PERFORMANCE AND CONTROL OF BIOFILM SYSTEMS WITH PARTIAL NITRITATION AND ANAMMOX FOR SUPERNATANT TREATMENT

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June 2007
**Acknowledgements**

I would like to express my deepest gratitude to all of those who contributed either directly or indirectly to bring this thesis to its final form. I would not have been able to achieve it without your help and support.

My sincere appreciation goes to:

My supervisors – for their scientific guidance and encouragement. In particular to Associate professor Elżbieta Plaza, who has given me the opportunity to become PhD student. I appreciate your support and time that you sacrificed to me.

My assistant supervisor, Dr. Eng. Józef Trela for giving me the opportunity to participate in a very interesting and stimulating research project on nitrogen removal.

My second assistant supervisor, Professor Bengt Hultman for all your advices and never-ending flow of ideas. I admire your extraordinary abilities of seeing things in a wider perspective.

I am deeply indebted to Professor Vladimir Cvetkovic for being a part of supervision committee and for your efforts to make things going smoothly, HVALA!

My friends Luiza Gut, Maja DługolecKa, Ewa Stobnicka and Grzegorz Cema for all your help, support and the most important- for your great friendship, which is my treasure.

Monica Löwen for your help and patience during my laboratory experiments and being so friendly to me.

Aira Saarelainen for pleasant help with administrative matters.

Jerzy Buczak for your help with computer issues and being so friendly.

Professor Roger Thunvik, Associate professor Ann-Catrine M Norrström and Associate professor Joanne M. Robison Fernlund for special support in finalising my PhD.

Staff of the Himmerfjärden WWTP for your collaboration and making facilities available to my research. I am especially grateful to Jan Bosander, who friendly assisted me during the pilot plant experiments, Director Lars Gunnarsson for great support in difficult moments, former Director Alf-Göran Dahlberg for being a part of supervision committee, Anders Olsson for nice chats 😊 and being always supportive, Malin Tuvesson for your exceptional kindness.

Staff of the Bromma WWTP for collecting supernatant for the laboratory-scale pilot plant.

Swedish Institute (SI), SYVAB, VA-Forsk and J. Gust. Richert Foundation for your generosity in financial support.

Professor Joanna Surmacz-Górski for valuable and very competent comments.

Master students Ania Bąkowska, Agnieszka Comber, Dorota Sosnowska, Ewelina Basiak and Ewelina Bator for your help in experiments.

Professor Krystyna Mędryszek at the Gdańsk University of Technology for keeping the faith and support.

PURAC for the construction of the pilot plant at the Himmerfjärden WWTP.

Last but not least, my family for being always supportive and especially my beloved husband – Paweł for his patience, encouragement, understanding and just for being there.

I wish to acknowledge all people whom I might have not mentioned here and who have – either directly or indirectly – affected my professional life.

Thank you!

Beata Szafkowska

Stockholm, June 2007
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LITERATURE
Acronyms and abbreviations

A – Anammox
ADEPT – Anaerobic Digestion Elutriated Phased Treatment
Anammox – Anaerobic ammonium oxidation
BABEL – Bio Augmentation Batch Enhanced
C – substrate concentration (g m$^{-3}$)
CANON – Completely Autotrophic Nitrogen Removal Over Nitrite
$c_i$ – the molarity concentration of ion
COD – chemical oxygen demand (mg l$^{-1}$)
DEAMOX – DEnitrifying AMmonium OXidation
DO – dissolved oxygen (mg l$^{-1}$)
Ea – activation energy (kJ mol$^{-1}$)
EC UWWT – European Commission Urban Waste Water Treatment
EU – European Union
$\Delta E$ – redox potential (volts)
F – the Faraday constant (96,500 coulombs mole$^{-1}$)
$\Delta G$ – Gibbs free energy change (kJ mol$^{-1}$)
HRT – hydraulic retention time (d or h)
ICA – instrumentation, control and automation
$I S$ – ionic strength
K – saturation constant (g m$^{-3}$)
$K_{a1}, K_{a2}$ – equilibrium constants
$K_i$ – inhibition constant (g m$^{-3}$)
$K_w$ – equilibrium constant of water
$K_{H}$ – the Henry's Law constant for CO$_2$ = $10^{-1.5}$ at 25°C
M – mol
MARE – Marine Research on Eutrophication
MBBR – moving-bed biofilm reactor
MISTRA – The Swedish Foundation for Strategic Environmental Research
n – stoichiometry coefficient
NAR – nitrite-to-ammonium ratio
OLAND – Oxygen-Limited Autotrophic Nitrification-Denitrification
OSPAR – The Convention for the Protection of the Marine Environment of the North-East Atlantic
OUR – oxygen uptake rate
P – gas pressure (kPa)
$P_{CO_2}$ – partial pressure in atmosphere
p.e. – person equivalent
R – molar gas constant = 8.314 J mol$^{-1}$ K$^{-1}$
RBC – rotating biological contactor
SBR – sequencing batch reactor
SEPA – Swedish Environmental Protection Agency
SHARON – Single reactor system for High activity Ammonia Removal Over Nitrite
SNAP – Single-stage Nitrogen removal using Anamox and Partial nitritation
SRT – sludge retention time (d or h)
SS – suspended solids (mg l$^{-1}$)
TAN – total ammonium nitrogen (mg l$^{-1}$)
TSS – total suspended solids (mg l$^{-1}$)
UASB – upflow anaerobic sludge bed
V – gas volume (m$^3$)
VSS – volatile suspended solids (mg l$^{-1}$)
WFD – Water Framework Directive
WWTP – wastewater treatment plant
Y – biomass yield (g VSS g substrate$^{-1}$)
$y$ – activity coefficient
$z$ – the number of electrons transferred in the reaction
$z_i$ – the charge of $i$ ion
$\mu$ – specific growth rate (h$^{-1}$)
$\mu_m$ – maximum growth rate (h$^{-1}$)
List of papers

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


Other publications related to this research are not appended. An additional list is provided for those who are interested:

International conference proceedings

Licentiate thesis


Swedish journals


Polish-Swedish seminar proceedings


Reports


Reprint and accepted papers for publication are published in this thesis with kind permission of the journals concerned.
Abstract

Separate treatment of supernatant with dewatering of digested sludge with application of partial nitritation/Anammox process is assessed to be a cost-effective way to remove about 10-15% of influent nitrogen and, thereby, facilitate possibilities to reach required effluent requirements from the plant. The combined partial nitritation/Anammox process can be performed in two separate reactors or in one-stage. Both process options have been investigated in technical- and laboratory-scale pilot plants with moving-bed biofilm reactors (MBBR) filled with Kaldnes rings. Use of the two-stage process resulted in a very stable partial nitritation with a suitable nitrite to ammonium ratio (NAR) for the following Anammox step. Dissolved oxygen (DO) and pH value were identified as key factors for the partial nitritation process. The Anammox process could also be operated in a stable way. A high nitrite concentration, however, inhibited the process and the time for recovering the process at low nitrite concentration was about four months. Seeding of the partial nitritation reactor with Anammox bacteria (the recirculation of Anammox effluent to the nitritation reactor) turned out to be a simple and easy method to enable creation of an oxic-anoxic biofilm in one reactor. Studies have shown that such a one-stage system would be the best choice for full-scale implementation due to significantly higher nitrogen removal rates and easier operation. The partial nitritation process was found to be the rate-limiting reaction to perform the overall nitrogen removal.

Measurements of conductivity and pH were suitable parameters for monitoring of the nitrogen reactions. A control and monitoring system was developed both for two-stage and one-stage technology. The system was mainly based on relationships between conductivity and inorganic nitrogen components, while in the one-stage technology measurements are used of both conductivity and pH and their relationships with inorganic nitrogen compounds. Alkalinity was an additional measured parameter suitable for process control and monitoring. Theoretically calculated values of conductivity were in good agreement with experimentally obtained results.

Keywords: alkalinity, Anammox, conductivity, partial nitritation, pH, removal rate, supernatant
Sammanfattning

Separat behandling av rejkvatten efter avvattnning av rötslam med partiell nitritation och Anammox är en ny teknik som bedömts vara en kostnadseffektiv väg att avlägsna ca 10-15% av inkommande kvävemängd och därmed underlätta möjligheter att nå uppställda krav på utgående kvävehalt från ett avloppsverk. Kombinerad partiell nitritation med Anammox processen kan genomföras i två separata reaktorer eller i en reaktor. Båda möjligheterna har undersöks i teknisk pilot-skala och i kontinuerlig laboratorieanläggning med rörligt bärarmaterial (Kaldnes). Vid partiell nitritation önskas oxidation av ammonium till nitrit med en kvot mellan nitrit- och ammoniumkväve (NAR) på teoretiskt ca 1,3:1 för att åstadkomma anammoxprocessen med oxidation av ammonium med nitrit främst till kvävgas.


Recirkulation av utgående flöde från anammoxsteget leddes till bildning av oxisk/anoxisk biofilm (partiell nitritation med syretillförsel i det yttre skiktet och anammoxprocessen utan syretillgång i det inre skiktet). Kvävereduktionen kunde därmed genomföras i ett steg. Partiell nitritation var det hastighetsbegränsande steget för hela kvävereduktionsprocessen och därför bedömdes enstegsteknik vara den kostnadseffektivaste lösningen vid användning av biofilmteknik.


Nyckelord: alkalinitet, Anammox, konduttivitet, kvävereduktion, partiell nitritation, pH, rejkvatten
1. INTRODUCTION

Eutrophication problem

The nitrogen cycle is one of the most important processes appearing in nature for living organisms. Bacteria present in the soil are capable to fix nitrogen into usable form acting as fertilizer for plant and for plant synthesis. Eutrophication is a natural process occurring in water bodies, but unfortunately human activities can greatly accelerate this process. The flow of nutrients and organic substances into aquatic systems can be increased by nutrients coming from agricultural runoff, urban runoff, leaking septic systems, sewage discharges and other similar sources. Excess of nitrate in the soil is most often found in rural and agricultural areas. Nitrate travels easily through the soil, carried by rain or irrigation water into groundwater basins. Specific health risks appear when fresh water, extracted from eutrophic areas, is used for the production of drinking water; such water becomes unacceptable for human consumption if nitrate removal is not accomplished. Drinking water with high concentrations of nitrate creates health problems because inside the human body nitrate is converted partly to nitrite, which can cause a reduction in the blood’s oxygen-carrying capacity.

Overloading with nutrients can cause health problems for fishes, animals and humans and can also stimulate growth of algae that later on lead to disturbances in the water system. Algal blooms are influencing the water system in two ways. First, they hamper the penetration of sunlight, causing death of underwater grasses. Secondly, the decomposition of died algae causes depletion of oxygen, which is normally essential to most organisms living in water. Moreover, excess of nutrients and eutrophication creates uncomfortable conditions as odours that influence the recreational use of lakes and estuaries.

Eutrophication in Baltic Sea

Eutrophication is also a serious problem appearing in the Baltic Sea waters. The production and abundance of phytoplankton has increased, creating surface accumulations and decreasing visibility. Biodegradation of dead algae consumes oxygen and is a factor contributing to the creation of anoxic and anaerobic bottoms.

In order to improve knowledge on the quality of the Baltic Sea, the Swedish Foundation for Strategic Environmental Research, MISTRA funded eight-year (1999-2006) research program called Marine Research on Eutrophication (MARE). The program was addressed to decision-makers working on Baltic Sea environmental issues. Around Baltic Sea are living 85 millions persons whose wastewater come to sea via different watercourses and land runoffs. Residence time for pollutants and nutrients in seawater is long due to restricted water exchange. Measurements taken in one country are not sufficient and cooperation between countries is needed. Thus, to provide one basis for decision-making at international negotiations and to achieve a desired environmental quality in the Baltic Sea, decision support system - Baltic Nest – within MARE program was developed (MARE, 2007).

Himmerfjärden Bay

The investigations concerning eutrophication are also regularly performed at the Himmerfjärden wastewater treatment plant (WWTP) collecting sewage from southern Stockholm region. The WWTP has been in operation from 1974 and is located at southern part of Stockholm archipelago. In total, 250 000 persons are connected to this WWTP. The recipient of discharged and treated wastewater is Himmerfjärden, an enclosed bay of the Baltic Sea. Wastewater is discharged to the natural environment by a 1.6 km long tunnel ending at a depth of 25 m in the bay. WWTP has an average inflow of 110,000 m$^3$ d$^{-1}$, with the optimal capacity of 108,000 (maximum of 132,000 m$^3$ d$^{-1}$). Data of discharged nitrogen indicated that in 2006 as much as 60,000 kg of nitrogen per month was discharged to the bay (Himmerfjärden eutrophication studies, 2007).

Discharged concentration of course undergoes dilution in the recipient and it varies within the depth of the bay. However, registered ammonium concentration in the depth...
of 20-30 m, in one out of nine research stations in August 2006, was equal to 35 mg N m$^{-3}$ (Himmerfjärden eutrophication studies, 2007). Such ammonium concentration was linked at the same time to low (2 mg l$^{-1}$) dissolved oxygen concentration. High ammonium concentration, discharged to the bay, causes oxygen depletion and therefore improvements are necessary.

**Eutrophication and environmental law**

National and European legislations have been introduced to protect environment from human damaging activities. They regulate the maximum allowed concentrations of carbon, nitrogen and phosphorus in purified wastewater discharged to the rivers and other water bodies. Enforcement of the environmental law is not always very strict; it varies between different countries and is usually related to the economy.

In order to protect the environment, the European Union (EU) started to issue different regulations in the seventies. A recent directive from EU is the Water Framework Directive (WFD) (2000/60/EC) that regulates the water management throughout the EU.

Since eutrophication process became a serious global problem, there is a need to prevent or substantially reduce negative effects of nutrients on the environment and it is necessary to involve effective control of nutrient discharges.

The increasing awareness of society concerning environmental protection results in implementation of stringent wastewater effluent standards. According to the European Union Directive 91/271/EEC of 21 May 1991 on Urban Waste Water Treatment (UWWT), there is a general need for secondary treatment of urban wastewater to prevent the environment from being adversely affected by the disposal of insufficiently treated urban wastewater. Member states shall ensure that urban wastewater entering collecting systems should be subjected to secondary treatment or equivalent treatment, before discharge. Moreover, application of more stringent treatment technologies is required in sensitive areas.

**Nutrient restrictions in Sweden**

Implementation of nutrient removal in Sweden started at the beginning of 1960s with phosphorus removal from wastewater, since it was recognized as necessary to be performed in all wastewater treatment plants. Later on, since 1990s, nitrogen removal was introduced. The European Commission Urban Wastewater Treatment (EC UWWT) directive established (in 1991) a new nitrogen discharge permit for more than 70 large (> 10 000 p.e.) sewage treatment plants located near the coastline in the southern and middle parts of Sweden. Since then, the treatment plants in Sweden have subsequently been upgraded for nitrogen removal. Sweden as one of the European Union countries that also introduced its own strict directive concerning nitrogen discharge (SNFS 1998:7 of 23 September 1998), which covers European requirements of 10 and 15 mg N l$^{-1}$ with minimum reduction of 70%. However, on April 2, 2004 the European Commission of the European Union sent the government of Sweden a written warning, for allegedly failing to treat urban wastewater as required by the 1991 EU UWWT. Sweden did not comply with directive rules in several locations and contributed to nutrient enrichment of the Baltic Sea. Moreover, in December 2006, the Commission initiated an European Court action against Sweden due to exceeded nitrogen discharge into the rivers in the Baltic catchments. As it was mentioned before the Baltic Sea is known as eutrophication sensitive to nitrogen discharges but nitrogen in Sweden is removed only from 60% of sewage discharging into Baltic catchment rivers (SCOPE Newsletter, 2007).

In order to reduce the negative effects of nutrients, Sweden set an ambition goal - "Zero eutrophication". To obtain the target different steps of improvement of sewage systems were taken. Moreover, to reduce the load, municipalities were requested to consider source separation techniques and to improve small-scale treatment plants. It was predicted that rebuilding of WWTPs and control of agricultural sectors connected with implementation of WFD will result in
nitrogen reduction of about 40% by 2010 (OSPAR, 2006).

Moreover, to take steps to reduce eutrophication a special committee was formed in 2005 by initiative of the Swedish Environmental Protection Agency (SEPA) to examine different data for Swedish coastal seas. One of the aspects concerned the increasing problem with Cyanobacteria blooms. The investigations resulted in the written report “Eutrophication of Swedish seas”. The main issues of the report were: the reduction of phosphorous input to the open Baltic, reduction of atmospheric nitrogen deposition, reduction of nitrogen inputs to the waters of the Swedish west coast and reduction of nutrient inputs in enrichment-sensitive areas of the Swedish east coast (Boesch et al. 2006).

The conducted investigations within SEPA report showed that eutrophication problems are met with contradictory opinions and it is still not clear which nutrient has the highest priority to be removed or if both have similar level of importance. The long-lasting effect of nutrient removal is difficult to predict. However, the problem has global scale and further investigations are required.

**Anammox as a new pathway for nitrogen removal**

Large efforts are today made in the field of nitrogen removal since nitrogen is one of the main indicators in the (WWTP) effluents. Future new solutions should consider both decrease in external nitrogen load to the head of WWTPs coming from human activities, and internal reuse or treatment of internal processes. In order to follow new solutions, existing WWTPs should be soon upgraded. At the same time, to avoid high costs of upgrading, one must consider new techniques without changing volume needs.

In nature, at least two mechanisms of biological ammonium oxidation can occur under anoxic conditions. One of them can be carried out by nitrifiers like *Nitrosomonas eutropha* indicating ability to oxidise ammonium with nitrite as an electron acceptor. The other recently discovered and more effective group is autotrophic bacteria responsible for Anammox (Anaerobic ammonium oxidation) reaction. Bacteria belong to the order of the *Planctomycetales* (Strous et al. 1999a). Before they were identified by microbiologists, the existence of Anammox bacteria was preliminary predicted by Engelbert Broda in 1977. He based his assumption on thermodynamic considerations and stated that if a chemical reaction yields energy, it is probable that there exist bacteria that are able to use it. Depending on electron acceptor, this reaction can yield enough energy to make it possible for chemolithoautotrophs to live (Table 1). He, as the first one, pointed out anaerobic ammonium oxidisers as “missing in nature”.

The Anammox bacteria have been detected in natural environments such as marine sediments (Dalsgaard et al. 2002, 2003; Engström, 2004; Kuypers et al. 2003). In these environments, Anammox reaction is often responsible for 30-50% of nitrogen gas formation.

The presence of Anammox bacteria has also been studied in the Baltic Sea. Gebhardt et al. (2004) investigated the pathway of hydroxylamine formation (NH$_2$OH), which is an intermediate in two important microbial processes of the nitrogen cycle: as nitrification and anaerobic ammonium oxidation. It turned out that nitrification is mostly responsible for its formation in the Baltic Sea. The contribution to the total N$_2$ production by Anammox fluctuates between different parts of the Baltic Sea. In the Gulf of Finland, it differs in time from 10 to 15% in spring and in autumn, respectively (Hietanen and Kuparinen, 2007). In sediments of Skagerrak, it amounted to 24-67% and it drops down to

<table>
<thead>
<tr>
<th>Electron acceptor</th>
<th>Reaction</th>
<th>Gibbs free energy [kJ mol$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxygen</td>
<td>2NH$_4^+$ + 3O$_2$ = 2NO$_2^-$ + 2H$_2$O + 4H$^+$</td>
<td>-241</td>
</tr>
<tr>
<td>nitrite</td>
<td>NH$_4^+$ + NO$_2^-$ = N$_2$ + 2H$_2$O</td>
<td>-335</td>
</tr>
<tr>
<td>nitrate</td>
<td>5NH$_4^+$ + 3NO$_3^-$ = 4N$_2$ + 9H$_2$O + 2H$^+$</td>
<td>-278</td>
</tr>
</tbody>
</table>
2% in coastal waters of the bay where denitrification plays a dominant role.

The identification of the Anammox bacteria was preceded by observation of Anammox in denitrifying pilot plant (Mulder et al. 1995). Along the time, its occurrence was affirmed in different reactor configurations (Jetten et al. 1999; Helmer et al. 1999; Strous et al. 1999a).

The discovery of Anammox became a revolution in nitrogen cycle pathways since it appeared that nitrogen can be removed not only during the well-known nitrification/denitrification process but also during the Anammox process. This finding changed the approach to the biological nitrogen removal processes. Anammox bacteria started to play an important role in sustainable wastewater treatment and since it occurred to be attractive to wastewater treatment, the processes involving Anammox bacteria are widely studied around the world. Nowadays, about 40 research groups are working with the Anammox process (Gut, 2006a).
2. AIM OF THE THESIS

The purpose of these studies was to investigate nitrogen removal from ammonium-rich supernatant coming from dewatering of digested sludge with an application of the partial nitritation/Anammox system. The process of total oxidation of ammonium during nitrification is well-known but prevention of further oxidation of nitrite to nitrate is still needed to investigate. Moreover, the second Anammox step is carried out by Anammox bacteria, which were quite recently identified and the knowledge about them is still under development. In addition, for full-scale process implementation, an advantage would be development of a process performance monitoring system.

The main goals of this thesis were:

- To investigate whether it is possible to obtain a long-lasting and stable Anammox process.
- To better understand mechanisms governing the partial nitritation and Anammox processes.
- To determine suitable conditions leading to maximum nitrogen removal rates.
- To recognize environmental parameters influencing both partial nitritation and Anammox processes.
- To assess different system configurations: one- and two-stage.
- To evaluate possibilities to control and monitor the partial nitritation/Anammox system.
- To better understand mechanisms governing the partial nitritation and Anammox processes.
- To determine suitable conditions leading to maximum nitrogen removal rates.
- To recognize environmental parameters influencing both partial nitritation and Anammox processes.
- To assess different system configurations: one- and two-stage.
- To evaluate possibilities to control and monitor the partial nitritation/Anammox system.

More specific aims in particular papers are presented in Table 2.

Table 2. Objectives of the following papers.

<table>
<thead>
<tr>
<th>Paper No</th>
<th>Objective:</th>
</tr>
</thead>
</table>
| I        | - Estimation of different control strategies that allows obtaining about equal amounts of ammonium and nitrite and low nitrate content in the effluent from the partial nitritation.  
- Identification of suitable conditions for Anammox bacteria culture.  
- Preliminary examination of conductivity as a monitoring tool for two-step process. |
| II       | - Evaluation of experiences with long-term partial nitritation/Anammox process performed in two separate stages. |
| III      | - Evaluation of conductivity parameter as monitoring tool and its interaction with other process parameters in two-stage process. |
| IV       | - Assessment of influence of temperature changes on both one- and two-stage partial nitritation/Anammox process. |
| V        | - Studies of process performance and estimation of nitrogen removal rates in one-stage partial nitritation/Anammox process. |
| VI       | - Investigations of factors influencing the activity of combined reactions in one stage partial nitritation/Anammox process.  
- Estimation of efficiency and nitrogen removal rates with special focus on oxygen conditions. |
| VII      | - Evaluation of conductivity, pH and alkalinity as tools to follow-up the one-stage process performance and their interactions with other process parameters. |
3. TREATMENT OF NITROGEN-RICH STREAMS

3.1 Examples of streams

**Supernatant**

Anaerobic sludge digestion is commonly used at WWTPs to reduce bacteria levels and odours. Moreover, it decreases the amount of solids present in the sludge and produces energy, which can be further used. However, this process has also disadvantages because usually during municipal sludge digestion about 50% of the nitrogen, bound in the sludge, is released. As a result, after sludge dewatering, an ammonium-rich stream called supernatant is formed (Siegrist, 1996). The ammonium concentration in the supernatant can be high even up to 2 kg m\(^{-3}\) (Strous et al 1997). Recycling of such stream to the biological step or to the inlet of the plant leads to an increase in nitrogen load by about 15–20% on an average, with reference to the total inlet nitrogen load (Jansen et al 1993). Interest of separate supernatant treatment is recently increasing because treatment of such internal side stream can significantly reduce the total load in WWTP. Performed research in Sweden showed that to remove nitrogen 48 out of 66 plants are using traditional activated sludge. In order to improve their nitrogen removal, 25 plants add external carbon source and 7 of them use internal carbon source from hydrolysis of sludge. Moreover, 23% (16 plants) have a separate supernatant treatment and apply sequencing batch reactor (SBR) technology (Nikolić and Sundin, 2006). These numbers indicate that there is a need for separate treatment of supernatant.

Different methods have been used and tested among the processes available for nitrogen removal e.g. air stripping (Budzianowski and Kozioł, 2005), chemical precipitation (Li et al 1999) and the most common biological methods. Physical-chemical methods have some drawbacks if compared to biological methods e.g. higher treatment costs, small capacity and release of secondary pollutants, which are difficult to handle. The high nitrogen concentration and temperature of supernatant enable to apply many biological techniques. However, in the last decade, since the Anammox bacteria were identified in both marine ecosystems and different wastewater streams, one of the most promising applications for supernatant treatment is the Anammox process (Caffaz et al 2006; Gut, 2006b) that can be combined with partial nitritation. Then, the first step - partial nitritation relies on ammonium oxidation that should be stopped at nitrite level and is followed by Anammox as second step, which is an anaerobic oxidation of ammonium with nitrite to dinitrogen gas. Introduction of this fully autotrophic process to the operational practice would make the wastewater treatment technology more sustainable since the process has less oxygen demand compared to traditional nitrification/denitrification and no need for external source of carbon.

A technology that combines supernatant treatment with upgrading of existing WWTP by increase in nitrifying activity is BABE (Bio Augmentation Batch Enhanced) process (Berends et al 2005). The idea of the concept is to return the fraction of the activated sludge from one of the main process stages - secondary clarification - to the BABE reactor, which is also supplied by supernatant and where nitrifying bacteria grow in the biomass flocs. In BABE reactor ammonium is oxidized to nitrite and nitrate and acid production can be compensated chemically or biologically by denitrification. The activated sludge, carrying a higher load of nitrifying organisms, is returned to the mainstream process. Hence, the nitrification capacity in the main line of activated sludge process is augmented and reduction of nitrogen load is improved. Salem et al (2004) reported a full-scale BABE process, which was applied at Garmerwolde WWTP in the Netherlands.

**Landfill leachate**

Another stream with high ammonium content is landfill leachate. The leachate arise from rain that passes through a landfill and polluted liquids that are generated due to break down of wastes within the landfill. Leachate contains high concentration of humic acids, ammonia nitrogen, heavy metals, xenobiotics and inorganic salts. Due to their toxicity or negative effect on the envi-
nvironment, they need to be removed before discharge into environment. Traditional nitrification/denitrification is probably the most efficient and cheapest method to remove nitrogen from leachate. However, biological treatment can be inhibited by the specific toxic substances and/or by the presence of bio-refractory organics (Wiszniowski et al 2006). Other methods as air stripping, coagulation, flocculation and settling are often expensive (energy consumption, addition of chemicals). Therefore, there is a need to search for new treatment methods. Helmer and Kunst, (1998) proposed as an alternative leachate treatment, the simultaneous aerobic nitrification/denitrification. The experiments were performed in RBC and an efficiency of 90% nitrogen removal was obtained.

A process, which also used a RBC for landfill leachate treatment is OLAND (Oxygen-Limited Autotrophic Nitrification-Denitrification) technology. The process is autotrophic conversion of NH$_4^+$ to N$_2$ gas that involves two steps in one reactor volume. First step is aerobic nitrification of NH$_4^+$ to NO$_2^-$ or NO$_3^-$ with oxygen as the electron acceptor and the second step is anoxic denitrification of NO$_2^-$ or NO$_3^-$ to dinitrogen gas with NH$_4^+$ as the electron donor. Nitrite oxidisers are out-competed at low oxygen and high NH$_3$ concentration. The whole process can be simply expressed by reaction 1:

$$2\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{N}_2 + 3\text{H}_2\text{O} + 2\text{H}^+$$ (1)

From stoichiometry of the reaction implies that process consumes 4.4 mg of alkalinity (HCO$_3^-$) per mg of NH$_4$N removed. OLAND system does not require NO$_2^-$ supply and does not need strictly anaerobic conditions. Inhibition by trace O$_2$ exposure is therefore not a serious problem (Kuai and Verstraete, 1998). The start-up was significantly improved by adding anaerobic sludge that resulted in a removal rate 20 times higher (Pynaert et al 2003). In subsequent studies the process was tested in two membrane-assisted bioreactors (MBR) with suspended biomass for oxygen-limited nitrification (or partial nitritation) and autotrophic denitrification (or anaerobic ammonium oxidation), respectively. It worked without extra carbon source with an efficiency of 82% and nitrogen removal rate of 0.55 kg N m$^{-3}$ day$^{-1}$ (Wyffels et al 2004a).

**Piggery manure**

Piggery manure is characterized by very high nitrogen and high chemical oxygen demand (COD) and total phosphorous content. For removal of nitrogen from such kind of wastewater the Anammox process was tested by Ahn et al. (2004). COD and total nitrogen concentration reported in these studies amounted to 56 g COD l$^{-1}$ and 5 g T-N l$^{-1}$. In the experiments granular sludge in lab-scale upflow anaerobic sludge bed (UASB) reactor was used. Hwang et al (2005) applied SHARON-Anammox system for treatment of piggery manure with very low C/N ratio (see description of SHARON process in chapter 3.3).

**Digested fish canning effluents**

The effluent from fish-canning industry has salinity similar to sea water. The concentration of particular ions amounts up to 19,000 g Cl$^-$ m$^{-3}$, 12,000 g Na$^+$ m$^{-3}$ and 2,500 g SO$_4^{2-}$ m$^{-3}$. Moreover, it has high COD contents equal to 10,000-60,000 g COD m$^{-3}$ and high protein concentration ranging of 3,500-15,000 g m$^{-3}$. During process digestion COD is reduced and high level of ammonium is produced (up to 5,000 g m$^{-3}$) which results in a stream with low C/N ratio (Dapena-Mora et al 2006). It occurred that it was possible to remove nitrogen from such kind of stream applying SHARON-Anammox process in sequencing batch reactor (SBR) lab-scale reactor. Despite high salt content and variable characteristics of supplied media an average removal efficiency of 67% was obtained.

**Tannery wastewater**

High nitrogen content occurs also in tannery wastewater (150-250 mg l$^{-1}$). It is characterized by presence of sulphide, chromium and especially chloride, which has negative effects on nitrification. For treatment of tannery wastes SBR reactor were in use (Banaš et al 1999; Murat et al 2006). In 2004 also SHARON-Anammox process as a full-scale application was used in the largest tannery in
the Netherlands, Koninklijke Hulshof’s Verenigde Fabrieken BV, in Lichtenvoorde. The process combines anaerobic COD breakdown and sulphate reduction, sludge separation, sludge digestion and dewatering, heat and power generation, biological sulphide oxidation to elementary sulphur and finally partial nitrification and Anammox (Paques, 2007).

Potato starch wastewater also has a high concentration of nitrogen. However, application of the Anammox for its treatment has not been reported. The nitrogen concentration varied between 1000-1100 mg l\(^{-1}\) and for its removal, activated sludge with nitrification/denitrification via nitrite was used (Abeling and Seyfried, 1992). Very high nitrogen content also occurs in the sludge liquid of slaughterhouse waste. Siegrist et al. (2006) reported nitrogen concentration in these streams up to 15 g N l\(^{-1}\), which leads to inhibition of the digester performance. To reduce so high nitrogen content he proposed continuous separation of a fraction of the sludge and the liquid. For that purpose first, he stripped excess of nitrogen and then he used the nitrification/denitrification in SBR. However, application of partial nitrification/Anammox process was also mentioned as one of another alternatives.

### 3.2 Traditional nitrification and denitrification

Nitrogen in water and wastewater is present in inorganic and organic forms. The ammonia as the main inorganic nitrogen compound in raw wastewater is mainly derived from urine according to reaction 2:

\[
\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3
\] (2)

To the organic nitrogen compounds in wastewater belong heterocyclic compounds and proteins. During different mechanisms of ammonification as hydrolysis, oxidative, reductive and desaturative deamination, they are converted to ammonia.

\[
\text{R-NH}_2 + \text{H}_2\text{O} \rightarrow \text{R-OH} + \text{NH}_3
\] (3)

\[
\text{R-CHNH}_2\text{COOH} + \text{H}_2\text{O} \rightarrow \text{R-CO-COOH} + 2(\text{H}) + \text{NH}_3
\] (4)

\[
\text{R-CHNH}_2\text{COOH} + 2(\text{H}) \rightarrow \text{R-CH}_2\text{-COOH} + \text{NH}_3
\] (5)

\[
\text{R-CH}_2\text{-CHNH}_2\text{-COOH} \rightarrow \text{R-CH=CH-COOH} + \text{NH}_3
\] (6)

Both, ammonia coming from urea and from ammonification are used during aerobic treatment processes for bacteria growth. It was estimated that bacteria consist roughly of 50% of protein and the nitrogen content of protein makes up of 16%. Therefore, for synthesis of 1 g of bacterial biomass about 0.08 g of ammonia nitrogen is needed (Gallert and Winter, 2005).

To remove ammonia that was left after cell synthesis the processes of nitrification followed by denitrification are commonly used. Ammonium oxidation during nitrification is carried out in the two following steps. During the first step called nitritation, ammonium is oxidized to nitrite. A process intermediate is \( \text{NH}_2\text{OH} \). In the second – nitratation – nitrite is oxidized to nitrate. For the first step of ammonium oxidation 3.16 g \( \text{O}_2 \) is required to oxidize 1 g \( \text{NH}_3\text{-N} \) nitrogen while for the second step to oxidize 1 g nitrite to nitrate 1.11 g \( \text{O}_2 \) is needed (excluding cell synthesis) (Ahn, 2006). To oxidize 7 mg of ammonium to nitrate 1 mmol alkalinity is needed (Auterská and Novák, 2006). Bacteria catalyzing ammonia oxidation belong to the genera Nitrosomonas, Nitrosococcus, Nitrosolobus, Nitrospira and Nitrosovibrio. Organisms responsible for nitratation are Nitrobacter, Nitrococcus, and Nitrospira. Both steps can be expressed by simplified reactions:

\[
\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + \text{H}^+ \] (7)

\[
\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^- \] (8)

Nitrification results in a pH value decrease. If wastewater buffer capacity is week, the pH value can drop below 7, which prevents further oxidation from nitrite to nitrate.

During the autotrophic ammonia oxidation process, energy is produced. However, the metabolism of nitrifying bacteria is complicated and can accomplish low profit of energy, which results in quite low growth rate.

Moreover, it has been discovered that some bacteria are able to perform heterotrophic nitrification of organic nitrogen (reaction 9). To the group of such bacteria belong Arthrobacter, Flavobacterium, and Thiosphaera.
R-NH₂→R-NHOH→R-NO→NO₃⁻ (9)

In contrast to autotrophic nitrification no energy is generated by nitrate formation. Some heterotrophic nitrifiers as Thiosphaera can also denitrify nitrate under aerobic conditions (Stouthamer et al. 1997). Moreover, many aerobic bacteria are able to switch from oxygen respiration to nitrate oxidation. Then, to perform nitrate respiration heterotrophic bacteria for denitrification require an electron donor as carbon source.

\[ \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \]  

(11)

The normally used process that follows nitrification to transfer nitrogen from wastewater to atmosphere is **denitrification**. It starts from nitrate reduction to nitrite and then NO and N₂O are formed, finally to be converted to dinitrogen gas.

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow 0.5\text{N}_2 \rightarrow 0.5\text{N}_2 \]  

(12)

Denitrification requires an electron donor in raw wastewater, endogenous respiration or an external source as methanol, ethanol or acetate. Stoichiometrical reaction of denitrification can be expressed by the following reactions with methanol or acetate as carbon source, respectively:

\[ 5\text{CH}_3\text{OH} + 6\text{HNO}_3 \rightarrow 5\text{CO}_2 + 3\text{N}_2 + 13\text{H}_2\text{O} \]  

(13)

\[ 5\text{CH}_3\text{COOH} + 8\text{HNO}_3 \rightarrow 10\text{CO}_2 + 4\text{N}_2 + 14\text{H}_2\text{O} \]  

(14)

For reduction of 1 g NO₃⁻-N during denitrification, more than 2.85 g COD is required (Bernet et al. 1996). Denitrifying bacteria also can use as electron acceptor NO₂⁻, NO or N₂O instead of nitrate. Under specific conditions, these intermediates can be generated during denitrification of nitrate. Especially nitrous oxide is formed, which is a greenhouse gas.

A proper value of the C/N ratio is essential for efficient denitrification (Plaza et al. 1989; 1990). Denitrification is a less temperature-sensitive process than nitrification. The optimum temperature is similar to nitrification. Temperature range varies between 35-50°C while the optimum pH value ranges from 7 to 9. Conversion of nitrates to nitrogen gas results in alkalinity increase. Per 1 mole of NO₃⁻-N reduced to nitrogen gas, 1 mole of alkalinity is produced.

Denitrification of nitrogen coming from supernatant (15-20% of total nitrogen inlet) requires enlargement of anoxic volume in the main stream by about 20% and increase in sludge retention time (SRT) from 11 to 15 days. The entire volume increases from about 140 to 190 l/p.e. taking into account sludge production of 45 g TSS/p.e. per day and an activated sludge concentration of 3.5 kg TSS m⁻³. Annually, supernatant gives 0.6-0.7 kg N/p.e. that corresponds to about 13 kg N denitrified y⁻¹ m⁻³ and additional reactor volume (501/p.e.) (Siegrist, 1996).

The processes of nitrification and denitrification have a significant contribution of the production of nitrous oxide (N₂O) also called laughing gas. N₂O has a great impact on the greenhouse effect and the destruction of the ozone layer. N₂O is about 320-fold more efficient than CO₂ in contributing to global warming. At different stages of the anthropogenic nitrogen cycle also emission of N₂O takes place. It is reported by Mulder (2003), that this emission occurs largely in agriculture (50%), fertiliser production (6%) and wastewater treatment (21%). The emission of N₂O in wastewater treatment is largely determined by operational conditions. Under unfavourable conditions, like a high load and a low COD/N ratio, the emission of N₂O can contribute to 10% in the total N-balance (Mulder, 2003). Unfortunately, both N₂O and NO gases are produced during denitrification or nitrification as intermediates (Renner and Becker, 1970). At one of the France WWTP, N₂O emission during nitrogen removal processes was estimated approximately to 38 kg N-N₂O day⁻¹ (Tallec et al. 2006a). It was shown that under low oxygen concentration nitrifiers can shift to nitrifier denitrification, then nitrite plays the role instead of oxygen as the electron acceptor. The end-product of nitrifier denitrification is N₂O (Tallec, 2006b). C/N ratio was reported by Kishida et al. (2004) as an important factor influencing N₂O emission during denitrification phase in the swine manure treatment process.
3.3 SHARON process

In some cases, it is useful to stop nitrification on level of ammonium to nitrite. There are three ways to prevent further oxidation of NO$_2^-$ to NO$_3^-$. The first one takes advantage of differences in temperature dependency between NH$_4^+$ and NO$_2^-$ oxidizers. Use of low or high pH value can also inhibit NO$_3^-$ formation. The third one is a proper adjustment of the oxygen to the N-load and biofilm thickness (Hao et al 2002). Comparing partial nitritation to traditional nitrification 25% less aeration is needed (Van Loosdrecht and Salem, 2006).

Conventional NH$_4$-N removal:

\[
2\text{NH}_4^+ + 4\text{O}_2 + 8\text{COD} \rightarrow \text{N}_2 + 2\text{H}^+ + 3 \text{ g sludge} \quad (15)
\]

Nitrite as intermediate:

\[
2\text{NH}_4^+ + 3\text{O}_2 + 4.8\text{COD} \rightarrow \text{N}_2 + 2\text{H}^+ + 1.8 \text{ g sludge} \quad (16)
\]

A system that takes advantage of the difference between ammonium and nitrite oxidizers maximum growth rate at higher temperature is SHARON (Single reactor for High activity Ammonia Removal Over Nitrite) process. The process is performed in a single, completely mixed reactor. SRT of about 1 day enable ammonium-oxidizing bacteria to grow and at the same, oxidation of nitrite to nitrate is eliminated (Hellinga et al 1998). Then, the nitrite is anoxically converted to nitrogen gas. The process requires high temperature in the range of 30-40°C, control of the hydraulic retention time (usually 1 day), as well as pH and dissolved oxygen control (Hellinga et al 1998). The biochemistry of the process results in significant reductions in the required oxygen needed for oxidation (25%) and in the carbon addition required for bacterial growth in denitrification (40%) (Fux et al 2002). However, external carbon source is still needed. The SHARON process has been applied for sludge digestion supernatant treatment in full-scale at WWTP Dokhaven in 1998, at the Rotterdam Sluisjesdijk sludge treatment plant in 1999, The Netherlands (Mulder et al 2000, Van Kempen et al 2001). At present, there are seven WWTPs in operation or under construction applying SHARON process (van Loosdrecht and Salem, 2006).

3.4 Anammox as sustainable process

When Anammox bacteria were discovered, a new alternative appeared for treatment of highly concentrated ammonium streams. The traditional biological methods as nitrification/denitrification applied for treatment of such wastewaters are not suitable due to the lack of carbon source available for denitrification. Feeding of wastewater with synthetic carbon as methanol would be relatively expensive. Moreover, application of Anammox for separate treatment of ammonium-rich supernatant coming from dewatering of the digested sludge that usually is returned back to the head of WWTP would decrease the total nitrogen load. In consequence, it would lead to a lower need for volume of nitrification and denitrification chambers. Additionally, separate treatment of the supernatant might be an advantage if supernatant inhibits nitrification in WWTP. Inhibition can appear when nitrogen load from supernatant exceeds 20% of total load (Siegrist, 1996).

<table>
<thead>
<tr>
<th>Saves:</th>
<th>Negatives:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Compared to traditional nitrification/denitrification 40% of oxygen demand is needed</td>
<td>• Slow start-up period</td>
</tr>
<tr>
<td>• No chemicals are used: neither external carbon source nor chemicals for pH corrections</td>
<td>• Production of NO$_3^-$</td>
</tr>
<tr>
<td>• Lower energy is consumed due to lower usage of oxygen</td>
<td>• Preceding step as partial nitritation required</td>
</tr>
<tr>
<td>• Applicable for the streams with high NH$_4$-N concentrations</td>
<td></td>
</tr>
<tr>
<td>• Versatility of applications (see chapter 3.1)</td>
<td></td>
</tr>
<tr>
<td>• Lower NO$_x$ emission compared to nitrification/denitrification</td>
<td></td>
</tr>
<tr>
<td>• Reduction of the CO$_2$ emission during waste water treatment by 88%</td>
<td></td>
</tr>
<tr>
<td>• Low amount of sludge production</td>
<td></td>
</tr>
</tbody>
</table>
Anammox for ammonium removal requires partial nitritation as the preceding step. The combined partial nitritation/Anammox has a much lower oxygen demand if compared to nitrification/denitrification processes. A price estimate for a combination of the partial nitrification with the Anammox process amounts to approximately 0.75 Euro per kg of N removed (van Dongen, 2001). Savings and negative factors are presented in Table 3. Application of the Anammox process would reduce emission of greenhouse gases if compared to nitrification/denitrification since during Anammox process, apart from nitrogen gas, only traces of N$_2$O, NO, NO$_2$ are produced. Moreover, the CO$_2$ emission can be reduced due to the fact that during this process CO$_2$ is consumed. The experiments with technical-scale pilot plant where partial nitritation and Anammox in two following steps were investigated, showed that concentration of N$_2$O above the surface of partial nitritation reactor was >100 ppm while above the Anammox reactor it amounted to 4.5-7 ppm (Trela et al 2005).

### 3.5 Physical-chemical methods

Other processes (than biological), which can be applied for removal of nitrogen from supernatant, are air or steam stripping. They both involve physical and chemical methods. During the first chemical part, a proper pH is achieved and next the physical process of stripping the ammonia gas in a stripping tower or column takes place.

**Air stripping**

According to EPA, 2000 ammonia stripping is defined as a simple desorption process used to lower the ammonia content of a wastewater stream. In order to reach a pH value of about 10.8-11.5 lime or caustic is added to wastewater. At such pH value, ammonium hydroxide ions are converted to ammonia according to the following reaction 17:

$$\text{NH}_4^+ + \text{OH}^- \rightarrow \text{H}_2\text{O} + \text{NH}_3 \quad (17)$$

Formed sludge flocs and precipitated CaCO$_3$ which are the effect of pH increase, should be removed in a sedimentation step (Siegrist, 1996).

Advantages or disadvantages of ammonia stripping are presented in Table 4.

**Steam stripping**

If excess steam from plant incineration is available, steam stripping might be another alternative. The main difference between air and steam stripping processes is in the final treatment of the ammonia-rich gaseous phase (Janus and van der Roest, 1997). During air stripping, the air with high ammonia content is either scrubbed with acid or combusted. When steam stripping is taking place, aqueous ammonia is produced which then can be concentrated by reflux. Such concentrated free ammonia solution may be used for reduction of nitrous oxide in flue gas cleaning.

**Precipitation of Struvite/MAP**

The chemical precipitation of magnesium-ammonium-phosphate $\text{MgNH}_4\text{PO}_4\cdot6\text{H}_2\text{O}$

### Table 4. Advantages and disadvantages of ammonia stripping (EPA, 2000).

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• relatively simple operation not affected by wastewater fluctuation if pH and temperature remain stable.</td>
<td>• need for re-pumping of water to the stripping tower, which requires maintenance and power requirements</td>
</tr>
<tr>
<td>• creates no backwash or regeneration</td>
<td>• cannot be performed in freezing conditions, fogging and icing result in reduction in process efficiency</td>
</tr>
<tr>
<td>• unaffected by toxic compounds (as it is in case of biological methods)</td>
<td>• does not remove nitrite, nitrate and organic nitrogen</td>
</tr>
<tr>
<td>• controlled process for selected ammonia removals</td>
<td>• possibility of air pollution resulting from reaction of ammonia and sulfur oxide</td>
</tr>
<tr>
<td></td>
<td>• probability of noise</td>
</tr>
</tbody>
</table>
(MAP) or struvite is another alternative to remove nitrogen from supernatant (Ek et al., 2006; Çelen and Türker, 2001; Siegrist, 1996; Yoshino et al., 2003). The concept of this method is based on the following reaction when thermodynamic solubility product, $K_s$, is exceeded:

$$Mg^{2+} + NH_4^+ + PO_4^{3-} \rightarrow MgNH_4PO_4 \cdot 6H_2O$$

$$pK_s = 12.6 \ (T = 25^\circ C) \quad (18)$$

Since MAP is soluble in acid solutions, to perform precipitation, the pH value of supernatant must be increased but at the same time, it should not exceed a value of 9.5 to avoid odor coming from free ammonia. The raise of pH can be obtained by aeration (removal of dissolved carbon dioxide) or addition of NaOH. Precipitation procedure performed by Siegrist, (1996) starts with the removal of remaining suspended solids (SS) in the digester supernatant by flocculation with a highly cationic polyelectrolyte. Later on, phosphoric acid, magnesium oxide (Mg: P: N ratio of 1.3:1:1), and NaOH were added in three following steps. Çelen and Türker, (2001) used a two-step purification process to recover MAP crystals from impurities of supernatant. First, precipitates were dissolved in acid and removed by centrifugation. Then, supernatant was re-precipitated by adjusting pH with caustic. In this way, 85% of MAP recovery was obtained. The calculated cost of ammonia recovery from supernatant amounted to $7.5-8.0$/kg $NH_4^+$-N. The similarities in availability of Mg, P and N between MAP soil and commercial fertilizers give the possibility to utilize MAP (after drying) as additive to compost, garden soil or dried sewage.
4. APPLICATIONS OF ANAMMOX REACTION

4.1 Systems involving Anammox process

**Two-stage process: partial nitritation/Anammox, SHARON-Anammox**

The combination of partial nitritation in the first step followed by Anammox in the second step is a recently proposed technology for total nitrogen removal. The overall process is autotrophic and nitrogen removal requires 1.71 kg O\textsubscript{2} (for oxidation of ammonium to nitrite) to remove 1 kg N. Continuously stirred reactor for nitritation and the SBR reactor for Anammox were used by Fux et al. (2002) to test such process configuration. Two aspects were given as essential for partial nitritation. First, it was necessary to suppress nitrate oxidizers and secondly, to obtain nitrite-to-ammonium ratio (NAR) about 1.3 in the effluent. Such ratio was essential to perform efficiently the following Anammox step.

For operation of the two steps it is also possible to use moving bed biofilm reactors (MBBR). In such option, the partial nitritation was stable with a proper NAR in the effluent due to adequate dissolved oxygen adjustment and resulted in a pH drop (Gut, 2006b; Szatkowska, 2004). It was also shown that high efficiency of Anammox reactor can be obtained due to the presence of *Brocadia anammoxidans* or *Kuenenia stuttgartiensis* even if the Anammox population is estimated only as 20-30% of the whole bacterial community (Gut, 2006a). The batch test studies on Anammox showed that the highest Anammox efficiency was obtained under dissolved oxygen concentration in the bulk liquid within the range of 0.2-0.4 mg l\textsuperscript{-1}. Then, ammonium and nitrite nitrogen were removed simultaneously (Szatkowska, 2004).

The partial nitritation/Anammox process is often named SHARON-Anammox by Dutch researchers. The SHARON-Anammox process combines two technologies, which were developed independently. Combination of both processes rely on conversion of half of ammonium to nitrite in the first step (partial nitritation) and then on anaerobic transformation of ammonium and nitrite mixture to nitrogen gas in the second following step. SHARON combined with Anammox process is one of the methods that was developed for supernatant treatment. The SHARON reactor can be operated either under oxic and anoxic conditions, or continuously with limited oxygen supply to prevent oxidation of nitrite to nitrate. Consumed oxygen during the partial nitritation generates proper conditions for Anammox in the next step. Combination of these two autotrophic processes causes that there is no need for external source of carbon. In 2002, at WWTP Dokhaven in Rotterdam, The Netherlands, the Anammox anaerobic granular sludge reactor was build to follow already existing SHARON reactor from 1998.

**One-stage process: partial nitritation/Anammox, CANON, deammonification, SNAP**

Many research studies indicate that there is a possibility to remove nitrogen under low dissolved oxygen condition and low concentrations of organic substances (COD) (Siegrist et al 1998, Helmer et al 2001). It was ascertained that utilization of nitrogen is not related to COD changes which indicates that autotrophic bacteria are responsible for the process. It is possible that nitrifiers can oxidize ammonium nitrogen to nitrite under limited oxygen conditions, while produced nitrite can be used to oxidize remaining ammonium by Anammox bacteria. Conducted investigations with rotating biological contactor (RBC) in Germany showed that 90% of inorganic nitrogen was removed during oxic conditions (Helmer et al 1998). Treated wastewater contained low COD to N ratio of about 2 and nitrogen removal could not be performed by denitrification.

Cooperation of two groups of organisms removing nitrogen was possible due to the diversity of biological layer, where both organisms were developed. Bacteria oxidizing ammonium to nitrite play a dominating role in the outer-layer. The produced nitrite penetrates into the biofilm depth where Anammox bacteria use them for anaerobic oxidation (Figure 1). The nitrogen removal rate depends on oxygen mass transfer efficiency.
Under oxygen-limited conditions nitrite is accumulated due to lower oxygen affinity for nitrite oxidizers than for ammonium oxidizers (Wyffels et al. 2004b).

The name of CANON is coming from Completely Autotrophic Nitrogen Removal Over Nitrite and is often used for such kind of system. The reaction describing the two processes that take place simultaneously in one reactor volume can be expressed as follows:

\[
\text{NH}_3 + 0.85\text{O}_2 \rightarrow 0.44\text{N}_2 + 0.11\text{NO}_3^- + 1.43\text{H}_2\text{O} + 0.14\text{H}^+ \quad (19)
\]

The CANON system is also called “deammonification” by German researchers (Helmer and Kunst, 1998; Hippen et al. 1997; Rosenwinkel et al. 2005) or SNAP (single-stage nitrogen removal using Anammox and partial nitritation) by Japanese researchers (Furukawa et al. 2006). It is an example where both partial nitritation and Anammox can be accomplished in one reactor volume.

**DEAMOX**

DEAMOX (Denitrifying Ammonium Oxidation) is another combined system involving Anammox process. In the first stage of this process, anaerobic mineralization of organic nitrogen takes place. Next in the nitrifying reactor, ammonium is oxidized to nitrate. In the final DEAMOX stage, nitrite is produced mainly from nitrate using sulfide as an electron donor. This reactor is partially fed with effluent from nitrogen mineralization step (Kalyuzhnyi et al. 2006). The process was tested in laboratory scale and it was applied for baker’s yeast effluent treatment. The system is characterized by the following advantages: no need for complex process control and absence of high nitrite level (no danger to exceed toxic level).

**ADEPT**

The combined ADEPT (Anaerobic Digestion Elutriated Phased Treatment) – SHARON - Anammox lab-scale processes was performed for the purpose of resources recovery and nitrogen removal (concentration varied within the range of 3240–4960 mg N l\(^{-1}\)) from slurry-type piggery waste. ADEPT consists of an acid elutriation slurry reactor for hydrolysis/acidification, followed by a high rate methanogenic reactor such as the upflow anaerobic sludge bed (UASB) reactor. After ADEPT process, low C/N ratio was obtained in the effluent, which was treated by SHARON-Anammox in following steps. Steady state conditions to produce nitrite in the effluent were reached after 65 days from the start-up operation. To start-up the Anammox, reactor was fed with stock nitrite solution and piggery wastewater for about 300 days. A steady state of reactor was reached after the 66\(^{th}\) day of operation (Hwang et al. 2006).

4.2 Reactor types

The Anammox process, in practice, requires preceding step, which converts half of ammonium to nitrite. These two reactions can be performed in one-stage (Third et al. 2005) or two-stage (Fux et al. 2002; Gut, 2006a, b) process. Moreover, different concepts of biomass growth systems can be used as activated sludge, biofilm and granular sludge. Additionally, process can be run in different reactor types (see Table 5).

The highest efficiency of Anammox bacteria of approx. 9 kg of N m\(^{-3}\) per day was
Table 5. Different concepts of bacteria growth systems and different reactor types.

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>granular sludge</td>
<td>Dapena-Mora et al 2004; Sliekers et al 2003</td>
</tr>
<tr>
<td></td>
<td>Ahn et al 2004; Schmidt et al 2004</td>
</tr>
<tr>
<td></td>
<td>Arrojo et al 2005</td>
</tr>
<tr>
<td>upflow anaerobic sludge bed UASB</td>
<td></td>
</tr>
<tr>
<td>air lift</td>
<td>Third et al 2005; Strous et al 1998</td>
</tr>
<tr>
<td>sequencing batch reactor SBR</td>
<td></td>
</tr>
<tr>
<td>activated sludge</td>
<td></td>
</tr>
<tr>
<td>membrane bioreactor MBR</td>
<td></td>
</tr>
<tr>
<td>biofilm</td>
<td></td>
</tr>
<tr>
<td>moving bed biofilm reactor MBBR</td>
<td></td>
</tr>
</tbody>
</table>

achieved in gas lift reactors (Sliences et al 2003).

The intensive investigations in the Anammox process have also resulted in different applications for patents (Patent Storm, 2007; the online Anammox resources, 2007).

4.3 Start-up in full-scale

Due to the slow growth rate of the Anammox process, start-up period is a weak point for full-scale implementation. Its speeding up was tested in altered reactor configurations and influence of different factors was investigated. Experiences with SBR reactor seeded from activated sludge showed that Anammox activity (in one-stage process) can be obtained after several months (Li et al 2004). Oxygen was pointed out as an important factor for process acclimatisation and nitrite and hydrazine as intermediates were tested to speed-up the start-up period and acclimatisation. Scaling-up the process in 3 steps was used in STRASS WWTP, Austria where partial nitritation/Anammox was implemented in full-scale. However, total start-up period lasted 2.5 years (Wett, 2006). The research experiences from implementation of the Anammox process in full-scale at Hattingen WWTP (Rosenwinkel et al 2005) showed that the start-up period can last up to more than 1 year, when strategy of Anammox development on the previously created nitrifying biofilm is used.

A similar strategy of start-up has been used at Himmerfjärden WWTP in Stockholm, Sweden. There, the largest in the world full-scale plant (1398 m³) for supernatant treatment based on one-stage process principle started to be implemented in April 2007.

Intensive research and increasing experiences with Anammox process have led to a decrease in start-up time. The Table 6 presents full-scale application and start-up periods obtained for particular cases.

Table 6. Full-scale application of Anammox and improvement of start-up period (modified after Abma et al 2007).

<table>
<thead>
<tr>
<th>Place</th>
<th>Application</th>
<th>Capacity kg N day⁻¹</th>
<th>Achieved kg N day⁻¹</th>
<th>Start-up time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterboard Hollandse Delta,Rotterdam, NL (two-stage)</td>
<td>Municipal (supernatant)</td>
<td>490</td>
<td>750</td>
<td>3.5 years</td>
</tr>
<tr>
<td>Strass WWTP, AT (one-stage)</td>
<td>Municipal (supernatant)</td>
<td>300</td>
<td>300</td>
<td>2.5 years</td>
</tr>
<tr>
<td>Hattingen WWTP, Ruhr, DE (one-stage)</td>
<td>Municipal (supernatant)</td>
<td>120</td>
<td>120</td>
<td>more than 1 year</td>
</tr>
<tr>
<td>IndustrieWater Lichtenvoorde, NL (one-stage)</td>
<td>Tannery</td>
<td>325</td>
<td>150</td>
<td>1 year</td>
</tr>
<tr>
<td>Waterstromen, Olburgen, NL (one-stage)</td>
<td>Potato processing</td>
<td>1250</td>
<td>700</td>
<td>6 months</td>
</tr>
<tr>
<td>Semiconductor Plant, Mie prefecture, JP (two-stage)</td>
<td>Semi-conductor</td>
<td>220</td>
<td>220</td>
<td>2 months</td>
</tr>
</tbody>
</table>
5. STOICHIOMETRY OF PARTIAL NITRITATION AND ANAMMOX REACTIONS

Nitrification
The stoichiometry of nitrification is well known and can be expressed by the simplified reaction:
\[ \text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+ \]  
(20)
Most of nitrifiers belong to autotrophs, which use carbon dioxide as source of carbon. Before carbon is incorporated in cells it must be reduced. Reduction results from oxidation of nitrogen source. The biomass growth can be described by reaction (21):
\[ 15\text{CO}_2 + 13\text{NH}_4^+ \rightarrow 10\text{NO}_2^- + 3\text{C}_5\text{H}_7\text{NO}_2 + 23\text{H}^+ + 4\text{H}_2\text{O} \]  
(21)
General reaction of nitrification where biomass yield can be seen is given as reaction 22 (Henze et al 2002):
\[ \text{NH}_4^+ + 1.86\text{O}_3 + 1.98\text{HCO}_3^- \rightarrow 0.020\text{C}_5\text{H}_7\text{NO}_2 + 0.98\text{NO}_3^- + 1.88\text{H}_2\text{CO}_3 + 1.04\text{H}_2\text{O} \]  
(22)
Anammox
An aerobic autotrophic ammonium oxidation can be generally expressed as transformation where substrates are carbon dioxide, ammonia and nitrite and products are nitrogen gas, nitrates and biomass (Szewczyk, 2005):
\[ n_{\text{NH}_3}\text{NH}_3 + n_{\text{NO}_2^-}\text{HNO}_2 + n_{\text{CO}}\text{CO}_2 \rightarrow n_p\text{N}_2 + n_{\text{NO}_3^-}\text{HNO}_3 + n_{\text{CH}_3\text{O}_x}\text{CH}_3\text{O}_x + n_{\text{H}_2}\text{H}_2\text{O} \]  
(23)
Researchers propose different stoichiometrical quotients. Strous et al (1998) determined stoichiometry of Anammox reaction based on experimental measurements and proposed the following equation:
\[ \text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_3\text{O}_x\text{CH}_3\text{O}_x + 2.03\text{H}_2\text{O} \]  
(24)
Van de Graaf et al (1996) gave the following reaction, where nitrogen in produced biomass is not considered:
\[ \text{NH}_4^+ + 1.31\text{NO}_2^- + 0.0425\text{CO}_2 \rightarrow 1.045\text{N}_2 + 0.22\text{NO}_3^- + 0.0425\text{CH}_2\text{O}_x\text{CH}_2\text{O}_x + 1.87\text{H}_2\text{O} + 0.09\text{OH} \]  
(25)
Irrespective of the differences in both reactions above, important for the process NAR is a value equal to 1.3. Moreover, similarities are visible in released nitrogen; per 2.3 moles of ammonium and nitrite, about 1 mole of nitrogen gas (N\textsubscript{2}) is generated.

Partial nitritation/Anammox
When Anammox is combined with partial nitritation and is established in two separate reactors then, the following subsequent reactions can describe the overall process:
Partial nitritation (including cell synthesis):
\[ 2.34\text{NH}_4^+ + 1.85\text{O}_2 + 2.66\text{HCO}_3^- \rightarrow 0.024\text{C}_2\text{H}_4\text{O}_5\text{N}_x\text{N}_x + \text{NH}_4^+ + 1.32\text{NO}_2^- + 2.54\text{CO}_2 + 3.94\text{H}_2\text{O} \]  
(26)
Anammox (including cell synthesis):
\[ \text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{CO}_2 + 0.066\text{H}^+ \rightarrow 0.066\text{C}_2\text{H}_4\text{O}_5\text{N}_x\text{N}_x + 1.02\text{N}_2 + 0.26\text{NO}_3^- + 1.96\text{H}_2\text{O} \]  
(27)
When the partial nitritation/Anammox process takes place simultaneously in one reactor volume, the reaction may be written based on reaction 26 and 27 as their resultant:
\[ 2.34\text{NH}_4^+ + 1.85\text{O}_2 + 2.59\text{HCO}_3^- \rightarrow 0.024\text{C}_2\text{H}_4\text{O}_5\text{N}_x\text{N}_x + 0.066\text{C}_2\text{H}_4\text{O}_5\text{N}_x\text{N}_x + 1.02\text{N}_2 + 0.26\text{NO}_3^- + 2.47\text{CO}_2 + 5.83\text{H}_2\text{O} \]  
(28)
Predictions based on stoichiometry
Based on stoichiometry several conclusions may be drawn:
- The oxygen needed for partial nitratation (reaction 26) is only 56% (1.8 g O\textsubscript{2} per g NH\textsubscript{4}+) of what is needed if all ammonium had been oxidized to nitrite (3.16 g O\textsubscript{2} per g NH\textsubscript{4}+).
- In order to accomplish partial nitratation (reaction 26) 1.1 mole of alkalinity per mol ammonium is needed and this ratio is typical for digester supernatant.
- The partial nitratation process consumes alkalinity and pH drop can be expected.
- If the influent alkalinity to the Anammox reactor is low, a certain pH value increase is expected due to the consumption of hydrogen ions during cell synthesis (reaction 27).
- A decrease in conductivity can be expected during the partial nitratation due to
transfer of hydrogen carbonate to carbon dioxide (reaction 26) and in the Anammox reaction (reaction 27) due to transfer of ammonium and nitrite into nitrogen gas both in one- or two-stage processes.

- The nitrogen assimilated in biomass is low compared with the initial nitrogen concentration (about 1.45%) in simultaneous partial nitritation/Anammox (reaction 28).
- The highest nitrogen removal efficiency in simultaneous partial nitritation/Anammox is equal to about 89% (reaction 28).

\[
\text{The sludge production is low both in the nitritation } \left( \frac{0.024 \times 113}{2.34 \times 14} \right) = 0.08 \text{ g/g } \text{NH}_4\text{-N; (reaction 26) and Anammox } \left( \frac{0.066 \times 24.1}{1 \times 14} \right) = 0.114 \text{ g/g } \text{NH}_4\text{-N (reaction 27).}
\]
6. FACTORS AFFECTING PARTIAL NITRITATION/ANAMMOX RATE

6.1 Kinetic factors

Growth rate function

Growth rate of nitrification is determined by concentration of substrate (electron donor), which is ammonium, and concentration of oxygen (electron acceptor). The equation for growth rate can be presented as follows:

$$\mu = \frac{K_{O_2} C_{NH_4}}{K_{O_2} + C_{O_2}} \frac{C_{NH_4}}{K_{NH_4} + C_{NH_4}}$$  \hspace{1cm} (29)

The growth rate of anaerobic ammonia oxidizers is depending on concentration of ammonia, nitrite and dissolved oxygen which acts as inhibitor for Anammox reaction (Szewczyk, 2005):

$$\mu = \frac{K_{1,0_2}}{K_{1,0_2} + C_{O_2}} \frac{C_{NO_2}}{K_{NO_2} + C_{NO_2}}$$  \hspace{1cm} (30)

For this process the yield value is equal to 0.066 mol biomass produced/mol ammonia nitrogen reduced, while an ammonium consumption rate per biomass amounted to 45 mmol/mg protein/min. The enrichment time of Anammox bacteria varies within the range of 100 - 600 days (van Dongen et al, 2001; Sliekers et al, 2003) (see also chapter 4.3). The high activity of Anammox is recorded within the range of pH between 6.4 and 8.2 and temperature of 20-43°C (Strous, 1999b). The physiological parameters of Anammox bacteria with comparison to ammonium and nitrite oxidizers are summarized in Table 7.

Inhibition of nitrification

The activity of nitrifiers is influenced by different factors such as substrate concentration, sludge retention time (SRT), temperature, pH value, DO and presence of toxic substances. In order to perform successfully nitrification the proper difference between sludge age and bacteria growth must be used. The sludge age must be higher than bacteria doubling time in order to avoid wash-out of the nitrifiers from the system.

Nitrification bacteria indicate high temperature dependence and the process with activated sludge can be performed within the range of 5-40°C (Henze et al, 2002). The optimum is at 30-35°C. A drop of rate is observed at lower temperatures and nitrification cannot proceed when temperature is 55-60°C. Moreover, there is a difference in the maximum specific growth rate depending on temperature between Nitrosomonas and Nitrobacter. At the low ammonium concentration in WWTP, nitrite oxidation normally proceeds faster than ammonia oxidation due to a high substrate utilization rate of the nitrite oxidizers (Jianlong and Ning, 2003).

When ammonium concentration is high at temperatures over 30°C an accumulation of nitrite can be observed. Then, nitrite oxidizers are suppressed and mainly Nitrosomonas bacteria are active.


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Ammonium oxidation</th>
<th>Nitrite oxidation</th>
<th>Anammox</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta G$</td>
<td>kJ mol substrate$^{-1}$</td>
<td>-272</td>
<td>-74</td>
<td>-357</td>
</tr>
<tr>
<td>$E_a$</td>
<td>kJ mol substrate$^{-1}$</td>
<td>68</td>
<td>44</td>
<td>70</td>
</tr>
<tr>
<td>$Y$</td>
<td>C-mol mol substrate$^{-1}$</td>
<td>0.08</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Aerobic maximum specific conversion rate</td>
<td>mM/ mg protein/min</td>
<td>0.2-0.6</td>
<td>0.07-2.6</td>
<td>0</td>
</tr>
<tr>
<td>Anaerobic maximum specific conversion rate</td>
<td>mM/ mg protein/min</td>
<td>0.002</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>h$^{-1}$</td>
<td>0.04</td>
<td>0.04</td>
<td>0.003</td>
</tr>
<tr>
<td>minimum doubling time</td>
<td>days</td>
<td>0.73</td>
<td>0.71</td>
<td>10.6</td>
</tr>
<tr>
<td>temperature</td>
<td>°C</td>
<td>up to 42</td>
<td>up to 47</td>
<td>up to 43</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>6-8.5</td>
<td>6-10</td>
<td>6.7-8.3</td>
</tr>
</tbody>
</table>

C-mol of biomass equal to 15 g
A favorable pH value for *Nitrosomonas* is 7.9-8.2, while for *Nitrobacter* 7.2-7.6. The nitrification rate decreases if pH level remains outside this range. Since free ammonia and free nitrous acid is a function of ammonium and nitrite concentration, respectively, and also a function of pH and temperature, nitrification activity can be inhibited by the presence of these two forms. This also means that pH influences on bacteria activity, which changes with NH$_3$ and HNO$_2$ concentartions. The first step of nitrification starts to be inhibited by free NH$_3$ in the range of 10-150 mg l$^{-1}$. At free NH$_3$ concentration of 0.1-1.0 mg l$^{-1}$, the reaction rate in the second step decreases and nitrates are accumulated. Free ammonia can be calculated according to formula (31):

$$\text{Free NH}_3 \ (\text{mgNH}_3 \text{l}^{-1}) = \frac{17 \times TAN(\text{mgNH}_4 - N \text{l}^{-1}) - 10^{9.5}}{14} e^{0.644(1273-T)} + 10^{9.5}$$

Moreover, *Nitrobacter* activity can be inhibited by free HNO$_2$ concentration in the range of 0.2-2.8 mg l$^{-1}$ (Anthonisen et al. 1976). Additionally, nitrification can be inhibited by excess of substrate. Kindaichi et al. (2004) investigated the influence of NH$_2$OH on nitrification and it occurred that the addition of NH$_2$OH stimulates the oxidation of NH$_4^+$ (ammonium consumption rate was even two times higher) whereas the addition of low concentrations of NH$_2$OH (250 μM) completely inhibited the growth of nitrite oxidizers.

Nitrifying bacteria are sensitive to inhibiting compounds in wastewater but experiences from many Swedish WWTPs with activated sludge proved that bacteria could adapt to the toxic substances present in the influent due to protective activity of heterotrophs (Jönsson et al. 2000).

**Anammox process inhibition and the influence of organic substances**

Due to the fact, that the Anammox bacteria are characterized by a very low maximum growth rate of 0.0027 h$^{-1}$ and a doubling time of at least 11 days (Strous et al. 1998), the system where Anammox culture is cultivated requires long biomass retention time. It is supposed that slow growth of Anammox bacteria is caused by slow rate of ammonium compounds assimilation resulting from low activity of proper enzymes in anoxic conditions.

Anammox bacteria indicate partial decrease or total inhibition of the activity under high dissolved oxygen conditions, too high nitrite concentration and presence of certain organic compounds.

Chemolithoautotrophs such as nitrifiers use carbon compounds as an additional carbon source. In case of Anammox, alcohols that are usually used at WWTPs as an external carbon source or are present in wastewater due to incomplete fermentation, strongly and irreversible inhibit the Anammox process. Methanol was found out as the strongest inhibitor and it influences the Anammox process at concentration as low as 0.5 mM. The organic acid acetate and propionate were oxidized parallel to ammonium oxidation. Propionate was consumed at higher rate (0.8 nmol min$^{-1}$ mg of protein$^{-1}$) and it was oxidized to CO$_2$ with NO$_3$ and/or NO$_2$ as an electron acceptor (Güven et al. 2005). Moreover, it was shown that Anammox bacteria could compete successfully with heterotrophic denitrifying bacteria for propionate in the presence of excess ammonium.

The process is completely irreversible inhibited by nitrite when its concentration is higher than 10 mM (Strous, 1998, 1999b). This finding is contradictory due to the fact that nitrite is one of the main substrate needed for reaction occurrence. Another factor inhibiting the process is dissolved oxygen concentration (higher than 1μM). Although, the bacteria are anaerobic, their activity is only reversibly inhibited by oxygen.

The inhibiting influence of sulphanilamide on Anammox process was reported by Bąkowska et al. (2006). Anammox exposed to these compounds (in milligram concentrations) for short-time period indicated low decrease in process efficiency. However, exposure lasting three or four weeks was causing rapid process breakdown. The effect was increasing parallel to an increase in dosed concentrations.
*Predictions based on thermodynamics*

The combination of the stoichiometry of individual reactions with classical thermodynamics helps to estimate such important results as cell biomass production, respiration and heat as well as nutrient requirements, pH, alkalinity effects and energy flow throughout the system.

The relationship between synthesis reactions and energy is crucial to understand the balance of inputs and outputs of overall process. Moreover, cell yield is a fundamental biological parameter. Correlations between thermodynamics and biomass yields have been developed by Perry McCarty in 1972. In order to estimate cell yield using thermodynamics it is assumed that electron and energy balances control the behaviour of biological systems. An electron-donor substrate is oxidized (in case of nitrification and Anammox it is ammonium). Electrons are transported to the carbon and nitrogen sources to reduce them to the degree necessary for incorporation into cell.

To account electrons and energy needed for activation of reaction and the electron balance, the fraction of electron-donor, which is an electron equivalent, is investigated in the calculations. Using equilibrium and balancing the reactant and product of half-reactions, it is possible to calculate energy of particular reactions based on thermodynamic principle. To account for the energy lost in the reaction, the Gibbs energy associated with the electron donor half-reaction is adjusted. Adjustments can be made either for the overall reaction by summing up all the sub-reaction steps or for each step separately.

In Table 8 half-reactions for nitritation, Anammox and cell synthesis are presented and corresponding values of free Gibbs energy are calculated.

Energetic gain in anaerobic ammonium oxidation amounts to $\Delta G = -363 \text{ kJ mol}^{-1}$ and is higher than change of Gibbs free energy for aerobic oxidation (as in Table 7). Calculated Gibbs free energy for Anammox is in general agreement with values given by other authors (see Table 1 and 7).

Biomass yield can be calculated based on thermodynamics. McCarty (1972) stated that the yield coefficient based on free energy during oxidation of the energy source can as maximum be transferred by 55% to the produced cells based on free energy. The free energy in oxidation of one electron equivalent ammonium nitrogen is $-60.5 \text{ kJ}$ and for one electron equivalent sludge $120 \text{ kJ}$ (Table 8). The sludge yield for Anammox bacteria is then $0.55 \times 60.2/120 = 0.276$ electron equivalent sludge/electron equivalent nitrogen. The equivalent weight is $113/20 = 5.65$.

**Table 8. Half-reactions and free Gibbs energy calculations for nitritation, Anammox and cell synthesis (based on values from Faust and Hunter, 1971).**

<table>
<thead>
<tr>
<th>Half reaction</th>
<th>$\Delta G^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nitritation:</strong></td>
<td></td>
</tr>
<tr>
<td>(I) $1/6$NH$_4^+$ + $1/3$H$_2$O = $1/6$NO$_2^-$ + $4/3$H$^+$ + e$^-$</td>
<td>32.8 kJ/equ</td>
</tr>
<tr>
<td>(II) $1/4$O$_2$ + H$^+$ + e$^-$ = $1/2$H$_2$O</td>
<td>-78.1 kJ/equ</td>
</tr>
<tr>
<td>(I-II) $1/6$NH$_4^+$ + $1/4$O$_2$ = $1/6$NO$_2^-$ + $1/2$H$_2$O + $1/3$H$^+$</td>
<td>-45.3 kJ/equ×6 = -272 kJ/mol</td>
</tr>
<tr>
<td><strong>Anammox:</strong></td>
<td></td>
</tr>
<tr>
<td>(I) $1/6$NH$_4^+$ + $1/3$H$_2$O = $1/6$NO$_2^-$ + $4/3$H$^+$ + e$^-$</td>
<td>32.8 kJ/equ</td>
</tr>
<tr>
<td>(III) $1/3$NO$_2^-$ + $4/3$H$^+$ + e$^-$ = $1/6$N$_2$ + $2/3$H$_2$O</td>
<td>-93.3 kJ/equ</td>
</tr>
<tr>
<td>(I-III) $1/6$NH$_4^+$ + $1/6$NO$_2^-$ = $1/6$N$_2$ + $1/3$H$_2$O</td>
<td>-60.5 kJ/equ×6 = -363 kJ/mol</td>
</tr>
<tr>
<td><strong>Cell synthesis:</strong></td>
<td></td>
</tr>
<tr>
<td>(IV) $1/20$ HCO$_3^-$ + $1/5$CO$_2$ + $1/20$NH$_4^+$ + H$^+$ + e$^-$ = $1/20$C$_3$H$_7$O$_2$N + $9/20$H$_2$O</td>
<td>41.2 kJ/equ</td>
</tr>
<tr>
<td>(V) $1/2$H$_2$O = $1/4$O$_2$ + H$^+$ + e$^-$</td>
<td>78.2 kJ/equ</td>
</tr>
<tr>
<td>(IV-V) $1/20$HCO$_3^-$ + $1/5$CO$_2$ + $1/20$NH$_4^+$ + $1/20$H$_2$O = $1/20$C$_3$H$_7$O$_2$N + $1/4$O$_2$</td>
<td>120 kJ/equ×20 = 2400 kJ/mol</td>
</tr>
</tbody>
</table>
and \(14/6 = 2.33\) for sludge and nitrogen, respectively. The biomass yield is then equal to \(0.276 \times 2.33/5.65 = 0.114\) g VSS/g N in agreement with predictions based on stoichiometry in chapter 5.

### 6.2 Diffusion factors

#### Cell membrane

The Anammox reaction takes place inside the anammoxosome: an intracytoplasmic compartment bounded by, unique in biology, a single ladderane lipid-containing membrane (van Niftrik et al. 2004). Membranes are taking part in the energy and transport processes necessary for cell growth and survival and are as major barrier highly impermeable to diffusion of solutes that keeps cellular components inside a cell and toxins outside it.

For the Anammox reaction it was postulated that nitrite is reduced to hydroxylamine, which together with ammonium forms hydrazine as an intermediate. Then, hydrazine is oxidized to nitrogen gas (Schalk et al. 1997; van Loosdrecht and Jetten, 1998, Jetten et al. 2005). Both hydrazine and hydroxylamine are very toxic and reactive intermediates. Presumably, the ladderane membranes help to contain the noxious Anammox intermediates (DeLong, 2002). Therefore, the specific structure of Anammox cell membranes plays an important role in Anammox process mechanism.

#### Biofilm

Biofilm concept presents a range of opportunities for application in wastewater technologies. Its application in biological treatment processes provides grounds for optimisation of the volumetric conversion capacity. Reactors with biofilm can assure high biomass residence time even if the hydraulic influent loading is low. This makes them especially suitable when treatment requires slow growing organisms with low biomass yield.

Biofilm is formed when the carrier material is submerged in a liquid that contains nutrients and microorganisms. Then, microorganisms attach to the surface and start to grow. Created polymeric matrix causes that they become immobilized on the surface. Biofilm contains a variety of microorganisms, located in its different layers, which have an impact on the biofilm structure.

Biofilm diffusion is influenced by biofilm density, age, thickness, porosity, the presence of filamentous organisms, microbial speciation and electrostatic interactions (Bishop, 1997). Moreover, biofilm structure varies within the biofilm depth. Porosity and pore diameter decrease with depth, while biofilm density increases with depth. The biofilm structure has a significant impact on the stable operation of a plant. It results in decrease in effective diffusion of substrates and decrease in metabolic activity in the deeper layers of biofilm (Wuertz et al. 2003).

**Effect of diffusion in one-stage biofilm reactor**

According to the concept of the moving bed biofilm reactor applied for one-stage process the biofilm growing on Kaldnes rings have two different layers: an outer layer where aerobic bacteria oxidizes ammonium and an inner layer with Anammox bacteria presence. The limiting factor for biofilm systems is diffusion within the biofilm depth, which is depending on concentration and diffusivity. The processes that are taking place in biofilm are mainly redox processes. The main components for the partial nitritation/Anammox process are energy source, which is ammonium, and oxidant (electron acceptor), which is oxygen for the partial nitritation and nitrite for the Anammox. The basis of biofilm kinetics is determination of limiting substrate. The process becomes diffusion limited if energy source or electron acceptor only partly penetrates the biofilm, and limiting substrate is the component that penetrates the biofilm in lower degree (Henze et al., 2002). Since ammonium nitrogen concentration in the bulk liquid is much higher than the oxygen or nitrite nitrogen concentration, ammonium diffusion will not limit the process. Nitrite is produced in outer layer by nitrifiers but is mainly consumed by Anammox in inner layer, which means that oxygen therefore is the main limiting factor controlling the overall rate of the partial nitritation/Anammox process in biofilm reactors. The value of oxygen concentration outside the biofilm is important and crucial. From one hand, too
low oxygen will suppress the partial nitritation rate and thereby necessary production of nitrite for the Anammox step. On the second hand, higher values of oxygen can increase oxidation of nitrite to nitrate and additionally, it may inhibit the Anammox reaction.

Moreover, if a system with biofilm is considered the influence of biofilm kinetics should not be omitted. In one-stage partial nitritation/Anammox system, ammonium oxidizers in the outer layer of biofilm can co-exist with the Anammox organisms present in the inner layer. In this way, oxygen that inhibits the Anammox process is consumed in the outer layer of biofilm and Anammox bacteria are protected from oxygen.

There are in the system also suspended bacteria and therefore, to cultivate a biofilm in a bioreactor, a competition between biofilm bacteria and suspended bacteria must be taken under consideration. Growth rate of biofilm cells is always lower than the growth of suspended cells due to diffusion limitation.

In the partial nitritation process, it is important to avoid further oxidation of nitrite to nitrate. Increase in ammonium oxidation rate and limitation in nitrite oxidation rate can be obtained in different ways. One way can take advantage of the use of temperature dependence and the other of oxygen-limited condition.

The rate of Anammox process is small due to factors as substrate limitations, diffusion limitations, inhibition reactions etc. In order to assure high rate of the Anammox reaction anaerobic conditions are important since it was discovered that the Anammox rate is in fact reversible, but inhibited by oxygen. Moreover, the other strong inhibitor for the Anammox process is nitrite concentration. If, however, nitrite would be consumed at about the same rate, as it would be produced during the partial nitritation this inhibition effect would not appear at all.
7. Monitoring and Control of Partial Nitritation/Anammox

Requirements of environmental protection law have become more stringent. Therefore, the application of more advanced treatment systems, which would be able to comply with tighter standards, is necessary. Complex treatment technologies increase parallel to increasing effluent water quality. A significant increase in the use of on-line measurements can be observed in the field of water and wastewater treatment. Technologies for instrumentation, control and automation (ICA) has become less expensive and are therefore more often applied. Modern WWTP are richly equipped with different automatic devices. Implementation of monitoring equipment instead of increase in reactor volume is often given as an alternative for treatment plants that need considerable upgrading investments (Vanrolleghem and Lee, 2003). Control-monitoring system improves the knowledge concerning the process performance and can lead to better management of the process.

For nitrogen removal, the process control should be implemented with a number of stated purposes:

- to reduce the operational costs
- to improve the outlet quality
- to improve the nitrogen removal capacity of the plant.

7.1 Measurements based on stoichiometry

**Involved reactions**

The knowledge about stoichiometrical coefficients of particular reactions involved in entire treatment process allows prediction of the contents of treated wastewater and evaluation process performance. In the partial nitritation process applied for supernatant treatment main substrate is ammonium, which is produced during anaerobic digestion. In the effluent from partial nitritation about equal amounts of nitrite and ammonium can be expected. Both ammonium and nitrite from partial nitritation constitute substrate for the following Anammox step. The effluent of efficiently working process should contain mainly nitrates (about 11% of influent) and some remaining ammonium and nitrite. The reactions involved in both partial nitritation and Anammox processes are presented in Table 9.

**Analytical methods**

It can be seen from Table 9, that in the process of the partial nitritation/Anammox the following ions are involved: NH\(_4^+\), NO\(_2^-\), HCO\(_3^-\) and NO\(_3^-\). Thus, their analytical analyses are the basis for evaluation of process progress. Nowadays, different analytical techniques are accessible to perform analysis of these forms.

For Anammox activity detection experiments can be performed with \(^{15}\)N-labelled NH\(_4^+\) and NO\(_2^-\). Under anoxic conditions labeled \(^{15}\)NH\(_4^+\) reacts with unlabeled \(^{14}\)NO\(_2^-\) in ratio around 1:1 forming \(^{29}\)N\(_2\) (\(^{14}\)N\(^{15}\)N). Following labeled and unlabeled N allows stating about Anammox reaction.

**Use of conductivity**

One of the parameters that is easy to measure and can be used as control-monitoring indicator is conductivity. It is often applied for controlling chemical (Garcia, 2005; Nikolic and Hultman, 2003) or physical (Kieniewicz, 2006) removal processes when reduction in pollutants is giving a significant response in conductivity. However, recently conductivity is also used to monitor biological processes (Barat et al 2005, Gaul et al 2006, Serralta et al

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**Table 9. Reactions involves in process monitoring.**

<table>
<thead>
<tr>
<th>Process</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>digestion</td>
<td>(\text{C}_6\text{H}_7\text{O}_2\text{N} + 4\text{H}_2\text{O} = 2.5\text{CH}_4 + 1.5\text{CO}_2 + \text{HCO}_3^- + \text{NH}_4^+)</td>
</tr>
<tr>
<td>partial nitritation</td>
<td>(55\text{NH}_4^+ + 76 \text{O}_2 + 109\text{HCO}_3^- = \text{C}_6\text{H}_7\text{O}_2\text{N} + 54\text{NO}_2^- + 57\text{H}_2\text{O} + 104\text{H}_2\text{CO}_3)</td>
</tr>
<tr>
<td>Anammox</td>
<td>(1 \text{NH}_4^+ + 1.32 \text{NO}_2^- + 0.066 \text{HCO}_3^- + 0.13 \text{H}^+ = 1.02 \text{N}_2 + 0.26 \text{NO}<em>3^- + 0.066\text{CH}<em>2\text{O}</em>{0.8}\text{N}</em>{0.15} + 2.03 \text{H}_2\text{O})</td>
</tr>
</tbody>
</table>
Nitrogen and phosphorus removal are the stages where changes in conductivity can be observed (Levlin, 2006) and therefore application of that parameter for monitoring of the nitrogen removal processes is possible. The combined partial nitritation and Anammox process used for supernatant treatment has recently been investigated by many researchers but, only few WWTPs applied it in full-scale. Thus, if upgrading of process performance is considered, an important issue is investigation in the field of process control and monitoring. One of the possibilities of process behavior prediction is based on modeling (Gut, 2006a, Tauhid-Ur-Rahman, 2006). Another approach can be based on conductivity changes resulting from transformations of ammonium and hydrogen carbonate ions into molecules and its relation with nitrogen forms.

The conductivity is a parameter that can be easily and simply measured and therefore it can be used as monitoring tool. It provides information concerning chemical composition of water and is directly linked to the concentration of the ions and their mobility. The conductivity parameter gives information about the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, phosphate, sodium, magnesium, calcium, iron and aluminum cations. Non-ionic organic compounds do not conduct electrical current very well and therefore have a low conductivity value in water.

Considering conductivity, the knowledge about conductance, ionic strength, activity coefficient, and temperature compensation is important.

*Conductance* is the ability of a material to pass electrons. The specific conductance or conductivity is the sum of the individual ion conductivities and is approximately linearly proportional to the concentration of ion. Therefore, conductivity can be used to obtain a rapid, approximate value of dissolved salts in water. The unit of conductance is “ohm”, which recently has been changed for Siemens (S). The concentration of total dissolved salts (if less than 1000 mg\textsuperscript{-1}) in water where pH varies between 5-9, and temperature T is between 10-40°C can be calculated according to the formula (Fair *et al* 1968):

\[
C_{\text{dissolved salts}} \text{ [mg l}\textsuperscript{-1}] = (4.5 \times 10^5)(1.02)^{T-25} \times \text{conductivity of water [ohm}\textsuperscript{-1} \text{cm}\textsuperscript{-1}]
\]  

The properties of particular ions are depending on other ions present in solution and therefore effective concentration (activity) of each ion is less than its real molar concentration. The *ionic strength* of a solution is a function of the concentration of all ions present in a solution and can be defined as:

\[
IS = \frac{1}{2} \Sigma c_i z_i^2
\]  

where: \(IS\) is the ionic strength, \(c_i\) is the molarity concentration of ion \(i\), \(z_i\) is the charge of that ion. The summation must be done for all charged particles present in the solution both negative and positive.

In very dilute solution, activity and molar concentrations are similar. However, in more concentrated solutions the measured concentration of a substance may not be an accurate indicator of its effectiveness. Thus, *activity coefficient*, \(\gamma\), which is the ratio of the activity of any substance to its molar concentration, must be introduced. It can be calculated from the following equation, which is valid for all types of ions in solution up to about 0.1 ionic strength (Fair *et al* 1968):

\[
-\log \gamma = 0.5 z_i^2 IS/(1+\sqrt{IS})
\]  

Activity coefficient for all ions having the same charge is identical. In Figure 2 are presented values of activity coefficient, \(\gamma\) calculated from formula 34, for ions with different charge as a function of ionic strength, \(IS\).

The conductivity of a solution is strongly dependent on temperature. Thus, the conductivity readings must be referred to a common reference temperature (25°C) for comparability. The term “temperature compensation” is a mathematical conversion of measured conductivity at any given temperature to the corresponding conductivity value that would be taken at the reference temperature (25°C). The conductivity of most aqueous solutions varies more or less linearly with temperature. In these cases, a linear correction function to compensate for the influence of temperature can be used. The degree of temperature influence on conductivity varies.
Performance and control of biofilm systems with partial nitritation and Anammox for supernatant treatment

from solution to solution and can be calculated according to the following formula:

\[ K_T = K_{T_{\text{cal}}} \{1 + \alpha(T - T_{\text{cal}})\} \]  (35)

where: \( K_T \) is conductivity at any temperature \( T \) in °C; \( K_{T_{\text{cal}}} \) is conductivity at calibration temperature \( T_{\text{cal}} \) in °C and \( \alpha \) is temperature coefficient of solution at \( T_{\text{cal}} \) in °C (Barron and Ashton, 2005; Cole-Parmer, 2007).

**Alkalinity and carbonate system**

Almost all natural waters contain carbon dioxide and carbonate. In the natural environment carbonate alkalinity tends to make up most of the total alkalinity due to the common occurrence and dissolution of carbonate rocks and presence of carbon dioxide in the atmosphere.

Dissolved carbon dioxide (carbonic acid) is a diprotic acid. This acid can yield more than one proton in steps. The basic equilibrium and their constants within the carbonate system are:

\[ \text{CO}_2 (g) \leftrightarrow \text{CO}_2 \text{ (aq)} \]  \hspace{1cm} (36)
\[ \text{CO}_2 \text{ (aq)} + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \]  \hspace{1cm} (37)
\[ \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \]  \hspace{1cm} (38)
\[ [\text{H}^+] [\text{HCO}_3^-] = K_{\text{a1}}[\text{CO}_2] \]  \hspace{1cm} (39)
\[ \text{HCO}_3^- \leftrightarrow \text{H}^+ + \text{CO}_3^{2-} \]  \hspace{1cm} (40)
\[ [\text{H}^+] [\text{CO}_3^{2-}] = K_{\text{a2}}[\text{HCO}_3^-] \]  \hspace{1cm} (41)

\[ \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{OH}^- \]

\[ K_w = [\text{H}^+] [\text{OH}^-] \]  \hspace{1cm} (42)

Carbon dioxide gas is dissolved in water and is determined by its partial pressure and its interaction with other solutes in the water. In acid solution, the \( \text{CO}_2 \) concentration is normally expressed by Henry’s Law:

\[ [\text{CO}_2] = K_{\text{HH}}P_{\text{CO}_2} \]  \hspace{1cm} (43)

where: \( K_{\text{HH}} \) is the Henry’s Law constant and is equal to about \( 10^{-1.5} \) at 25°C when \([\text{CO}_2]\) is in moles per liter, \( P_{\text{CO}_2} \) is a partial pressure in atmospheres. The Henry’s Law constant depends on presence of other ions in solution and on temperature. At higher temperatures, \( \text{CO}_2 \) is less soluble, and at 50°C it decreases to \( 10^{-1.7} \) (Butler, 1982).

Carbon dioxide dissolved in water hydrates to \( \text{H}_2\text{CO}_3 \). In basic solution, hydrated carbon dioxide is ionized giving hydrated protons \( [\text{H}^+] \), bicarbonate ion \( [\text{HCO}_3^-] \) and carbonate ion \( [\text{CO}_3^{2-}] \). Equilibrium of uncharged \( \text{CO}_2 \) is not affected at constant \( P_{\text{CO}_2} \) however, the total amount of dissolved carbonate increases parallel to increase in pH value because of the ionization equilibrium. At temperature of 25°C in dilute solution, \( K_{\text{a1}} \) is equal about \( 10^{-6.3} \) and \( K_{\text{a2}} \) is approximately \( 10^{-10.3} \). Both equilibrium constants are depended on temperature and the presence of other ions.

In natural water very important is a concept of alkalinity, which can be expressed by the formula:

\[ A = [\text{OH}^-] + [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] - [\text{H}^+] \]  \hspace{1cm} (44)

The forms of alkalinity in water and wastewater are strongly depended on pH value. Figure 3 presents carbonate alkalinity forms under different pH values together with values of \( K_{\text{a1}} \) and \( K_{\text{a2}} \).

In order to understand natural processes running in the environment it is necessary to know transformation of carbon dioxide and
carbonate minerals. Thereby, the processes involving acid-base equilibrium of CO\(_2\) during the treatment of wastewater should also be considered.

7.2 Measurements based on oxygen uptake rate (OUR)

Another method for assessing the process performance is oxygen uptake rate tests (OUR). OUR tests for biofilm bacterial cultures on Kaldnes have been developed by Gut et al (2005). The method is a modification of the OUR tests proposed for activated sludge given by Surmacz-Górska et al (1996). The tests rely on measurements of uptaken oxygen concentration by the biofilm bacterial culture and measurements of used oxygen during the subsequent addition of selective inhibitors of the nitrifying bacterial populations. Sodium chlorate (NaClO\(_3\)) inhibits the nitrite oxidation by *Nitrobacter* species, whereas allylthiourea (ATU) inhibits the nitrification process.

7.3 Measurements based on microbiology

In order to confirm the presence of the Anammox bacteria, FISH analysis are commonly used. A FISH (fluorescence in situ hybridisation) test uses fluorescent molecules to vividly paint genes or chromosomes and is an excellent tool to collect both qualitative and quantitative data of Anammox bacteria. Anammox bacteria belong to the order of the *Planctomycetales* (Strous et al 1999a). The best known are *Brevibacterium ammoniagenes* and *Kuenenia stuttgartiensis*. The others are: *Jettenia asiatica*, *Anammoxoglobus propionicus*, *Scalindua wagneri*, *Scalindua brodae*, *Scalindua sorokinii* (Op den Camp et al 2007).

7.4 Additional possibilities for improved control

**UV absorption**

Conductivity measurements give possibilities to evaluate the nitrogen removal during the partial nitritation/Anammox process performance. However, it is not possible to estimate based on conductivity the value of produced nitrate that normally is an effect of Anammox reaction. Unfortunately, nitrate production can be also an unwanted effect of nitrite oxidation during nitrification. Therefore, additional knowledge about nitrate formation would improve information about process progress.

An alternative for NO\(_3^-\) detection, given by Vanrolleghem and Lee, (2003), is UV absorption. In order to measure the quantity of nitrate, UV absorption at 210 nm can be applied. Due to the low maintenance need and short response time (about 10 seconds), the UV absorption NO\(_3^-\) analysers are useful. The UV technique for wastewater is suitable with low organic matter (as supernatant). However, the presence of organic substances being absorbed in the same UV-region can cause interference to the measurements.

**Gas production**

The high activity of Anammox bacteria is reflected in the flotation of Kaldnes rings due to production and accumulation of dinitrogen gas between rings.

From Anammox reaction (Table 9) it can be seen that from 2.31 moles of sum of NH\(_4^+\) and NO\(_2^-\), 1.02 moles of nitrogen gas are produced. From gas equation, gas volume can be calculated as follows:

\[
V = \frac{nRT}{P}
\]

where \(RT/P = 22.4\) dm\(^3\) because \(R=8.314\) J \(\text{mol}^{-1}\) \(\text{K}^{-1}\), \(T=273\) K and \(P=101.325\) kPa

Thus, volume of nitrogen gas produced during Anammox reaction is equal to \(V_A = 1.02 \times 22.4 = 22.848\) dm\(^3\). Therefore, measurements of gas production could be one of the methods, which could be applied for additional process monitoring.

**Use of redox potential (ORP)**

The free energy depends on the energy difference between the reactants and the products. Knowing Gibbs free energy, it is possible to determine which reactions are most energetically favorable. When an oxidant interacts with reducing agent, the difference between their respective redox potentials \(\Delta E\) is related to the Gibbs free energy \(\Delta G\) according to the formula:

\[
\Delta G = -zF \Delta E
\]

where \(\Delta G\) is measured in joules per mol, \(\Delta E\) is measured in volts, \(F\) is the faraday constant
[96,500 coulombs mole⁻¹], and z is the number of electrons transferred in the reaction.

The use of redox potential (ORP), to control wastewater treatment processes, was reported by Mędrzycka et al. 2004 and Vanrolleghem and Lee, 2003. ORP can be an indicator of the oxidation processes performance. Therefore, application of ORP electrodes for monitoring of biological oxidation processes provides information about the oxidation progress in anoxic and anaerobic conditions. The detection of breakpoints in ORP profiles is a basis for interpretation of ORP measurements. The end of nitrification is indicated by DO breakpoint in the aerobic phase while the end of denitrification and disappearance of NO₃⁻ is shown by the NO₃⁻ breakpoint.

The oxidation process is involved in both partial nitritation and Anammox process therefore, the use of ORP could be another possibility to monitor these processes. Calculations of ORP based on formula 46 are presented in Table 10.

<table>
<thead>
<tr>
<th>Process</th>
<th>z</th>
<th>ΔG  (kJ mol⁻¹)</th>
<th>ΔE  (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitritation:</td>
<td>×6</td>
<td>-272</td>
<td>0.470</td>
</tr>
<tr>
<td>Anammox:</td>
<td>×6</td>
<td>-363</td>
<td>0.627</td>
</tr>
<tr>
<td>Cell synthesis:</td>
<td>×2</td>
<td>2400</td>
<td>-1.243</td>
</tr>
</tbody>
</table>
8. MATERIAL AND METHODS

8.1 Experimental equipments

In order to evaluate nitrogen removal by the partial nitritation/Anammox process the following equipments were used:

- A laboratory-scale pilot plant with volume of 17 l, operating with 2 reactors in series, located at the Royal Institute of Technology (KTH). The process performance in reactors and factors influencing the process like dissolved oxygen, pH, initial concentration of nitrite and especially effect of temperature were studied (paper IV). The detailed description of the pilot plant can be found in paper IV, Gut and Plaza, (2003), Szatkowska et al (2005).

- A technical-scale pilot plant (2 reactors of 2.1 m$^3$) located at the Himmerfjärden WWTP. Pilot plant had been run since 2002. The Anammox process was preliminary developed in reactor 2 and it was combined with the preceding partial nitritation step in reactor 1. Such two-stage system was studied and evaluated (paper I, paper II, and III). After experiments with recirculation, Anammox bacteria were seeded in reactor 1 and one-stage system with diverse biofilm (oxic-anoxic conditions) was arisen and investigated (paper V, paper VI, and paper VII).

Complete description and chart of pilot plant reactors (for two-stage process) is presented in Gut et al (2006b) and Trela et al (2004). The flow-diagram of one reactor under operation of one-stage process is shown in paper VI and description can be found in both papers V and VI. The technical details of pilot plant are presented in Table 11.

**Table 11. Technical parameters of technical-scale pilot plant.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of pilot plant (m$^3$)</td>
<td>2×2.1</td>
</tr>
<tr>
<td>Volume of supernatant (m$^3$)</td>
<td>3.66</td>
</tr>
<tr>
<td>Volume of Kaldnes rings R1 (%)</td>
<td>46</td>
</tr>
<tr>
<td>Volume of Kaldnes rings R2 (%)</td>
<td>51</td>
</tr>
<tr>
<td>Protected surface area in R1 (m$^2$)</td>
<td>483.5</td>
</tr>
<tr>
<td>Protected surface area in R2 (m$^2$)</td>
<td>530.2</td>
</tr>
<tr>
<td>Volume of settle tank (m$^3$)</td>
<td>0.8</td>
</tr>
<tr>
<td>Volume of clarifiers (m$^3$)</td>
<td>0.125</td>
</tr>
</tbody>
</table>

- Among the entire experimental period batch tests with medium taken out from both pilot plants were conducted to estimate the nitrogen removal rates (paper V and VI) and different parameters influencing the process as temperature (paper IV). The procedures of batch tests performance are presented in paper IV (the influence of temperature) and paper V and VI (nitrogen removal rates).

8.2 Analytical procedures

Process performance at the technical-scale pilot plant was followed up by chemical analyses and both manual and on-line measurements of parameters. The measurements of pH, conductivity, temperature have been done in the influent supernatant and in the reactors, while chemical analyses concerning supernatant in the influent and effluent from reactors. Analyses of nitrogen forms (ammonium, nitrite, and nitrate), alkalinity, COD, PO$_4$-P and organic acids were performed with time intervals taking into consideration the hydraulic retention time. SS and VSS was analysed in the supernatant and reactors (Table 12). The analytical procedure of controlling the process performance at laboratory-scale pilot plant can be found in paper IV.

8.3 On-line measurements

In two-stage partial nitritation/Anammox process an important factor of process performance is pH drop during partial nitritation. Therefore an on-line pH electrode was installed in the third zone of reactor one at technical-scale pilot plant. In early stage of the Anammox process performance dosage of chemicals was applied to keep pH in optimal range. Thus, in this reactor, second pH on-line electrode was used for indication of the proper pH level. Moreover, on-line temperature electrodes placed in reactors allowed for fast intervention in case of heater breakdown, during the period when process was run with heat supply. Another DO on-line electrode, in the partial nitritation reactor, adjusted air supply adequate to influent load and prevented the system from too high or low process values of DO.
Table 12. Measurements and analysis conducted at technical-scale pilot plant.

<table>
<thead>
<tr>
<th>Place</th>
<th>Measurements</th>
<th>Analysis</th>
<th>On-line measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>pH, conductivity, flow, temperature</td>
<td>N-forms, alkalinity,</td>
<td>conductivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COD, PO₄-P*, SS, VSS,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>organic acids**</td>
<td></td>
</tr>
<tr>
<td>Partial nitritation reactor</td>
<td>DO, temperature, pH, conductivity</td>
<td>SS, VSS</td>
<td>DO, temperature, pH</td>
</tr>
<tr>
<td>Anammox reactor</td>
<td>DO, temperature, pH, conductivity</td>
<td>SS, VSS</td>
<td>DO, temperature, pH</td>
</tr>
<tr>
<td>Simultaneous partial nitritation/</td>
<td>DO, temperature, pH, conductivity</td>
<td>SS, VSS</td>
<td>DO, temperature, pH</td>
</tr>
<tr>
<td>Anammox reactor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effluent</td>
<td>conductivity</td>
<td>N-forms, alkalinity,</td>
<td>conductivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COD, PO₄-P*, organic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>acids**</td>
<td></td>
</tr>
</tbody>
</table>

* sporadically, ** performed only when two-stage process was run

The significant part of this work was devoted to process monitoring. Thus, among the list of measurements taken at technical-scale pilot plant, the conductivity can be found. Conductivity measurements were introduced in daily routines to evaluate whether the process can be monitored by use of this parameter (paper I, paper III, and paper VII). Moreover, to evaluate the reliability of online instruments, conductivity electrodes were installed. In two-stage process they monitored the influent and effluent from Anammox reactor. In further studies the electrodes controlled the influent and effluent in one-stage partial nitritation/Anammox process.

8.4 Investigated parameters

Performed analyses and measurements allowed evaluating their influence on process performance. The investigated factor and parameters in particular papers are listed in Table 13.

8.5 Biofilm carriers

The investigated process was based on the biofilm principle and moving bed biofilm reactors (MBBR) were studied. Kaldnes rings used as the biofilm carrier elements were made from polyethylene with a density of 0.95 g cm⁻³. They were designed to provide a

Table 13. Investigated parameters in particular papers.

<table>
<thead>
<tr>
<th>paper no</th>
<th>scale</th>
<th>I tech-scale</th>
<th>II tech-scale</th>
<th>III tech-scale</th>
<th>IV lab-scale</th>
<th>V tech-scale</th>
<th>VI tech-scale</th>
<th>VII tech-scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>stage no</td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2 and 1</td>
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<td>2 and 1</td>
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<tr>
<td>supernatant characteristics</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>NO₂-N/NH₄-N (NAR)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>alkalinity</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>HCO₃⁻/N ratio</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

● the highest priority in the paper
large protected surface for the bacterial culture growth. At both pilot plants, Kaldnes rings filled about 50% of reactor’s volume. More information about Kaldnes rings can be found on following webpage: http://www.kaldnes.com.

8.6 Strategies and process performance

Two-stage partial nitritation/Anammox process at technical-scale pilot plant was started-up in March 2002 by inoculation of two reactors with activated sludge from a nitrification basin at Himmerfjärden WWTP. The reactors were initially operated separately to develop nitrifying and Anammox culture in reactors. In August 2002, reactor one was connected directly to supernatant, while reactor two which indicated higher Anammox activity was connected to the nitritation reactor effluent in April 2003. When the reactors were connected, the influent load to the Anammox reactor was step-by-step increased by decreasing the dilution of the partial nitritation effluent (paper I). A temporary Anammox process inhibition occurred in the end of September 2003. Rebuilding of Anammox lasted 4 months. The second successful trial of Anammox run was carried out during the period from January-December 2004 (paper III). The two-step process was operated for 2-year period (paper II). Later on, different strategies of internal and external recirculation were tested (Gut, 2006a). The external recirculation of the Anammox effluent to the first zone of reactor 1 resulted in seeding of the Anammox bacteria in the partial nitritation reactor and occurrence of significant nitrogen removal. Investigations, therefore, started of one-stage system with simultaneous partial nitritation/Anammox (paper V). The process with new configuration have been run and examined for one-year period (paper VI). The main strategies of process start-up and performance at technical-scale pilot plant with reference to particular papers are presented in Figure 4. Paper IV concerned investigations at laboratory-scale pilot plant and therefore, is not seen in Figure 4.

![Diagram of process strategies and events](image)

**Fig. 4.** The main strategies and events during technical-scale pilot plant experiments performance and time schedule for adequate papers.
Fig. 5. Technical-scale pilot plant. Inlet tank and reactor 1 (partial nitritation) to the left side, reactor 2 (Anammox) to the right side.

Fig. 6. Kaldnes rings and granules in reactor 2 (Anammox).
9. Results and Discussion

9.1 Operational strategies, conditions and mechanisms governing the process

9.1.1 Two-stage process

The technical-scale pilot plant experiment was initiated with the purpose to cultivate and examine the abilities of the Anammox process. Two reactors filled with Kaldnes rings were inoculated by activated sludge. Both reactors were operated as “batch” and were supplied by ammonium and nitrite through addition of ammonium chloride and sodium nitrite solutions. No oxygen was provided for a period of 9 months. After three months in reactor 2 and five months in reactor 1, parallel utilization of both forms of nitrogen was observed. It indicated Anammox process activity. At first, reactor one was directly connected to the supernatant from dewatering of the digested sludge. Next, when stable partial nitritation was established, the second reactor was connected to the effluent from reactor one. The first results of continuously working partial nitritation followed by the Anammox reactor were obtained almost immediately and are reported from 6 May 2003 (paper I).

Partial nitritation

The pilot plant was supplied directly by supernatant and therefore, supernatant characteristics and its influence on process performance were studied (paper I, II, III). It was found out that important factors in the influent supernatant were the content of hydrogen carbonate and especially the quotient of hydrogen carbonate to ammonium expressed in mmol. For the whole experimental period with operation of two-stage process it amounted to 1.1. Such ratio is in agreement with the ratio given by stoichiometry of anaerobic digestion (see Table 9). During the partial nitritation, high consumption of $HCO_3^-$ was noted (86%, on average). In consequence about 50% of ammonium was oxidised to nitrite (paper II). Moreover, alkalinity utilization resulted in a pH decrease (drop from about 7.8 to 6.5), which caused inhibition of Nitrobacter and prevented significant nitrate formation (paper I and II). Therefore, the $HCO_3^-/NH_4^-$ ratio in the influent supernatant and hydrogen carbonate consumption during ammonium oxidation resulted in a pH decrease and gave a stable operation of the partial nitritation process (paper I, II and III). Long-lasting and steady process with an average of 56% of ammonium to nitrite conversion was obtained at temperature in the range of 30–35°C, and dissolved oxygen conditions of 0.5–2.0 mg l$^{-1}$ and average biofilm loading rate of 1.3 g NH$_4^-$N m$^{-2}$ d$^{-1}$ (paper I and II).

Obtained results are in agreement with results achieved by other researchers. Fux et al (2002) emphasized the importance of the $HCO_3^-/NH_4^-$ ratio, as well. He indicated that $HCO_3^-/NH_4^-$ ratio in the influent supernatant is the factor determining appropriate nitrite to ammonium mixture in the effluent from the partial nitritation. According to him a ratio of $HCO_3^-/NH_4^-$ in the supernatant equal to 1.2 (on molar basis) should result in 60% of ammonium oxidation and in a NAR value of about 1.5 in the effluent.

Similar to these studies the value of ammonium to NO$_x$-N oxidation was equal 58% during the SHARON process (partial nitritation) was reported by Hwang et al (2006). However, 15.8% of ammonium removal by NH$_3$ stripping and cell synthesis was observed. Udert et al (2003) showed that other methods of nitrite oxidation inhibition are possible. The nitrous acid formation and inhibition by hydroxylamine were given as other possible alternatives.

Experiences from presented studies showed that supernatant characteristics was suitable to perform partial nitritation. However, most of the problems and disturbances appearing in process performance resulted from variable supernatant characteristics (paper II). Special troubles resulted from high concentrations of suspended solids (SS) in the influent. However, not measured when extremes appeared cannot be presented in this work. In Table 13, performed analyses of SS and VSS in the supernatant and in the partial nitritation reactor are shown. As it can be seen from Table 14, the values were rather low and there were only small differences.
between influent values and values in the reactor, probably due to biomass growth and biofilm detachment.

An important issue was, in operation of the two-stage process, **nitrite-to-ammonium ratio** in the effluent from reactor one, which constitutes substrate for the Anammox reactor. The experiments showed that the following sequence of parameters as **HRT** and **DO** → **pH drop** resulted in sufficient NAR in the effluent (paper II). The investigations proved that there is a strong linear correlation between pH and NAR in the effluent (paper I). It was also found that DO concentration had a great impact on NAR. The correlation between pH and NAR had different slope depending on the DO values. Lower NAR was obtained under DO higher than 1 mg l\(^{-1}\) while higher NAR was observed when DO was lower than 1 mg l\(^{-1}\). Moreover, in further studies a logarithmic relation between pH drop and NAR was established (paper II). From obtained correlation it can be concluded that pH drop of about 1.6 units would result in a stoichiometrical NAR value of 1.3.

Table 15 presents parameters of process performance and obtained results from partial nitritation carried out in MBBR.

**Anammox**

From performed experiments, a **NAR** value of 1.13 (as average from 2 years) was achieved in the effluent from partial nitritation. This value is close to stoichiometry of the Anammox reaction. However, effluent from the reactor one was originally diluted with water to step-by-step build up Anammox culture. The dilution diminished the ratio, which finally in the influent to the Anammox reactor amounted to 0.9 (paper I). Despite this fact that the NAR value was lower than the stoichiometrical one, it was possible to successfully achieve a high Anammox process efficiency with a maximum removal rate of 0.9 g m\(^{-2}\) d\(^{-1}\) (paper I and II). The importance of NAR was also confirmed by a strong relationship between reduced nitrite to reduced ammonium ratio and process efficiency (paper II). Higher NAR value in the influent equal to 1.2 was obtained in the second run (see Table 21b) when Anammox, after process breakdown, was restarted again. Moreover, a relationship between nitrite reduction (in moles) and ammonium reduction (in moles) gave a linear equation curve \((y = 1.2x + 0.2)\) close to the theoretically constructed curve \((y = 1.3x)\) (paper II).

Higher nitrite-to-ammonium removal ratio in

---

**Table 14. SS and VSS in the supernatant and in the partial nitration reactor.**

<table>
<thead>
<tr>
<th></th>
<th>SS in*</th>
<th>VSS in*</th>
<th>SS z3**</th>
<th>VSS z3**</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>188</td>
<td>161</td>
<td>234</td>
<td>198</td>
</tr>
<tr>
<td>st dev</td>
<td>87</td>
<td>74</td>
<td>89</td>
<td>72</td>
</tr>
<tr>
<td>min</td>
<td>102</td>
<td>82</td>
<td>80</td>
<td>73</td>
</tr>
<tr>
<td>max</td>
<td>463</td>
<td>380</td>
<td>452</td>
<td>365</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>21</td>
<td>39</td>
<td>39</td>
</tr>
</tbody>
</table>

* data for period Feb-Dec 2004; ** data for period Apr 2003-Dec 2004

---

**Table 15. Parameters of the partial nitration process in MBBR.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent ammonium concentration</td>
<td>g NH(_4)-N m(^{-3})</td>
<td>642±147</td>
</tr>
<tr>
<td>Influent alkalinity: ammonium ratio</td>
<td>mol mol(^{-1})</td>
<td>1.45±0.63</td>
</tr>
<tr>
<td>Biofilm loading rate</td>
<td>g NH(_4)-N m(^{-2}) d(^{-1})</td>
<td>1.29±0.56</td>
</tr>
<tr>
<td>NO(_2)-N production rate</td>
<td>g NO(_2)-N m(^{-2}) d(^{-1})</td>
<td>0.57±0.21</td>
</tr>
<tr>
<td>Effluent produced NO(_2)-N</td>
<td>%</td>
<td>46±12</td>
</tr>
<tr>
<td>Effluent NAR</td>
<td>mol mol(^{-1})</td>
<td>1.13±0.33</td>
</tr>
<tr>
<td>pH in→out</td>
<td>-</td>
<td>7.8→6.5</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>g O(_2) m(^{-3})</td>
<td>1.1±1.0</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>32±2.3</td>
</tr>
<tr>
<td>HRT</td>
<td>d</td>
<td>1.9±0.3</td>
</tr>
</tbody>
</table>
the Anammox process equal to 2.13 was reported by Hwang et al. 2006. However, in these studies NAR in the influent was also higher and amounted to 1.56.

Since it was discovered that Anammox process is reversibly inhibited by dissolved oxygen, efforts were made to keep DO in the reactor as low as possible. For that purpose, a deoxidizing column filled with Kaldnes rings was installed after the clarifier of reactor one to get rid of oxygen from the liquid (paper I and II). With time Kaldnes rings caused problems with blocking of the pipe supplying the Anammox reactor and, therefore, they were removed from the column. It was, however, still possible to maintain a low oxygen concentration in the reactor. Moreover, after a while biofilm development was observed on the walls of both column and pipe and formation of red granules as well. Probably, Anammox bacteria found favourable conditions such as continuous access of substrate, low oxygen and protected surface area. The average value of the DO in Anammox reactor amounted to 0.3 and to 0.13 mg l\(^{-1}\), for the first and second process trial, respectively. Perhaps such values of the DO allowed nitrifying bacteria, present in the system to some degree, using DO and produce additional nitrite. Such phenomenon could even support the Anammox reaction.

Since it has been known that the optimum pH range for Anammox is around 8 (Egli et al. 2001), the pH value in the Anammox reactor was originally corrected. However, later on (May 2004) dosage of chemicals (HCO\(_3\)- and CO\(_3^{2-}\) mixture) was stopped and it occurred that the system can work without chemicals with the same process efficiency (paper II). Moreover, a pH value increase was observed during the process performance.

The Anammox process was not highly loaded with nitrogen. However, a strong correlation between load and nitrogen removal was obtained. Removal rates were increasing parallel to increase in load (paper II). This confirms high ability of nitrogen removal by Anammox process.

Insufficient nitrite supplied to the Anammox reactor would suppress the reaction, but at the same time too high concentration can inhibit the Anammox reaction. The Anammox process exposed to a nitrite concentration of above 70 mg l\(^{-1}\) for longer period resulted in a process breakdown. A correlation between process efficiency and NO\(_2\)-N concentration was found (paper II). A nitrite concentration in the reactor equal to 28 mg l\(^{-1}\) led to an Anammox efficiency reduction of about 50%. The process recovery took four months (paper II). After that a stable process was again obtained in the Anammox reactor.

The hydraulic retention time in the second step was about three days (varying between 1.8-3.8 days). Such adjusted value gave good results of nitrogen removal.

The process disturbances, occurring during the Anammox process performance, were strongly correlated with the quality of supernatant, similar as it was with operation of reactor one.

The main goal of the partial nitritation process performance is to obtain a proper NAR in the effluent and for the Anammox process is to achieve high nitrogen removal efficiency. Important factors (according to experiences collected during these studies) and their interactions that must be taken into account are:

---

**Table 16. Important interactions between factors influencing operation of two-stage partial nitritation/Anammox process.**

<table>
<thead>
<tr>
<th>Partial nitritation</th>
<th>Anammox</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT $\rightarrow$ low NO(_3) _ in the effluent</td>
<td>NAR $\approx$ 0.9-1.2 in the influent $\rightarrow$ high N removal</td>
</tr>
<tr>
<td>DO $\rightarrow$ drop in pH $\rightarrow$ inhibition of Nitrobacter (free NH(_3) and HNO(_2) $\rightarrow$ NO(_2) _ accumulation $\rightarrow$ proper NAR</td>
<td>low DO $&lt; 0.3$ mg O(_2) l(^{-1}) $\rightarrow$ high N removal</td>
</tr>
<tr>
<td>HCO(_3)/NH(_4)^+ = 1 $\rightarrow$ about 50% NH(_4) oxidation and 85% HCO(<em>3)</em> consumption $\rightarrow$ proper NAR</td>
<td>NO(_2)-N in the reactor $&lt; 20$ mg l(^{-1}) $\rightarrow$ 60-88% N removal</td>
</tr>
</tbody>
</table>

---
account to successfully perform both processes and are given in Table 16.

9.1.2 One-stage process
Recirculation and diverse biofilm formation

In the period from January-May 2005 different strategies of Anammox effluent recirculation were tested. According to one of the intentions it was aimed to denitrify nitrate from Anammox effluent in the first reactor supported by remaining supernatant organic acids from the anaerobic digestion. The average value of COD measured in supernatant, in the period before recirculation, amounted to 369 mg l\(^{-1}\). Such amount would allow denitrification of about 130 g N (2.85 g COD is needed per reduction of 1 g N). From results of Anammox reaction in 2004 about 14 mg l\(^{-1}\) (6 %) of NO\(_3\)-N, on average, was produced. Thus, it was enough COD to perform denitrification. Hence, in the period from 13 April - 6 June 2005 effluent from reactor two was recirculated to zone 1 of reactor one (in two-stage process, both reactors were divided into 3 zones) where aeration was turned off. However, the target was not accomplished due to seeding of the Anammox bacteria in the partial nitritation reactor, which resulted in a high ammonium nitrogen removal. As it can be seen from Figure 7, the Anammox bacteria activity was observed in reactor one after one-month period of recirculation. Nitrite concentration produced by nitrifiers was disappearing parallel to increasing utilization of ammonium nitrogen. Even if aeration was turned on again, nitrogen consumption was observed. Nitrogen removal took place both under oxygen-limited as well as under oxic conditions. It seems that the created conditions turned out to be favourable for coexistence of two kinds of bacteria. The Anammox bacteria found suitable conditions in inner layer of biofilm whereas nitrifiers could grow in outer layer.

Experiments with recirculation of Anammox effluent showed that in already created nitritation biofilm it is very easy and fast (1 month of recirculation) to seed Anammox bacteria when oxygen limited conditions are assured. The same result was described by Rosenwinkel et al (2005). Deammonification in the nitritation tank surprisingly occurred at the full-scale WWTP at Hattingen, Germany. There, one-stage process was performed with intermittent aeration to assure alternately aerobic and anoxic conditions in one reactor volume.

Due to the high nitrogen removal in technical-scale pilot plant, a decision (in June 2005) was made to change process operation to one-stage reactor. HRT was shortened and two-operation modes of 1 day and 16 hours were tested. The wall between zones was removed in November 2005 and since then one-stage process in completely mixed reactor was tested.

Performance and achievements

Experiments during one year indicated average nitrogen removal of 63% varying between 43 and 90%. Nitrogen utilization was mainly an effect of simultaneous partial nitritation and Anammox that proceeded in one reactor. However, observed decrease in COD

<table>
<thead>
<tr>
<th>COD (mg l(^{-1}))</th>
<th>in</th>
<th>out</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>206</td>
<td>165</td>
</tr>
<tr>
<td>st dev</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>min</td>
<td>177</td>
<td>127</td>
</tr>
<tr>
<td>max</td>
<td>262</td>
<td>202</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 17. COD values during one-stage process performance, the influent and effluent.

data for period June-December 2005

Fig. 7. Nitrogen conversions in reactor 1 in the period when Anammox recirculation took place.
(20%) indicated that less than 5% of nitrogen could have been removed due to heterotrophic denitrification (Table 17).

The $\text{HCO}_3^-/\text{NH}_4^-$ mol ratio in the supernatant was controlled as it was done in two-stage process and its average amounted to 1.33. During the process, per mol of removed ammonium, 1.16 moles of alkalinity were utilized on average (paper VI). However, the percentage reduction of alkalinity decrease was lower compared to two-stage process. The average removed alkalinity value under the entire period when $\text{HRT}$ was equal to 1 day amounted to 37 mM, while under $\text{HRT}$ equal to 16 hours it was only 20 mM. Shorter $\text{HRT}$ resulted in higher load, prevented nitrifiers from performing further partial nitritation and the alkalinity was consumed to a lower degree (Figure 8). Remaining alkalinity in the system (under $\text{HTR}$ of 16 hours) resulted in higher pH values. For the whole period, relationship between removed alkalinity and removed ammonium was found (paper VI).

Another relationship was found between the process efficiency and the influent load. The average load to the system amounted to 2.2 g N m$^{-2}$ d$^{-1}$ for $\text{HRT}$ equal to 1 day and to 3.3 g N m$^{-2}$ d$^{-1}$ during shorter period when lower $\text{HTR}$ of 16 hours was tested. Even if higher load (lower $\text{HRT}$) to the system resulted in higher removal rates, such phenomenon was only temporary and soon a decrease in nitrogen removal rates was observed. The increase in load led to higher values of ammonium measured in the effluent and a relationship between load and effluent ammonium for one-stage system was established (paper VI).

The experiment was run at an average $\text{DO}$ of 1.9 mg l$^{-1}$, which varied between 0.3 to 5.4 mg l$^{-1}$. The studies showed that in simultaneous partial nitritation/Anammox process, the $\text{DO}$ is the crucial factor in process performance (see also chapter 9.3). During the process, an increase in $\text{DO}$ can be observed (Figure 8). Surprisingly, no relationship was found between $\text{DO}$ and other process parameters from pilot plant experimental data. However, batch test studies indicated that the overall reaction was suppressed by insufficient $\text{NO}_2$-$\text{N}$ production during the partial nitritation. This process bottleneck was strongly related to biofilm diffusion and $\text{DO}$ concentration (see later chapter 9.2 and 9.3.1).

Most of the period, the process was run under natural temperature of supernatant and only during the wintertime supernatant was heated up. The temperature remained rather stable during the entire period and the average temperature of process performance amounted to 25°C (Figure 8) (paper VI).

9.2 Factors influencing the process

Dissolved oxygen (DO) concentration is one of the most often discussed parameter
when economical factors are considered in treatment plant operation. Moreover, it is a key parameter influencing the nitrogen removal processes. Dissolved oxygen demand and its consumption by microorganisms are determined by factors such as: type of medium; the amount and accessibility of substrate that must be oxidised (e.g. in case of a biofilm its diversity into inner anoxic/anaerobic and outer aerobic layer).

Performed experiments, both at technical- and laboratory-scale, indicated the importance of oxygen concentration during performance of one- and two-stage partial nitritation/Anammox processes (paper I, II, V and VI). Proper adjustment of dissolved oxygen in the technical-scale pilot plant, when two-stage process was tested, gave satisfactory results of partial ammonium oxidation. Equal millimolar amounts of ammonium and nitrite with low nitrate concentration were registered at the effluent for long time period which confirms that the concentration of DO in reactor one was provided adequately to nitrogen load. It was found that DO is especially strongly correlated to pH value (paper II). The trend of distributed points, obtained from correlation between DO in the reactor and pH drop, showed that DO concentration higher than 1.2 g m\(^{-3}\) did not result in higher pH drop due to the fact that no more alkalinity needed for further oxidation was available.

Dissolved oxygen is also an important factor affecting Anammox process efficiency since it is a process inhibitor. In this step, nitrite was used under anaerobic conditions instead of oxygen as an electron acceptor. Higher values of oxygen significantly decreased efficiency of the process due to process inhibition. Nitrifiers that were present in the system probably used trace amounts of DO in the Anammox reactor as indicated by earlier studies performed with application of oxygen uptake rate (OUR) tests by Gut et al (2005).

The influence of DO was also investigated in one-stage system. On-line adjustment of DO at technical-scale pilot plant was tested. The lowest and the highest critical range of DO were set. Aeration was automatically turned on when DO dropped below the lowest point, whereas DO exceeded the highest value the aeration was turned off. Such aeration system assured adequate adjustment of oxygen to the influent ammonium concentration. Maximum nitrate nitrogen production amounted to 16% of the influent ammonium nitrogen and was in average 6.4%. The one-stage process was performed at DO concentration of 1.94 mg l\(^{-1}\) as an average from one-year period.

The other factor that plays an important role in process performance is the temperature. The effect of temperature on the removal rates is a key issue when operational costs are considered. The partial nitritation/Anammox process is performed by two different kinds of bacteria, which have different growth rates and temperature coefficients, and therefore temperature dependence for both of them must be considered.

Partial nitritation and Anammox are autotrophic and exothermic processes. At the beginning of the experiment, the medium in the reactor was heated. However, later on, lower temperatures were tested and temperature was gradually decreased. Finally, the process was run at the temperature of supernatant. Two-stage process was performed under average temperature of 32 and 33\(^{\circ}\)C for one and second reactor, respectively, while one-stage process was run at considerably lower temperature of 25\(^{\circ}\)C. One-stage process operation period, as it was mentioned before, was carried out with heat supply only during the wintertime.

The pH value had a significant influence on process performance. Typical drop of pH, resulting from the partial nitritation reaction (Table 9), was also observed in the technical-scale pilot plant experiment. Decrease in pH value was an effect of alkalinity consumption (carbon dioxide stripping and cell synthesis actually increases the pH value of wastewater). For the entire period when the partial nitritation was performed in reactor one the average drop of pH value amounted to 1.3 (paper III). Such drop was sufficient to obtain a nitrite-to-ammonium ratio at the effluent equal to 1.1, which was close to the required value of 1.3. The pH dependency can be related to inhibition (chapter 6.1). For
high ammonium concentrations a control of pH value is recommended, as pH is a parameter preventing nitrate formation. Due to proper pH value, inhibition of Nitrobacter by free ammonia (FA) and free nitrous acid (FNA) can be obtained (Anthonisen, 1976). Free ammonia in partial nitritation reactor was calculated according to formula 31 in chapter 6.1 and is presented in Figure 9. The average value of free ammonia amounted to 3.6 mg l$^{-1}$. Most points corresponded to a pH value within the range of 5.5-6.5 and free ammonia below 3 mg l$^{-1}$. The average free HNO$_2$ concentration was equal to 1.84 mg l$^{-1}$.

9.3 Nitrogen removal rates

9.3.1 Influence of DO

In order to investigate nitrogen removal rates in one-stage process, batch test studies were carried out with medium taken from reactor at technical-scale pilot plant. Obtained results proved that the bottleneck of overall reaction was production of nitrite by the partial nitritation (paper V and VI). The supply of reactor by addition of nitrite significantly increased the nitrogen removal rates in comparison with tests where ammonium nitrogen was the only substrate. Then, removal rate was as high as 4.4 g N m$^{-2}$d$^{-1}$. The maximum rate was 2.96 g N m$^{-2}$d$^{-1}$ for simultaneous partial nitritation/Anammox (ammonium as substrate) obtained in batch tests with medium taken from pilot plant and run under DO of 1.4-1.6 g O$_2$ m$^{-3}$. However, the highest removal rates were obtained when Anammox reaction only occurred. Anaerobic conditions and the presence of both ammonium and nitrite resulted in a removal rate equal to 5.2 g N m$^{-2}$d$^{-1}$.

It was shown that possibilities of removal rates, investigated in batch tests simulating one-stage process, seem to be (under optimal conditions) even three times higher than removal rates obtained in pilot plant studies. Moreover, the batch test studies allowed identifying factors influencing the removal rates in one-stage process. The first factor was related to inhibition of Anammox bacteria by excess of substrate. The second one was inhibition of nitrifiers by free ammonia (paper V).

\[
y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]

\[y = 1 \times 10^{-13}x^{15.997} \\
R^2 = 0.9652
\]
The batch test with ATU (chapter 7.2), which is a selective inhibitor of Nitrosomonas, showed that Anammox bacteria are responsible for nitrogen removal. ATU added to the batch run under aerobic conditions stopped oxidation of ammonium to nitrite and at the same time made the course of Anammox reaction impossible (paper V).

The main results of batch test studies on removal rates are shown in Table 18.

9.3.2 Influence of temperature

Tests were run with medium taken both from technical- as well as from laboratory-scale pilot plants. The effect of temperature on removal rates was assessed in laboratory-scale pilot plant both in two-stage and one-stage process option (paper IV). In two-stage process, the studies on Anammox showed that the highest inorganic nitrogen removal rate of 2.5 g N m\(^{-2}\) d\(^{-1}\) was obtained at highest investigated temperature of 35°C. It was found that a sudden decrease in temperature had a negative impact on process efficiency and results in a decrease in nitrogen removal rates. Short-term decline in temperature from 35 to 20°C decreased inorganic nitrogen removal rate by 43%. A special effect of temperature was noticed when nitrite nitrogen removal rate was analyzed in the Anammox process. Nitrite removal rate decreased parallel to decline in temperature and to inorganic removal rate. It dropped from 1.45 to 0.5 g N m\(^{-2}\) d\(^{-1}\) at 35 and 20°C, respectively. The influence of temperature was investigated also in one-stage system. The highest ammonium removal rate amounted to 0.715 g N m\(^{-2}\) d\(^{-1}\) and was obtained at highest investigated temperature of 31°C for simultaneous partial nitritation/Anammox. Step-by-step decrease in temperature resulted in decline of removal rates. Investigations indicated that after some period bacteria are able to adapt to new conditions and work more efficiently.

It was found that biofilm with both nitrifying and Anammox bacteria exposed to temperature of 20°C for longer time period (four months) are able to remove nitrogen efficiently if compared to the results obtained under the same temperature but decreased suddenly. This would be in agreement with phenomenon that takes place in natural ecosystem where Anammox was found to work under low temperature in marine environment. Moreover, it was proved that the addition of NO\(_2\)- solution significantly increased reaction rate in one-stage process while addition of ATU (inhibitor of Nitrosomonas) inhibited partial nitritation reaction, and stopped completely the overall reaction.

Generally, removal rates in simultaneous partial nitritation/Anammox at laboratory-scale pilot plant were lower if compared to

<table>
<thead>
<tr>
<th>Process</th>
<th>Description</th>
<th>Temperature</th>
<th>Removal rate g N m(^{-2}) d(^{-1})</th>
<th>Reduction in rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anammox reactor 2</td>
<td>sudden decrease from 35°C to 20°C</td>
<td>2.50 inorganic 1.42 nitrite 1.48 inorganic 0.50 nitrite</td>
<td>(N\text{\textsubscript{inorg}} 43% N\text{\textsubscript{NO2-N}} 65%)</td>
<td></td>
</tr>
<tr>
<td>One-stage reactor 1</td>
<td>step-by-step decrease from 31°C to 27.8°C</td>
<td>0.71 ammonium 0.40 ammonium 0.66 ammonium 0.23 ammonium</td>
<td>(N\text{\textsubscript{NH4-N}} 43.6% ) (N\text{\textsubscript{NH4-N}} 65.1%)</td>
<td></td>
</tr>
<tr>
<td>reactor 2</td>
<td>after 4 months adaptation period to 20°C</td>
<td>0.51 ammonium 0.26 ammonium</td>
<td>(N\text{\textsubscript{NH4-N}} 28.2% ) (N\text{\textsubscript{NH4-N}} 60%)</td>
<td></td>
</tr>
<tr>
<td>reactor 1</td>
<td>after 4 months adaptation period at 20°C</td>
<td>1.72 inorganic 0.86 ammonium 1.51 nitrite</td>
<td>(N\text{\textsubscript{NH4-N}} 69% ) (increase)</td>
<td></td>
</tr>
<tr>
<td>reactor 2</td>
<td>after 4 months adaptation period and addition of NO(_2) at 20°C</td>
<td>2.40 inorganic 1.17 ammonium 1.55 nitrite</td>
<td>(N\text{\textsubscript{NH4-N}} 350% ) (increase)</td>
<td></td>
</tr>
</tbody>
</table>
the obtained rate when processes were run separately (paper IV). Summary of different studies on temperature influence on nitrogen removal efficiency is presented in Table 19.

These results can be compared to experiments performed by Basiak (2006). Batch tests with medium taken from technical-scale pilot plant when one-stage process was tested were carried out at three temperature values of 10, 22.5 and 35°C (four tests at each temperature). The best results of calculated nitrogen removal rates for simultaneous partial nitritation/Anammox process were achieved at the highest temperature (35°C). It amounted to 2.5 g N m⁻² d⁻¹ and was 1.5 times higher than removal rate at temperature of 22.5°C and almost 10 times higher than at 10°C. The rate at temperature 22.5°C corresponds to the rates obtained in pilot plant (annual average equal to 1.49 g N m⁻² d⁻¹).

Additionally, from Figure 10, it can be seen that the difference between temperature dependence of inorganic nitrogen removal rate and ammonium nitrogen removal rate increased parallel to the decrease in temperature.

![Fig. 10. Relation between temperature and nitrogen removal rates obtained from batch test experiments with medium taken from pilot plant with one-stage partial nitritation/Anammox process (based on data from Basiak, 2006).](image)

### 9.4 One- and two-stage processes – comparison

Tables 21 a, b and 22 compile results from performed studies with operation of one- and two-stage partial nitritation/Anammox systems. The period when two-stage system was investigated is divided into 2 sub periods when high activity of Anammox process was recorded (the breakdown of Anammox process is omitted).

Conducted studies showed that supernatant characteristics play an important role in supernatant treatment by partial nitritation/Anammox process irrespective of process configuration.

As it can be seen from Table 21 and 22, the supernatant characteristics varied during particular periods. When two-stage process was carried out, ammonium concentration was higher than its value in the period with operation of one-stage system. It can also be noticed (from standard deviation values) that in the period with two stages higher fluctuations were recorded. Moreover, supernatant was also characterized by higher HCO₃⁻ to NH₄-N ratio. These differences could be caused by periodic disturbances in process digestion and discharges of process waters from centrifuge cleaning. One-stage system proceeded with lower (64%, on average) than in two-stage process (86%, on average in partial nitritation) consumption of carbonate alkalinity.

Obtained values of removal rates in conducted studies showed that one-stage simultaneous partial nitritation/Anammox process is a better option for supernatant treatment than two-stage system. Removal rates were 5.2 times higher in one-stage (average equal to 1.4 g N m⁻² d⁻¹) than in two-stage (average for entire successful period amounted to 0.27 g N m⁻² d⁻¹) system.

Higher values of removal rates in one-stage system corresponded to higher decrease in conductivity values. In two-stage process average drop in conductivity in the partial nitritation reactor was equal to 1.4 mS cm⁻¹, while in the Anammox reactor it amounted to 0.8 and 0.7 mS cm⁻¹ for the first and the second trial period, respectively. High utiliza-
tion of nitrogen in one-stage system was accompanied by conductivity removal as much as 3 mS cm\(^{-1}\).

It was proved that both systems can be operated without chemical additions. The Anammox process in two-stage system was tested both with and without correction. Therefore, it can be seen from Table 21b that in period from 6 May – 20 August, 2003 (with pH correction), only insignificant pH changes between influent and effluent were recorded. In period 16 December, 2003 – 30 December, 2004 when the process was run most of the time without chemical addition, an increase in pH value was noticed. One-stage process was run without any chemical addition for pH control during entire operational period. Due to the simultaneous processes of the partial nitritation (pH decrease) and the Anammox (pH increase) in one reactor, pH compensation took place.

Moreover, obtained results from operation of two-stage process indicated importance of NAR in the effluent from the partial nitritation, this is at the same time the influent to the Anammox reactor. The performance of partial nitritation in such way to achieve proper and close to stoichiometry of Anammox reaction NAR in the effluent, assures high efficiency of Anammox reaction. However, there were no difficulties to obtain such a proper ratio during two-stage process. Successfully, long-lasting partial nitritation with a ratio of 1.1 in the effluent was obtained, as an average from 2-year period. Control of NAR was not required for one-stage process, therefore operation of one-stage process turned out to be easier and less complicated than operation of two separate reactors. Anammox bacteria in inner layer were consuming nitrite that was produced in outer layer of biofilm. Thereby, there was no risk to overload the system with nitrite concentration, which in the Anammox process was a crucial factor in process operation. Moreover, no pH control was necessary. Processes carried out in one reactor indicated compensation of H\(^+\) ions, preventing from too high drop of pH value (paper VII).

Higher value of free ammonia with an average value equal to 9.13 mg l\(^{-1}\) resulted from higher nitrogen load and higher pH values and lower temperature was registered for one-stage process. NH\(_3\) value above 10 mg l\(^{-1}\) inhibits *Nitrosomonas* bacteria and then ammonium oxidation is suppressed. Thus, to prevent inhibition it is important to keep NH\(_3\) below that critical level. The NH\(_3\) concentration in nitrogen-rich streams is a function of pH and temperature (see equation 31). Higher registered values of NH\(_3\) in one-stage process could result from high pH (not decreased as it was in the partial nitritation) and low temperature (8°C lower than in two-stage process performance). The high standard deviation values of free ammonia indicate that the level of 10 mg was many times exceeded and it could result in process inhibition.

Advantages of one-stage system over two-stage system are presented in Table 20.

### Table 20. Advantages of one-stage system process performance in comparison with two-stage system.

- Higher removal rates
- No need to control NAR
- Less complicated in operation
- No risk to overload the system with NO\(_2\)-N
- No pH control
- Lower alkalinity consumption
- Lower HRT

Conducted experiments both with two- and one-stage partial nitritation/Anammox system demonstrated that one-stage system is a better option for full-scale implementation since it has many advantages. The process seems to be not only easier to operate but also its application can lead to reduction of exploitation costs. The expenses could be lowered due to ability of the process to be carried out at natural temperature of produced supernatant as shown by performed experiments (paper V and VI). Then, no need for heat supply would be required. Moreover, combination of Anammox with partial nitritation in one reactor volume allows applying short retention time (as it was proved in this work). Thus, smaller reactor volume would be needed to perform the reaction. Addition-
ally, high reaction rate could result in higher system capacity and at the same in lower need for reactor volume (Figure 11). Therefore, separate treatment of supernatant by use of one-stage process brings opportunity to create more efficient method for nitrogen removal from ammonium-rich streams.

Independently, whether the process of supernatant treatment is run in one or two stages implementation of separate treatment of supernatant at WWTP can significantly contribute to decreasing the nitrogen load in the mainstream. The effect of separate supernatant treatment was studied by Wett and Alex, 2003. They indicated that implemented separate treatment of supernatant at STRASS WWTP resulted in observed increase of the yearly nitrogen elimination efficiency of the mainstream by 11% (from 79%-89%). Significant reduction in both the nitrogen return load and problematic load variations was noted.

Separate supernatant treatment with partial nitritation/Anammox process can lead to the decrease in contribution of supernatant to overall load at mainstream from 15-20% to 1%. In consequence it can result in lower requirements for efficiency of the biological treatment of the mainstream at the WWTP. Nitrogen removal in a biological step could be improved (paper II).

\[\text{Fig. II. Decrease in operational costs with application of one-stage partial nitritation/Anammox process.}\]
Table 21a. Results from two-stage process performance - Partial nitritation (6 May 2003- 30 Dec 2004).

<table>
<thead>
<tr>
<th></th>
<th>NH₄-N in</th>
<th>NHCO₃⁻</th>
<th>NO₂⁻-N/NH₄⁺-N</th>
<th>oxid NH₄⁻-N</th>
<th>prod NO₃⁻-N</th>
<th>NH₃</th>
<th>rem HCO₃⁻</th>
<th>DO</th>
<th>Temp</th>
<th>pH</th>
<th>conductivity**</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>642</td>
<td>1.45</td>
<td>1.13</td>
<td>55.7</td>
<td>2.5</td>
<td>3.60</td>
<td>86</td>
<td>1.1</td>
<td>32</td>
<td>7.8</td>
<td>6.5</td>
</tr>
<tr>
<td>st dev</td>
<td>147</td>
<td>0.63</td>
<td>0.33</td>
<td>9.95</td>
<td>2.01</td>
<td>6.53</td>
<td>15</td>
<td>1.0</td>
<td>2.3</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>min</td>
<td>323</td>
<td>0.37</td>
<td>0.20</td>
<td>16.2</td>
<td>0.16</td>
<td>0.09</td>
<td>27</td>
<td>0.2</td>
<td>25</td>
<td>7.4</td>
<td>5.4</td>
</tr>
<tr>
<td>max</td>
<td>918</td>
<td>3.20</td>
<td>2.20</td>
<td>78.7</td>
<td>9.35</td>
<td>30.39</td>
<td>100</td>
<td>5.3</td>
<td>39</td>
<td>8.1</td>
<td>8.2</td>
</tr>
<tr>
<td>n</td>
<td>107</td>
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<td>107</td>
<td>107</td>
<td>94</td>
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<td>348</td>
<td>348</td>
<td>347</td>
<td>348</td>
<td>346</td>
</tr>
</tbody>
</table>

Table 21b. Results from two-stage process performance - Anammox.

<table>
<thead>
<tr>
<th></th>
<th>N load [g m⁻² d⁻¹]</th>
<th>N rem rate [g m⁻² d⁻¹]</th>
<th>NO₂⁻-N/NH₄⁺-N</th>
<th>N rem [%]</th>
<th>prod NO₃⁻-N</th>
<th>NO₃⁻-N out [mg l⁻¹]</th>
<th>rem HCO₃⁻ [mM l⁻¹]</th>
<th>DO [mg l⁻¹]</th>
<th>Temp [°C]</th>
<th>pH</th>
<th>conductivity**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 6 May 2003 – 20 Aug 2003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>average</td>
<td>0.401</td>
<td>0.274</td>
<td>0.925</td>
<td>70.7</td>
<td>-5.9</td>
<td>9.6</td>
<td>n.d.</td>
<td>0.3</td>
<td>33</td>
<td>8.11</td>
<td>8.12</td>
</tr>
<tr>
<td>st dev</td>
<td>0.337</td>
<td>0.229</td>
<td>0.285</td>
<td>14.0</td>
<td>6.5</td>
<td>15.4</td>
<td>0.1</td>
<td>1.6</td>
<td>0.1</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>min</td>
<td>0.093</td>
<td>0.051</td>
<td>0.229</td>
<td>42.5</td>
<td>-26.6</td>
<td>0.1</td>
<td>0.1</td>
<td>29</td>
<td>7.8</td>
<td>7.8</td>
<td>0.8</td>
</tr>
<tr>
<td>max</td>
<td>1.227</td>
<td>0.905</td>
<td>1.814</td>
<td>89.8</td>
<td>12.5</td>
<td>62.5</td>
<td>0.7</td>
<td>37</td>
<td>8.4</td>
<td>8.4</td>
<td>4.0</td>
</tr>
<tr>
<td>n</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>114</td>
<td>114</td>
<td>38</td>
<td>38</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period 16 Dec 2003 – 30 Dec 2004</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>0.231</td>
<td>0.188</td>
<td>1.21</td>
<td>81.8</td>
<td>5.1</td>
<td>6.7</td>
<td>0.12</td>
<td>0.14</td>
<td>33</td>
<td>7.2*</td>
<td>8.21</td>
</tr>
<tr>
<td>st dev</td>
<td>0.083</td>
<td>0.090</td>
<td>0.32</td>
<td>17.3</td>
<td>2.4</td>
<td>11.7</td>
<td>2.10</td>
<td>0.07</td>
<td>3.0</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>min</td>
<td>0.103</td>
<td>0.026</td>
<td>0.46</td>
<td>25.0</td>
<td>0.1</td>
<td>0.2</td>
<td>-4.35</td>
<td>0.02</td>
<td>24</td>
<td>6.5</td>
<td>7.6</td>
</tr>
<tr>
<td>max</td>
<td>0.430</td>
<td>0.394</td>
<td>2.21</td>
<td>97.4</td>
<td>8.3</td>
<td>47.9</td>
<td>6.60</td>
<td>0.44</td>
<td>46</td>
<td>8.6</td>
<td>8.8</td>
</tr>
<tr>
<td>n</td>
<td>63</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>45</td>
<td>242</td>
<td>345</td>
<td>245</td>
<td>245</td>
<td>242</td>
</tr>
</tbody>
</table>

Table 22. Results from one-stage simultaneous partial nitritation/Anammox process performance (July 2005 – June 2006).

<table>
<thead>
<tr>
<th></th>
<th>NH₄-N in</th>
<th>NHCO₃⁻</th>
<th>N rem rate [g m⁻² d⁻¹]</th>
<th>N rem [%]</th>
<th>prod NO₃⁻-N</th>
<th>NH₃</th>
<th>rem HCO₃⁻</th>
<th>DO [mg l⁻¹]</th>
<th>Temp [°C]</th>
<th>pH</th>
<th>conductivity**</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>564</td>
<td>1.22</td>
<td>1.395</td>
<td>63.3</td>
<td>6.4</td>
<td>9.13</td>
<td>64</td>
<td>1.94</td>
<td>25</td>
<td>7.82</td>
<td>7.75</td>
</tr>
<tr>
<td>st dev</td>
<td>58.8</td>
<td>0.09</td>
<td>0.246</td>
<td>12.6</td>
<td>3.0</td>
<td>8.07</td>
<td>16</td>
<td>0.86</td>
<td>2.1</td>
<td>0.11</td>
<td>0.27</td>
</tr>
<tr>
<td>min</td>
<td>450</td>
<td>1.07</td>
<td>0.930</td>
<td>43.1</td>
<td>1.5</td>
<td>0.11</td>
<td>28</td>
<td>0.30</td>
<td>17</td>
<td>7.57</td>
<td>6.41</td>
</tr>
<tr>
<td>max</td>
<td>714</td>
<td>1.46</td>
<td>1.889</td>
<td>90.4</td>
<td>18</td>
<td>29.53</td>
<td>94</td>
<td>5.40</td>
<td>31</td>
<td>8.30</td>
<td>8.19</td>
</tr>
<tr>
<td>n</td>
<td>60</td>
<td>39</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>49</td>
<td>599</td>
<td>580</td>
<td>202</td>
<td>200</td>
</tr>
</tbody>
</table>

*rem - removal; n.d. - no data; oxid - oxidized; prod - production; rem - removal; * when no pH correction was done, ** conductivity in mS cm⁻¹
9.5 Process control

9.5.1 Conductivity

General approach

In order to improve process understanding and to optimize process control, relationships between process functions of partial nitritation/Anammox and conductivity were investigated. Preliminary presumption that conductivity can be used for this process monitoring was made due to the fact that high concentrations of ammonium and hydrogen carbonate ions in the supernatant have the highest contribution to the total value of conductivity and that the entire process is based on their conversions. Theoretical considerations gave additional confirmation.

The equivalent ionic conductance of \( \text{HCO}_3^- \) and \( \text{NH}_4^+ \) ion measured as \( \text{S cm}^{-2} \text{equivalent}^{-1} \) amounts to 44.5 and to 73.4, respectively. These two ions have opposite but the same absolute value of the charge (paper VII). During the partial nitritation reaction, both ions undergo changes: ammonium is partially converted into nitrite while hydrogen carbonate is removed (paper I-VII). Conductivity of removed part of ammonium is close to the conductivity value of produced nitrite because charge (-1) and ionic conductance (71.8) of nitrite are similar to the respective values for ammonium. The conductivity value in the effluent from the partial nitritation is diminished by conductivity value resulting from alkalinity depletion (paper III). In the next step, both remaining ions of ammonium and nitrite are converted to nitrogen gas molecules under anaerobic conditions by Anammox bacteria and therefore a conductivity decrease is expected. In the effluent only nitrate is remaining ion (about 11% of influent) according to stoichiometry of Anammox reaction.

As transformations of ammonium and hydrogen carbonate ions during supernatant treatment lead to conductivity changes, the whole process can be followed by very easy and simple conductivity measurements (paper III and VII). Table 23 presents ions in millimolar amounts according to the stoichiometry of reactions and their theoretical conductivity values in the influent and effluent from both partial nitritation and Anammox processes.

**Supernatant**

Supernatant characteristics is an important factor influencing the process. The knowledge about supernatant contamination and the influence of its particular components on conductivity are the basis for process control. Table 24 presents measurements of ammonium and alkalinity collected from a two-year period, the ratio between them, conductivity of supernatant and the ratio of \( \text{HCO}_3^- \) and \( \text{NH}_4^+ \) ions with conductivity.

Results show that ammonium fluctuations compared to alkalinity variations were lower. It resulted in higher alkalinity to conductivity ratio. \( \text{HCO}_3^-/\text{NH}_4^+ \)-N ratio was in general agreement with stoichiometry of the anaerobic digestion (Table 9).

Assuming equal amounts of ammonium and alkalinity and linear correlation between ions concentration and conductivity (From Table 23, 2.32 mmoles of \( \text{NH}_4^+ \) and 2.32 mmoles of \( \text{HCO}_3^- \) give a conductivity value of 0.246 mS cm\(^{-1} \)), for average \( \text{NH}_4^+ \) concentration equal to 44 mM (from Table 24), a theoretical conductivity value amounts to 44/2.32×0.246 = 4.66 mS cm\(^{-1} \). Such value is close to average conductivity experimental data from Table 24.

**Table 23. Ion conversions and conductivity changes in the partial nitritation and the Anammox process.**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Influent</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ions [mmol]</td>
<td>conductivity value</td>
</tr>
<tr>
<td>Partial nitritation</td>
<td>2.32( \text{NH}_4^+ ), 2.32( \text{HCO}_3^- )</td>
<td>0.246 mS cm(^{-1} )</td>
</tr>
<tr>
<td>Anammox</td>
<td>1( \text{NH}_4^+ ), 1.32( \text{NO}_2^- )</td>
<td>0.156 mS cm(^{-1} )</td>
</tr>
</tbody>
</table>
Table 24. Selected measurements and analyses of supernatant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Average</th>
<th>Min</th>
<th>Max</th>
<th>St dev.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄-N</td>
<td>mM l⁻¹</td>
<td>44</td>
<td>23</td>
<td>65.6</td>
<td>7.35</td>
<td>167</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>mM l⁻¹</td>
<td>64</td>
<td>15.8</td>
<td>189.3</td>
<td>34</td>
<td>106</td>
</tr>
<tr>
<td>Alkal./NH₄-N</td>
<td>mM l⁻¹ / mM l⁻¹</td>
<td>1.45</td>
<td>0.37</td>
<td>3.20</td>
<td>0.51</td>
<td>104</td>
</tr>
<tr>
<td>Conductivity</td>
<td>mS cm⁻¹</td>
<td>4.92</td>
<td>1.04</td>
<td>6.83</td>
<td>0.92</td>
<td>667</td>
</tr>
<tr>
<td>HCO₃⁻/cond.</td>
<td>mS cm⁻¹</td>
<td>12.3</td>
<td>3.6</td>
<td>36.26</td>
<td>6.32</td>
<td>104</td>
</tr>
<tr>
<td>NH₄-N/Cond.</td>
<td>mS cm⁻¹</td>
<td>8.7</td>
<td>6.4</td>
<td>11.81</td>
<td>1.56</td>
<td>114</td>
</tr>
</tbody>
</table>

Table 25 compiles comparisons between the theoretical and experimental alkalinity to ammonium ratio and ratios of both alkalinity and ammonium to conductivity. Experimental data are presented in two columns: one where all data are shown and the second where only data for HCO₃⁻/NH₄-N (C/N) ratio in the range of 0.9-1.2 are considered. It can be seen that if HCO₃⁻/NH₄-N is close to the theoretical ratio (1:1), the experimental ratio of HCO₃⁻/conductivity (9.5) is close to theoretically calculated ratio (9.1). However, any disturbances in process digestion result-ing in higher alkalinity give higher HCO₃⁻/NH₄-N and higher than theoretical ratio of HCO₃⁻/conductivity. The NH₄-N to conductivity ratio was found as similar to the theoretical value.

**Two-stage process**

Preliminary investigations for process monitoring with application of conductivity were performed for two-stage process (paper I). Similar progress of inorganic nitrogen time series curve to the conductivity curve was observed (paper III). It was shown that the process performance can be followed up by conductivity. High correlations were obtained between conductivity and inorganic forms of nitrogen values. The values of conductivity measured in the influent ammonium-rich supernatant were proportional to analysed concentrations of ammonium. The same result was observed in the effluents from the partial nitritation and Anammox reactors where inorganic nitrogen as a sum of ammonium, nitrite and nitrate was correlated with conductivity (paper I). Table 26 shows main expected and found correlations in two-stage process that gave the basis for consideration of conductivity as monitoring tool.

Moreover, in the partial nitritation, removed conductivity corresponded to oxidized nitrogen, while in the second - Anammox step, to removed inorganic nitrogen (paper III and paper VII). A correlation of conductivity with alkalinity was found as well (paper III).

Table 25. Comparison of experimental and theoretical values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Experimental value-average</th>
<th>Theoretical value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Unit</td>
<td>all data</td>
<td>data when C/N 0.9-1.3</td>
</tr>
<tr>
<td>HCO₃⁻/NH₄-N</td>
<td>mM/mM</td>
<td>1.45</td>
<td>1.2</td>
</tr>
<tr>
<td>NH₄-N/Conductivity</td>
<td>(mM l⁻¹)/(mS cm⁻¹)</td>
<td>8.7</td>
<td>8.3</td>
</tr>
<tr>
<td>HCO₃⁻/Conductivity</td>
<td>(mM l⁻¹)/(mS cm⁻¹)</td>
<td>12.3</td>
<td>9.5</td>
</tr>
</tbody>
</table>

* sum of 1mM NH₄-N and 1mM HCO₃⁻ divided by corresponded conductivity value = 0.110 mS cm⁻¹ from paper VII (Table 3).

Table 26. Main correlations with conductivity in two-stage partial nitratation/Anammox process.

<table>
<thead>
<tr>
<th>R1 partial nitritation</th>
<th>NH₄⁺ in R1 + HCO₃⁻ in R1</th>
<th>N iorg out R1 + HCO₃⁻ out R1 + (NH₄⁺ in R1 + HCO₃⁻ in R1)(N iorg out R1 + HCO₃⁻ out R1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity in</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Conductivity out</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Conductivity removed</td>
<td>=</td>
<td></td>
</tr>
</tbody>
</table>

R2 Anammox

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity in</td>
<td>=</td>
<td>N iorg in R2</td>
</tr>
<tr>
<td>Conductivity out</td>
<td>=</td>
<td>N iorg out R</td>
</tr>
<tr>
<td>Conductivity removed</td>
<td>=</td>
<td>(N iorg in R2)-(N iorg out R2)</td>
</tr>
</tbody>
</table>
Table 27. Linear equations describing conductivity correlations in two-stage process (paper III and VII).

<table>
<thead>
<tr>
<th>y axis</th>
<th>x axis</th>
<th>relationship</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Partial nitritation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>influent NH$_4$-N [mM l$^{-1}$]</td>
<td>influent conductivity [mS cm$^{-1}$]</td>
<td>$y = 10.3x - 6.7$</td>
<td>[1] $0.93^*$</td>
</tr>
<tr>
<td>influent HCO$_3$-[mM l$^{-1}$]</td>
<td>influent conductivity [mS cm$^{-1}$]</td>
<td>$y = 7.5x - 20.5$</td>
<td>[2]  $0.67^{**}$</td>
</tr>
<tr>
<td>effluent N inorg. [mM l$^{-1}$]</td>
<td>effluent conductivity [mS cm$^{-1}$]</td>
<td>$y = 11.6x + 4.2$</td>
<td>[3]  $0.88^*$</td>
</tr>
<tr>
<td>oxidized NH$_4$-N [mM l$^{-1}$]</td>
<td>removed conductivity [mS cm$^{-1}$]</td>
<td>$y = 12.4x + 8.6$</td>
<td>[4]  $0.70^{***}$</td>
</tr>
<tr>
<td><strong>Anammox</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>effluent N inorg. [mM l$^{-1}$]</td>
<td>effluent conductivity [mS cm$^{-1}$]</td>
<td>$y = 12.8x + 7$</td>
<td>[5]  $0.97^*$</td>
</tr>
<tr>
<td>removed N inorg. [mM l$^{-1}$]</td>
<td>removed conductivity [mS cm$^{-1}$]</td>
<td>$y = 15.0x + 3.3$</td>
<td>[6]  $0.83^{**}$</td>
</tr>
</tbody>
</table>

* May-August 2003, **Jan-Dec 2004, *** May 2003- Dec 2004

The presence of other ions in the supernatant certainly had also an influence on total measured conductivity value. However, their impact in experimental search for correlation was ignored.

The conducted studies allowed for establishing a quite high correlation between nitrogen, alkalinity and conductivity. The obtained equations from experiments describing correlations with conductivity for two-stage process are presented in Table 27.

Ammonium nitrogen coexists in supernatant with about the same amount of alkalinity in a form of hydrogen carbonate. 10 mM of these ions gives together a theoretical value of conductivity equal to 1.16 mS cm$^{-1}$. From equation [1] in Table 27 experimental value of conductivity ($x$) for ammonium ($y$) equal to 10 mM, amounts to 1.62 mS cm$^{-1}$. Similarity in obtained conductivity values confirms agreement between theory and experimental data. However, higher values of conductivity in experiment can indicate the presence of other ions present in the supernatant.

For the two-stage process, a control-monitoring system was proposed separately for each reactor (partial nitritation and Anammox) (paper III and VII). According to such system for the partial nitritation, knowledge about conductivity measured in supernatant allows prediction of ammonium nitrogen concentration in the influent. Automatic adjustment of oxygen to the nitrogen load (calculated from conductivity) would result in a proper pH decrease and finally in adequate NAR in the effluent. The information about the Anammox activity and removed nitrogen could be obtained from the difference between measured conductivity values in the influent and the effluent in the Anammox reactor. Moreover, installation of on-line electrodes can make process control more automated and easier (paper III).

**One-stage process**

The application of conductivity for process monitoring was investigated in one-stage process performance, as well. Measurements of conductivity were done parallel to collections of samples and analysis of inorganic nitrogen forms and alkalinity.

Apart from achieved results from pilot plant experiments, theoretical background concerning conductivity was given (paper VII). Theory was compared with experimental results and it occurred that the experimentally obtained data were in good agreement with theoretical considerations (paper VII).

The influence of ionic strength on conductivity value was emphasized (paper VII). In Figure 12, the relationship is drawn between ionic strength and calculated activity coefficients $y$. The trend of curve corresponds to the theoretical curve for single charged ions in Figure 2 (chapter 7.1). Ammonium and hydrogen carbonate ions were taken into account for $y$ calculations in the influent, and ammonium, hydrogen carbonate, nitrite and nitrate for the effluent. As it can be seen from Figure 12, higher ionic strength corresponds to the ions in the influent and lower activity coefficient, while lower IS is associated with the effluent and with higher values of the activity coefficient.

Using experimental data of temperature and conductivity that were measured in the reac-
tor effluent and using formula 32 in chapter 7.1, the concentration of total dissolved salts in the effluent was calculated. In Figure 13 results of calculations are presented together with experimental sum of analyzed nitrogen forms (NH$_4^-$-N, NO$_2^-$-N, NO$_3^-$-N) and alkalin-

ty (HCO$_3^-$).

It can be seen that both time series calculated (Sum out theory) and experimental (Sum out) run parallel and that theory and performed experiments are in good agreement. Moreover, it proves that use of formula 32 and known value of conductivity and tempera-
ture, the total salts concentration is possible be to predict and therefore formula 32 can be applied in process monitoring as supporting process indicator.

Evaluation of conductivity as monitoring tool led to finding of strong relationships with other process functions. Based on the experimental data many correlations were estimated for the influent and effluent (paper VII). A compilation of all relations is shown in Table 28.

The highest correlations that were obtained reflected relationships between conductivity and ammonium and conductivity with hydrogen carbonate. Calculated from theory conductivity of one mmol NH$_4^+$ at infinite dilution and at 25°C is 0.07 mS cm$^{-1}$ (one mmol NH$_4$HCO$_3$ gives a value of 0.110 mS cm$^{-1}$). The same value of conductivity obtained from linear correlation [1] amounted to 0.584 mS cm$^{-1}$. The experimental data are closer to the value of conductivity for one mmol of salt NH$_4$HCO$_3$ than the one mmol of NH$_4^+$ ions. It is due to the fact that the measured conductivity value reflects all ions present in supernatant not only ammonium. The slopes of curves from [1] to [5] are similar.

The correlation coefficient in linear curve [6] is rather low although, the slope is in general agreement with production of 1 mM alkalin-

![Fig. 12 Activity coefficient versus ionic strength calculated from experimental data for one-stage system.](image1)

![Fig. 13. Time series of dissolved salts concentrations in the effluent, experimental data (Sum out) and calculated data from formula C$_{diss.}$ salts [mg l$^{-1}$] = (4.5 10$^9$)(1.02)$^T$ conductivity [S cm$^{-1}$] (Sum out theory).](image2)
Table 28. Different relationships between process functions in one-stage system.

<table>
<thead>
<tr>
<th>y axis</th>
<th>x axis</th>
<th>relationship</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>influent NH₄-N [mM l⁻¹]</td>
<td>influent conductivity [mS cm⁻¹]</td>
<td>y = 8.02x - 5.5</td>
<td>0.77</td>
</tr>
<tr>
<td>effluent N inorg. [mM l⁻¹]</td>
<td>effluent conductivity [mS cm⁻¹]</td>
<td>y = 7.55x - 5.4</td>
<td>0.94</td>
</tr>
<tr>
<td>removed N inorg. [mM l⁻¹]</td>
<td>removed conductivity [mS cm⁻¹]</td>
<td>y = 7.31x + 4.27</td>
<td>0.83</td>
</tr>
<tr>
<td>influent alkalinity [mM l⁻¹]</td>
<td>influent conductivity [mS cm⁻¹]</td>
<td>y = 10.53x - 11.6</td>
<td>0.74</td>
</tr>
<tr>
<td>effluent alkalinity [mM l⁻¹]</td>
<td>effluent conductivity [mS cm⁻¹]</td>
<td>y = 9.9x - 9.65</td>
<td>0.87</td>
</tr>
<tr>
<td>influent alkalinity [mM l⁻¹]</td>
<td>influent NH₄-N [mM l⁻¹]</td>
<td>y = 0.80x + 17.06</td>
<td>0.46</td>
</tr>
<tr>
<td>effluent alkalinity [mM l⁻¹]</td>
<td>effluent N inorg. [mM l⁻¹]</td>
<td>y = 1.0x + 3.73</td>
<td>0.78</td>
</tr>
<tr>
<td>effluent pH &lt;7.4, 8.2&gt;</td>
<td>effluent conductivity [mS cm⁻¹]</td>
<td>y = 0.82lnx + 6.98</td>
<td>0.81</td>
</tr>
<tr>
<td>effluent pH &lt;7.4, 8.2&gt;</td>
<td>effluent alkalinity [mM l⁻¹]</td>
<td>y = 32.4x 234.5</td>
<td>0.85</td>
</tr>
<tr>
<td>effluent pH &lt;7.4, 8.2&gt;</td>
<td>effluent N inorg. [mM l⁻¹]</td>
<td>y = 25.9x-187.44</td>
<td>0.71</td>
</tr>
</tbody>
</table>

The carbonate system plays a dominant role in all transformations taking place during both partial nitritation and the Anammox process in treatment of supernatant. During degradation of organic matter in anaerobic digestion by heterotrophic biomass CO₂ (in abundance) and HCO₃⁻, NH₄⁺ ions are produced (Table 9). As an effect, (after process dewatering) the supernatant has a low COD/NH₄ ratio and contains ammonium and hydrogen carbonate as the two main ions. H₂CO₃ is a diprotic acid and can yield more than one proton in following steps and its ionic form depends on pH value (Figure 3). In the supernatant, the main fraction of inorganic carbon is HCO₃⁻ since pH of supernatant is about 8 (see Figure 3) (paper VII).

During the partial nitritation when ammonium is oxidized to nitrite, HCO₃⁻ is almost completely consumed (degree of consumption depends if entire process is performed in one- or two-stages). The HCO₃⁻/CO₂ ratio is a good indicator for pH-changes but as CO₂ is stripped off, it would have been necessary to know both alkalinity and acidity to calculate pH-changes.

Supernatant interacts with atmosphere due to diffusion to the surface water and by the air bubbles supplied to the reactor with the purpose of aeration. The low partial pressure of CO₂ (expected since CO₂ in the atmosphere is equal to about 0.03%) in the aerated liquid limits CO₂ concentration in the supernatant according to Henry’s law (formula 41). This means that air bubbles can strip more CO₂ then they can transfer into the
water. The effect of CO₂ stripping depends on efficiency of supplied oxygen.

During reaction, HCO₃⁻ is converted to acid H₂CO₃, which next undergoes changes into dissolution of CO₂ and H₂O. The entire way of HCO₃⁻ transformation can be expressed in combined reactions 36, 37, 38

\[ H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow CO_2(aq) + H_2O \leftrightarrow CO_2 \quad (45) \]

Moreover, from the reaction describing the partial nitritation (Table 9), it can be seen that trace amounts of carbon coming from HCO₃⁻ ions are used for cell synthesis. Nitrifying bacteria require proper level of hydrogen carbonate to convert ammonium to nitrite. The lack of adequate hydrogen carbonate would inhibit and decrease rates of nitritation.

The studies showed that the alkalinity was very essential to perform nitritation (paper I, II, III and VII); it provides buffering capacity for the water. A reduction in alkalinity resulted in rapid shifts in pH (paper I, II and III). However, due to CO₂ consumption for autotrophic growth during nitrification alkalinity of wastewater increases, but then in counter reaction of nitric acid formation from ammonia, it decreases. As a final effect the activity of *Nitrosomonas*, generates considerable acidity. The decrease in alkalinity reflects in measured pH value (paper I, II and VII).

HCO₃⁻ ions are also involved in Anammox reaction (Table 9) and are necessary to perform the process. This process is based on the activity of autotrophic bacteria, which transforms under anaerobic conditions ammonium with nitrite to dinitrogen gas. Thus, the process does not need dissolved oxygen and only inorganic carbon (carbon dioxide or bicarbonate) as the main source of carbon is used for new cell synthesis. In the Anammox process, part of NO₂⁻ is oxidized to NO₃⁻ to generate electrons that are needed for CO₂ fixation since Anammox is an autotrophic process. Fixation of CO₂ is accomplished via either Calvin cycle or acetyl-CoA pathway (Schouten *et al* 2004). When CO₂ is used for growth, H⁺ is consumed. Therefore, small increase in pH value could be expected and was noticed in the performed experiments (paper VII).

The studies showed that alkalinity changes played a dominant role in both partial nitritation and Anammox process irrespective of process configuration (paper VII). However, the highest correlations of alkalinity with other process parameters were found for one-stage process (Table 28) and therefore, alkalinity as one of the main components in supernatant can additionally be a supporting measurement to follow up the one-stage partial nitritation/Anammox process (paper VII).
10. RECOMMENDATIONS

10.1 Process selection
For a proper type of treatment method the influence of limiting factors should be evaluated. At first, it must be estimated which process at existing plant is the limiting step. For nitrification, it can be SRT or aeration capacity, while denitrification can be limited by anoxic tank volume or insufficient C-source. Additionally, choosing the process, one must also consider the length of start-up period, risk of process failures, process flexibility and so on.

One of the alternatives, for improvement of WWTP standards effluent, is separate treatment of supernatant. The variety of available biological and physical-chemical methods for nitrogen removal from supernatant can cause difficulties in decision making. The Anammox process as one of possibilities seems to require less volume if compared to traditional nitrification/denitrification and has lower operational costs. Moreover, simple control methods as conductivity and pH, studied in this work, makes combined partial nitritation/Anammox a very attractive alternative.

10.2 Process start-up
Start-up of a full-scale Anammox process is a bottleneck in all system configurations. Systems with the Anammox culture developed as biofilm (e.g. moving-bed biofilm reactor) or as activated sludge (e.g. SBR technology) would be suggested for application as performed studies presented in this thesis (biofilm) and other research (activated sludge) indicated their high possibilities of nitrogen removal.

The experiences from this work showed that the start-up period can last up to one year with the strategy of developing the Anammox culture on previously established nitrifying biofilm. However, literature studies and experiences obtained by other researchers proved that start-up can be shorter.

In process scaling-up, good results gave systematic “step-by-step” approach. In this way, it was possible to recognize and then eliminate different problems appearing in small-scale, in earlier step before full-scale implementation.

One of the alternatives (tested in this study) is development of Anammox on already existing nitrifying biofilm. It is not easy for Anammox bacteria to create own structure of biofilm but it can be seeded into already created biofilm. Therefore, at first the operation of combined partial nitritation/Anammox process can be started-up with development of nitrifying culture under proper aerobic conditions and with good access to the substrate. Since it is well known that Anammox bacteria are present in all wastewater streams it can be expected that mixture of both nitrifiers and Anammox bacteria will create the biofilm structure. However, nitrifiers will play a dominating role under aerobic conditions. Secondly, to intensify the Anammox growth an anaerobic conditions should be assured or alternatively if one-stage process is wanted oxygen limited conditions for Anammox development in the inner layer of biofilm.

If Anammox is already well developed in one reactor and nitrifying biofilm in a second one, there is another possibility of development of combined nitrifying/Anammox bacteria culture as was proved in this study. It can be accomplished by recirculation of Anammox effluent to the nitrification reactor with parallel assurance of low oxygen conditions. Results showed that such strategy could lead to development of diverse biofilm (outer layer nitrifiers and inner layer Anammox) in one reactor during one-month period.

The start-up of the Anammox process can be supported (as it was done in these studies) by dosing “best substrate” as ammonium and nitrite nitrogen combined accordingly to the stoichiometry of Anammox reaction. However, applying this strategy it must be remembered that nitrite can inhibit Anammox reaction at concentration above 20 N mg l^{-1}.

Important aspect in development of Anammox in system with simultaneous partial nitritation/Anammox is diffusion within biofilm depth.
Diffusion can be a limiting factor especially when transfer of oxygen in diverse biofilm is considered. In early stage of development, biofilm is very thin and therefore proper adjustment of DO to assure oxygen-rich outside and oxygen-free inside conditions are important issues. A good solution seems to be application of intermittent aeration. It assures better access to DO in all phases and at the same time can increase the partial nitritation rate, which is a limiting step in simultaneous partial nitritation/Anammox system. In the growing biofilm, diffusion will diminish its significance.

Start-up in moving bed biofilm reactor can also be performed without inoculation. Then a short hydraulic retention time should be assured to wash out suspended cells competing with biofilm. Moreover, oxygen should be properly adjusted to the nitrogen load to prevent further oxidation of nitrite to nitrate and pH control supporting nitrite suppression can be applied.

Additionally, to speed-up the start-up of Anammox process probably hydroxylamine can be used to catalyze the initiation of the Anammox reaction. Hydroxylamine is the first oxidation product of aerobic ammonium oxidation but also the reduction product of nitrite in the Anammox process. Its addition can contribute to a faster Anammox biofilm development.

The main and supporting strategies that can be used in start-up of combined partial nitritation/Anammox process are compiled in Table 29.

10.3 Monitoring and control

Important for operators is quick evaluation of process progress. Sometimes simple and easy measurements are the best and the most useful method for fast evaluation of process. Collected experiences from conducted experiments allows for giving advices and recommendations for different check points and their interference on the process performance. The following measurements for control and monitoring of the partial nitritation/Anammox are recommended:

**Temperature**

Due to the fact that both processes, partial nitritation and Anammox, are strongly depended on temperature its measurement is necessary. Despite the fact that Anammox bacteria culture in natural ecosystem occurs in arctic water, it is very sensitive to sudden changes in temperature. Considering influence of temperature on the partial nitritation process, the difference in growth of nitrite and nitrate oxidizers must be taken into account.

**pH**

pH on-line electrodes are widely applied at wastewater treatment plants for control and monitoring different processes where high proton changes are registered. Its measurements are advisable for monitoring of partial nitritation in case of two-stage process to control performance of ammonium oxidation. Then, pH changes are depending on proper dissolved oxygen supply. As a result of pH free ammonia and free nitrous acid are formed and may inhibit the nitrate oxidizers. In one-stage process, high buffering capacity was observed. However, its strong correlation with process functions in the effluent was found (paper VII). Therefore, installation of pH electrode in the effluent is strongly recommended.
**Alkalinity**

Alkalinity has a major role in the partial nitritation transformations. Therefore, the control of acid capacity is a very important issue. In order to perform successfully partial nitritation $\text{HCO}_3^-/\text{N}$ ratio around 1:1 should be assured. During ammonium oxidation, alkalinity is almost totally consumed and its lack can cause stop of process progress. In case of one-stage process, alkalinity consumption is lowered. However, here the relationships between alkalinity, conductivity and pH were found as very strong. Thus, alkalinity can be an important process performance indicator. Its regular control is suggested.

**Conductivity**

On-line conductivity measurements can be used in automatic control of the system to provide information to operator about state of the process and support daily operation. Continuous measuring electrodes were employed to monitor the influent and at the effluent of the systems. In conducted studies, it was proved that both in one and two-stage processes conductivity was an excellent indicator of process performance (paper III and VII). Conductivity measurements can be, therefore, used in an early warning system. The application of conductivity for the partial nitritation/Anammox is strongly recommended.

**DO**

Dissolved oxygen (DO) control is a very important issue especially in operation of biofilm systems governed by diffusion phenomenon. The aeration control by on-line adjustment to the level of nitrogen supply was tested in this study. However, the intermittent aeration successfully tested in full-scale Hattingen WWTP operating one-stage system led to higher removal rates. Therefore, such possibility in operation of the partial nitritation and Anammox in one reactor would be recommended.

**Suspended solids**

Suspended solids (SS) concentration is an important parameter, which is usually controlled at WWTPs. Its measurement was also investigated during the experiments with partial nitritation/Anammox process. It occurred that the most important is SS concentration in the supernatant. Too high concentrations of SS in the influent supernatant caused clogging of pipes and sticking suspension all over Kaldnes material which resulted in decrease in removal efficiency. Values of SS in the process reactors remained rather stable. It would be reasonable to provide regular control of SS.

**10.4 Other recommendations**

Performed studies indicated that varying characteristics of supernatant played an important role. Therefore, quality and quantity changes of the supernatant must be taken into account when full-scale design for particular WWTP is considered.

Experiences obtained from operation of technical-scale pilot plant showed that sludge digestion and improper work of centrifuges can cause interferences of supernatant composition. They can lead to: stops in supernatant supply and consequently to lowering the surface level of the reactor if clogging at dewatering devices occurs; or to rising in surface level of the reactor and even to overflow if clogging in the reactor effluent occurs.

In order to assure good diffusion and penetration of both energy source (ammonium) and electron acceptor (DO) within biofilm, the biofilm carrier material should be in movement. For full-scale design, a choice of proper stirrers is an important issue.

In order to meet effluent standards it is necessary to take into account the nitrate nitrogen production in the Anammox reaction (about 11% of the influent nitrogen load to the Anammox reactor according to stoichiometry). An alternative solution to get rid of nitrate can be post- or pre-denitrification before or after a partial nitritation/Anammox system. The “nitrate-rich” effluent alternatively can be directed to the denitrification in the main process line of a WWTP. Simultaneous seeding of Anammox bacteria may influence in a positive way the nitrogen removal efficiency in the main stream.
11. Conclusions

The combined partial nitritation and Anammox process was investigated in two system configurations as one- and two-stage. During these studies three different tools were used to evaluate operation of the process: laboratory-scale and technical-scale pilot plants as well as batch tests. From the performed experiments it can be concluded that:

For two-stage process:
- Equal amounts (on molar basis) of ammonium and hydrogen carbonate ions in the supernatant and further almost complete utilization of alkalinity ensure oxidation of half of ammonium during the partial nitritation process.
- The long-lasting and very stable operation of the partial nitritation process was obtained with average oxidation of ammonium to nitrite equal to 56%. An important factor influencing stability was supernatant composition.
- A proper adjustment of HRT and dissolved oxygen concentration in the bulk liquid resulted in pH value drop which next prevented further oxidation of nitrites to nitrates in the partial nitritation step.
- A proper nitrite-to-ammonium ratio (NAR) (on average equal to 0.9 and 1.2, for the first and second trials, respectively) obtained in the diluted effluent from the partial nitritation was essential for an efficient Anammox process performance.
- The Anammox step turned out to be the most critical step in operation of the partial nitritation/Anammox in two following steps.
- The Anammox performed under dissolved oxygen below 0.3 mg l\(^{-1}\) resulted in high nitrogen removal (81%, on average).
- Nitrite is both an electron acceptor and inhibitor of Anammox bacteria. For nitrite nitrogen concentrations below 20 mg l\(^{-1}\) the obtained nitrogen removal efficiency was above 60%. Higher values of NO\(_2\)-N will lead to a decrease in process efficiency.
- In case of nitrite inhibition and in consequence the Anammox process failure, 4 months period was needed for process recovery.
- No effect on process efficiency was observed when process was run without pH correction; therefore process performance without dosage of chemicals is possible.
- It was demonstrated that conductivity measurements are easy and a simple tool to monitor the ammonium oxidation processes (both the Anammox and the partial nitritation). The decrease in conductivity during the partial nitritation resulted from hydrogen carbonate removal while its decrease in the Anammox step was an effect of ammonium and nitrite nitrogen utilisation. Moreover, online conductivity electrodes gave a good correlation with manual electrodes readouts.

For one-stage process:
- The studies showed that development of diverse biofilm with oxic/anoxic dual layer is possible due to recirculation of the Anammox effluent into already working nitrification reactor.
- The system with the partial nitritation/Anammox carried out in one reactor gave better results of nitrogen removal rates than the process performed in two separate stages. The maximum removal rate was equal to 1.9 g N m\(^{-2}\) d\(^{-1}\). However, batch test investigations showed that the system had higher potential and removal rate of 3 g N m\(^{-2}\) d\(^{-1}\) (under DO in the bulk liquid of 1.4-1.6 mg l\(^{-1}\)) are possible to obtain.
- The HRT combined with proper DO and nitrogen load are the key factors to obtain high removal rates in one-stage process.
- The process can be performed at temperature of the supernatant. Alternatively, some heat can be supplied only during winter time.
- Accomplished research indicated that the bottleneck in simultaneous process is the
partial nitritation. Insufficient production of nitrite is the rate-limiting step to perform overall reaction.

- The influence of temperature on simultaneous partial nitritation/Anammox removal rates turned out to be significant in case of sudden changes of temperature. When longer period of adaptation to new temperature conditions was assured, bacteria culture was able to remove nitrogen at higher degree comparing to the results of nitrogen removal at the same temperature but decreased suddenly.

- For one-stage process conductivity as well as pH turned out to be excellent parameters for process progress control. Relationships between pH value (range 7.4-8.2) in the effluent and removed nitrogen, removed alkalinity and removed conductivity were found as linear with high correlation coefficients and they can function as process indicators. Moreover, alkalinity could act as supporting parameter in evaluation of process performance. Implementation of conductivity and pH as monitoring tool could lead to savings in operational costs.

**General:**

- The partial nitritation/Anammox process can be applied for separate treatment of supernatant that surely would improve the WWTP’s effluent quality.

- Application of Kaldnes rings as carrier material enabled bacteria to develop a biofilm and assured efficient bacterial growth.

- Experiences and results gathered from investigations at laboratory-scale and technical-scale pilot plants can be used as guidelines to design full-scale partial nitritation/Anammox systems for nitrogen removal.
**12. Future Research**

The investigations have shown that the systems combining partial nitritation and Anammox processes are not only able to remove high ammonium concentrations from supernatant but also have many advantages. Increasing interest in Anammox process and numbers of its implementation in-full scale, indicate that it may play an important role in the field of nitrogen removal in future. However, some biological, physical-chemical, environmental and technical aspects are still a challenge to future research. Therefore, the following topics are proposed for further studies:

- Production of N\(_2\)O is not expected in the Anammox process but its production in the partial nitritation can be significant. During the entire experimental period, the N\(_2\)O concentration was measured once when the partial nitritation and Anammox were run in separate reactors (chapter 3.4). The investigation showed that higher concentrations of N\(_2\)O were obtained in the partial nitritation than in the Anammox. Similar investigations concerning dinitrogen oxide production in one-stage system should be performed.

- Thickness of biofilm is an essential parameter related to the removal rates. The measurements of biofilm thickness should be performed and the influence of biofilm thickness on removal rates should be investigated.

- The studies in marine sediment showed that manganese cycles can have a positive effect on Anammox activity due to elimination of competing denitrifiers. This phenomenon may also have a potential in wastewater treatment. The application of material with manganese dioxide or water treatment sludge from ground water treatment as biofilm carriers for the Anammox development could be an interesting study.

- The use of redox potential was not investigated in this study. However, it seems that ORP application may be used for monitoring and control of the process performance. It can be especially useful in evaluation of NO\(_3^-\) production by Anammox reaction. Therefore, the use of ORP can be a valuable task for investigations.

- A diffusion process mainly governs a biofilm system. Both external and internal biofilm diffusion can be a limiting factor for the nitrogen conversion rate. Biofilm kinetics should be examined.

- Despite intensive research, the bottleneck of the Anammox in full-scale implementation is start-up process. Therefore, it still remains a challenge for future work to speed-up this stage. Different options and strategies should be investigated. A promising method seems to be addition of hydroxylamine as it can catalyze the initiation of the Anammox reaction. This can be a subject of future studies. Moreover, possibilities of seeding of nitritation and Anammox bacteria on WWTP mainstream would be an important and interesting issue.

- In regions with a cold climate, separate treatment of supernatant (with its high temperature) may be a suitable way to remove part of the nitrogen.

- Looking on commercial aspect it is worth to see, if selling of Anammox bacteria would be possible in future. Thus, investigations in survival abilities of Anammox bacteria e.g. suitable freezing methods (as use of glycerol) etc. would be an interesting task.
LITERATURE


Performance and control of biofilm systems with partial nitritation and Anammox for supernatant treatment


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