POLITECNICO DI MILANO

POLO TERRITORIALE DI COMO

A.A. 2011 - 2012

Master of Science in Environmental and Geomatic Engineering

State of the Art of ANAMMOX-based Processes For Biological Nitrogen Removal

Supervisor: Prof. ing. Roberto Canziani

M. Sc. Thesis of Thalapaneni Damodaranaidu Viswanathan
ID number: 764922

The Thesis Committee for Thalapaneni Damodaranaidu Viswanathan
Certifies that this is the approved version of the following thesis:

State of the art of ANAMMOX based processes

For Biological Nitrogen Removal

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

Prof. ing. Roberto Canziani, Ph.D.

State of the Art of ANAMMOX-based Processes For Biological Nitrogen Removal

by

Thalapaneni Damodaranaidu Viswanathan

Thesis

Presented to the Faculty of the Graduate School of

POLITECNICO DI MILANO

in Partial Fulfillment

of the Requirements

for the Degree of

ENVIRONMENTAL AND GEOMATIC ENGINEERING

POLITECNICO DI MILANO

July 2012

Acknowledgements

It would not have been possible to write this Master thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here.

This thesis would not have been possible without the help, support and patience of my supervisor, **Prof. Roberto Canziani**, I owe sincere thankfulness to my Supervisor, who made me believe in myself and guided me through the whole process of dissertation writing. I am sure that this dissertation would not have been possible without his support, understanding and encouragement I felt when working on my paper.

I would like to acknowledge, the library facilities of the Politecnico di Milano University have been indispensable. I would also like to show my gratitude to my parents, friends and colleagues for their help and moral support.

For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own.

Abstract

State of the Art of ANAMMOX-based Processes For Biological Nitrogen Removal

Thalapaneni Damodaranaidu Viswanathan, degree sought: M.Sc. in Environmental and Geomatic Engineering

POLITECNICO DI MILANO, 2012

Supervisor: Prof. Ing. Roberto Canziani

Abstract:

Nitrogen is one of the most abundant elements. About 80 percent of the air we breathe is nitrogen. It is found in the cells of all living organisms and is a major component of proteins. Inorganic nitrogen exists in the form of free-state as a gas N_2, or as reactive nitrogen in the form of nitrate NO_3^-, nitrite NO_2^-, or ammonia NH_3^+. Organic nitrogen is found in proteins and is continually recycled by plants and animals. Since wastewater discharges containing reactive nitrogen can be toxic to aquatic life, cause oxygen depletion and eutrophication in receiving water, and affect chlorine disinfection efficiency, reducing reactive nitrogen levels from the discharges is necessary. We use various technologies to treat wastewater which contains reactive nitrogen. Over the past few years, new technologies for nitrogen removal have been developed mainly because of the increasing costs of traditional wastewater treatment technologies.

Newly discovered biochemical pathways, such as the anaerobic oxidation of ammonium (ANAMMOX), and uses for nitrogen removal technologies are under discussion.

Processes and technologies such as: Partial nitrification; Single reactor systems for High Ammonium Removal Over Nitrite (SHARON); Anammox; Aerobic/anoxic deammonification; Oxygen Limited Autotrophic Nitrification- Denitrification (OLAND); Completely Autotrophic Nitrogen Removal Over Nitrite (CANON); all have a high potential for nitrogen removal. These processes are suitable for treatment of high strength ammonia wastewaters such as reject water from dewatering of digested sewage sludge and wastewater from sludge digesters.

This paper summarizes different aspects and experiences of several nitrogen removal processes and also the comparison of different nitrogen removal process. The main objective is to summarize various treatment techniques used for nitrogen removal and compare their performance. A simple design example of one-step reactor or two-step reactor configurations to treat digested sludge supernatant is also reported.

Table of Contents

List of Tables

List of Figures

List of ABBREVATIONS

ABF - Anaerobic biologically filtrated reactor

ART- Aerated retention time

AS - Activated sludge

ASBR - Anaerobic Sequencing Batch reactor

DHS - Down-flow Hanging Sponge reactor

FBA - Fixed Bed Anammox reactor

FBR - Fed batch reactor

MBBR - Moving bed biofilm reactor

MSBR - Membrane sequencing batch reactor

RBC - Rotating biological Contactor

SBR - Sequencing batch reactor

SNAD - Simultaneous partial nitrification, ANAMMOX and denitrification in 1- reactor system

SNAP – Single stage partial Nitritation and Anammox Process

UASB - Upflow Anaerobic Sludge Blanket reactor

UCR - Upflow Column Reactor

UGBR - Upflow Granular Bed Reactor

USFF - Upflow Stationary Fixed Film reactor

VSBR - Vertical Submerged Biofilm Reactor

CHAPTER 1: INTRODUCTION

WASTEWATER is any water that has been adversely affected in quality by anthropogenic influence and it comprises liquid waste discharged by domestic residences, commercial properties, industry, and/or agriculture areas. In the most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewaters from different sources as follows:

- Human waste (faeces, urine), also known as black water, usually from lavatories,
- Sewage treatment plant discharge,
- Washing water (personal, clothes, floors, dishes, etc.), also known as greywater,
- Rainfall collected on roofs, yards, hard-standings, etc,
- Surplus manufactured liquids from domestic sources,
- Urban rainfall runoff from roads, car parks, roofs, sidewalks, or pavements,
- Industrial waste, which includes
- Industrial site drainage, Cooling waters, Process waters.
- Drainage water from agricultural activities.

The wastewater should be treated in order to reduce the heavy use of water resources and to reduce the further impacts to the environment that will be developed by the wastewater.

1.1 Nitrogen

Nitrogen is one of the most abundant elements. About 80 percent of the air we breathe is nitrogen. It is found in the cells of all living things and is a major component of proteins. Inorganic nitrogen exist in the form of free-state as a gas N_2, or as reactive nitrogen in the form of nitrate NO_3^-, nitrite NO_2^-, or ammonia NH_3^+. Organic nitrogen is found in proteins and is continually recycled by plants and animals.

Nitrogen is a dietary requirement for all organisms, because it is a constituent of all proteins and nucleic acids. Plants consist of approximately 7.5% nitrogen (dry mass). Nitrogen is essential for plants, and can be found in air in large amounts. This elementary nitrogen cannot be taken up directly. Nitrogen must first be bound and

converted, for instance to nitrate. This so-called nitrification process is carried out by bacteria, which convert ammonia and ammonium to nitrate and nitrite. This releases energy, and establishes a nitrate stock in soils that can be applied by plants.

When nitrogen fertilizers are applied, the plant nitrogen amount increases. A number of crops, such as spinach, even accumulate nitrogen compounds.

Seawater contains approximately 0.5ppm nitrogen (dissolved inorganic nitrogen compounds without N_2). The amount is clearly lower at the surface, being approximately 0.1ppb. River water concentrations vary strongly, but are approximately 0.25ppm in general. Depending on water properties, various inorganic nitrogen compounds may be found.

In aerobic waters nitrogen is mainly present as N_2 and NO_3^-, and depending on environmental conditions it may also occur as N_2O, NH_3, NH_4^+, HNO_2, NO_2^- or HNO_3. Ammonium, nitrate and nitrite play the most important role in biochemical processes, but some organic nitrogen compounds in water may also be of significance.

Total nitrogen represents the sum of organic and inorganic nitrogen compounds. For wastewater Kjeldahl-nitrogen is generally applied as a measure. The TKN value (Total Kjeldahl Nitrogen) represents a total nitrogen concentration, which is the sum of organic nitrogen compounds and ammonium nitrogen. Nitrogen mainly occurs in wastewater in this form. After biological wastewater treatment, it mainly occurs as oxidized nitrite.

- TKN = org-N + NH_4-N (mg L^{-1})
- Nitrogen gas does not react with water. It does dissolve in water.

1.2 Sources of Nitrogen

1.2.1 Natural Sources

- Nitrogen is an essential plant nutrient. It is a key component in plant proteins and chlorophyll.

Some plants “make their own nitrogen”. If a legume (i.e., soybeans, alfalfa, clovers) is colonized by certain strains of Rhizobium bacteria, nodules will form on the plant

roots where the bacteria live and reproduce. Within these nodules, a symbiotic relationship develops between the bacteria and the host plant. The bacteria utilize plant sugars as a source of energy and in turn "fix" nitrogen, converting nitrogen gas into forms that can be used by the plant. Once nodules form, the plant usually receives all of the nitrogen necessary for growth from that "fixed" by the bacteria. Other crops, including all grass crops (e.g., corn, sorghum, wheat, forage grasses, etc.) and non-leguminous broadleaf crops (e.g., sunflowers, potatoes, sugar beets, cotton, etc.) are not colonized by nitrogen fixing bacteria and therefore must obtain the nitrogen they need from the soil. In addition to nitrogen fixed by Rhizobium bacteria, other natural sources that contribute to the soil nitrogen include (Smith et al., 1999):

- Mineralization of organic matter and nitrogen released as plant residues are broken down in the soil.
- Animal waste is a good source of natural nitrogen as well.

Barnyard or poultry manure and other animal waste products (e.g., bat guano) were used as a source of supplemental nitrogen long before inorganic nitrogen fertilizer came into popular use. Composted plant residues, legumes plowed under as green manure, and animal wastes continue to be used today, especially by organic crop producers, as a source of nitrogen (Smith et al., 1999).

A small amount of nitrogen is also contributed by rainfall in the form of nitric acid (HNO_3), which when dissolved in the soil water disassociates into hydrogen and nitrate ions. The nitric acid is formed when nitrogen and oxygen gases are combined with water by the intense heat of a lightning bolt during a thunderstorm (Smith et al., 1999).

While all these natural sources can make significant contributions to soil nitrogen levels, they usually do not supply enough nitrogen to meet all of the needs of high yielding non-leguminous crops in what are now considered "conventional" agricultural systems. Additional nitrogen in the form of added fertilizer is usually required for optimum yield (Smith et al., 1999).

The air we breathe is about 78% nitrogen in the form of N_2 gas and about 21% oxygen in the form of O_2 gas. The remaining one percent of the atmosphere is a combination of all the other gases, including carbon dioxide that is the source of

carbon used by green plants. Even though there is 33,000 tons of nitrogen in the air over every acre, the nitrogen gas is so chemically stable; plants cannot directly use it as a nutrient (Smith et al., 1999).

Plants readily take up and use two forms of soil nitrogen, ammonium (NH_4^+) and nitrate (NO_3^-). Other forms of nitrogen must be converted to one of these compounds by natural or artificial means before plants can utilize them directly as a source of nitrogen for plant growth (Smith et al., 1999).

1.2.2 Production by human activities

Nitrogen ends up in the environment mainly through agricultural processes and industrial processes, and thereby also ends up in water, which contains a large amount of nitrogen which is formed due to the anthropogenic activities.

Human activity has profoundly altered the global biogeochemical cycle of N (Galloway et al., 1995; Vitousek et al., 1997a, b). Humans have approximately doubled the rate of N input into the terrestrial N cycle, and these rates are still increasing (Vitousek et al., 1997a). Over all, anthropogenic inputs currently add at least as much fixed N to terrestrial ecosystems as do all natural sources combined, and humans mobilize more than 50 million metric tonnes of N via land trans formations (Vitousek et al., 1997b). The global production of agricultural fertilizers increased from <1 0 million metric tonnes of N in 1950 to ca. 80 million metric tonnes in 1990, and its production is predicted by some authors to exceed 135 million metric tonnes of N by 2030 (Vitousek et al., 1997b). Substantial addition al N is applied to croplands in the form of animal manures, for which regulatory standards are generally far less stringent than those applied to human sewage (Carpenter et al., 1998). A small but significant fraction of the total agricultural N applied to land is in excess of plant requirements for growth, and this surplus N may:

- Accumulate in soils;
- Move from the land into surface waters;
- Migrate into ground waters;

The combustion of fossil fuels causes an addition al emission of >20 million metric tonnes of N into the atmosphere (Vitousek et al., 1997a), a significant fraction of which subsequently return s to the land and ocean surface via wet and dry deposition.

This atmospheric deposition of N can have strong effects on the structure and function of both terrestrial and marine ecosystems.

The external supplies of N to aquatic ecosystems are derived from a wide variety of sources, including groundwater, fluvial, and atmospheric inputs. The sum of these three sources can be termed the external load. As can be seen in Table 1, the external supplies of nutrients to a water body can originate both as point sources, which are localized and more easily monitored and controlled, and as nonpoint sources, which are diffuse and much more difficult to monitor and regulate. The relative contributions of these two types of sources can differ substantially from watershed to watershed, depending upon local human population densities and land use. (Akpor .O.B et al., 2010)

Table 1: Sources of point and nonpoint chemical inputs (from Carpenter et al., 1998, modified from Novotny and Olem, 1994)

Point Sources	Non-point Sources
• Wastewater effluent (municipal and industrial), • Runoff and leachate from waste disposal sites, • Runoff and infiltration from animal feedlots, • Runoff from mines, oil fields, and unsewered industrial sites, • Storm sewer outfalls, • Overflows of combined storm and sanitary sewers, • Runoff from construction sites with an area >2 ha.	• Runoff from agriculture, • Runoff from pastures and rangelands, • Urban runoff from unsewered areas and sewered areas, • Septic tank leakage and runoff from failed septic systems, • Runoff from construction sites with an area <2 ha, • Runoff from abandoned mines, • Atmospheric deposition over a water surface, • Generation of contaminants by logging, wetland conversion, construction and development of land or waterways. • A significant amount of nitrogen can be found in domestic wastewater.

1.3 Effects of nitrogen contamination

1.3.1 Effects on health

The human body consists of approximately 2.6% nitrogen, which is a constituent of most proteins and nucleic acids. This means nitrogen is a dietary requirement. Nitrogen is the main constituent of the air we breathe. We mainly absorb nitrogen as proteins. These cannot be stored and are therefore directly converted to energy when not required. We also release nitrogen through the skin and the intestinal tract. When kidney failure occurs, one is incriminated with protein decomposition products.

- Increased nitrogen concentrations in air may cause asphyxiation, because it results in a lower oxygen concentration.
- Nitrogen is excreted through the kidneys as urea.

Nitrates are not generally considered toxic, but at high concentrations the body may convert nitrate to nitrite. Nitrites are toxic salts that disrupt blood oxygen transport by disrupting haemoglobin to methemoglobin conversion. This causes nausea and stomach aches for adults. For young infants it may be extremely risky, because it rapidly causes blood oxygen deprivation.

Nitrites and amines from protein-rich food form so-called nitrosamines, which are carcinogenic substances. This reaction may be prevented by the reducing and anti-oxidant properties of vitamin C. Examples of toxic nitrogen compounds are PAN-compounds, which are fifty times more toxic than the nitrogen compounds these are converted from (nitriles and nitrilo compounds). NTA is not absorbed in the stomach, because it is complexed with heavy metals. It may however still disrupt electrolyte metabolism.

Nitrogen oxides play a more significant role in air than in water. These can cause breathing disorders. Nitrogen hydrogen acid fumes may cause irritations, heart problems and collapsing.

1.2.2 Effects on environment

When nitrogen fertilizers are applied outside the growing season, this is completely useless and will negatively affect the environment. The fertilizers cannot be taken up

or immobilized, causing them to end up in groundwater and drinking water. Nitrogen has a high spreading potential. A number of plants are relatively susceptible to NO_2. Nitric acid is an important constituent of precipitation. Together with H_2SO_4 it causes acid rain, which negatively affects crops and soils.

Nitrogen itself is not hazardous when present in water, and therefore does not cause any environmental damage.

In seawater nitrates, nitrites and ammonia are dietary requirements for plankton, causing nitrogen concentrations to be lower at the surface than in the deep. At increasing nitrogen concentrations in surface layers, plankton production increases, leading to algal blooms. This may occur in any type of surface water. Large amounts of nitrate may cause eutrophication, which means an excess of nutrients.

Effects of eutrophication on lakes and reservoirs: (Smith et al., 1999).

- Increased biomass of freshwater phytoplankton and periphyton
- Shifts in phytoplankton species composition to taxa that may be toxic or inedible (e.g. bloom-forming cyanobacteria)
- Changes in vascular plant production, biomass, and species composition
- Reduced water clarity
- Decreases in the perceived aesthetic value of the water body
- Taste, odor, and water supply filtration problems
- Possible health risks in water supplies
- Elevated pH and dissolved oxygen depletion in the water column
- Increased fish production and harvest
- Shifts in fish species composition towards less desirable species
- Increased probability of fish kills

Nitrogen does not limit algal growth, because phosphorus is generally a limiting factor in water bodies. This means that phosphorus is the determining factor of algal spreading through surface waters. Oxygen deficits in surface water generally result in nitrate reduction to elementary nitrogen or nitrous oxide. In some cases nitrate can be biologically reduced to ammonia.

Nutrient-induced production of aquatic plants in receiving water bodies has the following detrimental consequences (Akpor et al., 2010):

- Algal clumps, odour and decolouration of the water, thus interfering with recreational and aesthetic water use;
- Extensive growth of rooted aquatic life interferes with navigation, aeration and channel capacity;
- Dead macrophytes and phytoplankton settle to the bottom of a water body, stimulating microbial breakdown processes that require oxygen, thus causing oxygen depletion;
- Extreme oxygen depletion can lead to the death of desirable aquatic life;
- Siliceous diatoms and filamentous algae may clog water treatment plant filters and result in reduced backwashing;
- Algal blooms may shade and submerge aquatic vegetation, thus reducing or eliminating photosynthesis and productivity.

Maximum recommended concentration for nitrogen varies widely from national and international regulations. For example, the maximum allowable total nitrogen (nitrate-N + nitrite-N + ammonium-N + organic-N) concentration in effluents coming from a plant serving more than 100.000 population equivalent is 10 mg L^{-1}.

In this review, the general principle of the Conventional biological wastewater treatment methods and the Innovative Nitrogen removal methods are explored and particularly with ANAMMOX nitrogen removal process. The exploration is mainly based on the literature available and finally presented with what can be done in the future for the improvement of the process.

Chapter-2: CONVENTIONAL BIOLOGICAL NITROGEN REMOVAL PROCESSES

2.1 Introduction

Water resources have been described as the limiting factor for the human development. Growing population demand more water for a range of uses, while at the same time existing water resources are being polluted (Bodalo et al., 2005). Over the last century, continued population growth and industrialization have resulted in the degradation of various ecosystems on which human life relies on. In the case of ocean and river quality, such pollution is primarily caused by the discharge of inadequately treated industrial and municipal wastewater. The extent of water contamination has risen due to the large quantities of industrial and domestic water discharges to the environment. Increased nitrogen concentrations are becoming significant among the pollutants. Ammonium is the most commonly encountered nitrogenous compound in the wastewater (Bodalo et al., 2005).

Since wastewater discharges containing nitrogen can be toxic to aquatic life, cause oxygen depletion and eutrophication in receiving water, and affect chlorine disinfection efficiency, reducing nitrogen levels from the discharges is necessary. To combat this increasing burden on our aquatic environment, increasingly strict regulation on pollution discharge is being implemented by various governmental bodies, with focus primarily on waste reduction (Chan Y.J. et al., 2009).

The removal of total nitrogen (TN), i.e., ammonium, nitrate, and nitrite, is an increasingly important goal for municipal and industrial wastewater treatment plants. The typical, conventional nitrogen cycle is shown in Fig.1.

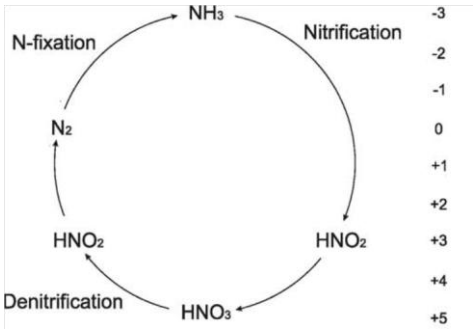


Fig.1. Nitrogen cycle - Classical N-cycle (Young H.A, 2006)

Practical and cost-effective technologies are needed, especially for older plants with limited space for expansion. Nitrogen compounds can be removed from wastewater by a variety of physicochemical and biological processes. Biological nitrogen removal is more effective and relatively inexpensive; it has been widely adopted in favour of the physicochemical processes (Young H.A, 2006).

Anoxic–aerobic systems have been found to perform well in sequential nitrogen removal including aerobic nitrification and anoxic denitrification.

These advantages have prompted the rapid development of anoxic–aerobic systems in the treatment of both industrial wastewater and municipal wastewater, designed for nutrient removal.

Nitrogen compounds are found in high concentrations in the wastewaters of many industries, while the average nitrogen composition in domestic sewage is approximately 60% ammonia, 40% organic nitrogen, with trace amounts of nitrite and nitrate (less than 1% – Barnes and Bliss, 1983, cited in William et al., 2000).

Aerobic treatment of highly nitrogenous compounds results in the mineralization of the organic nitrogen compounds present and the formation of ammonia by microorganisms. Under appropriate conditions, ammonia is further oxidized to nitrite and finally to nitrate. A variety of factors govern the latter process, pH, aeration, and SRT being the most important. In order to obtain an acceptable effluent, the oxidized nitrogenous products formed generally must be partly or totally eliminated (Voets et al., 1975).

At present, the combination of biological nitrification and denitrification is the most economic process to accomplish nitrate removal from wastewater (Khin et al., 2009).

The conventional biological nitrogen removal (thus, nitrification and denitrification) proceeds slowly due to low biological nitrification activity and yield. The process is generally performed on wastewater containing relatively low nitrogen concentration (of the order of up to 100 mg L^{-1}). In addition, the operational control of aerobic and anaerobic conditions needed for nitrification and denitrification, respectively, can be difficult.

2.2 Nitrification Process

Nitrification implies a chemolithoautotrophic oxidation of ammonia to nitrate under strict aerobic conditions and is conducted in two sequential oxidative stages:

- Ammonia to nitrite (ammonia oxidation) and
- Nitrite to nitrate (nitrite oxidation).

Each stage is performed by different bacterial genera which use ammonia or nitrite as an energy source and molecular oxygen as an electron acceptor, while carbon dioxide is used as a carbon source.

Equations for synthetic-oxidation using a representative measurement of yield and oxygen consumption for Nitrosomonas and Nitrobacter are as follows:

$$55NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow C_5H_7O_2N + 54NO_2^- + 57H_2O + 104H_2CO_3 \quad (1)$$

$$400NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195O_2 \rightarrow C_5H_7O_2N + 3H_2O + 400NO_3^- \quad (2)$$

By using equations (1) And (2), the overall synthesis and oxidation reaction in nitrification can be represented as follows:

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \quad (3)$$

In these equations, yields for Nitrosomonas and Nitrobacter are 0.15mg cells $mgNH_4\text{-}N^{-1}$ oxidized and 0.02mgcells $mgNO_2\text{-}N^{-1}$ oxidized, respectively. Oxygen consumption ratios in the equations are $3.16mgO_2$ $mgNH_4\text{-}N^{-1}$ oxidized and $1.11mgO_2$ $mgNO_2\text{-}N^{-1}$ oxidized, respectively. Also, it can be calculated that 7.07 mg alkalinity as $CaCO_3$ is required per mg ammonia-N oxidized. However, severe pH depression can occur when the alkalinity in the wastewater approaches depletion by the acid produced in the nitrification process. The significance of pH depression in the nitrification process is that the reaction rates are rapidly depressed as the pH is reduced below 7.0. Therefore, in cases where the alkalinity of the wastewater will be depleted by the acid produced by nitrification, the proper alkalinity must be supplemented by a chemical addition, such as lime (Young H.A, 2006).

2.2.1 Microbiology and Physiology of Nitrification

2.2.1a. Proteobacterial ammonia oxidizers

These ammonia oxidizing bacteria form two monophyletic groups, one within the beta- and one within the gamma-proteobacteria. They are generally considered as aerobic chemolithoautotrophs, but recently organic compounds have been described

that can serve them as carbon and energy source (Schmidt I. et al., 2003).The most commonly recognized genus of bacteria that carries out ammonia oxidation is Nitrosomonas; however, Nitrosococcus, Nitrosopira, Nitrosovibrio, and Nitrosolobus are also able to oxidize ammonium to nitrite. These ammonium oxidizers are genetically diverse, but related to each other in the beta subdivision of the Proteobacteria (Young H.A, 2006).

The beta-ammonia oxidizers comprise the well known genera Nitrosomonas and Nitrosospira, Nitrosococcus is the gamma-proteobacterial genus, but does not include Nitrosococcus mobilis, that is related to Nitrosomonas. Different members of these genera have been found to dominate different wastewater treatment plants or natural ecosystems, but general relationships between the ecological niche and evolutionary position are often still obscure. Salty wastewaters were found to be dominated by N. mobilis. The genome project of Nitrosomonas europaea nears completion. Although the relevance of this organism for wastewater treatment is disputable, it will still provide an invaluable source of information (Schmidt et al., 2003).

The physiology of conventional, 'aerobic' ammonia oxidizers is not completely understood. Only recently, it was discovered that these organisms also have an anaerobic metabolism. The proteobacterial ammonia oxidizers can obtain their energy for growth from both aerobic and anaerobic ammonia oxidation. Most likely ammonia (NH_3) and not ammonium (NH_4^+) is the substrate for the oxidation process. The main products are nitrite under oxic conditions and dinitrogen, nitrite and nitric oxide under anoxic conditions. Aerobic and anaerobic ammonia oxidation is initiated by the enzyme ammonia mono oxygenase (AMO) that oxidizes ammonia to hydroxylamine. Oxygen and dinitrogen tetroxide (dimer of NO_2) are the most likely electron acceptors for this enzyme (Eq. 4) (Schmidt et al., 2003).

$$NH_3+O_2+2H^++2e^-\rightarrow NH_2OH^++H_2O\ (\Delta G^o\text{-}120\ kJ\ mol^{-1}) \quad (4)$$

$$NH_3+N_2O_4+2H^++2e^-\rightarrow NH_2OH+2NO+H_2O\ (\Delta G^o\text{-}140\ kJ\ mol^{-1}) \quad (5)$$

The hydroxylamine resulting from ammonia oxidation is further oxidized to nitrite (Eq.7) by the hydroxylamine oxidoreductase (HAO).

$$NH_2OH+H_2O\rightarrow HNO_2+4H^++4e^-\ (\Delta G^o\text{-}140\ kJ\ mol^{-1}) \quad (6)$$

The four reducing equivalents derived from this reaction enter the AMO reaction (Eqs.4 and 5), the CO_2 assimilation, and the respiratory chain. The reducing

equivalents are transferred to the terminal electron acceptors O_2 (oxic conditions) or nitrite (anoxic conditions).The reduction of nitrite under anoxic conditions leads to the formation of N_2 resulting in the N-loss of 45 ± 15%. Under anoxic conditions the ammonia oxidation activity is relatively low (2.5 nmol NH_3 (g protein)$^{-1}$ min^{-1}).The doubling time is about 30 days at best and the biomass yield is 0.13 N 0.019 g dry weight (g NH_3-N)$^{-1}$.The Ks value for the substrate ammonia is about 20 µM at pH values between 6.7 and 8.3. These organisms are reversibly or irreversibly inhibited by various carbon compounds (Schmidt et al., 2003).

In contrast to aerobic ammonia oxidation, ammonia oxidation under anoxic conditions is not inhibited by acetylene. In the presence of oxygen, the produced NO can be oxidized to NO_2.Therefore, only small amounts of NO are detectable in the gas phase of N.eutropha cell suspensions. N_2O_4 is the oxidizing agent also under oxic conditions. Hydroxylamine and NO are produced as intermediates. While hydroxylamine is further oxidized to nitrite (Eq.6), NO is (re)oxidized to NO_2 (N_2O_4) (Eq.7) (Schmidt I. et al., 2003).

$$2NO+O_2 \rightarrow 2NO_2\,(N_2O_4) \qquad (7)$$

Recently, a model was developed to explain the role of NO_x in the metabolism of the ammonia oxidizers. Under oxic conditions (> 0.8 mgO_2 L^{-1}) aerobic nitrifiers convert ammonia to nitrite. At an oxygen concentration below 0.8 mgO_2 L^{-1} they use small amounts of the produced nitrite as terminal electron acceptors producing NO, N_2O, and N_2. In the absence of nitrogen oxides, up to 15% of the converted ammonia can be denitrified. N. eutropha was shown to nitrify and simultaneously denitrify under fully oxic conditions in the presence of NO_2 or NO. Interestingly, there is no fixed stoichiometry measurable between ammonia and NO_2 (NO) consumption under oxic conditions. The ratio of ammonia to NO_x consumption range between 1000:1 and 5000:1.Obviously, nitrogen oxides have a regulatory function in the metabolism of nitrifiers under oxic conditions, stimulating the denitrification activity (Schmidt et al., 2003).

Influenced by nitrogen oxides, ammonia oxidizers convert ammonia to gaseous dinitrogen (about 60% of the converted ammonia) and nitrite (just about 40% of the converted ammonia). The specific aerobic ammonia oxidation activity is stimulated by NO_2, with values increasing from 33 µmol NH_3 (g protein)$^{-1}$ min^{-1} without NO_x

addition to 280 µmol NH_3 (g protein)$^{-1}$ min^{-1} and a denitrification activity of 150 µmol NO_2^- (g protein)$^{-1}$ min^{-1} in the presence of 50 ppm NO_2. The biomass yield and the affinity for ammonia remain unchanged. Control experiments with N. europaea and Nitrosolobus multiform have yielded similar results. The reaction mechanism is the same, but the activities vary. Nitrogen oxides are toxic for many other microorganisms (nitrite oxidizers, heterotrophic bacteria) (Schmidt et al., 2003).

Reducing the cell number and the activity of the nitrite oxidizers by adding NO_x can be desirable in wastewater treatment, because the nitrite formed by the ammonia oxidizers is not further oxidized to nitrate (i.e. nitrite oxidizers). This is important since the nitrite is needed for the denitrification by the ammonia oxidizers (Schmidt et al., 2003).

2.2.1b. Aerobic nitrite oxidizers

The second step of nitrification, the oxidation of nitrite to nitrate, is performed by nitrite oxidizing bacteria, in the nitrite oxidation stage, several genera such as Nitrospira, Nitrospina, Nitrococcus, and Nitrocystis are known to be involved. However, the most famous nitrite oxidizer genus is Nitrobacter, which is closely related genetically within the alpha subdivision of the Proteobacteria (Young H.A, 2006). The two genera Nitrobacter, Nitrococcus are part of the alpha-proteobacteria, while Nitrospira is phylogenetically unrelated to any other cultivated species and forms a separate division. Several strains of Nitrobacter and one strain of Nitrospira are the only nitrite oxidizers that are not restricted to marine environments. There is some evidence that Nitrospira is the more specialized nitrite oxidizer. The other genera are more versatile, being facultative autotrophs and anaerobes, able to grow on heterotrophic substrates such as pyruvate and also capable of the first step of denitrification (the reduction of nitrate to nitrite).It appears that the genomes of nitrite oxidizers will not become available in the near future (Schmidt I. et al., 2003).

As mentioned above, nitrite oxidizers are often more versatile than ammonia oxidizers. When growing autotrophically with nitrite, the biomass yield is 0.036 g dry weight (gNO_2-N)$^{-1}$, at a maximum growth rate of 0.04h^{-1}. The apparent activation energy of nitrite oxidation is 44kJ mol^{-1}. Like the ammonia oxidizers, these bacteria can have high substrate affinities (around <70µM for nitrite and <25 µM for

oxygen).It has been reported that hydroxylamine, ammonia and NO can inhibit nitrite oxidizers, but a mechanism for such inhibitions has not yet been proposed.

The key enzyme of nitrite oxidizing bacteria is the membrane bound nitrite oxidoreductase which oxidizes nitrite with water as the source of oxygen to form nitrate. The electrons released from this reaction are transferred via a- and c-type cytochromes to a cytochrome oxidase of the aa_3-type. However, the mechanism of energy conservation in nitrite oxidizers is still unclear.

Thus, NADH is thought to be produced as the first step of energy conservation. Nitrite oxidizers are generally lithoautotrophic organisms. Higher growth rates are obtained when the cells are growing mixotrophically. Several strains of Nitrobacter are capable of heterotrophic growth under oxic as well as anoxic conditions.

Heterotrophic growth is significantly slower than lithoautotrophic growth, although 10-50-fold higher cell densities are obtained. Some strains of Nitrobacter were shown to be denitrifying organisms as well.

Since the oxidation of nitrite is a reversible process, the nitrite oxidoreductase can reduce nitrate to nitrite in the absence of oxygen. Nitrite oxidation occurs obligatory under oxic conditions. The involved organisms are much more sensitive to oxygen limitation than ammonia oxidizers are. Already at dissolved oxygen concentrations of about 0.5mg L^{-1} nitrite oxidation is completely inhibited. Additionally, Nitrobacter is inhibited at high oxygen concentrations. Thus, the oxygen content of a nitrite oxidizing nitrification vessel has to be maintained carefully to avoid accumulation of nitrite. With sufficient oxygen supply nitrite oxidation proceeds at a faster rate than conversion of ammonia to nitrite, therefore, high nitrite concentrations are found neither in natural environments nor in wastewater treatment plants (Schmidt et al., 2003).

2.3 Denitrification Process:

As the second step, denitrification is generally performed by a heterotrophic bioconversion process under anaerobic (anoxic, precisely) conditions. The oxidized nitrogen compounds (NO_2^- and NO_3^-) are reduced to gaseous dinitrogen by heterotrophic microorganisms that use nitrite and/or nitrate instead of oxygen as electron acceptors and organic matter as carbon and energy source.

Denitrifiers are common among the Gram-negative alpha and beta classes of the Proteobacteria, such as Pseudomonas, Alcaligenes, Paracoccus, and Thiobacillus. Some Gram-positive bacteria (such as Bacillus) and a few halophilic Archaea (such as Halobacterium) are able to denitrify. The process in environmental biotechnology is accomplished with a variety of electron donors and carbon sources such as: methanol, acetate, glucose, ethanol, and a few others (Table 5). Because methanol (CH_3OH) was relatively inexpensive, it gained widespread use. Combined dissimilation synthesis equations for denitrification using methanol as an electron donor are as follows:

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7O_2N + 0.47N_2 + 1.68H_2O + HCO_3^- \quad (8)$$

In these equations, the theoretical methanol requirement for nitrate is 2.47mg CH_3OH per mgNO_3-N. Neglecting synthesis, the requirement is decreased to 1.9. Eqs.(9) and (10) can be used for the calculation of methanol requirements for nitrite reduction and deoxygenation to allow a combined expression to be formulated for the methanol requirement (Young H.A, 2006).

$$NO_2^- + 0.53CH_3OH + 0.67H_2CO_3 \rightarrow 0.04C_5H_7O_2N + 0.48N_2 + 1.23H_2O + HCO_3^- \quad (9)$$

$$O2 + 0.93CH_3OH + 0.056NO_3^- \rightarrow$$

$$0.056C_5H_7O_2N + 1.04H_2O + 0.59H_2CO_3 + 0.056HCO_3^- \quad (10)$$

Generally denitrification is nearly exclusively a facultatively anaerobic or microaerophilic trait. With some exceptions, there are no examples where denitrification occurs in an obligate anaerobic bacterium. The complete denitrification at high dissolved oxygen (DO) concentrations was first observed in Paracoccus species. In batch culture experiments, Thiosphaera pantotropha which is an autotrophic bacterium produced dinitrogen gas from ammonium and/or nitrite and nitrate in well-mixed aerobic cultures.

It was also observed that Pseudomonas stutzeri SU2 which was isolated from the activated sludge of a sequencing batch reactor treating piggery wastewater rapidly reduced nitrate to nitrogen gas without nitrite accumulation under aerobic conditions. Oguz stated that in batch and complete mixed reactor with activated sludge 0.07 kgN $m^{-3}d^{-1}$ of nitrogen conversion and 56.7% removal rate were observed producing most equivalent amounts of N_2 and N_2O. Bacteria which co respire nitrate and oxygen are

widespread in the environment. The aerobic denitrification process may offer several potential advantages because

- It occurs directly in aerated bioreactors under the presence of readily biodegradable organics (Young H.A, 2006).

Table 2: Organic Requirement in Heterotrophic denitrification (Young H.A, 2006)

	Carbon Source	Organic requirement ($gCOD\ gN^{-1}$)
NO_2-N	Acetic acid	1.56
	Acetic acid	2.0
	Lactic acid	2.8
	Methanol	2.3
NO_x-N	Raw sewage	5.2
	Piggery waste	8.44
	Acetate	2.07
	Methanol	4.2
	Piggery waste	6.42
NO_3-N	Methanol	2.1-2.6
	Acetic acid	2.08
	Acetic acid	3.7
	Lactic acid	4.1
	Methanol	3.75-4.5
	Methanol	7.35

Biological denitrification enables the transformation of oxidized compounds by a wide spectrum of heterotrophic bacteria that convert nitrate to harmless nitrogen gas. The necessary condition for denitrification to take place in activated sludge systems is the presence of a facultative microbial mass. These organisms are characterized by the fact that they can use either oxygen or nitrate as an oxidant for organic matter.

2.3.1 Microbiology and Physiology of Denitrification

Many common denitrifiers found in activated sludge systems appear to be capable of heterotrophic denitrification, which appears to occur simultaneously with nitrification. Earlier workers have reported the effectiveness of several heterotrophic bacteria in denitrification. Common bacteria genera that have been reported to be denitrifiers in activated sludge systems include Achromobacter, Aerobacter, Alcaligenes, Bacillus, Brevibacterium, Denitrobacillus, Flavobacterium, Lactobacillus, Micrococcus, Brevibacterium, Pseudomonas, Spirillum, Proteus, Xanthomonas, Staphylococcus and Paracoccus.

Although there is a lack of data on the role of protozoa in nitrogen removal in activated sludge systems, studies carried out in aquatic ecosystems have shown that ciliates and phagotrophic microflagellates regenerate and mineralize nitrogen in large quantities while grazing.

Wastewater denitrification describes the use of NO_3^- or NO_2^- ions by denitrifiers to degrade carbonaceous biological oxygen demand (cBOD). Most denitrifiers are facultative anaerobic-heterotrophs that transfer redox equivalents from the oxidation of a carbon source to an N-oxide under anaerobic conditions. The modular organization of denitrification respiratory systems utilizing NO_3^-, NO_2^-, NO and N_2O is shown in Fig. 2

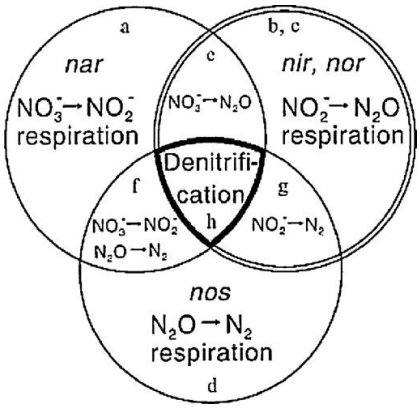


Fig.2: Modular organization of denitrification (four modules representing the respiratory systems utilizing (a) NO_3^-, (b) NO_2^-, (c) NO and (d) N_2O. Complete denitrification (h) is achieved only when all four modules are activated. Pair wise overlaps (e–g) of the individual respiratory modules occur naturally in denitrifying or other N oxide-utilizing bacteria (Kumar and Lin, 2010).

In addition, the overall energy yielding (catabolism or dissimilation) and cell synthesis (anabolism or assimilation) reactions of denitrification in the presence of acetic acid are shown as Eqs. (11) and (12), respectively. The hydroxyl ion (OH^-) and some of the carbon dioxide (CO_2) produced during denitrification are returned in the system as alkalinity (Eq. (12).

$$1.6NO_3^- + CH_3COOH \rightarrow 0.8N_2 + 2CO_2 + 1.2H_2O + 1.6OH^- \ (\Delta G^\circ = -843 kJ\ M^{-1}) \quad (11)$$

$$1.2NO_3^- + CH_3COOH + 0.1NH_4^+ \rightarrow 0.1C_5H_7O_2N + 0.6N_2 + 1.5CO_2 + 1.1OH^- + 1.3H_2O \quad (12)$$

Denitrifying bacteria degrade cBOD in the absence of free molecular oxygen to obtain energy for cellular activity and carbon for cellular synthesis under a redox potential range from +50 to −50 mV. Most denitrifiers reduce NO_3^- via NO_2^- to molecular nitrogen without accumulation of intermediates. Four enzymes are involved in a complete denitrification system, i.e. reduction of NO_3^- to N_2. The reduction of NO_3^- to NO_2^- is catalyzed by the enzyme nitrate reductase (Nar).

This is a membrane-bound molybdenum–iron–sulphur protein that is found in denitrifiers as well as in other dissimilatory nitrate reducing organisms.

Both the synthesis and activity of nitrtate reductase are inhibited by oxygen. The second enzyme in this pathway is nitrite reductase (Nir), which catalyzes the conversion of NO_2^- to N_2O. Nitrite reductase is unique to denitrifying organisms, which is found in the periplasm.

Nitric oxide reductase (Nor), a membrane-bound protein, is the third enzyme in the pathway, catalyzing the conversion of N_2O to NO. Nitrous oxide reductase (Nos), a periplasmic copper-containing protein, is the last enzyme in the pathway and converts NO to N_2.

Both the synthesis and activity of all four denitrification enzymes are controlled by oxygen. Nitrous oxide reductase is the most sensitive denitrification enzyme and it is inhibited by DO concentrations less than 0.2mg L^{-1}. However, some denitrifiers lack key enzyme systems to denitrify completely, and the lack of these enzyme systems can allow the production and accumulation of free intermediates.

Organisms with the capability of denitrification belong to a variety of groups and encompass a wide range of physiological traits. Many genera of denitrifying bacteria

can use $NO_3^-$and NO_2^- to degrade cBOD, some genera such as Enterobacter and Escherichiacan use only NO_3^-. On the other hand, some genera of denitrifying bacteria including Thiosphaera pantotropha or Paracoccus denitrificans, Magnetospirillum magnetotacticum and Pseudomonas stutzeri SU2 can denitrify under aerobic or microaerophilic conditions.

In addition, Nitrosomonas-like microorganisms including Bacillus cereus, Bacillus subtilisand Bacillus licheniformis, nitrify and denitrify simultaneously even under fully oxic or anoxic condition with N_2 as main final product, which has been reviewed previously.

Nitrosomonas eutropha is an obligate lithoautotrophic nitrifying bacterium and also a denitrifying organism that uses hydrogen as the electron donor and nitrite as the electron acceptor. The denitrification activity of N.eutropha could be stimulated by adding gaseous nitrogen oxide under anaerobic conditions. Mostly, the denitrifying nitrifiers are detected in a bioreactor operating with the coupling of aerobic and anaerobic ammonia oxidation; for example, SNAP (Kumar and Lin, 2010).

To distinguish between the productions of nitrites, nitrates, nitrites plus nitrates, the terms nitrification, nitrification, nitrification were used respectively. Similarly, denitritation, denitratation, and denitrification refer to the reduction of nitrites, nitrates, and nitrites plus nitrates to nitrogen gas, respectively. This nomenclature was proposed by Anthonisen et al. (1976).

2.4 Simultaneous Nitrification and Denitrification:

Simultaneous nitrification–denitrification (SND) can occur in continuously-fed MBR system by cyclical (on/off) aeration. Under low DO, diffusional limitations may create an anoxic zone within the biological floc where denitrification can take place (Pochanna et al., 1999). Furthermore, if SND is achieved through the shortened pathway, i.e. through nitrites, it is advantageous over conventional nitrogen removal processes.

Advantages of SND via nitrite are reduced aeration, COD, alkalinity requirements and lower biomass yield. Most of the SND studies have been conducted in sequential batch reactors (SBR), continuous flow extended aeration plants, and oxidation ditches (Holakoo L. et al., 2007).

The factors that affect SND are primarily ambient DO concentration and floc size (Pochanna et al., 1999). Pochanna et al. (1999) in their intermittently aerated SBR observed 52% N removal through SND with a median floc diameter of 80 lm and only 21% SND at a floc size of 40–50 lm at similar DO during the aerobic period (0.3– 2.5 mg L^{-1}). Floc size is influenced by many factors including sludge age, aeration intensity and shearing (Galil et al., 1991) (Holakoo L. et al., 2007).

Floc sizes in MBRs are reported to be smaller than in conventional activated sludge (CAS) despite high operating mixed liquor suspended solids concentration (MLSS). However because of the relatively low a values (ratio of oxygen transfer coefficient in mixed liquor ''Kl_a'' to oxygen transfer coefficient in water ''Kl_a'') of 0.2–0.4 at high MLSS concentration (11– 16 g L^{-1}), operation of MBR at low DO values can reduce aeration requirements and may also be conducive to SND (Holakoo L. et al., 2007).

The SND studies in MBRs reported so far are all in anoxic/oxic (A/O) systems operating under intermittent aeration mode. To our knowledge, there are very limited studies on the achievability of SND in continuously aerated MBRs (Holakoo L. et al., 2007).

2.5 Shortcut Nitrification-Denitrification:

The removal of nitrogen from wastewater has become one of the most important concerns in water pollution control. Biological nitrification–denitrification is commonly used for nitrogen removal from wastewater. The conventional treatment processes are achieved either by separate aerobic and anoxic reactors or by temporal division of systems such as the sequencing batch reactor (SBR). However, there were many problems associated with traditional nitrification–denitrification processes, with the most outstanding problems of high construction investment, operational cost and unstable performance (Gao et al., 2009).

In order to reduce the operational cost and enhance nitrogen removal efficiency, several studies have been carried out to shorten nitrification and denitrification processes by inhibiting the activity and growth of Nitrobacter (NOB) in phase II nitrification (Verstraete and Philips, 1998). As the consequence, both nitrification

and denitrification processes were shortened, with NH_4^+ oxidized to NO_2^- and NO_2^- directly reduced to nitrogen gas.

Compared with traditional nitrification–denitrification, shortcut nitrification – denitrification via NO_2^- has several advantages, as it:

- saves organic carbon and alkalinity,
- shortens reaction time and
- Reduces the amount of excess sludge production (Gao D., et al., 2009).

However, due to the rapid conversion of NO_2^- to NO_3^- by nitrite oxidizing bacteria (NOB), there is rarely successful application of shortened nitrogen removal via NO_2^-. For example, even though the nitrosation ratio (NO_2^- –N/NO_x^-–N) is beyond 96%, and (NO_3^-) is lower than 1 mg L^{-1}, there are still NOB present in treatment systems (Wang et al., 2004), which could gradually turn short-cut nitrification to full nitrification when operational condition (e.g.: long aeration time) become favorable for the growth of NOB (Gao et al., 2009).

Gao et al. (2009) reported the results of several studies: some have used temperature, dissolved oxygen (DO) or pH to control shortcut nitrification–denitrification. Others achieved DO control for shortcut nitrification at 25°C using substrate containing 80mg L^{-1} of NH_3–N. DO of 0.5mg L^{-1} had no adverse effect on NH_4^+ oxidation, but inhibited NO_2^- oxidation with the accumulated (NO_2^-) almost 60mg L^{-1}. Others found that NO_2^- oxidization was inhibited at pH greater than 7.5 in submerged biofilters, and the selective inhibition of NOB resulted in a significant accumulation of NO_2^- at the specific inhibitory concentrations greater than 1.5 mgNH_3free–N g^{-1}_{VS}.

However, the short-cut nitrification–denitrification controlled by temperature and pH usually requires higher temperatures (30–40°C) and pH values (pH 7–8) (such as SHARON, Single reactor system for high activity ammonia removal over nitrite, Hellinga et al. (1998). Therefore, it is critical to achieve shortcut nitrification–denitrification at neutral pH value (pH 6–8), normal temperature (25–27°C) and DO (2–5 mg L^{-1}) in order to make it easy to operate (Gao et al., 2009).

Several parameters (such as ORP, pH) have been studied for real-time control for nitrification/denitrification. Oxidation–reduction potential (ORP) is the electromotive force developed when oxidizers or reducers are present in aqueous solution. Compared with DO-based regulation, ORP regulation is more in tune with the

process dynamics, since many biological substances correlate with ORP values. In addition, protons generated in nitrification lowered pH values. So the (ORP) and pH have been commonly studied for real-time monitoring and control of activated sludge process (Gao et al., 2009).

Excess aeration was observed as a key factor to convert shortcut nitrification to full nitrification. The results showed that after excess aeration for 13 cycles, a shortcut nitrification with nitrosation ratio of 96% converted to full nitrification with nitrosation ratio of 29%.

A stable shortcut nitrification lasted for two months at room temperature (25 ± 0.5^{o}C), with ORP and pH as real-time control parameters, and the nitritation ratio was more than 96%. This good stability demonstrated that real-time control could not only avoid the negative impact of excess aeration, but also maintain shortcut nitrification stable (Gao et al., 2009).

Table-3: Advantages and Disadvantages of Biological Nitrogen Removal-Suspended Growth

Advantages	Disadvantages
• Low capital and operating cost. • Reduction of aquatic toxicity. • Operational flexibility. • Reduction in sludge production. • Reduction in filamentous growth. • Improved sludge settleability. • Improved sludge dewatering. • Reduction in oxygen requirement.	• Inhibition from non-biodegradable compounds. • Slow digestion rates. • Large storage tanks requirement. • Provision of enabling environment for survival of microorganisms.

2.6 Biological fixed film Systems (Fitch et al., 1998)

Biological fixed film systems offer several advantages when compared to Activated-sludge process such as

- Handling convenience,
- Little residual sludge and
- Ease of use in small scale treatment.

- Capacity to handle shock loads.

Biological nitrogen removal has become common because of its low cost and high efficiency as compared to physical and chemical treatment (van Dongen et al., 2001). However, nitrogen removal by biological processes is seldom used in wastewater with high NH_4^+ but low carbon content (Noophan P. et al., 2009).

Microbial cell aggregates, such as flocs and biofilms, are of great interest in biotechnology.

They offer advantages, with respect to suspended single cells, in downstream processing, by facilitating cell–liquid separation by sedimentation or filtration. The term floc is used to refer to an assemblage of individual cells and micro-colonies occurring under specific reactor conditions or after addition of various agents to the medium (Boonaert et al., 1999, cited in Nicolella et al., 2000).

A biofilm can be defined as a complex coherent structure of cells and cellular products, like extra-cellular polymers, which either form spontaneously as large, dense granules, or grow attached on a static solid surface (static biofilms) or on suspended carriers (particle supported biofilms; Nicolella et al., 2000).

Microbial aggregates (either in the form of biofilms, granules or flocs) and the bulk culture medium constitutes two distinct phases. This key feature has three major consequences (Nicolella et al., 2000):

1) Biomass retention can be used to improve reactor volumetric conversion capacity when the conversion is limited by the amount of biomass present. If no biomass retention is applied (e.g. in the standard chemostat), the biomass concentration depends only on the substrate concentration in the feed, and consequently large retention times are required in the presence of diluted feeds. Depending on the settling characteristics of the aggregates, biomass can be readily separated (e.g. by sedimentation) from the bulk liquid and retained in the bioreactor. In this respect, granules and particle-supported biofilms have an extra advantage in that they can be more easily separated than flocs (i.e. higher biomass concentration possible) and have a high reactor specific surface area (i.e. a large mass transfer area than static biofilms).
2) Substrates (e.g. oxygen, carbon and nitrogen sources) have to cross the aggregate–liquid interface and be transported through the aggregate to reach the

microbial cells and be consumed. This transport is in general by diffusion and results in a concentration gradient within the aggregate. The penetration depth of substrates in biofilms mainly depends on the porosity of the biofilm, substrate concentration in the bulk liquid, mass transfer at the biofilm–liquid interface and reaction rate in the biofilm. For poorly soluble substrate (e.g. oxygen) the penetration depth is shallow (typically 100–150 mm for oxygen) (Denac et al., 1983, cited in Nicolella C. et al., 2000).

3) Due to diffusional substrate concentration gradients, a growth rate gradient also exists within the aggregate. In multi-species biofilm systems this will lead to a biofilm with a layered structure, where the organisms with the highest growth rate will be found at the outside of the biofilm, whereas slower growing organisms will be found inside (Heijnen et al., 1989, cited in Nicolella C. et al., 2000). As a result of this organization, slower-growing organisms will be protected from external shear forces, and are less likely to be lost due to detachment and wash-out. In this case not the absolute maximum growth rate of the organisms should be considered but the maximum growth rate under conditions in the reactor (e.g. in the presence of an inhibitor) (Nicolella C. et al., 2000).

An extensive use of biofilm processes is made within the field of environmental biotechnology for three main reasons:

- Compared with most other industrial bioprocesses, large volumes of dilute aqueous solutions can be treated more easily than with suspended growth processes;
- Natural, mixed populations of microorganisms, which readily form biofilms, are used;
- The process can be operated at high biomass concentration in the reactor, without the need for settlers for biomass retention and recirculation; a polishing step of the effluent is usually needed to remove remaining suspended (detached) biomass.

2.7 Physical-chemical treatment: Ammonia stripping

Ammonia stripping means that the ammonium is first converted to slightly volatile gaseous ammonia that is readily soluble in water. This gas is then physically stripped off from the water. A balanced ammonium-to-ammonia ratio is a function of

temperature and pH (Jardin N. et al., 2006). At a pH of 10 and a temperature of 70°C, the dissociation equilibrium will be completely on the side of ammonia. That means that there is no ammonium in the water phase.

If this status is to be obtained at a temperature of only 20°C, a pH greater than 11 will be required (Jardin N. et al., 2006).

Subsequently, the ammonium dissolved in the water is to be transferred to the gas phase (desorption). For this process step, air and steam stripping in packed columns has proved to be the solution of choice in industrial-scale applications. Also desorption is temperature-dependent (Jardin N. et al., 2006).

Consequently, rising temperatures will accelerate the rate of reaction. That means on the other hand, the volumetric flow rate of the gas required for stripping will decrease if temperatures rise. But this eminent advantage compares with the unfavorably elevated energy demand typical of steam generation. Hence, large-scale application of steam stripping will only be economically viable at sites where sufficient steam is available, like, for example, in sewage sludge incineration or drying plants (Jardin N. et al., 2006).

Downstream of desorption, the ammonia stripped into the gas phase has to be converted to a recyclable or disposable product. For ecological reasons, the ammonia should not be directly released to the atmosphere, which as a matter of fact would hardly be permitted by the authorities. For large-scale applications, acidic scrubbing involving the production of ammonium sulphate and rectification to aqueous ammonia have made their way into practice as reliable options. By rectification, a 25- to 35%-aqueous ammonia is produced that can be readily reutilized in flue gas scrubbers (Jardin N. et al., 2006).

2.8 Different Reactor Configurations used in Conventional nitrogen removal Processes

As stricter environmental regulations are imposed, advanced and cost effective techniques for nitrogen removal from wastewater become more and more important. To cope with these problems, various kinds of bioreactors have been studied for enhancing the efficiency of nitrogen removal. Many modifications and processes had

been developed and implemented for nitrogen removal from wastewater. Basically, these processes for nitrogen removal can be classified as

- Suspended sludge and
- Fixed-film cultures.

The suspended sludge systems suffer from

- Sludge bulking,
- Large reactor volume required,
- Sensitive to shock loading.

While the fixed-film systems usually encounter

- Biofilm-associated clogging and
- Excessive sloughing problems.

Meanwhile, due to the sensitivity of nitrifying bacteria to environmental factors as well as their lower growth rates, it is difficult to obtain and maintain sufficient nitrifying biomass in conventional suspended or fixed culture-based wastewater treatment systems, while nitrification is the first step towards denitrification that converts nitrate and nitrite to nitrogen gas (Yang et al., 2003).

2.8.1 Fluidized Bed Reactor

Fluidized bed reactors are packed with mobile supports in which particles covered with biofilm are fluidized by the recirculation of liquid. They eliminate substrate diffusion limitations, which are usually inherent in stationary bed process. A schematic of a fluidized bed (FB) system is illustrated in Fig. 3

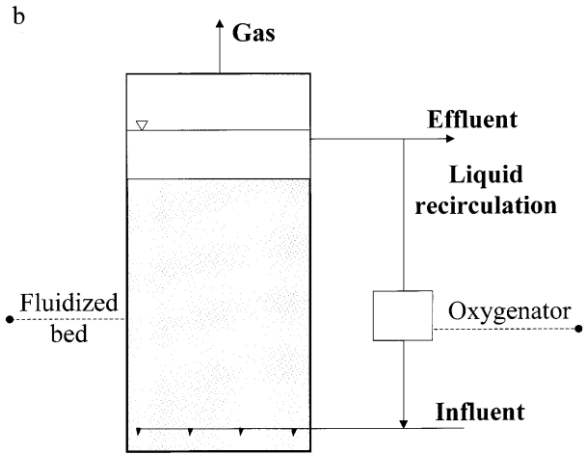


Fig.3. Schematic representation of a FB reactor (Nicolella C. et al., 2000)

The most common operational mode of an AFB reactor in wastewater treatment contains three phases:

- The discrete solid phase of inert particles with immobilized microbial cells,
- The discrete air bubbles and
- The continuous aqueous solution.

The AFB reactor exhibits numerous advantages such as

- A high biomass concentration,
- High OLR, Short HRT,
- No bed clogging,
- Small external mass transfer resistance and
- Large surface area for mass transfer.

Conversely, there are some problems which inhibit their applicability on a large industrial scale such as

- Control of the bed expansion,
- Thickness of the biofilm and
- Oxygen distribution system as well as
- High-energy consumption due to the very high liquid recirculation ratio.

2.8.2 Rotating biological contactors (RBC)

In a rotating biological contactor (RBC) system, microorganisms attach to an inert support medium and form a biological film. The support medium, with a sequential disc configuration, is partly or totally submerged and rotates slowly around a horizontal axis in a tank through which the wastewater flows.

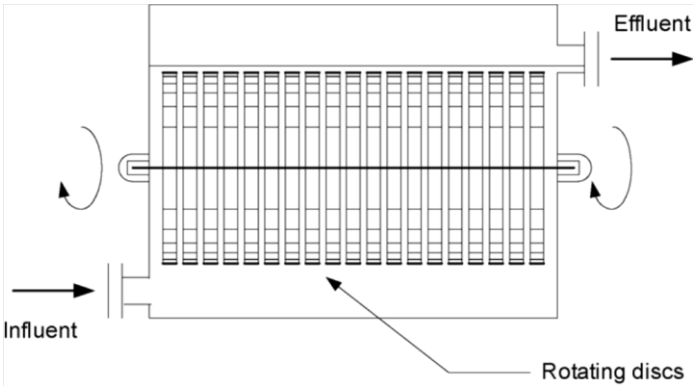


Fig.4. Schematic representation of an anaerobic RBC reactor (Chan et al., 2009)

Advantages of the RBC system are (Chan Y.J. et al., 2009)

- Low energy requirements, short retention time,
- Excellent process control, low operating costs and
- Capability of handling a wide range of flows.

Disadvantages include (Chan Y.J et al., 2009)

- Process performance is susceptible to wastewater characteristics, resulting in:
  - Limited operational flexibility to varying loading and operating conditions and
  - Frequent maintenance on its shaft bearings and mechanical drive units.

2.8.3 Baffled Reactors

In recent decades, anaerobic biological treatment of high-strength industrial wastewaters has become an established pollution control technology, and several anaerobic reactor configurations were available. Anaerobic baffled reactor (ABR) is known as a high-rate bioreactor (Hu et al., 2009).

Nitrogen removal via nitrification and denitrification is possible by combining aerobic and anoxic units with recycle; however, anoxic ammonium oxidation may become a promising alternative (van de Graaf et al., 1996). Potentially, the costs involved with having separate anaerobic and aerobic reactors can be greatly decreased by modifying an anaerobic baffled reactor (ABR) (William et al., 2000).

The has numerous advantages (William P.B et al., 2000) over other reactors and these include;

- Better resilience to hydraulic and organic shock loadings,
- Longer biomass retention times,
- Lower sludge yields.

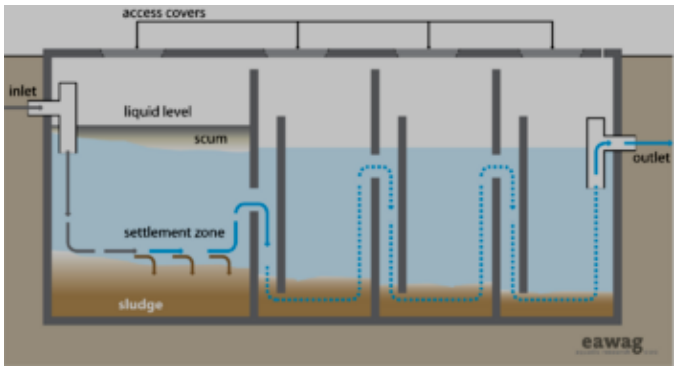


Fig.5: Schematic of Anaerobic baffled reactor.

2.8.4 Sequencing Batch Reactor:

Biological nitrogen removal by the use of Sequencing Batch Reactors (SBRs) is today an accepted and well proven model, (Morling, 2010). The development of tertiary treatments for internal flows from wastewater treatment plants (WWTP) has become very common. An example of this is the biological nitrogen removal (BNR) of the reject water from anaerobic sludge digesters (800-1000mg of NH_4^+-N L^{-1}).

In the absence of treatment it is re-circulated to the head plant due to its low flow rate. When developing tertiary treatment, the economic aspect is an important factor to consider (Gale et al., 2006).

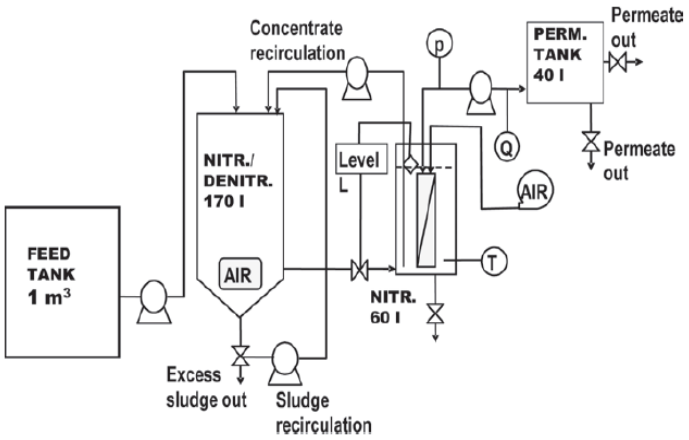


Fig.6: A flow sheet of used SBR and MBR equipment (Laitinen et al., 2006). Abbreviations: Nitr. = nitrification, Denitr. = denitrification, p = pressure, T = temperature, and Q = flow.

Sequencing batch reactors (SBR) is one of the most extended reactors to develop BNR for low flow rates. The main features of the SBR are

- flexibility and compactness and
- BNR via nitrite can be achieved by combining low DO and controlled pH range (Gale et al., 2006).

Leachate from sanitary landfills is a hazardous waste, especially as the deposit of solid waste was initially more or less uncontrolled, with little attention being paid to the separation of different refuse types. The use of Sequencing Batch Reactors (SBRs) soon became established based on the relatively small footprint that such a

treatment would require, and also the fact that the amount of leachate is normally rather limited.

SBR is a viable technology for leachate treatment, as long as the main objective is to reduce nitrogen and especially ammonia nitrogen (Morling S., 2010).

Compared to conventional aerobic wastewater treatment systems, the granulation system offers several advantages, such as (Yang S.F. et al., 2003)

- A denser and stronger microbial structure,
- Good settle ability, high biomass retention, and
- Ability to withstand high organic loading rate.

The aerobic granulation technology appears to have the potential to respond to the challenges of nitrogen removal from wastewater.

Microbial granules cultivated at different substrate N/COD ratios in SBRs are capable of simultaneously removing organic and nitrogen. It was found that heterotrophic, nitrifying, and denitrifying populations can co-exist in the granules, and shifts in microbial population in granules were closely related to the substrate N/COD ratio. The microbial granules developed at high substrate N/COD ratios exhibited enhanced nitrifying and denitrifying activities, while the activity of heterotrophic bacteria in granules showed a decreasing trend.

This is the advantage of microbial granules over conventional activated sludge, i.e.

- Different species may co-exist in the same microbial matrix, which provides a platform for bacteria to function synergically (Yang S.F. et al., 2003).

Compared to the conventional bioflocs, granules offer:

- Excellent settleability, which can ensure easy and effective separation of biosolids from the effluent (Yang S.F. et al., 2003),
- Dense microbial structure,
- High biomass retention and the ability to withstand a high organic loading rate (Yuan et al., 2010)

The advantages of the SBR process are (Chan Y.J. et al., 2009):

- Flexibility in the treatment of variable flows,
- Minimum operator interaction,
- Option for aerobic or anaerobic conditions in the same tank,

- Good oxygen contact with microorganisms and substrate,
- Small floor space and good removal efficiency.

These advantages justify the recent increase in the implementation of this process in industrial and municipal wastewater treatment.

2.8.5 Fixed-film bioreactor (FFB) system:

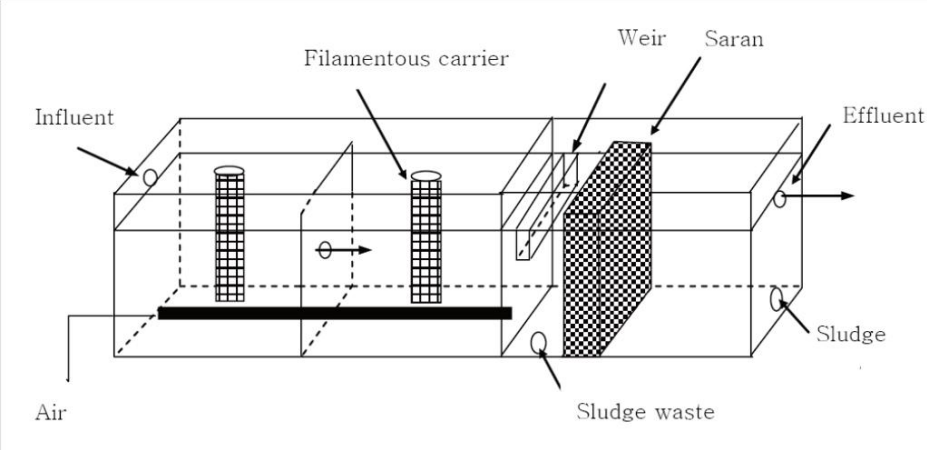


Fig.7. A schematic of a fixed-film system (Ahmed et al. 2009)

Immobilized cells on the surface (fixed-film) of the media offer some advantages over cultures in suspension such as;

- A greater variation in population;
- Less sensitivity to environmental variations (temperature, pH, and toxic substances);
- Higher growth rate;
- Faster utilization of the substrate in relation to free biomass.

This is attributed to physiological modification of the fixed cells undergo, due to either the increase in the local concentration of nutrients and enzymes, or the selective effect of the extracellular polymeric matrix in relation to inhibitory or toxic substances (Chan et al., 2009).

Among the biological processes for leachate treatment, fixed film bioprocesses offer some advantages compared to the suspended growth systems such as (Ahmed et al., 2010)

- Lower hydraulic retention time,
- Higher biomass retention time,

- Higher volumetric conversion rates,
- Higher resistance to toxic agents,
- Lower sensitivity to temperature, and
- Less sludge production rate.

2.8.6 Membrane Bioreactor:

Membrane bioreactor (MBR) technology, which combines biological-activated sludge process and membrane filtration has became more popular, abundant, and accepted in recent years for the treatment of many types of wastewaters, whereas the conventional activated sludge (CAS) process cannot cope with either composition of wastewater or fluctuations of wastewater flow rate.

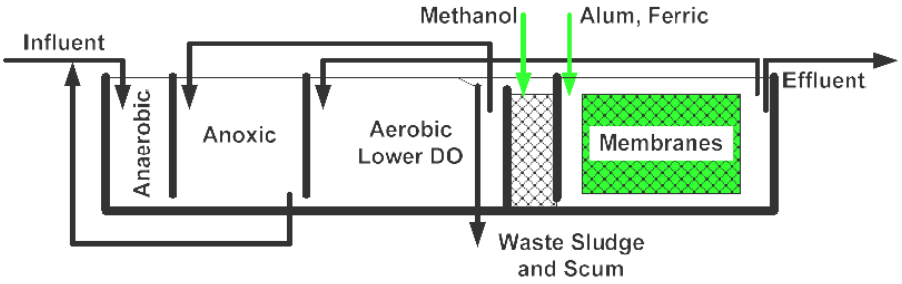


Fig.8. MBR Configurations with attached growth second anoxic zone.

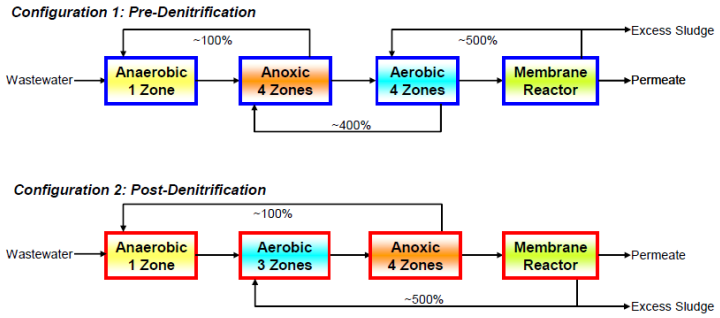


Fig.9. MBR Configurations for nitrogen removal (Gnriss, 2006)

The limiting step in the conventional treatment is the separation of sludge from the treated water. Without good sedimentation in the secondary settler, parts of the sludge end up in treated water which leads to poor efficiency of the treatment process. Sedimentation of the sludge is influenced by the characteristics of the microbial flocs as a function of their physiological state.

In order to overcome the limitations of ASP, MBR technology can be successfully employed to treat wastewater in conditions which do not allow successful sedimentation of activated sludge. Since an MBR uses membrane filtration instead of sedimentation to separate bacteria from the treated water, biomass concentration within the bioreactor can be maintained at a much higher level, thus reducing the size of the bioreactor (Radjenovi J. et al., 2008).

MBR technology is also used in cases where demand on the quality of effluent exceeds the capability of CAS. Although MBR capital and operational costs exceed the costs of conventional process, it seems that the upgrade of conventional process occurs even in cases when conventional treatment works well. It can be related with increase of water price and need for water reuse as well as with more stringent regulations on the effluent quality. Along with better understanding of emerging contaminants in wastewater, their biodegradability, and with their inclusion in new regulations, MBR may become a necessary upgrade of existing technology in order to fulfill the legal requirements in wastewater treatment plants (WWTPs) (Radjenovi J. et al., 2008).

Advantages are the possibilities to work at high biomass concentrations (concentrations up to 35 gSS L^{-1} are feasible) and at temperatures of 35 to 40^{o}C which is often the optimum for biological processes.

However one of the major drawbacks of MBRs is membrane fouling and subsequent membrane cleaning and associated costs. Membrane fouling has been related to sludge concentration (Germain et al., 2005, cited in Holakoo L. et al., 2007), supernatant chemical oxygen demand (COD) (Nuengjamnong et al., 2005, cited in Holakoo L. et al., 2007) and colloidal matter in the 0.01–1.0 lm range (Holakoo L. et al., 2007).

MBRs offer numerous advantages which include

- The high quality of the effluent,
- The separation of solid retention time (SRT) from HRT,
- The reduced sludge production due to endogenous respiration in long SRT,
- Low sludge loading rate.

2.8.7 Membrane Aerated Biofilm Reactor:

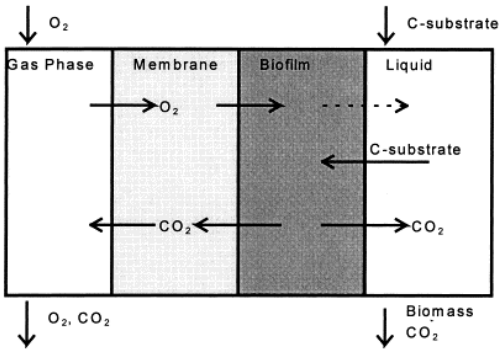


Fig.10. A schematic of MABR (E. Casey et al. 1999)

Another approach to TN removal is through membrane aerated bioreactors, which use oxygen-supplying, biofilm-supporting membranes in a non-aerated, well-mixed tank. Oxygen is supplied at the base of the biofilm, producing aerobic conditions deep in the biofilm and anoxic conditions in the outer biofilm. Nitrifying bacteria grow in the deep, aerobic portions of the biofilm where BOD concentrations are low, and heterotrophic denitrifying bacteria grow in the outer, anoxic portions where they use nitrate or nitrite as electron acceptors (Downing et al., 2008).

More accurate control of oxygen supply flux can be achieved by using a membrane-aerated bio film reactor (MABR). MABR, as one of the most promising membrane bioreactors for wastewater treatment, has been intensively investigated and developed in the last decade for treating domestic and industrial wastewaters, especially for its nitrogen removal performance (Wang et al., 2011). In an MABR, biofilm is naturally immobilized on an oxygen permeable membrane. Oxygen diffuses through the membrane into the biofilm where oxidation of pollutants supplied from the biofilm-liquid interface takes place. This means that oxygen and pollutants are supplied from opposite sides of the biofilm (Wang R. et al., 2011).

MABRs achieving TN removal were shown to have low levels of nitrate in the biofilm (Hibiya et al., 2003 cited in Downing et al., 2008), and an oxygen mass balance indicated that the majority of oxygen transferred to a MABR was used for ammonia oxidation, with little oxygen remaining for nitrite oxidation (Downing et al., 2008).

A major challenge with membrane aeration for wastewater treatment is biofouling. In MABRs used for TN removal, the biofilm can include 60% heterotrophic biomass resulting in thick biofilms, with mass transfer limitations. The effect is exacerbated at higher BOD: N loading ratios, significantly decreasing nitrification rates due to increased competition between heterotrophic and nitrifying bacteria in the biofilm (Downing et al., 2008).

Advantages of the MABR process include (Downing et al., 2008)

- Passive aeration,
- Providing energy savings of up to 70%;
- Reduced tank volume; elimination of internal water recycle; and
- Maximized use of influent BOD for denitrification.

2.8.8. Redox Stratified Membrane Biofilm Reactor

RSMBR has several advantages over conventional nitrogen removal technologies, such as

- Higher oxygen transfer and utilization efficiencies with consequent energy savings and cost reduction;
- Reduced emission of volatile pollutants by air stripping;
- Protection of AOB and NOB located at the base of biofilm from erosion or grazing at the biofilm surface.

The most significant advantage for MABRs over conventional biofilm reactors is the controllability of oxygen flux supplied to the biofilm.

The oxygen to nitrogen supply ratio was found to be a key regulator for biofilm short-cut nitrogen removal performance, based on the results of sensitivity analysis of MABs and conventional biofilms (Wang et al., 2011).

Attached growth on surfaces of support materials has many advantages as compared to suspended growth in flocs or granules, for instance (Sudarno et al., 2011)

- A long sludge retention time,
- Prevention of washout of biomass and
- Better process stability in terms of withstanding shock loadings or short-term disturbing effects (Nogueira et al., 1998).

Table 4: Advantages and Disadvantages of particulate biofilm Reactors (Nicolella C. et al., 2000)

Advantages	Disadvantages
High Terminal settling velocity of solids leading to possible elimination of external clarification/separation stages.	Biofilm formation on carriers poses problems leading to long start-up times.
High reactor concentration	Control of biofilm thickness is difficult.
High biofilm surface area	Overgrowth of biofilms leads to elutriation of particles.
High biomass concentration and mass transfer result in high conversion capacities.	Liquid distributors of fluidized system are costly for large-scale reactors
Compact reactor with small area requirements	Have problems with respect to clogging and uniform fluidization
High biomass age and minimization of excess sludge production.	

2.8.9 Moving Bed Bio Reactor:

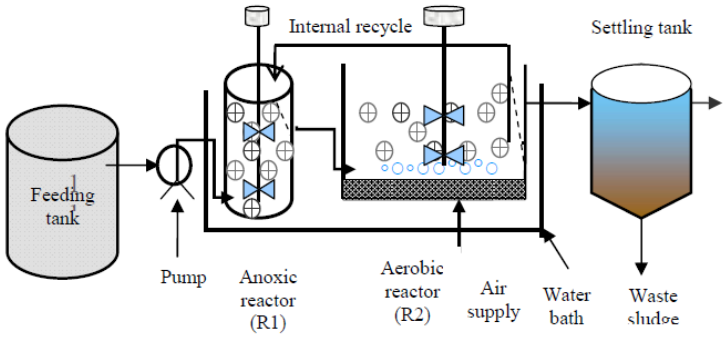


Fig.11. Schematic representation of a lab-scale MBBR

The MBBR process is based on the biofilm principle that take advantage of both activated sludge process and conventional fixed film systems without theirs disadvantages. Reactor can be operated at very high load and the process is

insensitive to load variations and other disturbances (Odegaard et al., 1994; Delenfort and Thulin, 1997 cited in Xiao et al., 2007).

Unlike most biofilm reactors, the reactor volume in the MBBR is totally mixed and consequently there is no dead or unused space in the reactor. In addition, this system has a small head loss and no need for recycling of biomass or sludge (Xiao et al., 2007).

An important advantage of MBBR is that the filling fraction of biofilm carriers in the reactor may be subject to preferences. In order to be able to move the carrier suspension freely, it is recommended that filling fractions should be below 70%.

2.9 Conclusion

The conventional nitrification/denitrification reactions have been known for a long time. The nitrification reaction consumes a large amount of oxygen, requiring 4.2 g of oxygen for each gram of NH_4-N nitrified (Gujer and Jenkins, 1974; EPA, 1975, cited in Khin et al., 2009). During denitrification, the requirement of organic carbon is significant. For example, 2.47g of methanol is required per gram of nitrate nitrogen for complete denitrification (McCarty et al., 1969, cited in Khin et al., 2009). The requirement of added electron donors such as methanol makes full-scale denitrification quite expensive.

Relatively low-cost electron donor methane is commonly used for denitrification in the presence of oxygen (Davies, 1973; Sollo and Mueller, 1976; Werner and Kayser 1991; Thalasso et al., 1997; Costa et al., 2000; Lee et al., 2001, cited in Khin et al., 2009). Methane is generally readily available in large amounts in wastewater treatment facilities through the anaerobic digestion of sludge. Denitrification with methane is brought about by the methanotrophic/methylotrophic association. Methanotrophs are strict aerobes and are capable of growth only on methane. An association of methanotrophs oxidizes methane to carbon dioxide and water (Mechsner and Hamer, 1985, cited in Khin et al., 2009). This process does not denitrify per se but produces organic intermediate compounds under suitable environmental conditions (Megraw and Knowles, 1989; Roy and Knowles, 1994; Amaral and Knowles, 1995, cited in Khin et al., 2009).

It is these organic intermediates that serve as the carbon source for aerobic or anoxic denitrifying bacteria (Rhee and Fuhs, 1978; Mechsner and Hamer, 1985; Werner and Kayser, 1991; Thalasso et al., 1997; Costa et al., 2000; Lee et al., 2001, cited in Khin et al., 2009). Methanol, formaldehyde and formate are the major known intermediate metabolism substrates of methane oxidation by methanotrophs (Hanson and Hanson, 1996). Unfortunately, although denitrification with methane is possible, it is a very slow process (Werner and Kayser, 1991, cited in Khin et al., 2009).

Because the organic carbon present naturally in the wastewater is quite limited, the complete removal of nitrogen from wastewaters that contain a high nitrogen concentration requires a large amount of an added carbon source for denitrification (van Dongen et al., 2001, cited in Khin et al., 2009). Furthermore, most existing wastewater treatment facilities were not designed for nitrogen removal, and meeting the demands of the nitrification/denitrification steps in these facilities can be difficult. Thus, many wastewater treatment plants do not meet the current discharge standard of 10 mg N L^{-1} (Jetten et al., 2002, cited in Khin et al., 2009). This was what drove the development of the new low-cost biotreatments for nitrogen-rich wastewaters (Khin et al., 2009).

Chapter – 3: INNOVATIVE NITROGEN REMOVAL PROCESS

3.1 Introduction

3.1.1 Nitrogen elimination in WWTPs

In wastewater treatment plants with anaerobic sludge digestion, 15-20% of the inlet nitrogen load is recycled with the return liquors from sludge dewatering. Separate treatment of this digester supernatant, containing 600-1000 gNH_4^+-N m^{-3}, would significantly reduce the nitrogen load of the main stream and improve nitrogen elimination (Fux et al., 2002).

Chemical elimination of ammonium with magnesium/ammonium/phosphate (MAP) precipitation or with air stripping is feasible but much more expensive than classical nitrification and denitrification with addition of an organic carbon source (Siegrist, 1996). In the late 1990s, Hellinga et al. (1998) presented the SHARON process for nitrogen elimination from concentrated waste streams. At relatively high temperatures (35^oC) and without sludge retention, nitrite oxidation was permanently prevented and denitrification with nitrite could begin. As a result, 25% of the oxygen and 40% of the carbon demand can be saved compared with complete nitrification/denitrification but an external electron donor for denitrification is required such as methanol and as well as an effective aeration system are still necessary (Fux et al., 2002).

3.2 Partial Nitrification and Denitrification

Several efforts have been made in order to optimize biological nitrogen removal. New processes have been developed such as nitrification/denitrification via nitrite accumulation (Ruiz et al., 2003). This process is based on the fact that, since nitrite and nitrate are intermediary compounds in both steps (nitrification and denitrification), a partial nitrification to nitrite and a denitrification from this nitrite, instead from nitrate, would be feasible (Fig. 12).

This approach will produce savings in oxygen demands during nitrification, a reduction of the organic matter requirements in the denitrification process, plus a decrease in surplus sludge production.

Partial nitrification is the oxidation of wastewater ammonium to nitrite, but not to nitrate. To achieve partial nitrification, the subsequent oxidation of nitrite to nitrate must be prevented. Partial nitrification can be combined with the anammox process, but even if it is combined with conventional denitrification (the so called 'nitrite route'), already a significant benefit is achieved in terms of use of resources (Schmidt et al., 2003).

The process needs less aeration, the subsequent denitrification consumes less COD (chemical oxygen demand), since only nitrite and not nitrate has to be reduced to molecular nitrogen (N_2).This is cost-effective if the low C/N ratio of the wastewater necessitates the addition of a synthetic electron donor, such as methanol. In that case the process also emits less CO_2 to the atmosphere (Schmidt I. et al., 2003).

The oxidation of nitrite to nitrate can be prevented in at least two ways, by making use of the difference in activation energy between ammonia and nitrite oxidation (68 kJ mol^{-1} and 44 kJ mol^{-1}, respectively) (Ingo Schmidt et al., 2003).

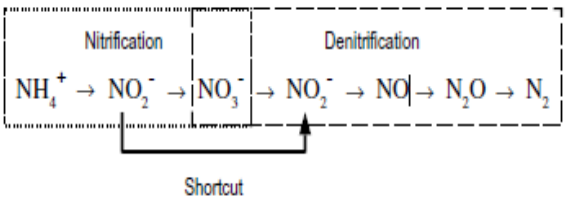


Fig.12. Nitrification - denitrification with nitrite accumulation.

In order to perform this process, two conditions must be fulfilled: nitrification must be stopped before nitrite oxidation and denitrifying sludge must be adapted to nitrite, which is toxic at low concentrations. To achieve partial nitrification it is necessary to selectively reduce the activity of the nitrite oxidizing bacteria without affecting the ammonia oxidizers. Some operational conditions may produce nitrite accumulation during nitrification, such as pH, temperature, and DO concentration.

The last operational variable seems to be the most interesting alternative. On the other hand, some researchers have proved that denitrifying sludge can be acclimated to nitrite, after an adaptation process (Jones et al., 1990; Chung and Bae, 2002).

Most of the research papers focused on nitrification denitrification through nitrite accumulation are applied to wastewaters with low nitrogen concentrations (Yoo et al., 1999; Bae et al., 2002; Peng et al., 2004).

3.2.1 Short-cut nitrification-denitrification: the SHARON process

The SHARON process (Single reactor High activity Ammonia Removal over Nitrite) was developed at the Technical University of Delft (Hellinga et al., 1997). The principle is based on a short circuit in the denitrification pathway. This process is operated without any biomass retention in a single aerated reactor at a relatively high temperature (35°C) and pH (above 7) (Brouwer et al., 1996; Hellinga et al., 1997) and full-scale experience has recently been gained in its operation (Mulder et al., 2001; van Kempen et al., 2001). The process involves partial nitrification of ammonium to nitrite, and this greatly reduces the expense of aeration. SHARON is the first successful process in which nitrification/denitrification with nitrite as an intermediate has been achieved under stable conditions (van Kempen et al., 2001) (Khin et al., 2004).

To obtain the stable partial nitrification, the operating variables (temperature, pH, hydraulic retention time, substrate concentration, dissolved oxygen) are controlled in a chemostat operation (Beccari et al., 1979; Randall and Buth, 1984; Hellinga et al., 1998, all cited in Khin. et al., 2004).

In the SHARON process, one can carefully makes use of the fact that at high temperatures, Nitrobacter has a distinctly lower growth rate than Nitrosomonas (Fig. 13). By implementing completely mixed reactor at short residence time e.g. one day and high temperatures, one can achieve wash out of Nitrobacter. By imposing intermittent aeration, both denitrification and concomitant pH control are possible. The overall process is schematized in Fig. 14 and illustrates that savings in oxygen supply and reductant are in the order of 25 and 40%, respectively (Verstraete W. et al., 1998).

Unfortunately, control of these process variables may be difficult in large-scale operations (STOWA, 1995). Hunik (1993) reported that the ammonium oxidizers grow faster than the nitrite oxidizers at elevated temperatures (>15^{o}C).

At the operational temperature of 35°C, the maximum specific growth rate of nitrite oxidizers is approximately only half of that for the ammonium oxidizers (0.5 and 1 day 1, respectively) (Hunik 1993).

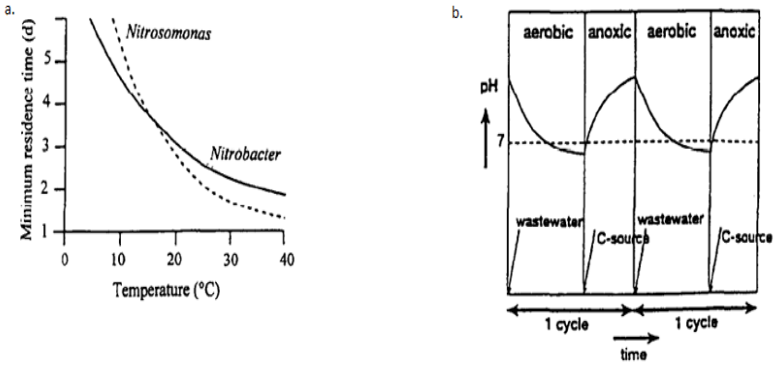


Fig.13., a. Growth rate of Nitrosomonas and Nitrobacter as function of temperature and residence time. The higher the temperature, the higher the growth rate and the lower the minimum residence time needed to avoid was out (after Mulder and Kempen, 1997); b. pH-pattern as a result of pH-control by intermittent aeration. Arrows indicate supply of wastewater and carbon source (after Mulder and Kempen, 1997).

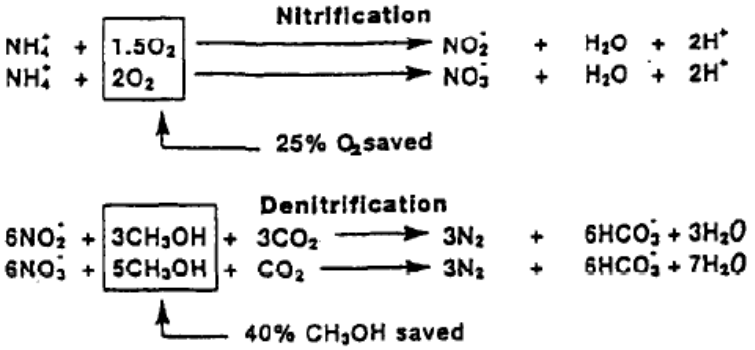


Fig.14. Reduction of oxygen and carbon requirements by N - removal via nitrate (after Mulder and Kempen, 1997)

Only at temperatures above 25°C is it possible for the ammonium oxidizers to effectively out compete the nitrite oxidizers (Brouwer et al., 1996). The ammonium oxidizers have a shorter minimum required sludge age at temperatures of >20°C. The sludge retention age of course can be controlled by the hydraulic retention time.

When faced with a short hydraulic retention time, the nitrite oxidizers are selectively washed out (Hellinga et al., 1998, cited in Khin et al., 2004).

Because SHARON depends on high temperature, it is not suitable for all wastewaters (but many wastewaters high in ammonium also have a high temperature, such as sludge liquor). Furthermore, because there is no sludge retention and the hydraulic retention time is fixed, the volumetric ammonium reactor loading depends on the ammonium concentration. Thus, the process costs also depend on the ammonium concentration (rising costs with decreasing ammonium concentration, Schmidt et al., 2003).

The high activation energy of ammonia oxidation makes the rate of this process more dependent on temperature. The SHARON process makes use of the different growth rates of ammonia and nitrite oxidizers at sufficiently high temperatures (more than 26^{o}C). It works at a hydraulic retention time higher than the growth rate of nitrite oxidizers but lower than ammonia oxidizers (about 1 day).Because this process has no sludge retention nitrite oxidizers are not able to remain in the SHARON reactor and they are washed out (Schmidt et al., 2003).

The oxidation of ammonium is an acidifying process. Therefore, the control of pH is important for preventing process inhibit ion (van Kempen et al., 2001). The nitrite oxidizers are particularly susceptible to a changing pH (Anthonisen et al., 1976; Truk and Mavinic, 1989; Abeling and Seyfried, 1992 cited in Khin et al., 2004). When the pH drops below 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^+ (Khin et al., 2004).

Aeration is not only necessary for oxygen supply, but also to strip CO_2 from the reactor to control the pH. SHARON still makes use of denitrification (with added methanol) to reduce the nitrite to dinitrogen gas. Methanol is supplied periodically while the aeration is switched off (Schmidt et al., 2003).

When pH drops too low, the free ammonium concentration becomes too low for sufficient growth of the ammonium oxidizers. Although the nitrite oxidizers do grow faster than the ammonium oxidizers at low pH values, the opposite is the case at high pH values. Therefore, a high pH is preferred for obtaining an effluent that is low in

NH_4^+ concentration (Hellinga et al., 1998). Above pH 8, nitrification also declines (Khin et al., 2004).

This is because too much NH_3 is apparently toxic for the nitrite oxidizers in this process (Anthonisen et al., 1976). The ammonium/nitrite ratio in the effluent of the SHARON process can be sensitively influenced by changing the reactor pH between 6.5 and 7.5 (van Dongen et al., 2001). Typically, for the sludge liquors the ratio of HCO_3/NH_4^+ is 1.1:1 (Hellinga et al., 1998), and consequently, about half of the ammonium in the liquor can be converted without any pH control and this depletes the alkalinity of water. This leads to a pH drop and prevents further nitrification (Jetten et al., 2002, cited in Khin et al., 2004).

The nitrite oxidizers have a lower affinity for oxygen than the ammonium oxidizers (Hunik, 1993; Picioreanu et al., 1997); therefore, a low DO concentration is restrictive for the growth of nitrite oxidizers (Truk and Mavinic, 1989; Hanaki et al., 1990; Laanbroek and Gerards, 1993, cited in Khin et al., 2004). Depending on the aerobic retention time, different concentrations of ammonium are achieved in the effluent (van Kempen et al., 2001). The ammonium oxidizers have a low affinity for ammonium (affinity constant 20 – 40 $mgNH_4^+$-N L^{-1}). In addition, HNO_2 inhibits the ammonium oxidizers, but they can tolerate high concentrations of nitrite (>0.5 gNO_2-N L^{-1}) at pH 7 (Jetten et al., 1997; van Dong en e t al., 2001) (Khin T. et al., 2004).

A variation on the SHARON process does make use of sludge retention. Instead of the hydraulic retention time, here the sludge age is controlled (in SHARON, the hydraulic retention time equals the sludge age).This allows higher ammonium loading rates and more efficient aeration. The process also makes use of a second principle to prevent nitrite oxidation; at low oxygen concentrations (< 0.4mg L^{-1} or 5% air saturation) and with surplus ammonium, nitrite oxidizers are unable to grow, and nitrite becomes the stable end product of nitrification. It is unclear why nitrite oxidizers are inhibited; inhibition of nitrite oxidizers by ammonia and a lower affinity for oxygen and/or nitrite have been suggested as possible explanations, but we still lack mechanistic evidence (Schmidt et al., 2003).

The SHARON process should be regarded as a pretreatment or side-stream treatment, e.g. for the handling of sludge digestion water, as is currently under design (Hellinga et al., 1997). In processes where nitrite is accumulated at a certain

point, one has to pay attention to the fact that nitrite can be involved in side reactions, e.g. forming of nitroanilines in the presence of aniline, nitrite and hydroxyl radicals (Chan and Larson, 1991, cited in Verstraete W. et al., 1998).

Of the various processes, the SHARON process appears to be the most practicable for substantially reducing the concentration of ammonium in wastewater that is relatively high in ammonium content. This can be achieved so long as operations are carried out at an elevated temperature and pH. A nitrogen removal efficiency of 90 % can be achieved (van Kempen et al., 2001, cited in Khin et al., 2004).

The process requires relatively little initial investment because a simple well-mixed tank reactor of modest dimensions without sludge retention is sufficient (Hellinga et al., 1998). The process does not produce chemical sludge and has a relatively low production of biological sludge. It requires relatively little oxygen because the oxidation is stopped at the nitrite stage, and this saves on energy and the added carbon source. Compared to the traditional nitrification and denitrification via nitrate, the SHARON process demands 25% less aeration energy and 40% less added carbon (Khin et al., 2004).

Van Hulle et al. (2005) described the start-up and operation of a lab-scale SHARON reactor operated at 35◦C without pH-control. An Anammox-suited influent was obtained with synthetic influent containing an ammonium loading rate up to 1.5 kgN $m^{-3}d^{-1}$. Udert et al. (2003) described also good SHARON performance with urine as influent. In the CSTR an ammonium: nitrite ratio of 1:1 was obtained at a HRT of 4.8 days and a pH of 9.2 (Stijn W.H, 2010).

The SHARON technology is nowadays successfully used at full scale to treat effluents from sludge digesters. Full-scale SHARON reactors are currently in operation at the sludge treatment site Sluisjesdijk of the WWTP of Rotterdam and Utrecht (The Netherlands). Fux et al. (2002) also operated a 2.1m^3 CSTR-reactor in Zurich at a HRT of 1.1 days and a temperature of 30°C without pH control.

Although the SHARON process is successfully started up at full scale, there are still some disadvantages connected to this process.

Sludge digesters operate at high HRT values guaranteeing a stable composition of its effluents for the subsequent SHARON process (low biodegradable organic matter and bicarbonate to ammonia molar ratio of 1). When the HRTs in the digesters are

lower than usual or when industrial wastewaters are used, fluctuations of the influent composition into the SHARON reactor will occur (Stijn W.H et al., 2010).

Therefore, operational parameters such as DO or pH must be controlled in the preceding SHARON process to obtain an optimal nitrite: ammonium ratio. Another disadvantage is the limited maximum volumetric loading rate of SHARON reactor, as sludge is constantly withdrawn. To assure stable operation, the minimum HRT of a chemostat is limited to 1–1.2 days. In MBR, SBR or biofilm systems biomass is retained giving the advantage that HRT can be uncoupled from SRT and HRT lower than 1 day is possible resulting in much higher loading rates (i.e. smaller reactors with similar treatment capacity) (Stijn et al., 2010).

Protozoa can cause problems in the operation of a SHARON reactor mainly if real wastewater is used. A possible solution is to lower the reactor pH to 6 for 2 h or to incorporate non-aerated periods. A pH-lowering can be obtained by reducing the influent flow under constant aeration (Stijn et al., 2010).

Non-aerated periods, however, clearly have a negative effect on the nitrogen conversion by nitrifiers and the SHARON reactor has to be 30% larger to maintain good nitrite formation (Stijn et al., 2010).

Moreover, the required performance temperature of SHARON is higher. When the effluent of the treated stream is lower than 24◦C the maximal growth rate of AOB turns lower than that of nitrite oxidizers and ammonium is fully oxidized into nitrate (Stijn et al., 2010). Therefore, to achieve partial nitritation at temperature lower than 24◦C other strategies such as inhibition of NOB by ammonia and nitrous acid or operation at low oxygen concentrations should be applied (Stijn et al., 2010).

Wyffels et al. (2004) used a MBR as a first step of the autotrophic nitrogen removal process at low dissolved oxygen concentrations ($<0.1 mgO_2\ L^{-1}$). The membrane had to be regularly cleaned to prevent clogging. The pH was controlled at 7.9 and the temperature was set to 35◦C, although an experiment at room temperature was conducted as well. Lowering the temperature had no significant effect on the obtained nitrite: ammonium ratio. Similarly, lowering the NH_3 concentration, and possibly lowering the NH3 inhibition on nitrite oxidizers, had no significant effect on the obtained nitrite: ammonium ratio. This indicates that oxygen limitation is the most important operational factor.

Feng et al. (2007) and Xue et al. (2009) also used the MBR to obtain good partial nitritation performance at low dissolved oxygen concentration. Feng et al. (2007) stated that alkalinity also played an important factor to achieve a nitrite: ammonium ratio of 1.3:1 while Xue et al. (2009) reported that free ammonia inhibited the nitrite oxidizers (Stijn et al., 2010).

3.3 Anammox processes

3.3.1 History of Anammox:

So far, only aerobic processes have been discussed for ammonium oxidation. A novel biological process was also discovered. In 1990, the Kluyver Laboratory of Biotechnology of Delft reported a new process in which ammonium is converted to dinitrogen gas with nitrate serving as the electron acceptor under anaerobic conditions (van de Graaf et al., 1990) (W. Verstraete et al., 1998). Because ammonium is oxidized in the absence of oxygen, this novel process has been named anaerobic ammonium oxidation (Anammox). This autotrophic process allows over 50% of the oxygen to be saved and no organic carbon source is needed. In addition, the biomass yield is very low so that little sludge is produced (Fux C. et al., 2002).

More recently, it has become clear that nitrite is the key electron acceptor (Strous et al., 1997). This so-called ANAMMOX process (Anaerobic AMMonium Oxidation) is autotrophic and hence there is no need for COD (Chemical Oxygen Demand) addition to support denitrification. Furthermore, if the ANAMMOX process is combined with a preceding nitrification step, preferably blocked at nitrite, only part of the ammonium needs to be nitrified to nitrite since the ANAMMOX process combines the remaining ammonium with this nitrite to yield dinitrogen gas (Verstraete et al., 1998).

$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H_2$ (van de Graff et al. 1996) (13)

The overall reaction for this anaerobic ammonium oxidation (Anammox) process is exergonic and thus could, in theory, supply energy for growth. It was therefore postulated that the removal of ammonium observed to occur in the denitrifying reactor was carried out by bacteria using ammonium as an electron donor for nitrate reduction (van de Graff et al. 1996).

This permits to reduce both oxygen demand in the nitrification reactor and COD demand in the denitrification phase (Strous et al., 1997). Aerobic nitrifiers are reported to be present in the ANAMMOX enrichment cultures, they are considered, on the basis of their densities, not to be responsible for the ANAMMOX process (van de Graaf et al., 1996).

This process can remove ammonium from high-concentrated stream with addition of nitrite, but more substantial experiments showed that oxygen and low-organic carbon can completely inhibit the ANAMMOX activity when it is exposed to the enrichment culture (Strous et al., 1997). Thus, ANAMMOX process can be obtained under strictly anoxic and devoid of organic carbon source conditions (Chen H. et al., 2009).

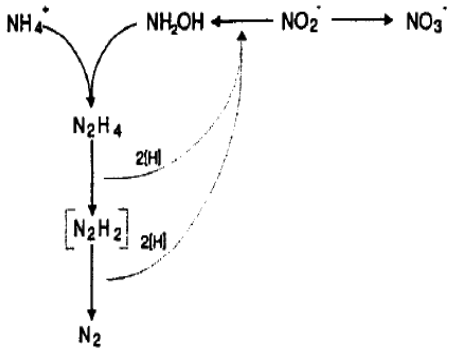


Fig.15. Pathway of the ANAMMOX - process

3.3.2 Growth of Anammox Bacteria

Anammox bacteria grow slowly. Measured doubling times in laboratory are under optimum conditions 11 days and in average 2-3 weeks (Strous et al., 2002). The biomass-yield is 0.07 C-mol fixed per mol ammonia oxidized which consists with the anammox catabolism's Gibbs free energy change (Strous et al., 1998; Strous et al., 2004). We can therefore eliminate inefficient energy conservation as the reason for the slow growth-rate. The real reason is a low substrate-conversion rate. The temperature-optimum has been determined for different habitats. In waste water treatment the optimum was at 37 °C (Kuenen et al., 2001). Under environmental conditions, the optimum was lower. In the sediment of Young Sound, Greenland it was 12°C (Rysgaard et al., 2004, cited in Hertach M., 2008) and in Skagerrak 15°C (Dalsgaard and Thamdrup, 2002, cited in Hertach M., 2008).

Researchers recently have claimed they optimized the reactor conditions to such an extent that a doubling time of 1.8 days was achieved (Isaka et al., 2006). A possible explanation for this high variation in growth rate could be the method to determine the growth rate, as Isaka et al. (2006) determined the growth rate by direct counts of Anammox bacteria, while other studies rely on biomass yield and nitrogen removal rate. This low growth rate and the difficulty in obtaining axenic (or "pure") cultures strongly hindered Anammox research (Stijn et al., 2010).

3.3.3 Biodiversity of Anammox Bacteria

A range of studies have been conducted for the detection of anammox bacteria and activities in variable environments from natural to man-made ecosystems (Risgaard-Petersen et al., 2003; Schmid et al., 2005, all cited in Hertach M., 2008). Anammox activity was found in marine environments, such as the Black Sea, the coast of Namibia, Chile, Peru and some freshwater and estuarine systems like, Lake Tanganyika and mangroves (Kuypers et al. 2003; 2005; Risgaard-Petersen et al., 2004; Meyer et al., 2005; Thamdrup et al., 2006; Schubert et al., 2006; Hamersley et al., 2009, all cited in Hertach M., 2008).In addition to widespread distribution, the activity of anammox bacteria in the environments also be substantial. The maximum reported contribution of anammox is 67-79%, occurring in sediments at a depth of 700m of the Norwegian Trench (Engström et al., 2005). Considerable supporting evidences have confirmed that anammox has global importance (Hertach M., 2008).

The microbes responsible for anammox process were identified as members of the bacterial order Planctomycetales (Strous et al., 1999). The first genome sequence of a representative anammox bacterium was published in 2006 (Strous et al., 2006). To date, five anammox genera have been described, Candidatus Brocadia, Candidatus Kuenenia, Candidatus Scalindua, Candidatus Anammoxoglobus and Candidatus Jettenia (Yangping and Clark, cited in Hertach M., 2008).

The three genera are monophyletic and branch off inside the planctomycete lineage. They have a similar ultrastructre and the same metabolism what leads to the conclusion that the anammox-feature has evolved only once in the history of life (Schmid et al., 2003). All anammox species which have been detected in marine and estuarine systems belong to the genus Scalindua (Schmid I. et al., 2007).

The only anammox bacteria found so far in a lacustrine system had a similarity of 95.7% to the know anammox bacteria Cadidatus Scalindua brodae in the 16S rRNA gene sequence (Schubert et al., 2006, cited in Hertach M., 2008).

3.3.4 Characterization of anammox bacteria

The coccoid anammox bacteria have usually a diameter of less than 1 m and a generation time of 10 - 30 days. They belong to the order of Planctomycetes and are therefore anaerobic chemolithoautotrophs (van Niftrik et al., 2004, cited in Hertach M., 2008). So far all trials to get pure cultures of anammox bacteria failed because it is very hard to isolate them. Thus there are just enrichment cultures available and all we know about anammox bacteria is derived from those cultures (Hertach M., 2008). They all possess one anammoxosome, a membrane bound compartment inside the cytoplasm which is the locus of anammox catabolism. Further, the intra cytoplasmic is surrounded by unique lipids, called ladderanes (Sinninghe Damsté et al., 2004, cited in Xing and Clark, 2011). Due to their unique characteristics, ladderane lipids have also been used as a biomarker for the presence of anammox bacteria (Kuypers et al., 2003, cited in Xing and Clark, 2011). Thus far, ladderanes have been found only in association with anammox bacteria that could be used to positively identify anammox organisms (M Kumar et al., 2010). Besides, an interesting special feature is the turnover of hydrazine (normally used as a high-energy rocket fuel and poisonous to most living organisms) as an intermediate (Xing and Clark, 2011).

3.3.5 Physiology of Anammox Process

The pathway of N_2 formation clearly distinguishes anammox from denitrification which combines N from two NO_3^- molecules to form N_2 and presents as an elegant shortcut in the natural nitrogen cycles (Fig. 16). Physical purification of the anammox microbes from the multispecies biofilms yielded a 99.6% pure culture that was capable of carrying PCR amplification of the DNA (Xing and Clark, 2011).

Anammox is characterized by slow growth and its cell doubles only once per 11 days under optimum conditions and 2- 3 weeks on average (Strous et al., 2006). The low growth rate of anammox bacteria is not caused by inefficient energy conservation but by a low substrate-conversion rate.

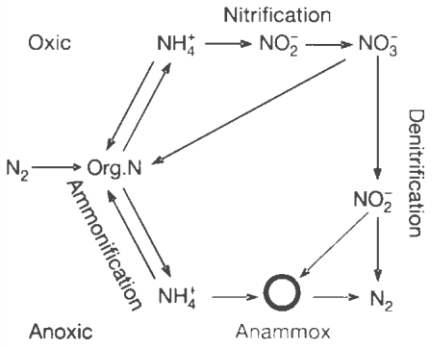


Fig.16. Anammox in the context of N - cycle (Kuypers et al., 2003).

Furthermore, anammox bacteria are obligate anaerobes and their metabolism is reversibly inhibited when oxygen concentration is above 2 μM and nitrite is higher than 10 mM (Strous et al., 1997). The temperature range suitable for anammox bacteria has been reported between -2^{o}C (sea ice, Rysgaard & Glud, 2004, cited in Xing and Clark, 2011) and 43^{o}C (Strous et al., 1999). A recent study has observed anammox activity at temperature from 60^{o}C to 85^{o}C at hydrothermal vents located along Mid-Atlantic Ridge (Byrne et al., 2008, cited in Xing and Clark, 2011). At optimal condition, Anammox biomass could be enriched from activated sludge within hundred days (Xing and Clark, 2011).

In addition, anammox bacteria have been found to be metabolically flexible, exhibiting alternative metabolic pathways. For instance, anammox can subsequently reduce NO_3^- to NO_2^- to NH_4^+, followed by the conversion of NH_4^+ and NO_2^- to N_2 through Anammox pathway, allowing anammox bacteria to overcome NH_4^+ limitation. Anammox bacteria are also a potential source of N_2O production by nitric oxide detoxification (Kartal et al., 2007). Apart from NO_2^- and NO_3^-, anammox bacteria also employ Fe_3^+, manganese oxides as electron acceptors (Strous et al., 2006), which further expended the metabolic diversity of the anammox bacteria.

3.3.6 Factors Inhibiting the Growth of Anammox Bacteria and the Process

3.3.6.1 Inhibition of substrates and products

The nitrite concentration is an important parameter to control since Anammox activity is inhibited by it. However, no uniformity is found about the threshold values of nitrite inhibition. Dapena-Mora et al. (2004) found that 350mgNL^{-1} nitrite

correspond to 50% inhibition of the Anammox process performing activity tests. In the presence of more than 100mgNL^{-1} nitrite, Strous et al. (1999) found that the Anammox process was completely inhibited. Fux (2003) showed in a long-term experiment that maintaining a nitrite concentration of 40mgN L^{-1} over several days led to the irreversible inactivation of the Anammox organisms. This decreased activity due to nitrite inhibition can be restored by adding trace amounts of the Anammox intermediates hydroxylamine and hydrazine, even after long-term exposure to high concentrations of nitrite (Stijn et al., 2010).

Furthermore, experiments by Strous et al. (1999) showed that increasing the nitrite concentration changed the stoichiometry of ammonium and nitrite consumption from 1.3 g nitrite per gram ammonium at 0.14gN L^{-1} nitrite to almost 4 g nitrite per gram ammonium at 0.7gN L^{-1} nitrite. From the distorted stoichiometry at high nitrite concentrations, it can be concluded that the microorganisms under these conditions did not only use ammonium as the electron donor but also must have generated an internal electron donor to reduce the nitrite. This changing stoichiometry was also noticed at higher temperatures. Dosta et al. (2008) observed a nitrite: ammonium consumption ratio of 1.38:1 at a working temperature of 30 ◦C but this ratio decreased to 1.05:1 when the reactor was operated at 18^{o}C (Stijn et al., 2010).

The Anammox process is not inhibited by ammonium or by the by-product nitrate up to concentrations of at least 1gN L^{-1}. Dapena-Mora et al. (2007) observed a 50% activity loss with high concentrations of ammonium and nitrate (770 and 630mgN L^{-1}, respectively).

3.3.6.2 Phosphate and sulphide

Similarly to nitrite inhibition a difference in tolerance for phosphate exists between different Anammox species. Dapena-Mora et al. (2007) observed at the phosphate level of 620mgP L^{-1} 50% inhibition of Anammox activity. In batch tests using sludge from a highly loaded lab-scale rotating biological contactor containing C. Kuenenia stuttgartiensis, phosphate was shown to partially inhibit the Anammox process. Anammox activity decreased to 63% of the normal activity at 55mgP L^{-1} and further to 20% at 110mgP L^{-1}. At 285mgP L^{-1} no further decrease was observed (80% inhibition).

The effect of sulphide on the activity was also tested since SO_4^{2-} reduction often takes place in anaerobic digestion mainly transformed into H_2S. In anaerobic conditions, sulphate reducing bacteria produce sulphide with organic carbon as electron donor (Stijn et al., 2010).

Wastewaters such as seafood processing, leather tanning, oil-refining and alcohol fermentation not only contain organic carbon and nitrogen but also sulphur compounds. Dapena-Mora et al. (2007) showed an Anammox inhibition of 50% at low sulphide concentration of 9.6mgS L^{-1}.

While van de Graaf et al. (1996) showed a resistance of Anammox to at least 64mgS L^{-1} in continuous and batch experiments. This large difference in sulphide inhibition could be explained by the addition of nitrate as electron donor for the Anammox biomass in van de Graaf et al. (1996) since sulphide could reduce nitrate to nitrite, which is the preferable electron donor of the process. Recently, simultaneous removals of ammonium and sulphate by Anammox have been reported (Stijn et al., 2010).

3.3.6.3 Oxygen

Anammox bacteria are strictly anaerobic and are inhibited by dissolved oxygen. Inhibition caused by low concentration of oxygen was demonstrated however to be reversible. Egli et al. (2001) stated that oxygen inhibits Anammox metabolism reversibly at low oxygen levels (air saturation of 0.25–2%) but probably irreversibly at high levels (>18% air saturation). Strous et al. (1997) concluded from experiments with intermittent oxygen supply that the Anammox process was reversible inhibited by oxygen, making partial nitritation and Anammox possible in one reactor (Stijn et al., 2010).

3.3.6.4 Organic carbon

Landfill leachate and wastewaters from digested animal waste contain high nitrogen concentration but also high organic carbon levels. Still, there are considered to be good influent streams for Anammox reactor. During anaerobic digestion fast biodegradable organic content is converted to biogas. As such, only slow biodegradable organic matter will be present in these wastewaters. Ruscalleda et al. (2008) found that Anammox and denitrifiers could co-exist and play an important

role in treating streams with high quantities of slowly biodegradable organic carbon such as digested liquor and landfill leachate. In such streams, heterotrophic denitrifying growth is limited by the low availability of easily biodegradable organic carbon (Stijn et al., 2010).

Several other studies reported that presence of organic matter has a negative impact on Anammox growth. In presence of certain amounts of organic carbon, Anammox organisms are not longer able to compete for nitrite with denitrifiers. This could be due to the fact that the growth rate of denitrifiers is higher than Anammox bacteria. Moreover, the denitrification reaction is thermodynamically more favourable than anaerobic ammonium oxidation (the Gibbs free energy of Anammox bacteria is −355 kJ mol^{-1}), while the Gibbs free energy of denitrifying bacteria is −427 kJ mol^{-1}) (Stijn et al., 2010).

Therefore, heterotrophic denitrifiers would grow faster when organic carbon is present in combination with ammonium and nitrite eliminating place for Anammox organisms. The threshold concentration for organic carbon in which denitrifiers out compete Anammox bacteria differs from report to report (Stijn et al., 2010).

Anammox activity is completely and irreversible inhibited by low concentrations of methanol (15mg L^{-1}) and ethanol. This aspect must be taken in account since methanol is often used to remove nitrate in a post-denitrification step. A possible explanation for the methanol inhibition is the formation of formaldehyde by the Anammox enzyme hydroxylamine oxidoreductase (Stijn W.H, 2010).

3.3.6.5 Temperature and pH

Several authors found that the optimum temperature for the operation of Anammox bacteria was around 30–40◦C. Dosta et al. (2008) used batch tests to observe the short-term effect of temperature on Anammox activity. They found that the maximum activity of non-adapted Anammox biomass ranged between 35 and 40◦C,

While a temperature of 45◦C caused an irreversible decrease of the Anammox activity due to biomass lysis. Small differences in optimal temperature were found for K. stuttgartiensis and B. anammoxidans. B. anammoxidans showed highest activity at 40◦C while the highest activity of K. stuttgartiensis was observed at 37◦C at an optimal pH of 8 (Stijn et al., 2010).

However, Cema et al. (2007) and Isaka et al. (2006) proved that the Anammox process in a RBC and anaerobic biological filtrated reactor respectively could be successfully operated at a low temperature of 20◦C. The slow adaptation of the Anammox sludge seems a key factor in order to operate an Anammox reactor at low temperatures since a drastic change in the operational conditions could lead to a destabilization of the biological system (Stijn et al., 2010).

An advisable start up strategy is needed to operate an Anammox system at low temperatures. First, the required amount of biomass must be produced in a separate reactor at a temperature close to the optimum temperature. Then, the biomass can be gradually adapted to low temperatures in the same reactor and finally the low-temperature adapted biomass can be inoculated in the low-temperature reactor. The optimal pH interval for Anammox is 6.7–8.3 with an optimum of 8.0 (Stijn et al., 2010).

3.3.6.6 Biomass concentration

Biomass concentration plays a crucial role for the Anammox activity. Strous et al. (1999) found that Anammox is only active when cell concentrations are higher than 1010-1011 cells m L^{-1}, even in purified cultures. This could be explained by the need for intercellular communication for activity. Another possible explanation is that hydrazine diffuses relatively easy to the outside of the cell and a minimum internal concentration is necessary for Anammox activity (Stijn et al., 2010).

Perhaps the presence of contaminating cells, 1 on 200–500, is necessary to sustain long term growth, because these cells can guarantee vitamin supply and the removal of toxic components. Pynaert et al. (2004) put forward the hypothesis that the presence of ammonium oxidizers is necessary for the re-activation of Anammox organisms after disturbance of the system. By the production or accumulation of hydroxylamine or hydrazine by ammonium oxidizers, Anammox organisms can re-activate their metabolism (Stijn et al., 2010). On the other hand, Dapena-Mora et al. (2007) did not observe a notable effect on the activity at different initial biomass concentration of 0.25–2.0 g VSS L^{-1}.

3.3.6.7 Suspended solids

Flocculants are often used to remove colloidal organic and inorganic substances from wastewater previous to the Anammox process.

Therefore, the effects of these flocculants on the Anammox process are tested in batch tests by Dapena-Mora et al. (2007). Concentrations up to 1g L^{-1} polymeric positively charged compound used as flocculant did not cause a detrimental effect on the Anammox activity. In the study of Yamamoto et al. (2008), a large amount of influent suspended solids present in the partial nitrified digested liquor attached to the nonwoven materials covering the Anammox biomass growing on the carries. This caused a decrease in Anammox activity and became the main reason responsible for the unsatisfactory performance of the Anammox process.

The use of a flocculant improved the settle ability of the influent suspended solids and reduced their accumulation inside the reactor but the flocculant itself attached also on the surface of the nonwoven carriers and hence reducing Anammox activity (Stijn et al., 2010).

3.3.6.8 Other influencing factors

Anammox activity was also found to be sensitive to visible light. A decrease in activity of 30 to 50% was observed by van de Graaf et al. (1996). As a result the equipment for further experiments by these researchers was covered with black plastic and paper to eliminate this light effect. Arrojo et al. (2006) showed the effect of shear stress on the Anammox process in a SBR. They stated that stirring speeds up to 180rpm had no negative effect on the performance of the Anammox process. Anammox activity and the average diameter decreased to 40% and 45%, respectively while nitrite accumulated in the reactor when a rotating speed of 250rpm was tested (Stijn et al., 2010).

3.4 Practical implementation of ANAMMOX nitrogen removal Process

An Anammox step has to be preceded by a partial nitritation step. This can be accomplished

- In the same reactor (1-reactor system) or
- By using 2 separate reactors (2-reactor system).

The nitritation–anammox process has been mainly configured as either a one-biomass, one-reactor system or a two-reactor, two-biomass system.

Examples of one-biomass, one-reactor processes are

- DEMON (DEamMONification),

- OLAND (Oxygen-limited Autotrophic Nitrification / Denitrification),
- CANON (Completely Autotrophic Nitrogen removal Over Nitrite), and
- SNAP (Single-stage Nitrogen removal using Anammox and Partial nitritation).

The use of a single reactor has some advantages with respect to the partial nitritation–Anammox configuration. Single-stage processes generally have higher volumetric nitrogen removal rate and lower capital costs than 2-stage systems since no additional nitritation reactor volume is required for ammonium oxidation without nitrogen removal.

Hao et al. (2001) and Nielsen et al. (2005) stated that for high loaded waste streams the relatively high investment costs for a partial nitritation–Anammox process will be compensated by lower operational costs and efficient nitrogen removal performance.

A major disadvantage of these autotrophic nitrogen removal processes is the low growth rate of AOB and Anammox bacteria. The performance of reactors involving slow growing bacteria can be enhanced by applying high sludge retention time. This could be achieved by applying carrier materials to develop biofilms or by self-aggregation in granules (Stijn W.H et al., 2010).

These processes have reported considerable benefits, such as reduced energy cost, biomass production, and carbon requirements. The primary limitation of these processes is the long startup period for anaerobic ammonium oxidation, typically a few months after inoculation with ammonium oxidizing bacteria. However, after a suitable startup period, the system is inexpensive to maintain and is simple to operate.

Table 5: Process names for Nitrogen Removal systems involving the ANAMMOX process, (van der Star et al., 2007)

Process name	Source of Nitrite	Alternative process name	Reference
One Reactor Nitritation-Anammox	Nitritation of NH_4^+	OLAND CANON SNAP DEMON	Kuai&Verstraete,1998 Third et al, 2001 Lieu et al., 2005 Wett, 2006

Process name	Source of Nitrite	Alternative process name	Reference
2-reactor Nitritation-Anammox process	Nitritation of NH_4^+	SHARON-Anammox	Van Dongen et al., 2001

3.4.1 One-Reactor System

3.4.1.1 Introduction

In a 1-reactor system, a co-culture of aerobic and anaerobic ammonium-oxidizing bacteria is established under microaerobic conditions to avoid inhibition of Anammox bacteria by oxygen and to achieve appropriated conditions to obtain partial nitritation. In those systems, the growth of NOB (and subsequent nitrate production) is prevented due to their lower affinity to oxygen compared to AOB and for nitrite compared to Anammox bacteria. Possible inhibition of nitrite oxidizers by free ammonium has also been suggested (Stijn W.H et al., 2010).

A one-reactor system may have a configuration with or without attached biomass, but a biofilm environment must be provided where anammox bacteria will be protected from oxygen, which is toxic to them. A probable model of this phenomenon would be that the outer layer of the biofilm would be occupied by oxygen-consuming organisms, such as AOBs, and the inner layer would be occupied by the anammox bacteria. In this configuration, both partial nitrification and the anammox reaction would occur simultaneously (Jaroszynski et al., 2011).

Full scale examples demonstrate volumetric SNRR similar to overall two-reactor systems. Typical SNRRs are 0.4–0.5 kg N m^{-3} d^{-1}, with the highest value reported to be 1.1 kg N m^{-3} d^{-1}.

Among the many configurations, the DEMON process that consists of an SBR configuration for full autotrophic ammonium removal has a relatively high volumetric SNRR of around 0.6 kg N m^{-3} d^{-1}. The DEMON process is controlled by pH and dissolved oxygen (DO). The simplicity of this control strategy allows a significant level of reliability.

The DEMON process is very sensitive to pH bandwidth, causing destabilization if it is greater than 0.02. Dissolved oxygen above 0.5 $mgO_2\ L^{-1}$ may lead to nitrite accumulation and substantial activity loss; a temperature change from 35-37°C was shown to slow down the process (Jaroszynski et al., 2011).

The one-reactor system works with a relatively high SRT of between 20 and 30 days; therefore it has a high potential for inert solids accumulation. This may necessitate installation of a presedimentation tank.

Various names are used to describe the 1-reactor systems:

1. The OLAND-process (Oxygen Limited Autotrophic Nitrification and Denitrification),
2. The CANON process (Completely Autotrophic Nitrogen removal Over Nitrite),
3. Aerobic/anoxic deammonification or DEMON Process.
4. Single-stage nitrogen removal using anammox and partial nitritation (SNAP).

Different kinds of systems were used to obtain the microaerobic conditions for the 1-step process.

- Sequencing Batch Reactor,
- Gas-lift Bioreactor,
- Rotating Biological Contactors and
- Moving bed reactors.

3.4.1.2 Sequencing Batch Reactor

For the SBR experimental set-up no special equipment is required, apart from the usual equipment used for continuous cultivation, making this cultivation technique accessible to many microbiology laboratories. SBR was a very suitable experimental set-up for the cultivation, enrichment and study of a very slowly growing microbial community. It is more reliable operation. The Experimental setup of the sequencing batch reactor is shown in the Fig.15 (Strous M. et al., 1998).

A homogeneous distribution of substrates and aggregates made representative sampling and the performance of experiments under defined bulk conditions possible for the first time for all chemical and biological assays.

Stable conditions (comparable to a steady state in a chemostat) can be achieved, enabling for the first time mass balancing under defined conditions at low substrate concentrations (Strous et al., 1998).

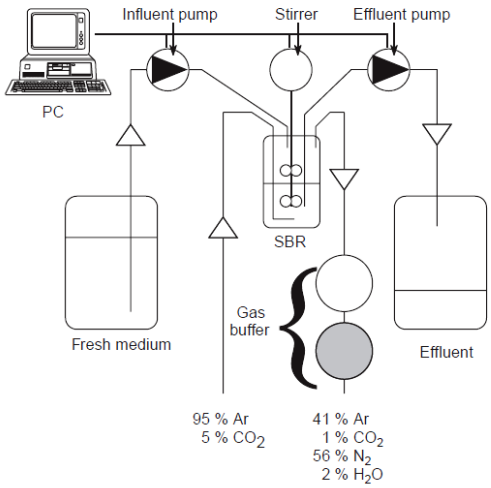


Fig.17. Experimental setup of SBR (M. Strous et al., 1998)

Several techniques have been developed for the culture and study of aggregated or slowly growing organisms, such as the microstat (Caldwell, 1995), the RotoTorque (Characklis, 1990), the retentostat (Chesbro et al., 1979), the fixed bed reactor (Strous et al. 1997a) and the fluidized bed reactor (Van de Graaf et al., 1996). In the RotoTorque, the fixed bed reactor and the fluidized bed reactor, biomass, substrates and products are not distributed homogeneously and representative experiments are not possible (Gjaltema et al. 1994; Strous et al. 1997a; Van de Graaf et al. 1996, cited in Strous et al., 1998). Even if the fluidized bed reactor could be optimized to improve mixing, biomass retention and reliability would still be less optimal compared to the SBR (Strous et al., 1998).

In both the microstat and the RotoTorque only very small amounts of biomass can be accumulated, compared to the SBR. The conditions in the retentostat are more defined than in the SBR, but operation of the retentostat is not reliable over the required long periods of time because the biomass is retained by a membrane that is easily clogged. Furthermore, the retentostat is not suitable for the study of aggregated micro-organisms (Strous et al., 1998).

In the settling period, the aggregates settled rapidly and efficiently, leading to 90% retention of the growing biomass. This means that of every 10 g protein generated in

the reactor, only 1 g was washed out. The settling properties of the aggregates may have been improved during the enrichment by selection for well-settling aggregates. Due to the reliable operation and the efficient biomass retention, large amounts of enriched Anammox biomass can be produced (Strous et al., 1998).

The development of SBR cultivation is essential for the microbiological and applied research of the Anammox process because it generates a steady supply of large amounts of highly enriched Anammox biomass of constant composition (Strous et al., 1998).

In the following figures different configurations of Anammox-based reactors are shown.

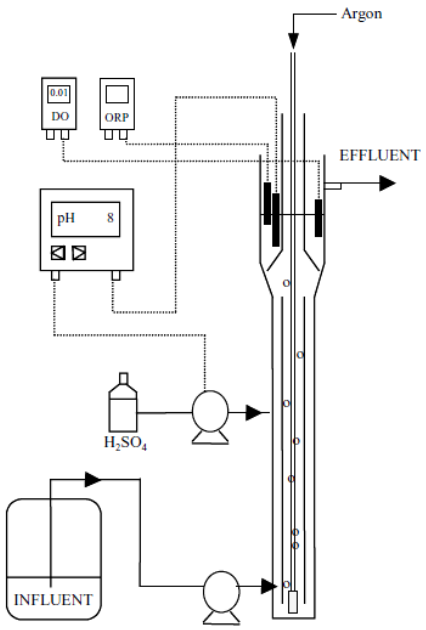


Fig.18. A schematic of Gas lift Bioreactor (Strous et al., 1998).

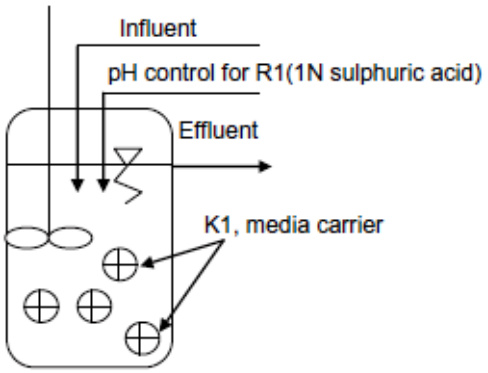


Fig.19. A schematic of MBBR (Jaroszynski et al., 2011a)

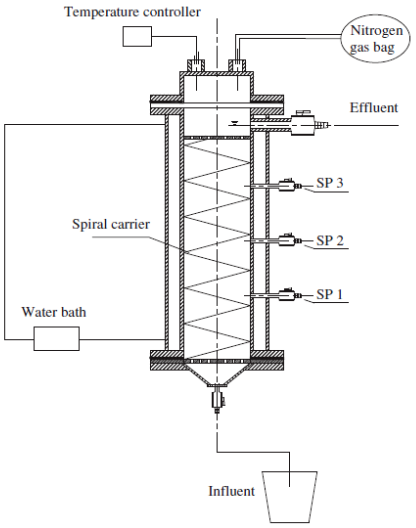


Fig.20. Schematic representation of a lab-scale Upflow reactor (Yang et al., 2001)

In biofilm or granule reactors the ammonium oxidizers are active in the outer layers of the biofilm (or granule), producing a suitable amount of nitrite for the Anammox organisms that are active in the inner layers. This way the Anammox organisms are protected from oxygen, which is consumed in the outer layers. A variation on the classic biofilm reactor is then membrane aerated biofilm reactor (MABR) (Stijn et al., 2010).

In MABR systems hydrophobic, gas-permeable membranes are used for bubbleless oxygen transfer. In the oxygen rich region near the membranes ammonium oxidizers are converting ammonium to nitrite, while in the ammonium rich region near the water phase Anammox organisms are active (Stijn et al., 2010).

When these biofilms and granular systems are used to perform the process, mass transfer resistance uses to be the limiting step. As long as ammonium concentration outside the biofilm is much higher than the oxygen or nitrite concentration, ammonium diffusion into the biofilm will not limit the process rate. If the nitrite produced in the outer layer is mainly consumed in the inner layer, oxygen is the main limiting factor controlling the overall rate. Sliekers et al. and Szatkowska et al. reported that oxygen transfer was indicated as the limiting factor for a lab scale air-lift and a pilot-scale moving bed reactor, respectively. This oxygen limitation can be attributed to the slow diffusion into the biofilm/granule or from a not-efficient gas–liquid transfer (Stijn et al., 2010).

Two strategies are possible to start-up a one-reactor autotrophic nitrogen removal system. The first method is the inoculation of nitrifying biomass into a well performing Anammox reactor and supplying air into the reactor to maintain microaerobic conditions. Otherwise, a partial nitritation reactor can be operated under oxygen limited conditions obtaining an ammonium: nitrite ratio of 1:1 before Anammox biomass is inoculated into the reactor (Stijn et al., 2010).

The second strategy seems to be more appropriated since an important decrease of Anammox activity will be observed when the first method is applied. This high nitrifying activity can protect the Anammox bacteria from oxygen and provides them enough nitrite. The inoculation of Anammox enriched biomass in a partial nitritation reactor accelerates the start-up and allows increasing the ANR after 1 or 2months instead of the several months or even years without inoculation. Moreover, only a limited amount of Anammox biomass is necessary to start-up the CANON process with this second strategy (Stijn W.H et al., 2010).

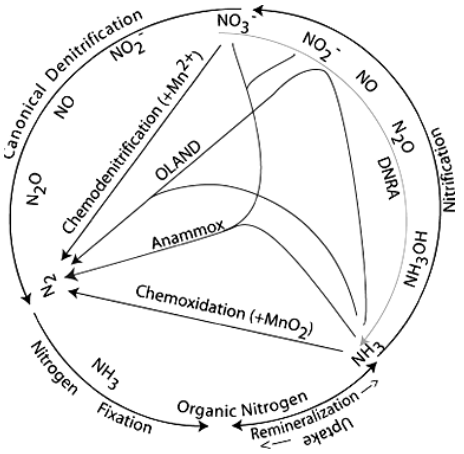


Fig.21. The nitrogen cycles as it is known today (Hertach, 2008).

The difference lies in the organisms that were originally assumed to be responsible for anaerobic ammonium oxidation. In both the OLAND-process and the aerobic/anoxic deammonification process nitrifiers were assumed to perform this ammonium oxidation under microaerobic conditions. In the CANON process Anammox bacteria were assumed to be responsible. Studies with FISH analyses confirmed that anaerobic ammonium oxidation in all reactors was performed by Anammox organisms, although Pynaert et al. did not exclude a specific role for the aerobic ammonium oxidizers (Stijn et al., 2010).

3.4.1.3 The OLAND-process

Kuai and Verstraete first introduced the term OLAND describing lab-scale research with a SBR reactor fed with synthetic influent in which only 0.050 kg ammonium-N m^{-3} d^{-1} was removed. The OLAND process (oxygen-limited nitrification and denitrification) is described as a process for one-step ammonium removal without addition of COD. The basic fact is that the nitrifiers are involved, and the ammonium loading rates are low (Schmidt et al., 2003).

Sliekers et al. (2003) conducted experiments in lab-scale completely mixed reactors using a specific start-up pattern consisting of anoxic inoculation with Anammox biomass followed by oxygen supply to develop nitrifying microorganisms. Ammonia was mostly converted to nitrogen gas (85%) while the remainder was recovered as nitrate.

Stoichiometry of oxygen limited autotrophic nitrification- denitrification

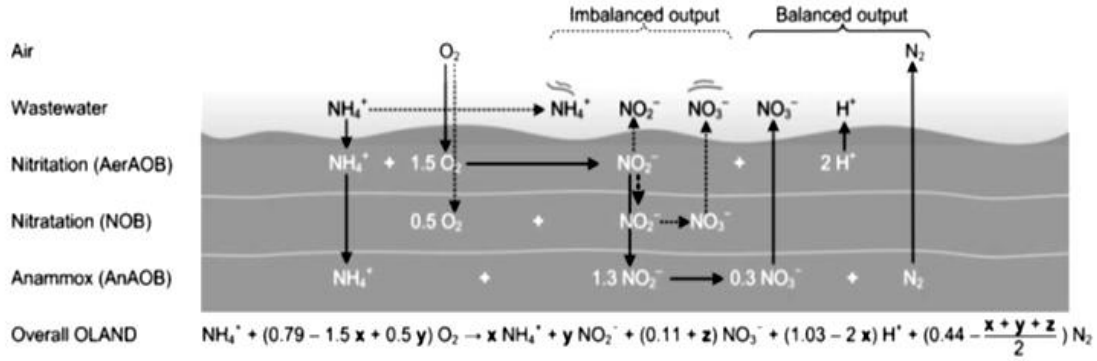


Fig.22. Conversion of nitrogen species, oxygen, and protons in OLAND, showing balanced and imbalanced contributions of three bacterial groups, i.e., aerobic ammonium-oxidizing, nitrite-oxidizing, and anoxic ammonium-oxidizing bacteria (AerAOB, NOB, AnAOB, respectively) (Clippeleir et al., 2011)

$$0.5\ NH_4^+ + 0.75O_2 \rightarrow 0.5NO_2^- + 0.5H_20 + H^+ \quad (14)$$

$$0.5\ NH_4^+ + 0.5\ NO_2^- \rightarrow 0.5\ N_2 + H_20 \quad (15)$$

$$NH_4^+ + 0.75\ O_2 \rightarrow 0.5\ N_2 + 1.5\ H_20 + H^+ \quad (16)$$

The reactions summarized demonstrate that it must be possible for the Nitrosomonas species to obtain sufficient energy for cell maintenance out of this combined action. Moreover, it indicates that the key parameter to control the process is the oxygen. The fact is that the autotrophic bacteria enriched sludge at the temperature in the range of 5 to 45°C. Now, it has been demonstrated that at 53°C methanotrophs can oxidize ammonium in the absence of methane, provided that some oxygen is present. The nitrate formed is rapidly denitrified (Verstraete et al., 1998).

In the laboratory (Laboratory of Microbial Ecology, Gent), an active enrichment culture of autotrophic nitrifiers is grown. Subsequently, this nitrifying autotrophic sludge is used as a biocatalyst to treat water rich in ammonium. The key feature is to provide oxygen so that the nitrification only proceeds to nitrite, and subsequently, due to shortage of electron acceptor, consumes its own nitrite to oxidize another mole of ammonium (Verstraete et al., 1998).

In contrast to the Anammox process, oxygen limited autotrophic nitrification-denitrification (OLAND) does not require anoxic conditions, but can proceed under microaerophilic conditions (Kuai and Verstraete 1998). Furthermore, the latter process is considered to be catalyzed entirely by autotrophic ammonia oxidisers (Philips et al., 2002).

Although the literature provides indications for the existence of this kind of process in artificial environments, it is not yet clear what could be the ecological niche in nature, where these processes are advantageous to traditional nitrification and opportunistic for the nitrifiers, and why bacteria should shift to using these processes (Philips et al., 2002).

A possible ecological niche could be the sediment of lakes and rivers. These sediments are environments where high amounts of nitrifiers can be found (e.g. Hastings et al., 1998; Pauer and Auer, 2000, cited in Verstraete et al., 1998), and where oxygen-limited conditions can occur. Moreover, when organic matter such as plant detritus and dead fauna are deposited at the sediment surface, the organic carbon is rapidly removed by aerobic heterotrophic bacteria, leaving relatively high amounts of ammonium, and depleting oxygen levels. The sparse remaining oxygen could allow some of the ammonium to be nitrified to nitrite. Subsequently, the OLAND process could take place in such sites. Indeed, there are reports in the

literature of unexplained nitrogen deficits and N_2 fluxes from sediments (Stief, 2001; Van Luijn et al. 1998 cited in Verstraete et al., 1998).

The mechanism of nitrite dismutation by Nitrosomonas species has been reported by Abeliovich and Vonshack (1992). This process of oxidative-reductive N removal, brought about by straight forwardly enriched autotrophic nitrifiers as biocatalysts is labeled OLAND (Oxygen Limited Autotrophic Nitrification Denitrification) (Verstraete et al., 1998).

Moreover, operation of the OLAND system has no requirement for an NO_2^- supply. A NH_4^+ -rich wastewater can be fed directly at a suitable loading rate. Although the process requires limited oxygen conditions, it does not require strictly anaerobic conditions. Therefore, inhibition by trace O_2 exposure is not a serious problem of concern in practice. The process operated by a pH controller is simple and reliable for practical operation (Kuai et al., 1998).

There are, however, some inherent challenges to obtain good OLAND process performance. First, AnAOB double only every 11 days, and this slow growth can result in very long reactor start-up periods and requires a high biomass retention, which must be ensured by growth in biofilms or flocs and granules. Second, high nitrogen removal efficiency of the OLAND process relies on limited nitrite accumulation, obtained when the AerAOB activity does not exceed the AnAOB activity. Third, high efficiency requires a limited nitrate production. Due to the anabolic nitrate production of AnAOB, OLAND typically converts 11% of the oxidized ammonium into nitrate, a value which is not exceeded in case the nitrate consumption by heterotrophic denitrifiers is larger than the nitrate production by nitrite oxidizing bacteria (NOB), i.e., nitratation (Siegfriede et al., 2009).

3.4.1.4 OLAND – Rotating Biological Contactor

Pynaert et al. (2003) constructed, operated and characterized an OLAND RBC system which high removal rates could be achieved after inoculation of a granular anaerobic sludge a maximum ammonium removal of 1.80 kgN m^{-3} d^{-1} was achieved. In Sliekers et al. a gas lift reactor with high conversion rate of up to 1.5kgN m^{-3} d^{-1} was easily maintained. Recently also artificial wetlands were used as autotrophic nitrogen removing systems resulting in 50–60% nitrogen removal (Stijn et al., 2010).

A mature OLAND biofilm under high NH_4^+ loading rate consists primarily of two major groups of bacteria responsible for autotrophic N removal. The aerobic ammonium oxidizing bacteria convert NH_4^+ to NO_2^- with oxygen as the electron acceptor (nitritation) and the anaerobic ammonium oxidizing bacteria (subsequently oxidize NH_4^+ with NO_2^- as the electron acceptor (anammox) (Strous et al., 1998; Pynaert et al., 2003; Wyffels et al., 2003).

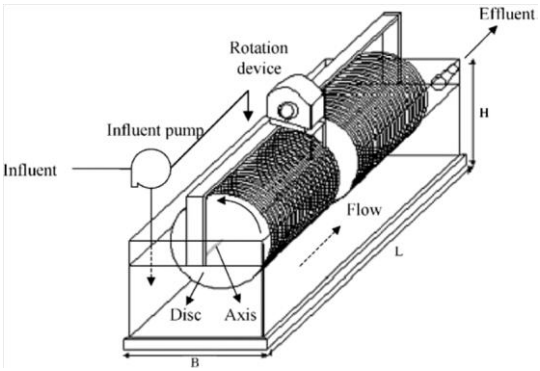


Fig.23. A Schematic representation of RBC reactor (Windey et al., 2005).

Autotrophic nitrogen removal of high-salinity wastewater with high nitrogen concentrations can be effectively achieved at loading rates upto 725 mg N L^{-1} d^{-1}, reaching N removal efficiencies of 84%. The N removal capacity was 31% lower at a salt level of 30 g NaCl L^{-1} compared to the reference period without salt addition (Windey et al., 2005).

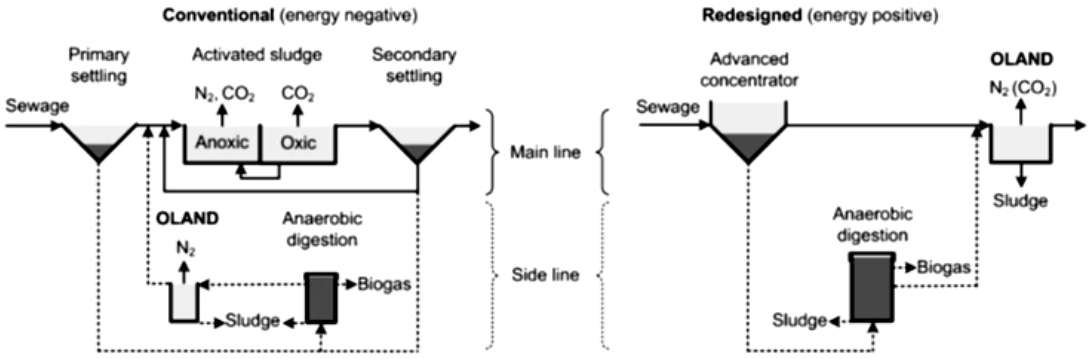


Fig.24. Conventional and redesigned sewage treatment schemes with OLAND in the side and main line, respectively (Clippeleir H.D. et al., 2011).

The energy requirement for OLAND is influenced by the reactor configuration: active aeration in sequencing batch reactors requires 1.2 kWh kg^{-1} N (Wett et al. 2010), whereas passive aeration in rotating biological contactors (RBC) requires down to 0.4 kWh kg^{-1} N (Mathure and Patwardhan 2005). Depending on the dilution, sewage is typically composed of 30–100 mgN L^{-1} and 450–1200 mg COD L^{-1} rendering a COD/N ratio of about 12 to 15 (Tchobanoglous et al. 2003; Henze et al. 2008, cited in Clippeleir et al., 2011).

Until now, the OLAND process has been applied for medium and high-strength nitrogen wastewaters (>0.2gN L^{-1}) such as landfill leachate and digestives from sewage sludge, specific industrial streams, and concentrated black water at relatively high hydraulic residence times. To obtain reasonably high nitrogen removal rates (400mgN L^{-1} d^{-1}), the treatment of low nitrogen levels (<80mgN L^{-1}) has to occur at low HRT, in the order of some hours, rendering biomass retention an important requirement (Clippeleir et al., 2011).

3.4.1.5 The CANON process

CANON is an acronym for 'Completely Autotrophic Nitrogen removal Over Nitrite'. This concept is the combination of partial nitrification and anammox in a single, aerated reactor. The name 'Canon' also refers to the way the two groups of bacteria cooperate (Schmidt et al., 2003). The entire nitrogen removal can be achieved in a single reactor with very low aeration, greatly reducing space and energy requirements. The autotrophic process consumes 63% less oxygen and 100% less reducing agent than traditional nitrogen removal systems.

A high amount of nitrogen loss as elemental nitrogen has been observed from wastewaters that are highly loaded with ammonium and contain low concentrations of organic carbon (Hippen et al., 1997; Helmer and Kunst, 1998; Kuai and Verstraete, 1998; Siegrist et al., 1998; Helmer et al., 1999, 2001; Koch et al., 2000, all cited in Khin et al., 2004). The microorganisms responsible for this are autotrophic populations that denitrify under low dissolved oxygen conditions. Along similar observations, Dijkman and Strous (1999) described a new biological nitrogen removal process named the CANON process for completely autotrophic nitrogen removal over nitrite (Khin T. et al., 2004).

They perform two sequential reactions simultaneously

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$ (17)

$NH_4^+ + 1.3NO_2^- \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O$ (18)

The process can be carried out in a single reactor or biofilm under oxygen-limited conditions. This process is based on a partial nitrification and anoxic oxidation of ammonia. These autotrophic cultures convert ammonia directly to dinitrogen gas with nitrite as an intermediate. Application of this concept to wastewaters can potentially lead to complete ammonia removal in a single autotrophic reactor. The two groups of microorganisms interact and perform the two sequential reactions simultaneously. As the nitrite also serves as an electron donor for the formation of biomass from carbon dioxide, the formation of nitrate in the reaction is stoichiometrically coupled to growth (Khin et al., 2004).

The nitrifiers oxidize ammonia to nitrite, consume oxygen and so create anoxic conditions the anammox process needs. Canon has been tested extensively on laboratory scale. The volumetric loading rate 1.5kgN $m^{-3}d^{-1}$ in a gas-lift reactor is lower than for anammox and also somewhat lower than has been achieved with high-end dedicated nitrification reactors (Schmidt et al., 2003).

The combination of the above two reactions results in nitrogen removal as follows (Strous, 2000):

$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.3H_2O + 1.4H^+$ (19)

Under oxygen-limited conditions (< 0.5% air saturation) a co culture of aerobic and anaerobic ammonium-oxidizing bacteria can be established (Strous, 2000), and this system is responsible for the CANON activity. The process relies on a stable interaction between the two groups of autotrophic microorganism populations: Nitrosomonas-like aerobic bacteria and Planctomycete-like anaerobic ammonium-oxidizing bacteria, under oxygen limited conditions (Khin et al., 2004).

The interaction of aerobic and anaerobic ammonium-oxidizing bacteria under oxygen limited conditions results in an almost complete conversion of ammonium to dinitrogen gas. Small amounts of nitrate are also produced. A dissolved oxygen (DO) concentration of up to 0.5 mg/l has no effect on ammonia oxidation, but nitrite oxidation is strongly inhibited in suspended growth reactors (Hanaki et al., 1990). In the oxygen-limited conditions, nitrite oxidizers have to compete for oxygen with the

aerobic ammonia oxidizers and for nitrite with anaerobic ammonia oxidizers. Possible inhibition of nitrite oxidizers by free ammonia has been suggested (Abeling and Seyfried, 1992, cited in Khin et al., 2004).

Considering this, ANAMMOX processes are feasible at low bulk oxygen concentrations. ANAMMOX bacteria are reversibly inhibited by low (0.5% air saturation) concentration of oxygen (Strous et al., 1997). The combined process (Eq.19) can occur under oxygen-limited conditions.

The effect of ammonium limitation in the CANON system was investigated at the laboratory scale in two different reactor types (sequencing batch reactor and chemostat). The lower limit of effective and stable nitrogen removal to dinitrogen gas was 0.1kgN $m^{-3}d^{-1}$. At this loading rate, 92% of the total nitrogen was removed. If the influx of nitrogen is lower than the critical NH_4^+ influx, the stoichiometry of the CANON reaction is affected, and this causes a temporary decrease of nitrogen removal from 92% to 57% (Khin et al., 2004).

In studies with a sequencing batch reactor operated with an ammonium-rich wastewater under oxygen-limited conditions at a suitable loading rate with aerobic nitrifying bacteria and ANAMMOX bacteria, a nitrogen removal rate of up to 0.3kgN $m^{-3}d^{-1}$ has been reported for the CANON process. In this reactor, heterotrophic denitrification did not occur, and no aerobic nitrite-oxidizing bacteria were detected (Sliekers et al., 2002). The CANON process has been carried out in gas lift reactors. Nitrogen removal rates up to 1.5 kgN $m^{-3}d^{-1}$ were achieved. This removal rate was 20 times higher compared to the removal rates achieved in the laboratory previously (Sliekers et al., 2003). Gaslift reactors are easy to operate stably, and a lot of information has become available for designing those (Khin et al., 2004).

The CANON process is an economic and efficient option for wastewater treatment, especially for wastewaters rich in ammonium but devoid of organic carbon (COD). The CANON process is completely autotrophic and therefore requires no added COD. In addition, the entire nitrogen removal can be achieved in a single reactor with little aeration. This greatly reduces the space and energy requirements. The autotrophic process consumes 63% less oxygen and 100% less reducing agents than does a conventional nitrogen removal process (Kuai and Verstraete, 1998).

Most CANON systems reported in literature were operated at 30–35◦C with a maximal nitrogen removal rate of 0.075–1.5 kgN $m^{-3}d^{-1}$.At these temperatures AOB grow faster than NOB and also the growth of Anammox bacteria is stimulated since this temperature range lies close to their optimal temperature. However, in an air pulsating SBR operated at 20-24◦C a similar maximal nitrogen removal of 0.5 kgN $m^{-3}d^{-1}$ are reported while only a slightly activity of NOB was observed. The feasibility of achieving a quick start-up and high nitrogen removal rates in autotrophic nitrogen removing systems at temperature around 20◦C was already reported by Isaka et al. (2006) and Dosta et al. (2008) in a two stage system and Pynaert et al. (2001) in one stage system.

The efficient retention of biomass in a SBR makes it possible to cultivate slowly growing bacteria. However, higher nitrogen removal rates were obtained in a RBC and in an air lift reactor. Model simulations indicated that the maximum nitrogen removal rate was achieved only when the dissolved oxygen concentration kept pace with the ammonium surface load.

Unlike other autotrophic nitrogen removal systems, such as the SHARON-anammox process (Jetten et al., 1997) where the nitrite is generated in a separate reactor, there is no requirement for nitrite addition in the CANON system. Thus, an ammonium-rich wastewater can be fed directly to a single oxygen-limited reactor at a suitable loading rate. Nitrogen removal rates of up to 0.3 kgN_{total} m^{-3} d^{-1} have been reported for the CANON process (Sliekers et al., 2001).

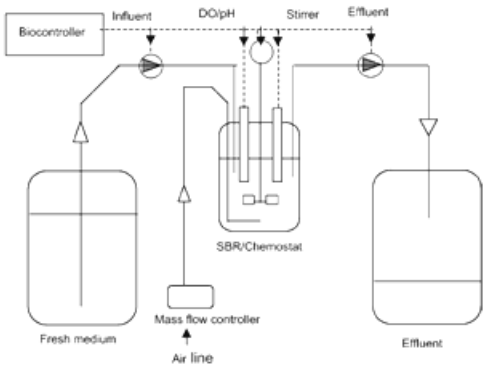


Fig.25. Experimental set-up of and SBR/Chemostat System (Third et al, 2004)

The CANON system could effectively remove nitrogen in a single oxygen-limited treatment step. The lower limit for stable nitrogen removal to dinitrogen gas was

0.4mmoles L^{-1} h^{-1} (0.12kgN m^{-3} d^{-1}). At and above this ammonium influx, a stable interaction existed between aerobic and anaerobic ammonium oxidizing bacteria under oxygen limitation (Third et al., 2004).

Other autotrophic systems for removing ammonium from wastewater include the SHARON-Anammox process (van Dongen et al., 2001) and the OLAND (oxygen limited autotrophic nitrification-denitrification) process (Kuai and Verstraete, 1998). The nitrogen-removal rate observed in this study (0.12kgN $m^{-3}d^{-1}$) compares well with the rates reported in the other autotrophic systems (0.8 and 0.05kgN $m^{-3}d^{-1}$, respectively). Under ammonium-saturated operation of CANON in the SBR and chemostat, the process was limited principally by the oxygen transfer rate to the aerobic ammonium-oxidisers (Third et al., 2004).

A recent investigation looked at increasing the oxygen transfer rate by using a gas-lift reactor for the CANON system. Nitrogen removal rates of up to 1.5kgN m^{-3} d^{-1} have been reported when the gas-transfer rate is optimized, showing that the CANON could be a very useful nitrogen removal process for very high strength ammonium wastewaters (Third et al., 2004).

The ability of the CANON system to withstand ammonium limitation for up to one month without irreversible damage shows that the CANON system could be a robust and effective industrial system to remove ammonium from wastewater with a very low organic load (Third et al., 2004).

FISH analysis confirmed the absence of nitrite oxidizers and the presence of aerobic ammonia oxidizers (45%) and anaerobic ammonium oxidizers (40%) in the CANON biomass. Recently, De Clippeleir et al. (2009) and Vazquez Padin (2009a) observed high nitrogen removal rates in a SBR provided that granulation occurred. The operation of these granular sludge reactors is very similar to biofilm reactors (Stijn et al., 2010).

3.4.1.6 The DEMON process

Deammonification represents a short-cut in the N-metabolism pathway and comprises 2 steps. About half the amount of ammonia is oxidized to nitrite and then residual ammonia and nitrite is anaerobically transformed to elementary nitrogen (Wett, 2007).

The significance of this process has not even been recognised despite its massive occurrence in natural habitats – it contributes up to 50% to the removal of fixed N from the oceans (Arrigo, 2005). Under anaerobic conditions an autotrophic metabolism can directly oxidise ammonia by means of nitrite (Fig.27) (Wett, 2007).

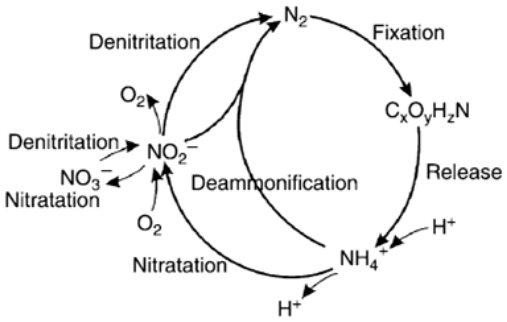


Fig.26. Nitrogen cycle presenting deammonification as a metabolic short-cut of N-conversion

Strous et al. (1999a) managed to identify the missing lithotroph as a new planctomycete which catalyses anaerobic ammonia oxidation according to following equation (20):

$$NH_4^+ + 1.32NO_2 + 0.066HCO_2 + 0.13H^+ \rightarrow$$
$$0.26NO_3 + 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (20)$$

Stoichiometric coefficients of this reaction have been derived in closer detail on base of an elemental balancing approach (Takacs et al., 2007). The appropriate molar ratio of the two reactants has to be provided by partial nitritation of ammonia by ammonia oxidisers AOBs. Further oxidation of nitrite to nitrate has to be repressed by ammonia inhibition of nitrite oxidisers NOBs (Turk and Mavinic, 1987). While high ammonia influent concentration facilitates optimised metabolic routing, accumulation of nitrite concentrations endangers process stability due to toxic impact on anammox organisms (Strous et al., 1999b). Finally specifically AOBs are the autotrophic organisms showing highest sensitivity to inorganic carbon limitation (Wett and Rauch, 2003; Guisasola et al., 2007 cited in Wett, 2007). Both consecutive process steps – partial nitritation and anaerobic ammonia oxidation–are referred to as deammonification (Wett, 2007).

The concept has not been "purposefully tested on pilot or full scale, but is known to occur accidentally in sub-optimally functioning full-scale nitrification systems" (Schmidt et al., 2003; cited in Wett, 2007).

Deammonification appears as an attractive option for treatment of high-strength ammonia streams and provides a high resource saving potential. In terms of the nitrogen cycle - the starting point of this presentation–deammonification has reduced the specific energy requirement for nitrogen conversion towards the range of the highly developed industrial N-fixation process. Long start-up periods and lack of operational reliability have frequently been reported as major short-comings of deammonification technology (Wett, 2007).

Presented full-scale case-studies could demonstrate the importance of a robust control strategy in order to integrate a side-stream deammonification system into an every-day routine operation operators are confident with. Applied volumetric loading rates up to 0.7 kgNH_3-N m^{-3} showed even higher removal efficiency than low-load situations (Wett, 2007).

3.4.1.7 DEMON Process-Full Scale application:

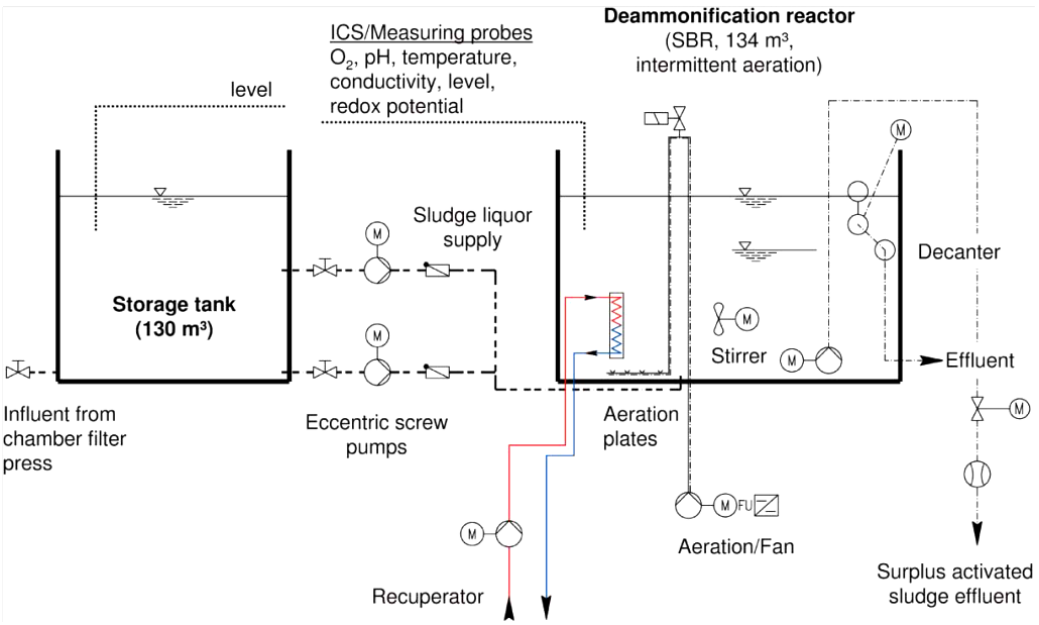


Fig.27. Flow scheme and process control layout of the deammonification plant.

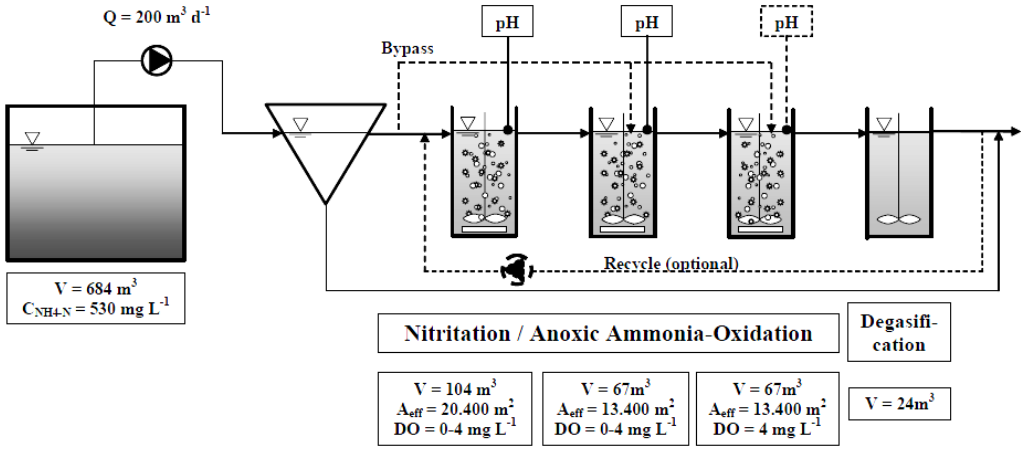


Fig.26. Flow sheet of full – scale deammonification plant at Hattingen WWTP (Jardin et al., 2006).

Separate treatment of reject water by deammonification improves nitrogen removal efficiency and operation of the wastewater treatment plants. At Plettenberg WWTP a deammonification using a SBR system with suspended biomass was built in 2007 and has been operated successfully by Ruhrverband with a modified operating strategy since 2008. More than 85% total nitrogen and up to 95% NH_4-N removal can be achieved (Jardin et al., 2012).

Sludge wash-out may be caused by system operation and control failure, but also may be due to low-load operation. So far there are two full-scale DEMON process implementations for high-nitrogen streams with ammonium concentration around 1800mgNH_4^+-N L^{-1}, but there are no available data on how the system would behave under lower concentrations in the range of 500 to 800mgNH_4^+-N L^{-1}. Washed-out sludge has to be regenerated, which may take a significant amount of time. Removed nitrogen load increased from 0.07kgN m^{-3} d^{-1} to 0.2kgN m^{-3} d^{-1} in four weeks (from 0.1 kgN $m^{-3}d^{-1}$ to 0.5kgN $m^{-3}d^{-1}$ in 12 weeks) in the DEMON reactor in the Strass wastewater treatment plant (WWTP). Similar results were obtained from the DEMON reactor in the Glarnerland WWTP, where the removed nitrogen load increased from 0.3 to 0.56kgN $m^{-3}d^{-1}$ in around four weeks (Jaroszynski et al., 2011). Nitrite accumulation seems to be very critical for the DEMON process, as accumulation up to 80mgNO_2^- -N L^{-1} may destabilize the system for a week and cause subsequent irreversible damage.

It was suggested that nitrite concentration as low as around 5mgNO_2^--N L^{-1} can negatively impact the DEMON process. Nitrite concentration increase in the reactor may be caused by many parameters, i.e. DO above 0.5 mgO_2 L^{-1} or a change in pH bandwidth from 0.02 to 0.04.

However, during the start-up phase of the deammonification plant the two-stage process of nitritation and anaerobic ammonium oxidation appears to be rather sensitive to external disturbances. In this phase, particular attention has to be paid to the sustained suppression of nitrite oxidisers. Key to a sustained inhibition on NOB is an appropriate selection of DO level, pH-value and cycle times for aerobic and anoxic phases. Once nitrite oxidation occurs in the system, reduction of aerobic cycle length at elevated DO levels proved to be effective in order to suppress growth of NOB (Jardin et al., 2012).

A start-up and control scheme with a specific strategy was developed to ensure a stable plant operation. This custom control scheme can also be applied in other wastewater treatment plants. Re-suppression of NOB after system disturbances is now possible by an adaptive control strategy. Furthermore, it can be said that though pH-control works sufficiently well, a back-up strategy using time control appears to be advisable.

Therefore, the deammonification process is a cost-effective method for reject water treatment. Calculating the economic value, there are additional benefits attributable to the decrease in oxygen consumption in the main stream of the WWTP as well as to lower wastewater discharge fees. At the deammonification unit in Plettenberg, it was possible to settle investment costs through wastewater discharge fees (Jardin et al., 2012).

After three years of successful operation at the Plettenberg WWTP, Ruhrverband can conclude that deammonification is ready for large scale operation but still this technology is not plug and play and needs at least during the start-up phase thorough supervision (Jardin et al., 2012).

The term aerobic/anoxic deammonification or DEMON was first used when significant losses of inorganic nitrogen of up to 90% were observed in the nitrification step of a rotating biological contactor (RBC) treating ammonium-rich landfill leachate under low oxygen condition.

Extended nitrogen loss was also observed in other RBCs in Switzerland and the UK. None of the plants were specifically built for deammonification, but nitrogen elimination was established over time. In the Swiss RBC about 50% of the bacteria population in the biofilm consisted of Anammox. Next to RBC's continuous flow moving-bed pilot plants were run as well. Optimal ammonium elimination was achieved at a bulk oxygen concentration of $0.7 mgO_2\ L^{-1}$. The end product is always N_2, although Gaul et al. reported up to 12% N_2O production caused by incomplete heterotrophic denitrification under anoxic or oxygen-limited conditions (Stijn et al., 2010).

The first full-scale application with deliberate deammonification in a moving bed reactor using Kaldnes® carriers was put into operation in April 2001 at the WWTP of Hattingen (Germany). Two identical reactors had a volume of 67 m^3 and an effective biofilm surface area of 13,400 m^2. The oxygen concentration was kept below 1 $mgO_2\ L^{-1}$ (Stijn et al., 2010).

3.4.1.7 The "SNAP" process

Single-stage Nitrogen removal using Anammox and Partial nitritation (SNAP) process was newly developed as an economical nitrogen removal process for ammonium rich wastewaters.

The stoichiometry of the anammox reaction is shown in the following equation (Strous et al., 1998):

$$1.0NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow$$
$$1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad (21)$$

For the application of the anammox reaction to the nitrogen removal process, half of the influent NH_4^+ must be converted to NO_2^-as a preceding step. Nitritation, in which NH_4^+ is oxidized to NO_2^- by ammonium oxidizing bacteria (AOB), was studied intensively by many researchers as an economically favorable process for wastewaters having high NH_4^+-N concentrations and low C/N ratios, such as landfill leachate and sludge digester liquor. The SHARON process enabled the nitritation by controlling the conditions (pH and temperature) under chemostat culture (Hellinga et al., 1998).

The combination of partial nitritation, by which half of the influent NH_4^+ is oxidized to NO_2^-, and anammox reaction will give a cost effective NH_4^+-N removal process (Furukawa et al, 2006).

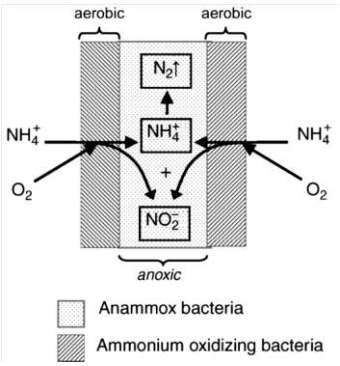


Fig.29. Conceptual demonstration of SNAP process (Furukawa et al., 2006)

3.4.2 "Two-Reactor" Systems

With a two-reactor system nitritation and Anammox are separated in space allowing flexibility and a more stable process performance since both steps can be controlled separately. In a first reactor half of the ammonium is converted to nitrite, while in a second reactor Anammox is active. It is important that the influent of the Anammox reactor has a constant composition in view of the nitrite toxicity, independent of the strategy used to obtain this Anammox-suited influent.

The application of the two-unit configuration would be appropriated when toxic or organic biodegradable compounds are present since these compounds will be degraded in the proceeding nitritation step avoiding its entrance to the Anammox reactor (Stijn W.H et al., 2010).

Examples of a two-reactor configuration include

- Partial nitrification–anammox and
- SHARON–ANAMMOX (Single-reactor High activity Ammonia Removal over Nitrite – Anaerobic AMMonium Oxidation).

3.4.2.1 Partial nitrification and anammox in separate reactors

A two-reactor system may consist of the first reactor being of chemostat type, i.e. where hydraulic residence time (HRT) equals solids residence time (SRT), as in the SHARON reactor, or of SBR type, for partial nitrification.

The second reactor may be an upflow-type reactor for anammox, where the SRT of the granular sludge is much longer than the HRT (Jaroszynski et al., 2011).

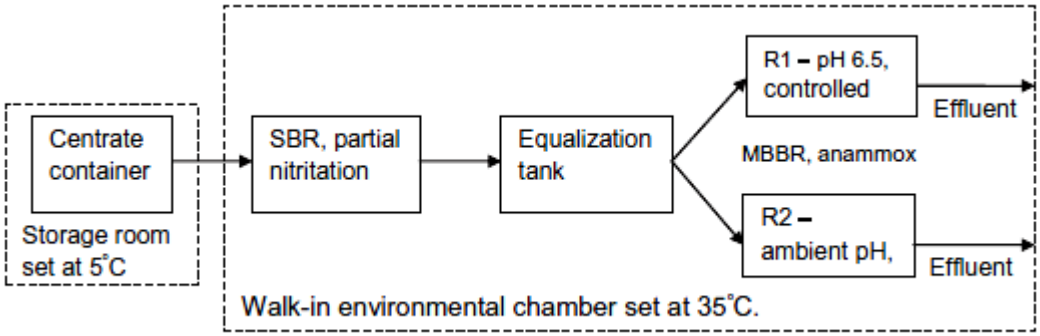


Fig.30. A schematic of PN and anammox process (Jaroszynski et al., 2011a)

3.4.2.1a The First Step: Partial Nitrification

For the proper optimization of a two-reactor system, it is necessary to balance the nitrification rate in the partial nitrification reactor with the anammox rate in the anammox reactor. In two-reactor configurations partial nitrification is achieved under high substrate concentrations in the range of 300 to 400 mgN L^{-1} for ammonium and nitrite, where nitrite concentration is determined by the available alkalinity. Therefore, fluctuations of ammonium and alkalinity strongly affect the nitrite to ammonia ratio, which may disturb the anammox process (Jaroszynski et al., 2011).

Digester effluent from two different WWTPs was tested obtaining an Anammox-suited ammonium: nitrite ratio of 1:1.32 at a pH between 6.6 and 7.2 (Stijn et al., 2010).

In a partial nitrification reactor under such high nitrogen concentrations, free ammonia (FA) and free nitrous acid (FNA) strongly affect ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Uncontrolled FA and FNA may stop the process as a result of inhibition of the energy production and growth processes. In an anammox reactor, nitrogen concentrations are relatively low when compared with the partial nitrification reactor; however, pH tends to increase significantly which may cause FA inhibition (Jaroszynski et al., 2011).

Ganigué et al. (2009) showed that the SBR is a feasible technology to achieve stable influent for an Anammox reactor when urban landfill leachate is treated.

At low pH values biological activity decreased due to an inhibitory effect by free nitrous acid and a lack of bicarbonate. On the other hand, high pH values indicated a decrease in oxygen uptake rate caused by free ammonia inhibition (Stijn et al., 2010). As such, pH is considered to be an important factor. Udert et al. (2003) used a SBR to treat urine at a temperature of 24.5◦C while varying the oxygen concentration between 2 and 4.5mgO_2 L^{-1}. The pH at the start of the reaction cycle was 8.8 and gradually decreased to a minimum of 6 as ammonium conversion continued. At this pH ammonium conversion stopped probably because NH_3 limitation and HNO_2 inhibition obtaining an ammonium: nitrite ratio of 1 (Stijn et al., 2010).

Another possible explanation is the inhibition of nitrite oxidizers by the intermediaries' hydroxylamine. Yamamoto et al. (2009) cited in Stijn et al., (2010) applied the partial nitritation and Anammox process to treat swine wastewater digester liquor. They observed that a stable conversion of ammonia into nitrite of 58% could be reached in a biofilm reactor due to inhibition of free ammonia and free nitrous acid. The inhibition of free ammonia was also brought forward by Liang et al. (2008) and Qiao et al. (2010) cited in Stijn et al., (2010) who treated landfill leachate and digested liquid manure, respectively in a biofilm reactor achieving a nitrite: ammonia molar ratio near 1.3 (Stijn et al., 2010).

In the different experiments described above, different conditions were used to favour the growth of ammonium oxidizers over nitrite oxidizers in order to produce an Anammox-suited-influent. Four principles can be distinguished:

- The operation of the reactor at low DO concentration (<0.5mgO_2 L^{-1}),
- The operation of the reactor at high pH (7.5–8.5), which increases the ammonia availability and decreases the nitrous acid availability,
- The operation of the reactor at high temperature (>25◦C), a limited nitrification time which stops ammonium oxidation before its depletion and
- Presence of a bicarbonate limitation which stops nitrification.

Compared to other techniques for treatment of nitrogen-rich wastewaters like steam stripping, the MAP-process or the air lift reactor, SHARON has several advantages:

- Low investment costs and operational costs
- No chemical by-products
- Simple operation and maintenance

- Easy start-up
- Insensitive to high influent SS levels
- Negligible odour emission.

The recovery procedure after a system upset may be similar to the start-up operation, because of the required biomass regeneration and build-up. The reasons for system failure are similar for various system configurations:

- Sludge washout (except in the attached growth),
- Nitrite accumulation (for the anammox process),
- Nitrogen starvation and
- Toxic substances in the feed.

Sludge washout may occur either in partial nitrification or in suspended growth anammox, and may be caused by excessive flow due to, for example, valve failure. Such factors may be controlled by proper design, operation and system monitoring. Depending on how severe the washout was or how much seed was available, the startup and/or recovery may not take long (Jaroszynski et al., 2011).

This may lead to the conclusion that the SHARON – ANAMMOX process is not the right configuration in terms of overall nitrogen removal rate calculated per volume of both reactors, because of the low partial nitrification rate in the SHARON reactor (Jaroszynski et al., 2011).

Partial nitrification in the SHARON reactor would not be as affected by washout, as the system works without sludge settling. In the SBR system, it would probably take no more than one or two weeks as a result of low SRT. For the anammox reactor it was observed that when a sufficient amount of anammox bacteria was available, the increase in load could be very fast. The conversion rate in one case of an attached growth increased from 0.1 kgN $m^{-3}d^{-1}$ to 0.9 kgN $m^{-3}d^{-1}$ in three weeks. The conversion rate increased from 0.3 kgN $m^{-3}d^{-1}$ to 1.0 kgN $m^{-3}d^{-1}$ in four weeks in an SBR type reactor. In a full-scale Rotterdam case, after a sufficient amount of anammox bacteria was cultivated (conversion rate around 2 kgN $m^{-3}d^{-1}$), the design load of 7 kgN $m^{-3}d^{-1}$ was achieved in around three weeks (Jaroszynski et al., 2011).

Nitrite accumulation is not a problem for the partial nitrification reactor as it is operated under high nitrite concentrations, unless nitrite together with pH leads to too high an FNA (around 0.4 mgHNO_2-N L^{-1} d^{-1}), which may inhibit the reaction.

Nitrite may cause severe damage to the anammox process. Nitrite concentration of around 100 mg NO_2–N L^{-1} was shown to lead to loss of activity. Such occurrences are unlikely in full-scale systems as the anammox reactor is designed to be operated under nitrite-limiting conditions, unless there has been a monitoring failure or appearance of toxic substances, which would slow down the anammox reaction rate (Jaroszynski et al., 2011).

Starvation and biomass decay are the results of many factors, but the most probable scenario would be the periodical conservation or breakdown of the dewatering facility, leading to occasional lack of feed. In this situation lab-data for the partial nitrification reactor demonstrate a very fast recovery time for the SHARON reactor (<2 days) and the SBR reactor (3-4 days) for a five-day starvation period. For the anammox reactor, to our knowledge, there are no available reliable data, and biomass decay is hard to predict, as some anaerobic processes (i.e. H_2S formation) would cause inactivation of the anammox bacteria (Jaroszynski et al., 2011).

Two-reactor configurations can be control in a very simple way. Partial nitrification is controlled by the proper HRT/SRT design, which is between 1 and 1.5 days, where the oxygen is set around 1.5mgO_2 L^{-1}. The anammox reactor is designed to be operated under nitrite-limiting conditions. The suggested design load is 7.1kgN m^{-3} d^{-1}. However, 10.0kgN m^{-3} d^{-1} can be achieved in full-scale operation (Jaroszynski et al., 2011).

Based on the reviewed literature, there is no reported any information about instabilities in full-scale operation of two-reactor configurations. Lab-scale examples show very stable partial nitrification in the SHARON configuration. However, some instability was reported for the anammox reactors (Jaroszynski et al., 2011).

The challenge for the first reactor in a 2-reactor system is to obtain a stable, Anammox-suited effluent, i.e. with a molar ammonium: nitrite ratio of 1:1.32 according to the stoichiometry proposed by Strous et al. In practice, however, this ratio will be closer to 1:1 in view of the desire to prevent nitrite inhibition, i.e. by providing an excess of ammonium. Up to now three types of reactors were used to achieve this:

- Completely stirred tank reactors (CSTR),
- Membrane bioreactors (MBR) and

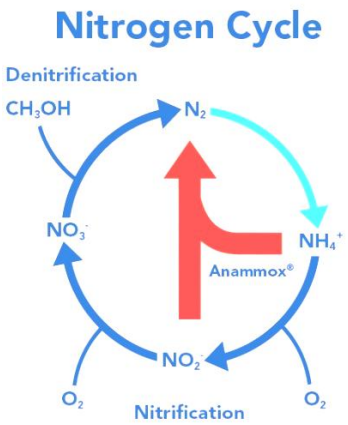
- Sequencing batch reactors (SBR).

In the MBR and SBR reactor high sludge retention times are obtained (50–75 days). In the MBR the SRT is difficult to manipulate unlike in suspended growth systems which bring difficulty to suppress nitrite oxidizers even under oxygen-limited concentrations. Fux et al. also stated that a long-term nitrite production without nitrate accumulation can be unreliable in biofilm systems since the control of the sludge age is difficult.

None of the selection criteria applied such as high free ammonia, low oxygen concentration or high ammonium loading rate led to selective suppression of nitrite oxidation in a long-term laboratory and pilot scale moving-bed biofilm reactor. For full scale applications, A CSTR or a SBR with suspended biomass is recommended (Stijn et al., 2010). Further, the footprint of an MBR system is reduced due to the absence of settling tanks and the reduction in bioreactor volume due to the higher biomass concentration.

The possibility to obtain an Anammox-suited effluent by SHARON process was tested by van Dongen et al. (2001) and Mosquera Corral et al. (2005) as cited in Stijn et al., (2010) in a CSTR with reject water as influent at a temperature of 35◦C and a HRT and SRT of 1 day. The ammonium was for 53% oxidized to nitrite without pH control resulting in a nitrite: ammonium ratio of 1.13:1. In the subsequent Anammox reactor nitrite was therefore the limiting component (Stijn et al., 2010).

3.4.2.2 – Second step: Separate Anammox Process:



So far a large range of bioreactor types have been evaluated for the enrichment of Anammox bacteria: Fixed bed reactors, Fluidized-bed-reactors, UASB-reactors, SBR, Gas-lift reactors. Among them, the SBR was accepted for Anammox enrichment for its simplicity, efficient biomass retention, homogeneity of mixture in the reactor, stability and reliability for a long period of operation, stability under substrate-limiting conditions and high nitrogen conversions. The SBR reached a biomass retention of 90% which was 1.4 times more than in a fluidized bed reactor. Strous et al. (1997) started up the Anammox process in a fixed-bed and fluidized bed reactor with glass and sand particles as carriers but could not prevent biomass loss due to floating sludge caused by entrapped gas bubbles. The same situation occurred in the gas lift reactor at increased nitrogen removal rate (Stijn et al., 2010).

Dapena-Mora et al. (2004) stated that mechanical stirring in a SBR could be more effective to eliminate the gas entrapped in the granules compared to the less abrasive stress in a gas-lift. Further, also the application of non-woven fibers can increase the biomass retention as several experiments with nonwoven fibers demonstrated a short start-up time and high nitrogen removal rates (Stijn et al., 2010).

An alternative for obtaining full biomass retention in Anammox systems might be the use of membrane bioreactors (MBR). Unlike the reactors with granular biomass, the MBR enables cultivation of slow growing bacteria with biomass retention and without a selection on settling ability. Van der Star pointed out that the MBR reactor is a more powerful tool for Anammox research as high production of almost pure suspended Anammox cells could be obtained avoiding the diffusion limitations within flocs or granules (Stijn et al., 2010).

A membrane SBR which is a combination of a SBR and a biofilm system was applied by Trigo et al. (2006) achieving a high nitrogen removal rate. Wang et al. (2009) used a stirrer in the MBR to make the Anammox bacteria suspended as free cells and a more homogeneous distribution of substrates and biomass can be achieved. However, for full-scale applications biofilm- or granular-based bioreactors are preferable over MBRs since anammox bacteria easily form sludge granules or biofilms obtaining a high biomass concentration in the reactor on a simple and economical way. Further, fouling of the membrane system could occur.

The operation costs due to backwashing (high energy consumption) or external cleaning with chemicals are inevitable in engineering practice. Moreover, wastewater always contains a certain amount of solids which are also retained in a MBR reactor. This accumulation of solids could decrease the activity in a full-scale MBR-based anammox process (Stijn et al., 2010).

From these studies the potential of the Anammox process can be seen since total nitrogen removal rates up to 26 kgN m^{-3} d^{-1} in a fixed bed reactor fed with synthetic wastewater. In contrast, the nitrogen removal rate is not so high in engineering. A possible explanation of the lower nitrogen removal rates in pilot plants is the limited availability of substrate in real waste waters. The efficiency of biomass retention is another factor which determines the maximum conversion while in biofilm reactors, nitrite flux to the biofilm is another potential limitation (Stijn et al., 2010).

Isaka et al. (2006) stated that HRT has an influence on the nitrogen removal rates. Under appropriate nitrite and ammonium concentrations nitrogen conversion rates can be increased by decreasing the HRT. Wyffels et al. (2004) stated that the maximum nitrogen removal rate of Anammox organisms is limited by the growth rate of ammonium oxidizers in the SHARON process since a minimum HRT of 1 day is needed (Stijn et al., 2010).

The concentration of nitrite during the start-up is of crucial importance for growth: a too low amount will result in substrate limitation and thus slower growth, while concentration above 20mgN L^{-1} can already lead to inhibition. As such, nitrite levels could increase even more leading to complete process failure. Start-up of Anammox reactors is often characterized by a gradual increase of nitrite concentration in the influent. The nitrite: ammonium ratio in the influent reaches 1 although often an excess of ammonium is used allowing lower overall nitrogen removal efficiency but guaranteeing a more stable process. Since the Anammox process is anaerobic, the absence of oxygen is an essential step especially during the start-up of reactors. Further, the impact of variability in real streams on the performance of Anammox in full-scale reactors is not well understood yet. To fasten up the start-up period, Anammox biomass is often used as inocula of Anammox reactors. The fast start-up time of 14 days in a SBR reactor by Sliekers et al. (2003) was due to the inoculation of the reactor with fully active Anammox sludge (Stijn et al., 2010)..

For the other reactors start-up time was significantly higher. Sequential addition of the pre-enriched Anammox sludge was also selected as a strategy for the engineering practice in the Netherlands. The 10 L lab scale reactor was directly scaled up to a full scale reactor of 70m^3 reactor. This reactor was initiated in Rotterdam in 2002 and the start-up took nearly 3.5 years. Now stable operation reached a nitrogen removal rate of 9.5 kgN $m^{-3}d^{-1}$ (Stijn et al., 2010).

3.4.3 Combined SHARON and ANAMMOX processes

The introduction of anammox to N-removal would lead to a substantial reduction of operational costs. Wastewater that contains high amounts of ammonium and little organic COD, such as sludge liquor or landfill leachate are prime targets for an Anammox application (Jetten et al. 1997; Strous et al. 1997a, cited in Jetten et al., 2002).

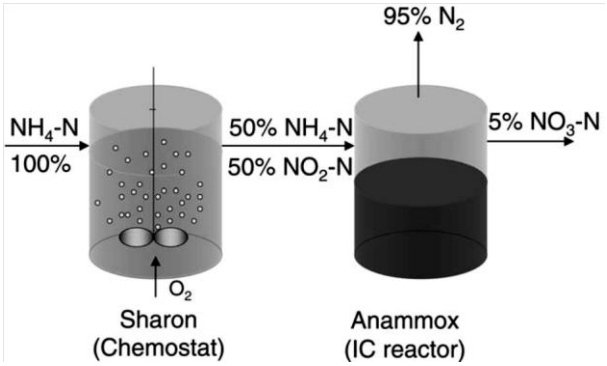


Fig.31. Schematic representation of the combined Sharon-anammox process for the removal of ammonium from sludge digestion effluents (Jetten M.S.M. et al., 2002)

The Sharon process was originally developed for the removal of ammonium via the so called nitrite route (see chapter 3.2.1). It was tested for 2 years in the laboratory and successfully scaled-up from 2L to full scale (1800 m^3) (Mulder et al. 2000, cited in Jetten M.S.M. et al., 2002).

As discussed previously, the ratio of ammonium and nitrite needed for the anammox process is 1.32. For sludge liquor, this ratio can be achieved without any pH control, because these effluents contain bicarbonate as the counter-ion for ammonium.

Thus, when half of the ammonium in the liquor is converted, the alkalinity of the water is nearly depleted; leading to a pH drop and preventing further nitrification (equation 22):

$$NH_4^+ + HCO_3^- + 0.75O_2 \rightarrow 0.5NH_4^+ + 0.5NO_2^- + CO_2 + 1.5H_2O \quad (22)$$

The feasibility of Sharon process for the production of approximately equal amounts of ammonium and nitrite was demonstrated in a 20 liter laboratory system with sludge digester effluent from the wastewater treatment plant, Dokhaven-Sluisjesdijk, in Rotterdam, the Netherlands. The ammonium in the liquor was oxidized for 53% to nitrite at nitrogen load of 1.2 kgN m^{-3} d^{-1}. It was not necessary to apply a pH control in the Sharon system. The ammonium and nitrite ratio in the effluent of the Sharon process could be fine-tuned by adjusting the pH between 6.5 and 7.5. The effluent of this Sharon reactor was used to start up an Anammox SBR system.

The characteristics of the biomass were consistent with those of B. anammoxidans. The cells produced hydrazine from hydroxylamine, converted ammonia and nitrite in the expected 1 to 1.3 stoichiometry, produced some nitrate, and reacted with 16S rDNA probes specific for anammox bacteria (Van Dongen et al. 2001, cited in Jetten et al., 2002).

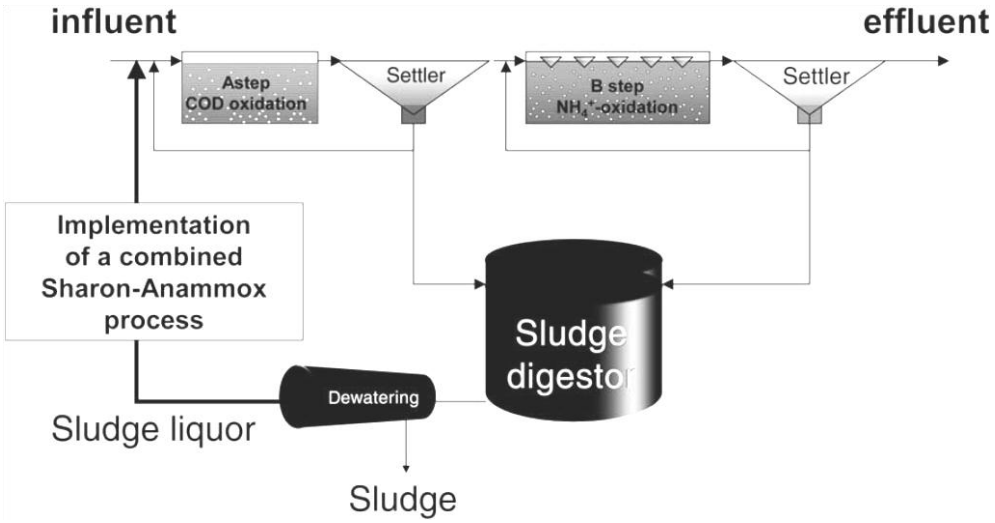


Fig.32. Schematic representation of the planned implementation of a combined Sharon-anammox process (Jetten M.S.M. et al., 2002)

3.4.4 Balancing Aerobic And Anoxic Ammonium Oxidation

About 50% of the ammonium needs to be oxidized to nitrite in the proposed system configurations. If the wastewater originates from an anaerobic sludge digestion process ammonium and bicarbonate are present in a one-to-one ratio. This gives an opportunity for a self-evident, natural control. If the aerobic ammonium oxidation has proceeded for 50% all alkalinity is consumed and the conversion will stop due to a drop in pH. Van Dongen et al. (2001) showed that indeed a stable and good N-removal is possible by this natural process control. For very strict effluent standards it is possible to adjust the pH in a minor way in order to adjust the ammonium/nitrite effluent of the Sharon process such that it matches exactly the requirements of the anammox process. Since in practice the HRT in the Sharon process is relatively long and in the anammox process short (van Dongen et al. 2001) it is most feasible to apply a feed back control to the fast anammox part of the integrated process. This could be based on ammonium and nitrite concentration measurements in the anammox effluent (Jetten M.S.M. et al., 2002).

If the influent doesn't contain the required alkalinity as for some industrial wastewaters, pH correction will be needed. In this case too pH control could be used most favorably to regulate the ammonia: nitrite ratio, this again based on, for instance, anammox effluent N-species concentration measurements (Jetten et al., 2002).

An alternative option would be to use measurements in the Sharon reactor. However, since the time constants of this process are considerably longer than the ones of the anammox process, a lower control performance can be expected. Of course feed forward control could anticipate the effect of disturbances, but this would require a thorough mathematical model and the measurement of these disturbances (Jetten et al., 2002).

An important aspect is of course that if the Anammox process is supplied with the correct feed no direct process control is needed, since the reaction will then run to completion. In case a reactor system is devised where the N-removal can occur in a single reactor process, the controllers main objective will have to be the balancing of the oxygen and ammonium flux into the biofilm in the proper stoichiometry.

If the requirements on the ammonium effluent are not too strict, this can probably be done by controlling the dissolved oxygen set point in a cascade way from a controller of the reactor ammonium concentration that uses ammonium concentration measurements in the reactor. Setting the ammonium set point to a value of about 0.5 mg N L^{-1} will yield good N-removal (Jetten et al., 2002).

From the modeling work it resulted the DO set-point and the ammonium surface loads are tightly coupled. From a controllability point of view it is then most beneficial to select an ammonium loading rate such that the DO can be controlled at around 1mg L^{-1}. If a very low N effluent is required (< 1mgN L^{-1}) correct control of the aeration becomes very critical and gradients in the reactor will result in many local variations that may negatively affect performance. It might be that the system is part of the time ammonium rather than oxygen limited. In such situation mixing properties of the reactor will need adequate attention (Jetten et al., 2002).

The process control system must also make sure that nitrite oxidizing bacteria are kept out of the system. In a suspended sludge system this can be done by setting the SRT such that nitrite oxidizers wash out (Hellinga et al. 1998). However, this only works for systems run at higher temperatures where the nitrite oxidizers grow slower than the ammonium oxidizers.

In oxygen limited biofilm systems on the other hand, ammonium can be partially oxidized to nitrite (Bernet et al. 2001; Garrido et al. 1997; Picioreanu et al. 1997) since in this system gradients occur due to the diffusion processes. Hence, anammox processes are also feasible at the lower bulk oxygen concentrations. In these cases, however, steady state analysis showed that a mixture of nitrite and nitrate is produced. Only when the nitrite is continuously removed from the system by denitrification, nitrite oxidizers can be competed out of the process. This can be done by conventional denitrification or by using anammox. Anammox has the advantage that these organisms can be positioned in the same biofilm as the aerobic nitrifiers. For heterotrophic denitrifiers this can be done by alternatingly recycling the reactor content over an aerated and a non aerated biofilm compartment (Jetten M.S.M. et al., 2002).

Jetten et al. (2002) report that experiments and simulations showed that the combined competition for the nitrite oxidizers (for oxygen with ammonium oxidizers and for

nitrite with anammox) can lead to a stable process. Again in this case cascade DO control based on effluent ammonium measurements seems to be the most appropriate.

3.4.5 Long term stability

A second purpose of process control is of course the need to keep the process stable. Due to the slow response of the biomass, it is important to prevent any significant deterioration of anammox population or in growth of nitrifiers. This is not only due to the slow growth rate of anammox bacteria but also due to the slow wash-out of nitrifiers once they have accumulated in the system. In the Sharon process this has been shown to be the reciprocal of the difference between the maximal specific growth rate and the wash out rate, whereas in biofilms this is mainly the biofilm turnover rate.

To achieve biomass stability, first, the anammox biomass has to be protected against high nitrite and oxygen concentrations. This can simply be achieved by redox or oxygen measurements and appropriate control actions. Secondly, one has to consider that most wastewater treatment plants are highly dynamic. It is essential that in growths of nitrite oxidizers is prevented under all circumstances, since it will take a very long time before they are washed out again (days to weeks,). Small daily variations do not seem to be a problem due to the slow response of the biomass, but a period of a few days in which no or little influent is supplied, requires special measures to be taken. Although it may be stated that these control strategies could be regarded as ‘trouble-shooting’, they are certainly not trivial for an anammox process in practice (Jetten et al., 2002).

Table 6: A comparison of the new process of nitrogen removal with the conventional Nitrification/Denitrification (Jetten et al., 2002)

System	Conventional Nitrification/Denitrification	SHARON	ANNAMOX	CANON
Number of reactors	2	1	1	1
Feed	Wastewater	Wastewater	Ammonium + nitrite	Wastewater
Discharge	NO_2^-, NO_3^-; N_2	NH_4^+, NO_2^-	NO_3^-, N_2	NO_3^-, N_2
Conditions	Oxic; anoxic	Oxic	Anoxic	Oxygen limited
O_2 Requirement	High	Low	None	Low
pH control	Yes	None	None	None
Biomass Retention	None	None	Yes	Yes
COD requirement	Yes	None	None	None
Sludge Production	High	Low	Low	Low
Bacteria	Nitrifiers + various heterotrophs	Aerobic NH_4^+ oxidizers	Planctomycetes	Aerobic NH_4^+ oxidizers + Planctomycetes

A one-reactor system that may be operated in the SBR mode seems to be an attractive option, providing reliable operation and being easy to evaluate. A one-reactor system is simple in configuration, but it is limited by complex interaction between AOBs, NOBs and anammox bacteria. It requires control in a very tight oxygen and pH range.

A two-reactor system, owing to operation under high nitrogen concentrations, may lead to instability. Such a system, on the other hand, has a very high potential for optimization and process intensification, as optimal conditions can be provided for each of the two consecutive operations.

This is in contrast with the two reactor system, where a more complex configuration may allow simpler system design and operation leading to higher efficiency and reliability. The two-reactor system is the most robust, which may shorten the recovery time after possible system upsets.

Table 7: Comparison of operational characteristics of Nitrogen removal Processes.

	Conventional Nitrification denitrification	OLAND	SHARON	ANAMMOX	CANON	Aerobic Deammonification
Aerobic ammonia oxidizers	Many	Unknown	N. eutropha	Absent	N. eutropha	Unknown salt tolerant ammonia oxidizer.
Aerobic nitrite oxidizers	Many	Unknown	Absent	Absent	Absent	
Anaerobic ammonia oxidizers	Absent	Unknown	Absent	B. anammoxidans, K. stuttgartiensis.	B. anammoxidans, K. stuttgartiensis.	Nitrobacter, K. stuttgartiensis.
Biofilms or Suspension	Biofilms/Suspension	Biofilms	Suspension	Biofilms	Biofilms	Biofilms
NH_4^+ loading rate ($kgN\ m^{-3}_{reactor}\ d^{-1}$)	2-8	0.1	0.5-1.5	10-20	2-3	1-2
Nitrogen Removal efficiency	95%	85%	0%	90%	90%	60%
Process Complexity	Separate oxic & anoxic compartments, Methanol dosing.	Aeration needs to be tuned to ammonia loading.	Separate oxic & anoxic compartments, Methanol dosing.	Preceding partial nitrification needed.	Aeration needs to be tuned to ammonia loading.	Aeration needs to be tuned to ammonia loading.
Investment Costs	Medium	Medium	Medium	Low	Medium	Medium
Operational costs	High	Unknown	Low	Very Low	Low	Low

Table 8: Summary of several lab scale experimental studies on one-reactor system for autotrophic nitrogen removal

Process	Reactor type	Volume (L)	Influent type	Inocula	HRT(d)	SRT (d)	DO ($mg\ L^{-1}$)	pH	T (^oC)	Removal rate ($kgN\ m^{-3}\ d^{-1}$)	N removal (%)	Reference
OLAND	RBC RBC	50 50	Synthetic Sludge liquor	RBC biomass RBC biomass	1 1	- -	0.3 1.0	7.85 7.85	30 14	1.80 0.42	88 42	[45]
OLAND	RBC	44	Synthetic	RBC biomass		-	0.6		29	1.05	89	[44]
OLAND	SBR	2.5	Synthetic	OLAND biomass	-	-	0.3-0.7	>7.4	32-34	1.1	-	[47]
OLAND	SBR	4	Synthetic	Nitrifying sludge from hospital WWTP	-	-	0.1-0.8	7±0.2	33	0.05	40	[50]
OLAND	FBR	2	Inorganic Substrate	Nitrifying culture	-	-	<0.1	7	28	-	76	[51]
OLAND	RBC	50	Synthetic		-	-	<1	7.5±0.02		-	84	[52]
OLAND	RBC	2.8	Black water	OLAND biomass	1.3±0.08		0.5	7-8	25.8±0.4	0.716	76	[53]
OLAND	RBC	44	Synthetic	Nitrifying culture-ABIL	1[c] 1.7[c]	- -	0.3±0.1 0.15±0.1	7.76±0.3 7.78±0.2	28±2 28±2	-	35±11 84±8	[55]
CANON	SBR	1.5	Rejected water diluted with tap water 1:1[a]	Nitrifying granular + 'Anammox'	0.5	30 - 110	0.5	7.5-7.9	21	0.5	78	[65]
CANON	SBR	2.0	Synthetic	'Anammox'	1	-	<0.1	7.8	30	0.06	50	[46]
CANON	SBR	10	Sludge liquor			-	0.6		30	0.06	76	[48]
CANON	MBBR	4	Synthetic	Nitrifying biofilm + anammox		-	0.5		35	0.77	89	[49]
CANON	Granular SBR	1.5	D. effluent of WWTP	Nitrifying granular biomass	0.25	-	2.1±1	7.5-8	20	1.1	-	[32]

Process	Reactor type	Volume (L)	Influent type	Inocula	HRT(d)	SRT (d)	DO (mg L^{-1})	pH	T (^{o}C)	Removal rate (kgN m^{-3} d^{-1})	N removal (%)	Reference
CANON	NRBC		Synthetic	PN-biomass	-	-	0.5-0.7	8	35	-	72	[25]
CANON	Chemostat	1	Synthetic	Activated sludge from WWTP	-	-	<0.1	-	36	0.08	-	[15]
CANON	Gas lift	1.8	Synthetic	Anammox+ nitrifying sludge	0.42	-	0.5	7.5	30	1.5	42	[18]
CANON	Upflow	10	Synthetic Sludge digester liquid	Anaerobic granules + anoxic AS	5 5 7		5.6-6.5	 7.1-7.9 7.1-7.9	30 30 30	-	92±1.5 76±1.5 94±1.7	[58]
CANON	Air pulsing SBR	1.5	Synthetic + Supernatant from anaerobic sludge digester	VSS of biomass from another reactor	0.5	150	0.5	7.7±0.2	18-24	0.45	85	[57]
SNAD	NRBC		Synthetic	PN biomass	-	-	0.4-0.6	8-8.2	35	-	70	[25]
CANON	SBR	18	Synthetic	From aeration tank of landfill leachate treatment plant.	9 4.5 3	Inf Inf Inf	0.5-1	7-8	35	-	85.7+8.7[b] 87.3+7.8[b] 85.5+7.3[b]	[30]
CANON	UGBR	50	Sludge liquor	Nitrifying : denitrifying activated sludge		-	1.8		30	0.36	60	[A]
SNAP	Immobilized reactor	5.43	Synthetic	Nitrifying activated sludge		0.4	2-3	7-8	35		60-80	[41]

Note: Some of the characteristics of the reactors were not reported. ; [a]To reach an ammonium concentration of 0.15-0.35 gNL^{-1}; bTN removal (%) – By Partial nitrification, Anammox(%) + Denitrification(%); [c]The process carried out in two different periods.
[nn]: See references with corresponding highlighted red numbers in the list of References at the end.

Table 9: Summary of several experimental studies using pilot-scale and full-scale operations of one-reactor type autotrophic nitrogen removal systems (Stijn W.H. et al., 2010).

Reactor type	Volume (m^3)	Influent type	pH	T (^oC)	DO ($mg\ L^{-1}$)	Removal rate ($kg\ N\ m^{-3}\ d^{-1}$)	N removal (%)
SBR	500	Sludge liquor	7.05-7.10	25-30	0.3	0.6	84
	400	Sludge liquor	7.05-7.10	25-30	0.3	0.4	90
	4.1	Sludge liquor	7.4-7.6	25	0.5-1.0	0.65	90
Upflow	600	Sludge Liquor	8.0	30-35	2.0-3.0	1.3	75-80
MBR	Full Scale	Landfill leachate	-	-	0.5	0.33	73
Moving bed	0.04	Sludge Liquor	8.0-8.5	-	0.3	0.33	84
	0.04	Sludge Liquor	8-8.1	27	<1.0	0.5	60-70
	21	Sludge Liquor	7.6-8	28-29	0.8-2.0	0.12-0.22	75-71
	Full scale	Sludge Liquor	7.8	23-27	1.2-2.6	0.38	62
	Full Scale	Sludge Liquor	8	30	3	0.35	64
RBC	265	Landfill leachate	8.3(7.4-8.7)	27	-	0.21	72
	33	Landfill leachate	7.3	28(27-30)	0.7-1.0	0.15-0.26	40-70
	240	Landfill leachate	8.1(7.2-8.8)	16	1.0-2.0	0.25-0.57	30-70
				14(10-28)	0.8-1.2	1.7	30-70

Note: Some of the characteristics of the reactors and about the processes were not reported.

Table 10: Comparative performances in aerobic ammonium oxidation (modified from (Ahn Y.H., 2006))

Process	Reactor	Substrate	pH	T (^oC)	Influent NH_4-N ($mg\ L^{-1}$)	AUR(Ammonia uptake rate)		SRT (d)	Reference
						mg NH_4-NO_x $L^{-1}\ h^{-1}$	mg NH_4-NO_x g $VSS^{-1}h^{-1}$		
Nitritation	SBR	Synthetic	7.2	32	400	-	4	40	[65]
					350-540	-	3.2	24	
					400-500	-	4.8-6.1	14	
					500	-	25.8	10	
					500	-	10.0	5	
					500	-	22.9	3	
Aerobic denitrification	SBR	Rural w/w	-	20	100-110	-	1.1-2.0	16-32	[66]

[nn]: See references with corresponding highlighted red numbers in the list of References at the end.

Table 11: Comparative performances of Nitrogen removal Process

Process	Reactor	Inocula	Substrate	pH	T (^{o}C)	Influent NH_4-N/NO_2-N (mg L^{-1})	SRT (d)	Nitrogen uptake rate		N removal (%)	Reference
								kgN $m^{-3}d^{-1}$	mg N g VSS^{-1} d^{-1}		
Aerobic denitrification	SBR	AS	Rural w/w	-	20	100/0	16-32	0.7-1.5	-	43-52	[66]
PN(Sharon)-Anammox	CSTR-FBR	Anammox	Digester liquor	6.5-7	35	1176/0	1	0.75±0.2	8.0[a]	-	[16]
	CSTR-SBR	Anammox	D. Supernatant	6.5-8	30-35	619-657/0	-	0.6-2.4	12.5[a]	90	[12]
	MBBR	Anammox, nitritation	Synthetic	7.8	26.2	350-720/0		1.9	-	-	[70]
DEMON	RBC	RBC biomass	Synthetic	8-8.5	27	-	-	0.3	-	-	[67]
	Bioflm	-	Synthetic	-	-	150/0	-	1.49	-	98.9	[48]
	MBBR	Kaldnes rings	Supernatant	7.83	25	130-355/0	-	1.4-1.6	-	-	[71]
DEMON	UASB	UASB granule	Synthetic			100-200/50	-	0.02-0.03	-	30-50	[48]
PN-denitrification	MBBR	Municipal WWTP	Synthetic	7-8	28.5±1	889/0	-	1.86	-	98.23	[68]
Short-cut nitrification/denitrification	[b]Artificial system	AS and denitrifying sludge	Synthetic	-	25-30	60	-	-	-	87	[69]

a- mg N mg SS^{-1} h^{-1}; b- Artificial bio-augmented system;

[nn]: See references with corresponding highlighted red numbers in the list of References at the end.

Table 12: Summary of several experimental studies concerning partial nitritation in view of coupling with an Anammox reactor described in the literature

Process	Reactor type	Volume (L)	Influent type	pH	T (^oC)	DO ($mg\ L^{-1}$)	SRT(d)	HRT(d)	N load ($kgN\ m^{-3}\ d^{-1}$)	NO_2^- : NH_4^+ ratio	Nitrate in effluent (%)	Refe-rence
SHARON	CSTR	2100	D. effluent from WWTP	6.6-7.2	29	2.7	1.05-1.18	1.05-1.18	0.56	1.4	Negligible	[12]
SHARON	CSTR	2	Synthetic	7.1	35	-	1-1.5	1-1.5	1.5	1	-	[A]
SHARON	CSTR	10	D. effluent from WWTP	6.7	35	-	1	1	1.2	0.74	113	[16]
SHARON	CSTR	1.5	Centrifuged D. effluent	6.5-8.5	35	-	1.5	1.5	-	-	-	[54]
SHARON	CSTR	2.8	Urine	9.2	30	2.5-4	4.8	4.8	1.580	1	Negligible	[A]
SHARON	CSTR	3.2	D.effluent of fish canning	7.5	35	>2	1	1	0.1	1	No nitrate	[39]
SHARON	Chemostat	4	Reject water	6.5-6.7	35±0.5	3	1	1	0.35±0.05		No nitrate	[64]
SHARON/DN	Chemostat	4	Reject water	6.8-8	33±0.5	<3	2	2	0.7		Negligible	[56]
BNR via nitrite	SBR	3	Reject water	7.3-8.1	32±0.5	<1	11	1	0.8		Negligible	[56]
Partial nitritation	SBR	7.5	Urine	6-8.8	24.5	2-4.5	>30	4	0.560	1	Negligible	[A]
Partial nitritation	SBR	20	Landfill leachate	6.8-7.1	36	2	3-7	1.5	1.5	0.6-1.5	<5	[40]
Partial nitritation	Biofilm	10.8+2.5	Digested liquor of swine water[a]	-	25	5	13	1	1.0	1.38	<5[b]	[41]
Partial nitritation	UF-fixed biofilm	11	Landfill leachate	8.4	30	0.8-2.3	-	Varying	0.27-1.2	58.3		[21]

Process	Reactor type	Volume (L)	Influent type	pH	T (^{o}C)	DO (mg L^{-1})	SRT(d)	HRT(d)	N load (kgN m^{-3} d^{-1})	NO_2^- : NH_4^+ ratio	Nitrate in effluent (%)	Refe-rence
Partial nitritation	Column with PEG carrier	8.0	Digested liquid manure	7.5-8	30	2.5-6.5	-	-	3.8	1.22	2.3	[19]
Partial nitritation	MBR	1	Synthetic	8	35	<0.6	35	0.23	-	1.30	Trace	[42]
Partial nitritation	MBR	14	Synthetic	8	35	0.3-0.5	-	0.67	0.450	1	-	[A]
Partial nitritation	MBR	1.5	D. effluent of WWTP	7.9	30	<0.2	Varying	0.58-1	0.73-1.45	1.13	-	[43]
Partial nitritation	Airlift	1.6	Sewage sludge	7-8	30	1.5-5.0		Varying	0.6-3.0	1-1.4	-	[28]
Partial nitritation	Swim Bed Reactor	0.95	Digestion liquor	9	15-30	-	-	1	1.9	1.32	-	[38]
Partial nitritation	SBR	250	Raw leachate	8	36±1	2	3.1-12	3-6	0.85-1.2	1.32	-	[31]
Partial nitritation	Immobilized reactor	5.43	Synthetic	7.5-7.7	35		0.7-1	0.4	0.48	-	-	[41]
Partial nitrification	SBR	1.5	D. Supernatant	7.5-8	20	2.7	-	0.25	-	-	-	[32]
	SBR	24	Landfill leachate	-	20±1	-	20-25	-	0.1	-	<5	[35]
	SBR	1	Reject water	6.5-8	30±0.5	3	5	0.35	0.8±0.05	-	No nitrate	[64]
Partial Nitrification	SBR	18	Synthetic	7-8	35	0.5-1	Infinite	Varying	0.222	-	0.1-1	[30]

Note: Some of the characteristics of the reactors and about the processes were not reported.
[a]Diluted with tap water to achieve a NLR of 1 kgN m^{-3} d^{-1}; [b]Conversion efficiency of ammonia in nitrate.
[nn]: See references with corresponding highlighted red numbers in the list of References at the end.

Table 13: Full Scale application of Sharon process (Mulder et al., 2006)

Treatment plant	In operation since	Load (kgN/day)	Tanks	Volume (m3)	ART (day)	Inlet concentration (mg NH_4-N L^{-1})	NH4-N removal efficiency (%)	Wastewater application
Utrecht	1997	900	Two	3,000/1,500	3 – 6	600 – 900	90 – 95	Sludge dewatering
Rotterdam-Dokhaven	1999	850	Single	1,800	1.3 – 1.8	1,000 – 1,500	85 – 98	Sludge dewatering
Zwolle	2003	410	Two	900/450	1.3 – 1.8	400 – 600	85 – 95	Sludge dewatering
Beverwijk	2003	1,200	Two	1,500/750	1.3 – 1.8	700 – 900	85 – 95	Sludge dewatering/drying
The Hague-Houtrust	2005	1,300	Single	2,000	1.5 – 1.8	900 – 1,200	85 – 98	Dewatering
Groningen-Garmerwolde	2005	2,400	Two	4,900/2,450	1.5 – 1.8	700 – 800	≥ 95	Sludge dewatering/drying

Table 14: Summary of several experimental studies concerning ANAMMOX process described in the literature.

Reactor type	Volume (L)	Influent type	Inocula	HRT (d)	pH	T (^oC)	NLR ($gNL^{-1}d^{-1}$)	NRR ($gNL^{-1}d^{-1}$)	SNR (gN $gVSS^{-1}$ d^{-1})	N removal (%)	Reference
SBR	20	Anaerobic digester centrate	Anammox	-	7.5-7.8	35	0.380	0.2[a]	-	85	[A]
SBR	1600	Digester effluent from WWTP	Anammox	-	7.45-7.59	30.4-31.8	0.650[a] (2.6)	0.56-0.64(2.4)	0.075	85-99	[12]
SBR	1	Anaerobic digester supernatant	Anammox	1	7.5	19-21	0.28	0.08	0.13	69	[13]

Reactor type	Volume (L)	Influent type	Inocula	HRT (d)	pH	T (^{o}C)	NLR ($gNL^{-1}d^{-1}$)	NRR ($gNL^{-1}d^{-1}$)	SNR (gN $gVSS^{-1}$ d^{-1})	N removal (%)	Reference
SBR	1.2	Synthetic	Digested sludge of WWTP	0.1	7.5-8	30	2.7	2.0	-	-	[14]
SBR	1	Synthetic	Activated sludge	1	7.8	35-36	0.6	0.6	0.21	-	[15]
SBR	10	Digester liquor	Anammox	1	7-8	30-37	1.0	0.55-0.95	0.18	-	[16]
SBR	3	Effluent from a fish-canning industry	Anammox sludge	1.8	7.5-8.2	35	0.34-0.67	0.3	0.44	40-80	[A]
Airlift	70,000	Digester effluent from WWTP	Activated sludge + anammox biomass	-	7-8	30-40	9.5	9	-	-	[17]
Gas lift	7+3	Synthetic	Anammox granular sludge	1	7.9-8.1	30	2.3	2.0	1.15	88	[2]
Airlift	1.8	Synthetic		0.28	7.5	30	10.7	8.9	-	-	[18]
MBR	1.5	Digester effluent from WWTP	Anammox biomass	0.75-1.1		20-30	0.65-1.1	0.55	-	82	[A]
MBR	5	Synthetic	Anammox granular sludge	1	8	35	0.74	0.71	0.45	73.6	[7]
MBR	4.8	Synthetic	Nitrifying and denitrifying AS	2	8	35	-	-	0.35	90	[5]
CSTR	0.73	Liquid manure digester liquor	Anammox granular sludge	0.2	7.5	30	3.73	2.60	-	70	[19]
Fixed bed	0.8 0.8	Synthetic Synthetic	Activated WTP-sludge Concentrated WWTP - AS	0.06-0.3 0.01-0.3	7.0-7.5 7.0-7.5	37 37	0.1-9.4 0.1-58.5	6.2 26.0	- 1.6	- -	[20]

Reactor type	Volume (L)	Influent type	Inocula	HRT (d)	pH	T (^{o}C)	NLR ($gNL^{-1}d^{-1}$)	NRR ($gNL^{-1}d^{-1}$)	SNR (gN $gVSS^{-1}$ d^{-1})	N removal (%)	Reference
Upflow	36	Landfill leachate	AS + Anammox		7.5-8	30	-	0.11	-	62	[21]
Upflow	2.85	Liquid manure	Anammox sludge	1	7.2-7.6	35	0.39	0.22		55	[22]
Upflow	2.85	Synthetic	Anammox sludge	1	7.2-7.6	35	0.67	0.5		73	[22]
FB	2.5	Synthetic	Denitrifying sludge	0.9-1.75	8	36	2.0	1.8	0.18	-	[11]
FB	2.5	Effluent of digested WWT sludge	Denitrifying sludge	0.14-11	8	36	2.5	1.5	0.15	-	[11]
Continuous flow	19.4	Digested effluent from WWTP	Anammox biomass	-	7.5-7.8	35	0.14-0.38	0.33	-	85-91	[A]
ABF	0.2	Synthetic	Anammox biomass	0.03 0.06	7.2 7.2	20-22 37	12 19.1	8.1 11.5	- -	- -	[23]
UBF	200	Effluent of partial nitritation		0.38	7.5	30	7.0	6.4			[A]
UBF	1.2	Synthetic	Digested sludge of WWTP	0.12	7.5-8	30	2.5	2.0	-	-	[14]
UASB	1+0.5	Piggery waste	Granular sludge	5 5	8.2-8.5 8.2-8.5	35 35	1.02[b] 0.84[b]	0.66 0.59	0.08 0.06	80 82	[A]
SBR	1	Synthetic	Anammox granular sludge	0.625	7.8-8	35	1	0.7	0.65	78	[2]
UASB	6	Synthetic	Granular sludge	3.5	Neutral	30±2	0.09	-	-	60	[4]

Reactor type	Volume (L)	Influent type	Inocula	HRT (d)	pH	T (^{o}C)	NLR ($gNL^{-1}d^{-1}$)	NRR ($gNL^{-1}d^{-1}$)	SNR (gN $gVSS^{-1}$ d^{-1})	N removal (%)	Reference
MBR	4.8	Synthetic	Aerobic AS + nitrifying AS	2	8±0.1	35	-	-	-	90	[5]
MSBR	5	Synthetic	Anammox granular sludge	1	8	35	0.39	0.71	-	73.6	[7]
FBR	2.5 2.5	Synthetic D. effluent	Denitrifying Denitrifying	0.91-1.75 0.14-11	8 8	36 36	0.2-1.0 0.2-1.0	1.8 1.5	0.18 0.15	83-85 81-99	[11]
Fixed bed	2	Synthetic	Denitrifying	0.25-0.95	8	36	0.02-0.4	1.1	-	92-99	[11]
AnMBR	15	Synthetic	Anaerobic seed	1-3	6-8	30	Varying	-	-	96	[8]
NRBC		Synthetic	Anammox biomass	0.21-1.08	8-8.2	35	2.1	-	-	82-93	[25]
Upflow	7.0	Synthetic	Anammox sludge	0.075	-	23±2-26±1	5.2-8.2	17.5	-	85±2	[26]
VSBR	25.5	Synthetic	Activated Sludge	-	7.8±2	30±1	0.06-0.276	-	0.134	52	[27]
Airlift	8	Digester liquor	Anammox Sludge	0.1-0.262	7-8	30	5.3	>4.0	-	80-95	[28]
Upflow	7.0	Synthetic	Anammox sludge	1.1-1.3	7.2	23±2	20.5	17.5	-	90	[29]
SBR	1	Synthetic	Anammox biomass	1	7.5	20±1	-	0.08	0.28	-	[32]
Hybrid anaerobic+ Upflow	0.745+ 0.790	Pretreated Slaughter house effluent	Anaerobic stabilization pond		7.5-8.0	35±0.5	0.033-0.067	0.05	-	40-65	[33]
MBR	36	Synthetic	Activated Sludge	1.5-4	8	>30	0.075-0.09	0.071	-	30-70	[34]

Reactor type	Volume (L)	Influent type	Inocula	HRT (d)	pH	T (^{o}C)	NLR ($gNL^{-1}d^{-1}$)	NRR ($gNL^{-1}d^{-1}$)	SNR (gN $gVSS^{-1}$ d^{-1})	N removal (%)	Reference
UASB	1.5	Synthetic	Granular Sludge	5		35	0.5	0.023	-	48	[36]
FBA	18.5	Synthetic	Anammox sludge	1 2	No control	28-32 28-32	5400 3700	4.15 3.23		76.97 87.35	[37]
Fixed bed	0.8	Synthetic	Nitrifying sludge	-	8	35		0.35[c]	-	-	[59]
SBR	3	Synthetic	Anammox	18	7.5-8	-	0.60	-	-	67-99[d]	[60]
CSTR		Synthetic	Anammox	120	7-8	30	0.004-0.662	0.003-0.58		90-94	[62]
UASB	1.5	Synthetic	Activated sludge	12	-	35	2.18	-	-	33-86	[61]
USFF	1.5	Synthetic	Activated sludge	12	7.5-8	30	1.28-2.57	-	-	28-90	[61]
ASBR	1.5	Synthetic	Activated sludge	16	7.5-8	30	0.86-1.60	-	-	54-97	[61]
DHS	2.5	Synthetic	AN (80%) + AS[e] (20%)	0.7-0.2	7.6-8.2	30-35	0.48-5.96	0.26-2.27	-	38-95	[62]
Upflow	10	Synthetic	Enriched biomass	-	-	30	1.735 ± 0.137	1.477 ± 0.168	-	85±7	[2]
ABF	0.2	Synthetic	Anammox sludge	0.125	7.2	37	0.08	0.98	-	-	[24]

Note: Some of the characteristics of the reactors and about the processes were not reported.
[a]A nitrite surplus in the effluent of the nitritation reactor is balanced by adding raw digester effluent; [b]With addition of synthetic nitrite; [c]0.35 g $(NH_2)_2CO$-N L^{-1} d^{-1}; [d]Nitrite (Limiting substrate) removal percentage; [e]AN (80%) + AS (20%): Anammox (80%) + activated sludge (20%).
[nn]: See references with corresponding highlighted red numbers in the list of References at the end.

4.1 Comparison of nitrogen removal process:

Compared with the conventional nitrogen removal process, the process using anaerobic ammonium oxidation appears to be a sustainable biotechnology to remove ammonia. The combined process offers various advantages such as less oxygen and alkalinity demand, no need for organic carbon, less nitrite and nitrate production, no production of undesirable by-products like N2O, and negligible sludge production. These advantages will lead to substantial saving of energy and resources in ammonium-rich wastewater treatment.

The process coupled with the PN process remains a challenge for future application in the removal of ammonium from wastewater with high ammonium concentrations, because it is difficult to manage the system properly due to the high sensitivity of anammox bacteria to operational conditions such as temperature, salinity, substrate concentrations, presence of dissolved oxygen, inhibitors, and so on.

These novel processes were mainly investigated in laboratory and pilot scale. Most Anammox reactors used the Anammox enrichment as inocula, originated from an Anammox-SBR reactor in which 80% of the biomass consisted of Anammox bacteria. The processes showed better nitrogen conversion rate. The combination of the partial nitritation and Anammox processes and its applications are currently underway. Since the Canon process has a compact reactor configuration with excellent biomass retention, it may be a more economic and efficient option for wastewater treatment. In spite of its high potential for successful application, only a few researchers have studied the Canon process, particularly by using synthetic wastewater in laboratory scale reactors.

The nitrogen loading and removal rate was quite lower for the one-reactor system than for two-reactor system however. Nitrogen conversion rates represent quite different trends according to type of inocula, substrate, type of process, reactor configuration and concentration of influent ammonium, etc. The nitrogen removal process using denitrification nitrifier (thus, Nitrosomonas-like microorganisms) represents even lower nitrogen conversion, relatively.

Many challenges yet remain for the optimization and application of Anammox and its combination process on pilot or full-scale plant. One of the main challenges in the

Anammox process is to decrease the long start-up time because the Anammox bacteria have very low biomass yielding rate.

Although several types of bioreactors including sequencing batch reactor (SBR) have been successfully applied for the cultivation of the Anammox microorganisms in different laboratories, many others conducting studies on this process agree that the detection and mass enrichment of the Anammox bacteria are still remaining a significant obstacle. The accumulation of enough Anammox biomass is required for fast process start-up. Although the effect of hydrazine as a driving force of Anammox process has been described (reference), control methods to stimulate the Anammox activity and new process control strategies have to be developed and evaluated.

During process start-up procedure, the competition between autotrophic Anammox bacteria and heterotrophic denitritation bacteria (or other autotrophic denitrifiers) will be also another problem. Because the Anammox process requires nitrite as an electron donor, a pre-partial nitritation process should be initiated. Also, denitrification is a faster process than the Anammox process. This situation could result in competition between the Anammox bacteria and the combined microbial mixture. Actually, this will be a common problem in treatment of high strength wastewater containing carbon compounds as well as nitrogen.

Under favourable conditions, various autotrophic nitrogen removal bacteria involving Anammox bacteria could be co-cultivated in the anaerobic or oxygen-limited bioreactor such as the Canon reactor, giving a promise of complete nitrogen removal by elimination of undesirable by-products (NO3-N and N2O, etc.). In a co-culture of denitrifying nitrifier and anaerobic ammonium oxidizing bacteria under these conditions, denitrifying nitrifier (Nitrosomonas-like microorganisms) can be the predominant species due to the difference of both bacteria in physiological characteristics unless the process has enough Anammox biomass (reference). From this point of view, operation of two-in series partial nitritation and Anammox process appears to be more attractive. The phase separation may provide a better environment to maintain process stability and to decrease start-up time of the process.

However, the discharge of the ammonia oxidizer (Nitrosomonas-like microorganisms) from the pre-nitritation reactor needs to be controlled when the phase separated system is adopted.

Even though there are still many unknown reactions in novel nitrogen removal process, it appears that the autotrophic nitrogen removal microorganisms can contribute to sustainable nitrogen removal under anaerobic/or anoxic conditions, depending on the type of electron donor in the substrate.

Because most lithoautotrophic organisms, including Anammox bacteria, are characterized by a low maximum growth rate, a reactor with high biomass retention, such as immobilization or granulation process (biofilm or UASB) is required. The lithoautotrophic granular sludge reactor will provide more stable and higher nitrogen conversion rate although a longer start-up time is required. The development and application of microbial Anammox granulation remain another challenge towards implementation of the sustainable nitrogen removal process in a future wastewater treatment plant.

4.2 Conclusion

The conventional nitrogen removal process comprised of autotrophic nitrification and heterotrophic denitrification are often used for treating nitrogen in wastewater. Since the process requires significant energy and carbon source, more research should be directed toward development and application of a more economical process.

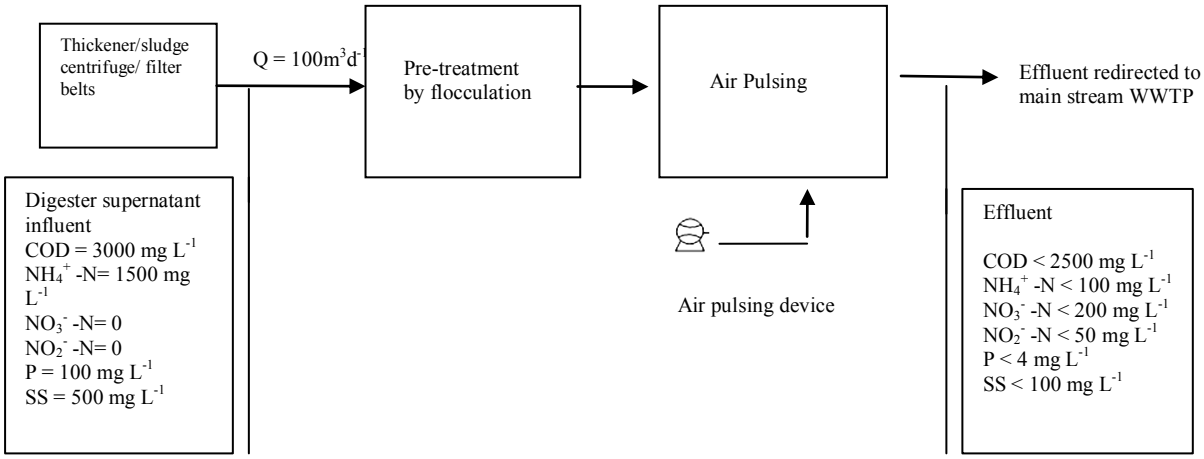
The discovery of Anammox microorganisms has greatly improved the understanding of the nitrogen cycle. In environmental biotechnology, the Anammox provide great promise for removal of nitrogen from wastewater, containing high concentration of ammonium. The discovery of the versatility of aerobic ammonium oxidizer in denitrification also led to the development of new processes such as CANON, SHARON and OLAND. The combination of the different groups of nitrogen elimination microorganisms and the process optimization will provide better performance in nitrogen removal. Even though novel technologies like those described in the review meet a few challenges for the introduction and application in

full-scale plant, it might offer a better solution for treatment of high strength wastewater.

In physiological characteristics of bacteria, two-in series operation of partial nitritation and Anammox process appears to be more attractive. The phase separation may provide a better environment to maintain process stability and to decrease startup time of the process. Research to stimulate the activity of Anammox is required with accumulation of Anammox biomass as the predominant species. In the future, full-scale introduction of lithoautotrophic nitrogen elimination biotechnology will lead to substantial savings in energy and resources and better management of water environment.

Chapter 5: Design Example: One- and Two-reactor Systems Comparison

5.1 Design of a One-reactor System



Preliminary design Calculations

General considerations on effluent concentration:

1. SS and phosphorus have to be removed in the pretreatment step, to avoid interferences with the anammox process; only the particulate COD fraction can be removed as the filterable fraction is mostly non-biodegradable.
2. Effluent nitrogen compounds concentration values have been derived from the following assumptions :
    a. At least 90% ammonium removal should be achieved.
    b. Nitrite concentration should be kept at the lowest possible values to avoid anammox inhibition.
    c. Nitrate concentration can be derived from stoichiometry of partial nitrification and subsequent anammox process.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_2^- + 0.13H+ \rightarrow$$

$$0.26NO_3^- + 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

Effects of Phosphorus concentration on the activity of Anammox:

A difference in tolerance for phosphate exists between different Anammox species. Van de Graaf et al. (1996) experienced a loss of activity for C. Brocadia anammoxidans at phosphate concentrations above 155mgP L^{-1}, while Egli et al.

(2001) did not see any inhibitory effect of phosphate when a culture of C. Kuenenia stuttgartiensis was supplied with up to 620mgP L^{-1}.

Dapena-Mora et al. (2007) observed at the same phosphate level of 620mgP L^{-1} 50% inhibition of Anammox activity. In batch tests using sludge from a highly loaded lab-scale rotating biological contactor containing C. Kuenenia stuttgartiensis, phosphate was shown to partially inhibit the Anammox process.

Anammox activity decreased to 63% of the normal activity at 55mgP L^{-1} and further to 20% at 110mgP L^{-1}. At 285 mgP L^{-1} no further decrease was observed (80% inhibition).

Due to the inhibition effect, a pretreatment of supernatant is required to remove phosphorus and the Suspended solids.

Treatment of Supernatants by CANON process:

A feasible treatment for digester supernatants comprises two processes: one aerobic, the partial nitrification, where 50% of ammonia is oxidized to nitrite and an anoxic one, the Anammox process, where ammonia and nitrite are converted to nitrogen gas producing a small amount of nitrate.

The concept of the Canon process is also the combination of partial nitritation and Anammox. However, this process performs two sequential reactions in a single and aerated reactor, implying that the two groups of bacteria (Nitrosomonas-like aerobic microorganisms and Planctomycete-like anaerobic bacteria) cooperate in the whole process.

The partial nitritation which is a key process in the Canon process is quite sensitive process in microbial environment, such as DO, nitrogen loading, pH and presence of toxic substance, etc. the anaerobic ammonium oxidation process had little impact due to low DO conditions.

Most research on Canon process has been studied with synthetic substrate, but not real wastewater like sludge digester liquids. Therefore, process performance of Canon reactor treating identical substrate was not reported yet.

Because of this at first, a synthetic media can be used as feeding media in order to check the operation of the pulsing reactor since no information is available

about nitrification in aggregates formed in this kind of reactors. And then if the reactor is working properly then we can use the supernatants for the process.

According to Vázquez-Padín et al. (2009) it is mentioned that, several strategies have been tested for the start-up and for the optimization of the performance of reactors where autotrophic nitrogen removal takes place. Among them, two can be pointed out:

- To inoculate an Anammox reactor with nitrifying biomass and to supply air to maintain microaerobic conditions (or)
- To operate a nitrifying reactor under oxygen-limited conditions to obtain the desired ammonia to nitrite molar ratio inside the system and then inoculate Anammox biomass.

The obtaining of the microaerobic conditions for the CANON process can be achieved in different kind of systems like SBR, gas-lift, etc. and the air pulsing flow reactor. The use of pulsing air flow can be advantageous compared to the continuous mode due to

- The reduction of the aeration costs and
- Better control of the required low dissolved oxygen concentrations.

When biofilms and granular systems are used to treat wastewaters, external mass transfer resistance uses to be the limiting step. In this sense, pulsing reactors could be a suitable technology to improve mass transfer.

According to Vázquez-Padín et al. (2009), Anammox activity observed at $25^{o}C$ is 0.05gN $gVSS^{-1}$ d^{-1}.

Due to high fluctuations of temperature during the year according to seasonal changes, this kind of operation will help to have very good operational conditions during the winter season, when temperature is low. So, the air pulsing SBR can be operated in a simple and robustic way at $30^{o}C$, because of a very good activity of anammox bacteria at that temperature.

In synthetic wastewater treatment by lab-scale Canon reactor in which enriched Anammox (80%) biomass was seeded, N conversion rate and removal efficiency were 0.04–0.11 gN L^{-1} d^{-1} for SBR type configuration and 0.06–1.5 gN L^{-1} d^{-1} for gas lift type configuration (Jetten et al., 2002), respectively.

According to the physiological data of Anammox bacteria (Jetten et al., 2001), the Anammox bacteria have a very low yielding rate (0.07 g protein $gNH_4–N^{-1}$) and long doubling time (10.6 days). This means that a considerable long start-up time will be required at full-scale plant. Therefore, how to increase (or how fast to accumulate) the biomass in the reactor is a big challenge for the success of the full-scale application.

According to Schmid et al., (2003) Anammox organisms have doubling time of 11 days (which corresponds to a specific growth rate of 0.065 d^{-1}) and a biomass yield of 0.13 g dry weight g NH_4^+-N^{-1} oxidized. However, van der Star et al. (2008) concluded that the doubling time of Anammox bacteria is at most 5.5–7.5 days calculated on the basis of maximum conversion capacity, but possibly as low as 3 days. Researchers recently have claimed they optimized the reactor conditions to such an extent that a doubling time of 1.8 days was achieved (Isaka et al., 2006).

According to Strous et al. (1998), the maximum specific growth rate (μ_{max}) of ANAMMOX organisms is 0.0027 h^{-1}(equivalent to 0.065 d^{-1}) at 30°C, and a specific growth rate of 0.072 d^{-1} measured at 32°C by Jetten et al., 1997 and by K. Isaka et al., (2006) the doubling time of anammox bacteria was calculated as 1.8 days, and the specific growth rate (μ) was 0.39 d^{-1}.

According to Dosta et al. (2008) Anammox organisms settle well as SVI of Anammox biomass is as low as 58mL $gVSS^{-1}$ (< 100mL g VSS^{-1}).

Half-saturation constant is negligible (0,2 - 3 $\mu g\ L^{-1}$, van der Star, 2008; < 5 $\mu g\ L^{-1}$, Jetten et al., 2004, in Ekström S., 2010) so that Anammox process kinetics can be considered as zero-order.

From Vázquez-Padín et al., (2009) the maximal nitrogen removal rate obtained for an air pulsing SBR is 0.45gNL^{-1} d^{-1}., which is in the range of 0.06–1.5gNL^{-1} d^{-1} mentioned in different research activities.

Reactor Volume calculation:

Ammonium nitrogen removal efficiency = $\eta = 0.94$

Ammonium nitrogen load to be removed = $Q * \eta * NH_4\text{-}N_{in}$

= 100 $m^3\ d^{-1} * 0.94 * 1500\ g\ m^{-3}$

$= 141000 \text{ g } d^{-1} = 141 \text{kg } d^{-1}$

Anammox volumetric removal rate: v_A $= 0.05 \text{gN } gVSS^{-1} \ d^{-1} * 0.04 \text{ gVSS}^{-1} \ L^{-1}$

$= 200 \text{ g } m^{-3} \ d^{-1}$

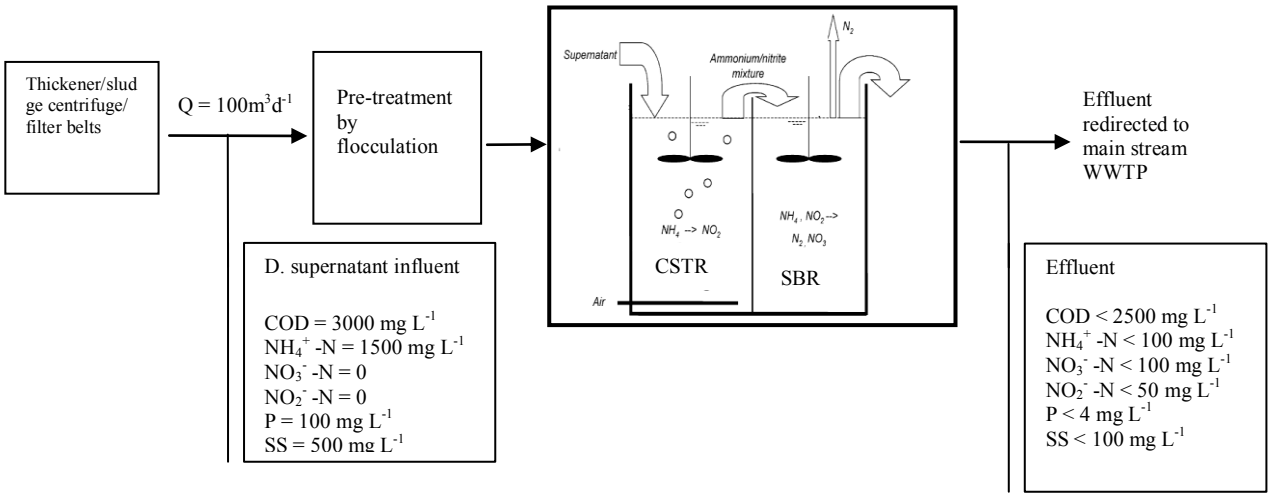
Reactor volume: $V_{reactor}$ **$= 141000 / 200 = 705 \ m^3$.**

The good retention capacity of the reactor and the low VSS concentration in the effluent will minimize the size or even the need of a posterior settler. The air pulsing flow would reduce the requirements of aeration with a consequent decrease of aeration costs and would allow an easy control of the dissolved oxygen level by changing the pulsing frequency or the amount of air pulsed.

Finally, the high H/D ratio of the reactor will reduce the surface needed and makes this technology promising under an economical point of view. Nevertheless, more studies are necessary to study the maximal ANR that can be reached and the feasibility of a pulsing device at an industrial scale.

The operation of the air pulsing SBR allows simple and robust regulation of the DO concentration of the liquid bulk for the stable operation of the CANON process.

5.2 Design of a pilot scale two-reactor system:



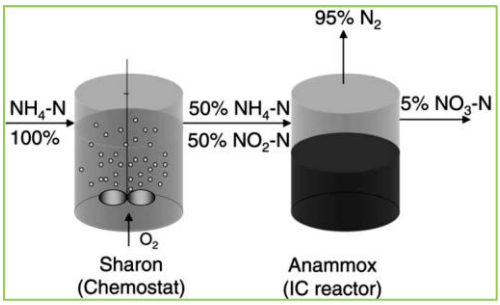
Partial nitrification and Anammox processes can be performed in two different units as the SHARON-ANAMMOX combined system.

Preliminary design Calculations

General considerations on effluent concentration:

1. SS and phosphorus have to be removed in the pretreatment step, to avoid interferences with the anammox process; only the particulate COD fraction can be removed, as the filterable fraction is mostly non-biodegradable.
2. The ratio of ammonium and nitrite needed for the anammox process is about one.
3. About 50% of the ammonium needs to be oxidized to nitrite in the proposed system configurations. Generally, the wastewater originating from an anaerobic sludge digestion process contains ammonium and bicarbonate in one-to-one ratio. This gives an opportunity for a self-evident, natural control.
4. If the aerobic ammonium oxidation has proceeded for 50% all alkalinity is consumed and the conversion will stop due to a drop in pH. Van Dongen et al. (2001) showed that indeed a stable and good N-removal is possible by this natural process control.
5. Two-reactor configurations can be control in a very simple way. Partial nitrification is controlled by the proper HRT/SRT design, which is between 1 and 1.5 days, where the oxygen is set around $1.5 mgO_2\ L^{-1}$. The anammox reactor is designed to be operated under nitrite-limiting conditions.
6. Effluent nitrogen compounds concentration values have been derived from the following assumptions :
    a. At least 90% ammonium removal should be achieved.
    b. Nitrite concentration should be kept at the lowest possible values to avoid anammox inhibition.
    c. Nitrate concentration can be derived from stoichiometry of partial nitrification (SHARON) and subsequent anammox process.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_2^- + 0.13H^+ \rightarrow$$
$$0.26NO_3^- + 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$



Effects of Phosphorus concentration on the activity of Anammox:

A difference in tolerance for phosphate exists between different Anammox species. Van de Graaf et al. (1996) experienced a loss of activity for C. Brocadia anammoxidans at phosphate concentrations above 155mgP L^{-1}, while Egli et al. did not see any inhibitory effect of phosphate when a culture of C. Kuenenia stuttgartiensis was supplied with up to 620mgP L^{-1}.

Dapena-Mora et al. (2007) observed at the same phosphate level of 620mgP L^{-1} 50% inhibition of Anammox activity. In batch tests using sludge from a highly loaded lab-scale rotating biological contactor containing C. Kuenenia stuttgartiensis, phosphate was shown to partially inhibit the Anammox process. Anammox activity decreased to 63% of the normal activity at 55mgP L^{-1} and further to 20% at 110mgP L^{-1}. At 285mgPL^{-1} no further decrease was observed (80% inhibition).

Due to the inhibition effect, a pretreatment of supernatant is required to remove phosphorus and the Suspended solids.

Treatment of the Supernatant by SHARON-ANAMMOX Process:

The Sharon Process will be carried out in CSTR and the Anammox process will be carried out in an SBR. The combination of the Anammox process and a partial nitrification (SHARON) process has been successfully tested using sludge digester effluent by many researchers.

The SHARON reactor was operated, the ammonium present in the sludge digester effluent was converted to nitrite for 50%. In this way an ammonium-nitrite mixture suitable for the Anammox process was generated. The effluent of the

SHARON reactor was used as influent for the Anammox - SBR. In the nitrite limited Anammox reactor all nitrite was removed, the surplus ammonium remained.

In the SHARON process, one can carefully makes use of the fact that at high temperatures, Nitrobacter has a distinctly lower growth rate than Nitrosomonas. By implementing completely mixed reactor at short residence time e.g. one day and high temperatures, one can achieve wash out of Nitrobacter.

Fux et al. (2002) also operated a 2.1m^3 CSTR reactor in Zurich at a HRT of 1.1 days and a temperature of 30◦C without pH control. Digester effluent from two different WWTPs was tested obtaining an Anammox-suited ammonium: nitrite ratio of 1:1.32 at a pH between 6.6 and 7.2

The overall nitrogen removal in the Sharon-Anammox process, when compared to the conventional nitrification–denitrification processes, the process requires less oxygen supply (1.9 kg O_2 (kgN) $^{-1}$ instead of 4.6 kg O_2 (kgN) $^{-1}$), no presence of carbon source (no need of 2.6 kg BOD (kg N) $^{-1}$) and has a lower sludge production (0.08 instead of approximately 1.0 kg VSS (kgN) $^{-1}$) (Van Loosdrecht et al., 1998).

According to Munz et al., (2011) the maximum specific growth rates of the AOB and NOB were tested in different DO conditions and found the results which are too far away as was mentioned before in the literature, and the values for $\mu_{max,\ AOB}$ and $\mu_{max,\ NOB}$ at 20^{o}C are 1.05 $d^{-1} < \mu_{max,\ AOB} < 1.4\ d^{-1}$ and 0.91 $d^{-1} < \mu_{max,\ NOB} < 1.31\ d^{-1}$. The decay coefficients of both AOB and NOB were much higher in aerobic (from 0.22 d^{-1} to 0.28 d^{-1}) than in anoxic (0.04 d^{-1} to 0.16 d^{-1}).

The maximum growth rate of the ammonium oxidisers 0.85 d^{-1} to 0.95 d^{-1} at 30^{o}C limited the nitrite production rate to 0.35 kg NO_2-N $m^{-3}_{reactor}$ d^{-1} or 1.2 kg NO_2-N kg^{-1} TSS d^{-1}, respectively. This is in accordance with the observations of van Dongen et al. (2001), who reported stable nitritation for over 2 years in a 10 L CSTR with dilution rate of 1d^{-1} at temperatures above 30^{o}C.

According to Jubany et al., (2008) the maximum growth rate and the decay rates of AOB and NOB at 25^{o}C is 1.21d^{-1}, 0.21d^{-1} for AOB and 1.02 d^{-1}, 0.17d^{-1} for NOB respectively. Y_{AOB} and Y_{NOB} are the growth yield constants for AOB and NOB, respectively. The values are Y_{AOB} = 0.18 g COD gN^{-1} and Y_{NOB} = 0.08 g COD gN^{-1}

(Jubany et al., 2008). From Van Dongen et al., 2001, affinity constant for ammonia and nitrite is 0.1mg N L^{-1}.

According to the van't Hoff–Arrhenius equation, the growth rate of microorganisms doubles with each 10°C increment in temperature.

Reactor volume for SHARON reactor:

$K_{S,AOB} = 1\, mgNL^{-1}$ $\quad Q = 100 m^3 d^{-1}$ $\quad \begin{cases} HRT_{\min} \cdot \left(\mu_{AOB}^{T} - k_{d\,AOB}^{\ T}\right) = 1 \\ HRT_{\min} = 1/(1.21 - 0.21) = 1\,\text{d} \\ HRT_{\max} \cdot \left(\mu_{NOB}^{T} - k_{d\,NOB}^{\ T}\right) = 1 \\ HRT_{\max} = 1/\left(1.02 - 0.17\right) = 1.17\,\text{d} \end{cases}$

$\mu_{AOB}^{25^oC} = 1.045\, d^{-1}$ $\quad NH_4^{+} - N_o = 1500 mgL^{-1}$

$k_{d\,AOB}^{\ 25^oC} = 0.21\, d^{-1}$ $\quad T = 25^oC$

$\mu_{NOB}^{25^oC} = 1.02\, d^{-1}$ $\quad \eta_{NH_4-N} = 0.5$

$k_{d\,NOB}^{\ 25^oC} = 0.17\, d^{-1}$

$Y_{AOB} = 0{,}18\ \text{gVSS gN}^{-1}$

$HRT = 1.2d$

$$S = \frac{K_s \cdot (1 + k_d \cdot HRT)}{HRT \cdot (\mu - k_d) - 1} = \frac{1*(1 + 0.21*1.2)}{1.2*(1.045 - 0.21) - 1} = 750\, mgNL^{-1}$$

$$C_V = \frac{S_0}{HRT} = \frac{1500}{1.2} = 1250 \frac{gN}{m^3 d}$$

$$x = \frac{(S_0 - S) \cdot Y}{1 + k_d \cdot HRT} = \frac{(1500 - 750)*0.18}{1 + 0.21*1.2} = 108\, mgVSS_{nitr} L^{-1}$$

$$V = q * HRT = 100 * 1.2 = 120\, m^3$$

I have chosen growth rate of AOB at lower temperature, to be robust in the process and to be safe when it is operated during winter seasons at which the temperatures are really low and can have better growth of the bacteria at higher temperature, since the optimal temperature range for the growth of AOB is from 30°C-35°C.

An advantage of the Anammox process is the low sludge production. However, systems with efficient biomass retention such as the used SBR system will be necessary to keep all the Anammox biomass in the reactor and long start-up times will be required to grow enough biomass since, The growth rate (doubling time 11 days) and growth yield (0.11 gVSS gNH4-N^{-1}) of the ANAMMOX microorganisms is very low.

The high maximum specific nitrogen consumption rate (0.82gN $gVSS^{-1}d^{-1}$), the very high affinity for ammonia and nitrite (Ks < 0.1 mg N L^{-1}) and the granular growth allowing efficient biomass retention, makes the design of very compact installations possible.

Previous studies have shown that some Nitrosomonas species were also capable of ammonium oxidation with nitrite as the electron acceptor.

Under anoxic or oxygen-limiting conditions the reaction rate was less than 0.08gN $gVSS^{-1}$ d^{-1} (Bock et al., 1995; Jetten et al., 1999; Kuai, Verstraete, 1998; Schmidt, Bock, 1997; Schmidt, Bock, 1998; Zart, Bock, 1998).

The anammox activity reached values as high as 0.8 kg N kg dry $weight^{-1}$ d^{-1} (Van Dongen et al., 2001). The Anammox yield value found by Graaf et al. (1996) is 0.07 kg VSS (kg NH_4^+-N)$^{-1}$.

According to Van der Star et al., (2007) the concentration of nitrite during the startup is of crucial importance for growth: a too low amount will result in substrate limitation and thus slower growth, while concentrations above 50–150 mg-N L^{-1} can already lead to inhibition (Strous et al., 1999b; Egli et al., 2001; Dapena-Mora et al., 2007). These inhibition values are especially low compared with the nitrite concentration in a nitritation reactor running on sludge digestate (ca 600 mg-N L^{-1}).

Strous et al. (1998) estimated the stoichiometric parameters for the ANAMMOX microorganisms and obtained a yield value expressed as biomass produced per ammonia nitrogen reduced of 0.066 mol $(mol)^{-1}$, an ammonium consumption rate per biomass expressed as protein of 45 nmol mg^{-1} min^{-1} and a maximum specific growth rate of 0.0027 h^{-1}. This means a doubling time of at least 11 days.

The practical application of the Anammox process is still limited by its long start-up periods due to the very low growth rates (0.072 d^{-1} measured at 32^{o}C) and biomass yield generated per ammonia nitrogen consumed (0.088 g g^{-1}) of these microorganisms (Jetten et al., 1997).

On the other hand, Strous et al. (1998) showed that the SBR is a suitable system to grow ANAMMOX micro-organisms, obtaining an enrichment of 74% of ANAMMOX microorganisms, thanks to the strong selective conditions achieved in this system.

From Suneethi et al., (2011), Start up of ANAMMOX process is challenge to the researchers and practitioners owing to the slow growth rate (0.072 d^{-1} at 32^{o}C) and low biomass yield (0.13 g dry weight g Amm-N^{-1} oxidized) (Chamchoi and Nitisoravut 2007; Trigo et al., 2006; Third et al., 2005) of the Anammox bacteria.

From the results of Isaka K. et al., (2006) the doubling time of anammox bacteria was calculated as 1.8 days, and the specific growth rate (μ) was 0.39 d^{-1}. This result indicated that the anammox bacteria have higher growth rate than the reported value (doubling time, 11 days).

According to Dosta et al. (2008) Anammox organisms settle well as SVI of Anammox biomass is as low as 58mL $gVSS^{-1}$ (< 100mL g VSS^{-1}). Half-saturation constant is negligible (0,2 - 3 μg L^{-1}, van der Star, 2008; < 5 μg L^{-1}, Jetten et al., 2004, in Ekström S., 2010) so that Anammox process kinetics can be considered as zero-order.

The anaerobic ammonium oxidation was carried out in SBR, by Fux C. et al. (2002) and achieved with a maximum nitrogen elimination rate of 2.4 kgN $m^{-3}_{reactor}$ d^{-1} or 0.3kgN $kgTSS^{-1}$ d^{-1} were obtained at 30^{o}C.

However, the overall nitrogen elimination rate was only about 0.60±0.04 kgN m^{-3} d^{-1} due to limitation of the inlet nitrogen load from the nitritation reactor. This is somewhat lower than the 0.75 kgN m^{-3} d^{-1} or 0.18kgN $kgTSS^{-1}$ d^{-1} respectively reported in van Dongen et al. (2001) during a test period of 110 days in a granular-sludge SBR.

Helmer et al. (2001) achieved a nitrogen elimination rate of 1.5 kg N m^{-3} d^{-1} at 28^{o}C in an anoxic batch test with a moving bed, and the same maximum nitrogen conversion capacity was obtained in a fluidised-bed reactor fed with sludge digestion effluent (Strous et al., 1997).

Therefore, provided that the nitritation capacity is high enough, an overall nitrogen elimination rate of 0.6 kgN m^{-3} d^{-1} in the anammox reactor is feasible, resulting in a hydraulic dilution rate of 1-1.5 d^{-1}.

Anammox Reactor Volume calculation:

Overall Ammonium - N removal efficiency = η = 0.5

ANAMMOX Ammonium-N removal efficiency = η = 0.95

Ammonium nitrogen load to be removed $= Q * \eta * NH_4\text{-}N_{in}$

$= 100\ m^3\ d^{-1} * 0.95 * 750\ g\ m^{-3}$

$= 71250\ g\ d^{-1} = 71.25 kg\ d^{-1}$

Anammox volumetric removal rate: v_A $= 0.6\ kgN\ m^{-3}_{reactor}\ d^{-1}$

Reactor volume: $V_{reactor}$ **$= 71.25 / 0.6 = 118.75 m^3$.**

Total Reactor volume **$= 120\ m^3 + 120 m^3 \quad = 240\ m^3$.**

On average, above 95% of the nitrogen load can be eliminated in the anammox reactor with the correct nitrite/ammonium ratio of 1.3 in the influent. The overall sludge production will be negligible and part of the produced nitrate was denitrified by heterotrophs in the anammox reactor by using the carbon released from biomass decay and hydrolysis.

5.3 Conclusion:

A one-reactor system operated in an air pulsing-SBR mode is an attractive option, providing reliable operation and easy to evaluate.

The good retention capacity of the reactor and the low VSS concentration in the effluent will minimize the size or even the need of a posterior settler.

The air pulsing flow will reduce the requirements of aeration with a consequent decrease in the aeration costs and it also allows an easy control of the DO level.

The operation of the air pulsing-SBR allows simple and robust regulation of the DO concentration of the liquid for the stable operation of the CANON process.

A one-reactor system is simple in configuration, but it is limited by complex interaction between AOBs, NOBs and anammox bacteria. It requires control in a very tight oxygen and pH range.

A two-reactor system can be maintained in a stable condition by maintaining the required conditions for the operations in the both the reactors separately, which is easy to do when compared to do in a one-reactor system. However the drawback in the two-reactor system is the influent to the Anammox reactor, that has to be properly

controlled in the previous partial nitritation process which can produce 50% NH_4-N, and 50% NO_2-N, otherwise the anammox bacteria will get inhibited.

The reactor dimensions are small for the two-reactor system, in the above mentioned pilot scale design, which is economically better when compared to one-reactor system.

A two-reactor system has a very high potential for optimization and process intensification, as optimal conditions can be provided for each of the two consecutive operations.

The two-reactor system is the most robust, which may shorten the recovery time after possible system upsets.

References:

Ahmed Z., Sang-Eun Oh and In S. Kim, 2009, Effects of Organic loading rates on treatment performance in a Polyvinylidene media based fixed-film reactor, Environmental Engineering Research, 200, 14(4): 238-242.

Ahn Y.H., Jinwoo Lee, Hee-Chul Kim and Sooyoul Kwon, 2006, Lithoautotrophic nitrogen removal with anaerobic granular sludge as seed biomass and its microbial community, Environmental Engineering Research, Vol. 11, No. 4, pp 173-180 - **[36]**.

Ahn Y.H., Hoon-Chang Choi, 2006a, Autotrophic nitrogen removal from sludge digester liquids in upflow sludge bed reactor with external aeration, Process Biochemistry, 41, 1945–1950 – **[58]**.

Ahn Y.H., 2006b, Sustainable nitrogen elimination biotechnologies: A review, Process Biochemistry 41, 1709–1721- **[48]**.

Akpor O. B. and Muchie M., 2010, Bioremediation of polluted wastewater influent: Phosphorus and nitrogen removal, Scientific Research and Essays Vol. 5(21), pp. 3222-3230.

Anthonisen A.C., R.C. Loehr, T.B.S. Prakasam, E.G. Srinath, 1976, Inhibition of nitrification by ammonia and nitrous acid, J. Water Pollut. Control Fed, 48, pp. 835–852

Barber W.P. and David C. Stuckey, 2000, Nitrogen removal in a modified Anaerobic Baffled Reactor: 1, Denitrification, Water. Res. Vol. 34, No. 9, pp. 2413-2422.

Bodalo A., Gomez J., Gomez E., Gerardo L., Tejara M., 2005, Ammonium removal from aqueous solution by Reverse osmosis using Cellulose acetate membranes, Desalination, 184, 149-155.

Carpenter S.R., Caraco N.F., Correll D.L., Howarth R.W., Sharpley A.N., Smith V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8, 559-568.

Casey E., B. Glennon, G. Hamer, 1999, Review of membrane aerated biofilm reactors, Resources, Conservation and Recycling 27, 203–215.

Cema G., J. Surmacz-Górska and K. Miksch, Implementation of Anammox Process in the Membrane assisted bioreactor – **[34]**.

Cema G., B. Szatkowska, E. Plaza, J. Trela and J. Surmacz-Gorska, 2006, Nitrogen removal rates at a technical-scale pilot plant with the one-stage partial nitritation/Anammox process, Water Science & Technology Vol 54 No 8 pp 209–217 – **[70]**.

Chamchoi N., Suwanchai Nitisoravut, 2007, Anammox enrichment from different conventional sludges, Chemosphere 66, 2225–2232 - **[1]**.

Chan Y.J., Mei Fong Chong, Chung Lim Law, D.G. Hassell, 2009, A review on anaerobic–aerobic treatment of industrial and municipal wastewater, Chemical Engineering Journal, 155,1–18.

Chen H., Sitong Liu, Fenglin Yang, Yuan Xue, Tao Wang, 2009, The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal, Bioresource Technology 100, 1548–1554 – **[25]**.

Chuang H.P., Takashi Yamaguchi, Hideki Harada and Akiyoshi Ohashi, 2008, Anoxic Ammonium Oxidation by Application of a Down-flow hanging sponge (DHS) reactor, Journal of Environmental Engineering Management,18(6), 409-417 – **[62]**.

Clippeleir H.D., Siegfried E. Vlaeminck, Marta Carballa, Willy Verstraete, 2009, A low volumetric exchange ratio allows high autotrophic nitrogen removal in a sequencing batch reactor, Bioresource Technology 100, 5010–5015 – **[47]**.

Clippeleir H.D, Xungang Yan, Willy Verstraete, Siegfried Elias Vlaeminck , 2011, OLAND is feasible to treat sewage-like nitrogen concentrations at low hydraulic residence times, Applied Microbiology Biotechnology, 90:1537–1545.

Dapena-Mora A., Belen Arrojo, Jose Luis Campos, Anuska Mosquera-Corral and Ramon Mendez, 2004, Improvement of the settling properties of Anammox sludge in an SBR, Journal of Chemical Technology and Biotechnology 79, p 1417–1420 – **[60]**.

Dapena-Mora A., J.L. Campos, A. Mosquera-Corral, M.S.M. Jetten, R. Méndez, 2004a, Stability of the ANAMMOX process in a gas-lift reactor and a SBR, Journal of Biotechnology 110, 159–170 – **[2]**.

Dapena-Mora A., I. Fernandez, J.L. Campos, A. Mosquera-Corral, R. Mendez, M.S.M. Jetten, 2007, Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production, Enzyme and Microbial Technology 40; 859–865.

Dosta J. Fernandez I., Vázquez-Padín J., Mosquera-Corral A., Campos J.L., Mata-Alvarez J., Méndez R., 2008, Short and long-term effects of temperature on the Anammox process, Journal of Hazardous Materials 154, 688–693.

Downing L.S., Robert Nerenberg, 2008, Total nitrogen removal in a hybrid, membrane-aerated activated sludge process, water research 42, 3697 – 3708.

Egli K., U. Fanger, P.J.J. Alvarez, H. Siegrist, J.R. Van Der Meer, A.J.B. Zehnder, 2001, Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate, Archive of Microbiology 175; 198–207.

Eldyasti A., Nabin Chowdhury, George Nakhla, Jesse Zhu, 2010, Biological nutrient removal from leachate using a pilot liquid–solid circulating fluidized bed bioreactor (LSCFB), Journal of Hazardous Materials 181, 289–297.

Fatone F., Paolo Battistoni, David Bolzonella, Paolo Pavan, Franco Cecchi, 2008, Long-term experience with an automatic process control for nitrogen removal in membrane bioreactors, Desalination, 227,p-72–84.

Feng Y.J., Szu-Kung Tseng, Tsung-Hui Hsia, Chun-Ming Ho, and Wen-Po Chou, 2007, Partial Nitrification of Ammonium-Rich Wastewater as Pretreatment for Anaerobic Ammonium Oxidation (Anammox) Using Membrane Aeration Bioreactor, Journal of Bioscience and Bioengineering, Vol. 104, No. 3, 182–187 - **[42]**.

Fitch M.W., Natalie Pearson, Gene Richards, Joel G. Burken, 1998, Biological fixed film Systems-Literature review.

Furukawa K., P.K. Lieu, H. Tokitoh and T. Fujii, 2006, Development of single-stage nitrogen removal using anammox and partial nitritation (SNAP) and its treatment performances, Water Science & Technology Vol 53 No 6 pp 83–90 – **[41]**.

Furukawa K., Yasuhiko Inatomi, Sen Qiao, Lai Quan, Taichi Yamamoto, Kazuichi Isaka, Tatsuo Sumino, 2009, Innovative treatment system for digester liquor using anammox process, Bioresource Technology 100, 5437–5443 – **[28]**.

Fux C., Marc Boehler, Philipp Huber, Irene Brunner, Hansruedi Siegrist, 2002, Biological treatment of ammonium rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation **(**anammox**)** in a pilot plant, Journal of Biotechnology 99, 295-306 – **[12]**.

Galloway JN, Schlesinger WH,Levy H II,Michaels A, Schnoor JL. 1995.Nitrogen fixation:Anthropogenic enhancement—environmental response. Global Biogeochemical Cycles 9: 235–252.

Gali A., Joan Dosta, Mark C. M. van Loosdrecht, and Joan Mata-Alvarez, 2006, Biological Nitrogen Removal via Nitrite of Reject Water with a SBR and Chemostat SHARON/Denitrification Process, Ind. Eng. Chem. Res., 45, 7656-7660 – **[56]**.

Gali A., J. Dosta, M.C.M. van Loosdrecht, J. Mata-Alvarez, 2007, Two ways to achieve an anammox influent from real reject water treatment at lab-scale: Partial SBR nitrification and SHARON process, Process Biochemistry 42, 715–720 – **[64]**.

Ganigue R., H. Lopeza, M.D. Balaguer, J. Colprim, 2007, Partial ammonium oxidation to nitrite of high ammonium content urban landfill leachates, Water Research 41, 3317 – 3326 – **[40]**.

Ganigue R., Jordi Gabarro, Alexandre Sanchez-Melsio, Mael Ruscalleda, Helio Lopez, Xavier Vila, Jesus Colprim, M. Dolors Balaguer, 2009, Long-term operation of a partial nitritation pilot plant treating leachate with extremely high ammonium concentration prior to an anammox process, Bioresource Technology 100, 5624–5632 – **[31]**.

Gao D., Yongzhen Peng, Baikun Li, Hong Liang, 2009, Shortcut nitrification–denitrification by real-time control strategies, Bioresource Technology 100, 2298–2300.

Ghyoot W., Stefaan Vandele and Willy Verstraete, 1999, Nitrogen removal from sludge reject water with a membrane – assisted bioreactor, Water Research. Vol. 33, No. 1, pp. 23-32.

Gong Z., Fenglin Yang, Sitong Liu, Han Bao, Shaowei Hu, Kenji Furukawa, 2007, Feasibility of a membrane-aerated biofilm reactor to achieve single-stage autotrophic nitrogen removal based on Anammox, Chemosphere 69, 776–784 – **[49]**.

Grunditz C., Lena Gumaelius and Gunnel Dalhammar, 1998, Comparison of inhibition assays using Nitrogen removing bacteria: Application to Industrial wastewater, Water Research Vol. 32, No. 10, pp. 2995-3000 – **[39]**.

Hellinga C., A.A.J.C. Schellen, J.W. Mulder, M.C.M van Loosdrecht and J.J. Heijnen,1998, The SHARON process: An Innovative method for nitrogen removal from Ammonium-rich waste water, Water Science And Technology, Volume: 37, Issue: 9, Pages: 135-142 – **[54]**.

Helmer C., Tromm C., Hippen A., Rosenwinkel K.H., Seyfried C.F., Kunst S., 2001, Single stage biological nitrogen removal by nitritation and anaerobic ammonium oxidation in biofilm systems. Water Sci. Tech. 43 (1), 311-320.

Holakoo L., George Nakhla, Amarjeet S. Bassi, Ernest K. Yanful, 2007, Long term performance of MBR for biological nitrogen removal from synthetic municipal wastewater, Chemosphere 66, 849–857.

Hu S., Fenglin Yang, Sitong Liu, Lingqin Yu, 2009, The development of a novel hybrid aerating membrane-anaerobic baffled reactor for the simultaneous nitrogen and organic carbon removal from wastewater, water research 43, 381 – 388.

Isaka K., Y. Date, T. Sumino, S. Yoshie, S. Tsuneda, 2006, Growth characteristic of anaerobic ammonium-oxidizing bacteria in an anaerobic filtrated reactor, Applied Microbiology & Biotechnology 70, 47–52 – **[24]**.

Isaka K., Tatsuo Sumino, and Satoshi Tsuneda, 2007, High Nitrogen Removal Performance at Moderately Low Temperature Utilizing Anaerobic Ammonium Oxidation Reactions, Journal of Bioscience and Bioengineering, Vol. 103, No. 5, 486–490 – **[23]**.

Jardin N. and J. Hennerkes , 2012, Full-scale experience with the deammonification process to treat high strength sludge water – a case study, Water science and technology,65.6, 447- 455.

Jardin N., Dieter Thöle, Bernhard Wett, Ruhrverband, Treatment of sludge return liquors: Experiences from the operation of full-scale plants, Water Environment Foundation.

Jaroszynski L.W. and J.A. Oleszkiewicz, 2011, Autotrophic ammonium removal from reject water: partial nitrification and anammox in one-reactor versus two-reactor systems, Environmental Technology Vol. 32, No. 3, 289–294.

Jaroszynski L.W., N. Cicek, R. Sparling, J.A. Oleszkiewicz, 2011a, Importance of the operating pH in maintaining the stability of anoxic ammonium oxidation (anammox) activity in moving bed biofilm reactors, Bioresource Technology 102,7051–7056.

Jetten M.S.M., Horn S.J., van Loosdrecht M.C.M., 1997, Towards a more sustainable wastewater treatment system, Water Science Technology, 35, pp. 171–179.

Jetten M.S.M., Marc Strous, Katinka T. van de Pas-Schoonen, Jos Schalk, Udo G.J.M. van Dongen, Astrid A. van de Graaf, Susanne Logemann, Gerard Muyzer, Mark C.M. van Loosdrecht, J. Gijs Kuenen, 1999,The anaerobic oxidation of ammonium, FEMS Microbiology Reviews 22, 421-437.

Jetten M.S.M., Wagner M., Fuerst J., Van Loosdrecht M., Kuenen G., Strous M., 2001; Microbiology and application of the anaerobic ammonium oxidation ('anammox') process, Curr Opin Biotechnology; 12:283–8.

Jetten M.S.M., Schmid M., Schmidt I., Wubben M., Van Dongen U., Abma W., et al., 2002, Improved nitrogen removal by application of new nitrogen-cycle bacteria. Revised Environmental Science Biotechnology; 1:51–63.

Jetten M.S.M., Markus Schmid, Ingo Schmidt, Mariska Wubben, Udo van Dongen, Wiebe Abma, Olav Sliekers, Niels Peter Revsbech, Hubertus J.E. Beaumont, Lars Ottosen, Eveline Volcke, H.J. Laanbroek, Jose Luis Campos-Gomez, Jeff Cole, Mark van Loosdrecht, Jan Willem Mulder, John Fuerst, David Richardson, Katinka van de Pas, Ramon Mendez-Pampin, Katie Third, Irina Cirpus, Rob van Spanning, Annette Bollmann, Lars Peter Nielsen, Huub Op den Camp, Carl Schultz, Jens Gundersen, Peter Vanrolleghem, Marc Strous, Michael Wagner & J. Gijs Kuenen, 2002, Improved nitrogen removal by application of new nitrogen-cycle bacteria, Reviews in Environmental Science & Biotechnology 1: 51–63.

Jin R.C., Ping Zheng, An-Hui Hu, Qaisar Mahmood, Bao-Lan Hu, Ghulam Jilani, 2008, Performance comparison of two anammox reactors: SBR and UBF, Chemical Engineering Journal 138, 224–230 – **[14]**.

Jin R.C., Bao-lan Hu, Ping Zheng, Mahmood Qaisar, An-hui Hu, Ejazul Islam, 2008a, Quantitative comparison of stability of ANAMMOX process in different reactor configurations, Bioresource Technology 99, 1603–1609 – **[61]**.

Jubany I., Carrera J., Lafuente J., Baeza J.A., 2008, Start-up of a nitrification system with automatic control to treat highly concentrated ammonium wastewater: experimental results and modeling. Chem. Eng. J. 144, 407–419.

Khin T., Ajit P. Annachhatre, 2004, Novel microbial nitrogen removal processes, Biotechnology Advances 22, 519–532.

Kuai L. and Willy Verstraete, 1998, Ammonium Removal by the Oxygen-Limited Autotrophic Nitrification-Denitrification System, Applied and Environmental Microbiology, Nov. 1998, Vol. 64, No. 11, p. 4500–4506 – **[50]**.

Kuypers, M.M.M.; Sliekers, A.O. Lavik, G. Schmid, M. Jørgensen, B.B. Kuenen, J.G., Sinninghe Damsté, J.S. Strous, M. & Jetten, M.S.M., 2003, Anaerobic ammonium oxidation by anammox bacteria in the Black Sea, Nature. 422., 608-11.

Kumar M., Jih-Gaw Lin, 2010, Co-existence of anammox and denitrification for simultaneous nitrogen and carbon removal—Strategies and issues, Journal of Hazardous Materials, 178,1–9.

Laitinen N., Antero Luonsi, Jari Vilen, 2006, Landfill leachate treatment with sequencing batch reactor and membrane bioreactor, Desalination 191, 86–91.

Lan C.J., Mathava Kumar, Chih-Cheng Wang, Jih-Gaw Lin, 2011, Development of simultaneous partial nitrification, anammox and denitrification (SNAD) process in a sequential batch reactor, Bioresource Technology 102, 5514–5519 – **[30]**.

Liang Z., Junxin Liu, 2008, Landfill leachate treatment with a novel process: Anaerobic ammonium oxidation (Anammox) combined with soil infiltration system, Journal of Hazardous Materials 151, 202–212 – **[21]**.

Liang Z., Yingxu Chen, Wenhong Li, Shangyuan Yang, Ping Du, 2011, Autotrophic nitrogen removal in one lab-scale vertical submerged biofilm reactor, Physics and Chemistry of the Earth 36, 470–474 – **[27]**.

Liu S., Zheng Gong, Fenglin Yang, Hanmin Zhang, Lijun Shi, Kenji Furukawa, 2008, Combined process of urea nitrogen removal in anaerobic Anammox co-culture reactor, Bioresource Technology 99, 1722–1728 – **[59]**.

Magrí A., Vanotti M.B., Szogi A.A., ANAMMOX treatment of Swine water using Immobilized technology – **[3]**.

Martin Hertach Hildastrasse, 2008, Anaerobic Ammonium Oxidation (Anammox) – A new sink in the marine nitrogen cycle, ETH Zurich.

Matošić M., Ivana Prstec, Helena Korajlija Jakopović, Ivan Mijatović, 2009, Treatment of beverage production wastewater by membrane bioreactor, Desalination, 247, 286–294.

Morling S., 2010, Nitrogen removal and heavy metals in leachate treatment using SBR technology, Journal of Hazardous Materials 174, 679–686.

Mostafal M.G., Y. Kawakubo and K. Furukawa, 2011, Removal of nitrogen from ammonium-rich synthetic wastewater in an upflow column type anammox reactor, Water Resources and Environmental Engineering Vol. 3(9), pp. 189-195 – **[37]**.

Mulder A., A.A. van de Graaf, L.A. Robertson, J.G. Kuenen, 1995, Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor, FEMS Microbiology Ecology 16, p 177-184 – **[9]**.

Mulder J.W., J.O.J. Duin, J. Goverde, W.G. Poiesz, H.M. van Veldhuizen, R. van Kempen, P. Roeleveld, 2006, Full-Scale Experience with the Sharon Process through the eyes of the operators, Water Environment Foundation – **[63]**.

Munz G., Claudio Lubello, Jan A. Oleszkiewicz, 2011, Factors affecting the growth rates of ammonium and nitrite oxidizing bacteria, Chemosphere 83, 720–725.

Nicolella C., M.C.M. van Loosdrecht, J.J. Heijnen, 2000, Wastewater treatment with particulate biofilm reactors, Journal of Biotechnology 80, 1–33.

Novak J.T., Sarita Banjade, Sudhir N. Murthy, 2011, Combined anaerobic and aerobic digestion for increased solids reduction and nitrogen removal, water research 45, 618-624.

Noophan P., Siriporn Sripiboon, Mongkol Damrongsri, Junko Munakata Marr,2009, Anaerobic ammonium oxidation by Nitrosomonas spp. And anammox bacteria in a sequencing batch reactor, Journal of Environmental Management 90, 967–972.

Patel A., Jesse Zhu, George Nakhla, 2006, Simultaneous carbon, nitrogen and phosphorous removal from municipal wastewater in a circulating fluidized bed bioreactor, Chemosphere, 65, p 1103–1112.

Plaza E., J. Trela and B. Hultman, Swedish experience with Deammonification in Biofilm system – **[71]**.

Philips S., S. Wyffels, R. Sprengers, W. Verstraete, 2002, Oxygen-limited autotrophic nitrification/denitrification by ammonia oxidisers enables upward motion towards more favourable conditions, Applied Microbiology Biotechnology, 59:557–566 – **[51]**.

Pochanna K., Keller J., Lant P., 1999, Model development for simultaneous nitrification and denitrification. Water Sci. Technol. 39, 235–243.

Pollice A., Valter Tandoi, Carmela Lestingi, 2002, Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate, Water Research 36, 2541–2546 – **[65]**.

Pynaert K., R. Sprengers, J. Laeden and W. Verstraete, 2001, Oxygen-limited Nitrification and Denitrification in a Rotating Biological contactor, Environmental Technology, Vol.23, pp 353-362 – **[55]**.

Pynaert K., Barth Smets, Stijn Wyffels, Daan Beheydt, Steven D. Siciliano and Willy Verstraete, 2003, Characterization of an Autotrophic Nitrogen-Removing Biofilm from a Highly Loaded

Lab-Scale Rotating Biological Contactor, Applied and Environmental Microbiology, Vol. 69, No. 6, p. 3626–3635 – **[44]**.

Pynaert K., Barth Smets, Daan Beheydt and Willy Verstraete, 2004, Start-up of Autotrophic Nitrogen Removal Reactors via Sequential Biocatalyst Addition, Environmental Science Technology, 38, p.1228-1235 – **[45]**.

Qiao S., Taichi Yamamoto, Motoki Misaka, Kazuichi Isaka, Tatsuo Sumino, Zafar Bhatti, Kenji Furukawa, 2010, High-rate nitrogen removal from livestock manure digester liquor by combined partial nitritation–anammox process, Biodegradation, 21:11–20 – **[19]**.

Radjenovi J., Marin Matosi, IvanMijatovi, Mira Petrovic, Damià Barceló, 2008, Membrane Bioreactor (MBR) as an Advanced Wastewater Treatment Technology, Hdb Environmental Chemistry Vol. 5, Part S/2: 37–101.

Reginatto V., R. M. Teixeira, F. Pereira, W. Schmidell, A. Furigo Jr, R. Menes, C. Etchebehere and H. M. Soares, 2005, Anaerobic Ammonium Oxidation in a bioreactor treating slaughterhouse wastewater, Brazilian Journal of Chemical Engineering, Vol. 22, No. 04, pp. 593 - 600– **[33]**.

Schmidt I., Olav Sliekers, Markus Schmid, Eberhard Bock, John Fuerst, J.Gij Kuenen, Mike S.M. Jetten, Marc Strous, 2003,New concepts of microbial treatment processes for the nitrogen removal in wastewater, FEMS Microbiology Reviews 27, 481-492.

Seyfried C.F., A. Hippen, C. Helmer, S. Kunst, K.H. Rosenwinkel, 2001, One-stage deammonification: nitrogen elimination at low costs, Water Science and Technology: Water Supply Vol 1 No.1 pp 71–80 – **[67]**.

Sliekers O.A., N. Derwort, J.L.Campos Gomez, M. Strous, J.G. Kuenen, M.S.M. Jetten, 2002, Completely autotrophic nitrogen removal over nitrite in one single reactor, Water Research 36, 2475–2482-**[46]**.

Sliekers O. A., K.A. Third, W. Abma, J.G. Kuenen, M.S.M. Jetten, 2003, CANON and Anammox in a gas-lift reactor, FEMS Microbiology Letters 218, 339-344 – **[18]**.

Smith V.H., G.D. Tilman, J.C. Nekola, 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, Environmental Pollution 100, 179-196.

Spagni A., Stefano Marsili - Libelli, 2009, Nitrogen removal via nitrite in a sequencing batch reactor treating sanitary landfill leachate, Bioresource Technology 100, 609–614 – **[35]**.

Stijn W. H. Van Hulle, Pascal Boeckx, Eveline I. P. Volcke, Oswald Van Cleemput, Peter A. Vanrolleghem, Willy Verstraete, 2004, Modeling and Simulation of Oxygen-Limited Partial Nitritation in a Membrane-Assisted Bioreactor (MBR), Wiley InterScience – **[43]**.

Stijn W.H. Van Hulle, Helge J.P. Vandeweyer, Boudewijn D. Meesschaert, Peter A. Vanrolleghem, Pascal Dejans, Ann Dumoulin, 2010, Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams, Chemical Engineering Journal 162, 1–20 – **[A]**

Strous M., Eric van Gerven, Ping Zheng, J. Gijs Kuenen , M.S.M Jetten, 1997, Ammonium removal from concentrated waste streams with the ANAMMOX process in different reactor configurations, Water Research Vol 31. No.8, p 1955-1962 – **[11]**.

Strous M., J. J. Heijnen ,J. G. Kuenen, M. S. M. Jetten, 1998, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, Applied Microbiology Biotechnology, 50: 589-596.

Sudarno U., J. Winter, C. Gallert, 2011, Effect of varying salinity, temperature, ammonia and nitrous acid concentrations on nitrification of saline wastewater in FBR, Bio resource Technology 102, 5665–5673.

Suneethi S., Kurian Joseph, 2011, ANAMMOX process start up and stabilization with an anaerobic seed in Anaerobic Membrane Bioreactor (AnMBR), Bioresource Technology 102, 8860–8867 – **[8]**.

Tran H.T, Young-Joo Park, Mi-Kyeoung Cho, Dong-Jin Kim, and Dae Hee Ahn, 2006, Anaerobic ammonium oxidation process in an Upflow Anaerobic Sludge blanket reactor with Granular Sludge selected from an Anaerobic digestor, Biotechnology and Bioprocess Engineering, 11:199-204 – **[4]**.

Third K.A, Olav Sliekers .A, Kuenen J.G., and Jetten M.S.M, 2001, The CANON System (Completely Autotrophic Nitrogen-removal Over Nitrite) under Ammonium Limitation: Interaction and Competition between Three Groups of Bacteria, Systematic and Applied Microbiology 24, 588–596.

Third K.A., J. Paxman, M. Schmid, M. Strous, M.S.M. Jetten and R. Cord-Ruwisch, 2005, Enrichment of Anammox from Activated Sludge and Its Application in the CANON Process, Microbial Ecology, Volume 49, 236–244 - **[15]**.

Trigo C., J.L. Campos, J.M. Garrido, R. Mendez, 2006, Start-up of the Anammox process in a membrane bioreactor, Journal of Biotechnology 126, 475–487 – **[7]**.

Tsushimaa I., Yuji Ogasawaraa, Tomonori Kindaichib, Hisashi Satoha, Satoshi Okabe, 2007, Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors, Water Research 41, 1623 – 1634 – **[20]**.

Udert K., Fux C., Munster M., Larsen T., Siegrist H., Gujer W., (2003), Nitrification and autotrophic denitrification of source-separated urine, Water Science & Technology 48 (1), 119–130.

Vázquez-Padín J., Isaac Fernádez, Mónica Figueroa, Anuska Mosquera-Corral, Jose-Luis Campos, Ramón Méndez, 2009, Applications of Anammox based processes to treat anaerobic digester supernatant at room temperature, Bioresource Technology 100, 2988–2994 – **[13]**.

Vazquez-Padin J.R., I. Figueroa, A. Mosquera-Corral, J.L. Campos, R. Méndez, 2009a, Post-treatment of effluents from anaerobic digesters by the Anammox process, Water Science & Technology 60, 1135–1143-**[65]**.

Vazquez-Padin J.R., M.J. Pozo, M. Jarpa, M. Figueroa, A. Franco, A. Mosquera-Corral, J.L. Campos, R. Mendez, 2009b, Treatment of anaerobic sludge digester effluents by the CANON process in an air pulsing SBR, Journal of Hazardous Materials 166, 336–341 – **[57]**.

Vazquez-Padın J. R., I. Fernandez, N. Morales, J. L. Campos, A. Mosquera-Corral and R. Mendez, 2011, Autotrophic nitrogen removal at low temperature, water science and technology, 63.6, – **[32]**.

Van de Graff A.A., A. Mulder, Peter DE Bruijn, Mike S. M. Jetten, A Lesley, A. Robertson, and J. Gijs Kuenen, 1995, Anaerobic Oxidation of Ammonium Is a Biologically Mediated Process, Applied and Environmental Microbiology, p. 1246–1251.

Van de Graaf A.A., Peter de Brujin, Lesley A. Robertson, Mike S. M. Jetten and J. Gijs Kuenen, 1996, Autotrophic growth of anaerobic ammonium-oxidizing microorganisms in a Fluidized bed reactor, Microbiology,142, 2187-2196 - **[10]**.

Van Dongen U., M.S.M. Jetten and M.C.M. van Loosdrecht, 2001, The SHARON -Anammox process for treatment of ammonium rich wastewater, Water Science and Technology: Vol 44 No1 pp 153–160 – **[16]**.

Van der Star W.R.L, Wiebe R. Abma, Dennis Blommers, Jan-Willem Mulder, Takaaki Tokutomi, Marc Strous, Cristian Picioreanu, Mark C.M. van Loosdrecht, 2007, Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam, Water Research 41, 4149 – 4163 – **[17]**.

Van der Star W.R.L., Andreea I. Miclea, Udo G.J.M. van Dongen, Gerard Muyzer, Cristian Picioreanu, Mark C.M. van Loosdrecht, 2008, The Membrane Bioreactor: A Novel Tool to Grow Anammox Bacteria as Free Cells, Biotechnology and Bioengineering, Vol. 101, No. 2– **[6]**.

Van Hulle S.W.H., Van Den Broeck S., Maertens J., Villez K., Donckels B.M.R., Schelstraete G., Volcke E.I.P., Vanrolleghem P.A., (2005) , Construction, start-up and operation of a continuously aerated laboratory-scale SHARON reactor in view of coupling with an Anammox reactor, Water SA 31, 327–334.

Van Loosdrecht M.C.M., Jetten M.S.M., 1998, Microbiological conversions in nitrogen removal, Water Science Technology 38 (1), pg 1–7.

Vazquez-Padin J.R., M.J. Pozo, M. Jarpa, M. Figueroa, A. Franco, A. Mosquera-Corral, J.L. Campos, R. Mendez, 2009, Treatment of anaerobic sludge digester effluents by the CANON process in an air pulsing SBR, Journal of Hazardous Materials 166; 336–341.

Verstraete W., S. Philips, 1998, Nitrification-denitrification processes and technologies in new Contexts, Environmental Pollution 102, Sl, 717-726.

Vitousek, P.M., Mooney, H.A., Lubchenko, J., Melillo, J.M., 1997a, Human domination of Earth's ecosystems. Science 277, 494-499.

Vitousek, P.M., Aber, J., Howarth, R.W., Likens G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, G.D., 1997b. Human alteration of the global nitrogen cycle: causes and consequences. Ecological Applications 7, 737-750.

Vlaeminck S., Akhikoterada, Barthf Smets, Nicoboon, Willy Verstraete and Marta Carbella, 2009, Nitrogen Removal from Digested Black Water by One-Stage Partial Nitritation and Anammox, Environmental Science Technology, 43, 5035–5041 – **[53]**.

Voets J.P., H. Vanstaen, W. Verstraete, 1975, Removal of nitrogen from highly nitrogenous wastewaters, Water Pollution Control Federation, Water Environment Federation.

Wang R., Xinmin Zhan, Yalei Zhang, Jianfu Zhao, 2011, Nitrifying population dynamics in a redox stratified membrane biofilm reactor (RSMBR) for treating ammonium-rich wastewater, Environmental Science Engineering. China, 5(1): 48–56.

Wang T., Hanmin Zhang, Fenglin Yang, Sitong Liu, Zhimin Fu, Huihui Chen, 2009, Start-up of the Anammox process from the conventional activated sludge in a membrane bioreactor, Bioresource Technology 100, 2501–2506 – **[5]**.

Wang Y., Xia Huang, Qipeng Yuan, 2005, Nitrogen and carbon removals from food processing wastewater by an anoxic/aerobic membrane bioreactor, Process Biochemistry, 40, 1733–1739.

Wang, S.Y., Gao, D.W., Peng, Y.Z., Wang, P., Yang, Q., 2004. Nitrification - denitrification via nitrite for nitrogen removal from high nitrogen soybean wastewater with on - line fuzzy control. Water Sci. Technol. 49 (5–6), 121–127.

Wett B., 2007, Development and implementation of a robust deammonification process, Water Science & Technology Vol 56 No. 7 pp 81–88.

Windey K., Inge De Bo, Willy Verstraete, 2005, Oxygen-limited autotrophic nitrification–denitrification (OLAND) in a rotating biological contactor treating high-salinity wastewater, Water Research 39, p 4512–4520 – **[52]**.

Wyffels S., P. Boeckx, K. Pynaert, D. Zhang, O. Van Cleemput, G. Chen, W. Verstraete, 2004, Nitrogen removal from sludge reject water by a two-stage oxygen limited autotrophic nitrification denitrification process, Water Science &Technology 49 (5–6), 57–64.

Xing Y. and Ian D. Clark, 2011, Anaerobic Ammonium Oxidation in Waste Water an isotope hydrological perspective, in "Waste Water - Treatment and Reutilization", ISBN: 978-953-307-249-4, Department of Earth Science, University of Ottawa, Canada,

Xue Y., Yang F., Liu S., Fu Z., 2009, The influence of controlling factors on the startup and operation for partial nitrification in membrane bioreactor, Bioresource Technology 100, 1055–1060.

Yamamoto T., Keita Takaki, Toichiro Koyama, and Kenji Furukawa, 2006, Novel Partial Nitritation Treatment for Anaerobic Digestion Liquor of Swine Wastewater Using Swim-Bed Technology, Journal of Bioscience and Bioengineering, Vol. 102, No. 6, 497–503- **[38]**.

Yamamoto T., Keita Takaki, Toichiro Koyama, Kenji Furukawa, 2008, Long-term stability of partial nitritation of swine wastewater digester liquor and its subsequent treatment by Anammox, Bioresource Technology 99, p 6419–6425 – **[22]**.

Yang S.F., Joo-Hwa Tay, Yu Liu, 2003, A novel granular sludge sequencing batch reactor for removal of organic and nitrogen from wastewater, Journal of Biotechnology 106, 77–86.

Yang J, Li Zhang, Daisuke Hira, Yasuhiro Fukuzaki, Kenji Furukawa, 2011, Anammox treatment of high-salinity wastewater at ambient temperature, Bioresource Technology 102, 2367–2372 – **[26]**.

Yang J, Li Zhang, Daisuke Hira, Yasuhiro Fukuzaki, Kenji Furukawa, 2011a, High-rate nitrogen removal by the Anammox process at ambient temperature, Bioresource Technology 102, 672–676 – **[29]**.

Yuan L.M., Chuan-Yi Zhang, Yan-Qiu Zhang, Yi Ding, Dan-Li Xi, 2008, Biological nutrient removal using an alternating of anoxic and anaerobic membrane bioreactor (AAAM) process, Desalination 221, 566–575.

Yuan X., Gao D., 2010, Effect of dissolved oxygen on nitrogen removal and process control in aerobic granular sludge reactor, Journal of Hazardous Materials 178, 1041–1045.

Zafarzadeh A., B. Bina, M. Nikaeen, H. Movahedian Attar, M. Hajian nejad, 2010, Performance of Moving bed Biofilm reactors for biological nitrogen compounds removal from waste water by partial nitrification-denitrification process, Iran. J. Environ. Health Science Engineering, vol. 7, No. 4, p. 353-364 – **[68]**.

Note: red numbers in square brackets are the references cited in Tables 8, 10, 11, 12 and 14.