

WEF, IWA, EPA, and the Chesapeake WEA
Nutrient Removal Specialty Conference
Baltimore, MD, USA
4th – 7th, March, 2007
Session 6 Poster

Microbiology and Biochemistry of the Nitrogen Cycle
Process Applications: SHARON[®], ANAMMOX, and, InNitri[®] ©

Alphonse Warakomski
Mixing and Mass Transfer Technologies, INC
Lotepro Environmental Systems and Services
8833 North Congress, suite 818
Kansas City, MO, USA, 64153

Rogier van Kempen
Grontmij Water and Wastewater Management
(PO BOX 14) 3730AA DeBilt
The Netherlands

Peter Kos PhD
11 Karen Drive
Sunderland, MA, USA 01375

ABSTRACT: SHARON^R and InNitri^R are very cost-effective treatment processes for the total removal of nitrogen components, thru nitrification/denitrification, from wastewater flow streams containing high concentrations of nitrogen. The processes are used in the treatment of municipal wastewater side streams with high ammonia concentrations to achieve high total overall nitrogen removal.

SHARON^R is a high rate process for the removal of total nitrogen operating with minimal sludge retention time. Due to differences in growth rates of the bacterial species at the process design temperature (30-40 °C) a selection can be made wherein the nitrite oxidizing bacteria can be washed out of the system while ammonia oxidizing bacteria are retained along with denitrifying bacteria. Using this metabolic mode of operation allows for a 25% reduction in aeration energy required for ammonia removal, a 40% reduction in the amount of BOD addition needed for denitrification, and a 33% percent reduction in sludge production. In addition since the process is accomplished in a side stream there are savings in mainstream reactor costs.

InNitri^R is a high rate process for the conversion of ammonia to nitrate operating with a short sludge retention time allowable due to the temperature of the recycle stream. It converts the ammonia load in the side stream to nitrifying bacteria and nitrates. The nitrifying bacteria produced are used to seed the main stream reactors. The combined effect of this conversion and seeding of the main stream reactor is to reduce the reactor costs necessary for nitrification by 50%.

The pertinent biochemical principles of both nitrification and denitrification that apply to both processes are developed and illustrated. The process design procedures for implementing both SHARON and InNitri will be demonstrated with typical designs for wastewater plants with high strength ammonia side streams.

The process design parameters for the four SHARON plants in the Netherlands (Utrecht, Rotterdam, Zwolle, and Beverwijk) are listed and compared to actual operating data. The process design parameters for the InNitri^R at Tuscon AZ is listed and compared to the actual demonstration program operating results.

SHARON[®] acronym Stable reactor system for **H**igh activity **A**mmonium **R**emoval **O**ver **N**itrate

InNitri[®] acronym **I**nexpensive **N**itrification

ANAMMOX[®] acronym **A**noxic **A**mmonium **O**xidation

KEYWORDS: nitrification and denitrification metabolic pathways, nitrification and denitrification biochemistry, growth rates of Nitrosomonas and Nitrobacter, seeding

© Water Environment Federation

INTRODUCTION

Three relatively new processes have been developed to remove nitrogen in high concentration from wastewater, especially recycled side streams from biosolids processing- SHARON[®], InNitri[®], and ANAMMOX[®]. They each have unique characteristics. SHARON[®] incorporates a different metabolic pathway than is usually implemented in wastewater treatment, InNitri[®] uses the concept of bioaugmentation in a new way, and ANAMMOX[®] uses an entirely new group of bacteria to oxidize ammonia anaerobically; all to remove nitrogen more cost effectively. What we will find is that the processes, while unique, do fit into our understanding of the bacteria and biochemistry that propel the nitrogen cycle. Further we will see that, while unique, the processes use the same principles of biological design that we have known for years.

NITROGEN CYCLE

The nitrogen cycle is the continuous series of natural and man made processes which passes nitrogen thru its various oxidation-reduction states from nitrogen gas thru ammonia, carbon bound nitrogen, nitrogen oxides and back to nitrogen gas.

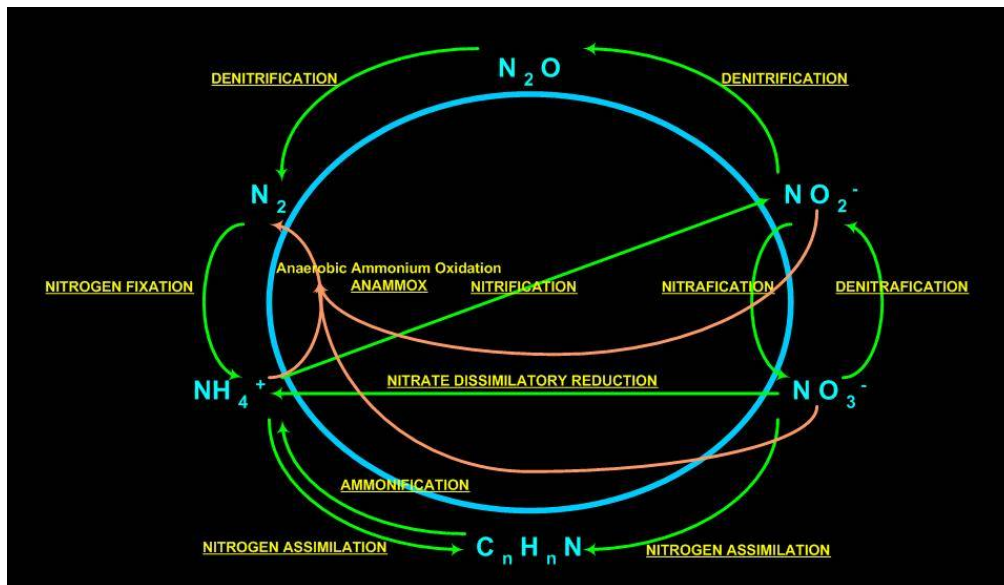


Figure 1 The Nitrogen Cycle

Many of the natural steps are biologically mediated, with plants, fungi, or bacteria conducting the chemical reactions. The bacteria are of special interest in that they can be used to remove nitrogen pollutants from waste water. The process of growing biomass in waste water treatment is based on ammonium and nitrate assimilation from the wastewater to make the carbon bound nitrogen compounds in the protoplasm of the biosolids. Then when we breakdown those biosolids in their disposal we release back nitrogen in the form of ammonium by ammonification to the waste water. To remove ammonium from the waste water other bacteria can be used as oxidizers to provide nitrification and nitrification and as reducers to provide denitrification and denitrification; returning, if necessary nitrogen back to the atmosphere. Recently a new group of bacteria have been found that increased our knowledge of the nitrogen cycle. They are the so called anammox bacteria which can accomplish an anaerobic ammonium oxidation and release as a product nitrogen gas back into the atmosphere. While

originally predicted by Broda ⁽¹⁾, then “discovered” by Mulder ^{(2) (3) (4)}, these bacteria are finally being uncovered in oceanic anaerobic zones such as the Baltic North Sea ⁽⁵⁾, the Black Sea ⁽⁶⁾, and the Thames Estuary ⁽⁷⁾. It is becoming known that these bacteria play a significant role in recycling large quantities of nitrogen from the sea beds of the world into the atmosphere.

CHEMICAL THERMODYNAMICS

The driving force of life is energy. The chemical compounds in wastewater contain the energy that bacteria use to synthesize the complex chemical compounds that is their own cellular protoplasm, which provides the force to transport nutrients into and within the cell that allows them to move and to reproduce themselves.

All chemical reactions can be described thermodynamically by the following equation:

$$\Delta H = \Delta G + T\Delta S$$

wherein: ΔH is the amount of change in enthalpy
 i.e. the amount of total energy content of the chemical system
 ΔG is the amount of change in free energy
 i.e. the change during a process in the energy available to do work
 ΔS is the amount of energy lost thru entropy
 i.e. the change during a process in the energy unavailable to do work do to the creation of disorder
 T is the absolute Temperature

Or simply stated the total energy change of a chemical system is equal to the sum of the changes in useful energy and energy that is no longer available

rearranged:

$$\Delta G = \Delta H - T\Delta S$$

This simply stated allows one to predict if a chemical process will proceed and the extent to which it will proceed. All spontaneous reactions must have a -, negative ΔG , that is the process must proceed to a state of lower free energy.

Exergonic processes are those that have a -, negative ΔG and proceed spontaneously; the magnitude of ΔG indicates the maximum amount of energy available to proceed.

Endergonic processes are those that have a +, positive ΔG and can not proceed spontaneously; the process can only proceed by coupling them to an energy releasing process.

A look at the free energies, ΔG s, of the nitrogen compounds in waste water shows that:

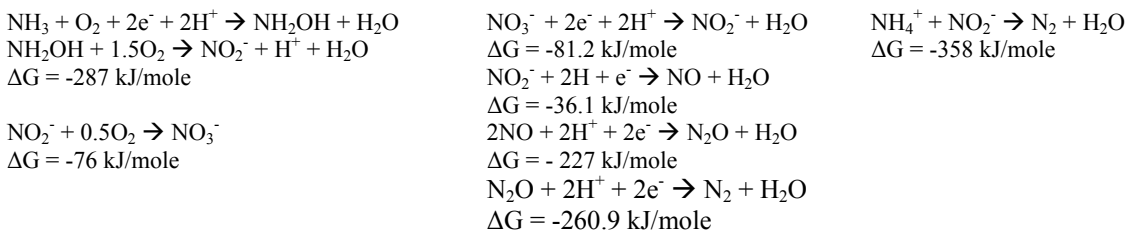


Figure 2 Gibb’s Free Energy for Nitrogen Cycle Reactions

The energy available for the oxidation of ammonia to nitrite is favorable although not great.

The energy for the oxidation of nitrite is available and slight.

The energy for the reduction of nitrate is available but slight.

The energy for the overall reduction of nitrite is favorable although not great, even with a first step ($\text{NO}_2^- \rightarrow \text{NO}$) that where energy is available but slight.

The energy for the anaerobic oxidation of ammonium is favorable.

Thus while not providing large amounts of energy to the bacteria both the oxidation sequence of ammonia to nitrite and nitrate and the reduction of nitrate/nitrite to nitrogen can occur spontaneously; provided there is a means of transporting the electrons and protons involved in the reactions. The cellular material in the membranes of the bacteria do provide for this transport.

ENZYMES

Enzymes are very complex proteins; they function as biochemical catalysts. The thermodynamics of the nitrogen reactions of interest in waste water treatment have been shown to be spontaneous. However they would occur very slowly if left to themselves within the bacterial cell because a certain amount of activation energy is necessary to bring the reacting molecules together in the proper orientation to form a transition state leading to the product molecules of the reaction. Enzymes accelerate the biochemical reactions within the cell. Enzymes accomplish this by lowering the activation energy of a chemical reaction. As stated all chemical reactions, even spontaneous reactions, need a boost of energy to occur; it is the energy needed to bring the molecules that are going to react together in a manner that causes the reaction to occur. Enzymes increase the rate of reaction by lowering the amount of activation energy necessary to bring the reacting molecules together in the proper orientation. They do this by binding chemically with the substrate molecules at an active site, when bound like this the substrate molecules are in close proximity to each other and oriented in such a manner that a transition state complex that leads to the final products readily occurs. The efficiency of enzymes is astounding; catalysis of as many as 10^2 to 10^6 molecules per minute can occur.

Of particular note in regard to enzymes is the fact that they have metal atoms incorporated in to their structures called metal cofactors at their physical centers. The metals in the enzymes used in nitrogen processing are iron, molybdenum, and copper. It seems that the iron enzymes are more common than molybdenum and copper; that is fortunate because iron is usually prevalent in waste water although there are cases of plants with iron shortages. Methods are just being developed in this area to determine if that is iron is indeed prevalent. However nitrification, nitratification, denitrification, and denitratification could falter in waste waters lacking sufficient metals for proper enzyme development ⁽⁸⁾. This importance of iron electron transport in the metabolic pathway is also illustrated. The cytochromes used to transport electrons in the metabolic pathway are iron based.

(HAO). One of the oxygen atoms in nitrite derives from oxygen, the other comes from water. Two of the four electrons generated by HAO are transferred via the tetra-heme cytochrome c_{554} either to AMO or diverted into an electron transport chain, where many of the carriers involved and the intermediates have not been completely characterized. The overall reaction converts ADP to ATP an energy rich compound.

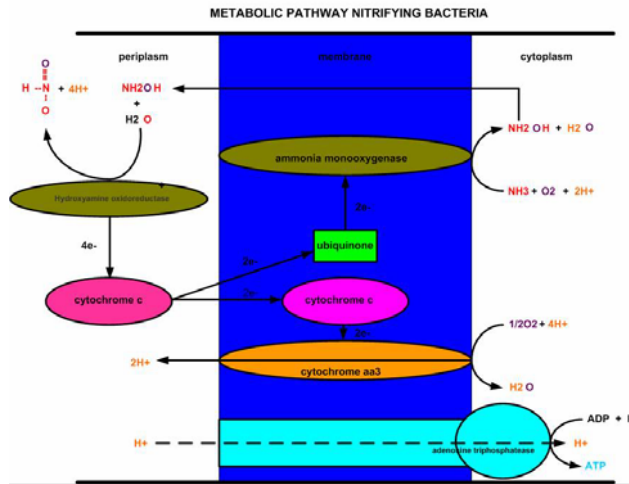


Figure 6 Metabolic Pathway Nitrifying Bacteria

METABOLIC PATHWAY OXIC NITRITE OXIDATION

A Nitrobacter bacterium is illustrated (Figure 4); note the folds in the membrane where the enzymes that catalyze the reaction are located. Nitrite is catalytically oxidized to nitrate by the enzyme nitrate oxidase. Again the over all reaction converts ADP to ATP.

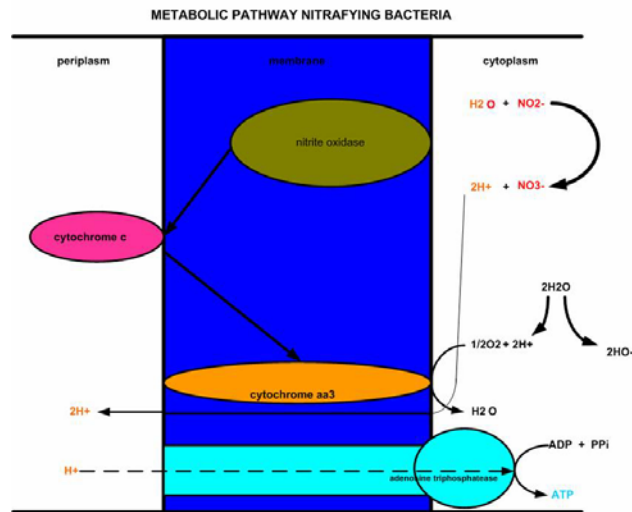


Figure 7 Metabolic Pathway Nitrifying Bacteria

METABOLIC PATHWAY ANOXIC NITRITE/NITRATE REDUCTION

Typical bacteria that accomplish denitrification and denitrification are illustrated (Figure 5); again note the folds in the membrane where the catalytic enzymes are located. To say denitrification and denitrification is complex is almost an understatement. Let us start with an overview of a complete metabolic pathway (Figure 8); the upper diagram illustrates the reduction of either nitrite or nitrate in the cell by the enzymes and the lower

diagram is for a gram negative bacterium, the model is based on research of the species *pseudomonas stutzeri*.

Nitrate moves thru the cell membrane's anti porter to the cytoplasm and is reduced catalytically at a nitrate reductase (NAR) site in the plasma membrane to nitrite. The nitrite then flows back thru the plasma membrane into the periplasm where it is reduced catalytically by nitrite reductase in the periplasm to nitric oxide. The nitric oxide is then catalytically reduced at a nitric oxide reductase site in the membrane to nitrous oxide and finally a nitrous oxide reductase in the periplasm reduces the nitrous oxide to nitrogen, which leaves the cell thru its outer membrane and enters the mixed liquor.

The lower diagram goes to a deeper mechanistic level and shows the flow of electrons and protons involved in the reaction. The energy for this flow is provided by the free energy, ΔG , released in each reaction step. This energy flow carried by the electrons and protons is used by the cell in other reactions to synthesize molecules and conduct other cellular functions.

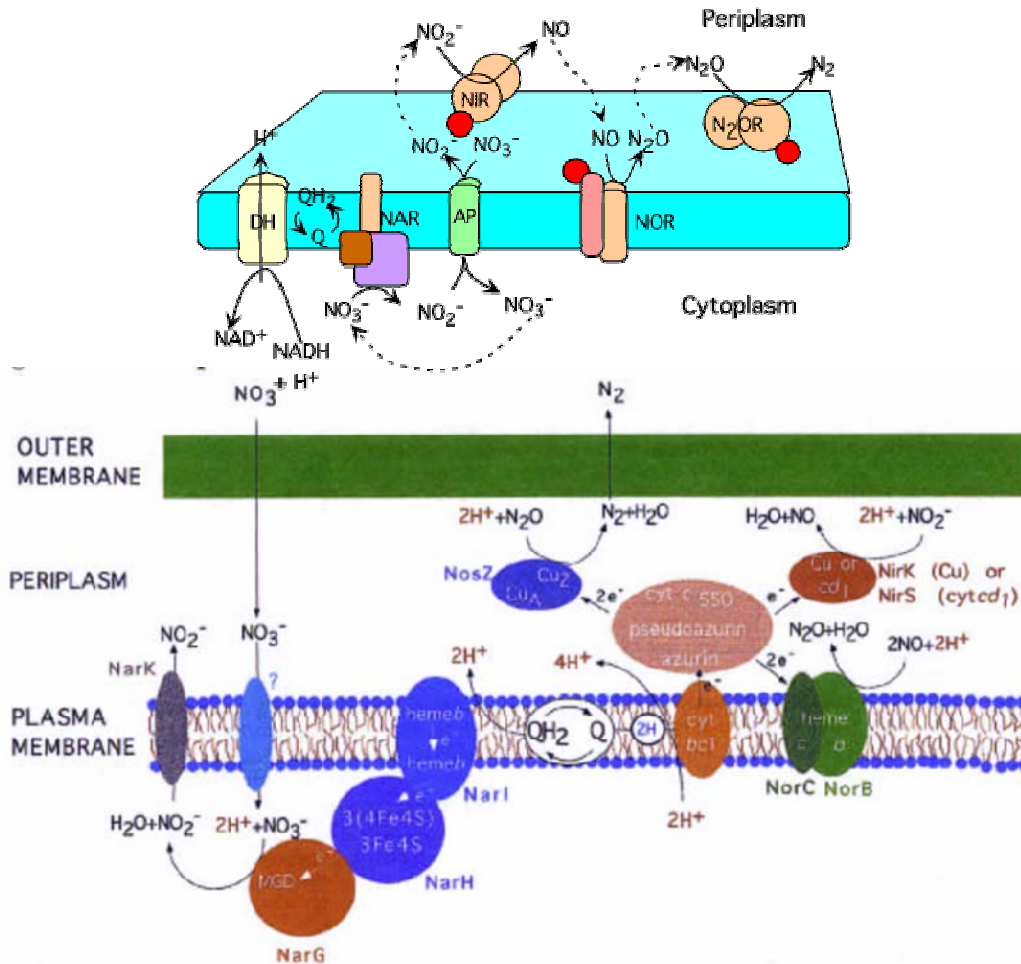


Figure 8 Metabolic Pathway for Denitri- and Denitra- fication

METABOLIC PATHWAY ANAEROBIC AMMONIA OXIDATION

At present, five bacteria have been identified as having an anammox metabolic pathway: *candidatus brocadia anammoxidans*, *candidatus kueneia stuttgartiensis*, *candidatus scalindua wagneri*, *candidatus scalindua brodae*, and *candidatus scalindua sorokinii* ⁽⁹⁾. An anammox bacterium is illustrated (Figure 5), note the unique structures of a dense fibrillar nucleoid, ribosome like particles, and crateriforms on the surface.

A plausible metabolic pathway has been proposed to account for the facts discovered by the research done so far. Ammonia and hydroxylamine, generated from the reduction of nitrite by nitrite reductase, are converted to hydrazine by the catalytic enzyme HZF. Subsequently the hydrazine is oxidized to nitrogen gas by the catalytic enzyme hydroxylamine oxidoreductase, which generates four electrons that can be utilized in the reduction of nitrite ⁽¹⁰⁾.

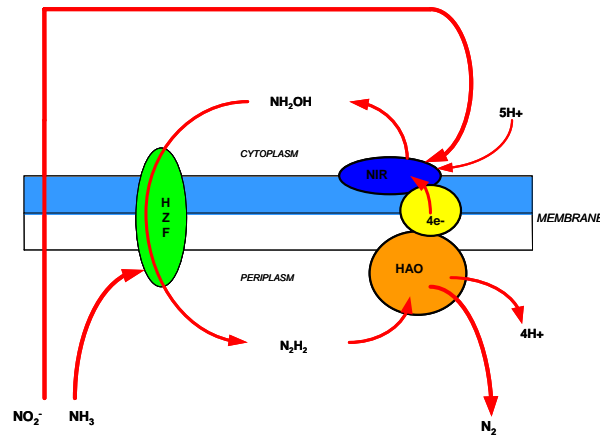


Figure 9 Metabolic Pathway for Anaerobic Oxidation of Ammonia

AMBIENT ENVIRONMENTAL EFFECTS ON BACTERIAL GROWTH

TEMPERATURE All chemical processes are effected by temperature, especially biochemical processes. The effect of temperature on the ammonia nitrogen utilization coefficient of the bacterium nitrosomonas is parabolic in shape. The optimum temperature for this bacterium is 26-28°C. Its growth rate will decline on either side of that optimum. In practical terms this means that for that bacterium to proliferate and establish a representative population at lower or higher temperatures a long sludge retention time is needed, or the nitrosomonas population will be washed out of the reactor.

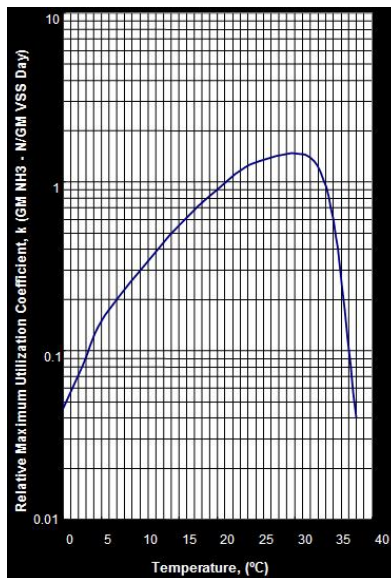
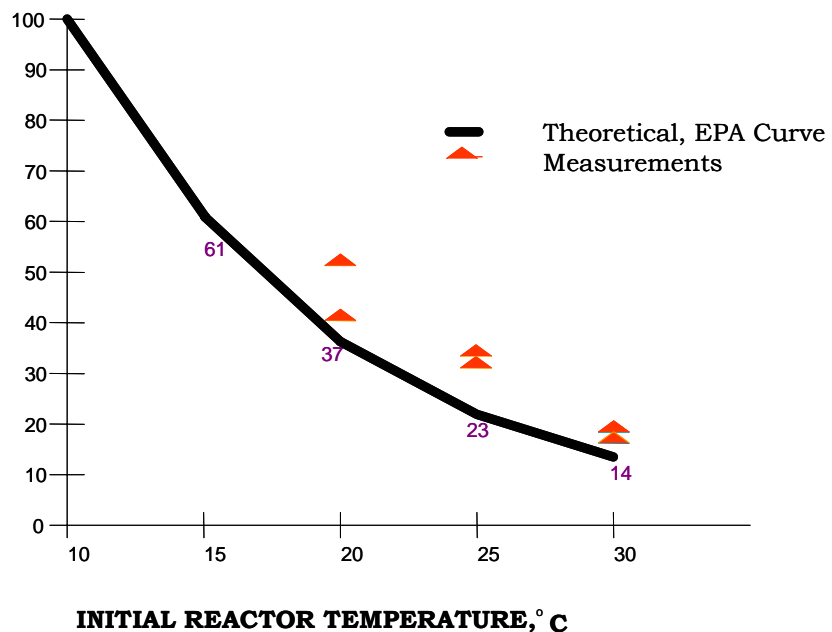


Figure 10 Effect of Temperature on Growth Rate

With InNitri[®], a new process concept, bioaugmentation of nitrifying biomass from one process reactor to another is proposed. That biomass transfer might be accomplished with a temperature differential between the reactors of as much as 20°C. Thus there is concern that the transferred biomass would be “shocked” and not function at the expected nitrification removal rate. This fear has been assuaged with a recent study.

A study ⁽¹¹⁾ was done at the University of Manitoba by Melanie Head PhD to determine the impact of a sudden temperature decrease on the nitrification rates of a nitrifying biomass grown at warm temperatures. The results indicate that nitrifying biomasses grown at 20^o, 25^o, and 30^o C and put into an environment of 10^o C respond with decreases in nitrification rates that are less than the USA EPA factor of $e^{0.098(T-15)}$. The observed decreases in nitrification rates were 58%, 71%, and 82% for nitrifying biomasses grown at 20^o, 25^o, and 30^o C and cooled to 10^o C. The USA EPA equation predicts nitrification rate reductions of 62.5%, 77%, and 85.9%.

NITRIFICATION RATE AT 10 ° C AS A PERCENTAGE OF THE RATE AT THE INITIAL TEMPERATURE, %



Work done @ University of Manitoba in Canada by Melanie Head²

Figure 11 Effect of Change in Temperature on Bacteria Growth Rate

DISSOLVED OXYGEN

The level of dissolved oxygen (DO) impacts the ability of the nitrifiers and nitrifiers to attain good removal of ammonia as illustrated (Figure 12). The optimum DO is dependent on floc size which in turn depends up on the amount of turbulence in the reactor. The larger the flocs the higher the DO that will be needed to fully penetrated it and provide a minimum of 0.1mg/l DO to the individual bacteria. It can be seen that good performance can be obtained with a DO of 3-4.

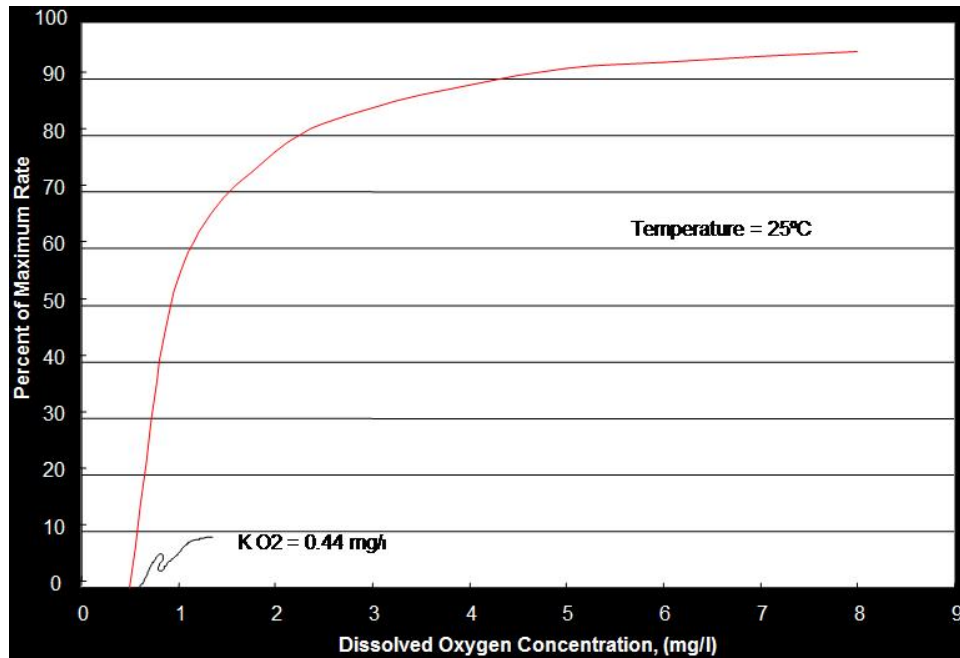


Figure 12 Effect of Dissolved Oxygen on Bacterial Growth Rate

The opposite effect is needed for denitrification, a low DO. This effect is illustrated (Figure 13). The catalytic enzyme nitrogen oxide reductases will operate only at low oxygen levels of DO. Figure 13 shows that with DO drop the bacteria start generating the enzymes to accomplish denitrification. Once a level of low threshold level of DO is occurs the bacteria utilize these enzymes to derive energy from the oxides of nitrogen.

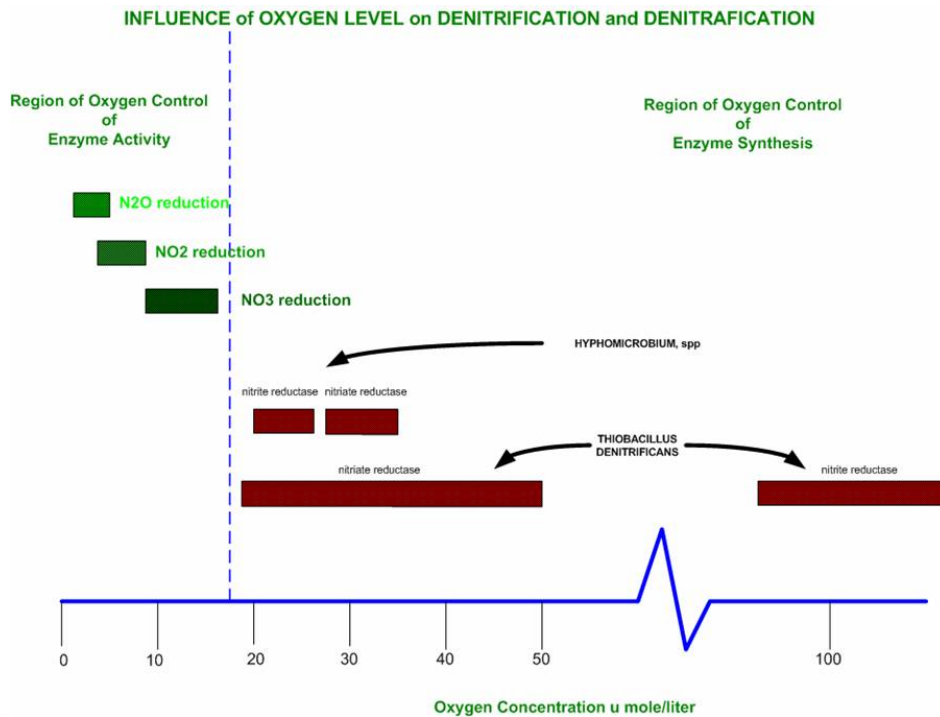


Figure 13 Effect of Dissolved Oxygen on Denitr*i*- and Dentra*a*- fication

pH, ALKLINITY

The growth rate of the nitrifiers and nitrifiers is influenced by the pH of the mixed liquor. Growth occurs at low pH but it is slow, nitrification can be accomplished at low pH, but only at extended sludge retention times. This is attested to by the 20 oxygen activated sludge plants that accomplish complete nitrification at a pH range of 5.6-6 on a continuous basis.

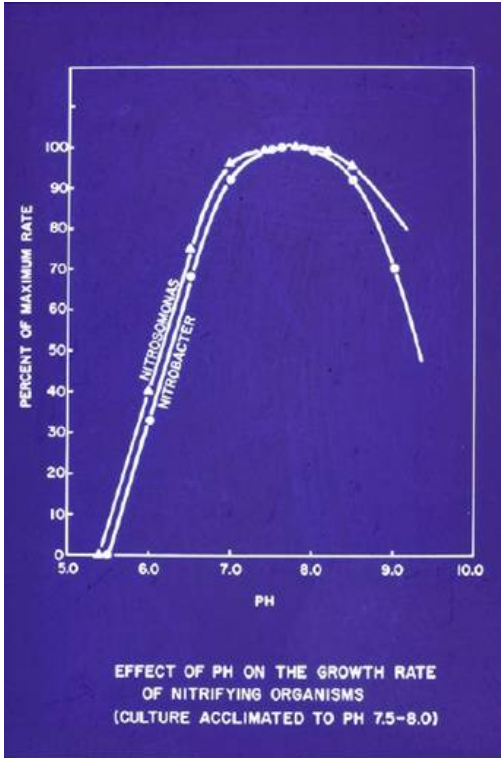


Figure 14 Effect of pH on Bacterial Growth Rate

NUTRIENTS

One of the limiting nutrients in nitrogen removal systems that sometimes is overlooked is carbon, many systems are carbon limited and the addition of carbon source is required. A popular commercial source is methanol; however high methanol prices have changed this and many plants are switching to other carbon sources.

As pointed out the catalytic enzymes that are required to accomplish nitrogen removal are metal based and the waste water must have a minimum amount of Fe, Cu, and Mo for those enzymes to be synthesized.

DIFFERENTIATING PROCESS DESIGN CHARACTERISTICS

BACTERIAL GROWTH RATE DIFFERENCES (SHARON[®])

The key to the development of the SHARON[®] process was the recognition that there was a substantial difference in growth rates between the two genera of

bacteria that perform nitrogen oxidations, the nitroso- and nitro- bacteria, over the range of temperatures used in the design of waste water treatment plants.

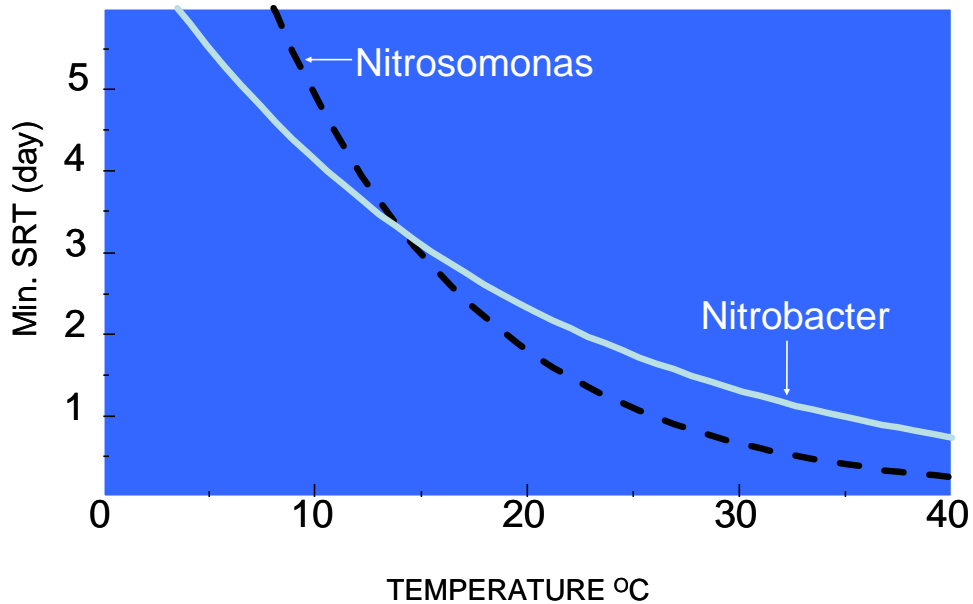


Figure 15 Effect of Temperature on Growth Rate of Nitroso and Nitro Bacteria

At temperatures below about 12°C the nitro- bacteria grow at a faster rate than nitroso- bacteria. At temperatures above about 12°C the nitroso- bacteria grow at a faster rate than nitro- bacteria. So at the temperatures that can readily be attained in a SHARON[®] reactor (25-40°C) if the sludge retention time (SRT) and hydraulic detention time (HRT) are set properly (0.5- 1.5 days) a situation results wherein nitroso- bacteria are preferentially grown; of course nitro- bacteria are present but are washed out of the reactor more rapidly than they can proliferate. This allows a different metabolic pathway to be used to convert ammonia to nitrogen gas.

BIOAUGMENTATION (InNitri[®])

Bioaugmentation of biological waste water treatment reactors has been known for a considerable period of time. Its most common form is the addition of bacteria or their enzymes to dysfunctional biological treatment reactors to improve their performance. In addition there was work done on moving a nitrifying biomass from one activated sludge reactor to another to observe if that would reduce the apparent sludge age required for nitrification⁽²²⁾; the results were positive in that the biomass in the reactor which was bioaugmented nitrified at a lower apparent sludge age than would be predicted. This method of bioaugmentation was never reduced to commercial practice; probably because main stream reactors do not have enough exportable nitrifying biomass to sustain that type of operation during cold weather due to the slow growth rates of both nitroso- and nitro- bacteria at low temperatures. Peter Kos recognized a special situation in regard to recycled side streams, especially those generated during biosolids processing; there the temperature of the waste water is warm and ammonia concentrations are high. Under these conditions a biological reactor can be designed and built to

produce an exportable nitrifying biomass that can be used to lower the apparent sludge retention time necessary for nitrification in the main stream reactors. The main stream reactors necessary for nitrification can be reduced by 40-50% using this process ⁽¹⁵⁾.

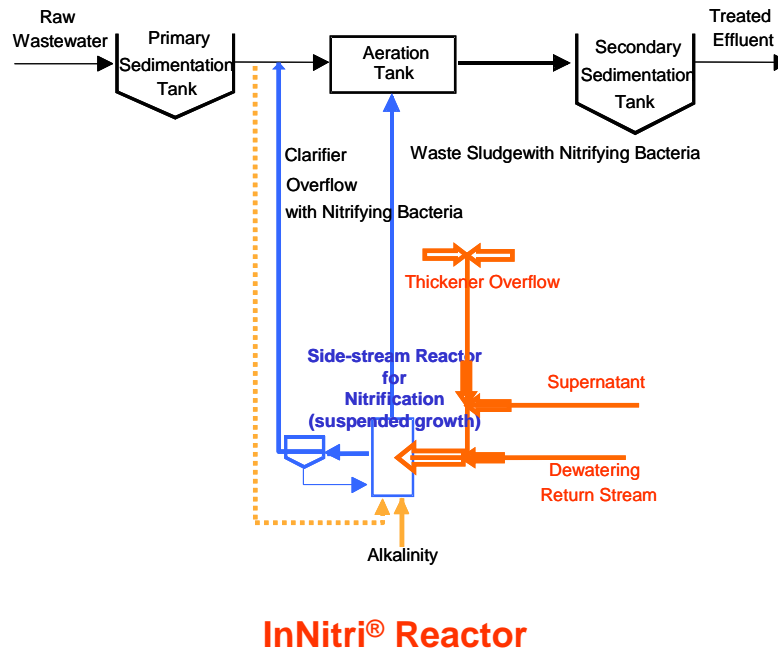


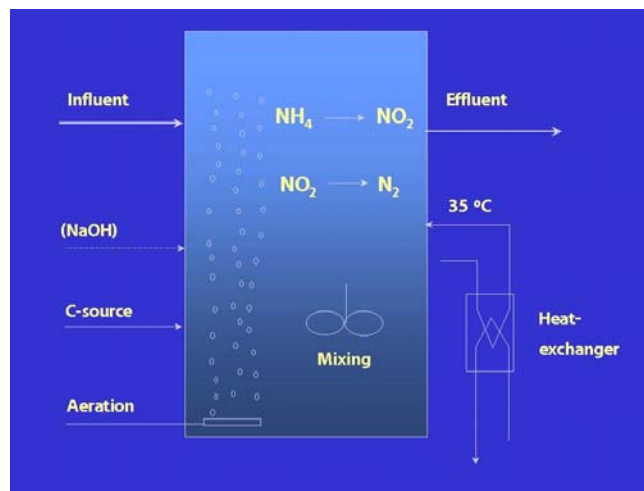
Figure 16 Bioaugmentation of the Main Stream Reactor with InNitri^R

NOVEL BACTERIAL SPECIES (ANAMMOX[®])

The recent research on anammox bacteria shows us that the window is not closed on new processes for wastewater treatment. There are now several commercial anammox reactors in operation; now to be proved is that the savings due to reduced aeration provided are greater than the cost of installation and operation of the reactor.

THE SHARON[®] PROCESS ⁽¹²⁾

The SHARON[®] process:



**Figure 17 The SHARON process:
The oxic (aeration) / anoxic (mixing) steps can be accomplished in time or space**

The SHARON process is a new and innovative process for total nitrogen removal which allows for nitrification/denitrification at minimal SRT values, resulting in a substantially smaller reactor volume than is currently required for conventional total nitrogen removal. In addition the process allows for both a saving of twenty five (25%) percent in oxygen transfer energy and forty (40%) in carbon feed for denitrifying bacterial growth as compared to conventional processes because of the unique metabolic pathway used.

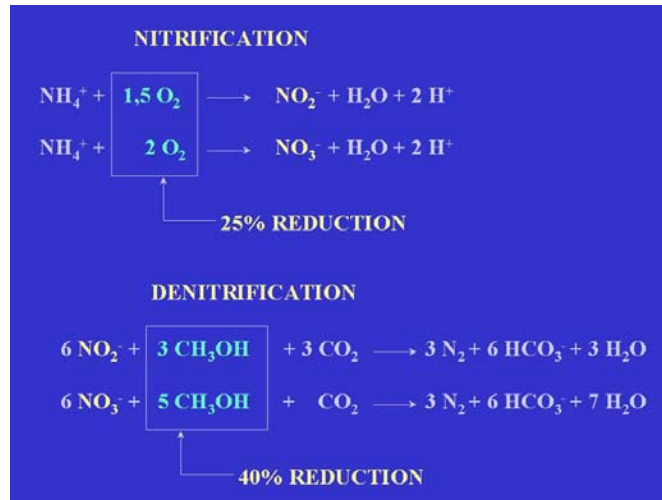


Figure 18 The SHARON Stoichiometry

There are associated capital cost savings. The pathway is conversion of ammonia to nitrite and conversion of nitrite to nitrogen, the chemical species nitrate is not produced or converted. The core concept on which the process is based is that at temperatures above 15°C, and especially between 30-40°C, the growth rates of the nitrifying bacteria are greater than the nitrifying bacteria. This allows for the design of a selector reactor where in nitroso-genera bacteria (nitrifying) predominate over the nitro-genera bacteria (nitrifying). The nitrite produced is converted to nitrogen gas by denitrifying bacteria under anoxic conditions. This two step reaction can occur in either space or time. That is either a reactor with a single stage and sequencing oxic-anoxic time periods or a reactor with two stages one oxic one anoxic can be used.

SHARON®'s CONTINUING OPERATION @ UTRECHT (13)



Figure 19 The UTRECHT SHARON, oxic/anoxic stages in space

A review of a full year's operating data for 1998 shows very stable operation. The influent ammonia varied from 400 to 750 mg/l during the period with a highly variable flow ranging from 0 to 1,375 m³/day, which is 0 to 363,275 gallons/day.

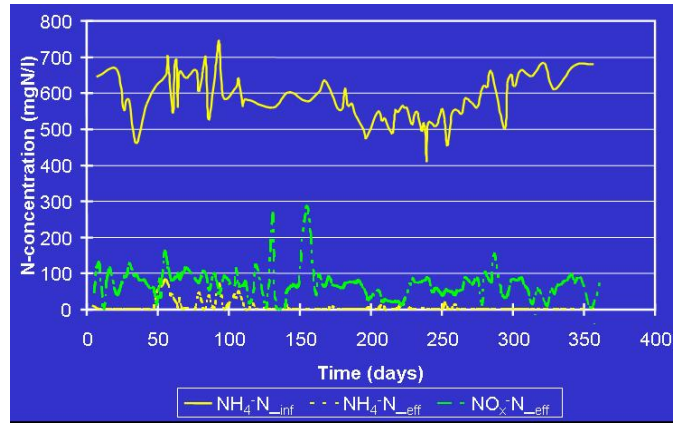


Figure 20 Nitrogen Load and Effluent Produced

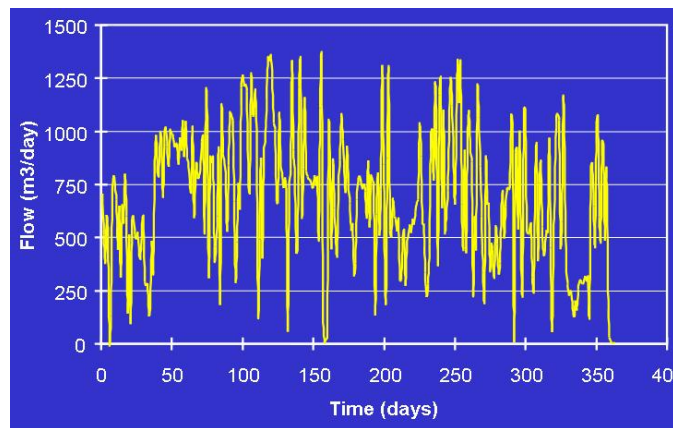


Figure 21 Flows Experienced

During this period the effluent ammonia averaged less than 5 mg/l, albeit with some peaks in the period from 0 to 400 days. The peaks were caused by insufficient pH control. The NO_x-N concentration in the effluent was generally under 100mg/l. Flows vary as a result of the variability of output from biosolids processing. The process was not upset by high influent suspended solids which at times reached concentrations of 30-50,000 mg/l and were often above 10,000 mg/l.

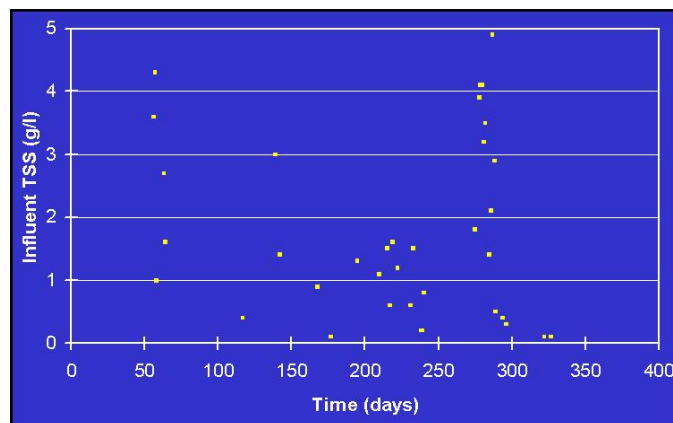


Figure 22 High Solids Levels Experienced

The control of pH was done thru recovered alkalinity from denitrification/denitratification caused by methanol addition in anoxic conditions. Occasionally supplemental sodium hydroxide, a backup ph control, is used in the case where insufficient methanol was dosed.

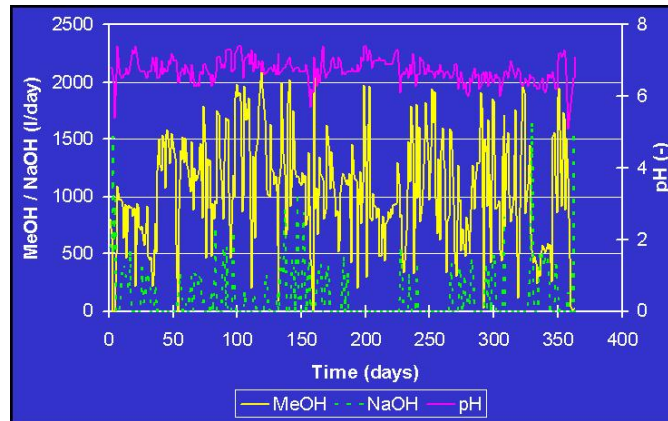


Figure 23 pH Control with Methanol and Sodium Hydroxide

The influent temperature varied over the year between 20 and 30^o C. The reactor temperature was raised to 38^o C by means of biochemical heat production, there is no need to provide supplemental heat. The installed supplemental heat exchange unit operation has been discontinued as the required reactor temperatures are maintained thru heat released by the reactions and the insulation of the reactors.

It was the choice of the operations group to maximize ammonia removal so there was partial denitratification via nitrate. The ammonia removal efficiency aimed for can only be achieved with an oxic retention time of more than 2 days and therefore the conversion is partially to nitrate and partially to nitrite. Thus there is only partial nitrogen removal via nitrite in this SHARON[®]. This is confirmed by the average ratio of COD/ N-denitrified which had a value of 3.3 g COD/ g N. In theory the minimal stoichiometric demand would amount to 2.86 g COD/g NO₃-N denitrified or 1.72 g COD/g NO₂-N denitrified. Considering biomass yield, the demand is expected to be between 3.5 and 2.2 g COD/g N removed.

Since installation of the SHARON[®] process the main stream plant has lowered the total nitrogen content in the effluent by 30%. During the first half of 1998 the total nitrogen content in the plant effluent dropped on average from 16 to 11 mg/l.

SHARON[®]'s CONTINUING OPERATION @ ROTTERDAM ⁽¹⁴⁾

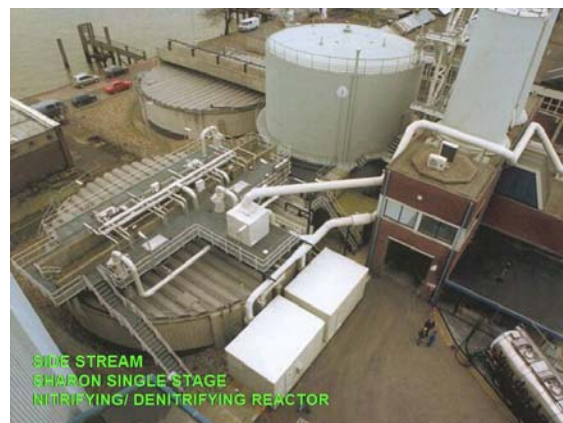


Figure 24 The Rotterdam SHARON, oxic/anoxic stages in space

The results for 2002 show continued stable efficient operation. The large fluctuations in feed nitrogen continued ranging from 200-750 kg/day, which is 441-1653 lbs/day in a random pattern. During the second half of 2002 denitrification efficiency was raised from 60-80% to approximately 90% by increasing the methanol dosage.

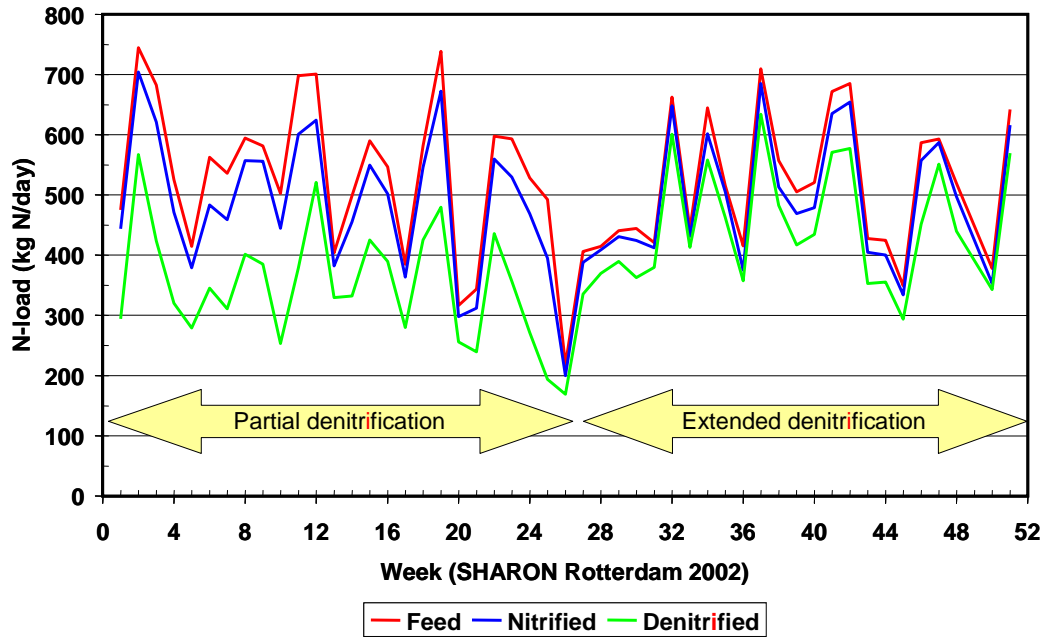


Figure 24 Effect of Methanol on Denitrification Efficiency

During that period ammonia removal was on average above 95%. The influent ammonia nitrogen varied from 700-1200 mg/l and was above 1000 mg/l the majority of the time. Effluent concentrations stayed in the range targeted by the operations staff. Depending on the process settings, effluent ammonia nitrogen concentrations ranged from 10-90 mg/l and averaged below 50 mg/l.

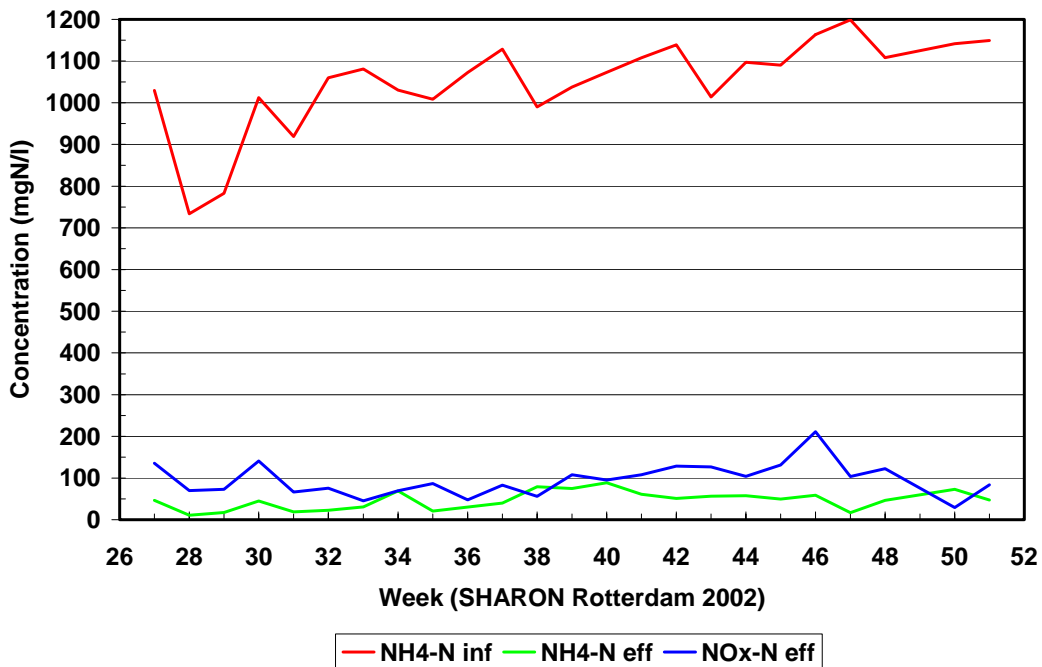


Figure 25 Load and Effluent of the Rotterdam, Dorkhaven Plant

The Rotterdam SHARON[®] system is controlled to remove nitrogen via nitrite which requires an oxic hydraulic retention below 2 days. At an oxic hydraulic retention time of approximately 1.5 days the COD/N ratio as methanol consumed illustrates clearly the metabolic pathway from ammonia via nitrite to nitrogen.

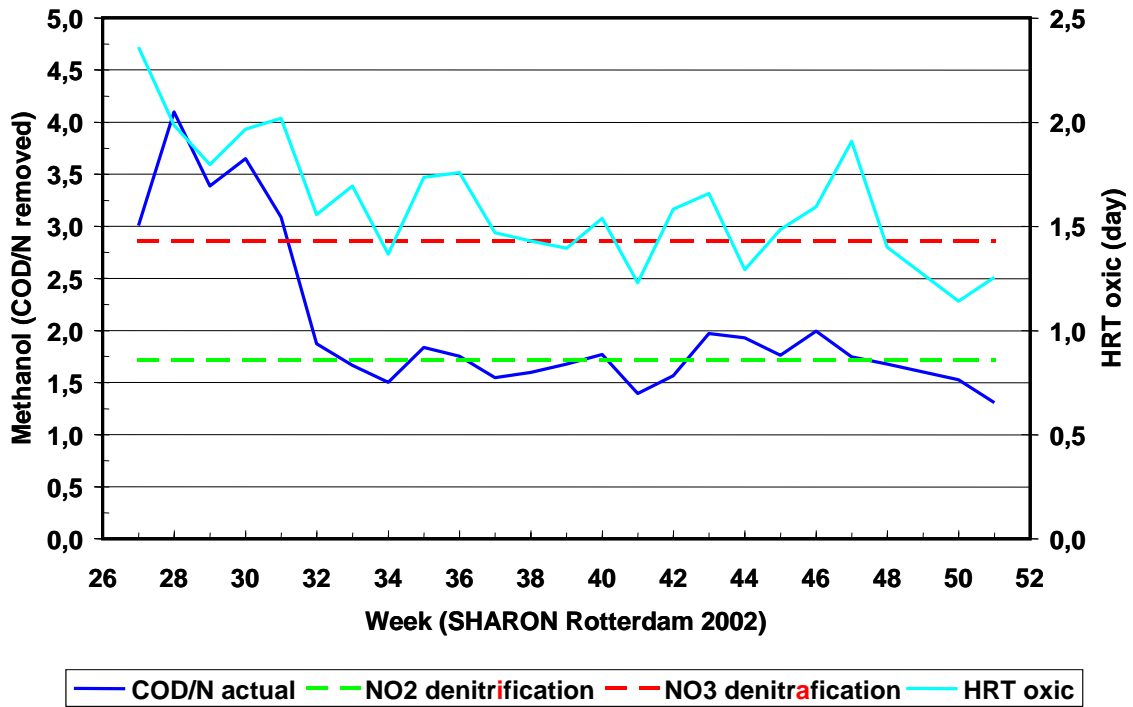


Figure 26 Stoichiometric Proof of the Nitrite Pathway

From 1999 thru 2000 the SHARON[®] process had a very beneficial effect on the main stream effluent lowering the effluent ammonia from an average 6.2 mg/l to 2.1 mg/l and total nitrogen from an average 7.5 mg/l to 3.9 mg/l.

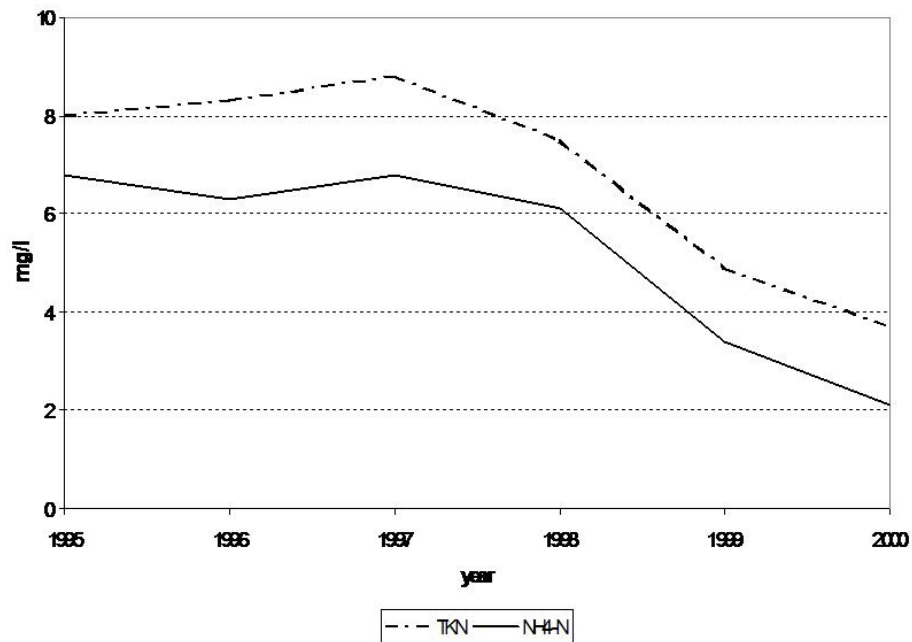


Figure 27 Improvements to the Overall Average Nitrogen Removal

OTHER SHARON[®] OPERATIONS

Sharon has been successfully implemented at Zwolle, Beverwijk, and Groningen in the Netherlands. All facilities are getting 90-95% nitrogen removal. There are plants designed with specifications and plans done and awaiting bid tender at Haag Houtrust in the Netherlands and New York, NY, Wards Island in America.



Figure 28 SHARON[™] Installations, Netherlands and United States as of March 2006

THE ANAMMOX[®] PROCESS (28)

The ANAMMOX process involves taking the effluent from a SHARON[®] reactor which is designed to produce a 50%/50% molar effluent of nitrite and ammonia and treating it with anammox bacteria to bring about an anaerobic oxidation of ammonia to nitrogen gas. The cost savings are the reduced costs for aeration by going with this metabolic pathway. To be cost effective the value of the ANAMMOX[®] reactor plus the savings for reduced aeration has to be less than the value of the reduced size SHARON[®] reactor plus the marginal cost of aeration to achieve a full oxidation of ammonia.

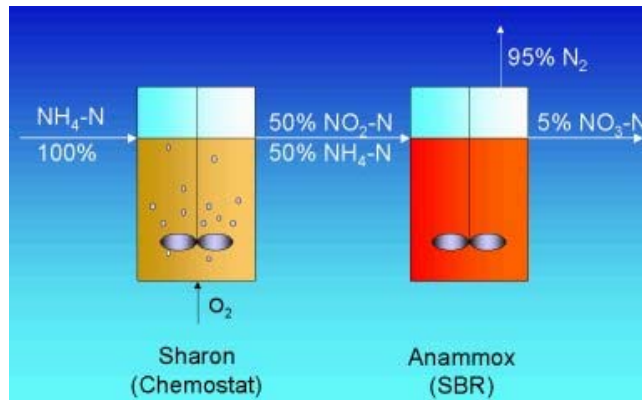


Figure 29 ANAMMOX Process Flow Sheet

An ANAMMOX[®] reactor was installed at Rotterdam to process the full flow from the SHARON[®] reactor, the plant has had a difficult startup never achieved more than 30% of its design capacity for several years. The problems were over come in early 2006 and it is now processing 100% of its design flow. Further testing is on going.



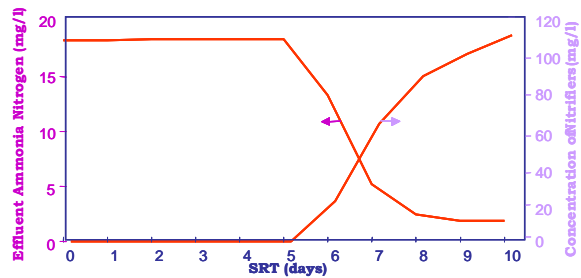
Figure 30 ANAMMOX Reactor at Rotterdam's Dorkhaven Plant

THE InNitri[®] PROCESS ⁽¹⁵⁾

The InNitri[®] process is a new and innovative process for nitrification which converts the recycled side stream ammonia into nitrate and generates excess nitrifying biomass which can be exported to the main stream reactors. The SRT used in the design of the InNitri reactor for nitrification will be significantly less than the SRT used for nitrification in the main stream reactors. This is because of the difference in temperature of the recycled side stream, warm, as compared to the temperature of the main stream, ambient cool to cold. The continual exportation of nitrifying biomass to the main stream reactor allows one to cut the SRT used for nitrification by 50%.

Buildup of Nitrifiers and the Effect on Effluent @ 10 °C

Legend — Conventional Nitrification Process



Buildup of Nitrifiers and the Effect on Effluent @10° C

Legend — Sidestream Nitrification Process

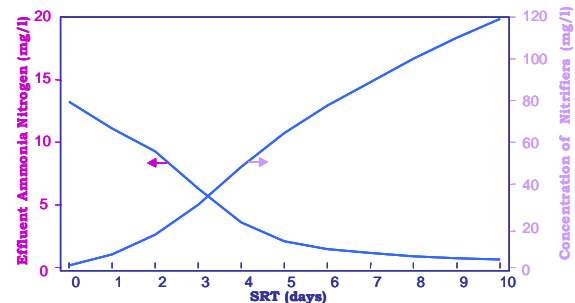


Figure 31 Nitrification at 10⁰ C Without and With Bioaugmentation

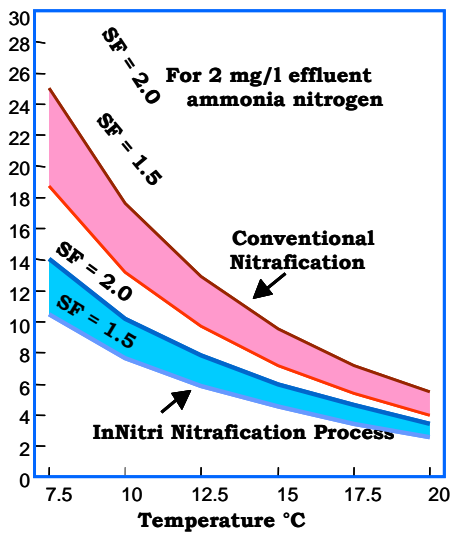


Figure 32 Main Stream SRT Reductions with Bioaugmentation

Therefore InNitri® is well suited for large plants, plants in cold climates, and is especially suited to upgrading UNOX and OASES high purity oxygen activated sludge plants. If denitrification is necessary the overflow stream from the InNitri® reactor's clarifier can be processed in a main stream denitrification reactor. While at present there are no full scale operating InNitri® systems enough work has been accomplished in regard to the concepts required for its successful implementation to believe the process is very viable. Therefore demonstration programs should be run where a cost effective analysis has shown InNitri® to have merit in its application.

There are many plants where nitrification is accomplished on high strength ammonia recycled side streams. The only requirement for successful operation at a facility is that sufficient SRT, provision for alkalinity addition, and pH control be provided.

The other concern about the seeding, bioaugmentation concept is will the transferred nitrifying biomass survive in the new environment. Again there are indications from other similar and same type studies that, that concern can be mollified. The effects have been studied both theoretically and empirically by many: Finnson 1994⁽¹⁶⁾, Lee 1997⁽¹⁷⁾, Li and Hultman 1997⁽¹⁸⁾, Rittman^{(19) (20)}, and Tendaj-Xavier⁽²¹⁾. But more importantly the concept has been studied empirically. A full scale demonstration⁽²²⁾ at Hillsboro OR's Unified Sewage Agency showed that seeding, bioaugmentation using a nitrifying waste activated sludge reduced the apparent sludge retention time need to achieve nitrification in a UNOX^R system. The study showed that at a seeding, bioaugmentation rate of 35% nitrification could be achieved at a sludge retention time of 4 days whereas normally a sludge retention time of 7 days was needed for nitrification. Pilot plant studies⁽²³⁾ were run by Plaza, Trela, and Hultman to simulate seeding, bioaugmenting from a reactor fed high concentration ammonia supernatant into a reactor that represented a main stream reactor cooled to an average temperature of 14.6^o C. The simulated main stream reactor achieved ammonia reductions at sludge ages well below the calculated sludge retention time necessary achieve nitrification. In addition other researchers have done experimental work that illustrates the same effect: Parker and Richards⁽²⁴⁾, Rittman and Whiteman⁽²⁵⁾, Sinkjaer⁽²⁶⁾, and Daigger⁽²⁷⁾.

TUCSON, ARIZONA DEMONSTRATION PLANT

Tucson, Arizona and Mixing and Mass Transfer Technologies LLC conducted a demonstration program at its Ina Road plant that started in the fall of 2003 to simulate the effect of an InNitri[®] system on its UNOX[®] carbonaceous removal system. The results of the pilot study have been presented at WEFTEC 2004⁽²⁹⁾.

The demonstration plant:



Figure 33 InNitri^R Demonstration Plant at Tucson, AZ, Ina Road Plant

The summary of operations:

CENTRATE TREATMENT SYSTEM SUMMARY PERFORMANCE

Operating Conditions	Phase 1	Phase 2
Duration, days	39	25
Average SRT, days	3.78	4.75
Average Feed NH ₃ -N, mg/l	767	940
Average Effluent NH ₃ -N, mg/l	12	4
Average Temperature, °C	27	29
Average Dissolved Oxygen, mg/l	3.1	3.1
Average / Maximum NH ₃ -N loading, lbs/1000ft ³ /day	26 / 38	35 / 42
Average Percent of Seed produced transferred to UNOX [™] System, %	60%	80%
Equivalent Centrate Flow for 25 MGD UNOX [™] Flow, gpd (Based on observed seed transfer, assuming a 50 mg/l TSS concentration in the final clarifier effluent of the centrate nitrification system.)	200,000	250,000

Table 1 Summary of InNitri^R Operating Conditions at Tucson, AZ, Ina Road Plant

The operating data indicates that centrate nitrification was demonstrated, nitrification was demonstrated in the UNOX[®] system at a shorter apparent SRT than would have occurred with out bioaugmentation, and denitrification was demonstrated. Bioaugmentation allowed the UNOX[®]-InNitri[®] system to provide significantly more nitrification and denitrification than would be expected at the apparent SRTs. The amount of nitrogen removed from the system during the first and second operational periods as compared to the amount of seed:

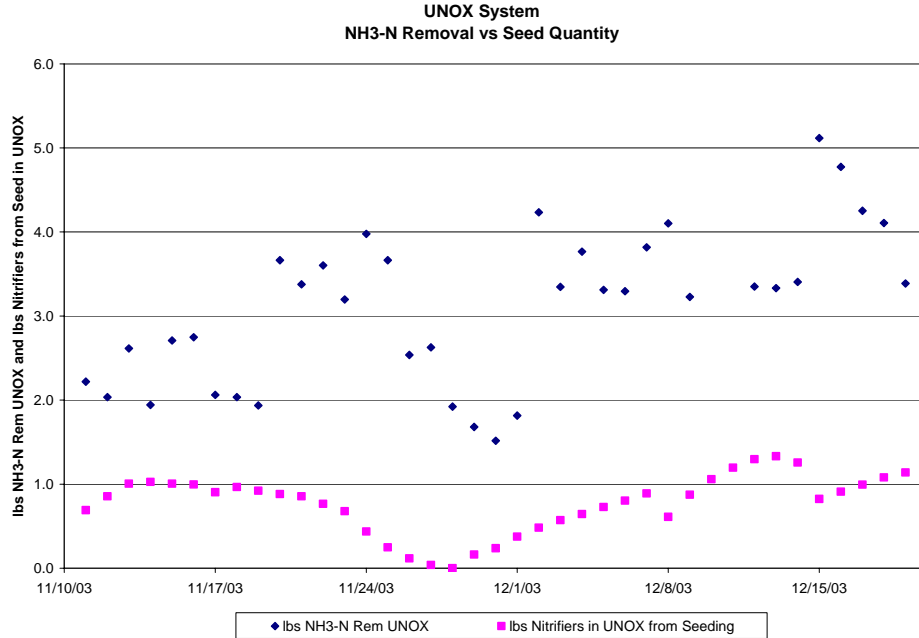


Figure 34 Effect of Amount of Bioaugmentation on Ammonia Removal Phase I

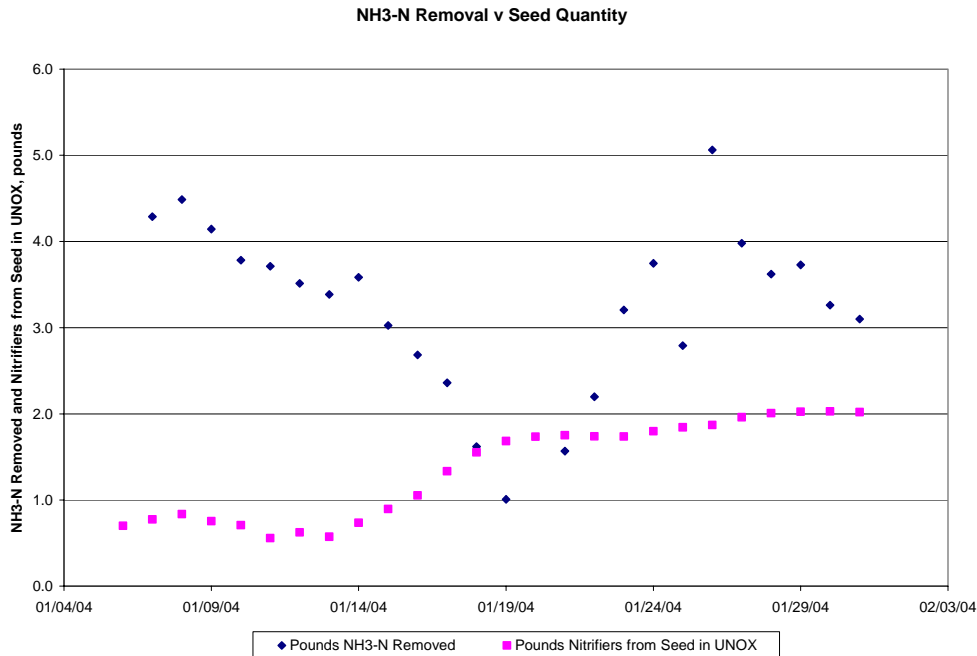


Figure 35 Effect of Amount of Bioaugmentation on Ammonia Removal Phase II

While the amount of nitrogen removed in the first phase.

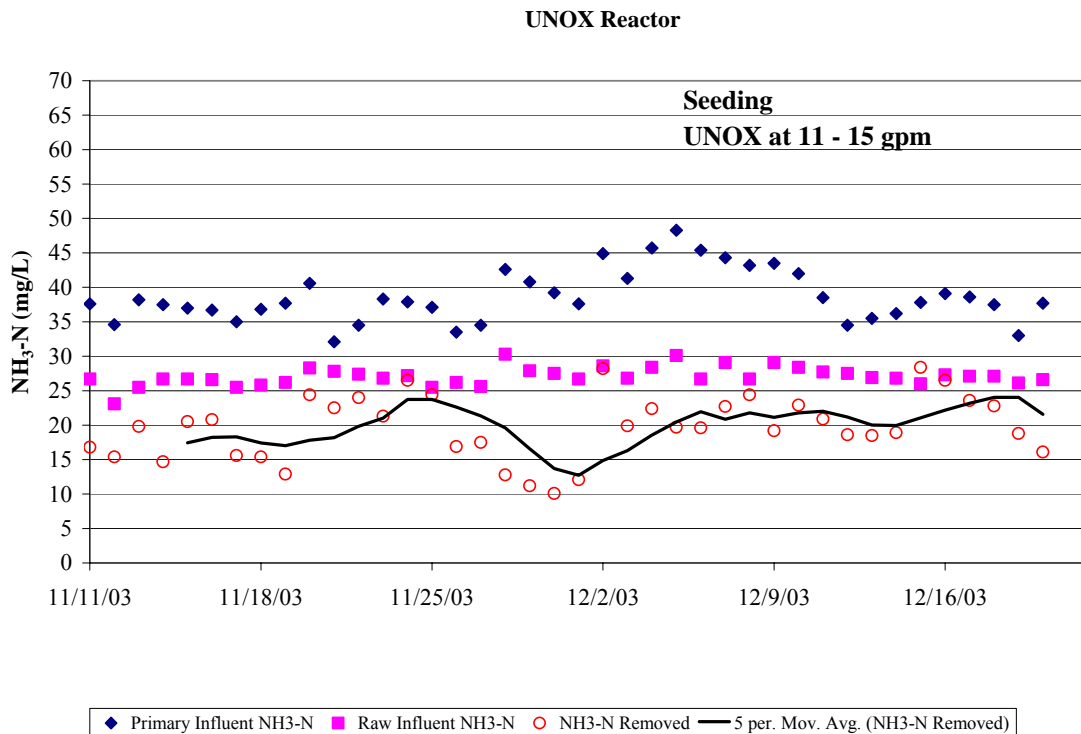


Figure 36 Ammonia Levels 1st Phase of Demonstration

CONCLUSIONS

- Both SHARON[®] and InNitri[®] are cost effective relative to other means of treating recycled side streams with high concentrations of ammonia. SHARON[®] has been fully commercialized with five plants running, InNitri[®] has been demonstrated and work is being done on its commercialization. Anammox[®] is still in the development stage.
- The SHARON[®] process, which preferentially selects nitroso-genera bacteria and removes total nitrogen by means of a hither to unutilized metabolic pathway, has been successfully demonstrated full scale at five facilities for a number of years. The hither to unutilized metabolic pathway is partial oxidation of ammonia to nitrite and then reduction of nitrite to nitrogen; the values for oxygen and methanol consumption using the hither to unused metabolic pathway (ammonia to nitrite to nitrogen) have been proven.
- Research indicates that the transport of nitrifying biomass from a warm side stream reactor to a cold main stream reactor behaves as would be expected in regard to nitrification rates; there is no “shock” effect on the nitrifying biomass.
- Full scale and pilot studies have been run which show that bioaugmentation from an InNitri[®] type reactor treating a supernatant make it possible to maintain a stable nitrification process in a mainstream reactor at low sludge ages, which would otherwise preclude nitrification.

ACKNOWLEDGEMENTS

The authors wish to thank the Dutch Foundation for Applied Water Research (STOWA) and the Delft University of Technology (TUD) for sponsoring the initial research on the SHARON[®] process; the operations personnel of Utrecht and Rotterdam WWTP for sharing the performance results of their SHARON[®] systems operation; the University of Manitoba for providing Ms. Melanie Head with the funding and facilities to conduct important research regarding nitrification bioaugmentation; and the City of Tucson for providing funding for the first North American InNitri[®] demonstration program.

LICENSEE

Mixing and Mass Transfer Technologies INC, Lotepro Environmental Systems and Services is the licensee from Grontmij of the SHARON[®] process and from Dr Peter Kos of the InNitri[®] process for North America. Please address all communications to:

Alphonse Warakomski Jr.
Mixing and Mass Transfer Technologies, LLC
8833 North Congress Avenue, suite 818
Kansas City, MO, USA 64153

office telephone	816-584-1969
office telefacsimile	816-584-1970
mobile telephone	816-665-1931
e- mail	awarakomski@m2ttech.com awarakomski@loteproesg.com

REFERENCES

- (1) Two Kinds of Lithotrophs Missing in Nature; Broda, E; Z. Allg. Mikrobiol. 17, 491-493
- (2) Anaerobic Ammonium Oxidation Discovered in a Denitrifying Fluidized Bed; A. Mulder; Biological Anaerobic Ammonium Oxidation, Chapter 2, Delft University Press
- (3) Process for the Purification of Wastewater and/or Wastewater Sludge; Mulder, A; European Patents 0,051,888 and 0,327,184
- (4) Anoxic Ammonia Oxidation; Mulder, A; US Patent 5,078,884
- (5) Factors Controlling Anaerobic Ammonium Oxidation within Marine Sediments; Dalsgaard, T. and Thamdrup, B; Applied and Environmental Microbiology 2002 August; 68(8):3802-3808
- (6) Anaerobic Ammonium Oxidation by Anammox Bacteria in the Black Sea; Kuypers, MM et al; Nature 2003 April 10; 422(6932):608-11
- (7) Anaerobic Ammonium Oxidation Measured in Sediment Thames Estuary, United Kingdom; Trimmer, M et al; Applied and Environmental Microbiology 2003 November; 69(11);6447-6454
- (8) Trace Metals, Enzymes, and Biogeochemical Cycles; Morel, F; note at Center for Environmental Bioinorganic Chemistry, Princeton University
- (9) New Vision on the Life Style and Application of Anammox Bacteria; Jettin M; website note on Einladung zum Seminar University of Nijmegen; September 2004
- (10) The Biochemical Mechanism of Anaerobic Ammonium Oxidation; Jettin M; website note on Environmental Biotechnology

- (11) Change in Nitrification Rates with Sudden Change in Temperature: It's Implication to Short-SRT Systems; Head M., Oleszkiewicz J., Lagasse P., Taniguchi D.; WCWWA 2001 Session 55 Selected Topics
- (12) Biological Treatment of Wastewater; Marinus C M van Loosdrecht assigned to Grontmij; US Patents 5,863,435 and 6,183,642 January 1999 and February 2001
- (13) Operating Experience in the Netherlands with a High Rate Process for Total Nitrogen Control SHARON at Rotterdam and Utrecht; Warakowski A., van Kempen R., van Loosdrecht M. C. M.; NEWEA 2003 Poster Session
- (14) IBID
- (15) Method and System for Improved Biological Nitrification of Wastewater at Low Temperature; Dr. Peter Kos; US Patent 5,811,009
- (16) Computer Simulations of Full Scale Activated Sludge Strategies and Possibilities; Finnson A; 1994 Licentiate Thesis Royal Institute of Technology Division of Water Resources Engineering TRITA-VAT 1941
- (17) Discussion of How Input Active Biomass Affects Sludge Age and Process Stability; Lee C. Y.; Journal of Environmental Engineering ASCE 1997 123 (1) pp101-103
- (18) Effects of Weighting Agents and Seeded Nitrification Bacteria on the Activated Sludge Process—Evaluation by the Use of Simple Models; Li P. and Hultman B; Vassen 1997 53 (1) pp 21-25
- (19) How Input Active Biomass Affects Sludge Age and Process Stability; Rittman B. E.; Journal of Environmental Engineering ASCE 1996 122 (1) pp 4-8
- (20) Closure of How Input Active Biomass Affects Sludge Age and Process Stability; Rittman B. E.; Journal of Environmental Engineering ASCE 1997 123 (1) pp103
- (21) Biologisk Behandling av Rejektvatten fran Centrifugering av Rotslam; Tendaj-Xavier M.; 1985 Licentiate Thesis Royal Institute of Technology Division of Water Resource Engineering TRITA-VAT 1851
- (22) Achieving Nitrification in Pure Oxygen Activated Sludge by Seeding; Neethling J. B., Spani C., Danzer J., Willey B.; Water Science and Technology Volume 37 Number 37 pp 573-577
- (23) Impact of Seeding with Nitrifying Bacteria on Nitrification Process Efficiency; Plaza E., Treia J., Hultman B.; Water Science and Technology Volume 43 Number 1 pp 155-164
- (24) Discussion of Process and Kinetic Analysis of Nitrification in Coupled Trickling Filter Activated Sludge Systems; Parker D. and Richards J. T.; Water Environment Research 1994 66 (7) pp 934-935
- (25) Bioaugmentation Comes of Age; Rittman B. E. and Whiteman R.; Water Quality International 1994 (1) pp 22-26
- (26) Running in the Nitrification Process with and without Inoculation of Adapted Sludge; Sinjar O et al; Water Science Technology 1996 24 (1-2) pp 261-268
- (27) Process and Kinetic Analysis of Nitrification in Coupled Trickling Filter Activated Sludge Processes; Diagger G. T. et al; Water Environment Research 1993 65 (6) pp 750-758
- (28) Anoxic Ammonia Oxidation; Mulder A; US Patent 5,078,884
- (29) Pilot Scale Tests of a Unique Approach for A BNR Upgrade of a Short SRT High Purity Oxygen System at Pima County; Johansen, R et al; Session 24 Side Stream Treatment WEFTEC 2004 New Orleans