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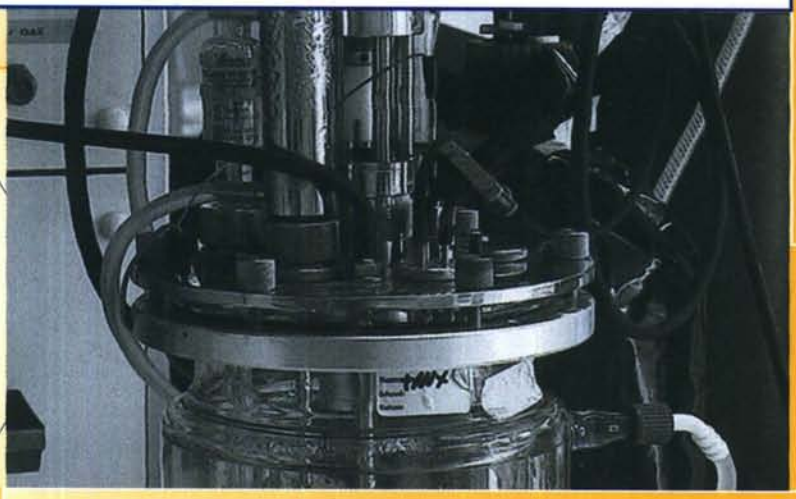
The Combined Sharon/Anammox Process

A sustainable method for N-removal from sludge water



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STOWA, the Dutch Foundation for Applied Water Research, is an organisation for the initiation, co-ordination and application of applied research for the benefit of all water authorities responsible for water management in The Netherlands. STOWA produces scientific publications in the applied fields of wastewater treatment, urban water management, sludge disposal, surface and groundwater quantity and quality, aquatic ecology and flood protection. The water authorities in the foundation provide the finances needed to carry out the research programme and to run the STOWA secretariat.

STOWA REPORT

The Combined Sharon/Anammox Process

A sustainable method for N-removal from sludge water

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Contents

Preface

Since the 1980s, significant experience has been built up regarding biological nitrogen removal at wastewater treatment plants. After the introduction of stringent worldwide effluent standards for nitrogen, many plants have been gradually upgraded in order to attain the required effluent quality. Biological nitrogen removal is a good example of how fast a certain technology can be classified as conventional. Undoubtedly this has been dictated by the fact that it elaborates on the good old activated sludge system.

The biochemical principles of the nitrogen removal process are widely recognised and there are several options for designing new plants or for adjusting existing plants to attain the required standards. Nevertheless, many plants are still unable to fulfil the requirements regarding total nitrogen in effluent. Reasons for this can range from a lack of space to enable the application of the conventional N-removal process or an unfavourable wastewater composition. One important factor that can negatively affect the wastewater composition is the recirculation of N-rich streams from sludge handling processes.

Every branch of industry is currently somehow involved in attaining a higher degree of sustainability for their processes. Wastewater treatment management is also seeking new alternative technologies that focus mainly on minimising the consumption of resources or even on recovering them from wastewater. The conventional N-removal process, which consists principally of the two sub-processes, nitrification and denitrification, cannot objectively be considered as a sustainable process. First of all nitrification requires a lot of energy for aeration and, due to the low growth rate of nitrifiers, large nitrification volumes are required. Second, denitrification requires organic carbon to be efficient. If the COD in the wastewater is not sufficient, an external carbon source (e.g. methanol) has to be supplied

which contributes to an increase of overall treatment costs, consumption of additional resources and consequently a decreased sustainability of the system.

Within the research programme of STOWA (the Dutch Foundation of Applied Water Research) two innovative processes for N-removal have recently been examined: the Sharon process and the Anammox process. Both processes focused on the removal of nitrogen from digested sludge water. In the Sharon process (Single reactor system for High Ammonium Removal Over Nitrite) ammonium is oxidised in one reactor system under aerobic conditions to nitrite, which in turn is reduced to nitrogen gas under anoxic conditions by using an external carbon source. In the Anammox process (Anaerobic Ammonium Oxidation) nitrite and ammonium are converted into nitrogen gas under anaerobic conditions without the need to add an external carbon source.

In comparison with conventional N-removal, the Sharon process results in a reduction of required aeration energy and carbon source. A partnership between Sharon and Anammox would contribute even more to a sustainable wastewater treatment. Compared with conventional N removal, 40% less oxygen (= energy) is necessary, an organic carbon source is not required and sludge production is negligible.

In the research described in this report, the feasibility of ammonium removal from digested sludge water was tested using the combined Sharon/Anammox process. The research confirms the sustainability of the process and the results offer good economic and operational perspectives. For these reasons, it has been recommended that a scale-up of the process in practical conditions forms a very realistic conclusion of this research. At the moment two full-scale Sharon reactors are operating in the Netherlands for the treatment of N-rich sludge water, and more are planned in the future. This combination has become an obvious option in the optimisation of existing plants and the design of new plants. A successful scale-up of the combined Sharon/Anammox process will make this even more favourable.

The research was carried out by the Technical University of Delft, The Netherlands. The research team consisted of Dr. ir. M. Jetten, Prof. Dr. ir. M.C.M. van Loosdrecht and ing. U. van Dongen. The project was for STOWA supervised by a steering committee formed by: ir. D.M.E. Anink (chairman), ing. R. van Dalen, ir. R. van Kempen, ir. J.W. Mulder, ir. P.J. Roeleveld, ir. P.C. Stamperius and ir. C.A. Uijterlinde.

The combined Sharon/Anammox is currently feasible for N-rich sludge water. It will be a challenge to make such a sustainable concept also suitable for the treatment of wastewater with lower nitrogen concentrations and low temperatures.

Paul Roeleveld
STOWA

Overview

Conventional removal of ammonium requires usually large amounts of energy for aeration and organic carbon for denitrification. The research described in this report focused on making the N-removal process more sustainable. This can be achieved by a partial oxidation of ammonium to nitrite, after which the nitrate produced can be converted into nitrogen gas with the rest of ammonium under anoxic conditions.

The formation of nitrite can take place in a Sharon-type reactor (without sludge retention). However, it is not necessary to intermittently aerate the reactor for denitrification. The reactor is continuously aerated and because pH control does not take place, the pH will decrease and approximately 50% of the ammonium available (originating from sludge water) will be oxidised into nitrite. This denitrification with ammonium (Anammox) can take place in a second reactor with sludge retention.

In this research the feasibility of ammonium removal from digester supernatant was tested using the combined Sharon/Anammox process.

The Sharon reactor was started within two weeks, using nitrifying sludge from a low-loaded activated sludge plant. The Sharon process was carried out in a continuous flow reactor with a hydraulic retention time of 1 day and a temperature of 35°C. Under these circumstances 50% of ammonium was converted into nitrite. The conversion rate of ammonium can be increased by slightly raising the pH. A prolonged continuous aeration in the Sharon reactor can lead to the germination of protozoa. This has a negative effect on the stability of the process. The presence of protozoa can be determined by using a microscope.

During periods of no or low influent flow (when aeration is normally switched off), if aeration is continued for one to two hours, these protozoa can be suppressed in the system, resulting in a decrease of pH to below 6. After this, aeration can be switched off until the influent is available.

For this research, the Anammox biomass was cultivated from activated sludge from a WWTP by feeding with synthetic wastewater or effluent from the Sharon reactor (the ammonium and nitrite concentrations remained lower than 70 mg N_{tot}/L). After that, the concentrations of ammonium and nitrite were gradually increased to 700 mg N_{tot}/L.

Using the FISH (Fluorescent In Situ Hybridisation) technique, certain specific bacteria or groups of bacteria (including Anammox) can be observed from sludge mixtures using a fluorescent microscope. Using this technique, Anammox cells from the sludge mixture were found four to six weeks before any Anammox activity was measured in the system.

The combined Sharon/Anammox system operated in a stable way for a period of 120 days, with a N_{tot}-conversion rate of 0.75 kg $N_{tot}/m^3_{reactor}$/day. The average specific conversion rate amounted to 0.18 kg N_{tot}/kg MLSS/day. During activity tests a maximum specific conversion rate was measured of 0.82 kg N_{tot}/kg MLSS/day.

In order to scale up the combined Sharon/Anammox system, the Anammox reactor can be inoculated with low-loaded activated sludge. Using the FISH technique it can be quickly determined whether the chosen method is correct. An on-line nitrite/nitrate analyser can be used in a control strategy for the Anammox process. A very low nitrite concentration means that more nitrite has to be formed in the Sharon reactor (which can be accomplished by correcting the pH or aeration time taken). A very high nitrite concentration in the Anammox reactor means that less nitrite has to be formed in the Sharon reactor (aeration off).

To apply the Anammox process, the choice of reactor type is very important. This study showed that the Anammox process, whether in pilot- or full-scale, can best be carried out in a biofilm reactor or granular sludge reactor. The advantage of a biofilm reactor is its relatively easy start-up and operation. The advantage of a granular sludge reactor is that a higher nitrogen loading can be applied, resulting in more compact systems. The disadvantage is that the start-up of such a system could take longer because of a lower sludge retention. When an Anammox granular sludge reactor is started up, new Anammox reactors can then be started more quickly than the first, analogously to Upflow Anaerobic Sludge Blanket (UASB) reactors.

The costs for treating digester supernatant using the combined Sharon/Anammox system were estimated to be 0.7–1.1 € per kg N-removed. From previous STOWA research, it was found that the costs of other techniques, based on the same type of calculation, were significantly higher. The costs of the Sharon process with methanol for pH correction were estimated to be 0.9–1.4 €/kg N removed, while other biological techniques cost 2.3–4.5 €/kg N and physical-chemical techniques cost 4.5–11.3 €/kg N removed.

1

Introduction and background

To reach a low concentration of nitrogen in effluent from wastewater treatment plants (WWTPs), one possible measure is to reduce the nitrogen content in nitrogen-rich return streams from the sludge treatment. This leads to a reduction of the overall nitrogen loading to the main treatment process.

In WWTPs, the nitrogen-rich stream is mainly produced during the sludge digestion process. With anaerobic digestion, organic carbon is partially converted to methane gas while organically bound nitrogen is converted to ammonium (STOWA 1996a). Digested supernatant is produced during thickening or dewatering of digested sludge. This supernatant, also called sludge water, sludge water, centrate or filtrate, contains a relatively high concentration of ammonium nitrogen and a relatively low content of biodegradable organic matter (COD (chemical oxygen demand) or BOD (biological oxygen demand)).

The sludge water is usually directly returned to the beginning of the WWTP and forms 10–20% of the overall nitrogen (N) loading to the main treatment process. When the main process at the WWTP has been designed in such a way that the effluent quality demand for N is fulfilled, the recycling of the nitrogen-rich stream does not constitute any problem. In many cases the treatment process is unable to meet the required standards and needs to be upgraded. Since there is often not enough space to extend the existing treatment process to fulfil the effluent standards, another solution has to be found. Separate treatment of the ammonium-rich sludge water is one of the existing possibilities (STOWA 1996a).

A process whereby ammonium can be biologically removed using less energy in aeration and without a COD demand is a very attractive option for making the whole treatment process more sustainable.

Several different STOWA research projects on ammonium removal from sludge water have been carried out in the past. One of these was on the Sharon process (Single reactor system for High Ammonium Removal Over Nitrite). In a single reactor, ammonium is oxidised under aerobic conditions to nitrite and this nitrite is, under anoxic conditions and with the addition of a carbon source, converted to nitrogen gas (STOWA 1996a). This denitrification is mainly used to control the pH of the process.

Another STOWA research project on the treatment of sludge water was a study on the feasibility of the Anammox process (Anaerobic AMMonium OXidation). During the Anammox process, nitrite and ammonium are converted under anaerobic conditions to N-gas and water while no additional carbon source is applied.

From the Anammox study, it was concluded that for the Anammox process to function optimally, a stable (constant) nitrite supply is necessary. This stable nitrite supply can be secured by the incorporation of partial nitrification in a Sharon reactor, placed in front of the Anammox reactor.

Within this study, it was tested whether this combined Sharon/Anammox system has the potential to treat sludge water. This was tested for a longer period in a Sharon/Anammox system consisting of two 10 L reactors. The system was fed with sludge water originating from the Sluisjesdijk sludge handling facility in Rotterdam, the Netherlands.

From the preceding feasibility study on the application of Anammox for the treatment of sludge water (Van Loosdrecht and Jetten 1996), the following recommendations for further research were proposed:

- research to combine the Anammox process with a stable supply of nitrite;
- larger-scale research into the application of the Anammox process.

As a follow-up of the feasibility study, this research was carried out, and was made up of the following subsequent phases:

1. **Start-up and stable operation of a Sharon reactor fed with sludge water such that the ratio NO_2-N:NH_4-N in the effluent was 1.3:1.**

After a successful start-up the relation between the conversion of ammonium and pH (in a range of 6.5–7.5) was studied. Affinity constants for ammonium and oxygen were also estimated. With an optimal NO_2-N:NH_4-N ratio of 1.3:1, no ammonium remains in the effluent of the Anammox process.

2. **Enrichment and stable operation of an Anammox reactor fed with synthetic wastewater, whereby activated sludge was used as inoculum.**

When an Anammox reactor has to be started up at full-scale, a large amount of seed sludge (inoculum) may be necessary. Because it is not possible to grow such large amounts in lab-scale, research was carried out on ways to enrich Anammox biomass from nitrifying activated sludge. This was carried out with synthetic wastewater to see whether it is possible to attain such an enrichment from activated sludge.

3. Enrichment and stable operation of an Anammox reactor with effluent from the Sharon reactor where activated sludge was used as the inoculum.

In a full-scale experiment, growth of Anammox biomass and the consequent start-up of the Anammox reactor could not be performed with synthetic wastewater. This would have to happen when using effluent from the Sharon reactor. An additional advantage of the start-up using effluent from the Sharon reactor is that the biomass does not need to be adapted to the wastewater.

4. Long-term operation of the combined Sharon-Anammox.

When Anammox biomass is once enriched by effluent of the Sharon reactor, the system is expected to operate in a stable manner. Both Sharon and Anammox systems can be operated stably for long periods of time; however, the combined process was never operated for a longer period of time. During this long-term operation the necessary parameters for the design of the full-scale combined Sharon/Anammox system were estimated. Because the Sharon process operates without sludge retention, all nitrifying biomass from the Sharon process is washed out and brought to the Anammox reactor. To predict the consequences of this, research was carried out on the effect of this biomass wash-out on processes in the Anammox reactor.

5 Formulation of the most important design parameters for Sharon/Anammox for pilot- or full-scale and economic evaluation.

The most important parameters for full-scale design were formulated during this research. An evaluation was also made as to the most suitable reactor type for a full-scale Anammox system. Finally, the economic aspects of the combined system were considered.

This report consisted of the following parts:

- theoretical background to both the Sharon and Anammox processes;
- research performance;
- results and discussion of experimental work;
- process design and economic feasibility;
- conclusions and recommendations.

2

Process description

This chapter describes the theoretical backgrounds of the Sharon and Anammox processes as well as the background to the combination of these processes.

2.1 THE SHARON PROCESS

In 1995, research was carried out on a number of treatment techniques to remove nitrogen (N) from N-rich return streams, for example, sludge water (STOWA 1996a). One of the tested treatment techniques was biological N-removal from N-rich wastewater using the Sharon (Single reactor system for High Ammonium Removal Over Nitrite) process. This process takes place in an intermittently aerated, completely stirred continuous flow reactor without sludge retention.

In the Sharon process, ammonium is converted to nitrite under aerobic conditions by ammonium-oxidising bacteria (nitritification). The following equation describes this process:

$$NH^{+}_{4} + 1.5O_2 \rightarrow NO^{2} + H_2O + 2H^{+} \qquad (2.1)$$

When nitrite oxidising bacteria are present in the reactor as well as ammonium-oxidising organisms, the following reaction takes place under aerobic conditions where nitrite is oxidised to nitrate (nitratification):

$$NO_2 + 0.5O_2 \rightarrow NO_3 \quad (2.2)$$

Due to a short retention time (approximately 1 day) and high temperature (35°C), the nitrite oxidisers are washed out and nitrite only is formed in the Sharon reactor.

For the oxidation of ammonium to nitrite, 25% less oxygen is necessary than for the oxidation of ammonium to nitrate.

Both nitrite and nitrate can be removed under anoxic conditions in the Sharon reactor by heterotrophic organisms (denitrification). The following, simplified reactions describe this process:

Denitrification of nitrite: $6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 6HCO_3^- + 3H_2O$ (2.3)

Denitrification of nitrate: $6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 6HCO_3^- + 7H_2O$ (2.4)

For nitrite or nitrate removal, methanol or another organic carbon source is necessary. For denitrification of nitrite, 40% less methanol is needed than for denitrification of nitrate.

Summarising, this means that the nitrite route for N-removal needs 25% less of oxygen and 40% less of methanol than the nitrate route.

For research on the combination of the Sharon process and the Anammox process, instead of a standard nitrifying/denitrifying Sharon reactor, a Sharon reactor was operated where ammonium was only partially converted to nitrite, to promote the growth of ammonium oxidisers based on sludge age.

In the following sections the process performance will be explained in the light of two important parameters, temperature and pH.

2.1.1 Temperature

The relation between temperature and maximum growth is different for ammonium-oxidising and nitrite-oxidising bacteria. Ammonium oxidisers require a shorter minimum sludge age at higher temperatures. In the Sharon reactor the environmental conditions are more beneficial for ammonium oxidisers (*Nitrosomonas* species) than for nitrite oxidisers (*Nitrobacter* species), mainly because of the higher operational temperature (35°C). Figure 2.1 shows that at 35°C the maximum growth rate (μ_{max}) of nitrite oxidisers is approximately two times lower than that for ammonium oxidisers (0.5 and 1 day^{-1} respectively). When impaired with a short hydraulic retention time, nitrite oxidisers are selectively washed out.

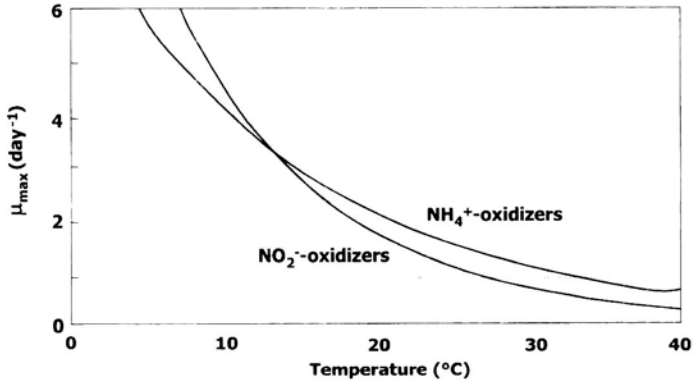


Figure 2.1. The effect of temperature on the maximal growth rate of ammonium and nitrite oxidisers (Hunik 1993). At higher temperatures impaired with a short retention time, nitrite oxidisers can be selectively washed out from the system.

2.1.2 pH

The oxidation of ammonium to nitrite (nitritification) is an acidifying process which can be seen in Equation (2.1). This acidifying effect is, however, partially neutralised by bicarbonate, which is present in sludge water. Bicarbonate works as a buffer. This buffering property can be explained using the following equation:

$$2HCO_3^- + 1.5O_2 + NH_4^+ \rightarrow 2CO_2 + 3H_2O + NO_2^- \quad (2.5)$$

In sludge water the ratio HCO_3^-:NH_4^+ is normally 1.1:1 (Hellinga *et al.* 1998). By conversion of approximately 50% NH_4^+ almost all HCO_3^- is utilised. When half of the free ammonium is converted, the pH will begin to decrease. When the pH drops below approximately 6.5, the ammonium oxidation will no longer take place, because of a pH-dependent equilibrium between the concentrations of NH_3 and NH_4^+. In fact, NH_3 will then be used as a substrate by ammonium oxidisers. The equilibrium between NH_3 and NH_4^+ is described by the following relation:

$$NH_4^+ \rightarrow NH_3 + H^+ \quad (2.6)$$

At too low a pH (<6.5) the equilibrium moves too far to the left and there is a too low concentration of nitrogen in the form of ammonia (NH_3) present in the reactor. When the pH drops too low, the free ammonium concentration becomes too low for the proper growth of ammonium oxidisers.

Since the influent contains bicarbonate, the pH will increase and again nitrification can take place. Because Sharon is a continuous flow reactor, a steady state would finally be established, whereby half of the ammonium provided could be converted to nitrite. When the Sharon reactor is operated to nitrify as well as denitrify, denitrification takes care of the production of HCO_3^-, contributing to an increase in the buffer capacity and consequently also in the pH.

In this research the Sharon reactor was only used to convert available 50% of the ammonium into nitrite. That is why base- or acid dosing was not necessary.

2.2 THE ANAMMOX PROCESS

Anammox is a biological process to remove ammonium from wastewater, whereby under anaerobic conditions ammonium is converted to nitrogen gas with nitrite as electron acceptor. Because the Anammox process is autotrophic, a complete conversion of ammonium to nitrogen gas can take place without the addition of methanol or another form of BOD.

The process can be characterised by a very high potential capacity (2.6 kg N_{tot}/ $m^3_{reactor}$/day) (Jetten *et al.* 1999), therefore the Anammox process seems suitable for the design of compact treatment systems (STOWA 1996b) (for comparison: N-loading of an activated sludge system is equal to approximately 0.1 $kgN_{tot}/m^3_{reactor}$/day).

The growth rate of Anammox bacteria is low (doubling time = 11 days). One big advantage of this is that a low amount of bacterial sludge is thus formed. One disadvantage is a long start-up period for the Anammox process. Recently the organisms responsible for Anammox process were identified as planctomycete-type bacteria (Strous *et al.* 1999). This had previously been unknown. The phylogenetic position of the organism in the cluster of the planctomycetes is shown in Figure 2.2.

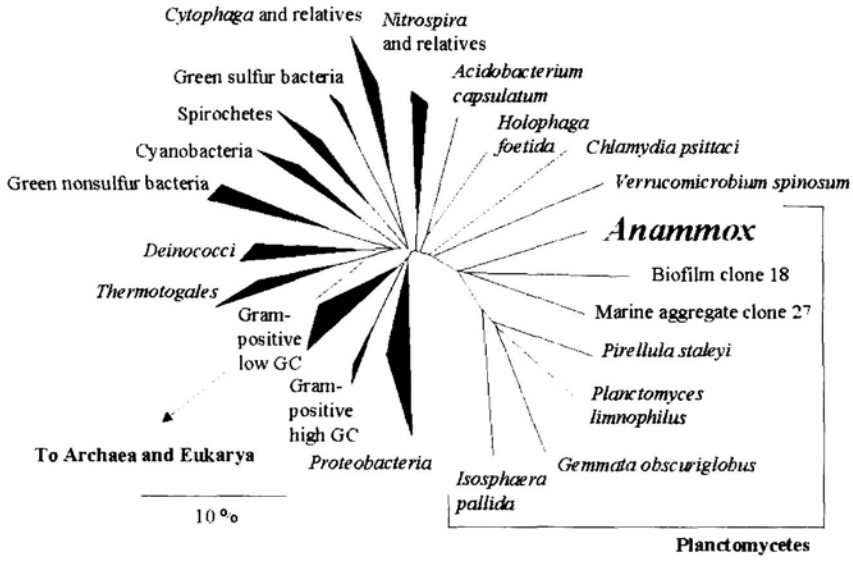


Figure 2.2. Phylogenetic position of a planctomycete-type bacteria responsible for the Anammox process.

In the Department of Biotechnology at the Technical University of Delft, the Netherlands, Anammox reactors (as sequencing batch reactors) have been operated for some time. These Anammox reactors were fed with synthetic wastewater with concentrations of 420 mg NO_2^--N/L and 420 mg NH_4^+-N/L. In these reactors, 100% of NO_2^- was removed, while over 80% of NH_4^+ was also removed. The stoichiometry of the Anammox reaction is given by the following reaction:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.066CH_2O_{0.5}N_{0.15} \quad (2.7)$$

The conversion of ammonium takes place without the presence of an organic carbon source (HCO_3^- serves instead as the C-source) and under anoxic conditions. The bacteria utilise the available ammonium as an electron donor to convert nitrite into nitrogen gas.

A small fraction of nitrite has to be oxidised into nitrate to provide the electrons necessary for cell growth.

As can be seen from Equation (2.7), the ratio between ammonium and nitrite present in wastewater should be as high as 1:1.3. This ammonium/nitrite ratio can be secured by using the Sharon process for partially treating the sludge water.

In current research on the applicability of the Anammox process for the treatment of sludge water (STOWA 1996b), the following important conclusions were drawn:

- Anammox seems a suitable process to remove ammonium from sludge water;
- the temperature and pH normally found for sludge water are optimal values for the Anammox process. The only concern is to prevent too rapid cooling down of the medium;
- different reactor configurations can be applied for the conversion of ammonium in the Anammox process;
- when using a fluidised-bed reactor, high nitrogen loadings can be applied while the sequencing batch reactor is simpler and more stable.

2.2.1 Reactor performance (design/construction)

Different reactor configurations have been tested for the Anammox process. These were fixed- and fluidised-bed biofilm reactors (STOWA 1996b). In this research, however, the Anammox process was however not run as a fixed- or fluidised bed but as sequencing batch reactor (SBR) with granular sludge. This was chosen because the SBR-type Anammox can operate in a stable manner for a long period of time, and high N-conversion rates can be achieved in this reactor type (Strous *et al.* 1998).

Unlike to the Sharon reactor (chemostat), the Anammox SBR is not continuously but sequentially operated. One cycle consists of three phases: filling, settling and a withdrawal phase. The cycle is presented in Figure 2.3.

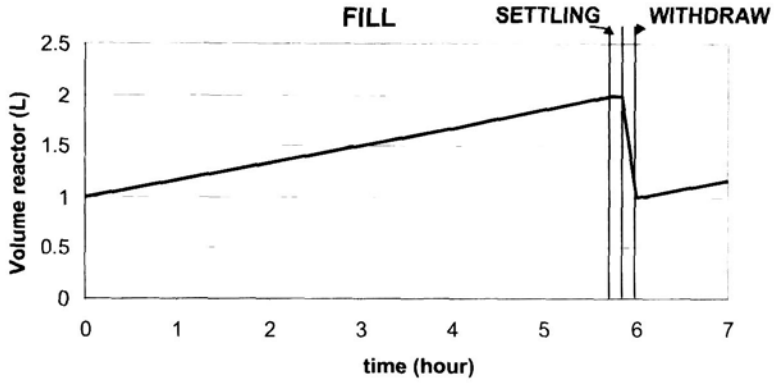


Figure 2.3. Schematic presentation of a six-hour SBR cycle.

2.3 COMBINED SHARON/ANAMMOX

In earlier research, the combined Sharon/Anammox process has been operated with the Anammox as a fluidised bed reactor (Jetten *et al.* 1997). Using this configuration it was proved that sludge water can be successfully treated using such a system.

A Sharon reactor without pH control was fed with sludge water at a loading rate of 1.2 kg N_{tot} /m^3 $_{reactor}$/day. In 53% of the incoming N-load, ammonium was converted: to nitrite (39%) and nitrate (14%), so the effluent contained a mixture of ammonium/nitrite in the ratio 1.3:1. This effluent was used as influent to the Anammox fluidised bed reactor. In the Anammox

reactor, all nitrite was removed, while some ammonium remained unconverted. During the research period, 83% of ammonium was removed from sludge water using the combined system.

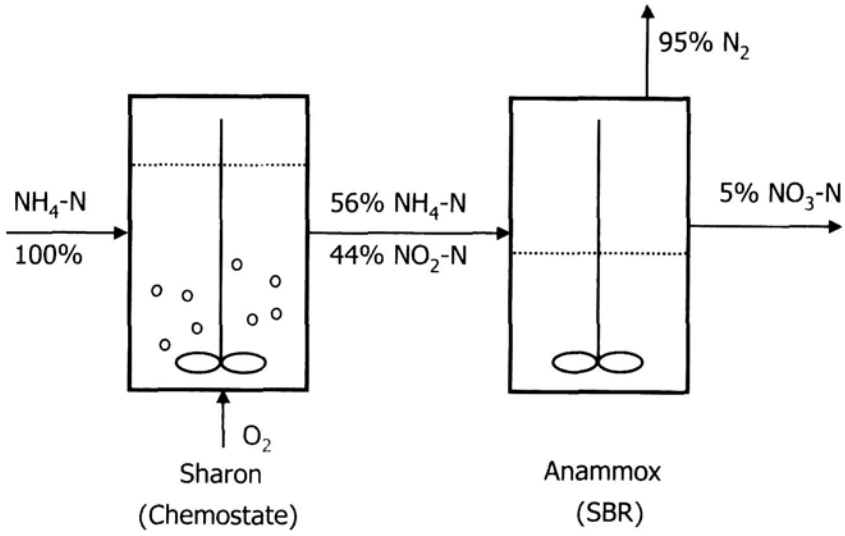


Figure 2.4. Schematic presentation of the combined Sharon/Anamox system.

In this research the Anammox reactor was a SBR (see section 2.2.1). Figure 2.4 shows the combined Sharon/Anammox configuration that was used.

3

Process performance

This chapter describes the construction/configuration of various reactors, as well as the type of analysis performed. Section 3.1 describes the performance of the Sharon process, and various process conditions and experiments. Section 3.2 gives the construction, start-up and process conditions of different Anammox reactors and describes experiments performed. Section 3.3 describes the process conditions of the combined Sharon/Anammox system and related analysis.

3.1 THE SHARON PROCESS

For the purpose of this research, a 10 L Sharon reactor was started up. The following paragraphs describe the experimental set-up, process conditions, analysis and experiments associated with this reactor.

3.1.1 Experimental set-up

This section describes the different materials used for the construction and start-up of a 10 L Sharon reactor (chemostat), fed with sludge water from the Sluisjesdijk sludge treatment plant in Rotterdam, the Netherlands. The average composition of this medium is given in Table 3.1.

Table 3.1. Average composition of sludge water from the Sluisjesdijk sludge treatment plant, Rotterdam, the Netherlands

Component	Concentration
COD	1184 (mgO_2/L)
BOD	230 (mg/L)*
Total N	1605 (mg/L)
NH_4^+-N	1156±160 (mg/L)
Total P	12 (mg/L)
Total Suspended Solids	56 (mg/L)*
NO_2^-N	<1 (mg/L)
HCO_3^-	5100 (mg/L)
PH	8.1–8.4

* These values were not determined for purpose of this research but originate from earlier research. It is assumed that the original values do not deviate significantly from current (actual) ones.

As can be seen from Table 3.1, the sludge water contains a relatively high concentration of ammonium nitrogen.

The reactor was inoculated with 4 L return sludge from the nitrifying B-step (AB-system) of the wastewater treatment plant (WWTP) Dokhaven in Rotterdam, The Netherlands. The pilot set-up is shown in Figure 3.1.

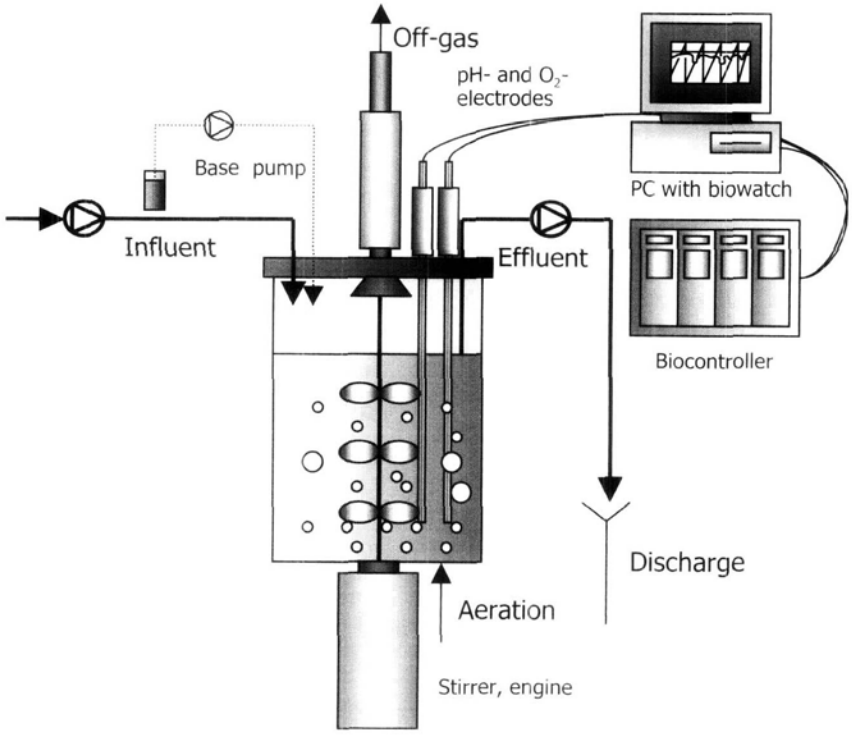


Figure 3.1. Schematic presentation of the Sharon reactor.

3.1.2 Process conditions in the Sharon reactor

The Sharon reactor operated during the entire period of research at a temperature of 35°C. To begin with, the hydraulic retention time (HRT) was 2 days. When the nitrification process had been established, the HRT decreased to 1 day. The combination of ammonium-rich sludge water as substrate and a short retention time meant that fast-growing ammonium oxidisers were favoured. In a later period, the HRT was increased in order to insert anaerobic periods to prevent the development of protozoa in the reactor. Regularly, the biofilm growing on the reactor wall was brought back into suspension. Finally, as well as base dosing, acid dosing was also applied in order to keep the pH at the level required.

3.1.3 Analysis and experiments

3.1.3.1 Components

During the research period, grab samples were taken from both influent and effluent at least three times per week. The samples were centrifuged for 3 minutes at 13,000 rpm. The NH_4-N and NO_2-N concentrations were determined in the supernatant. Ammonium (+ammonia) was calorimetrically determined at 623 nm, according to the Fawcett and Scott method (Fawcett and Scott 1960). Nitrite nitrogen was calorimetrically determined at 540 nm according to the Griess, Romijn and Eck method (Shinn 1941).

Using Merck test strips daily, the nitrite concentration in the reactor was semi-quantitatively estimated.

In the start of the research, the inorganic carbon concentration was regularly determined in the influent and effluent of the Sharon reactor using a total organic carbon (TOC) analyser.

3.1.3.2 pH and conversion rate

Because of the shift in equilibrium between NH_3 (the actual substrate for ammonium oxidisers) and NH_4^+ at different pH values, tests were carried out to discover at which rate ammonium is oxidised at different pH values. By dosing 4M NaOH, the pH in the reactor was repeatedly increased by a few tenths. Further, it was assumed that the system stabilised after four days at a given pH. After this period the ammonium conversion rate at the set pH was determined.

3.1.3.3 Respirometry

To obtain an insight into the conversion magnitudes of the nitrifying sludge, respirometric tests were performed using a BOM (Biological Oxygen Monitor) meter, which consists of an airtight vessel where dissolved oxygen is monitored.

Before each test, the sludge was washed with a phosphate buffer (20 mM KH_2PO_4 and 200 mM NaCl) and the pH was set at the level required using HCl and NaOH solutions. The aim of the washing was to separate the oxygen using substrates (among others, BOD, COD and NH_4^+) from the bacteria. The washing was carried out by centrifuging the sample for 10 minutes, and then decanting the supernatant. The remaining sludge was resuspended in the phosphate buffer and again centrifuged. The supernatant was once more decanted and the sludge resuspended in a fresh volume of phosphate buffer and was thus ready for tests. The washed sludge was saturated with oxygen by aeration with compressed air at 35°C. The pH was controlled before and after each measurement. A change in pH during measurement could have led to inaccurate results.

The dissolved oxygen concentration in the respiration meter was read and registered by a computer at each moment of the test. The oxygen consumption rate can be estimated from the slope of oxygen decline. When different oxygen consumption rates are plotted against the appropriate substrate concentrations, one obtains the conversion curve. From this the affinity or saturation constant (K_S) can be estimated. (K_S is a substrate concentration at which half the maximal conversion rate is attained).

The affinity to ammonium is estimated during the respiration experiment by the addition of a known amount of ammonium solution using a long thin needle. In this manner the oxygen consumption rate at varying ammonium concentrations per time unit can be obtained. The values of ammonium and oxygen consumption rates are imported to the Grafit 3.0 computer program (available from Erithacus Software, PO Box 274, Horley, Surrey, RH6 9YJ, England), and the affinity coefficients and maximal rates are estimated using non-linear regression.

As well as affinity to ammonium, the possible inhibition of ammonium conversion rate by nitrite was also determined in this way.

3.1.3.4 Bicarbonate content

To ascertain whether ammonium and bicarbonate are converted in a ratio of 1:2, grab samples were taken each week from the influent and effluent of the Sharon reactor over a two-month period when it was operating in a stable manner. Bicarbonate concentrations were determined using a TOC analyser.

It is not known whether the nitrification rate is reduced when CO_2 is stripped from the wastewater by, for instance, diffusion. When CO_2 is stripped, bicarbonate also disappears. Consequently, the acid equivalents will be withdrawn from the wastewater and the pH will increase according to the following reaction:

$$H^+ + HCO_3 \rightarrow CO_2 + H_2O \qquad (3.1)$$

When bicarbonate is stripped from the wastewater before the reaction with ammonium takes place, the pH will rise. This pH increase will be prevented by the oxidation of ammonium whereby oxygen equivalents are released. Thus, the total acid buffering activity remains constant.

3.1.3.5 Sludge characteristics

Using FISH (Fluorescent In-Situ Hybridisation) analysis, the specific bacteria types or groups can be observed under a fluorescent microscope and in this way the presence as well as the amount of given bacteria in the sludge sample can be ascertained.

Molecular research at the Kluyver Institute (Technical University of Delft, the Netherlands) indicated that the oxidation of ammonium in the Sharon reactor is carried out by *Nitrosomonas eutrophea* bacteria (Logemann *et al.* 1998). It was also proved, using FISH analysis, that this type of bacteria was also present in the operated Sharon reactor.

The FISH method is based on the hybridisation of the labelled probe with a specific part of the 16S RNA of a bacterium. A probe (chemically synthesised oligonucleotide) consists of 15 to 30 nucleotides (bases). The probe is labelled with a fluorescent colouring agent. The hybridised cells of a given type of bacterium can be than observed under fluorescent microscope, using a probe.

For the purpose of this research, the biomass from the Sharon reactor was analysed and controlled for the presence of nitrifiers and also, more specifically, *Nitrosomonas* species and/or *Nitrosoccus* species, using FISH.

3.1.3.6 Influence of anaerobic conditions on nitrifiers

During this research protozoa activity (exposing predation) was observed in the Sharon reactor. Because of this a decreased nitrification activity was measured in the reactor. Intermittent aeration of the Sharon reactor content is one action that can be taken against the development of protozoa. However, at the same aerobic retention time as during continuous aeration, the nitrification was less efficient. To evaluate whether the ammonium oxidisers suffer during the anaerobic period, the nitrification capacity reduction of the nitrifying biomass was determined during an anaerobic period. The experiment was performed with biomass from the Sharon reactor in the Sluisjesdijk sludge treatment plant.

During this period the reactor was operated at 35°C without aeration. At the same time the 50 L vessel was filled with the reactor's content and the temperature of the mixture was lowered to 5°C.

Nitrification capacity tests were used to determine whether there were differences at different temperatures. To estimate the nitrification capacity, a 500 mL vessel filled with water from the Sharon reactor was aerated at 35°C. With a pulse dose of ammonium, a concentration NH_4^+-N of 350 mg/L was obtained. After 10 minutes, samples were analysed for NH_4^+-N and NO_2^--N. During the experiment the pH, dissolved oxygen concentration and temperature were noted. The same experiment was repeated after 3 and 6 days in order to evaluate how fast the nitrification capacity was being reduced.

3.2 THE ANAMMOX PROCESS

When a full-scale Anammox reactor has to be started, a large amount of inoculating sludge is necessary. One option for acquiring such a large quantity of biomass is to grow (cultivate) it in laboratory reactors. A better solution is to enrich the Anammox biomass from, for instance, activated sludge (which is always available) fed with sludge water. In that way, no large quantities of lab-grown inoculum are needed.

To evaluate whether the enrichment of Anammox biomass from activated sludge is possible, two 2 L enrichment SBR reactors were started up and fed with synthetic wastewater. In a later phase one 10 L Anammox biomass enriching reactor was started up and fed with the effluent from the Sharon reactor instead of synthetic wastewater, thus simulating full-scale conditions.

This section discusses the build-up of different enrichment reactors together with associating process conditions, analyses performed and experiments.

3.2.1 Experimental set-up

As mentioned before, the first two 2 L reactors were put in operation in order to assess whether it is possible to grow Anammox biomass from activated sludge. The reactors were started up with thickened, nitrifying sludge from the B-step of the Dokhaven wastewater treatment plant in Rotterdam. The B-step was chosen because it is there that the conversion of ammonium via nitrite to nitrate takes place, which increased the possibility of the presence of Anammox.

To control the enrichment method, a 20 µl Anammox sludge (1: 100,000 of the maximum reactor content) was added to one of the two reactors. Both enrichment reactors were configured as SBR with a volume of 2 L each. They were controlled by a computer using the Biodacs program.

When one of the reactors (SBR1 without Anammox inoculum) was sufficiently enriched with Anammox biomass, the synthetic influent was replaced by effluent from the Sharon reactor. SBR2 was fed with synthetic wastewater for the entire experimental period.

In full-scale conditions, the enrichment of the Anammox biomass using synthetic influent is not possible. In such a case, effluent from the Sharon reactor should be used. A third of the enrichment reactor (SBR) of the maximal volume of 10 L was fed with diluted effluent from the Sharon reactor, to which a nutrient and nitrate solution was added. This 10 L enrichment reactor was inoculated with the biomass from Boskoop and Reeuwijk Randenburg WWTPs, also in the Netherlands. The first plant is an oxidation bed plant, in which ammonium is only partially nitrified. This, in combination with a long sludge age, increases the likelihood that Anammox cells are present in the biomass. The second plant is an activated sludge system with pre-denitrification, where the sludge age is approximately 15 days. Likewise, a 100 µl Anammox sludge (1: 100,000 of the maximum reactor content), originating from the 2 L enrichment reactor without added inoculum, was added to the sludge mixture. The additional inoculum was added because at start-up, it was not known whether this could have a possible enhanced effect.

The reactor was controlled and the pH and ORP (oxidation reduction potential) were registered by a computer using the BIODACS program. The complete Sharon/Anammox installation was configured as shown in Figure 3.2.

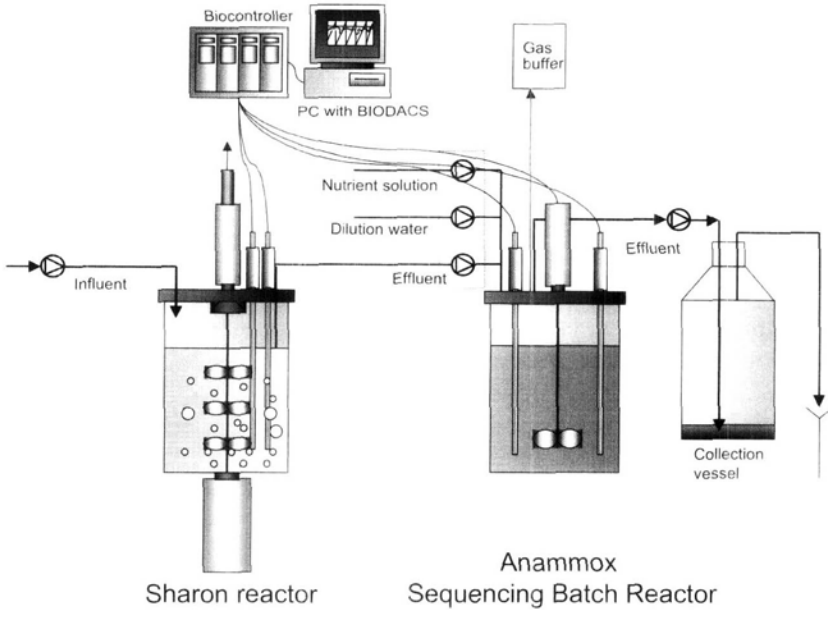


Figure 3.2. Schematic representation of the enrichment set-up where the Anammox was enriched using effluent from the Sharon reactor as feedstuff.

3.2.2 Process conditions

3.2.2.1 Enrichment of Anammox biomass with synthetic wastewater

During start-up the reactors were fed with synthetic wastewater (10 mM $NaNO_3$; 1 mM $(NH_4)_2SO_4$ (2 mM NH_4^+); 12.5 mM $KHCO_3$; 0.15 mM KH_2PO_4; 2 mM $CaCl_2 \cdot 7H_2O$; 1.9 mM $MgSO_4 \cdot 7H_2O$; 0.05 mM $FeSO_4$/EDTA; 25 ml solution micro-elements). It was important that the synthetic wastewater contained sufficient nitrate to prevent sulphate reduction. During sulphate reduction, sulphide is released, which is toxic to Anammox bacteria. To promote the growth of Anammox cells, after Anammox activity was observed, the ammonium concentration was increased to 15 mM while the nitrate decreased to 0 mM. At the same time, increasing amounts of nitrite were added. The synthetic wastewater was provided to the reactors with a flow of 4 L per day. The SBRs ran four cycles per day, where the settling and withdraw phase were set at 6 and 9 minutes respectively.

Regularly samples of 10 mL were taken, frozen and later analysed for dry solids concentration. Every two or three days the ammonium and nitrite content were calorimetrically determined. The anaerobic conditions in the reactors were kept steady by provision of a gas mixture of argon and carbon dioxide (CO_2) (synthetic wastewater does not contain any oxygen-consuming bacteria). This gas mixture also prevents a too rapid increase in pH. The mixing velocity in the two 2L reactors was 150 rpm. After an N conversion rate of 1 kg $N_{tot}/m^3_{reactor}$/day had been reached in both reactors, the number of cycles was lowered to 2 instead of 4, and the flow decreased from 4 to 2 L/day. Also at that time, the concentration of NH_4^+ and NO_2^- were increased from 15 to 30 mM, and thus the total nitrogen loading remained the same in both reactors. The reason for this was that in sludge water, ammonium and nitrite approach concentrations closer to 30 mM than 15 mM.

3.2.2.2 Enrichment of Anammox biomass with effluent from the Sharon reactor

The reactor was fed with effluent from the Sharon reactor even during its start-up phase. This effluent was diluted in such a way that the nitrite concentration was <70 mgN/L. The concentrated nutrient solution was also added (10 mM $NaNO_3$; 12.5 mM $KHCO_3$; 0.15 mM KH_2PO_4; 2 mM $CaCl_2 \cdot 7H_2O$; 1.9 mM $MgSO_4 \cdot 7H_2O$; 0.05 mM $FeSO_4$/EDTA; 1.25 ml/L solution micro-elements). The amount of effluent from the Sharon reactor was gradually increased. The total influent flow during the start-up amounted 10 L/day at 4 cycles per day, whereby the maximal reactor volume was 7 L. Settling and withdraw time were set at 12 and 6 minutes respectively. There was no gas used to keep the Anammox reactor content anaerobic because the nitrifying bacteria from the Sharon reactor may use the O_2 present, thereby ensuring a very low dissolved oxygen (DO). Higher pH values (>8) were corrected with 2 M H_2SO_4.

To begin with, the rotation speed of the stirrer was set at 130 rpm while in the later phase it was observed that the growth of Anammox bacteria was better at 65 rpm because too much turbulence in the reactor appeared to have a negative effect on the growth of Anammox biomass.

3.2.3 Analysis and experiments

3.2.3.1 Analysis

The NH_4^+-N and NO_2^--N concentrations were measured three times a week in the influent and reactors' content. Most weeks, 10 mL samples were taken and frozen in order to determine the dry solids concentration.

3.2.3.2 Activity tests

When the reactors operated in a stable manner, activity tests were performed to estimate the maximal conversion rates. For this type of experiment, the pumps were switched off and 2 mM anaerobically prepared NO_2^--N solution was added. Ammonium was then still present in the reactors because of nitrite limitation. Samples were taken every 5 minutes and analysed on NH_4^+-N, NO_2^--N and NO_3^--N. The dry solids concentration was determined at the same moment in order to estimate the specific conversion rates.

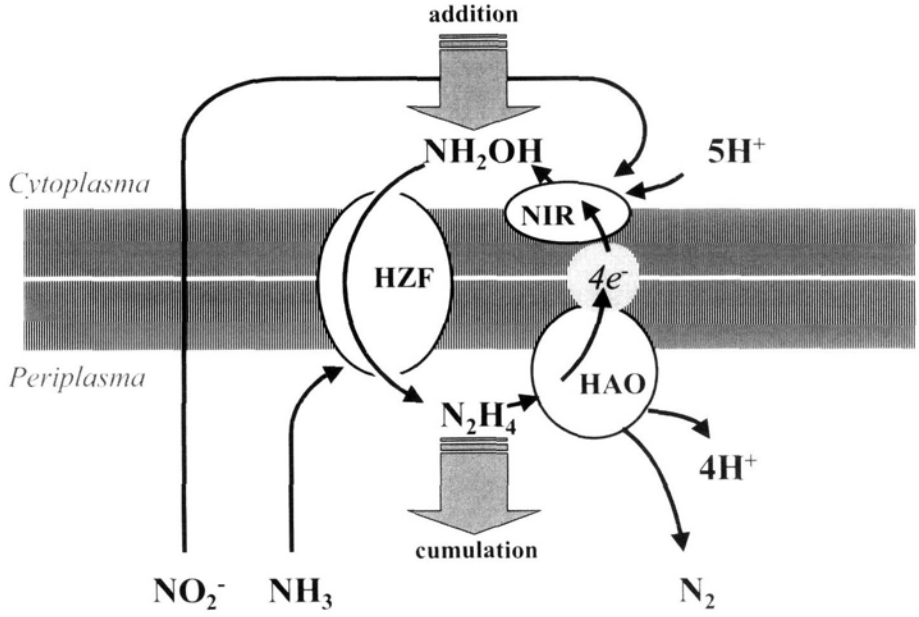
3.2.3.3 Tests with hydroxylamine (NH_2OH)

Hydrazine (N_2H_4) is a unique intermediate in the Anammox process. As far as it is known, this compound is not formed in any other biological process. The production of hydrazine from hydroxylamine in a system is a method to detect the active Anammox biomass. Aerobic ammonium oxidisers convert hydroxylamine to nitrate when sufficient amounts of oxygen are present or to nitric oxide (NO) or nitrous oxide (N_2O) when no oxygen is present. The latter conversion, however, takes place at least 50 times more slowly than in the Anammox process. To detect the active Anammox biomass the following experiment was carried out: when hydroxylamine is provided to the system, the enzyme hydrazinase converts it to hydrazine. Formed hydrazine is oxidised by hydroxyloamine-oxidoreductase (HAO) to nitrogen gas whereby four protons and four electrons are released. When nitrite is present in a system, those four electrons, together with nitrite, are converted to hydroxylamine by the enzyme nitrite reductase. When nitrite is absent from the system (Anammox operates under NO_2^--N limiting conditions) the electrons have to leave the system in another way. This usually happens by hydrazine disproportioning to ammonium and nitrogen gas according to the following reaction:

$$3N_2H_4 \rightarrow 4NH_3 + N_2 \tag{3.2}$$

The disintegration of hydrazine proceeds more slowly than the formation of hydroxylamine, so hydrazine should cumulate in the system. Because hydrazine disintegrates to ammonium and nitrogen gas, an accumulation of the ammonium concentration would be expected. The above is shown in Figure 3.3 as a reaction mechanism.

To perform this experiment, the influent pumps were switched off and a pulse of anaerobically prepared hydroxylamine was dosed to both reactors so that its concentration in both reactors was 1 mM. The samples were taken from the reactors every 5 minutes and analysed for NH_2OH, N_2H_4 and NH_4^+.



HAO = hydroxylamine oxidoreductase; NIR = nitrite reductase; HZF hydrazine forming enzyme

Figure 3.3. The possible conversion mechanism of the Anammox process.

3.2.3.4 Characterisation of sludge with the FISH (Fluorescent In-Situ Hybridisation) technique

Using the FISH technique, it is possible (among other things) to detect at a very early stage whether or not Anammox bacteria are present in the biomass. Furthermore, it is also possible to follow the growth and development of biomass and to visualise it with this technique.

FISH was used to test whether Anammox bacteria in the enrichment reactors were the same as those from reactors tested earlier by researchers from the Technical University of Delft (STOWA 1996b). The FISH analysis was performed using 12 different probes for biomass from the enrichment reactors and the original 'Delft' reactors. When the probe reacted with one Anammox cell but not with another, it may mean that they are different types of Anammox bacteria.

After feeding SBR1 with oxidised sludge water, the state of the nitrifiers brought to the reactor was determined using the FISH technique. The samples were fixated with the influent, the reactor content and the effluent from the Anammox reactor. Based on the amount of nitrifiers in the total biomass from different microscopic sections, it could be concluded whether the nitrifiers are washed out or remain in the reactor.

3.2.3.5 Influence of nitrifiers on Anammox

Because the Sharon process operates without biomass retention, all nitrifying biomass will end up in the Anammox reactor under anaerobic conditions. The influence of ammonium oxidisers on the Anammox process is not known. To determine this relation a decay experiment was performed. The nitrifying sludge was washed and incubated with the cells free of Anammox effluent. The bacteria mixture was divided into 20 jars and brought to

anaerobic conditions. These jars were kept for 10 weeks at a temperature of 32°C. Every two weeks the aerobic activity and the number of living and dead cells were determined. Aerobic activity was determined using the BOM meter. The number of living/dead cells was determined using a microscopic calorimetric method. From activity decline and/or reduction in the number of living cells, a decay curve can be plotted and the rate of decay can be estimated.

3.3 COMBINED SHARON/ANAMMOX PROCESS

During the research period two combined Sharon/Anammox processes were operated. The first combined system contained the Anammox biomass grown on synthetic wastewater until the required nitrogen loading was reached. After this the synthetic influent was replaced by effluent from the Sharon reactor.

The second combined system was directly started with diluted effluent from the Sharon reactor. In this case, nutrient- and nitrate solutions were dosed during the enrichment period. After a sufficient amount of the Anammox biomass had been cultivated, the addition of nutrient- and nitrate solutions was gradually reduced. In this way the Anammox reactor was finally fed only with the effluent from the Sharon reactor.

3.3.1 Process conditions

3.3.1.1 Operation of the Anammox reactor first fed with synthetic wastewater and later with effluent from the Sharon reactor

When the Anammox reactor fed with synthetic influent achieved a stable operation (N-loading of 1 kg $N_{tot}/m^3_{reactor}$/day), the oxidised sludge treatment water (effluent from the Sharon reactor) was gradually introduced as influent. The reactor operated with two cycles per day.

3.3.1.2 Operation of the Anammox reactor fed with diluted effluent from the Sharon reactor

After the required loading was reached the number of cycles was reduced from 4 to 2 per day. The stirring velocity was increased from 65 to 75 rpm. The settling and withdraw phases were set at 12 and 6 minutes respectively. During settling and the withdraw phase the effluent pump of the Sharon reactor was switched off.

Values for pH above 8 were corrected using an acid solution. No gas was used to keep the reactor's content anaerobic. During the operation of the combined system, effluent from the Sharon reactor was fed directly into the Anammox reactor. The diluted water and nutrient solution was not added to the effluent from the Sharon reactor of the combined system.

3.3.2 Analysis

During the operation of the two combined systems, levels of NO_2-N and NH_4-N were regularly determined, and the dry solids concentration in the Anammox reactor was also controlled. The maximal/(over) capacity of the 10 L reactor was determined by a pulse addition of nitrite. The maximal conversion rate was then compared with the conversion rate at normal process conditions.

4

Results and discussion

This chapter describes and discusses the results of various experiments, including the results of the Sharon process, the start-up of the Anammox reactors, the combined Sharon/Anammox process and various process parameters. Section 4.5 contains a short evaluation of all results obtained.

4.1 THE SHARON PROCESS

The Sharon reactor operated, for the purposes of this study, for over 1.5 years. Different parameters, such as conversion rates at different pH conditions, substrate and oxygen affinity constants, bicarbonate content and sludge characteristics were determined. The presence of protozoa in the Sharon reactor was also evaluated. Various strategies were undertaken to inhibit this development of protozoa, and the influence of these strategies on the nitrification process was then tested.

4.1.1 Conversions in the Sharon process

The majority of the research on the Sharon reactor took place in the first eight months after its start-up. During this period, the Sharon reactor was continuously aerated at a temperature of 35°C and a hydraulic retention time (HRT) of 1 day. The results from start-up and stable

operation are shown in Figure 4.1. In this figure six experimental periods are shown and these will be further discussed in this section.

In period 1 the reactor was started up using nitrifying sludge that originated from the B-step of the Dokhaven wastewater treatment plant (WWTP) in Rotterdam, the Netherlands. After 12 days, the nitrification was well established and the reactor had begun to operate in a stable manner (period 2). This stable operation lasted until day 45. Then pH experiments were carried out in the Sharon reactor (period 3), and these are further described in section 4.1.2. An increased ammonium conversion was observed in this period. After the pH experiments had finished, the reactor again reached a period of stable operation (period 4). In period 5 pH experiments were again carried out and from period 6 the Sharon reactor again operated in a stable way.

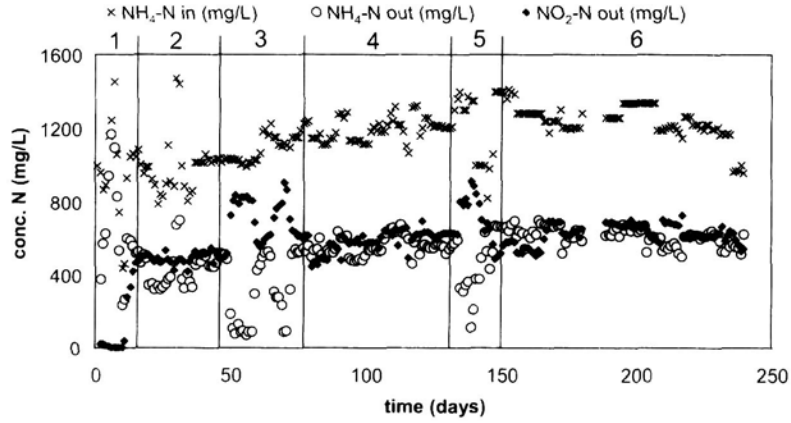


Figure 4.1. N-conversion in the Sharon reactor with continuous aeration. HRT = 1 day, T = 35°C. Periods were as follows: 1 = start-up of the Sharon process, 2 = stable operation 1, 3 = pH experiments, 4 = stable operation 2, 5 = pH experiments and 6 = stable operation 3.

The average nitrogen conversions in the three stable operation periods (periods 2, 4 and 6) are reported in Table 4.1. In the right-hand column the average conversion rates over the whole experimental period are given.

Table 4.1. Overview of the nitrogen conversions in the Sharon reactor during stable operation and over the whole experimental period

Parameter	Stable periods	Whole experimental period	Unit
NH_4-N influent	1.176 (±0.138)	1.168 (±0.247)	kg/m^3
NO_2-N influent	0.005 (±0.016)	0.007 (±0.018)	kg/m^3
NH_4-N effluent	0.548 (±0.101)	0.598 (±0.183)	kg/m^3
NO_2-N effluent	0.598 (±0.830)	0.547 (±0.183)	kg/m^3
pH	6.75 (±0.3)	6.83 (±1.2)	
NH_4-N conversion	53	49	%
NO_2-N : NH_4-N (ideal 1.3)	1.09	0.91	
N-loading	1.17 (±0.2)	1.05 (±0.3)	kg N_{tot}/m^3/day
N-conversion	0.63 (±0.1)	0.52 (±0.2)	kg N_{tot}/m^3/day

The differences between values of the three stable operation periods and the whole experimental period were caused by various experiments performed in between the stable periods, process disturbances and different measures for protozoa suppression.

During normal operation the conversion rates as given in the column 'stable periods' were achieved. The Sharon reactor seems to be an appropriate reactor configuration to convert 50% of the incoming ammonium load from sludge water into nitrite.

4.1.2 pH and conversion rates

The conversion rate of ammonium is strongly pH-dependent. That is why this research studied the rate at which ammonium is oxidised at different pH values.

The conversion rates in relation to the various pH values are given in Table 4.2. The results from Table 4.2 are also plotted in Figure 4.2.

Table 4.2. Different conversion rates at various pH values

pH	NH_4-N out (mg/l)	NH_3-N out (mg/l)	NO_2-N out (mg/l)	NO_2-N : NH4-N
6.8	431	3.1	493	1.1
7	277	3.1	764	2.8
7.1	90	1.3	886	9.8
7.3	84	1.9	811	9.7
7.5	184	6.4	725	3.9
7.8	71	4.7	840	11.83

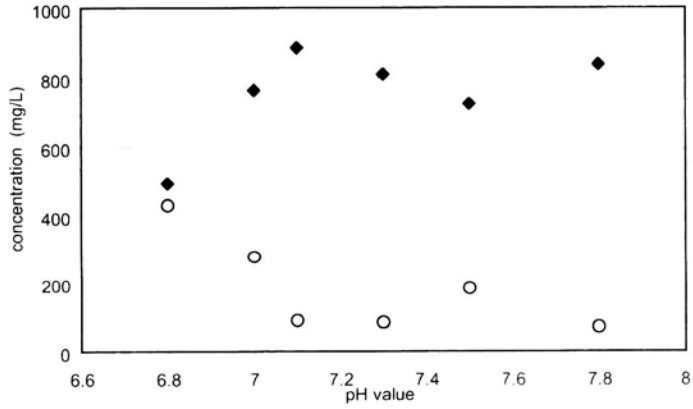


Figure 4.2. Ammonium conversion of sludge water at various pH values. ○ = NH_4-N out (mg/L); ◆ = NO_2-N out (mg/L).

No tests were performed at a pH lower than 6.8. These 'acid' conditions are not beneficial for nitrifiers, and the likelihood of a wash-out occurring under such conditions is high.

As can be seen from Figure 4.2, more NO_2 is formed at a higher pH. This result was expected because the fraction of NH_3, the actual substrate for ammonium oxidisers, increases with pH, which is shown in Figure 4.3.

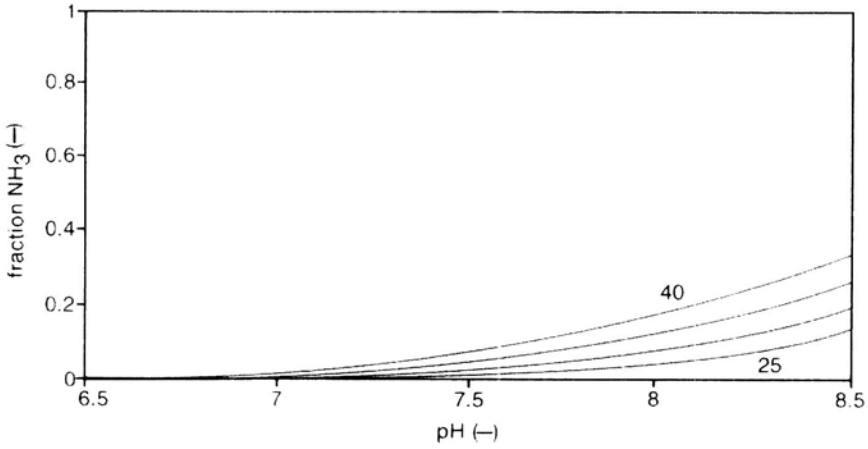


Figure 4.3. Fractions of NH_3 at different pH and temperature.

As can be seen from Table 4.2, the differences between NH_3 concentrations are very small. The relatively constant NH_3 concentrations can be explained as follows:

For a chemostat (a completely mixed, continuously fed reactor without any form of biomass retention), the following equation holds:

$$D = \mu = \mu_{max} \cdot \frac{C_{NH3}}{C_{NH3} + K_{NH3}} \tag{4.1}$$

The retention time does not change, thus μ remains constant. μ_{max} and K_{NH3} are not pH-dependent, thus C_{NH3} is also pH-independent. This means that if the ratio NH_4/NH_3 decreases because of a higher pH, the effluent ($NH_4 + NH_3$) concentration will be lower.

4.1.3 Maximum conversion rates and affinity constants

The most important kinetic parameters of the nitrifying biomass, affinity- or saturation constants and maximum conversion rate, were estimated for ammonium and oxygen. The affinity constant is a concentration at which half of the maximum conversion rate is reached.

This section presents the results of the estimations of ammonia (+ ammonium) and oxygen affinity constants. A number of experiments were also performed to determine if nitrite has an inhibiting effect on the conversion rate of ammonium.

4.1.3.1 Ammonium affinity constants

The affinity constants and maximal specific conversion rates of nitrifying biomass were estimated on days 26, 27, 39 and 40. During this period the pH in the reactor was not corrected. Using the stoichiometry from Equation (2.1) from section 2.1 (1.5 mole O_2 per mole NH_4^+) the conversion rate of ammonium was calculated.

The number of nitrifiers in the reactor was estimated using the yield coefficient (Y^{amm}_{NX}) of ammonium oxidisers. This value is 0.064 C-mole/N-mole (Hellinga *et al.* 1998). The amount of converted N is the amount of converted nitrogen per litre reactor volume.

The amount of moles C of nitrifying biomass were calculated using the general molecular formula for biomass $CH_2O_{0.5}N_{0.2}$ (molecular mass = 24.8 g/mole). There were 62.4 mg/L nitrifiers in the reactor.

The maximal conversion rates and affinity constants estimated for ammonium are given in Table 4.3.

Table 4.3. Estimated affinity constants for ammonium and the maximum specific conversion rates

Day (N^0)	$K_{NH3,4}$ (mg N/L)	V_{max} (kg N/kg DS/day)	V_{max} : $K_{NH3,4}$
25	18.9 (±7.8)	5.2 (±0.9)	0.28
26	23.3 (±2.2)	6.9 (±0.2)	0.30
39	36.7 (±3.3)	6.3 (±0.3)	0.17
40	26.0 (±2.9)	6.5 (±0.2)	0.25

The conversion rate in the reactor was 8.81 kgN/kg DS/day. This value is significantly higher than measured by the respirometer. It is possible that the respiration measurement was negatively influenced by the preparation procedure.

4.1.3.2 Nitrite influence

In the Sharon reactor ammonium is oxidised in the presence of high nitrite concentrations. That is why the possible negative influence of high nitrite concentrations on the conversion of ammonium was estimated. To attain this, known amounts of nitrite were brought to the reaction vessel before different ammonium doses were added. After that the oxygen consumption rate was measured. This value is diminished by the consumption rate measured at the moment when both ammonium and nitrite were present in the reaction vessel.

The oxygen consumption rate of ammonium oxidisers with nitrite as the only substrate was very low. In some cases the oxygen consumption rate after nitrite was dosed was even lower than the endogenous respiration rate. This indicates that nitrite has an inhibiting effect on the ammonium oxidisers. The pH in the reaction vessel during the experiment was 6.8.

Table 4.4 gives the different affinity constants and maximum conversion rates for ammonium at different nitrite concentrations.

Table 4.4. Measured maximum conversion rates and affinity constants for ammonium

NO_2-N concentration (g/L)	V_{max} (kg N/kg DS/day)	K_{NH4} (mg/L)	V_{max} : K_{NH4} (L/kg·DS·day)
0.0	6.2 (±0.4)	26 (±4.1)	0.25
0.15	4.1 (±0.3)	24 (±4.3)	0.18
0.18	4.0 (±0.4)	25 (±7.2)	0.16
0.30	5.4 (±0.3)	54 (±6.9)	0.10
0.46	5.4 (±0.53)	62 (±14.1)	0.09

From the results in Table 4.4, it can be concluded that a nitrite concentration of over 300 mg/L results in a lower affinity to ammonium (higher affinity constant). In other words, in the presence of nitrite bacteria are less capable of converting lower concentrations of ammonium than when only ammonium is present. In the right-hand column of Table 4.4 it can be seen that the ratio V_{max}:K_{NH4} decreases with increasing amounts of nitrite in the reaction vessel. From this it can be concluded that in the presence of nitrite a decreased conversion rate for ammonium will be observed.

Based on the fact that the oxygen consumption rate, measured when only nitrite was dosed, either does not differ or hardly differ from the endogenous respiration rate, it can be concluded that there were few or no nitrite oxidisers in the Sharon reactor.

4.1.4 Bicarbonate content

For a few weeks at the start of the research, the inorganic carbon content (originating from bicarbonate) was measured in the influent and effluent every week. In this way it could be estimated how much bicarbonate was utilised by the reactor.

Table 4.5 gives various measured bicarbonate concentrations of the influent and effluent.

Table 4.5. Bicarbonate concentrations in the influent and effluent (after each horizontal line the system was fed with a new batch sludge water)

Week	HCO_3^- influent (mmol/L)	HCO_3^- effluent (mmol/L)	HCO_3^- utilised (mmol/L)	NH_4^+ conversion (mmol/L)	HCO_3^- : NH_4^+
4	54	4	50	29	1.7
5	76	5	71	29	2.5
6	80	3	77	39	1.9
7	65	12	53	66	*0.8*
8	59	3	56	44	*1.3*
9	93	6	87	74	*1.2*
10	88	5	83	48	1.7
11	70	6	64	45	1.4
12	88	6	82	47	1.8

From the above table it can be seen that, per mole of ammonium converted, less than 2 mole of bicarbonate is utilised. The acid equivalents released during the oxidation of ammonium are buffered with bicarbonate according to Equation (3.1) (see section 3.1.3).

When bicarbonate is stripped from wastewater before the reaction starts, the pH increases. Because of this, the same amount of ammonium can be still converted. It does not matter whether bicarbonate is stripped before or after the reaction $NH_4^+ \rightarrow NO_2^-$. Lower concentrations of bicarbonate will be measured; the buffer capacity remains the same but the pH increases.

Values written in italics were measured in periods when the reactor was subject to increased pH values by base addition. A higher conversion of ammonium took place, by which more ammonium was converted per mole of bicarbonate. It can also be seen that in weeks 7 and 8 the conversion of bicarbonate decreased. When the pH is increased artificially, the solubility of CO_2 (thus also bicarbonate) is higher.

From Table 4.5 one can also see that the longer a batch influent is stored, the less bicarbonate is present in the influent. It is likely that CO_2 diffuses from the wastewater because of contact with open air. To minimise this stripping effect, the content of the influent vessel was not stirred through most of the research.

4.1.5 Sludge characterisation

To determine whether the nitritification was carried out by *Nitrosomonas species*, FISH analysis (see section 2.1.3) was performed with sludge from the Sharon reactor operating in steady state. For this the sludge was labelled with two specific probes; a Nso190 Fluos label for self-tolerant nitrifiers and a aN11Cy£ label for *Nitrosomonas* or *Nitrosococcus*.

From the FISH analysis it turned out that the labelled floc consists mainly of *Nitrosomonas* and/or *Nitrosococcus*. The results of research (Logermann *et al.* 1998) where *Nitrosomonas eutrophea* was found to be the dominant nitrifier in the Sharon reactor is then confirmed.

4.1.6 Influence of anaerobic conditions on ammonium oxidisers

Because of the growth of protozoa in the Sharon reactor in the later phase of the research, it was necessary to periodically switch from continuous to intermittent aeration. The question arose whether the anaerobic periods could have a negative effect on the nitritification process. To answer this, the nitritification capacity reduction was measured for the full-scale Sharon reactor of the Sluisjesdijk sludge treatment plant in Rotterdam when this was temporarily out of operation. During this period, the concentrations of nitrite and ammonium were 100 and 0 mgN/L respectively. On three different days (days 0, 3 and 6), the nitritification capacity of the reactor mixture (35°C) and the content of the separate cold vessel with the same reactor contents (ca. 3–10°C) was measured. The results of these experiments are given in Table 4.6.

Table 4.6. Decline of nitritification capacity in the content of the Sharon reactor (at 35°C) and in the non-heated vessel (at 3–10°C).

Day	NO_2-N formation (mg/L/min)		NH_4-N decline (mg/L/min)	
	Reactor	Vessel	Reactor	Vessel
0	0.75	0.75	0.72	0.72
3	0.39	0.66	0.40	1.08
6	0.15	0.88	Data not available	0.57

During these experiments, the dissolved oxygen concentration was maintained at a minimum of 20% of saturation. The temperature was 35°C and the pH at the start of the tests was ca. 8.5. For the of the test, the pH was not corrected.

As can be seen from Table 4.6, the nitritification capacity declines when the reactor is kept in anaerobic conditions. Cooling of the reactor content better preserves the original nitrification capacity. The possible inhibition of nitritification by ammonium under anaerobic conditions could not be verified based on this test.

4.1.7 Protozoa

The Sharon reactor was started up in the autumn of 1997 and operated in a stable manner for 9 months. After these 9 months a higher pH and reduced nitrification capacity were observed in the reactor. The presence of protozoa in the reactor turned out to be one reason for this. These protozoa consumed the free-swimming ammonium oxidisers.

Figure 4.4 shows a number of the protozoa found in the Sharon reactor.

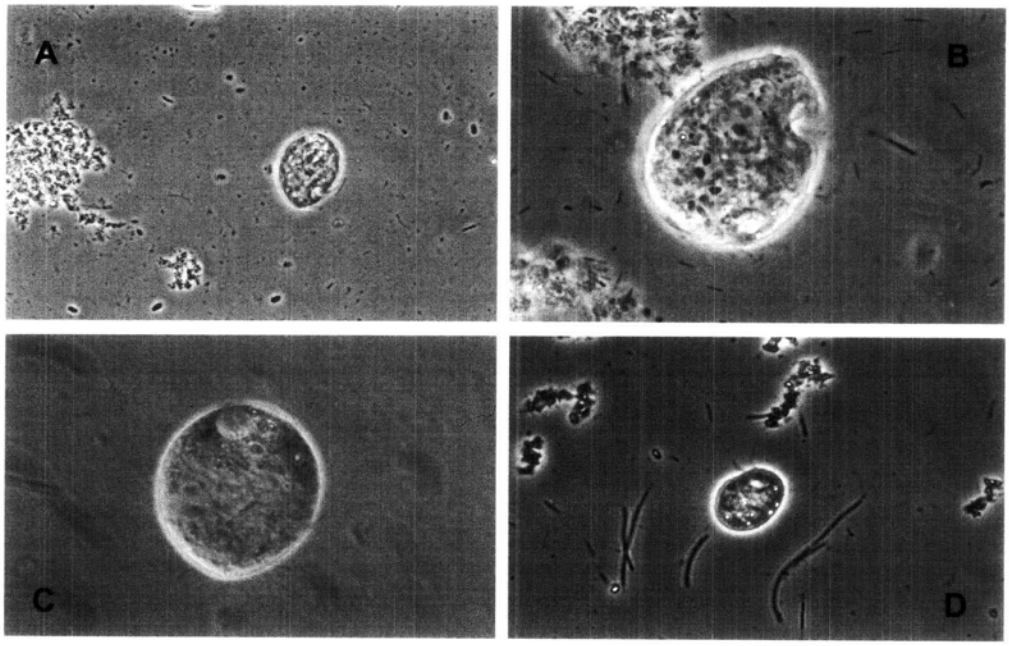


Figure 4.4. Examples of protozoa found in the Sharon reactor.

Since this problem occurred in a number of batches, it was assumed that the protozoa were present in the sludge water and entered the system in this way. It is possible that protozoa, grown in the A- or B-step of the AB system of the Dokhaven WWTP in Rotterdam, survived the digestion process in the form of cysts. In the research carried out by the Technical University of Delft (STOWA 1996a), the presence of protozoa was not confirmed. There were no operational problems relating to the presence of protozoa in the full-scale Sharon reactor operating at the Sluisjesdijk sludge treatment plant.

It is possible that the growth of protozoa in the Sharon reactor can be attributed to a small-scale type of research. For the purposes of this research 300 L of sludge water was brought each month. The batches were stored at room temperature. It is not known precisely what happens in a vessel that contains sludge water, and whether any living organisms can develop under such conditions. The ammonium- and nitrite concentrations remain constant under these conditions.

Two important differences between the 'conventional' Sharon process and the Sharon reactors discussed here are the ways in which they aerate, and the related pH fluctuations. 'Conventional' Sharon reactors are always intermittently aerated, leading to variations in DO and pH (which can be between 6.8 and 7.8). It is possible that protozoa do not grow under anaerobic conditions and/or pH fluctuations.

The origin and the growth pattern of protozoa are being further investigated: these experiments and results are presented in Appendix A. The most important conclusion is that protozoa enter the Sharon reactor in the form of cysts and under aerobic conditions germinate to protozoa, where they start to predate. The growth rate of protozoa estimated with batch tests was 1.62 day^{-1}.

There are different strategies for preventing protozoa growth in the reactor. These are presented in the following sections.

4.1.7.1 Influent pasteurisation

By placing a heating element in the influent tube, the influent was rapidly heated up to 80°C. By this treatment, the sludge water is pasteurised, and protozoa should be killed.

However, there was no reduction in protozoa in the reactor observed during the period when the influent was heated. It is difficult to believe that protozoa survive heating. Protozoa in the form of cysts, however, can survive such temperatures. These cysts will germinate under aerobic conditions.

4.1.7.2 Shorter HRT

There was also an attempt made to wash out the protozoa by shortening the retention time. Although the retention time was decreased to 0.8 day the protozoa remained in the reactor. Further shortening of HRT was not tested because the ammonium oxidisers could be washed out. At an HRT of 0.8 day and a pH of 7.7, the conversion of ammonium only amounted to 30%. The minimal HRT needed to wash out the protozoa is around 0.6 day. This is far too short a period to maintain the ammonium oxidisers in the reactor.

4.1.7.3 Incidental periods without aeration

An attempt was made to 'suffocate' protozoa by switching off aeration for a few hours. It could be seen under a microscope that after some time the protozoa changed (their cells lysed; see Figure 4.5). Perhaps this was caused by the lack of oxygen, so that the protozoa had too little energy to keep their salt content at the required level. Because of the osmotic pressure the protozoa burst.

Observing the cilia of protozoa, one can see when the oxygen content decreases and the osmotic pressure begins to rise. The movement of these cilia will terminate upon oxygen depletion. The time difference between the first and second photographs in Figure 4.6 being taken is three minutes while between the second and third photograph it was only one minute. At t = 0, their cilia just stopped moving.

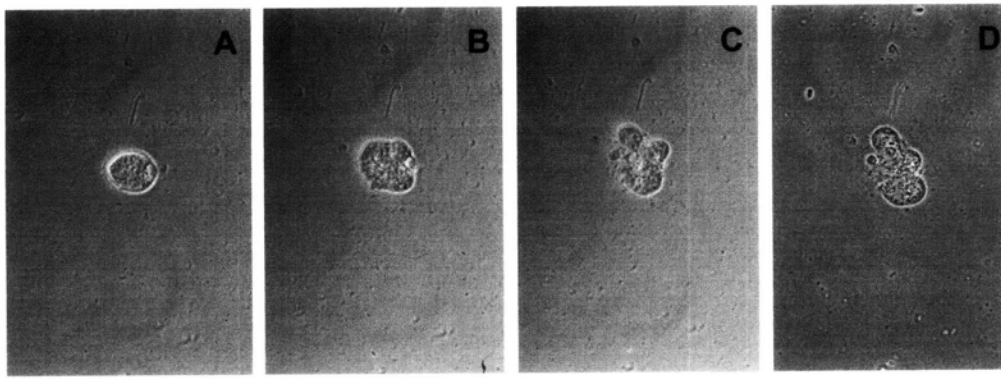


Figure 4.5. A protozoa bursting open under anoxic conditions (A: t = 0, B: t = 3 minutes, C: t = 4 minutes and D is photograph C under different lighting).

After an anoxic period (which varied from 0.5 to 6 hours) no protozoa were observed in the reactor. When the mixture was again aerated for a longer period (maximum 1 day) the protozoa returned. It seems that an incidental anoxic period is not sufficient to prevent protozoa growth over a longer period of time.

4.1.7.4 Intermittent aeration of the Sharon reactor

Protozoa were not found in nitrifying and denitrifying Sharon reactors. An essential difference between these 'conventional' reactors and the Sharon reactors from this research is the way they aerate the sludge. In the conventional system, the content is aerated intermittently, so the system is regularly subjected to anoxic conditions. Because of this, the cysts have no chance to germinate, or the decay rate of protozoa during this phase is too high.

To get rid of protozoa, the reactor was intermittently aerated for a longer period. This prolonged period of intermittent aeration turned out to be an effective method of preventing protozoa development. In the following/subsequent summer, however, protozoa were again observed in the Sharon reactor and stayed alive despite the strategy of intermittent aeration.

4.1.7.5 Acidification

Intermittent aeration of the Sharon reactor caused a fluctuation in pH during its normal operation. When the influent is temporarily shut off, the reactor content was acidified to a pH <6. Due to this, protozoa either did not germinate or they died. One possible reason for protozoa die-off at a lower pH is the shift in the equilibrium between NO_2^- and toxic HNO_2.

4.1.7.6 Summary

Protozoa enter the Sharon reactor in the form of cysts, which can germinate under aerobic conditions. The germination of protozoa can be prevented by intermittent aeration of the reactor content and/or by its acidification by longer aeration without influent flow. A combination of both strategies would lead to the best result. In practice both conditions may occur regularly because the influent of the sludge water does not have a constant flow.

There is a chance of reduced nitrite formation in the reactor caused by protozoa predation. This can easily be confirmed by examining a sample using a simple microscope.

It is possible that the growth of protozoa in the Sharon reactor is seasonal and that cysts are only present in the sludge water in the summer. The Sharon reactor was started up in autumn and it was continuously aerated. It operated for a period of approximately nine months, after which a reduced nitritification was measured as a result of protozoa predation. This was at the end of summer, around September. Whether the presence of protozoa is indeed seasonal can only be verified when a Sharon reactor has been operated for a longer period of time, preferably a few years. In present full-scale applications (including the site of sludge water collection in Rotterdam) no problems with protozoa have been encountered.

4.2 START-UP OF THE ANAMMOX REACTORS

Three Anammox reactors inoculated with activated sludge were started up for this research. In the first instance, two reactors were started up, where synthetic influent was used. This was to determine whether it was possible to enrich a robust Anammox biomass from activated sludge. As well as activated sludge, an additional 20 µL of enriched Anammox biomass was added to one of the two reactors as a control for the enrichment method.

When it turned out that the enrichment method worked well, the third, larger-scale Anammox reactor was fed with (diluted) effluent from the Sharon reactor and started up. The results from the start-up phase of these three reactors are described in this section.

4.2.1 Enrichment of Anammox biomass with synthetic wastewater

To enrich the Anammox biomass, two 2 L enrichment reactors were started up. Both reactors were inoculated with B-step activated sludge from the Dokhaven WWTP in Rotterdam. One of the reactors (SBR2) was additionally enriched with 20 µL Anammox inoculum and acted as a control of the enrichment method.

Shortly after the start-up phase, gas production was observed in both reactors. This may have been N_2 formed by denitrification with dead biomass as C-source. In first instance the settling and discharge phases were set at 9 minutes. Two weeks after start-up, it appeared that 6 minutes was sufficient to ensure good settling.

Samples were taken weekly from reactors 1 and 2 to determine the dry solids concentration. The amount of biomass in the reactors during the start-up phase decreased gradually. This reduction was mainly caused by wash-out of bad settling particles and partially by (heterotrophic) denitrification with dead organisms as C-source. On day 58 a large amount of biomass was sent back from the collection vessel (Figure 3.2) to SBR1 because the dry solids concentration was <0.5 g/L (experience has shown that 1 g/L constitutes a critical lower value for the Anammox process). After approximately 100 days the amount of biomass again increased, due to the growth of Anammox organisms.

SBR1 showed Anammox activity later than SBR2. This was not due to the addition of Anammox inoculum to SBR2, but because of a significant wash-out of biomass.

Figure 4.6 shows the conversions of ammonium and nitrite. After day 50 it can be seen that the nitrogen loading increased exponentially.

From day 110 both reactors operated with a nitrogen loading of approximately 1 $kgN_{tot}/m^3_{reactor}/day$. In this period it was switched from 4 to 2 cycles per day. The influent flows were halved and the nitrite and ammonium concentrations doubled (420 $mgN_{tot}/L \rightarrow$ 840 mgN_{tot}/L), so the nitrogen loading remained the same.

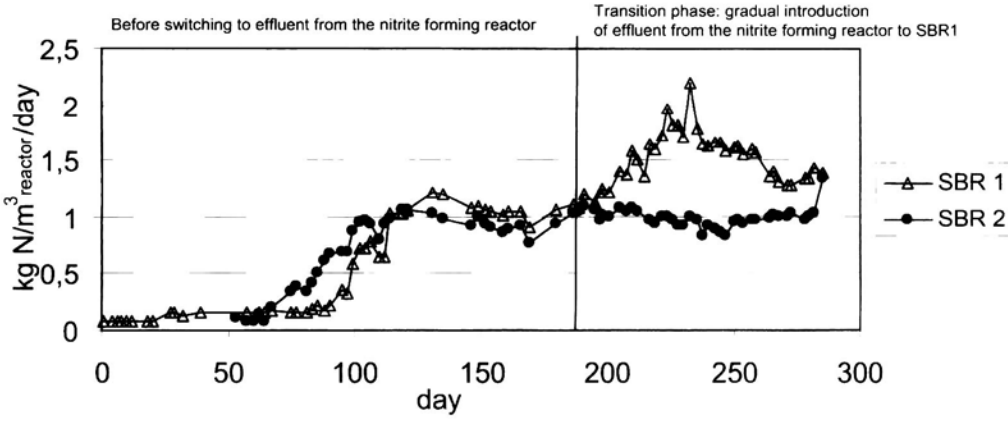


Figure 4.6. Anammox activity in SBR1 (without Anammox inoculum) and SBR2 (with Anammox inoculum).

4.2.2 The Anammox reactor during stable operation

Between days 50 and 110, the maximum growth rate (μ_{max}) of the Anammox organisms was estimated. In SBR1 this amounted $5.9 \cdot 10^{-2}$ ($\pm 0.8 \times 10^{-2}$) day^{-1}. The doubling time ($t_{1/2}$) was thus $\ln 2 / 5.9 \times 10^{-2} = 11.7$ days. In SBR2, μ_{max} was $4.2 \cdot 10^{-2}$ ($\pm 0.3 \times 10^{-2}$) day^{-1}, and the doubling time was 16.5 days. These values are comparable with a doubling time of 11 days found elsewhere (Strous *et al.* 1998).

The most important operational parameters of SBR1 and SBR2 are given in Table 4.7.

Table 4.7. Ammonium- and nitrite removal in SBR1 and SBR2 during periods of stable operation

Parameter	SBR1	SBR2
Test period (day)	155–190	155–190
NH_4^+-N in (kg × m^{-3})	0.45 (±0.02)	0.46 (±0.02)
NH_4^+-N out (kg × m^{-3})	0.068 (±0.02)	0.091 (±0.03)
NO_2^--N in (kg × m^{-3})	0.46 (±0.03)	0.44 (±0.04)
NO_2^--N out (kg × m^{-3})	1.25×10^{-4} ($\pm 3.5 \times 10^{-4}$)	8.75×10^{-4} ($\pm 3.5 \times 10^{-4}$)
NH_4^+-N removal (%)	85	80
NO_2^--N removal (%)	100	100
Total N removal at nitrite limitation (kg $N_{tot}/m^3_{reactor}$/day)	0.96 (±0.07)	0.91 (±0.09)
Average specific N_{tot} removal at nitrite limitation (kg N_{tot}/kg DS/day)	0.70 (±0.07)	0.70 (±0.06)

After day 90, the nitrogen loading in SBR1 was further increased (to 165 kg $N_{tot}/m^3_{reactor}$/day). This was done because this reactor was switched to effluent from the Sharon reactor where ammonium and nitrite concentrations were higher than those in the synthetic wastewater. After this, the synthetic influent was gradually replaced by effluent from the Sharon reactor. From day 260 onwards, SBR1 was fed only with effluent from the Sharon reactor.

The Anammox reactor treated the effluent of the Sharon reactor successfully for a period of 145 days. Levels of Anammox bacteria seemed not to change when the gradual switch-over was made from synthetic wastewater to effluent from the Sharon reactor.

4.3 COMBINED SHARON/ANAMMOX

A combined Sharon-/Anammox system was started in 10 L scale after the Anammox biomass had been enriched from activated sludge using synthetic influent. The Anammox biomass of the combined system was further enriched with effluent from the Sharon reactor.

4.3.1 Enrichment of Anammox biomass with effluent from the Sharon reactor

The reactor was inoculated with activated sludge mixture from the Boskoop and Reeuwijk Randenburg WWTPs (respectively, because of partial nitrification and a long sludge age). Further, 100 µL of enhanced Anammox biomass from SBR1 was added to the sludge mixture. The amounts of ammonium and nitrite (N_{tot}) converted in the reactor during the research period are shown in Figure 4.7.

In the beginning gas production was observed, probably N_2 gas formed by denitrification. Because until day 105 no Anammox activity had been measured, the stirring speed was reduced from 130 to 75 rpm. Shortly afterwards, Anammox cells were detected in the reactor

using the FISH technique. Starting from this time, a slight increase in the rate of nitrogen conversion can be seen in Figure 4.7.

A clear exponential growth did however not take place in the reactor system because the sludge retention was insufficient. Around day 150, the biomass concentration decreased to the critical value of 1 g/L. Samples of the reactor content and wall growth were taken for FISH analysis performance. This FISH analysis indicated that more Anammox clusters were present in the biomass attached to the wall than in the suspended aggregates. Anammox clusters seem to prefer growing on the surface (on a carrier).

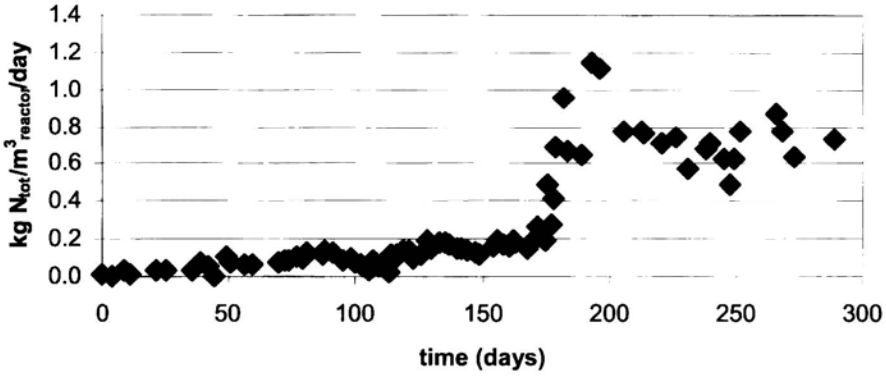


Figure 4.7. Nitrogen conversion in the enrichment reactor fed with effluent from the Sharon reactor. Around day 110, FISH analysis of the Anammox cells in the reactor was carried out.

On day 175, samples of the reactor content, effluent and the sediments in the collection vessel were taken in order to carry out FISH analysis. These three samples were controlled for the presence of Anammox cells. From this FISH analysis, it turned out that the Anammox cells were present in the reactor as well as in the effluent and in the sediments of the collection vessel. All biomass formed in the reactor was thus washed out and remained in the collection tank forming clusters.

Because the Anammox biomass formed in the reactor was discharged during the withdraw phase, the biomass from the collection vessel was returned to the reactor, where a strong increase in the nitrogen conversion was observed shortly afterwards.

4.3.2 Conversion(s) in a combined Sharon/Anammox system

From day 179, the 10 L Anammox reactor was fed with undiluted effluent from the Sharon reactor and from this day it operated in a stable manner for over 100 days. The conversion data of this combined 2 × 10 L Sharon/Anammox system are reported in Table 4.8. The results of the second combined Sharon/Anammox system are also given in this table. The second system was the 2 L Anammox reactor where the synthetic wastewater was gradually replaced by effluent from the Sharon reactor.

It can be seen from Table 4.8 that the ratio between converted ammonium and converted nitrite almost equals 1:1. The Anammox process, however, used nitrite and ammonium in a 1.3:1 ratio; theoretically 0.25 mole nitrate is formed per mole ammonium. In both reactors there was thus more ammonium converted than theoretically was possible according to the Anammox reaction. This can be explained by heterotrophic denitrification of formed nitrate

to nitrite. The nitrite that is released here could again be converted to ammonium in the Anammox process. Due to this, less nitrite had to be formed in the Sharon process.

When no denitrification took place, (0.36:1.25) × 0.25 = 0.072 kg NO_3-N/m^3 should be found in the reactor system. However, only 0.012 kg NO_3-N/m^3 was found (this was semi-quantitatively determined with test strips). There was thus 0.072 – 0.012 = 0.06 kg NO_3-N/m^3 denitrified to NO_2-N. 0.06 kg NO_2-N/m^3 reacts with 0.048 kg NH_4-N/m^3. The ratio ammonium and nitrite is then (0.36 + 0.06)/0.35 = 1.2. This number comes close to the well-known NO_2-N:NH_4-N ratio of 1.3.

Table 4.8. Ammonium- and nitrite removal in the Anammox of the two combined Sharon/Anammox systems during stable operation

Parameter	10 L Anammox	2 L Anammox
Test period (day)	179–289	234–400
NH_4-N removal (kg × m^{-3})	0.35 (±0.08)	0.38 (±0.2)
NO_2 N removal (kg × m^{-3})	0.36 (±0.01)	0.40 (±0.2)
NO_2-N : NH_4-N	1.03	1.05
NO_2-N removal (%)	100	100
Total N removal (kg $N_{tot}/m^3_{reactor}$/day)	0.75 (±0.2)	0.97 (±0.5)
Average specific N_{tot} removal at nitrite limitation (kg N_{tot}/kg DS/day)	0.18 (±0.03)	0.33 (± 0.2)
Maximal specific N conversion rate (kg N_{tot}/kg d.s./dag)	0.82	0.52

4.4 CHARACTERISTICS OF THE ANAMMOX SYSTEM

In this section, various process parameters of Anammox are discussed which were determined during this research.

4.4.1 Maximal activity

The maximal specific conversion rates were determined for the three Anammox reactors. Both 2 L reactors were fed with synthetic wastewater. The 10 L Anammox SBR was fed with effluent from the Sharon reactor.

The activity tests in SBR1 and SBR2 (two 2 L SBRs) were performed on the 125th day of the test period; on day 235 the maximal activity of SBR3 (a 10 L SBR) was estimated. After the influent pumps had been switched off, 2 mM NO_2^- was added to the reactors. The results of these activity tests are shown in Figure 4.8.

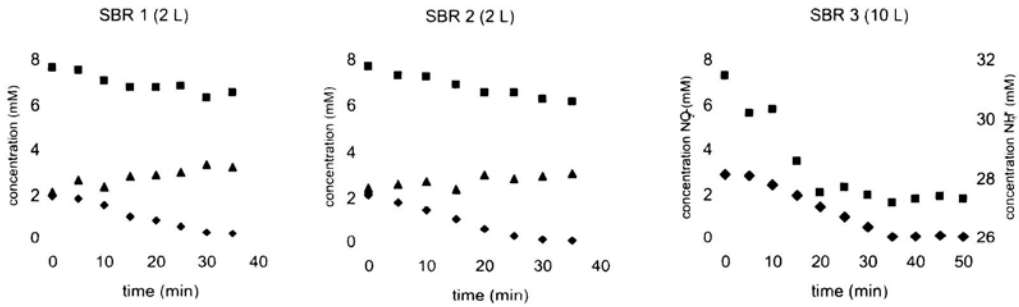


Figure 4.8. Activity tests in two 2 L SBRs and in the 10 L reactor (■ = NH_4^+, ▲ = NO_3^-, ◆ = NO_2^-)

The maximum specific conversion rates were estimated from these graphs, and the results are reported in Table 4.9. The conversion rates measured at the same time in various reactors (actual rates) are also given. Based on both data, the over-capacity (maximum capacity) of the reactors (from this time) was calculated, according to the following equation: $((V_{max}/V_{reactor}) - 1) \times 100\%$.

Table 4.9. Specific activity in the various SBRs

	SBR1 (2 L)	SBR2 (2 L)	SBR3 (10 L)
Test day	125	125	235
V_{max} kg NO_2-N/kg DS/day	0.32	0.94	0.46
V_{max} kg NH_4-N/kg DS/day	0.2	0.62	0.36
V_{max} kg N_{tot}/kg DS/day	0.52	1.56	0.82
N conversion in reactor (kg N_{tot}/kg DS/day)	0.5	1.3	0.17
Over-capacity (%)	4	20	380
Dry solids (gDS/L)	1.75	0.7	3.0

The dry solids concentrations of SBR1 and SBR2 differ significantly. The specific activity measured in reactor 2 is almost 2.5 times higher than in reactor 1. In reactor 1 the same amount of Anammox biomass was measured as in reactor 2; the rest of the dry solids content consists of dead or inert biomass. Furthermore, both reactors converted the same amount of nitrogen per volume reactor content.

The over-capacity, as calculated for the various reactors, is also given in Table 4.9. In SBR1 and SBR2 it was 4 and 20% respectively. In SBR3 it was 380%. These large differences in the over-capacities of 2 L and 10 L reactors can be explained by differences in running time of the test. In the time that the activity was determined in SBR1 and 2, both reactors had operated at the demanded nitrogen loading for a period of approximately one week. The biomass was thus converted at more or less maximum capacity. The maximum capacity of SBR3 was determined on day 235. This was approximately eight weeks after the reactor had operated at the demanded nitrogen loading.

4.4.2 Tests with hydroxylamine

To prove that Anammox bacteria were responsible for nitrogen conversion in the reactors, approximately 10 mgN/L of anaerobically prepared hydroxylamine solution was added to the reactors after the influent pumps had been switched off. The results of these experiments are presented in Figure 4.9.

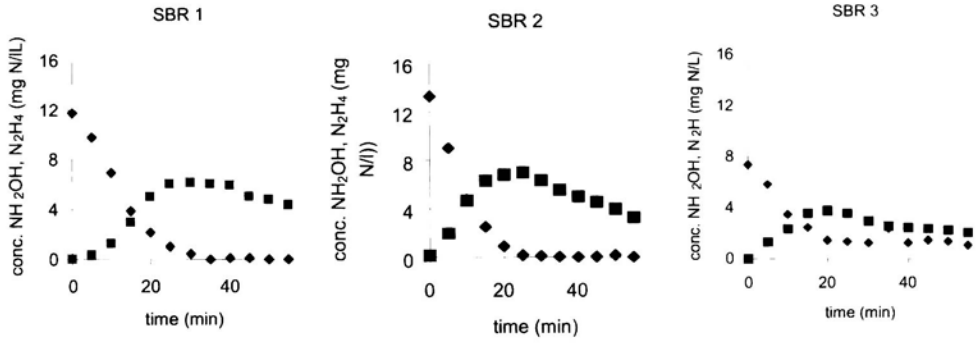


Figure 4.9. Results of hydroxylamine tests in SBR1, 2, and 3 (◆ = NH_2OH, ■ = N_2H_4).

After the addition of a hydroxylamine pulse to the reactors, a slight decrease of the pH was noted. As can be seen from Figure 4.11, the unique Anammox intermediary hydrazine was formed in all the reactors. This means that the nitrogen conversion in the reactors is carried out by anaerobic ammonium oxidisers. Hydroxylamine was converted in SBR1, 2 and 3 at 0.89; 1.69 and 0.67 $kgN_{tot}/m^3_{reactor}/day$ respectively.

A slight increase in the ammonium concentration was observed in SBR1 and 2. This is according to the expectations described in section 3.2.3. In SBR3 the ammonium concentration remained more or less constant. This was because the ammonium concentration was higher in this reactor and a potential slight increase was not easily detectable.

4.4.3 Sludge characterisation using FISH analysis

Ten specific FISH probes have recently been developed at the Technical University of Delft. All these probes were designed and developed based on the DNA structure of the previous Anammox research at this university. If the enhanced Anammox in the various reactors is the same as it was previously, all Anammox cells should be fluorescent with all probes. If the Anammox cells are different, the cells will be fluorescent with one probe but not with the other.

It turned out that the Anammox cells were fluorescent with six of the ten known Anammox probes in the various reactors. No or little signal was found by four of the ten probes. In the control sample all ten probes gave a clear signal.

It seems that in the enriched reactors another type of Anammox was present than in the previous Anammox system.

4.4.4 Nitrifiers in Anammox

Because all ammonium oxidisers formed in the Sharon reactor finally reach the Anammox reactor, their influence on the Anammox process was observed.

This section describes the results of the various decay tests, carried out with nitrifiers. Samples of effluent from the Sharon reactor, the content of the Anammox reactor and effluent from the Anammox reactor were examined using the FISH technique to evaluate the survival of nitrifiers under anaerobic conditions. Furthermore, the aerobic activity of the Anammox biomass fed with effluent from the Sharon reactor was compared with the activity of the biomass fed with synthetic wastewater.

4.4.4.1 Influence of nitrifiers on the Anammox process in batch tests

To predict what would happen to the washed-out nitrifiers reaching the Anammox reactor, a decay test was performed. The nitrifying biomass was washed and incubated with Anammox effluent. Twenty anaerobically prepared samples were stored for 10 weeks at a temperature of 32°C.

Every two weeks the amount of dead and living nitrifying biomass was measured using the live/dead staining method.

4.4.4.2 Aerobic activity

Every two weeks the aerobic activity of the nitrifiers was measured using a respiration meter. The affinity constant was also estimated. To estimate the respiration rate, the nitrifiers were first washed using a phosphate buffer. Ammonium was added as a substrate up to approximately 500 mg/L in the respiration meter. Various affinity constants and maximum

conversion rates as found over a period of 10 weeks are given in Table 4.10. These results are also depicted in Figure 4.10.

Table 4.10. Affinity constants for O_2 and maximal respiration rates during decay experiment

Week	K_o (mg/L)	V_{max} (mg/L/min)	V_{max} : K_o
0	1.03	1.70	1.65
2	1.10	1.41	1.30
4	0.50	0.82	1.64
6	0.18	0.35	1.94
8	0.21	0.12	0.57
10	0.13	0.14	1.0

It is easy to see from Table 4.10 that the affinity to oxygen after incubation with cell-free Anammox effluent increases with time. The maximum conversion rates decline over the same period. The nitrifiers thus remain alive. They seem to specialise in the consumption of minimal amounts of oxygen under anoxic conditions. Directly after opening the bottles, NH_4-N and NO_2-N were measured (Table 4.11).

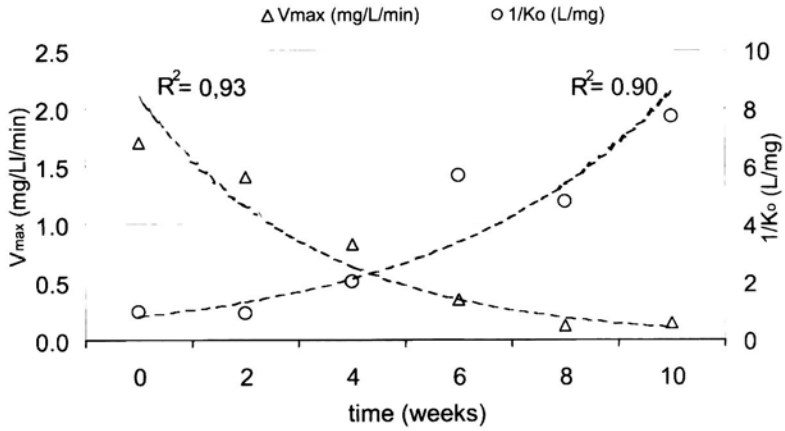


Figure 4.10. Aerobic activity of ammonium oxidisers after anaerobic incubation in Anammox effluent.

Table 4.11. Ammonium- and nitrite concentrations during anaerobic incubation

Time (weeks)	NH_4^+-N-concentration (mg/L)	NO_2^--N-concentration (mg/L)
0	33	12
2	51	3
4	28	29
6	41	23
8	59	2

No regular pattern in NH_4-N and NO_2-N concentrations can be seen in Table 4.11.

4.4.4.3 Activity of ammonium oxidisers in the Anammox reactors

The activity of the ammonium oxidisers in the Anammox reactor fed with effluent from the Sharon reactor was determined using a BOM meter. For comparison, the aerobic activity of the Anammox biomass fed with synthetic wastewater was also measured.

As expected, no aerobic activity was measured in the Anammox reactor fed with synthetic influent while in the Anammox reactor fed with Sharon effluent, the oxygen consumption rate was 0.94 (± 0.04) mg/L/min.

The oxygen consumption rate in the Sharon reactor during continuous aeration was 1.3 mgO_2/L/min. This value corresponds to values measured in the Anammox reactor. It can be concluded that the amount of active ammonium oxidisers in the Anammox reactor is similar to the amount found in the Sharon reactor.

4.4.4.4 Live/dead staining

The nitrifiers that were put under anaerobic conditions were subjected to a live/dead staining. No clear differences were observed between the number of living and dead cells over a period of 10 weeks.

It was not possible to evaluate whether the nitrifiers survived or died under Anammox conditions. The microscopic slides examined showed very few differences.

4.4.4.5 Fluorescent In Situ Hybridisation (FISH)

As well as being used for live/dead staining, FISH analysis was also performed for the combined Sharon/Anammox system to determine the level of nitrifiers in effluent from the Sharon reactor, in the Anammox reactor and effluent from the Anammox reactor. It turned out that the levels of nitrifiers in the Sharon effluent and in the Anammox reactor were the same. This proves that nitrifiers are not retained in the Anammox reactor.

4.5 EVALUATION OF THE EXPERIMENTAL PROGRAMME

It is possible to remove ammonium from sludge water using a combined Sharon/Anammox system. The Sharon process was performed in a chemostat. Without pH control at a hydraulic retention time of 1 day and temperature of 35°C, half of the available ammonium was converted into nitrite. Nitrate was then not formed.

The effluent from the partially nitrifying reactor is suitable as influent to the Anammox process. In the Anammox process, ammonium is converted under anaerobic conditions with nitrite into nitrogen gas and water. Neither nitrite formation nor the Anammox process needs an additional carbon source because these are both autotrophic processes.

The reactor configuration of the Anammox process should provide good sludge retention because the Anammox bacteria grow very slowly (doubling time of 11–16 days). For this research the Anammox process was carried out in a granular sludge sequencing batch reactor (SBR).

An Anammox reactor can be started up using diluted effluent from a Sharon reactor. To enrich the Anammox biomass, the effluent from the Sharon reactor should be diluted such that the ammonium and nitrite concentrations are lower than 70 mg/L. The dilution water should contain a sufficient amount of nitrate to prevent sulphate reduction in the system.

When Anammox activity is noted in the system, the nitrogen loading can be gradually increased by a gradual increase of the fraction of the Sharon effluent. The hydraulic retention time of the Anammox system within this research was always one day. The average nitrogen conversion after 120 days amounted to 0.75 $kgN/m^3_{reactor}$/day. After this period the test was stopped. The average specific conversion rate over a period of 120 days was 0.18 kgN_{tot}/kgDS/day.

The second combined Sharon/Anammox system operated without any problems for a period of 145 days, after which it was stopped. In this system, Anammox biomass was enriched using synthetic wastewater instead of effluent from the Sharon reactor.

The development of biomass can be measured and quantified precisely using the FISH (Fluorescent In Situ Hybridisation) technique. Long before any Anammox activity can be detected in the system, Anammox cells can be detected using FISH analysis, and in this way the growth conditions (process parameters) can be verified at an early stage of research.

Based on the results of this research, Chapter 5 was written. It discusses, among other things, how to make the choice between an Anammox reactor at pilot- or full-scale.

5

Process design and economic feasibility

5.1 GENERAL PERFORMANCE

In the combined partial nitrification/Anammox system two microbiological processes need to be combined with each other. The first step (partial conversion of ammonium to nitrite) requires oxygen, while the second step (conversion of ammonium with nitrite to nitrogen gas) is inhibited by oxygen. In principle, both steps can be carried out in a biofilm reactor. There, however, the amount of oxygen has to be precisely coupled with the amount of ammonium in the influent. Such biofilm reactors should be very well mixed. For a good control and process stability it is probably better for each conversion to be carried out in separate reactors.

Nitrite formation can take place in a suspended sludge mixture as well as in a biofilm reactor (van Benthum 1998). It was, however, never assessed whether the conversion of ammonium into nitrite is stable in a biofilm reactor over the long term. That is why, for the purposes of this research, a sludge suspension reactor was chosen which was comparable to the Sharon process at the Sluisjesdijk sludge treatment plant in Rotterdam, the Netherlands.

Sludge retention is an absolute prerequisite for the Anammox process because of the low growth rate of the micro-organisms involved.

5.1.1 Start-up

The start-up of the nitrite-forming system can be relatively fast. Within two weeks the required 50% of ammonium conversion to nitrite can be attained. For the Anammox process a longer start-up period (a few months) is necessary. Hereby it is necessary to prevent the presence of sulphate in the influent because it will be converted into sulphide. Sulphide is toxic for Anammox bacteria, and is formed when an excessive amount of BOD is present in the Anammox reactor. Sulphate reduction can be prevented by the addition of nitrate to the influent. The amount of nitrate has to be sufficient to remove BOD present in the influent or released from inoculum sludge via conventional, heterotrophic denitrification. If the nitrification process is present before the Anammox reactor, the amount of influent BOD is negligible, and it is only during the start-up phase that one has to prevent sulphate reduction.

The Anammox reactor can be inoculated with nitrifying low-loaded activated sludge. The chance that Anammox bacteria are already present in this sludge is high. Moreover, a small amount of BOD can be released from such mineralised sludge. The addition of a small amount of Anammox biomass as inoculum, originating from a laboratory, does not have any accelerating effect. When, however, the Anammox sludge can be obtained from a system in full operation, the start-up phase can be significantly shortened. This is analogous to the acceleration of the start-up of an UASB system by the addition of granular sludge from an already operating system.

It appears that there are many types of Anammox bacteria. This means that the choice of start-up inoculum should take into account the relationship between the type of wastewater and the type of Anammox bacteria related to it.

The start-up phase of the Anammox reactor can be followed with a FISH analysis of the reactor sludge or of the washed-out sludge. At an early stage, an increase in Anammox bacteria can be assessed by FISH probes. For an assessment of Anammox activity using nitrite- and/or ammonium removal rate batch tests, large amounts of Anammox cells are needed.

5.1.2 General process control and warning system

In a continuously operating process, the amount of ammonium converted in the nitrite-forming reactor has to be controlled in such a way that after the Anammox reactor all ammonium and nitrite are converted. To attain this, the ratio of NO_2-N/NH_4-N in the influent to the Anammox reactor should be approximately 1.3:1. Because of the possible disturbing effect of high nitrite concentrations on the Anammox activity, the nitrite concentration in the Anammox should be kept as low as possible. A nitrite measurement in the Anammox reactor using an online nitrite/nitrate analyser could serve as a control variable. Nitrite measurement is more sensitive than ammonium measurement. A supplementary ammonium measurement can be used to verify whether or not the N-removal is complete.

To control the nitrite concentration in the Anammox reactor at 10 mgN/L, a nitrite analysis should be taken. When the nitrite concentration in the Anammox reactor increases, the conversion (rate) in the nitrite-forming reactor has to be reduced. Conversely, when the nitrite content decreases, the conversion (rate) has to be increased. In the latter case, the influent flow will be controlled to determine whether ammonium is present.

The conversion in the nitrite-forming reactor can be controlled by pH or by adjustment of the aeration time. When the pH in the first reactor is not controlled, 53% of ammonium is transformed into nitrite. Without process control, 85% of total nitrogen reduction in both reactors is expected. If for the treatment of N-rich return streams at a WWTP, only the

majority of N has to be removed, then further process control may not be necessary. In such case redox electrodes can serve for process warning.

Ammonium conversion in the Sharon reactor can be controlled via the aerobic retention time and pH. The control can be based on the nitrite content in the Anammox reactor. It was found experimentally that to protect the Anammox biomass, the nitrite concentration should remain lower than 70 mg NO_2-N/L. For process control, 10 mg NO_2-N/L is recommended as the control value. When the nitrite concentration in the Anammox reactor is too high, firstit should be determined whether sufficient ammonium is still present. When too little influent is provided, the ammonium conversion in the Sharon reactor has to be reduced. Lowering the aeration capacity can achieve this. If the nitrite concentration in the Anammox reactor increases while too little ammonium is present, the provision of the Sharon effluent has to be periodically switched off until the nitrite concentration is sufficiently declined. If the nitrite content does not decrease or doesn't decrease quickly enough, washing the Anammox reactor with fresh sludge water can be an option. When the Anammox reactor is washed with fresh sludge water, the presence of toxic sulphide from the sludge supernatant has to be taken into account.

Suspended solids in the influent (caused, for example, by insufficient separation of sludge and water during the sludge treatment) do not seem to be a problem. In the nitrite forming process this matter should simply pass through. In the Anammox process, accumulation of suspended solids in the system should be prevented. Accumulation of inert material in the Anammox reactor leads to a 'dilution' of the Anammox sludge and consequently to a lower conversion. This is shown in Table 5.1, where different reactor configurations are evaluated.

5.1.3 Process disturbances

5.1.3.1 Protozoa

During this research it was found that protozoa may have a negative influence on the nitrite-forming process. In previous research on the Sharon process (STOWA 1996a), this effect was not observed. A big difference between the two research projects is that in later research the reactor was continuously aerated, while in earlier research it operated with longer, non-aerated denitrification periods.

In the current research, it was found that non-aerated periods or temporary lowering of the reactor pH from 6.8 to 6 prevents undesirable protozoa growth. This can be observed via simple microscopic examination. Non-aerated periods, however, clearly have a negative effect on the conversion by nitrifiers. A pH lowering in the nitrite-forming reactor can be attained when aeration is kept on (albeit temporarily) at a low influent flow. After one to two hours the pH will decline to approximately 6. This does not require large aeration intensity because conversion rates are relatively small. It seems possible that, by 'natural' variations in the influent flow, the pH can be lowered regularly.

When anaerobic periods have to be regularly provided to prevent protozoa growth, the nitrite-forming reactor has to be 30% larger to ensure good nitrite formation.

5.1.3.2 No feeding

After feeding has been stopped it is advised to switch off aeration. Optimally this should be done after the pH has declined to 6. Very precise pH control is not really necessary. If there is a long-lasting absence of influent (that is, several days) the aeration should be switched on periodically. The negative effect of anaerobic periods on the nitrite-forming bacteria is partially caused by high nitrite and ammonium concentrations in the reactor. This can be

reduced by circulating the reactor mixture between two reactors during periods of low influent flow. The nitrogen content can be lowered without washing out the nitrifiers.

For the Anammox process, longer periods without feeding do not represent any problem as long as nitrate is present in the reactor. Nitrate is formed during the Anammox process. In the absence of nitrate, the likelihood of sulphide forming is high, and this is toxic for Anammox bacteria.

5.1.3.3 Variable feeding

Practically, variable feeding is not problematic. The nitrite-forming process adapts to variations in concentrations because the biomass concentration is also variable. Variations in the flow can be compensated for by adjusting the aeration time in the nitritification reactor. Very high flows are not beneficial for the nitritification process because they can lead to a wash-out of nitrifiers.

Variations in loading do not represent any problem for the Anammox process. One should, however, ensure that the nitrite concentration in the Anammox reactor does not increase too much, as it did in the Sharon reactor. The addition of fresh sludge water can be a temporary solution.

5.2 CHOICE OF REACTOR

For the Sharon process a completely stirred tank reactor (CSTR) with an ejector aerator and mixers can be used. Retention of biomass has to be prevented (for instance by employing a small overflow in the tank).

For the Anammox process, the reactor configuration is defined in less detail. Table 5.1 gives an overview of possible options and their qualitative comparison.

Table 5.1. Possible reactor types for the Anammox process; the last four reactors are based on growth of granular sludge of approximately two millimetres

	Biofilm surface (m^2/m^3)	Operational	Stirring/ Mixing	Technical (performance)	Sensitivity for suspended sludge input
Activated sludge	5*	–	++	+	—
Membrane reactor	30*	+	++	0	——
Packed bed biofilm reactor	200	++	—	+	++
Moving bed reactor	350	+	+	+	++
Fluidised bed	2000	–	–	0	++
UASB/EGSB (Expanded granular sludge bed) reactor	2000	0	–	+	–
Internal circulation reactor	2000	0	++	+	+
Sequencing batch reactor	2000	0	++	0	+

* For the activated sludge system and membrane reactor the values in this table are given in g/L.

The input of suspended solids plays an essential role in deciding which reactor configuration to use. The growth rate and yield of the Anammox sludge are low: even a small input of solids can lead to a strong reduction of the volumetric capacity.

From all the reactor configurations given in Table 5.1, the standard activated sludge process can be directly eliminated because it gives volumetric conversions that are too low. The membrane reactor can also be eliminated because sludge in the influent is very efficiently retained. In the latter case, even suspended ammonium-oxidising bacteria from the nitrite forming process will be retained, and too low an amount of Anammox bacteria will be present.

Mixing is essential because incoming water contains a very high nitrite concentration. This has to be evenly distributed in the reactor. A relatively low gas production (1065 kg N_2/day or 1000 m^3/day) will not ensure good mixing. One can use liquid or gas circulation for badly mixed systems. This option was not discussed in the evaluation in Table 5.1.

After the above configurations have been eliminated, two types of reactor are left: a granular sludge reactor and a biofilm reactor (packed or moving bed). Separation of the input of suspended solids in the granular sludge reactor depends on the upflow velocity or settling time (during sludge/water separation).

Based on these preliminary operational considerations, a choice was made to carry out the Anammox process in a moving bed reactor. Such a reactor has a larger specific surface than a packed bed reactor, is well mixed (no liquid circulation is required) and the likelihood of the accumulation of suspended sludge is small.

5.3 DESIGN

The design is based on earlier STOWA research on N-elimination from sludge water. All basic assumptions were (as far as possible) the same as the assumptions from STOWA (1996a, b). In these reports an influent of 1200 kg NH_4^+-N/day was assumed. The cost calculation per kilogram of removed nitrogen was performed for three scenarios:

- scenario 1 – Low: situation where sludge water contains a relatively low nitrogen concentration (0.5 gN/L);
- scenario 2 – Average: situation with relatively high ammonium concentrations (1.2 gN/L);
- scenario 3 – High: situation where efficient mechanical thickening of the surplus sludge takes place before digestion (2 gN/L).

A high efficient ammonium and nitrite removal without pH correction with base dosing were assumed. With pH control, a very low concentration of ammonium in the effluent can be achieved, but the costs associated with base dosing are very high. The ammonium conversion in the Sharon reactor (53%) is assumed as a result from this research. Another assumption was that the loading was given 80% of the time.

The numbers presented in Table 5.2 mean that at a N-loading rate of 1200 kgN/day, 636 kg ammonium (53%) is oxidised to nitrite. For this conversion $636 \times 3.43 = 2181$ kg O_2 is necessary. After the Anammox $(636/1.3) \times 0.26 = 127$ kg NO_3-N and 75 kg NH_4-N per day are found in the effluent.

Table 5.2. Dimensioning (parameters)*

Parameter	Unit	Scenario 1	Scenario 2	Scenario 3
N-loading	kg/day	1200	1200	1200
N-concentration	g N/m^3	500	1200	2000
Flow	m^3/day	2400	1000	600

Parameter	Unit	Value
Reactor temperature	°C	32–38
Influent temperature	°C	27
pH		6.5–7.0
Aerobic retention time nitritification	day	1
O_2 demand	g O_2/g NH_4-N converted	3.43
Loading Anammox biomass	g N/g DS·day	0.6**
Ratio NO_3^- formed per NH_4^+	g N/g N	0.26
Ratio NO_2^- consumption per NH_4^-	g N/g N	1.3

* It was assumed that accumulation of influent dry matter (suspended solids) in the reactor does not take place.

** This number (0.6) results from research where the Anammox sludge is formed on sludge water. For designing the first Anammox application it is advised to take a lower activity; for the calculations below, the conversion rate of 0.3 g NO_2-N/g DS.day was used.

5.3.1 Calculations

The biofilm surface is a determining parameter for the design of a biofilm reactor. It is further assumed that in the Anammox reactor the nitrite concentration is lower than the ammonium concentration, that 53% of N-loading consists of nitrite, and that the reactor can be considered as a completely stirred system. Conversion can be calculated as follows:

Calculation of active layer depth of biofilm

$$\delta = \sqrt{\frac{D \bullet C_{Nitrite}}{k \bullet C_X^{biofilm}}} \tag{5.1}$$

where:

$C_X^{\text{Nitrogen rich sludge water}}$ = biomass density in biofilm (70 kgDS/m^3)
k = sludge activity (safely estimated at 300 gNO_2-N/kgDS/day)
D = diffusion coefficient (8.6×10^{-5} m^2/day)
$C_{nitrite}$ = nitrite concentration in liquid phase (10 gN/m^3)

When the above numbers are put into Equation (5.1) it is discovered that a biofilm thickness of 0.2 mm is completely active. Up to this depth the conversions can be calculated without taking into account diffusion limitation. Normally, the biofilms are thicker and thus limited by diffusion. This means that the conversion can be calculated per unit biofilm surface instead of sludge unit.

Calculation of conversion per biofilm surface:

$$N\text{-}flux = \sqrt{C_X^{Biofilm}} \bullet k \bullet D \bullet C_{nitrite} \tag{5.2}$$

where:

N-flux = nitrite conversion in gN/m^2 per day.

This results in a conversion of 4.2 g NO_2-N/m^2.day. Assuming that the average specific surface amounts to 350 m^2/m^3reactor and the nitrite loading to be converted is 1200 × 0.53=636 kgNO_2-N/day, the required reactor volume is equal to 432 (rounded up to 450) m^3.

When the reactor used is a granular sludge reactor, the specific surface is 2000 m^2/m^3, and the reactor volume approached 75m^3.

5.4 ECONOMIC EVALUATION

5.4.1 Assumptions

To define cost economic frames, a retention time in the nitrification reactor of 1.3 day and a tank dept of 5 m were assumed. The retention time is a combination of the necessary aerobic retention time of 1 day and additional volume to suppress protozoa by non-aerated periods. The dissolved oxygen can be kept at 1.5 mg/L. For the Anammox reactor, a loading rate of 3.5 g NO_2-N/m^2.day, a specific surface of carrying material of 350 m^2/m^3 (packed bed) and a minimal retention time of 4 hours were chosen. For the Anammox granular sludge reactor, a specific carrying surface of 2000 m^2/m^3 was assumed. Table 5.3 presents the results for the three scenarios.

Table 5.3. Dimensioning of Sharon- and Anammox reactors for various scenarios (numbers in thousands)

Reactor	Parameter	Unit	Scenario 1	Scenario 2	Scenario 3
Sharon reactor	N-loading	kg N/day	1200	1200	1200
	NH_4-N content	kg N/m^3	500	1200	2000
	Sludge water flow	m^3/day	2400	1000	600
	Volume aeration	m^3	3120	1300	780
	Oxygen demand based on ammonium oxidation	kg O_2/day	2181	2181	2181
	Air supply*	Nm^3/day	56,000	56,000	56,000
Moving bed Anammox reactor	Volume reactor	m^3	450	450	450
	HRT	hour	4.5	11	18
Anammox granular sludge reactor	Volume reactor	m^3	75	75	75
	HRT**	hour	0.75	1.8	3

* Calculated on the assumption that the oxygen consumption per metre of reactor height is 15 g/Nm^3.m.

** From the process stability point of view (variation in influent) a somewhat longer retention time may be required.

5.4.2 Estimate of costs

The estimate of costs (Table 5.4 was created by Grontmij Consultants, de Bilt, The Netherlands) given here is based on the assumptions given in Appendix B.

It is possible to compare a Sharon process (complete oxidation to nitrite and denitrification with methanol) with the combined Sharon/Anammox process. Because less oxygen (43%) and no methanol are required, significant savings can be reported. The total costs per kg N for scenario 2 are €1.35 (including rest tax) and €1.12 (excluding rest tax). Scenario 2 results in a total saving of €84,000 per year.

Table 5.4. Costs estimate (all prices given in thousands of euros)

Parameter	Unit	Scenario 1	Scenario 2	Scenario 3
N-loading	Kg N/day	1200	1200	1200
Flow	m^3/day	2400	1000	600
Concentration	Kg/m^3	500	1200	2000
Investment	K€	2261	1814	1635
Depreciation (D)	K€/year	240	196	178
Maintenance (M)	K€/year	46	41	38
Staff (S)	K€/year	11	11	11
Total of D + M + S	K€/year	296	248	227
Electricity	K€/year	82	76	74
Ammonium discharge to surface water	K€/year	100	100	100
Total costs (incl. rest charge)	K€/year	479	424	401
Costs per kg N incl. rest charge/tax*	€	1.32	1.17	1.11
Costs per kg N excl. rest charge/tax*	€	1.04	0.89	0.83

* Costs per kg N were calculated based on the removed amount nitrogen. For scenario 2 counts 83 % removal.

6

Conclusions and recommendations

In the Sharon process more than 50% of the ammonium from sludge water (digested sludge supernatant) can be oxidised to nitrite without applying a pH correction (base dosing). Ammonium and nitrite from the effluent of the Sharon reactor can be converted in an Anammox reactor to nitrogen gas. The Anammox reactor can be started up using activated sludge. The start-up period lasts usually a few months. After an Anammox is started up, it can then be operated for a long period.

The most important aspects of concern for system choice and operation of the combined Sharon-Anammox system are the input of suspended matter into the Anammox reactor and possible predation by protozoa in the Sharon reactor.

From a scale-up exercise it turns out that the Sharon-Anammox process offers a good economic and operative perspective. Costs for sludge water treatment are estimated to be 0.7–1.1 € per kg of nitrogen removed. Based on similar calculations, previous STOWA research showed that other techniques are significantly more expensive: for the Sharon process with denitrification with methanol the estimation amounts 0.9–1.4 €/kg N while other biological techniques (converting ammonium over nitrate to nitrogen gas) were between 2.3–4.5 €/kg N while physical-chemical techniques cost 4.5–11.3 €/kg N.

The Sharon-Anammox process is a more sustainable wastewater treatment. Compared with conventional nitrogen removal, 40% less oxygen (= energy) is necessary for this process, an organic C-source is not required, and sludge production is negligible.

For the above economical and environmental reasons, the scale-up of the system should be done as soon as possible. Since the Anammox process can use an existing (common) reactor type, it is possible to scale up the process directly to full-scale.

A successful scale-up can be followed by further steps towards a sustainable wastewater treatment plant (WWTP). This means, on one hand, a maximisation of the nitrogen load to the sludge digester and on the other hand the application of Anammox at low temperatures in the water line (Jetten *et al.* 1997).

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Appendix A: Growth tests with protozoa

The presence and the growth of protozoa were tested using two types of experiments. This Appendix describes these two tests.

Origin of protozoa in the Sharon reactor

To determine whether the protozoa originate from sludge water, growth tests were performed with sterilised and non-sterilised sludge water. Since cysts would not survive sterilisation, protozoa could not originate from this sterilised water. For germination of the present cysts, 10 mL of sludge water and 2 mL of bacteria suspension were added to 6 round flasks with a capacity of 200 mL. The flasks were placed in the shaker at 30°C. Each day the flasks were observed under a microscope to see if protozoa were present and, if they were, they were counted. Three flasks out of a total of six contained sterilised sludge water (flasks 4–6, where flask 6 was a control flask, containing no bacteria) and the other three contained non-sterilised sludge water (flask numbers 1–3, while flask 3 was also a control). The protozoa were counted in microscopic samples of 4 μL. When using a drop of 4 μL, all liquid stays under the cover glass of the microscopic sample. All protozoa found in this 4 μL sample were counted under a microscope and recalculated to discover the number of protozoa per 1 L. The results are shown in Table A.1.

Table A.1. Batch growth tests with sterilised and non-sterilised sludge water

Day	Non-sterilised sludge water			Sterilised sludge water		
	Flask 1	Flask 2	3 (control)	Flask 4	Flask 5	6 (control)
	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)
0	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
1	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
2	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
5	32.6	40.3	<0.25	<0.25	<0.25	<0.25
6	26.8	0.375	<0.25	<0.25	<0.25	<0.25
7	1.0	<0.25	<0.25	<0.25	<0.25	<0.25

Table A.1 shows that when the sludge water is sterilised, no protozoa germinated. Only in flasks 1 and 2 was protozoal growth observed, starting from day 5. Based on this it can be concluded that the protozoa originate from sludge water.

Growth pattern of protozoa during batch tests

To get a rough idea of the growth pattern and rate of protozoa, a growth test was used. In this test, five flasks containing non-sterilised sludge water were placed in a shaker. The growth conditions were identical to those in the first batch test. The results of the second test are given in Table A.2 and plotted in Figure A.1.

Table A.2. The growth pattern of protozoa in batch tests (shaker)

Day	Flask 1	Flask 2	Flask 3	Flask 4	Control
Number	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)	Number ($n \times 10^6$/L)
0	<0.25	<0.25	<0.25	<0.25	<0.25
1	<0.25	<0.25	<0.25	<0.25	<0.25
2	<0.25	<0.25	<0.25	<0.25	<0.25
3	<0.25	<0.25	<0.25	<0.25	<0.25
6	15.7	0.91	39.0	45.4	0.25
7	0.50	1.4	7.3	10.3	<0.25
8	<0.25	<0.25	0.50	1.0	<0.25
9	<0.25	0.25	0.25	<0.25	<0.25
10	<0.25	<0.25	<0.25	<0.25	<0.25
13	<0.25	<0.25	<0.25	<0.25	<0.25

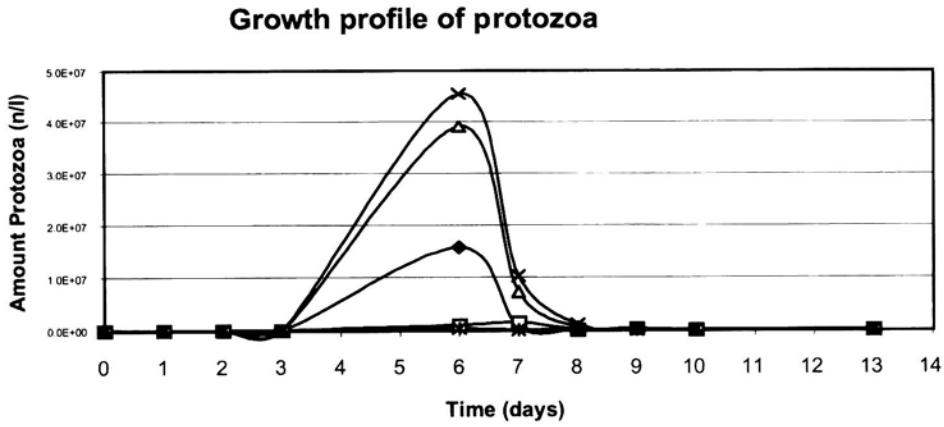

Figure A.1. Growth pattern of the protozoa.

Figure A.1 does not show a clear exponential curve, because at the beginning of the growth phase no measurements were performed. It is, however, clear that within three days the number of protozoa increased from $<0.25 \times 10^6$ to $\pm 40 \times 10^6$ per litre. There were thus seven generation times in three days. One generation time (t_g) is thus 3/7 = 0.42 day. The minimal growth rate (μ) is therefore $ln\ 2/t_g = 1.62\ day^{-1}$.

Flask 5 (control) did not show protozoa growth. No bacteria were added to this flask. In flask 3, limited protozoal growth was observed. There is no explanation for this fact. It was, however, obvious that the flasks with protozoa growth (1, 3 and 4) had a clearer colour than flask 2, which did not show any protozoa growth. There were still large amounts of bacteria in flask 2 after 13 days.

It can also be seen from Figure A.1 that the conversion of protozoa to cysts proceeds very quickly. Within two days, almost all protozoa were again in the form of cysts.

Appendix B: Assumptions for cost estimate

In the cost estimates the following assumptions were taken into account:

CIVIL ENGINEERING

Sharon and Anammox reactors:

- insulated concrete tank with a concrete roof founded on steel (with a manhole)
- influent pump
- NaOH dosing installation and storage
- operation room (small building)
- no ground/soil conditioning

Sharon reactor:

- methanol dosing installation and storage

Anammox reactor:

- carrier material

MECHANICAL ENGINEERING

Sharon and Anammox reactors:

- piping, stainless steel
- heating installation

Sharon reactor:

- 2 influent pumps (one in operation and one stand-by)
- blowers (cased), close to the tank

Anammox reactor:

- by-pass facilities

ELECTRO-TECHNICAL ENGINEERING

Sharon and Anammox reactors:

- high level of automation
- sufficient electrical feeding present

Sharon reactor:

- oxygen, pH and temperature measurement/control

Anammox reactor:

- nitrite-, pH- and temperature measurement/control.

BUILDING COSTS

The investment costs were calculated based on the design for different scenarios. The building costs include:

- total building costs inclusive of incompleteness surcharge are based on numbers used by an engineering company (Grontmij consultants, De Bilt, the Netherlands)
- incompleteness surcharge amounts to 10% of total building costs;
- additional costs include, for instance, insurance, taxes, permits/concessions, extensions, utilities, soil examination and legal costs. These costs are estimated at 10% of the total building costs plus the incompleteness surcharge;
- unforeseen costs are estimated at 10% of the total above costs;
- consulting costs are 10% of the total above amount'
- VAT of 17.5% is added to all the above costs.

OPERATIONAL COSTS

Annual expenses for each scenario relate to the operational costs of wastewater treatment. Costs as of the year 2000 were used. The following assumptions were made:

- for depreciation of the civil and technical engineering a period of 30 years is assumed while for mechanical and electro-technical engineering 15 years;
- capital expenses were calculated using the annuity method. The interest was kept at 8%;
- the maintenance costs for civil and technical works were calculated as 0.5% per year; maintenance costs for mechanical and electro-technical works – 3% per year;
- for the staff a € 36 302,- per year was taken (1.5 man-day);

To calculate the cost of energy and chemicals needed, we used the numbers given in Table B.1.

Table B.1. Prices of energy and chemicals

Feedstock		Price (excluding VAT)	
Energy	Electricity	(€/kWh)	0.068
Electron donor Denitrification	Methanol	(€/kg)	0.136

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Page numbers in *italics* refer to figures and tables.

Wastewater treatment management, alongside many other industries, is seeking to attain a higher degree of sustainability for its processes by focusing on new technologies that minimise the consumption of resources, or even recover them from wastewater.

Conventional removal of ammonium usually requires large amounts of energy for aeration and organic carbon for denitrification. This Report focuses on making the nitrogen-removal process more sustainable. This can be achieved by a partial oxidation of ammonium to nitrite, after which the nitrate produced can be converted into nitrogen gas with the rest of ammonium, under anoxic conditions.

The treatment of nitrogen-rich water can be carried out beneficially by a combination of the Sharon process with the Anammox process. In this combined process, less than 50% of the aeration energy is needed, no COD is required and an insignificant amount of sludge is produced. In this Report, the potential of using this technology for the treatment of water arising from sludge treatment at municipal wastewater treatment plants is evaluated and the results of the operation of the system are described in detail. This reject water contains a significant fraction of the N-load towards the wastewater treatment plant. The results are used in an economic evaluation of a potential full-scale installation.

The Combined Sharon/Anammox Process will provide an invaluable source of information for all those concerned with the efficient and sustainable treatment of wastewater including plant managers, process designers, consultants and researchers.

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