the supernatant contains about equimolar amounts of ammonium and bicarbonate. The carbon dioxide stripping could therefore balance the nitrite production by a concurrent pH drop, preventing further oxidation. The nitrite/ammonium ratio in the effluent from the 'partial' SHARON reactor can be consequently influenced by pH control (van Dongen et al., 2001a). The 'partial' SHARON concept can also be used as the preceding step for the Anammox process. The 'partial' SHARON process can be modified with the goal of obtaining proper effluent quality, which is an essential factor for the appropriate operation of an Anammox reactor. It is discussed further in the next section.

Anammox process

The anaerobic ammonium oxidation (Anammox) process is a promising pathway for removing nitrogen from wastewater (Mulder et al., 1995; de Graaf et al., 1995; Dijkman and Strous, 1999; van Dongen et al., 2001a; Strous et al., 1999; van Loosdrecht et al., 2004). The anaerobic character of the process (reaction 9) allows for considerable savings and no addition of chemicals are needed. The ammonium reacts with the nitrite acting as an electron acceptor to pro-
duce nitrogen gas. Intermediates of the process are hydrazine and hydroxylamine (Jetten et al., 2001). The catabolic reaction of fixing nitrite with one molecule of carbon dioxide leads to the anaerobic production of nitrate in the anabolism (Strous et al., 1998). According to Van Niftrik et al. (2004), it is typical for the Anammox bacteria, which belong to the phylum Planctomycetales, to have an intracytoplasmic compartment: anammoxosome. The exact function of the anammoxosome is currently under study and it is strongly believed to play a major role in the Anammox metabolism.

According to the stoichiometry of the reaction proposed by van Dongen et al. (2001a, 2001b) the nitrite nitrogen concentration should exceed the ammonium nitrogen concentration in the feed to the Anammox reactor. Thus, a quotient NO\textsubscript{2}-N/NH\textsubscript{4}-N is equal to 1.32. It was demonstrated that the Anammox bacteria have very low growth rate (Jetten et al., 2001) and equals 0.003 h\textsuperscript{-1}. This is the main obstacle in the process implementation in full scale. A doubling time of 11 days (Jetten et al., 1999) is a challenge in terms of starting-up an Anammox reactor. van Dongen et al. (2001b) show that it takes between 100 and 150 days for the Anammox activated sludge reactor to reach its full capacity. It has been reported however, that the doubling time would be closer to a month in full-scale application due to the kinetics of the process (Fux et al., 2002, 2004). It would imply a longer start-up period.

Methods for accelerating the acclimation of the Anammox bacteria as well as the recovery of its culture deserve a special interest. Li et al. (2004) reported the influence of the Anammox reaction intermediate, hydrazine, on speeding up the acclimating process. Egli et al. (2001) demonstrated that the Anammox bacteria found in a WWTP are active at temperatures within the range of 6-43°C and an optimum at 37°C. For the optimal temperature, the pH range is between 6.5 and 8.5. In the natural conditions of sea sediments, the optimal temperature was found to be substantially lower and amounted to 15°C (Dalsgaard and Thamdrup, 2002). The Anammox process is reversibly inhibited by oxygen and irreversibly by nitrite at concentrations exceeding 70 mg NO\textsubscript{2}-N l\textsuperscript{-1} for several days (van Dongen et al., 2001a). In case of Candidatus Kuenenia stuttgartiensis, the nitrite nitrogen concentration in the reactor can be raised to 180 mg NO\textsubscript{2}-N l\textsuperscript{-1} (van de Graaf et al., 1996; Strous et al., 1999). This nitrite inhibition can be overcome by the addition of trace amounts of either of the Anammox intermediates: hydrazine and hydroxylamine (Strous et al., 1999; Li et al., 2004). The exposure of the Anammox bacteria to even low concentrations of alcohols, methanol in particular, should be prevented due to the immediate, complete, and irreversible inhibition of the process (Güven et al., 2005). This research is highly relevant as methanol is often used to remove nitrate in the post-denitrification or to compensate for pH effects in partial nitrification. The results of a study by Schmidt et al. (2002a) provide strong indications that the anaerobic ammonia-oxidizing Planctomycetales (B. anammoxidans) are not sensitive to NO concentrations up to 600 ppm and that the nitrogen conversion rates of an Anammox reactor system increase about twofold in the presence of 50 ppm of NO\textsubscript{2}.

Feasibility studies about the granulation of the Anammox bacteria show that the high applicability to the wastewater treatment (Imajo et al., 2004, Schmidt et al., 2004). The granular sludge is maintained in reactor and such configuration is set in a flow-up reactor. This technique can result in shorter start-up periods due to using methanogenic granules as carrier material in the initial phase. Additionally, Jianlong and Jing (2005) presented applicability of an expanded granular sludge bed (EGSB) in the granulation of the Anammox bacteria.

Research about the Anammox process is nowadays directed towards defining the biochemical reaction with its intermediates as well as investigating the possibility of formation detrimental intermediate emissions of NO and N\textsubscript{2}O. New microbiological techniques for identifications of the Anammox bacteria are under development.
Combined partial nitritation/Anammox processes – deammonification process

The least resource consuming method to transform ammonium to nitrogen gas is the technology based on partial nitritation/Anammox processes. Anammox needs a preceding process to convert half of ammonium to nitrite (reaction 4), without subsequent oxidation of nitrite to nitrate. The oxygen uptake based on initial ammonium concentration is 1.72 g O$_2$ g$^{-1}$ N or just 38% of the oxygen demand for oxidation of all the ammonium to nitrate. After this process, the Anammox process (reaction 9) follows without need for organic material in a separate reactor. Modifications of the SHARON process ('partial' SHARON) by not supplying methanol and excluding anoxic periods are an alternative to generate the ammonium/nitrite mixture for the Anammox reactor. The crucial factor is the stoichiometrically correct ratio of nitrite to ammonium. This should be equal to 1.3 in the influent of the Anammox reactor (van Dongen et al., 2001a, 2001b; Volcke et al., 2003).

The research group at the University of Hanover introduced the 'deammonification' term in order to differentiate a novel process of nitrogen removal from the traditional denitrification observed in a rotating-disk plant treating leachate (Hippen et al., 2001). This term is also used to describe an ammonium removal process that does not depend on the supply of organic matter (Hippen et al., 1999, 2001; Helmer et al., 2001; Seyfried et al., 2002; Rosenwinkel and Cornelius, 2005). It employs aerobic and anaerobic ammonia oxidizers in converting the ammonia directly to nitrogen gas under oxygen limitation. Over time, it became apparent that the deammonification could be defined as a combination of nitritation and Anammox processes occurring in the biofilm and established in two separate reactors as well as in one single reactor. During the start-up of a single-stage deammonification process, it is necessary to develop a nitrifying biofilm culture in aerobic conditions to allow the Anammox bacteria to enrich the culture while the conditions are alternated into oxygen-limited. Such a procedure results in obtaining a mixed biocoenosis that coexists in the biofilm. The shear forces caused by the intense mixing limit the formation of the biofilm structure that allows for the development of anoxic zones. The moving-bed biofilm reactor (MBBR) with the Kaldnes® biofilm carriers was extensively applied in the development of the deammonification process (Seyfried et al., 2002; Trela et al., 2004b,c; Rosenwinkel and Cornelius, 2005).

CANON process

A new process configuration that allows for the combination of nitrifying cultures and Anammox bacteria was named the Completely Autotrophic Nitrogen removal Over Nitrite (CANON) process (reactions 4+9, Table 3). It is based on the concept of simultaneous nitrification and denitrification (SND) in the same reactor vessel at constant operating conditions (Keller et al., 1997; Surmacz-Górska et al., 1997; Helmer and Kunst, 1998; van Benthum et al., 1998; Yoo et al., 1999; van Loosdrecht et al., 2000; Third, 2003). With the discovery of the Anammox bacteria, the CANON process was proposed (Sliekers et al., 2002, 2003) and anaerobic ammonium oxidizers were used as denitrifies. Oxygen-limited conditions are obligatory to obtain the cooperation between aerobic and anaerobic bacteria. A sequencing batch reactor (SBR) was used to develop the CANON process (Third et al., 2001; Sliekers et al., 2002). Unlike the Anammox process, the CANON process can be fed directly with an ammonium-rich influent at an appropriate loading rate. In one reactor, nitrite oxidizers can be outcompeted due to differences in the affinity constants between nitrifying bacteria. In a biofilm bacterial culture it is possible to achieve a concurrent accumulation of nitrate in the outer aerobic part of the biofilm layer and attain Anammox reaction in the inner anaerobic part of the biofilm (Hao et al., 2002b; Hao and van Loosdrecht, 2003). Hao et al. (2002b) modelled this cooperation of bacteria in the biofilm layer. Schmidt et al. (2002b) assessed the harmonious and balanced interaction between the two groups of bacteria. *Nitrosomonas* can supply *Brocadia anammoxidans* with nitrite as an oxidant at the oxic-anoxic biofilm interface. The coopera-
tion seems possible despite the natural competition for the same substrate – ammonium. It is *Nitrosomonas* that limits the Anammox process in the CANON reactor configuration due to its role in preventing diffusion of oxygen into the deeper layers as well as supplying nitrite to the Anammox bacteria (Nielsen et al., 2005). However, according to the Gibbs free energy calculations, the Anammox bacteria should be more efficient than the *Nitrosomonas*.

A novel nomenclature for the combination of partial nitritation and Anammox processes in one reactor was recently established: Single-stage Nitrogen Removal Using Anammox and Partial Nitritation (SNAP) process (the SNAP process web page). It is a wholly autotrophic nitrogen removal process using acryl resin fibre as a biomass carrier.

**OLAND process**

Other publications concerning the unexplained nitrogen losses in full-scale denitrifying biofilm reactors led researchers to the development of the OLAND (Oxygen-Limited Autotrophic Nitrification-Denitrification) process. Unlike the CANON process, the ammonia-oxidizing bacteria are able to convert ammonium to nitrogen gas in one reactor under oxygen limitation (Kuai and Verstraete, 1980; Verstraete and Philips, 1998; Pynaert et al., 2002). The *Nitrosomonas* species can use, due to shortage of an electron acceptor, the produced nitrite (reaction 10, Table 3). A pH-controlled aeration of the enriched autotrophic nitrifiers forces bacteria to consume nitrite (Verstraete and Philips, 1998).

For practical purposes, the OLAND process could be easily applied due to the uncomplicated production of nitrifying inoculums from the activated sludge. This system does not need the direct supply of nitrite and the ammonium-rich stream can be treated directly. However, the current system capacity is still low.

**NOx process**

Schmidt et al. (2003) presented a new possibility of stimulating and controlling the activity of the *Nitrosomonas*-like microorganisms by the addition of nitrogen oxides. The *Nitro-

**BABE process**

Bio-augmentation was postulated as an option for upgrading existing WWTPs by the treatment of nitrogen-rich flows (Salem et al., 2003, 2004). The so-called BABE® (bio-augmentation batch enhanced) process aims at boosting the development of the nitrifying community by shortening the SRT in the reactor. The enhanced population of nitrifiers from the BABE reactor is used to feed a conventional activated sludge system. The effect of seeding the main nitrification reactor with separately cultivated culture was
studied previously (Plaza et al., 2001) and focused on treatment of the digester supernatant. The nitrification capacity can be substantially increased by the side-treatment of supernatant due to both the decreased influent load on the WWTP and the enhanced activity of the nitrifiers. The improvement of the effluent quality, creation of extra capacity and better potential for dealing with peak loads is also achieved.

Van der Zandt et al. (2005) report that the BABE process costs 1.75 Euro per kg of total N removed (2005), which corresponds to a 60% reduction of costs. In the Netherlands, the full-scale application of the process that treats reject water exists at the Garmerwolde WWTP, Groningen, and the outing of the next application is due September 2005 (the ‘s-Hertogenbosch WWTP).

Bio-augmentation may be of special interest in a partial nitritation/Anammox system as both nitritation and Anammox bacteria can be seeded into the main wastewater stream.

Comparison of the processes
The evaluation of the most important processes for nitrogen removal from wastewaters is presented in Table 4. Compared to the well-recognized combination of nitrification and denitrification, novel processes can be established in only one reactor. Considerable savings on aeration (energy) and chemical addition are typical for the SHARON and Anammox processes. Due to the fact that the Anammox process demands a preceding step, it was proposed to shorten the nitritation process using the ‘partial’ SHARON concept. Due to a very low growth rate, the Anammox bacteria need to be retained in the system. An advantage is the minor formation of excess sludge but the start-up period is long. The maintenance costs are reduced as well as the investment costs due to higher compactness of the reactors and there is no need for sophisticated devices for the process control.

The information concerning wastewater treatment processes for nitrogen removal is summarized in Table 4. It shows that there is a need to consider the application of novel processes on a full scale. The detailed assessment of the sustainability factors is given in Table 5. The objective of conserving energy and resources is met in the case of SHARON, Anammox, CANON and OLAND processes. The operability is a great advantage of the new processes. Even though the research studies have shown that it is possible to suppress nitrification at the level of nitrite formation (‘partial’ SHARON), there is still quite high uncertainty in applying this process on a full scale, as it is in the developing phase. A necessity is to scrutinise for possible undesirable side effects, e.g. highly reactive nitrite can react with aromatic molecules to produce nitroso- and nitro-derivatives.

Smaller and more compact installations can be used for the new processes. Special applicability for highly concentrated ammonium wastewater brings the wastewater management nearer to the source, which diminishes the impact from a sewer system. Moreover, the trend to keep the streams as concentrated as possible (e.g. collection of urine) has to be integrated with the consumption patterns, i.e. taking into account all parts of the system. As the sludge production is reduced to a minimum in the systems with the Anammox process, only the sludge from the nitrification/denitrification processes and processes for removal of organic compounds needs to be handled. The amount of the excess sludge will be substantially reduced if separate treatment of supernatant is employed. The new investigated concepts can be implemented in the existing infrastructure without difficulties (van Loosdrecht et al., 1997; Mulder, 2003). The examples of process selection cases can be found in van Loosdrecht and Salem (2005).

It can be stated that the combined partial nitritation/Anammox system is one of the most economical ways of removing nitrogen in a WWTP. In Table 6 the cost estimation of two applications were considered: a conventional nitrification/denitrification process and a novel combined partial nitritation/Anammox system. Fux (2003) performed the cost analysis for the separate treatment of sludge digester effluents of a WWTP for 100,000 p.e. The operational
**Table 4. Qualitative and quantitative comparison of several processes of nitrogen removal technology, modified after Mulder (2003) and Schmidt et al. (2003) (d.w. – dry weight).**

<table>
<thead>
<tr>
<th>Process/factor</th>
<th>Conventional nitrification/ denitrification</th>
<th>SHARON</th>
<th>Anammox</th>
<th>CANON</th>
<th>OLAND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of reactors</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Discharge</td>
<td>NO$_3^-$, N$_2$O, N$_2$</td>
<td>NH$_4^+$, NO$_2$, N$_2$</td>
<td>N$_2$, NO$_3^-$</td>
<td>N$_2$, NO$_3^-$</td>
<td>N$_2$</td>
</tr>
<tr>
<td>Conditions</td>
<td>Oxic; anoxic</td>
<td>Oxic/anoxic</td>
<td>Anaerobic</td>
<td>Oxygen-limited</td>
<td>Oxygen-limited</td>
</tr>
<tr>
<td>Oxygen demand (kg O$_2$ kg$^{-1}$ N)</td>
<td>High (4.6)</td>
<td>Low (3.4)</td>
<td>None</td>
<td>Low (1.5-2)</td>
<td>Low (1.5-2)</td>
</tr>
<tr>
<td>pH control</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Biomass retention</td>
<td>None</td>
<td>None</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>COD requirement</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Sludge/biomass production (kg d.w. kg$^{-1}$ N)</td>
<td>High (1-1.2)</td>
<td>Low (0.8-0.9)</td>
<td>Low (&lt;0.1)</td>
<td>Very low</td>
<td>Very low</td>
</tr>
<tr>
<td>N-removal efficiency (%)</td>
<td>95</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>Bacterial growth</td>
<td>Biofilm/ suspension</td>
<td>Suspension/ biofilm</td>
<td>Suspension/ biofilm/granules</td>
<td>Biofilm</td>
<td>Biofilm</td>
</tr>
<tr>
<td>Type of bacteria</td>
<td>NH$_4^+$ and NO$_2^-$ oxidizers, Various heterotrophs</td>
<td>Aerobic NH$_4^+$ oxidizers, N. eutropha, heterotrophs</td>
<td>Planctomycetales: Brocadia anammoxidans, Kuenenia stuttgartiensis, Scalindua brodae, S. wagneri, S. sorokinii</td>
<td>Aerobic NH$_4^+$ oxidizers, Planctomycetales</td>
<td>Autotrophic nitrifiers</td>
</tr>
<tr>
<td>Process complexity</td>
<td>Separate oxic and anoxic compartments or periods, methanol dosing</td>
<td>Separate oxic and anoxic compartments or periods, methanol dosing</td>
<td>Preceding partial nitritation needed</td>
<td>Aeration tuned to ammonia loading</td>
<td>Aeration tuned to ammonia loading</td>
</tr>
<tr>
<td>Application status</td>
<td>Established</td>
<td>Four full-scale plants</td>
<td>Two full scale plants</td>
<td>Laboratory</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Availability of performance data</td>
<td>High</td>
<td>Medium</td>
<td>Medium</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>Investment costs</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Operation and maintenance costs</td>
<td>High</td>
<td>Low</td>
<td>Very low</td>
<td>Low</td>
<td>Unknown</td>
</tr>
<tr>
<td>Management</td>
<td>Constant control of the process</td>
<td>Simple control by pH, conductivity and dissolved oxygen (DO) concentration measurements</td>
<td>Simple control by conductivity; nitrite nitrogen concentration monitoring</td>
<td>Oxygen transfer control</td>
<td>Oxygen transfer control</td>
</tr>
</tbody>
</table>
Table 5. Matrix for the assessment of the sustainability of biological nitrogen removal systems (*suitability to treat ammonium-rich streams*).

<table>
<thead>
<tr>
<th>Process/factor</th>
<th>Conventional nitrification/denitrification</th>
<th>Partial nitritation ('partial' SHARON)</th>
<th>Anammox</th>
<th>CANON</th>
<th>OLAND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suitability aspect <em>1</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential environmental impact from installation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy demand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sludge production</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area requirement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resource consumption</td>
<td></td>
<td></td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>N₂O, CO₂ emissions</td>
<td></td>
<td></td>
<td>Possible</td>
<td>None</td>
<td>Possible</td>
</tr>
<tr>
<td>Reliability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Public acceptance</td>
<td></td>
<td></td>
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<td>Acceptance among researchers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applicability at local treatment systems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scale:
- High
- Medium
- Low
- Very low


<table>
<thead>
<tr>
<th>Factors decisive for cost estimation</th>
<th>Nitrification/denitrification</th>
<th>Combined partial nitritation/Anammox</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EUR per kg Nₘₑₐₙ</td>
<td>EUR per kg Nₘₑₐₙ</td>
</tr>
<tr>
<td>Investment</td>
<td>1.35</td>
<td>1.30</td>
</tr>
<tr>
<td>Operation (60% NH₄ to NO₂)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy</td>
<td>0.35</td>
<td>0.15</td>
</tr>
<tr>
<td>Maintenance</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Control/staff</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Chemicals (0.2 EUR kg CH₃OH⁻¹)</td>
<td>0.50</td>
<td>0.05</td>
</tr>
<tr>
<td>Sludge disposal</td>
<td>0.30</td>
<td>Negligible</td>
</tr>
<tr>
<td>Total cost</td>
<td>3.50</td>
<td>2.50</td>
</tr>
</tbody>
</table>
costs for denitrification are heavily dependent on the biomass yield (estimated as 0.2 g COD\textsubscript{biomass} g\textsuperscript{-1} COD\textsubscript{dosed}) and the final electron acceptor (nitrate for values in Table 6). The overall costs estimated for the combined partial nitritation/Anammox process are almost 1.5 times lower than for the nitrification/denitrification alternative. A conventional extension of the activated sludge system consisting of introducing the nitrification and providing an additional anaerobic volume for the denitrification amounts to 8.0 EUR per kg nitrogen removed, whereas the overall costs estimated for a full-scale partial nitritation/Anammox plant are 2.5 EUR kg\textsuperscript{-1} N\textsubscript{removed}. All the data is for 2003. Consequently, the separate treatment of the digester supernatant is definitely more cost-effective for the assumed size of plant than a conventional extension of the activated sludge process.

During a scale-up of the biofilm system, the investment price of the biofilm carriers needs to be taken into account. The market price of Kaldnes rings is nowadays 3800 SEK m\textsuperscript{-3} (approx. 420 EUR m\textsuperscript{-3}) (Mele, 2005). It imposes additional initial costs but the choice of the option applied in the full scale should be taken in a broader context, considering the location and the size of the plant, energy consumption for aeration, cost of energy in a specific country, cost of mixing devices, pumps, heating, maintenance costs, etc. Kaldnes rings have been successfully applied in a full-scale deammonification plant at Hattingen WWTP, Germany and the solutions to the problems in the operation and maintenance have been reported (Rosenwinkel et al., 2005). At the other full-scale Anammox reactor in Rotterdam, the Netherlands, the initial problems with establishing the granular Anammox sludge culture in an up-flow reactor occurred (van Loosdrecht, 2004). Additionally, a study presented by Wett (2005) gives an account on solving scaling-up problems in a SBR full-scale deammonification plant at the Strass WWTP, Austria.

### 3.4. Applications of the Anammox process

The implementation of the Anammox process in different systems is presented in Table 7. It can be noticed that the highest nitrogen removal capacity was obtained in the gas-lift reactor with granular sludge (Sliekers et al., 2003) and amounted to 8.9 kg N m\textsuperscript{-3} d\textsuperscript{-1}. This was, however, shown only in a laboratory-scale pilot plant. Jetten et al. (1997) reported a high maximum nitrogen removal capacity in a fluidised-bed reactor as 2.6 kg N m\textsuperscript{-3} d\textsuperscript{-1}. Reactors with very efficient biomass retention need to be applied in order to mitigate the slow biomass yield of the Anammox bacteria. The above systems are especially suitable, but also a sequencing batch reactor (SBR) with the activated sludge has also been studied frequently (Strous et al., 1998; Fux et al. 2002; Fux, 2003). Dapena-Mora et al. (2005) performed also experiments on upgrading the SBR reactors by placing inside an internal hollow fibre membrane module or zeolite carrier materials. Furthermore, Hassanzadeh (2005) suggests the combined use of partial nitritation/Anammox and ion exchange or precipitation of magnesium ammonium phosphate.

Interestingly, van Dongen et al. (2001a) argues that biofilm (packed or moving-bed) and granular sludge reactors are the most appropriate alternative for the implementation of the Anammox process in full scale. In both types of reactor configuration, the pre-separation of the input suspended sludge is recommended.

Moving-bed bioreactors with biofilm Kaldnes carriers were applied as well (Beier et al., 1998; Hippen et al., 2001; Plaza et al., 2002; Trela et al., 2004a, 2004b; Rosenwinkel and Cornelius, 2005). Independent research centres obtained high specific nitrogen removal rates oscillating around 2 g N m\textsuperscript{2} d\textsuperscript{-1} (Beier et al., 1998; Helmer et al., 2000; Hippen et al., 2001; Seyfried et al., 2001; Szatkowska, 2004; Cema et al., 2005a). The AnoxKaldnes group (the AnoxKaldnes group web page) estimates the specific effective biofilm surface as 500 m\textsuperscript{2} m\textsuperscript{-3} (Kaldnes rings are 9.1x7.2 mm). van Dongen et al. (2001a) suggest that it is possible to decrease the reactor volumes with the
Table 7. Summary of different nitrogen removal systems with the use of the Anammox process (nd - no data).

<table>
<thead>
<tr>
<th>System type/scale</th>
<th>Influent</th>
<th>Support material/type of biomass</th>
<th>Nitrogen loading rate</th>
<th>Maximum nitrogen removal capacity</th>
<th>Specific reaction rates</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic medium</td>
<td>Glass beads/biofilm</td>
<td>1.3 kg N/m³ d</td>
<td>1.1 kg N/m³ d</td>
<td>nd</td>
<td>Strous et al. (1997)</td>
<td></td>
</tr>
<tr>
<td>Fixed-bed reactor (FBR)/lab-scale</td>
<td>Synthetic medium</td>
<td>Biofilm FBR 1 PCV 250 m³/m³</td>
<td>0.08-0.42 kg N/m³ d</td>
<td>0.35 kg N/m³ d</td>
<td>nd</td>
<td>Fux et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>FBR 2 PCV 250 m³/m³</td>
<td>0.07-0.55 kg N/m³ d</td>
<td>0.38 kg N/m³ d</td>
<td>3.5 kg N/m³ d</td>
<td>Fux et al. (2004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FBR 3 PP 90 m³/m³</td>
<td>0.14-0.44 kg N/m³ d</td>
<td>0.35 kg N/m³ d</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic medium</td>
<td>Sand/biofilm</td>
<td>1.0 kg N/m³ d</td>
<td>1.8 kg N/m³ d (0.8 kg NH₄⁺-N/m³ d)</td>
<td>0.18 kg N/kg VSS d</td>
<td>Strous et al. (1997)</td>
<td></td>
</tr>
<tr>
<td>Fluidised-bed reactor/lab-scale</td>
<td>Sludge digestion effluent</td>
<td>Sand/biofilm</td>
<td>1.2 kg N/m³ d</td>
<td>1.5 kg N/m³ d (0.7 kg NH₄⁺-N/m³ d)</td>
<td>0.15 kg N/kg VSS d</td>
<td>Strous et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Synthetic medium/sludge liquor</td>
<td>Sand/biofilm</td>
<td>0.2 – 2.6 kg N/m³ d</td>
<td>5.1 kg N/m³ d</td>
<td>0.04 - 0.26 kg N/kg SS d</td>
<td>Jetten et al. (1997, 1998)</td>
</tr>
<tr>
<td>Fluidised-bed reactor/full-scale</td>
<td>Baker yeast plant effluent</td>
<td>Sand/biofilm</td>
<td>nd</td>
<td>1.5 kg N/m³ d (0.4 kg NH₄⁺-N/m³ d)</td>
<td>nd</td>
<td>Mulder et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Leachate PVC disc/biofilm</td>
<td>1.4 – 3.2 g N/m² d</td>
<td>nd</td>
<td>0.4 – 1.2 g N/m² d</td>
<td>Siegrist et al. (1998)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leachate PVC disc/biofilm</td>
<td>1.5 – 3.3 g N/m² d</td>
<td>nd</td>
<td>nd</td>
<td>Hippen et al. (2001)</td>
<td></td>
</tr>
<tr>
<td>Rotating biological contractor/full-scale</td>
<td>Synthetic medium PVC disc/biofilm</td>
<td>2.3 g N/m² d</td>
<td>nd</td>
<td>1.55 g N/m² d</td>
<td>Pynaert et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>Moving-bed system/pilot-scale</td>
<td>Sludge liquor</td>
<td>Kaldnes rings/biofilm</td>
<td>4.8 g N/m² d</td>
<td>nd</td>
<td>2.2 g N/m² d</td>
<td>Hippen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Sludge liquor</td>
<td>Kaldnes rings/biofilm</td>
<td>4.6 g N/m² d</td>
<td>nd</td>
<td>2.0 g N/m² d</td>
<td>Beier et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Sludge liquor</td>
<td>Kaldnes rings/biofilm</td>
<td>4.8 g N/m² d</td>
<td>nd</td>
<td>2.0 g N/m² d</td>
<td>Seyfried et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Sludge liquor</td>
<td>160 kg d⁻¹</td>
<td>nd</td>
<td>2.0 g N/m² d</td>
<td>Rosenwinkel et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>Moving-bed system/lab-scale</td>
<td>Partial nitritation effluent/sludge liquor</td>
<td>Kaldnes rings/biofilm</td>
<td>0.5 – 2.3 g NH₄⁺-N/m² d</td>
<td>nd</td>
<td>0.6 – 2.3 g NH₄⁺-N/m² d</td>
<td>Szatkowska (2004)</td>
</tr>
<tr>
<td>SBR/pilot-plant scale</td>
<td>'partial' Sharon effluent/sludge liquor</td>
<td>Granular sludge</td>
<td>1.2 kg N/m³ d</td>
<td>0.75 kg N/m³ d</td>
<td>0.18 kg N/kg TSS d</td>
<td>van Dongen et al. (2001b)</td>
</tr>
<tr>
<td></td>
<td>Partial nitritation effluent/sludge liquor Activated sludge</td>
<td>nd</td>
<td>2.4 kg N/m³ d</td>
<td>0.3 kg N/kg TSS d</td>
<td>Fux et al. (2002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Activated sludge</td>
<td>nd</td>
<td>0.8 – 0.9 kg N/m³ d</td>
<td>0.12 – 0.16 kg N/kg TSS d</td>
<td>Fux et al. (2003)</td>
<td></td>
</tr>
</tbody>
</table>
application of the granular sludge as the specific surface of granules is 2000 m² m⁻³. High nitrogen elimination has also been feasible in fixed-bed reactors (FBR) with the polyvinyl chloride (PCV) carrier material (Fux et al., 2004) or glass beads (Strous et al., 1997). Other designs for Anammox reactors take advantage of the nitrogen gas produced during the process. A reactor analogous to a UASB (upflow anaerobic sludge blanket) reactor could be applied and nitrogen gas could be used for mixing. Surmacz-Górška et al. (2003) and Dapena-Mora et al. (2005) presented the possibility of attaining the Anammox process in membrane-assisted bioreactors.

Systems for the Anammox process can be used for the treatment of many types of high strength ammonia wastewater among which supernatant and leachate streams are the most appropriate. Both media have a comparable composition so can be treated by the same processes. A low value for the average biodegradable COD/N ratio is characteristic for the supernatant and leachate (leachate has

Table 7. Summary of different nitrogen removal systems with the use of the Anammox process (nd- no data) (contd).

<table>
<thead>
<tr>
<th>System type/scale</th>
<th>Influent</th>
<th>Support material/ type of biomass</th>
<th>Nitrogen loading rate</th>
<th>Maximum nitrogen removal capacity</th>
<th>Specific reaction rates</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBR/lab-scale</td>
<td>Synthetic medium</td>
<td>Activated sludge/Activated sludge+zeolite carrier material</td>
<td>40-150 g N/m³ d</td>
<td>52-130 g N/m³ d</td>
<td>0.30-0.34 g N/gVSS d</td>
<td>Dapena-Mora et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Synthetic medium</td>
<td>Granular sludge</td>
<td>0.75 g N/m³ d</td>
<td>nd</td>
<td>0.65 kg N/kg TSS d</td>
<td>Dapena-Mora et al. (2004)</td>
</tr>
<tr>
<td>Membrane-assisted bioreactor</td>
<td>Synthetic medium</td>
<td>Activated sludge in a SBR reactor</td>
<td>nd</td>
<td>100 g N/m³ d</td>
<td>0.24 g N/gVSS d</td>
<td>Dapena-Mora et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Synthetic medium</td>
<td>Activated sludge</td>
<td>0.05-0.08 g NH₄-N/MLSS d</td>
<td>nd</td>
<td>nd</td>
<td>Surmacz-Górška et al. (2003)</td>
</tr>
<tr>
<td>Up-flow reactor:</td>
<td>Synthetic medium</td>
<td>Granular sludge</td>
<td>nd</td>
<td>2.9 kg N/m³ d</td>
<td>nd</td>
<td>Imajo et al. (2004)</td>
</tr>
<tr>
<td>• Pilot-plant</td>
<td></td>
<td></td>
<td>nd</td>
<td>6.4 kg N/m³ d</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>• Lab-scale</td>
<td>Synthetic medium</td>
<td>Granular sludge</td>
<td>2.0 g N/m³ d</td>
<td>nd</td>
<td>1.15 kg N/kgTSS d</td>
<td>Dapena-Mora et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Synthetic medium</td>
<td>Granular sludge</td>
<td>10.7 kg N/m³ d</td>
<td>8.9 kg N/m³ d</td>
<td>nd</td>
<td>Sliekers et al. (2003)</td>
</tr>
<tr>
<td>Gas-lift reactor/lab-scale</td>
<td>Synthetic medium</td>
<td>Granular sludge</td>
<td>1.02 kg N/m³ d</td>
<td>0.7 kg N/m³ d</td>
<td>0.08 kg N/gVSS d</td>
<td>Ahn et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Synthetic medium</td>
<td>Granular sludge</td>
<td>0.52 kg N/m³ d</td>
<td>0.14 kg NH₄-N/m³ d</td>
<td>nd</td>
<td>Schmidt et al. (2004)</td>
</tr>
<tr>
<td>UASB/lab-scale</td>
<td>Piggery waste</td>
<td>Granular sludge</td>
<td>1.36 kg N/m³ d</td>
<td>0.72 kg N/m³ d</td>
<td>0.09 kg N/gVSS d</td>
<td>Hwang et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Effluent from a paper mill factory</td>
<td>Granular sludge</td>
<td>1.02 kg N/m³ d</td>
<td>0.7 kg N/m³ d</td>
<td>0.08 kg N/gVSS d</td>
<td>Ahn et al. (2004)</td>
</tr>
<tr>
<td>Granular up-flow reactor/lab-scale</td>
<td>Piggery waste</td>
<td>Granular sludge</td>
<td>1.36 kg N/m³ d</td>
<td>0.72 kg N/m³ d</td>
<td>0.09 kg N/gVSS d</td>
<td>Hwang et al. (2004)</td>
</tr>
<tr>
<td>Bench-scale upflow biological aerated filter (BAF)</td>
<td>Synthetic medium</td>
<td>Granular lava media</td>
<td>1.1 kg NH₄-N/m³ d</td>
<td>nd</td>
<td>nd</td>
<td>Wang et al. (2004)</td>
</tr>
</tbody>
</table>
only a periodically increased COD content), which makes traditional nitrification/denitrification treatment inapplicable or too expensive. Rotating Biological Contractor (RBC) systems with a biofilm bacterial growth have been mainly used for treatment of landfill leachates in the deammonification process (Hippen et al., 2001). As for the supernatant, new biological methods have recently been put in deployment. Among these, the applications of the Anammox process are the most promising for the future. In the supernatant treatment, the investigated systems were both for the activated sludge growth, e.g. in the SBR reactor (Fux, 2003) and for the biofilm development, e.g. in a Moving Bed™ Biofilm Reactor (Beier et al., 1998; Płaza et al., 2003a,b; Szatkowska, 2004).

3.5. Modelling of the systems with biological wastewater treatment

The modelling, optimisation and simulation of biological nitrogen removal processes has been the subject of many publications (Finnson, 1994; Jeppsson, 1996; Gujer et al., 1999; Ekman, 2005; Samuelsson, 2005; Van Hulle, 2005). An array of tests for the determination of stoichiometric and kinetic parameters for the microbial conversions (model calibration) is a necessity in constructing models for a full-scale process design (Henze et al., 1987). A reliable and validated model is a tool for simulating the different configurations of a biological system. Modelling tools can be used at different stages in the process development. They can be applied in advance of implementing a process in the full scale as well as can contribute to the optimisation strategy of an existing system. To give an example, Finsson (1994) investigated the usefulness of a computer simulation model at a full-scale activated sludge biological WWTP in Sweden. There were also studies using models to describe the biological processes in biofilms (Koch et al., 2000; Kreft et al., 2001).

In research concerning the novel processes of nitrogen removal from wastewater, the modelling tools were applied for the process design and simulation (Van Hulle et al., 2003; Van Hulle, 2005). In the case of the SHARON process, the insight gained by simulations resulted in removing the pilot plant tests and allowed a direct construction of the process in the full-scale (Hellöga et al., 1999; Mulder et al., 2001). The SHARON process modifications to couple with the Anammox process for treatment of ammonium-rich streams have also been modelled (Vołcke et al., 2002b; Van Hulle, 2005). What is more, the continuously aerated ‘partial’ SHARON reactor was examined by establishing a reliable model and simulating the behaviour of the process (Vołcke et al., 2002a, 2003). The influence of temperature and pH parameters on obtaining the desired nitrite-to-ammonium ratio in the effluent and the prevention of toxic nitrite concentrations was shown.

The partial nitritation process was also modelled in nitrifying membrane-assisted bioreactor (MBR) treating the digester supernatant (Wyffels et al., 2004). The modelling of the process start-up as well as the effects of changes in the process parameters allowed for further optimisations of the oxygen-limited partial nitritation process. It was argued that the modelling could provide a tool for scaling-up the process by performing scenario the analyses during simulations.

A mathematical model to evaluate the influence of ammonium surface load (ASL) and the temperature on a fully autotrophic nitrogen removal process in an aerated biofilm CANON reactor was studied by Hao et al. (2002a,b) and Hao and van Loosdrecht (2004). The ASL was associated with the biofilm thickness. Simulations with different loading rates (0.25-4 g NH4-N m-2 d-1) were run at a constant temperature of 20°C and a fixed biofilm depth of 0.7 mm. It has been proven that a thin biofilm has limited capacity for the activity of the Anammox process. At a constant temperature and defined ASL, there is always an optimal biofilm depth to achieve the maximum ammonium nitrogen removal efficiency. Alternatively, at a defined biofilm depth a lower temperature requests a lower ASL and a lower DO for a better nitrogen removal. Relatively high nitrogen removal efficiency along with the variable
ASL can be achieved in practice by controlling the dissolved oxygen concentration exactly on the requirement of the momentary ammonium load. On the other hand, at the defined ammonium surface load, a lower temperature needs a thicker biofilm and hence a higher DO concentration to maintain the nitrogen removal efficiency at a high level. Therefore, for the full-scale application, a careful control of the dissolved oxygen concentration in the bulk liquid along with a variable ASL in biofilm systems is obligatory to achieve high nitrogen removal efficiency.

A separate issue concerning modelling of the biological process is the area of a multivariate data analysis (MVDA). So-called projection methods (Eriksson et al., 2001) were studied extensively with the purpose of modelling the wastewater treatment systems. In the case of the biological processes, the MVDA approach is relevant due to the existence of the correlated variable groups and the necessity of understanding the covariances between them. Mossakowska (1994), van Dongen and Geuens (1998), Hallin (1998), Tomita et al. (2002), Miettinen et al. (2004) and MacGregor et al. (2005) studied the multivariate monitoring and the analysis and control of biological wastewater treatment processes. Aguado et al. (2005) presents a study of the MVDA methodology for detecting operational shifts in an SBR process. In the applied technology, the multivariate monitoring of variables describing large and correlated time series provide an insight into the historical data as well as the prediction of the effluent quality in terms of changing the design or the operational scheme.

4. METHODOLOGY

The deammonification system for nitrogen removal has been investigated at the technical-scale pilot plant at the Himmerfjärden WWTP, Grödinge, Sweden. In this section, a summary of the studies performed, the used materials and the experimental procedures will be presented. Further details are given in Papers I-IV. An extended description of the modelling tool used in Paper IV is included in this chapter. The chapter also includes a typical batch test procedure.

4.1. Pilot plant description

The technical-scale pilot plant was constructed by the PURAC Company and is located at the Himmerfjärden WWTP (SYVAB AB), southwest of Stockholm by the Himmerfjärden bay. It was continuously fed with a supernatant from the dewatering of a digested supernatant. A detailed description of the sludge handling at the Himmerfjärden WWTP can be found in Harabasz (2004). The system that was preceded by a buffer tank (0.8 m$^3$) consisted of two reactors in series (2.1 m$^3$ each), followed by settling tanks (0.125 m$^3$ each). The pilot plant was designed as a Moving Bed™ Biofilm Reactor (MBBR) and filled up to 45-50% with Kaldnes® carriers (AnoxKaldnes Company, the AnoxKaldnes group web page). The first reactor (R1) was operated to obtain a partial nitritation process whereas in the second one (R2) an Anammox process was established. The system was therefore named the two-step partial nitritation/Anammox. Both reactors were divided into three zones equipped with a mechanical vertical mixer (two-blade propeller) and a blower that was used only in R1. The first zone of each reactor had a heater installed. The pH correction in zone 1 of R2 could be done continuously with the use of an on-line pH-electrode and was applied at the beginning of the experiments. The pilot plant was built inside of a purposely-furnished container fully equipped with manual and on-line instruments such as a pH-meter, a dissolved oxygen meter, a conductivity meter, and a thermometer to control the process and collect the data.

The minimum efficient surface of the biofilm on Kaldnes rings (K1) was estimated as 500 m$^2$ m$^{-3}$ in the bulk liquid. Therefore, the filling rate results in an average 500 m$^2$ of an active biofilm surface in each reactor. The Kaldnes biofilm carrier elements K1 are made from polyethylene (PEHD). The material has a specific weight of about 0.95 kg l$^{-1}$, thus it floats in water. The density of the biofilm carrier elements in bulk is 160 kg m$^{-3}$.

The biofilm carrier elements are formed as tubes with an internal cross, and with 18 external fins with nominal diameter of 9.1 mm and a nominal length of 7.2 mm.
4.2. System configurations and operational approach

Figures 1 and 2 show the technical-scale pilot plant configurations and operational changes. The pilot plant was operated at subsequent aerobic and anaerobic conditions to obtain the two-step process. The reactors were initially operated separately for 1 year to develop nitrifying and Anammox culture in reactors 1 and 2, respectively (Horeglad, 2001; La Rocca, 2001). When the reactors were connected, the influent load to the Anammox reactor was increased during the June – August 2003 and February – July 2004 periods (Paper III). Stepwise decreases of the dilution rate of the influent flow to the Anammox reactor (Figure 3) and changes in the hydraulic retention time (Table 8) were the strategy aimed at varying loadings. The internal recirculation was introduced with the goal of substituting cold tap water used for the dilution of the influent to R2 with the effluent from R2. This strategy was discarded due to the temporal Anammox process inhibition in March 2005. It was assumed that toxic metabolites were accumulating in R2. Another strategy was implemented in April 2005. It consisted of the external recirculation of the system effluent to the first zone of R1 and simultaneous switching-off the aeration in that zone. The aim of this strategy}

Fig. 1 Pilot plant configurations; R1-partial nitritation reactor, R2-Anammox reactor (based on Trela et al., 2004d, 2005).

<table>
<thead>
<tr>
<th>Changes in parameters</th>
<th>Average temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH correction in R2</td>
<td>around 35°C</td>
</tr>
<tr>
<td>No aeration in R1</td>
<td>around 30°C</td>
</tr>
</tbody>
</table>

Fig. 2 Operational strategies for the partial nitritation/Anammox system over the period May 2003 – April 2005 (based on Trela et al., 2004d, 2005).
was to denitrify the nitrate nitrogen generated in the Anammox process.

Additionally, in 2005 the temperature in the system was gradually decreased from 35°C to 30°C (Figure 2) down to 27°C on average. Furthermore, the pH correction in the Anammox reactor was stopped in May 2004. This strategy resulted in savings of the chemicals without negative impacts on the process performance.

4.3. Measurements and analytical procedures

Table 8 shows a summary of the methods used in this study. The results from measurements, analyses and the derived variables presented in Table 10 were used in the papers appended. The grab samples taken from different points of the pilot plant were immediately filtrated with a 25-mm prefilter and a 0.45-μm filter and analysed. The equipment for the performance of measurements and analyses is shown in Table 11. The Sludge Volume Index (SVI) was determined after 30 minutes of sludge settling by measuring the volume occupied by the sludge.

The two-step process was operated steadily for 2 years (Paper I, III, IV). A substantial nitrogen removal observed in R1 in May 2005 directed the research towards investigating the reaction rates in R1 whilst maintaining the Anammox culture in R2.

### Table 8. Hydraulic retention time in the pilot plant operated in different configurations in the period 2003-2005.

<table>
<thead>
<tr>
<th>Date</th>
<th>Average HRT (d)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
<td></td>
</tr>
<tr>
<td>28 Apr – 31 Jun 2003</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1 July – 25 Jul 2003</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>26 Jul –29 Sep 2003</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>30 Sep - 17 Oct 2003</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>18 Oct 2003 – 31 April 2005</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

### Table 9. Summary of the methods and tools used in Papers I-IV and chapter 5 of the thesis.

<table>
<thead>
<tr>
<th>Method/tool</th>
<th>Papers:</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Chapter 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen Uptake Rate (OUR) tests</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch tests</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate data analysis</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Multivariate data analysis</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pilot-plant operation</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Two-step system</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Table 10. Overview of the measurement and analyses performed during pilot plant operation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Measurements (Weekdays)</th>
<th>Analyses (Different time spans)</th>
<th>Derived variables (Different time spans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent characteristics</td>
<td>pH, conductivity, temperature</td>
<td>NH₄-N, alkalinity, COD, PO₄-P, SS (total suspended solids), VSS (volatile suspended solids)</td>
<td>Flow rate (Weekdays), Load</td>
</tr>
<tr>
<td>Reactor 1 (R1) Zone 1, 2, 3 and out</td>
<td>pH, conductivity; only in zones: temperature, dissolved oxygen (DO)</td>
<td>NH₄-N, NO₂-N, NO₃-N, alkalinity, COD, PO₄-P, organic acids, profiles of N₃org forms, SS, VSS, SVI (Sludge Volume Index)</td>
<td>Flow rate (Weekdays), HRT, Load, NH₄, HNO₂, NO₂-N/NO₃-N, NH₄-N ratio</td>
</tr>
<tr>
<td>Reactor 2 (R2) Influent, zone 1, 2, 3 and out</td>
<td>pH, conductivity; only in zones: temperature, DO</td>
<td>NH₄-N, NO₂-N, NO₃-N, alkalinity, COD, PO₄-P, organic acids, profiles of N₃org forms, SS, VSS, SVI</td>
<td>Flow rate, dilution rate, HRT, Load, HNO₂ concentration, NO₂-N/NH₄-N ratio</td>
</tr>
</tbody>
</table>

Table 11. Equipment for measurements and analyses performance (N/A – not adequate).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Manual equipment</th>
<th>On-line equipment</th>
<th>Analytical devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>pH meter model WTW pH330</td>
<td>Analon pH 10, Contronic (HACH-Lange AB)</td>
<td>N/A</td>
</tr>
<tr>
<td>Temperature</td>
<td>Thermometer Hanna model HI9063</td>
<td>Analon pH 10, Contronic (HACH-Lange AB)</td>
<td>N/A</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Conductivity meter: HACH44600</td>
<td>Dr Lange Analon Cond 10</td>
<td>N/A</td>
</tr>
<tr>
<td>Dissolved oxygen (DO)</td>
<td>Oxygen meter YSI52CE</td>
<td>Cerlic BB2 - O2X</td>
<td>N/A</td>
</tr>
<tr>
<td>NH₄-N, NO₂-N, NO₃-N, alkalinity, COD, PO₄-P, organic acids</td>
<td>N/A</td>
<td>N/A</td>
<td>TECATOR-AQUATEC 5400 Analyser, Dr.Lange VIS Spectrophotometer XION 500, spectrophotometer HACH model DR/2010 (only COD)</td>
</tr>
<tr>
<td>Suspended Solids (SS)</td>
<td>Cylinder and stopper (SVI)</td>
<td>N/A</td>
<td>Vacuum filtration apparatus connected with a plate supporting the glass fibre (SS, VSS)</td>
</tr>
<tr>
<td>Suspended Solids (VSS)</td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Sludge Volume Index (SVI)</td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

4.4. Batch tests

To follow the reaction rates in the pilot-plant reactors some batch tests were performed. The test execution methodology was developed by Gut (2003) and extended by Gut and Plaza (2003), Szatkowska et al. (2003a,b; 2004a), Szatkowska (2004), Siembida (2004), Cema et al. (2005a,b) and Mele (2005).

Each batch test was run in a 1-litre bottle 50%-full with Kaldnes rings with the biofilm biomass and poured over with the supernatant from R2. On test days, the biomass was taken from the Anammox reactor of the continuously working pilot plant, put into batch vessels and placed in a water bath to keep the temperature as in the pilot plant. The covered test vessels were equipped with magnetic slow-stirring implements. Measurements of pH value, DO concentration and conductivity were performed and samples were taken. The test lasted 8 hours on average with hourly sampling patterns.

4.5. Oxygen Uptake Rate (OUR) tests

A methodology to execute an Oxygen Uptake Rate (OUR) test for biofilm bacterial cultures on Kaldnes has been developed (Paper I). The method included a modified procedure of the OUR tests for activated sludge proposed by Surmacz-Górska et al. (1996). Measurements of the dissolved oxy-
gen concentration during its uptake by the biofilm bacterial culture and during the subsequent addition of selective inhibitors of the nitrifying bacterial populations were carried out. The sodium chlorate (NaClO₃) inhibited the nitrite oxidation by *Nitrobacter* species whereas allylthiourea (ATU) inhibited the nitritation process. The respiratory activity of the heterotrophs was also calculated as the remaining oxygen uptake after the addition of a dose of the ATU. This method, however, does not allow for distinguishing between the oxygen consumption for the substrate oxidation and the endogenous respiration. For a more detailed description refer to Długolećka (2004).

An emphasis has been put on preparing the equipment for the OUR test, adjusting the optimal conditions during the test runs, selecting the optimal amount of Kaldnes carriers and checking the inhibitory concentrations of NaClO₃ and ATU (Paper I). Second, the nitrifying activity has been assessed in the partial nitritation reactor (Papers I and II) and in the Anammox reactor (Paper II). The proposed test procedure was successfully verified (Paper I and II). The inhibiting effect with 100 Kaldnes carriers has been achieved. 17 mmol l⁻¹ as a final concentration of NaClO₃ solution and 43 μmol l⁻¹ as a final concentration of ATU solution were injected into the bottle when the dissolved oxygen level was at 4 and 2-3 mg O₂ l⁻¹, respectively. Papers I and II present comprehensive descriptions of the test procedures.

4.6. Modelling of the process data with the SIMCA-P software

The multivariate data analysis (MVDA) is designed to extract information from a data set with the purpose of interpreting covariances and patterns in the variables. An overview of the multivariate character of the data can be obtained by the Principal Component Analysis (PCA) method (Wold et al., 1987). This method can discern deviations in the data set and gain an understanding of the relationship between variables. The PCA method can also reveal groupings amongst variables. The groups can be used as the established class models for classifying new observations. The PCA modelling has been extended by finding covariations in two blocks of data, denoted as X and Y with the aim of predicting Y from X for new observations (Wold et al., 2001). This method is called the Partial Least Squares projections to latent structures (PLS) and can be treated as a regression modelling tool for assessing how the factors in the X block influence the responses in Y, finding collineation and adjusting factors to get the desired profile for the responses. The nomenclature used for the PCA and PLS modelling approach together with the geometric representation of the methods is presented in Paper IV.

The PCA method has been used in Paper IV to show how the observations are related and hence find any group of observations that deviate or form separate classes. In the PCA approach, a line (or a component) is fitted in the direction of the greatest variability of the measured variable space. Then, the second line is approximated in the next greatest direction of the variability orthogonal to the first component; hence, a plane is obtained. If necessary, the subsequent lines orthogonal to the plane are found. During this process the goodness of prediction is simultaneously computed by a cross-validation method. This is repeated until no systematic variability remains. A trade-off between the explained and predicted variation determines the number of components. For details refer to Eriksson et al. (2001).

The most important difference between the PCA and PLS methods is that PCA describes the maximum variance in a least square projection of X, whereas PLS is a maximum covariance model of the relationship between X and Y (Eriksson et al., 2001). The importance of a given X-variable for Y, where Y can be a single variable or a block of variables, is computed as PLS regression coefficients (bₘₖ). This expresses the relation between the Y variables and all the terms in the model. The measure of a variable importance is identified by the large absolute values in the PLS regression coefficients. If a variable is important for the modelling of X (large loadings, pₘₖ), a variable importance for the projection (VIP) function should be used. The
VIP summarizes the importance of an X-variable for both variable data blocks X and Y. After the inspection of the regression coefficients and VIP plots, it can be decided to exclude unimportant variables.

In the Paper IV, the SIMCA-P package was used to perform the MVDA (Umetrics AB, Umetrics AB web page). All variables were pre-processed using the mean centring and auto scaling to unit variance. The scaling function gives variables equal influence in the model. It is necessary that variables are not expressed in different units and display substantially different numerical ranges. In this study, the data were both mean centred and scaled to unit variance (autoscaled). Additionally, the data excluded from the model form the residuals matrix and are of diagnostic interest (Wold et al., 2001). A Distance to Model in X-space (DModX) value is used to inspect the model residuals and enables to detect outliers in the X-space, i.e. process points that deviate from the stable operation of the process. In case of the PLS-regression modelling, the residuals of a Y-block should form a straight line in a normal probability plot. If a curvature is detected, the plot may be improved by transforming parts of the data, e.g. by using a logarithmic function. A normal distribution of the data for a single variable is the goal in the transformation procedure.

The validation of an output model is done by means of cross-validation (CV), response permutation testing for checking the statistical significance of the prediction in case of PLS modelling. An external validation can be also applied and consists of using a test data set that is obtained through the use of the multivariate design. The CV method for validation is commonly used in the SIMCA-P software and is applicable in both PCA and PLS approaches. Dividing the data into a number of groups, usually from five to nine, and developing a number of parallel models for each of the group deleted perform the CV validation.

5. RESULTS AND DISCUSSIONS

5.1. Bacterial identification and activity

5.1.1. FISH tests

The Fluorescence In Situ Hybridisation (FISH) is a technique that can be used to detect specific groups of microorganisms. It uses fluorescent probes that only bind selectively to the 16S rRNA. As a result of using probes for particular bacteria, it is possible to detect individual cells of a specific type of microorganisms. The FISH method is a useful tool in molecular ecology. Individual bacteria of targeted species are detected microscopically, located and quantified in the

*Fig. 4 Results from FISH analyses (photos Wouter van der Star).*
background of a complex population.

By courtesy of the research group from the Delft University of Technology, Kluuyver Laboratory for Biotechnology, the Netherlands, the FISH technique was applied to the samples taken from both reactors of the pilot plant in June 2004. Figure 4 shows the results from the FISH analyses. Only the biofilm bacteria were the subjects to the analyses; the formamide concentration was 35% at the hybridisation temperature 47°C.

The partial nitritation reactor was tested for the presence of nitrifiers. The sample from the biofilm mainly hybridised with the NSE-1472 probe, Figure 4A. This probe is very specific for *Nitrosomonas europeae*, *Nitrosomonas eutropha* and *Nitrosomonas halophila*, meaning that one of these three organisms is dominant in the nitrifying population. It was analysed that there are some Anammox bacteria present also in the partial nitritation reactor.

It has been proved that the population in the Anammox reactor indeed contained Anammox bacteria. The AMX-820 probe hybridises both with *Brocadia anammoxidans* and *Kuenenia stuttgartiensis* (Figure 4B). Therefore, it was not determined during these investigations which of these Anammox species are present. The Anammox population was roughly estimated as 20-30% of the whole bacterial community in the Anammox reactor.

Later tests performed in June 2005 by a research group of the Gothenburg University, Department of Chemistry, Sweden (Hulth, 2005) confirmed that *Brocadia anammoxidans* is present in the Anammox reactor. The BAN-162 probe was used to assert the presence of this bacteria strain.

### 5.1.2 Application of OUR tests

Paper I focused on establishing a reliable monitoring tool for the assessment of the nitrifying activity in the biofilm partial nitritation reactor. It was demonstrated that 100 Kaldnes carriers are an optimal number for achieving reliable results. Separate tests verified that the 17 mmol l⁻¹ final concentration of NaClO₃ was adequate. The ATU final concentration was kept at 43 μmol l⁻¹, as recommended by Surmacz-Górska et al. (1996). Daily aeration of the media in the initial tests resulted in achieving the predominance of nitrite oxidation over the ammonia oxidation. The enhanced *Nitrobacter* activity was therefore induced by the aeration procedure before the test. Such behaviour of the bacterial population did not however occur in the partial nitritation reactor as the nitrite oxidation was suppressed (Paper I, II, III). The shortened aeration showed the prevailing *Nitrosonomas* group activity in all the zones of R1 with the zone 1 being the most active. In Paper I, the most adequate ammonia concentration for oxidation of ammonia only to nitrite was calculated in that zone.

The successful development of the OUR methodology enabled to check the presence of nitrifiers in the Anammox reactor and compare the nitrifying activity results between reactors in Paper II. The nitrifying activity in R1 was related to the biofilm on the Kaldnes rings (Paper I and II), whereas in R2 the presence of nitrifiers was proved to predominantly exist in the activated sludge (Paper II). Occurrence of the *Nitrosonomas* community in the activated sludge of the Anammox reactor is advantageous due to its contribution in supplying nitrite for the Anammox culture in the biofilm and sustaining oxygen-limited conditions (Paper II). The main findings show that *Nitrosonomas* species are more active than *Nitrobacter* bacteria in both reactors of the pilot plant. The nitrifying activity in the Anammox reactor should not, however, be overestimated. The activities in R1 are on average 20 times higher when compared with the biofilm culture and 10 times higher in the activated sludge from R1 (Paper II). Irrespective of the order of magnitude, the *Nitrosonomas* activity was 2.5 times higher than the *Nitrobacter* activity, measured as OUR in both reactors.

The monitoring potential of the OUR tests to reflect the changes in the activity profile of the nitrifying bacterial culture in R1 was shown in Paper I. Paper II demonstrated also the applicability of the OUR tests to detect changes in the nitrification activity over time. A variable nitrifying activity was expected as the result of changes in the moving-bed
system configuration. Nitrifying activity in the activated sludge of the Anammox reactor showed an increasing tendency but it was low in comparison with activities in R1 over one year.

Surmacz-Górska et al. (2003) also applied the OUR tests to measure the respiratory activity of the nitrifying activated sludge present in the membrane-assisted bioreactor with the Anammox process during its start-up. It was found that after the introduction of the Anammox process, the ammonia- and nitrite-oxidizing bacteria increased their activity. It was suspected that the Nitrosomonas-like bacteria could find more suitable conditions in a mixed biocenosis or changed their metabolism to be able to denitrify under anoxic conditions. The enhancement of the Nitrobacter-like bacteria activity can be explained by the high persistence of these bacteria in the activated system. Moreover, it was deduced that other groups of bacteria can have the same vulnerability to sodium chlorate and for this reason the activity results could be overvalued with regards to the activity in the reactor. It however gives some insight into the high nitrite-oxidizing bacteria activity presented in Paper I.

5.2. Factors affecting system efficiency

Table 12 shows the factors that were recognized as the most significant for proper operation of the two-step partial nitritation/Anammox system.

The stoichiometrical demand of supplying the Anammox reactor with a proper influent directs the reliability of efficient nitrogen removal towards the stable operation of the partial nitritation reactor (Papers III, IV). Formation of nitrite at the suitable rate with ammonium (a nitrite-to-ammonium ratio about 1.3) is a result of the interplay between influent ammonium nitrogen concentration, DO concentration in the bulk liquid and adequate pH drop for a given reactor configuration (Papers I, III, IV). Conductivity measurements are highly applicable for monitoring of both processes (Papers III, IV). In case of R1, the oxidation of ammonium and consumption of alkalinity are parallel to the conductivity decrease, whereas in R2 the transformation of formed ions into molecules results in a conductivity decrease at a stable rate. During the operation of the fully mixed Anammox reactor, monitoring of the effluent nitrite nitrogen concentration is obligatory (Paper III, IV) as it is an inhibitory compound for the Anammox culture. The results of the univariate data analysis presented in Paper III were confirmed in Paper IV by applying the multivariate approach to the same data set. The optimisation strategy of shortening the HRT, introducing internal and external recirculation as well as changes

Table 12. Recognition of factors affecting the deammonification system performance.

<table>
<thead>
<tr>
<th>Process</th>
<th>Factors</th>
<th>Paper</th>
</tr>
</thead>
</table>
| Partial nitritation | • Supernatant characteristics  
• Oxygen supply to nitritation reactor  
• Effluent nitrite-to-ammonium ratio  
• pH decrease and alkalinity consumption  
• Hydraulic retention time  
• Temperature  
• Free ammonia and free nitrous acid concentrations | I, III, IV |
| Anammox     | • The influent nitrogen load  
• Oxygen-limited conditions  
• Nitrite-to-ammonium ratio in the influent  
• pH increase  
• Nitrite nitrogen concentration in the reactor  
• Activity of the Anammox bacterial culture (reaction rates)  
• Hydraulic retention time  
• Temperature | III, IV |
in the temperature in the system was successful. It allowed for assessing the different two-step system configurations.

5.2.1. Supernatant characteristics

The characteristics of the influent supernatant are presented in Papers I and III. Comparing the results from these papers, the average ammonium nitrogen concentration was over 600 and 700 mg l$^{-1}$, respectively and for the whole period described it varied in between 270 and 920 mg l$^{-1}$. The ammonium nitrogen variability occurs at the same time as the changes in the alkalinity, which was also confirmed in studies by Szatkowska et al. (2005). Variability of the influent ammonium nitrogen concentration determines to a high extent the nitrite-to-ammonium ratio (Figure 5). Based on the results presented in Paper IV (Trial 1) the relationship takes into account the effect of a pH drop and demonstrates a necessity of coping with the variable concentration of the ammonium nitrogen. Moreover, the modelling results from Paper IV repeatedly confirmed the stability of the pH parameter in the influent. It did not greatly influence the models describing both the start-up period and the stable operation of the partial nitritation/Anammox system.

The average alkalinity/NH$_4$-N ratio was 1.1 (Paper I) and 1.6 (Paper III) for the described periods, respectively, confirms the excess of alkalinity in the digester supernatant to obtain a stable oxidation of half of the ammonium to nitrite. An average alkalinity/NH$_4$-N ratio for the experimental period presented in this thesis is 1.4. Fux (2003) reported an alkalinity/NH$_4$-N ratio of 1.2 in the digester supernatant, which is consistent with the results presented in Paper I. The mean COD/N ratio in the influent supernatant for the whole period amounted to 0.5, which proved that the traditional nitrification/denitrification treatment is inapplicable for the supernatant stream. The temperature range was 19-26°C and depended on seasonal changes. The organic acids concentration

![Fig. 5 Relationship between the influent ammonium nitrogen concentration, effluent (out) pH value and nitrite-to-ammonium ratio (NAR) in the partial nitritation reactor.](image)
fluctuated in the supernatant and for the whole period described was equal to 61 mg l$^{-1}$. This value suggests that the digesters were periodically overloaded with the biological sludge. The sludge generation pattern at the Himmerfjärden WWTP, the fermentation efficiency and operation of the sludge dewatering units affect the quality and quantity of the supernatant.

Conductivity changes at the inflow reflected the variability of the influent ammonium concentration, which is presented in Paper III and IV, and confirmed in the studies by Szatkowska et al. (2004b, 2005). Moreover, Fux (2003) reported that conductivity measurements could be used to follow the nitrogen concentration in an SBR cycle.

The analyses of the total suspended solids (SS) and volatile suspended solids (VSS) were introduced to control the supernatant quality. The average values at the influent to the pilot for the period described in Paper I were 173 mg SS l$^{-1}$ and 145 mg VSS l$^{-1}$ whereas for the whole evaluated period the average values were very similar (178 mg SS l$^{-1}$ and 152 mg VSS l$^{-1}$). Additionally, there were periodical discharges of a large quantity of sludge from the centrifuges that resulted in disturbances in the operation of the pilot plant. The concentration of the suspended solids in the influent was varying in the range 100-5000 mg SS l$^{-1}$ and the discharge of solids was removed in the buffer tank.

5.2.2. Partial nitritation process

Figure 6 presents conversions of the inorganic nitrogen forms in the partial nitritation reactor. The partial nitritation reactor was operated as a preceding step to remove nitrogen in the Anammox reactor. Other authors (van Dongen 2001a; Volecke et al., 2003; Fux, 2004) argue also that a stable partial nitritation is an essential prerequisite for the Anammox process. The initial underproduction of nitrite to supply to the Anammox reactor in the start-up period was exchanged in 2004 with a stable oxidation of more than half of ammonia to nitrite (NAR=1.2 as average value for the 2004) (Paper III). Until March 2005, NO$_3$-N concentration at the effluent was on average 16 mg l$^{-1}$, which confirms stability of the process. An increase of the nitrate nitrogen concentration in the end of the described period was caused by the external recirculation of the system effluent. Occasional losses of nitrogen were calculated through the whole experimental period (Paper I, II, III). Examples of routine profiles of inorganic nitrogen forms presented in Paper I and II demonstrated that the most robust nitritation occurred in zone 1 and 2 of the reactor.

Seeding with the Anammox in the end of the described period and no aeration in zone 1

![Fig. 6 Variations of nitrogen forms in the partial nitritation reactor (based on Trela et al., 2004d, 2005).](image-url)
resulted in high nitrogen removal and changes in the distribution of the nitrifying activity in the system (Paper II). As a result of changes, a substantial nitrogen removal in R1 was obtained in the period May-August 2005 in oxygen-limited conditions as well as aerobic conditions. This phenomenon is now subjected to further investigations. Rosenwinkel et al. (2005) described a similar trend. Unscheduled deammonification in the nitrification reactor occurred at the full-scale deammonification plant at the Hattingen WWTP, Germany. As a consequence, the operation was changed into intermittent aeration to attain subsequent aerobic and anoxic periods for nitrification and Anammox, respectively, occurring in one reactor.

The mean concentration of SS in R1 for the whole period was equal to 294 mg l\(^{-1}\) with 85% of the organic part. The accumulation of the solid particles might have been due to hydraulic conditions in the reactor and occasional discharges of scum from centrifuges. The sludge volume index of the sludge generated in reactor 1 was checked to amount on average to 82 ml g\(^{-1}\), which indicates quite good settling properties.

Effluent nitrite-to-ammonium ratio (NAR) ratio

The importance of stable operation of the partial nitritation reactor with regard to the NAR influence on the nitrogen removal in the following step was emphasised in Papers I, III and IV. The results showed that the variability of the influent ammonium concentration could be coped with by adjusting the oxygen supply. On this basis, the aeration rate influences the pH drop. The drop of the pH value by the unit of 1.5 conditions the NAR equal to 1.3. Paper I and III describe successful operation of the reactor with regard to obtaining the NAR oscillating around 1.3.

pH parameter

A typical drop of the pH value during the nitritation process through using up the alkalinity and carbon dioxide stripping was confirmed in Paper I, III and IV. It appeared to be applicable to monitor the partial oxidation of ammonia to nitrite in the first reactor of the pilot plant and was included in the control-monitor system for the partial nitritation/Anammox system given by Szatkowska et al. (2005). The drop of pH value by the unit of 1.5 is sufficient to obtain the nitrite-to-ammonium ratio at the effluent around the required value of 1.3. The logarithmic correlation is characteristic for the pH parameter as the hydrogen ions are still produced when the buffering capacity is nearly depleted. Values lower than the pH value of 6 are not expected due to natural inhibition by free ammonia and free nitrous acid (Paper I). The results described by Fux (2003) concerning the drop of the pH value buffered by the alkalinity present in the supernatant to obtain the NAR of 1.3 was comparable with results presented in Paper I and III.

Hydraulic Retention Time (HRT)

In the partial nitritation reactor, the HRT fluctuated from 1 to 2 days and was changed in order to vary the influent load. The decrease of the HRT to 1 day did not substantially affect the system efficiency. However, during that time (July-September 2003) the average NAR was 0.8, which was caused by the underproduction of nitrite.
Temperature
The temperature parameter was kept in the range of 30-35°C for most of the period described in order to maintain stable oxidation of ammonia to nitrite (Paper III). Temperature was gradually lowered starting from February 2005 to reach values around 30°C in April 2005. It was aimed at operating the reactor without additional heat supply and making use of the natural temperature of the influent supernatant.

Free ammonia and free nitrous acid
Concentrations of free ammonia that exceeded 20 mg NH$_3$ l$^{-1}$ in the partial nitritation reactor did not cause inhibition of *Nitrosomonas* species (Paper I). This observation could signify the acclimation of ammonia-oxidizing bacteria as a result of long-term exposure to high free ammonia concentrations. Such observations should be expected in the partial nitritation reactors highly loaded with ammonium. Additionally, based on the results of routine profiles (Trela et al., 2004d, 2005) (examples presented in Paper I and II) performed through the whole operational period, it was calculated that free ammonia concentration was stratified along the zones with the average values of 22 mg NH$_3$ l$^{-1}$ (zone 1), 6 mg NH$_3$ l$^{-1}$ (zone 2) and 4 mg NH$_3$ l$^{-1}$ (zone 3). Free nitrous acid concentration was occasionally elevated up to 5 mg HNO$_2$ l$^{-1}$ in zone 2 and 3 of R1 that can be an additional inhibiting effect on further ammonia oxidation and suppression of nitrite oxidation. In Paper II, the correlation between the activity of ammonia-oxidizing bacteria and free ammonia concentration showed no short-term inhibition of ammonia oxidation during the OUR test performance. Fux (2003) calculated also nitrite nitrogen oxidation at levels as high as 80 mg NH$_3$ l$^{-1}$.

5.2.3 Anammox process
Figure 7 presents the results of the Anammox reactor performance over the investigation period. The initial total inorganic nitrogen concentration was gradually increased until the Anammox reactor capacity reached 0.9 g N m$^{-2}$ d$^{-1}$ in the initial months of the two-step system operation. Nitrite nitrogen concentrations exceeding 30 mg l$^{-1}$ appeared in the reactor at several instances and significantly affected the process. After process inhibition in August 2003, a slow enhancement of the reactor capacity was obtained during the year 2004. For the year 2004, the efficiency of the process was on average 84% (Paper III). The internal recirculation of the effluent started in November 2004 caused accumulation of the nitrate nitrogen, which was a probable cause of the process disturbance in March 2005. But no previous studies proved this effect (Strous et al., 1999; Fux,

![Fig. 7 Total inorganic nitrogen concentrations at the inflow and outflow of the Anammox reactor (based on Trela et al., 2004d, 2005).](image-url)
Stable nitrate nitrogen production at the rate \( \text{NO}_3^-/\text{NH}_4^+/\text{NO}_2^- \) around 0.6 (theoretical value of 0.11) confirmed that also a minor simultaneous nitrification could take place as the conditions were not strictly anaerobic. The presence of nitrifying activity in the suspension from the Anammox reactor was demonstrated in Paper II and confirms the previous calculations. Parallell removal of nitrite and ammonium nitrogen was analysed as well in Paper III. The average ratio \( \text{NO}_2^-/\text{NH}_4^- \) equal to 1.22 was calculated for the year 2004, which confirms the established Anammox process. The results are promising for the full-scale operation (Paper III and IV).

Total SS in the Anammox reactor established as a moving-bed reactor was high and was caused by the seeding with nitrifiers from R1 (Paper II). The changes in the hydraulics of the Anammox reactor resulted in fluctuations in the SS concentration from 65 to 6440 mg l\(^{-1}\) (53-89% of VSS). An average value of SVI amounting to 97 ml g\(^{-1}\) of the sludge accumulated in the Anammox reactor showed acceptable settling properties. To compare other values, Dapena-Mora et al. (2005) gives the SVI value of 123 ml g\(^{-1}\) to characterise an SBR Anammox biomass during the start-up phase. Wett (2005) showed also satisfying settling properties of the activated sludge in a SBR with SVI=116 ml g\(^{-1}\).

### Dissolved oxygen (DO) concentration

The oxygen-limited conditions were attained for the whole period described (Paper I, II, III). The DO concentration was on average 0.1 mg O\(_2\) l\(^{-1}\). Occasional enhancement of the DO concentration up to 0.5 mg O\(_2\) l\(^{-1}\) did not cause inhibition of the Anammox bacteria activity. The nitrifiers present in the activated sludge of the Anammox reactor (Paper II) coped with the increased oxygen concentration. A column installed after the partial nitritation reactor played a role of deoxidising the aerated liquid.

### Influent nitrite-to-ammonium ratio (NAR) ratio

Assessment of the stable operation of the Anammox reactor shown in Paper III demonstrated that the most efficient process performance (87% of process efficiency) was in the NAR range from 1.0 to 1.5. The concomitant removal of ammonium and nitrite for the results presented in Paper III did not deviate much from the theoretical value of 1.3, which confirmed the stable Anammox process. Moreover, modelling results presented in Paper IV asserted that the influent NAR is correlated well with high process efficiency. These outcomes of the study emphasise the importance of the partial nitritation process effectiveness.

### pH parameter

The pH value was initially corrected in the Anammox reactor by the addition of a base solution to keep the pH value around 8.2. According to the stoichiometry of the Anammox process, it was expected that the pH value increase in the Anammox process would compensate for the pH value decrease in the preceding step. Therefore in 2004, the experiment of ceasing the pH correction was performed. It appeared that the pH correction is not necessary in the system (Paper III). During highly efficient nitrogen removal the Anammox reactor was operated without the pH correction and during that time (2004) the pH increase between the influent and effluent by the unit of 1 was calculated. Similar to results presented in Paper III, Fux (2003) reported as well an increase of the pH value in the SBR with an established Anammox process. Wett (2005) demonstrated that a single-stage SBR system with the Anammox
process could be operated with an intermittent aeration controlled by the pH signal.

**NO$_2^-$N concentration in the reactor**

Over the period of the Anammox reactor operation the nitrite nitrogen concentration was maintained on average below 30 mg l$^{-1}$. An inhibition of the Anammox bacteria occurred when a long-term exposure to the concentrations above 100 mg l$^{-1}$ was analysed. The covariations between the variables describing the 20-month operation of the Anammox reactor presented in Paper IV showed that the enhanced nitrite nitrogen in the effluent decreases efficiency of nitrogen removal in the Anammox reactor. Paper IV scrutinized for the importance of monitoring the NO$_2^-$N concentration during the start-up period and the lessened inhibiting effect during the stable operation of the Anammox reactor. Fux (2003) demonstrated that the conductivity gradient between the cycles in the Anammox SBR reactor could be used as a warning indicator before nitrite nitrogen is measured in the effluent. Moreover, the author demonstrates that a temporal enhancement of nitrite nitrogen concentration up to 50 mg l$^{-1}$ did not cause long-term inhibition during the operation of an Anammox fixed-bed reactor. The exposure time seemed to have more importance in the nitrite inhibition of Anammox organisms.

**Hydraulic Retention Time (HRT)**

During the start-up period, the HRT was set at 3 days in order to ensure the efficiency of the process. The strategy of increasing loadings was done by the decrease of the HRT to 2 days in July 2003. It resulted in an overloading of the system and the inhibition of the Anammox culture by nitrite. As a result, the HRT was set again at 3 days and kept at that level until the end of the investigated period.

**Temperature**

The optimal temperature for the Anammox bacteria in the range of 30-35°C (Egli et al., 2001; Fux, 2003) was maintained in the Anammox reactor for most of the investigated period (Paper III). A decrease in the temperature value was imposed step-by-step in order to obtain values around 30°C in April 2005. The activity of the Anammox bacteria was not substantially lower due to changes. It brings possibilities of savings on heating.

**Presence of nitrifiers**

An initially assumed phenomenon of seeding the Anammox reactor with nitrifiers was proved in Paper II. Despite the fact that the Anammox reactor was established in a moving-bed system, the activated sludge gradually developed due to detachment of the biofilm. The nitrifying activity was mainly concentrated in the activated sludge present in the reactor. When the dissolved oxygen concentration in the suspension taken from the Anammox reactor was increased before the OUR test (Paper II), the nitrifying activity was analysed. This activity was on average 10 times lower than the activities in the partial nitritation reactor.

**5.2.4. Reaction rates**

Batch tests were performed to provide information on an actual velocity of the Anammox reaction in the system and to check the stability of the process (Siembida, 2004).

During the experiment of the increase in the influent nitrogen load in the period February – July 2004 (Figure 3) the series of batch tests was conducted with the supernatant and the Kaldnes rings taken from the Anammox reactor. An example of the course of reaction in a batch test is shown in Figure 8. The reaction rates for the Anammox culture obtained in this batch test were 0.7 g NH$_4^+$-N m$^{-2}$ d$^{-1}$ for ammonium nitrogen, 0.9 g NO$_2^-$-N m$^{-2}$ d$^{-1}$ for nitrite nitrogen and 1.4 g N m$^{-2}$ d$^{-1}$ for total inorganic nitrogen elimination. These are the highest values calculated during the whole experimental period. A slight nitrate nitrogen production was also observed at the rate of 0.22 g NO$_3^-$-N m$^{-2}$ d$^{-1}$. Table 13 summarizes the average values of the reaction rates for 6 consecutive tests. For these tests the dilution factor H$_2$O/supernatant of 1.5 was set in the Anammox reactor. The increasing tendency in nitrogen removal rates was observed with the parallel increase in the influent nitrogen load (Paper III). It indicates
an increase in the bacterial activity in the Anammox reactor.

To compare, in August 2003, when the highest removal of the influent nitrogen load was estimated, the nitrogen removal rate in the Anammox process amounted to 0.7 g N m\(^{-2}\) d\(^{-1}\). It is in agreement with the values from Table 13. The reaction rates presented in this study are somewhat lower that the rate of 2.2 g N m\(^{-2}\) d\(^{-1}\) obtained by Hippen et al. (2001) in a moving-bed Anammox reactor for sludge liquor treatment. Moreover, Beier et al. (1998) and Seyfried et al. (2001) investigated the single-stage deammonification process in moving-bed pilot plants and found values around 2 g N m\(^{-2}\) d\(^{-1}\). These are the rates expressing the simultaneous nitritation and Anammox processes. Rosenwinkel et al. (2005) also assumed 2 g N m\(^{-2}\) d\(^{-1}\) of the surface degradation capacity in the single-stage deammonification system. The comparison of the reaction rates is shown in Table 7 in chapter 3.4.

6. IMPLICATIONS FOR FULL-SCALE IMPLEMENTATION

In the full-scale operation, an interplay of two functional purposes of the system must be taken into account; namely, to optimise a sustainable and economically feasible full-scale system and to intensify biochemical reactions. The capacity of a moving-bed Anammox reactor should be recognised and therefore there is a need for an established and reliable reaction rate for the system in order to calculate the size of the reactor.

6.1. Proposal for system configurations

As a result of this licentiate study, the assessment of options for the moving-bed biofilm system for nitrogen removal from ammonium-rich wastewater has been done. Figure 9 presents the development degrees of a WWTP initially operated as a traditional nitrification/denitrification system (I) through nitritation/denitrification (II) to ob-
tain a two-step partial nitritation/Anammox system with case-specific modifications (III A-F).

I. Traditional nitrification/denitrification system represents a typical biological part of a WWTP. The existing infrastructure can be used as a base for introducing the following modifications presented in options II and III A-F.

II. An intermediate system for establishing the subsequent nitritation and denitritation processes is a solution for the plants aiming at introducing in the future a two-step partial nitritation/Anammox system. Another reason can be savings in operational costs on aeration and chemicals for the pH control and the addition of carbon source for heterotrophic bacteria.

III. Two-step partial nitritation/Anammox system.

As a result of the investigations presented in this work, the following options for process configurations are proposed:

III-A. This thesis assesses the most important findings concerning stable operation of a system consisting of two steps where a preceding step is operated as a preparatory phase for the Anammox reaction in the succeeding part. It is known that in order to establish a moving-bed biofilm Anammox bacterial culture the aerobic nitrifiers must be already enriched on the carrier elements. Therefore, in order to switch an operation mode from aerobic to the oxygen-limited must be done at a proper time during the start-up period by investigating the biofilm structure. The heterotrophic biofilm culture could be also initially developed on a support material but the inhibitory effect of alcohols on the Anammox culture should be accounted for. The start-up period can last up to 8 months. In this system, the effluent nitrogen concentration is expected at the amount of around 10% of the influent nitrogen load.

III-B. Pre-denitrification added to the system would enable higher total nitrogen removal efficiency through removal of the residual NO\textsubscript{3}-N concentrations in the effluent from the system. The bypassing with the step feeding of the influent supernatant is therefore necessary to steadily operate the partial nitritation process. The Anammox process follows and the additional bypassing of the supernatant to this reactor can mitigate higher nitrite nitrogen concentration in the reactor.

III-C. The B-option is modified with regard to make use of the production of nitrous oxide (N\textsubscript{2}O) and carbon dioxide in the nitritation stage. It was proved during the experiments that a substantial production of N\textsubscript{2}O in R1 of the partial nitritation/Anammox system occurred (Armand and Vikström, 2005). N\textsubscript{2}O could be decomposed into dinitrogen gas. A closed reactor system with collection of the gas flow to use it for mixing could be an operational option for the Anammox reactor. The shape of the reactor has to be accordingly adjusted in this case. It is not known, however, whether the Anammox bacteria can transform nitrous oxide. If N\textsubscript{2}O is still present in the effluent gas flow, it can be collected separately and treated with other gases. The excess gas system together with an internal gas recirculation rate should be taken into account. This proposition is an alternative for a mechanical stirring operational mode that is a cause of damaging the biofilm carriers.

III-D. With time, the Anammox reactor seeded with the nitrifying microorganisms could be switched to the reactor mode where a simultaneous partial nitritation/Anammox (SPNA) process takes place. In this option, the operation of the partial nitritation reactor as the first stage must be modified. Less aeration would be necessary as the effluent nitrite-to-ammonium ratio could be below 1.3. The bypassing of the influent supernatant to the inflow to R2 is again a safety measure to cope with the occasional peaks of nitrite nitrogen concentrations. As the effluent from the system contains nitrate nitrogen, a pre-denitrification step is proposed. The advantage of placing the denitrification step before R1 consists in using the denitrifying volume for the purpose of coping with occasional insufficient total inorganic nitrogen elimination. The organic acids present in the supernatant might be enough to denitrify the effluent nitrate nitrogen concentration when
Fig. 9 Proposed moving-bed biofilm systems for nitrogen removal from ammonium-rich wastewater (DN – denitrification; SPNA – Simultaneous Partial Nitritation/Anammox).
it is recycled at the inflow. If the pre-denitrification is used, the recycled effluent might contribute to seeding with the Anammox bacteria. Methanol and other alcohols cannot be used as an external source of carbon because it inhibits the Anammox bacteria.

III-E. In comparison to the D-option, the denitrification reactor is placed at the end of the system, after the settling tank. Technically, this option is much easier in operation. The addition of an external easy biodegradable material might be necessary. The introduction of the internal flow of gases for mixing and the removal of nitrous oxide together with the recirculation of gases (similar to the option III-C) is also possible in the post-denitrification step. The shape of the reactor must be therefore designed to enable the proper mixing regime. The aerobic conditions in the SPNA reactor are obligatory for the process, the DO range 1-2 mg O$_2$ l$^{-1}$. An inspection of the biofilm thickness is necessary in the SPNA system. The pH correction is not necessary; the pH value is the range 7.8-8.2. The temperature from 25°C to 35°C is optimal for the coexistence of ammonia oxidising and Anammox bacteria.

III-F. If a stable SPNA process can be established, the influent nitrogen load can be coped with by a system consisting of two deammonifying reactors in series. The reactors could be equally loaded with the nitrogen by means of bypassing of the supernatant flow or the second SPNA reactor might work as a safety volume. Setting oxygen-limited conditions by proper adjustment of a mixing regime would be the bottleneck of such system. A pre-denitrification is the best complement to the system to meet effluent standards.

In the options III-A – III-F, the sludge separation units collect the excess sludge with predominance of the nitrifying biomass. The nitrifying and Anammox sludge can be collected separately and used respectively to facilitate nitrification process in the main wastewater treatment system and seed the denitrifying units to support nitrogen removal in the case of deficiency of the organic material. The buffer tank is compulsory in all the options to avoid the influence of detrimental sludge and scum injections to the system.

It has to be emphasized that the presented modifications are not exclusive options for the Anammox system. Case-specific argumentation should be used to choose the most suitable option for a particular plant with regard to flexibility of dealing with different side-streams generated at the plant. Wett (2005) gives a successful example of a shift from a functioning nitritation/denitrification system towards a stepwise enrichment of the biomass with the autotrophic Anammox bacteria. The author argues that substantial savings on aeration, stirring and pumping energy were obtained. van Loosdrecht and Salem (2005) propose a decision support chart that motivates the choice of the sludge digester liquids treatment process with regard to three case-specific aspects: the limiting process, the limiting factor (e.g. sludge retention time, COD availability, aeration costs) and the presence of a counter ion for ammonium (normally bicarbonate, but sometimes chloride and fatty acids).

The research conducted at both the Royal Institute of Technology and the experience from the operation of a full-scale deammonification plant at the Hattingen WWTP in Germany (Rosenwinkel and Cornelius, 2005; Rosenwinkel et al., 2005) show possibilities to obtain an efficient nitrogen removal in one moving-bed biofilm reactor. Moving-bed biofilm reactors are recommended for the Anammox bacteria as in the biofilm it is possible to develop internal anoxic zones in the biofilm layer. Oxygen-limited conditions in a reactor initially operated in aerobic conditions and seeded with the Anammox bacteria can result in cooperation between aerobic and anaerobic ammonia oxidizers. Moving-bed reactors can be operated with intermittent aeration or different aeration rates can be set in separate reactors.
6.2. System technology with partial nitritation/Anammox

A system with a partial nitritation followed by an Anammox process as one- or two-step technology may interact with different steps of the WWTP and may be supplemented by various pre-treatment or post-treatment steps. The following examples are given below.

Pre-treatment of the influent:
- Increase of the ammonium content in the supernatant due to special handling of the excess sludge before digestion with the use of mechanical, physical, chemical or biological sludge minimization methods (or special handling methods of the digested sludge, like thermal or chemical conditioning methods).
- Removal of a part of ammonium before partial nitritation/Anammox by the use of methods like ammonia stripping, precipitation of magnesium ammonium phosphate or ion exchange.
- Use of a fraction of the supernatant to oxidize ammonium into nitrite to supply it to a one-step partial nitritation/Anammox in order to increase the reaction rates.
- Improved separation of suspended solids before a partial nitritation/Anammox system in order to increase the fraction of nitritation and Anammox bacteria in the suspended solids or biofilm in the reactors.

Special handling of the gas phase or produced sludge in the partial nitritation/Anammox system:
- The gas phase from the partial nitritation process may contain traces of nitrous oxides; if this stream cannot be handled internally, it may be transferred to a nitrification or denitrification step in the main stream (e.g. activated sludge basins, post-denitrification step, etc.) or handled separately for instance in a compost filter.
- Formed nitritation bacteria may be important to improve the nitrification process in the main stream and all of the return sludge or its fraction may be seeded into e.g. the aeration basin.
- Formed Anammox bacteria may also have an important role to improve nitrogen removal efficiency or reduce the necessary amount of carbon source in heterotrophic denitrification.

Post-treatment of the effluent:
- Excess ammonium may be treated by an ammonium separation method or be oxidized to nitrite and recycled back to the partial nitritation/Anammox step.
- Excess nitrite or nitrate may be removed by heterotrophic denitrification with an internal or external carbon source.

As a final point, a system with partial nitritation/Anammox may meet very high emission standards by the use of pre-treatment, post-treatment and special handling of the gas phase. Of course, additional treatment units imply investments and operational costs. Savings in the mainstream methods of nitrogen removal must therefore balance these costs. Some of the potential advantages of a partial nitritation/Anammox system are relatively low flows of water and air (in comparison to the main stream processes), high temperature of the supernatant, low energy demand, small usage of chemicals and possibilities to significantly improve the mainstream processes by reducing the nitrogen load and the use of seeding effects of nitritation and Anammox bacteria.

6.3. Overall recommendations

The performed pilot-plant experiments with the goal to study the influence of different parameters controlling the two-step partial nitritation/Anammox process enabled to give recommendations for full-scale implementations of nitrogen removal from ammonium-rich wastewater. The recommendations are grouped as follows.

General comments:
- Start-up of a full-scale Anammox is a limiting factor in all system configurations. System with the cultures developed both as the activated sludge (e.g. SBR technology) and as the biofilm (e.g. moving-bed biofilm reactor) can be applied.
• The research experience from this work and the full-scale Hattingen WWTP (Rosenwinkel et al., 2005) shows that the start-up period can last up to 8 months with the strategy of developing the Anammox culture on the previously established nitriﬁng biofilm. The experience from the STRASS WWTP (Wett, 2005) showed a step-by-step approach in scaling up the deammoniﬁcation process but the total start-up period took as much as 2.5 years.

• Kaldnes rings make the process more compact and less sensitive. In a moving-bed Kaldnes bioﬁlm reactor, the HRT can be lower than in the SBR reactors, which will result in savings on reactors’ volumes. The cost of carrier materials should be regarded as proﬁtable in the long way run because it results in future much higher operation ﬂexibility and robustness.

• Production of N₂O is expected in the partial nitriﬁcation reactor due to side reactions occurring together with the suppressed nitrite oxidation.

• It is 11% of the inﬂuent nitrogen load to the Anammox reactor that is by theory discharged from the system as nitrate nitrogen. It is thus obligatory to take into account the nitrate nitrogen production in the Anammox reactor to meet efﬂuent standards. Post- or pre-denitriﬁcation designed before or after a partial nitriﬁcation/Anammox system can be an option to remove nitrate nitrogen. The efﬂuent from an Anammox system can be even directed to the denitriﬁcation unit in the main stream of a WWTP.

• Biogas generated during sludge digestion or heat from heat exchangers can be used as energy source for aeration and heating purposes in the partial nitriﬁcation/Anammox system.

**Design:**

• A full-scale partial nitriﬁcation/Anammox system design is dependent on the sludge generation pattern and the size of a WWTP. Local conditions inﬂuence the choice of the system with the Anammox process.

• The design of the partial nitriﬁcation/Anammox system must complement the WWTP design. Quality and quantity changes of the supernatant must be taken into account.

• Mass balance calculations of inorganic nitrogen forms should be done with regards of designing a partial nitriﬁcation/Anammox system in full scale.

• The distribution of the reactors’ volumes in the partial nitriﬁcation/Anammox system is dependent on the growth rates of the respective bacterial cultures.

**Reaction rates and system capacity:**

• The reaction rates around 1.2 g N m⁻² d⁻¹ and HRT=3 d with 1 m³ of the Kaldnes carriers in the reactor of 2 m³ (50% of volumetric ﬁlling, 500 m² of the established bioﬁlm and 1.8 m³ of the liquid in the reactor) can cope with the load oscillating around 0.34 kg N m⁻³ d⁻¹; it corresponds to the inﬂuent nitrogen concentration of as much as 1000 g m⁻³. The increase of the reactor volume by around 13% should be taken into account in full-scale design due to Kaldnes ﬁlling.

• Higher load should be imposed as the strategy for extending system capacity with a simultaneous monitoring of the on-line conductivity readouts. A sudden peak of conductivity may be a signal of an enhanced nitrogen concentration in the Anammox reactor.

**System conﬁgurations:**

• The bypassing of the inﬂuent supernatant to further zones of the partial nitriﬁcation reactor (step feeding) can allow for more stable culture in that reactor.

• Bypassing of the inﬂuent supernatant at the inflow to the Anammox reactor can be used to fulﬁl stoichiometry of the Anammox process and as a safety measure in case of an overproduction of nitrite. The rate of the bypass flow can be adjusted with the help of conductivity measurements.
Avoidance of problems:

- A buffer tank is needed in front of the system to exclude the suspension in the supernatant and work as a safety step to prevent dumping of unwanted wastewater.
- Storing supernatant and supplying it during e.g. cleaning of centrifuges can cope with uneven flows of supernatant.
- It is advised to place the system as close to a sludge processing unit as possible to take advantage of high supernatant’s temperature, decrease losses of heat and avoid seasonal problems with frozen pipes.
- Frequent clogging of pipes requires regular maintenance procedure.
- Other authors (Seyfried et al., 2001; Dapena-Mora et al., 2005; Rosenwinkel et al., 2005) reported calcium, iron and phosphorous precipitation on the Kaldnes material. The content of Ca, Fe and P salts should be analysed in the influent stream to prevent the carriers’ damage by precipitation.

Equipment:

- Operation of a moving-bed reactor is dependent on the shear forces imposed on the biofilm carriers by mixing therefore, the mixing speed and a type of mixing unit in a full-scale moving-bed reactor should be chosen carefully.
- The mixing speed in both reactors should be different due to the fact that the aeration unit in the partial nitritation reactor puts carriers into mixing as well. In the Anammox reactor, however, keeping oxygen-limited conditions requires different type of mixers or lower speed of mixing to avoid the destruction of Kaldnes rings.
- Frequent exchange of electrodes and rotary piston elements in pumps is necessary due to the reactivity of the liquor.

Control and monitoring:

- On-line adjustment of the dissolved oxygen concentration in the bulk liquid based on the on-line pH readouts (proper drop of the pH value and alkalinity consumption) is a proposal for an optimal operation strategy for the partial nitritation reactor.
- Changes in the ammonium nitrogen concentration in the influent can be monitored by the conductivity parameter in the partial nitritation step.
- On-line control of the system should consist of a pH-electrode at the outflow from the partial nitritation reactor to maintain the proper effluent nitrite-to-ammonium ratio.
- On-line monitoring of the inorganic nitrogen removal in the Anammox reactor can be done by measurements of conductivity in the influent and effluent, which could detect deviations from the optimal nitrogen removal capacity.
- An increase of the pH value in the Anammox reactor can be a monitoring indicator of an undisturbed reaction.

Modelling:

- If the data for modelling are gathered purposefully, modelling of the historical data is a powerful tool in understanding of covariations in the process variables as well as allowing for the assessment of different system configurations.
- Scale-up problems can be simulated by the use of deterministic models. Calibration of the deterministic models requires however proper experimental pilot-plant configuration.
7. Final conclusions

The following conclusions concerning the partial nitritation reactor can be stated:

- Suitable adjustments of the parameters like pH, DO concentration, temperature, and HRT enabled to obtain two-year stable operation of the partial nitritation reactor.

- The proper nitrite-to-ammonium ratio at the outflow was obtained for most of the period investigated. OUR tests, profile performance, univariate and multivariate data analyses confirmed the stable process operation.

- The most robust partial oxidation of ammonia to nitrite was in the first zone of the reactor, which was demonstrated by the OUR tests and profile performance.

- A disturbance of the partial nitritation reactor operation could be due to almost total alkalinity consumption in zone 1 of the reactor.

The main findings concerning the Anammox process are as follows:

- The operation of the Anammox reactor over the period of two years gave efficient inorganic nitrogen removal.

- A proper nitrite-to-ammonium ratio (NAR) can be in the range 1.0-1.5 for the most efficient nitrogen removal in the Anammox reactor. The NAR is dependent on the partial nitritation reactor performance.

- Dilution of the effluent from the partial nitritation reactor was a reliable strategy for extending the capacity of the Anammox reactor.

- The pH correction appeared to be unnecessary in the Anammox reactor. During stable operation of the reactor an increase of the pH value was calculated.

The assessment of the efficient nitrogen removal in the whole system resulted in the following conclusions:

- All tools used in the study proved to be applicable in assessing the performance of the two-step moving-bed partial nitritation/Anammox system.

- Variable characteristics of the influent supernatant and an enhanced nitrite nitrogen concentration in the Anammox reactor are two factors that substantially influence stable operation of the pilot plant and can periodically decrease the efficiency of the process.

- FISH analyses proved the presence of ammonia-oxidizing bacteria in the partial nitritation reactor and the Anammox bacteria in the second reactor.

- Dynamic detachment of the biofilm and seeding of the reactor with the nitrifying sludge from the partial nitritation reactor can explain periodical increases of the suspended solids in the moving-bed biofilm Anammox reactor.

- Nitrifying activity in the partial nitritation reactor is present in the biofilm whereas in the Anammox reactor minor contribution of the nitrifying activity was detected only in the suspended solids.

- Process bottlenecks were recognised as the dissolved oxygen concentration in both reactors and nitrite nitrogen concentration increase in reactor 2.

- Profiles of inorganic nitrogen forms were a tool to monitor the system performance.

- The two-step process can be successfully monitored by conductivity measurements.

The literature review and the experimental work presented in this thesis proved that the technology with the application of the partial nitritation/Anammox system is a sustainable and cost-effective alternative for nitrogen removal from ammonium-rich wastewater.
8. Further Research Work

The following implications for further research work can be presented:

- The digestion process at the Himmerfjärden WWTP should be scrutinised with the aim of recognising factors influencing the quality of the influent supernatant.

- There is still lack of information concerning the inhibitory effect of nitrite on Anammox bacteria. The initial concentration of nitrite nitrogen over 100 mg l⁻¹ during short-term batch test was not inhibitory for the Anammox bacteria. Reliable on-line nitrite monitoring is a future research goal.

- Biochemical reactions of the Anammox bacteria could be scrutinized for the possibility of utilization of N₂O in their metabolism. If this phenomenon were proved, it would open possibilities of dealing with the production of an unwanted greenhouse gas.

- A possibility of symbiosis of Anammox and *Nitrosomonas* bacteria and the distribution of the activity between the activated sludge and biofilm in the reactor under oxygen-limited conditions should be checked in batch tests.

- Nitrifying activity in the Anammox reactor can positively affect the performance of the Anammox reactor, as the nitrite-to-ammonium ratio in the effluent from the partial nitritation reactor can be lower than the stoichiometric value of 1.3. It implies savings on aeration in converting ammonia to nitrite. It needs further investigations.

- The research could be extended into an option to perform partial nitritation and Anammox processes in one reactor. In the reactor configuration with a simultaneous partial nitritation/Anammox processes, the intermittent aeration operational mode could be applied.

- The ability of the Anammox culture to recover from the time of stress has not yet been investigated. A possibility of temporal acclimation of Anammox bacteria to lower temperatures could provide incentive for future commercial sale of the bacteria for seeding purposes.

- The main line of a WWTP can be constantly seeded with the developed Anammox sludge to increase capacity or to gradually change the system operational mode from traditional to more cost-effective. The upgrading procedure needs further investigations.

- The inspection of the biofilm thickness and structure could be a monitoring tool, especially during the start-up phase. The effect of changing the feed volume and agitation speed on the biofilm thickness is necessary to investigate.
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