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Study of Nitrification Rates in a Biofilm System

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ABSTRACT

The purpose of this degree project was to investigate the capacity for nitrification in a moving bed biofilm reactor (MBBR) at different oxygen and ammonium concentrations fed with wastewater from the trickling filter effluents at the Rya wastewater treatment plant (WWTP), located in Göteborg. Due to new stricter effluent limits, the present nitrification capacity at the Rya WWTP has to be increased. One promising solution is to build a post-denitrification step treating a fraction of the effluent from the existing trickling filters, thereby decreasing the load on the activated sludge system. Of particular interest for the study was the nitrification capacity at low oxygen concentrations and the corresponding ammonium concentrations that would give relatively high nitrification rates without leading to high ammonium discharges. From this investigation conclusions were to be drawn whether or not to build an additional non-aerated nitrification step in front of the planned post-denitrification step at the Rya WWTP to decrease the dissolved oxygen concentrations to such level that efficient denitrification could be obtained and to reduce the cost of added carbon source (methanol) to the post-denitrification step. From the investigation it was also to be concluded how to build the additional nitrification step in the best way with respect to the economical aspects.

For this investigation a laboratory-scale plant was built. The laboratory-scale plant consisted mainly of two nitrification reactors in which Kaldnes biofilm carrier elements, K1, from the fourth compartment of the nitrification pilot MBBR covered with biofilm containing nitrifying bacteria were placed. The water used for the experiments was nitrified wastewater taken from the effluent from the pilot MBBR. The nitrified water from the pilot reactor has a low ammonium concentration. To increase the ammonium concentration in the water for the experiment, ammonium rich sludge liquor from the sludge centrifuges was added. The feed water to the nitrification reactors was aerated to obtain a saturated oxygen concentration in the water. By filling the nitrification reactors with different amounts of carrier elements, different oxygen concentrations were obtained for the experiments.

Factorial design experiments were carried out to reduce the number of experiments, where the factors were ammonium nitrogen and oxygen concentration. Experiments were made at two different levels of the two factors: around 2 mg/l and 4 mg/l of ammonium nitrogen in the influent water to the nitrification reactors and 25 % and 50 % carrier element filling of the nitrification reactors, resulting in dissolved oxygen concentrations around 2.7-2.8 mg/l and 4.4-4.5 mg/l of oxygen respectively in the nitrification reactors.

By comparing the obtained nitrification rates for the factorial design experiments with nitrification rates obtained in experiments made by Hem et al. (1994), estimations of expected nitrification rates for different influent ammonium concentrations and biofilm areas in the nitrification step could be made. It was concluded that for experiments performed at environmental conditions at which the biofilm was acclimatized the same nitrification rates as obtained in Hem et al.'s experiments (1994) for the corresponding ammonium nitrogen and oxygen concentration were obtained. Since the biofilm in the future additional nitrification step will be acclimatized to the environment that will be obtained in that step, Hem et al.'s experimental results (1994) could be used to estimate

what nitrification rates that are to be expected in the additional nitrification step for different influent ammonium concentrations and biofilm areas.

The optimal biofilm area for the additional nitrification step was concluded to be around 500 000-600 000 m^2 which corresponds to a carrier element filling of 50-60 % of the 1920 m^3 large additional nitrification step. For an influent water flow of 2.5 m^3/s, which is the maximal water load allowed to the additional nitrification step, an influent ammonium nitrogen concentration around 2 mg/l seems to give a large reduction in methanol consumption without leading to high ammonium discharges. A reduction of about 1000-1200 kg of methanol can be obtained per day from building an additional nitrification step, resulting in an economical saving of about 1.1-1.3 MSEK/year. However, these calculated savings are made for maximal water load on the nitrification step and most of the time the influent water flow will not be that large, therefore the actual economical saving will be lower. For the additional nitrification step to be profitable to build the costs related to the building of it must not exceed the economical savings related to the use of it.

Keywords – nitrogen removal, nitrification rate, moving bed biofilm reactor, oxygen concentration, ammonium concentration, carbon source, substrate limitation

SAMMANFATTNING

Syftet med detta examensarbete var att undersöka nitrifikationskapaciteten i en reaktor med rörlig biofilmsbädd vid olika syre- och ammoniumkoncentrationer i avloppsvatten från biobäddarna på Rya avloppsvatten-reningsverk, beläget i Göteborg. Den nuvarande nitrifikationskapaciteten på Rya reningsverk måste ökas p.g.a. nya hårdare utsläppsgränser. En lovande lösning är att bygga ett efter-denitrifikationssteg för behandling av en delström av utgående vatten från de befintliga biobäddarna, vilket medför sänkt belastning på aktivslam-systemet. Av särskilt intresse för studien var nitrifikationskapaciteten vid låga syrekoncentrationer och motsvarande ammoniumkoncentrationer som skulle ge relativt höga nitrifikationshastigheter utan att leda till höga ammoniumutsläpp. Från denna undersökning skulle slutsatser dras om huruvida ett extra icke-luftat nitrifikationssteg ska byggas framför det planerade efter-denitrifikationssteg på Rya reningsverk, för att sänka koncentrationen på löst syre i vattnet till sådan nivå att effektiv denitrifikation skulle kunna erhållas och kostnaderna för tillsatt kolkälla (metanol) till efter-denitrifikationssteget skulle kunna reduceras. Från undersökningen skulle det också dras slutsatser om hur efter-denitrifikationssteget ska byggas på bästa sätt med avseende på ekonomiska aspekter.

För att undersöka detta byggdes en anläggning i laboratorieskala. Denna laboratorie-skale-anläggning bestod främst av två nitrifikationsreaktorer i vilka Kaldnes biofilm-bärarelement, K1, från det fjärde facket ifrån pilotreaktorn med rörlig biofilmsbädd, täckta med biofilm med nitrifierande bakterier, placerades. Vattnet som användes för experimenten var nitrifierat avloppsvatten taget från utgående ström från pilotreaktorn med rörlig biofilmsbädd. Det nitrifierade vattnet från pilotreaktorn har låg ammoniumkoncentration. För att höja ammoniumkoncentrationen i vattnet inför experimenten tillsattes ammoniumrikt rejekt från slamcentrifuger. Ingående vatten till nitrifikationsbehållarna luftades för att få en mättad syrekoncentration i vattnet. Genom att fylla nitrifikationsbehållarna med olika mängd bärarelement erhölls olika syrehalt för de olika experimenten.

Faktorförsök utfördes för att minska antalet experiment, där faktorerna var ammoniumkväve- och syrekoncentration. Experiment utfördes vid två olika nivåer för faktorerna, omkring 2 mg/l och 4 mg/l ammoniumkväve i inkommande vatten till nitrifikationsreaktorerna samt 25 % och 50 % bärarfyllnadsgrad av nitrifikations-reaktorerna, vilket resulterade i lösta syrehalter i vattnet på 2,7-2,8 mg/l samt 4,4-4,5 mg/l.

Genom att jämföra de erhållna nitrifikationshastigheterna för faktorförsöken med nitrifikationshastigheter erhållna i experiment utförda av Hem et al. (1994), gjordes uppskattningar över vilka nitrifikationshastigheter som kan förväntas för olika ingående ammoniumkoncentrationer och biofilmsareor i nitrifikationssteget. Slutsatsen drogs att för experiment utförda i en miljö till vilken biofilmen var acklimatiserad erhölls samma nitrifikationshastigheter som Hem et al. (1994) fått i sina experiment för motsvarande ammoniumkväve- och syrekoncentrationer. Eftersom biofilmen i det extra nitrifikationssteget, om det byggs, kommer att vara acklimatiserad till den miljö som kommer att erhållas i nitrifikationssteget, kunde Hem et al.:s experimentella resultat (1994) användas för att uppskatta vilka nitrifikationshastigheter som kan förväntas

erhållas i det extra nitrifikationssteget för olika ingående ammoniumkoncentrationer och biofilmsareor.

Den optimala biofilmsarean för det extra nitrifikationssteget bestämdes till omkring 500 000-600 000 m^2 vilket motsvarar en bärarfyllnadsgrad på 50-60 % av det 1920 m^3 stora extra nitrifikationssteget. För ett ingående vattenflöde på 2,5 m^3/s, vilket är den maximala tillåtna vattenbelastningen på det extra nitrifikationssteget, verkar en ingående ammoniumkvävekoncentration på omkring 2 mg/l ge en stor reduktion i metanolförbrukning utan att leda till höga ammoniumutsläpp. En reduktion på omkring 1000-1200 kg metanol kan erhållas per dygn genom att bygga ett extra nitrifikationssteg, vilket resulterar i en ekonomisk besparing på omkring 1,1-1,3 MSEK/år. Dessa beräknade besparingar gäller dock för maximal vattenbelastning på nitrifikationssteget och för det mesta kommer ingående vattenflöde inte vara så stort, därför kommer de verkliga ekonomiska besparningarna bli lägre. För att det extra nitrifikationssteget ska vara lönsamt att bygga får inte kostnaderna relaterade till byggnationen överstiga de ekonomiska besparningar som är förknippade med dess användande.

Nyckelord – kväverening, nitrifikationshastighet, reaktor med rörlig biofilmsbädd, syrekoncentration, ammoniumkoncentration, kolkälla, substratbegränsning

PREFACE

This thesis is a result of a twenty weeks Master's degree project performed at Rya wastewater treatment plant and Chalmers University of Technology, both located in Göteborg. The degree project was a compulsory final element in my Master of Science education in chemical engineering with specialization in environment and energy, an education read at Chalmers University of Technology.

During the time that I have performed this Master's degree project I have been given help and advice from a lot of people whom I would like to give my acknowledgements.

First of all I would like to thank my supervisor Britt-Marie Wilén at the division of Water Environment Transport (WET) at Chalmers University of Technology, for all her support, help and guidance in the work with this degree project. Your commitment has meant so much to me. Thanks also to my examiner Greg Morrison and remaining personnel at the WET department for kind treatment and help with the project.

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Lovisa Björnsdotter

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1. INTRODUCTION

1.1 BACKGROUND

Nitrification tests were carried out at the Rya wastewater treatment plant (WWTP) in Göteborg. The Rya WWTP is run by Gryaab (Göteborgsregionens Ryaverksaktiebolag = the Göteborg region's Ryaverk Incorporation.) owned by the municipalities of Göteborg, Ale, Härryda, Kungälv, Lerum, Mölndal and Partille. The Rya WWTP is a large plant and Gryaab has, as things stand today, close to 80 employees. The plant serves approximately 775 000 population equivalents (pe) including about 600 000 pe of domestic wastewater and 175 000 pe of industrial wastewater. The hydraulic load to the plant varies partly due to diurnal variations but mostly due to variations in inflow of storm water and infiltration water to the sewer system. The average flow of wastewater reaching the wastewater treatment plant is 4 m^3/s but varied between 2.0 and 16.5 m^3/s. The water goes from the households, industries and gully pots through pipes and tunnels leading to the plant. The total length of the tunnel system is 130 km and the pipe system is even longer. The time required for the water to reach the plant varies between 0-30 h depending on the distance of transport. The Rya WWTP was built in 1972 but has since then been reconstructed and other improvements have been made, all to keep up with the tightened regulations concerning allowed discharges of e.g. phosphorus and nitrogen (Gryaab, 2004).

The nitrogen in wastewater comes from urine and nitrogen containing organic matter. These organic compounds are oxidized, which among other compounds results in ammonium (NH_4^+) as a product. This process occurs in the sewer which means that the main part of all the nitrogen that reaches a wastewater treatment plant is in the form of ammonium (Henze et al., 2002). The nitrogen has to be removed from the wastewater, otherwise it may cause eutrophication and oxygen depletion if released in too large amounts into lakes, rivers and seas (Svenska kommunförbundet, 1975). The removal of ammonium is of special interest because it can be toxic to aquatic species (Khin & Annachhatre, 2004). The annual average discharge limit of total nitrogen from the Rya WWTP is 10 mg/l. Today the nitrogen is removed from the wastewater via a nitrification step followed by a denitrification step. Phosphorus is removed by simultaneous precipitation using ferrous sulfate ($FeSO_4$). Precipitated phosphorus is aggregated into the activated sludge and settled in a secondary settler, which is placed last in the wastewater treatment process. In the nitrification step, which takes place in the trickling filters, ammonium in the water is first transformed into nitrite (NO_2^-) and then further into nitrate (NO_3^-) by nitrifying bacteria present in the biofilm in the trickling filters (diagram 1.1). The two steps are carried out by two different groups of bacteria that are not related. In the denitrification step, which takes place in the first part of the activated sludge basins, nitrate is transformed to nitrogen gas (N_2) by denitrifying bacteria present in the sludge flocs. The nitrogen gas is released into the air and the water is thereby purified from nitrogen. Secondary settler effluent can be recirculated to the nitrifying trickling filters for nitrification. Due to hydraulic limitations of the secondary settlers, the flow of recirculated water to the trickling filters has to be adjusted to the flow of incoming wastewater to the plant. A balance between nitrogen and phosphorus removal has to be struck where high nitrogen removal requires a high

degree of recirculation and thus high settler hydraulic loadings whereas high phosphorus removal requires low settler loadings (Gryaab, 2004).

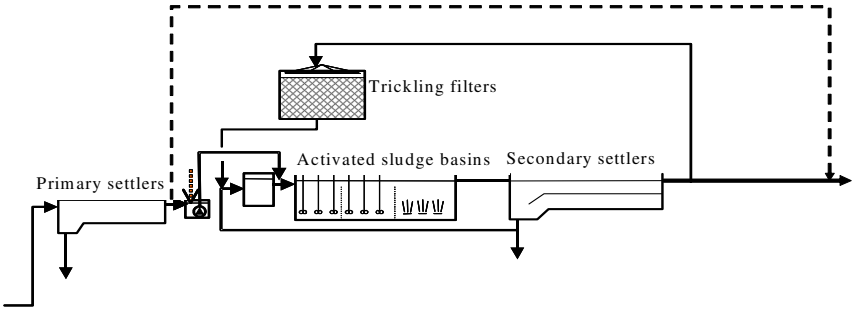

Diagram 1.1 Flowchart of the water purification process as it looks today.

To meet further stricter discharge limits for nitrogen, there are plans of building an additional post-denitrification step, separated from the existing activated sludge system. This treatment step has to be very compact due to limited area available and is designed as a moving bed biofilm system (MBBR) using Kaldnes biofilm carrier elements. Through this dentrification step a part of the effluent stream from the trickling filters will be led (diagram 1.2). By doing so, more nitrogen can be removed from the water and the discharge of total nitrogen could be reduced to about 8 mg/l. At present the flow through the trickling filters cannot be increased because of hydraulic limitations of the secondary settlers. With the additional post-denitrification step the flow through the trickling filters and therefore also the nitrogen removal could be increased without affecting the phosphorus removal. To this additional post-denitrification step a carbon source such as methanol (CH_3OH) or ethanol (C_2H_5OH) must be added. The carbon is used to obtain energy and to build up the bacterial cells. The denitrifying bacteria use the oxygen in nitrite and nitrate to break down the carbon source. However, if there is free oxygen present in the water it will be used first, before the denitrification starts. To obtain a maximal denitrification it is therefore important that there is not too much oxygen present in the water, since this will be used to oxidize carbon source. Consequently, the more free oxygen in the water, the more carbon source must be added. Addition of carbon source is expensive and it is therefore desirable to remove as much oxygen as possible from the water before it enters the anoxic denitrification zone. By introducing a non-aerated tank for nitrification before the additional post-denitrification tank, dissolved oxygen can be used up due to nitrification. This will lower the amount of carbon source needed for the denitrification and will thereby give Gryaab an economical saving if the investment costs for the additional nitrification step are not greater than the expected savings related to reduced carbon source-usage (Mattsson, 2004).

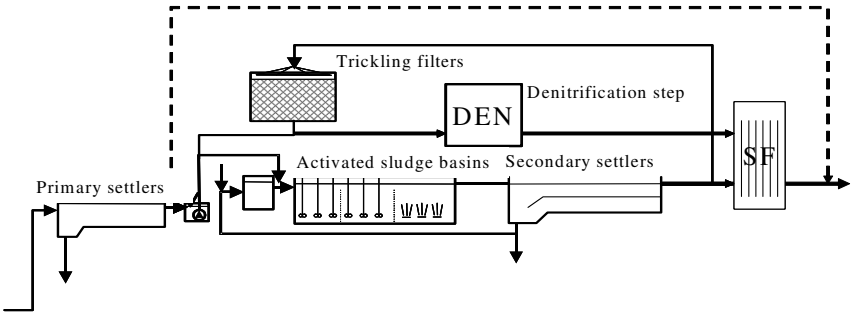

Diagram 1.2 Flowchart of the planned water purification process.

1.2 AIM

The purpose of this Master's degree project was to investigate the feasibility, both in terms of obtained nitrification rates and costs, of introducing a nitrification step in front of the planned post-denitrification step to decrease the dissolved oxygen concentrations to such level that efficient denitrification can be obtained and to reduce the cost of added carbon source. The study was aimed to measure what nitrification rates can be obtained at low dissolved oxygen concentrations as well as at different ratios between dissolved oxygen concentration and ammonium concentration that are expected to occur in the future nitrification-denitrification plant. An economical estimation of the most cost effective solution for the additional nitrification step was to be done to find out whether or not it is profitable to build the additional nitrification step and if so, how to build it in the best way with respect to the economical aspects.

1.3 DELIMITATIONS

A laboratory-scale plant was built to study the effects of different concentrations of ammonium and oxygen on the nitrification rate. Water from the last compartment of a pilot plant MBBR was used for the experiments. To vary the ammonium concentrations in the water, different amounts of ammonium rich sludge liquor was added. Two nitrification reactors were used for the experiments. Carrier elements covered with biofilm containing nitrifying bacteria were put into these containers. The carrier elements were taken from the fourth compartment of the pilot MBBR before the start of each day's experiments and were put back into the pilot plant after each day's finished experiments. By aerating the influent water to the nitrification reactors a saturated oxygen concentration in the influent water was obtained. Different oxygen concentrations were obtained in the water in the containers by adding different amounts of carrier elements. The experiments were carried out at water temperatures around 13-16 ºC. From factorial design four different combinations of influent ammonium nitrogen concentration and carrier element filling volume, expressed as percentage of total water and carrier element volume in the nitrification reactors, were chosen for the study; ammonium nitrogen concentrations around 2 mg/l and 4 mg/l for 25 % carrier element filling and the same ammonium nitrogen concentrations for 50 % carrier element filling. For each combination of influent ammonium nitrogen concentration and carrier element filling volume, experiments were carried out three times to make sure the test results were reliable. A comparison of the capacity of the biofilm on the carrier elements from the second and fourth compartment was also done to investigate the impact of the biofilm growth conditions on the nitrification capacity.

1.4 DISPOSITION

The thesis starts with a description of the Rya WWTP and the planned future additional nitrification step (chapter 2), followed by a theory section on nitrification (chapter 3). In subsequent chapters the performed experiments are described and the results from the experiments are presented and commented (chapters 4 and 5). After that comes an economical estimation of what the most cost effective solution for the building of the additional nitrification step is, concluded from the results of the experiments (chapter 6). This is followed by a discussion of the material presented in the thesis and conclusions made from the experiments (chapters 7 and 8). Placed last in the thesis are the appendices.

2. THE RYA WASTEWATER TREATMENT PLANT

2.1 THE WASTEWATER TREATMENT PROCESS

A flowchart of the Rya WWTP is shown in appendix 1. The wastewater that comes to the Rya WWTP reaches the wastewater treatment plant 20 m below ground level. There it passes through a 20 mm bar screen where bigger objects such as paper, rags, sanitary towels etc. are removed. The wastewater is then pumped to the primary settlers. On the way to the primary settlers the wastewater will, within the near future, go through another much finer screen than the first one and a sand trap where sand will be separated from the water. The grating trimmings will be burned and the sand will be washed and after that either sold or deposited. There are twelve primary settlers of which eight are constantly used and the remaining four will soon be used, but only when the flows of incoming wastewater are so high that water must be bypassed. This happens during large rain events or snow melting. In the four primary settlers that will be used when bypassing wastewater, precipitation of phosphorus, removal of heavier particles through sedimentation and removal of fat will be done, before the water will be led to the recipient which is the mouth of the river Göta älv at Rya nabbe, about 200 m from land. Today bypassed water is led straight to Göta älv after having passed through the 20 mm bar screen. The wastewater that is not to be bypassed is and will be led to the ordinary primary settlers in which heavier particles that can settle to the bottom of the basins are removed. With time a bottom layer called sediment builds up, consisting of sludge. On the surface of the basins another layer consisting of fat may form. The sediment is scraped off and if necessary, so also the fat layer and the water continues on into the plant for further purification (Gryaab, 2004).

The wastewater contains phosphorus, which comes from urine, feces and washing powder among other things. Phosphorus is a plant nutrition which can cause eutrophication and oxygen depletion if too big amounts are released into lakes, rivers and seas. The main part of the phosphorus in wastewater is bound to phosphate (PO_4^{3-}) which is the compound formed when phosphorus compounds such as e.g. proteins are decomposed. Ferrous sulfate is added to the wastewater to precipitate phosphate. In the presence of oxygen ferrous ion, Fe(II), is oxidized to ferric ion, Fe(III), which either reacts with phosphate forming ferric phosphate ($FePO_4$) or with hydroxide forming ferric hydroxide ($Fe(OH)_3$). Ferric hydroxide precipitates into voluminous particles onto which phosphates, polyphosphates and other phosphorus containing particles can attach to. Ferric phosphate precipitations also coagulate and form flocs which can be separated from the water through sedimentation. Sometimes ferrous sulfate is dosed before the primary settlers and sometimes after. When the ferrous sulfate dosing is taking place before the primary settlers, the ferric phosphate partly sediments in the primary settlers. When the ferrous sulfate dosing is taking place after the primary settlers, the flocs are separated from the water in a later sedimentation step in the secondary settlers described later on (Gryaab, 2004).

After the primary settlers the water is led to the activated sludge basins. These basins are divided into two zones, one aerated and one non-aerated. First the water is led to the non-aerated part, called anoxic zone. In the inlet of the anoxic zone, recirculated water from the trickling filters, return sludge and primary settled wastewater are mixed. Both

the trickling filters and the secondary settlers will be described later on. In the activated sludge basins the organic matter in the water is broken down. The recirculated water from the trickling filters contains nitrogen in the form of nitrate. In the first part of the activated sludge basins no oxygen is supplied so the bacteria in the basins use the oxygen from nitrate to break down the organic matter in the water, this causes nitrate to be transformed into nitrogen gas which is released into the air. This process is called denitrification. The water continues to the aerated part of the activated sludge basins, which is called the aerobic zone. Here the remaining part of the organic matter is degraded. To maximize the processes, air is blown into the water through diffusers so that the bacteria have a constant supply of oxygen (Gryaab, 2004).

When the water has passed through the activated sludge basins it continues on to the 24 secondary settlers. In these basins, water and sludge are separated from each other through sedimentation. In some cases, before the water leaves the aerated activated sludge basins, a polymer is added that helps the sludge to flocculate. This improves the clarification properties of the sludge, i.e. small particles can easier attach to larger flocs. The main part of the settled sludge is recycled to the entrance of the activated sludge basins to once again let the bacteria within it transform nitrate to nitrogen gas and to break down organic matter. The excess sludge is pumped on for further treatment, described below. About half of the water that has passed through the secondary settlers is led to Göteborg Energi, where the heat in the water is taken care of by Göteborg Energy's heat pump plant and it covers 20 % of the needed district heating in Göteborg. After having passed through the heat pumps the water is let out as an effluent to the bottom of the mouth of the river Göta älv. The other half of the water, the one that is not being led as an effluent to Göta älv, is led to the two trickling filters together with the ammonium rich sludge liquor from the centrifuges. The centrifuges will be described later on. The trickling filters are filled with corrugated plastic material, offering a large surface area of 230 m^2/m^3. Nitrifying bacteria grow in a biofilm on the plastic material. The bacteria transform ammonium to nitrite and further on to nitrate. Water is sprinkled over the trickling filters and air is blown into the filters from below. When the water has passed through the trickling filters it is sent into the anoxic part of the activated sludge basins where the nitrogen removal process continues (Gryaab, 2004).

The sludges from the primary settlers, primary sludge, and the excess sludge from the secondary settlers are thin and have a dry matter of around 1-3 %. Before the sludge is thickened a polymer is added to the sludge. The polymer repels the water, which makes the sludge thickening easier. First the sludge goes to one of the two thickeners, where it is transported on a rolling hoop made of straining cloth, allowing water to run through. When the sludge has passed through the thickeners it has a dry matter content around 5 %. The separated water, called reject, goes to the primary settlers for purification and the sludge is pumped to one of the two silos for thickened sludge. Here the sludge is stored before it is pumped to one of the two digesters. The Rya WWTP receives fat and leftovers from restaurants, which are also pumped into the digesters. In the digesters a biological degradation of the sludge, the fat and the leftovers in an anaerobic environment takes place. The sludge is stirred and the temperature is kept around 37 ºC. During the digesting process biogas is formed. The biogas consists of about 60 % methane gas (CH_4) and the remaining 40 % consists mainly of carbon dioxide (CO_2). After about 15-30 days of digesting the organic sludge is reduced by about half. The sludge is then pumped out of the digesters and is aerated to make the digesting process stop. The sludge continues on to a digested sludge silo where it is stored until

dewatered. The dewatering is done with centrifuges. Before the sludge reaches the centrifuges, a polymer is added to the sludge. This polymer also has the function of repelling water, making the dewatering process of the sludge easier. When the sludge has passed through the centrifuges it has a dry matter content around 30 %. The water separated from the sludge is called reject. The sludge is now called biosolids. The biosolids are nutritious and can therefore be used as soil improvement in the agriculture. Today the biosolids are used as filling material at golf courses, banks built for noise protection, roadwork etc. The biogas produced in the digesters is equalized in a small gas holder. The biogas plant produces about 20 000 m^3 biogas during a day. Some of the biogas is purified to 95-98 % methane consistence. The methane gas is compressed and used as fuel for the company's cars. The rest of the biogas is sold to Göteborg Energi (Gryaab, 2004).

Within the near future reconstructions of the Rya WWTP will be made to improve the nitrogen and phosphorus removal. The outgoing water will pass through filters to further improve the phosphorus removal. There are different possible solutions to how to improve the nitrogen removal of which the building of an additional post-denitrification step is the most likely solution to be implemented (Mattsson, 2004).

2.2 THE ADDITIONAL DENITRIFICATION AND NITRIFICATION STEPS

The more secondary settled water that is recycled to the trickling filters, the better the nitrogen removal. The problem is that with an increased amount of recycled water, more water will be led through the activated sludge basins and the secondary settlers, which will be higher hydraulically loaded. Most of the time, the secondary settler capacity is limiting the amount of water that can be recycled to the trickling filters. Consequently, the maximal settler capacity is determining the efficiency of the whole plant. An attractive solution to improve the nitrogen removal is to build a post-denitrification step preceded by a non-aerated nitrification step. According to those plans part of the water leaving the trickling filters will pass through these additional nitrification and denitrification steps and the rest of the water will pass through the activated sludge basins as usual. The water leaving the post-denitrification step will pass through a filter and then be led out directly to Göta älv, thereby the recycling of water can be increased thus also the nitrogen removal, without an increase in the amount of water passing through the secondary settlers (Mattsson, 2004).

The denitrifying bacteria need carbon to build up their cells. This carbon is taken from organic matter that is degraded into carbon dioxide. The denitrifying bacteria use the oxygen in nitrite and nitrate to break down the carbon source. However, if there is free oxygen present in the water it will be used first, before the denitrification starts. Most denitrifying bacteria have this ability to change their metabolism from using oxygen as a final electron acceptor to use nitrate instead (facultative bacteria). When the water from the trickling filters is sent to the activated sludge basins, the organic matter in the water from the primary settlers is usually enough to get a more or less complete denitrification. If the organic matter in the water is insufficient a carbon source in the form of ethanol is added. In the case of post-denitrification the water hardly contains any organic matter, since it has already passed through the activated sludge basins where organic matter is degraded. Therefore a carbon source must be added, this in the form of methanol due to its lower cost compared to ethanol. The denitrifying bacteria use the oxygen in nitrate and nitrite to break down the methanol, as a result the nitrogen in the nitrate and the nitrite is transformed into nitrogen gas. If however there is free

oxygen in the water this oxygen will be used as electron acceptor instead of nitrite and nitrate, with the consequence that a fraction of the methanol will be used up before denitrification can start. This is of course not desirable due to the high cost of carbon source. Instead, the idea is to build a tank where the free oxygen in the water can be consumed by nitrifying bacteria grown on biofilm carrier elements before the additional denitrification step. Thus, there will be no oxygen added to the additional nitrification step through aeration, which is usually the case for nitrification reactors. The goal is to let the nitrifying bacteria proceed with the nitrification until the oxygen concentration is reduced to a level where it is still economically profitable. In the effluent from the trickling filters most of the original ammonium is transformed into nitrate. To feed the nitrifying bacteria in the additional nitrification step ammonium will be added in the form of sludge liquor from the centrifuges. Today this sludge liquor is sent to the trickling filters and even with the additional nitrification step some of the sludge liquor will still have to be sent there, since the sludge liquor flow is too high for the additional nitrification step to handle all of it (Mattsson, 2004).

Both the additional post-denitrification and nitrification reactor will be a MBBR filled with carrier elements (Mattsson, 2004). These carrier elements will have a high specific surface area per volume and a density slightly below that of water. On these carriers a biofilm will grow containing the denitrifying and nitrifying bacteria respectively. Carrier elements are designed to provide a large protected surface for the bacteria (Kaldnes, 2004). The additional nitrification step is planned to consist of six reactors. The total flow into the nitrification step will be about 2.5 m^3/s. Each additional nitrification reactor will have a length of 8 m, a width of 4 m and a height of 10 m, resulting in a volume of 320 m^3. Thus will the total volume for all six reactors be 1920 m^3. To have a good mixing of the water in the reactors and to make the carrier elements move around stirrers will be used (Mattsson, 2004).

3. NITRIFICATION

3.1 THE MICROBIOLOGICAL PROCESS

Nitrification is a microbiological process that converts ammonium into nitrite and eventually nitrate. The main part of the nitrogen in the water reaching the wastewater treatment plant is in the form of ammonium. The ammonium in the wastewater is a product formed when organic matter containing nitrogen is oxidized. The main part of the bacteria that perform the nitrification is chemoautotrophic although some heterotrophic nitrifying bacteria exist (Henze et al., 2002). Chemoautotrophic bacteria obtain their energy from chemical bound energy in non-organic compounds and they use carbon dioxide as carbon source, i.e. building material for their cells. Heterotrophic bacteria obtain their energy and carbon from organic matter. Nitrifying chemoautotrophic bacteria are chemolitotrophs (Water Environment Federation, 2001). The nitrification process takes place in two steps. First ammonium is oxidized to nitrite by a group of bacteria known as Nitrosomonas and then nitrite is oxidized to nitrate by two other groups of bacteria known as Nitrospira and Nitrobacter. Nitrosomonas bacteria are called ammonium oxidizers and Nitrospira and Nitrobacter are called nitrite oxidizers (Lydmark, 2004a). The main nitrite oxidizer is Nitrospira (Daims et al., 2000). Other bacteria like Nitrococcus and Nitrosocystis are also involved in the nitrifying process (Henze et al., 2002). In the biofilm in the trickling filters at the Rya WWTP different kinds of Nitrosomonas have been found; Nitrosomonas oligotropha, Nitrosomonas communis and Nitrosomonas europaea, which perform the oxidation of ammonium. However Nitrosomonas europaea was only found in the top of the trickling filters. The oxidation of nitrite in the trickling filters at Rya is performed by Nitrospira spp i.e. unknown species of Nitrospira. Anammox bacteria have also been found in the trickling filters. Anammox bacteria are bacteria that perform both nitrification and denitrification in anaerobic environments. These bacteria were found in areas of the trickling filters to which oxygen was believed not to have reached for some reason (Lydmark, 2004a). Since the composition of the nitrifying bacterial community in a nitrification reactor depends largely on the water composition itself it is likely that the composition of nitrifying bacteria in the additional nitrification step will be similar to the one in the trickling filters at the Rya WWTP (Water Environment Federation, 2001). In full-scale wastewater treatment plants the variation in active nitrifying species is considerable. However the various nitrifying bacteria do not seem to have treatment process performances that deviate very much. The process for the ammonium oxidizing bacteria is:

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + H_2O + 2H^+ \qquad (3.1)$$

The energy released when this reaction occurs is 270 kJ/mol ammonium nitrogen. Ammonium nitrogen is sometimes denoted NH_4^+-N. The process for the nitrite oxidizing bacteria is:

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \quad (3.2)$$

The energy released when this reaction occurs is 80 kJ/mol ammonium nitrogen. The released energies for these reactions are relatively low compared to the energies released through the processes of other bacteria and therefore the nitrifying bacteria are characterized by a low growth rate. The carbon dioxide used as carbon source for the nitrifying bacteria has to be reduced before the carbon can form part of the cell mass and this reduction takes place through the oxidation of a nitrogen source. For oxidation of ammonium the expression for growth is:

$$15\,CO_2 + 13NH_4^+ \rightarrow 10NO_2^- + 3C_5H_7NO_2 + 23H^+ + 4H_2O \quad (3.3)$$

For oxidation of nitrite, the corresponding growth expression is:

$$5CO_2 + NH_4^+ + 10NO_2^- + 2H_2O \rightarrow 10NO_3^- + C_5H_7NO_2 + H^+ \quad (3.4)$$

The oxidation of ammonium to nitrite takes place in several steps; ammonium is oxidized to hydroxylamine (NH_2OH), which is transformed into an unknown intermediate that is oxidized into nitrite. The oxidation from nitrite to nitrate on the other hand takes place in a single step (Henze et al., 2002).

Nitrous oxide (N_2O) is produced as a by-product in the nitrification process, but the mechanism of nitrous oxide production is not clarified. It seems that this production cannot be avoided, although higher dissolved oxygen concentrations in the water results in less nitrous oxide production. Nitrous oxide is a greenhouse gas and wastewater treatment plants make a contribution to the global nitrous oxide emissions through the nitrification process but also through the denitrification process (Zheng et al., 1994).

3.2 THE BIOFILM

Reactors for nitrification, like trickling filters and moving bed biofilm reactors, are characterized by nitrifying bacteria being attached to a solid surface in the form of a biofilm (Henze et al., 2002). Biofilm structures are very heterogeneous. Many biological, physical and chemical factors affect the structure to various extents. The biological factors that influence the biofilm structure depend on the composition of bacteria in the biofilm. Since the environmental conditions inside biofilms vary due to consumption of substrates (a substrate is a substance that the bacteria use to get energy) and diffusion limitations, the bacterial community structure can also be stratified. The hydraulic erosion, detachment, mass transfer and shape of the substrate are among the physical forces whereas physico-chemical environment, substrate concentration and type of substrate are some of the chemical factors that influence the biofilm structure (Suren et al., 2004). A biofilm is commenced if a reactor medium is in contact with water containing the bacteria needed to build up the film. It takes approximately 14 days under aerobic conditions to develop a biofilm. The building up is selective and the bacteria that are not attached will simply be washed out of the biofilm reactor. Biofilms are a dense layer of bacteria characterized by their ability to adhere to a solid medium and form a fixed film of extracellular polymers in which the bacteria are protected against being washed out of the reactor. During nitrification the nitrifying bacteria grow continuously and hence there is a growth of the thickness of the biofilm. If this is not

balanced by a corresponding detachment of the biofilm for trickling filters, the biofilm will be too thick resulting in clogging of the trickling filters. It is therefore desirable to have a stable state with equilibrium between growth and detachment. (Henze et al., 2002). The advantage of the MBBR is that it does not clog due to the fact that the carrier elements are constantly circulating inside the tank (Hem et al., 1994). Detachment of biofilm is caused by hydraulic erosion, degradation of starved out bacteria, super saturation and gas bubble formation. Hydraulic erosion acts continually on the surface of the biofilm and leads to a steady detachment on the outer side. In trickling filters the flow of water is not strong enough to cause detachment all by itself through hydraulic erosion, but other factors release the biofilm so that it can be detached hydraulically. Degradation of starved out bacteria in the inner layer of a biofilm may cause a weakening of the adhesion to the carrier element. Biofilms will in practice have a tendency to grow to a thickness where they are only partially penetrated with substances needed for continues growth and nitrification. In the nitrification reactor whose nitrification reaction rate is controlled by the oxygen supply, anaerobic conditions may occur at the inner layer of the biofilm. This will degrade the bacteria in the biofilm and destroy the adhesion to the carrier element. Sufficiently weakened, the biofilm will be completely detached from the carrier element over a smaller area by hydraulic erosion. Super saturation and bubble formation at the inner layer of the biofilm may also destroy the adhesion, also causing the biofilm to be completely detached. When the biofilm is completely detached, a naked area is left on the filter medium where new growth starts. Because of the continuous detachment and regrowth, biofilms never have a well-defined thickness that applies to the entire film (Henze et al., 2002).

For high substrate loads a compact biofilm is built up, while a more porous one is built up when the supply of substrate is more limited. Availability of substrate for growth makes the biofilm saturated with bacteria leading to structures with very low porosity and high cell density. At lower substrate concentrations the substrate is quickly exhausted at the base of the biofilm where competition among growing bacteria is most intense. The lack of substrate retards the growth and a highly porous structure results. When the biofilm is compact with low porosity, the ammonium oxidizers tend to be restricted to the surface of the biofilm. When the biofilm is increasingly porous the ammonium oxidizers can be located in the interior of the biofilm. High loading rates of organic matter favors the faster growing heterotrophic bacteria, which has a higher yield factor and a faster growth rate than the nitrifying bacteria. It suppresses the nitrification potential of the biofilm due to the spatial competition between the heterotrophic and nitrifying bacteria (Suren et al., 2004).

3.2.1 The hydrodynamic and concentration boundary layers

Before the substances needed for nitrification can reach the nitrifying bacteria in the biofilm, they must be transported through the hydrodynamic boundary layer between the surface of the biofilm and the liquid phase surrounding it. The liquid bulk flow in the nitrification reactor is turbulent and mass is transported by eddies present within the turbulent streams. This mass transport is convective. Fluid particles immediately adjacent to the solid surface are stationary and a thin layer of fluid close to the surface is in laminar flow, regardless of the nature of the free stream. The mass transfer through this film is diffusional. Figure 3.1 illustrates how the thickness of the boundary layer, δ, increases with the distance, x, from the edge of a flat plate. At relatively small values of x flow within the boundary layer is laminar and this is designated as the laminar

boundary layer region. At larger values of x the transition region is shown where fluctuations between laminar and turbulent flow occur within the boundary layer, when the liquid bulk flow is turbulent. For a certain value of x and above, the boundary layer will always be turbulent when the bulk liquid flow is turbulent. In the region in which the boundary layer is turbulent there exists a very thin film of fluid called the laminar sublayer wherein flow is still laminar. Depending on the degree of turbulence, the thickness of the diffuse boundary layer will vary; high turbulence gives lower values of the diffuse boundary layer (Welty et al., 2001).

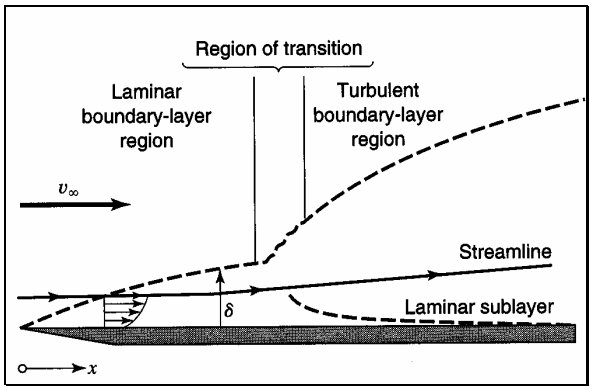

Figure 3.1 Hydrodynamic boundary layer on a flat plate (Welty et al., 2001).

The hydrodynamic boundary layer looks a bit different for the carrier elements planned to be used in the additional nitrification step at the Rya WWTP compared to what it looks like in figure 3.1, since the carrier elements are not flat. However the principle with the hydrodynamic boundary layer is the same (Welty et al., 2001).

Due to diffusion limitations, there is also a concentration gradient between the liquid phase surrounding the biofilm and all the way into the carrier element, causing a concentration boundary layer. The concentrations of the substances used in the nitrification process decrease with the depth of the biofilm and the concentrations of the products produced in the nitrification process increase with the depth of the biofilm (Welty et al., 2001). The thickness of the concentration layer depends on the substrate load and the Reynolds number in the liquid bulk in the reactor during biofilm growth, Re_{growth}. The higher the liquid bulk flow velocities the higher the Reynolds number. For a biofilm at high Re_{growth} and small substrate loadings a thin concentration layer develops. When Re_{growth} is larger than 3000 i.e. at high bulk flow velocities during biofilm growth, the thickness of the concentration layer becomes more and more independent from the substrate load during biofilm cultivation and the mass transfer becomes more and more dominated by the hydrodynamic shear stress. At high substrate loadings and low Re_{growth} the biofilm surface will be very rough. There will be several different concentration profiles, which can be explained with the open biofilm structure near the liquid phase surrounding the biofilm. The substrate load has the highest influence on mass transfer at high substrate loads and laminar hydrodynamic conditions (Wäsche et al., 2000).

According to Henze et al. (2002) the transport of a substance from the liquid bulk to the surface of the biofilm can be described in a simplified manner by a proportionality between the difference in transport and concentration:

$$F = h(c_b - c_i) \quad [3.1]$$

F = transport of substance through the cross section of the biofilm [g/(m^2·d)]
h = transfer coefficient [m/d]
c_b = concentration of substance in the bulk liquid [g/m^3]
c_i = concentration of substance at the interface [g/m^3]

3.3 THE INFLUENCE OF ENVIRONMENTAL FACTORS

A number of factors influence the nitrification process, of which some of the most important ones are alkalinity, pH, toxic substances, concentration of substrates, oxygen concentration and temperature (Henze et al., 2002).

3.3.1 Alkalinity

Alkalinity in water is a measurement of the water's sensitivity to acidification. It describes the buffering capacity at acid addition, i.e. the waters ability to stand addition of hydrogen ions (H^+) without lowering the pH. If the alkalinity is zero, pH is lowered at each addition of hydrogen ions. If the alkalinity is larger than zero the lowering of the pH is not proportional to the addition of hydrogen ions, but the alkalinity is lowered when hydrogen ions are added. The equilibrium between the carbonate compounds carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) determines the alkalinity of the wastewater. There are other compounds too that have this buffering capacity like for instance various phosphates, but in wastewaters these play a minor roll (Olsson & Newell, 1999).

$$CO_3^{2-} \xrightleftharpoons{H^+} HCO_3^- \xrightleftharpoons{H^+} H_2CO_3 \quad (3.5)$$

Which carbonate compound that is formed is dependent on the pH. At high pH-values alkalinity consists of carbonate and at pH-values below 8.3 the alkalinity consists mainly of bicarbonate. At pH-values around 4.5 the alkalinity is almost consumed and most of the carbonate is in the form of carbonic acid (Olsson & Newell, 1999). When the pH reaches the point where the buffering capacity is consumed, the carbonic acid equivalence point is reached. There will always be some bicarbonate and carbonate present in the water regardless of the pH, but at some pH there are enough hydrogen ions in solution that if they were combined with the bicarbonate and carbonate present, it would all be converted to carbonic acid and this is where the carbonic acid equivalence point lies (Skoog et al., 1996). Most waters, wastewaters included, have a pH-value between 5 and 8 and therefore almost all alkalinity from carbonate compounds consists of bicarbonate. The bicarbonate in the wastewater has mainly three sources. One of the sources is from the oxidization of organic matter in the wastewater resulting in bicarbonate as a product among others (Henze et al., 2002). Another source is the production of bicarbonate when carbon dioxide is dissolved in the water. The controlling of the alkalinity of the drinking water at the water purification plant contributes to a third source for bicarbonate in the wastewater, since the drinking water eventually reaches the wastewater treatment plant (Wilén, 2004).

During nitrification hydrogen ions are released, causing a reduction in the alkalinity. For every mole of ammonium that is oxidized to nitrite, approximately two moles of bicarbonate are consumed, corresponding to 2 equivalents (eqv) of alkalinity. Other

processes in the treatment of the wastewater, such as denitrification and chemical precipitation, change the alkalinity. Normal municipal wastewaters with alkalinity over 5 meqv/l will not cause problems in connection with nitrification, whereas lower alkalinity may cause a drop in the pH resulting in a low efficiency and inhibition of the nitrification (Henze et al., 2002). However, earlier studies of the effect of alkalinity on nitrification in a trickling filter at Rya WWTP have shown that as long as the ratio between bicarbonate and ammonium in the influent water to the trickling filter is larger than 2.5 eqv HCO_3^-/mole of ammonium, the nitrification is not limited due to low alkalinity (Andersson & Mattsson, 1996).

3.3.2 pH

The nitrification process is pH dependent with an optimum in nitrification rate in the range 8-9. The diagram below is presenting this dependency (Henze et al., 2002).

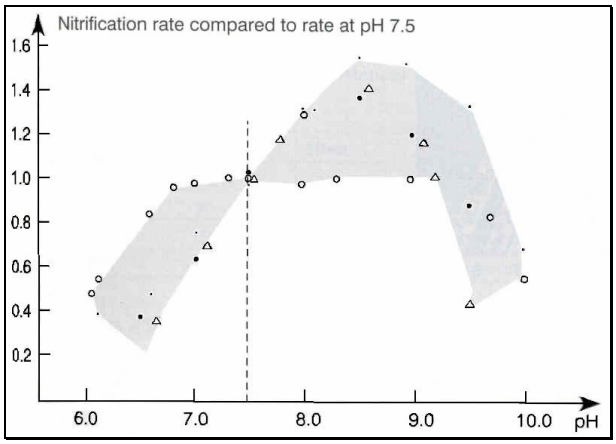

Diagram 3.1 The nitrification rate's dependency of the pH (Henze et al., 2002).

When the pH lies between 6.45 and 8.95 the nitrification is complete. At pH lower than 6.45 and above 8.95 a complete inhibition of both ammonia and nitrite oxidizing bacteria takes place (Ruiz et al., 2003). The release of hydrogen ions during nitrification causes a lowering of the pH and the pH in the biofilm is normally lower than the pH in the liquid bulk surrounding the biofilm. If the alkalinity is low it can be exhausted, resulting in a substantial reduction of the pH, which will inhibit the nitrification process. It is possible that the pH dependency is linked to the inhibition caused by ammonia (NH_3) and nitrous acid (HNO_2) (Henze et al., 2002). Ammonia is formed from ammonium at high pH-values and nitrous acid is formed from nitrite at low pH-values (Anthonisen et al., 1976).

$$NH_4^+ - N + OH^- \leftrightarrow NH_3 + H_2O \tag{3.6}$$

$$NO_2^- + H^+ \leftrightarrow HNO_2 \tag{3.7}$$

It is the concentrations of nitrous acid and ammonia that affects the degree of inhibition of the nitrification process, not the concentration of ammonium and nitrite. The nitrous acid and ammonia concentrations are not just affected by pH but also temperature, number of active nitrifying bacteria and acclimation of the nitrifying bacteria to nitrous acid and ammonia. Both ammonia and nitrous acid can be toxic to ammonium and nitrite oxidizing bacteria, although ammonia is more toxic to nitrite oxidizing bacteria

than ammonium oxidizing bacteria and vice versa for nitrous acid. An operational chart may be used to assess the performance of nitrifying bacteria depending on the pH, ammonia and nitrous acid concentrations (Anthonisen et al., 1976).

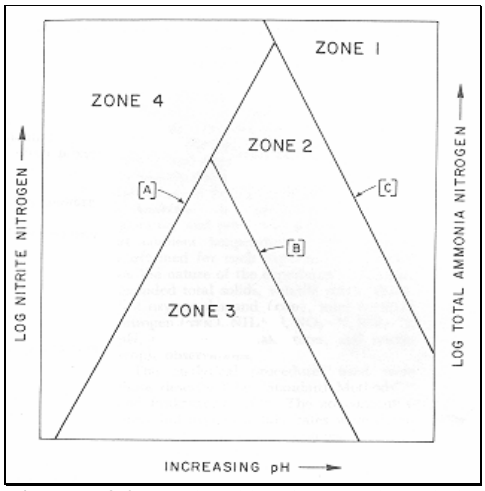

Diagram 3.2 Relationship of ammonia and nitrous acid inhibition to nitrifying bacteria (Anthonisen, et al., 1976).

The diagram consists of four different zones. In zone 1 ammonia inhibits both ammonium and nitrite oxidizing bacteria. In zone 2 ammonia inhibits nitrite oxidizing bacteria. In zone 3 no inhibition of the nitrification occurs. In zone 4 nitrous acid inhibits nitrite oxidizing bacteria. The lines A, B and C in the diagram are not strict lines as they may appear in the picture, they are ranges within which a change in inhibition of nitrifying bacteria are expected to occur. Nitrous acid inhibition of nitrifying bacteria begins at concentrations between 0.22-2.5 mg/l (line A). Ammonia inhibition to nitrite oxidizing bacteria begins between 0.1-1.0 mg/l (line B) and ammonia inhibition to ammonium oxidizing bacteria begins at concentrations between 10-150 mg/l (line C) (Anthonisen et al., 1976).

3.3.3 Toxic substances

Ammonia and nitrous acid may be toxic to the nitrification process, so may also high concentrations of nitrite, which can inhibit the ammonium oxidizing bacteria from performing the first step in the nitrification process, where ammonium is transformed into nitrite. In a study of an unknown specie of Nitrosomonas, nitrite toxicity occurred at nitrite concentrations higher than $30 \cdot 10^{-3}$ mol/l. For Nitrosomonas europea this toxicity occurs at nitrite concentrations around $5\text{-}20 \cdot 10^{-3}$ mol/l (Stein & Arp, 1998).

Some substances are always more or less toxic to the nitrifying bacteria, some of these are complex organic substances, heavy metals, pesticides, inorganic solids and surges of disinfectants such as chlorine. These have the potential of either greatly reducing the performance or causing a biological kill of the nitrifying bacteria. The results of this toxicity are either poor treatment performance or massive detachment of the biofilm (Water Environment Federation, 1996). When a toxic substance has inhibiting effect on the nitrifying bacteria, the stop of the nitrification process will not take place instantaneously but over a period of several weeks. Nitrifying bacteria is however not more sensitive to toxic substances than other bacteria (Henze et al., 2002).

3.3.4 Concentration of substrates and other vital substances

Like already mentioned a substrate is a substance the bacteria use to get energy. For the ammonium oxidizing bacteria the substrate is ammonium and for the nitrite oxidizing bacteria the substrate is nitrite. Closely related to the substrate is the substance from which the bacteria get their carbon. In the case of nitrifying bacteria this carbon source is carbon dioxide (Lydmark, 2004b). Phosphate is another important substance that is also needed for the survival of the nitrifying bacteria. Some of the trace elements needed for the growth and activity of the nitrifying bacteria are magnesium, molybdenum, calcium, copper and iron. The presence of these substances is a basic condition for nitrification to take place and their concentrations affect the nitrification rate (Hem et al., 1994).

3.3.5 Oxygen concentration

The supply of oxygen affects the nitrification. An excess of oxygen is necessary for a well functioning nitrification process. If the oxygen concentration in the water is too low it might inhibit the nitrification. The nitrifying bacteria are more sensitive to low oxygen concentrations than the heterotrophic bacteria. The oxidation of ammonium is often the rate-limiting step in the overall nitrifying process since the ammonium oxidation requires more oxygen then the oxidation of nitrite. The oxygen and substrate effect on the growth rate of the nitrifying bacteria can be described by a Monod expression:

$$\mu_{obs} = \mu_{max} \frac{c_s}{c_s + K_{s,s}} \cdot \frac{c_{O_2}}{c_{O_2} + K_{s,O_2}} \qquad [3.2]$$

μ_{obs} = observed nitrifying bacteria growth rate [d^{-1}]
μ_{max} = maximum nitrifying bacteria growth rate [d^{-1}]
c_s = substrate concentration in the reactor [g/m^3]
c_{O_2} = oxygen concentration in the reactor [g/m^3]
$K_{s,s}$ = saturation constant for substrate [g/m^3]
K_{s,O_2} = saturation constant for oxygen [g/m^3]

The saturation constant for oxygen depends on the biofilm thickness and on the temperature as it reflects diffusional limitations for oxygen into the biofilm (Henze et al., 2002).

3.3.6 Temperature

The rate of a chemical reaction is increased with increasing temperature. This is true also for biochemical reactions such as the nitrification process, which in the bacterial cell use enzymes as catalysts. At high temperatures enzymes are damaged. The with temperature increasing reaction rate for a biochemical process, is counteracted by the with temperature increasing enzymatic destruction. The sum of the effects gives a curve for the reaction rate with an optimum in reaction rate at a certain temperature. Microorganisms with an optimum around 15-20 ºC are called psychrophilic and the once with optimum around 30-35 ºC and 50-55 ºC are called mesophilic and thermophilic respectively (Svenska kommunförbundet, 1978). The dependency of the temperature on the nitrification rate can be expressed as:

$$r_{max,T} = r_{max,T_0} \cdot 10^{(\kappa_T (T-T_0))} \qquad [3.3]$$

$r_{max,T}$ = maximum reaction rate at temperature T [g/(m^2·d)]
r_{max,T_0} = maximum reaction rate at temperature T_0 [g/(m^2·d)]
T = temperature [ºC]
T_0 = reference temperature [ºC]
k_T = temperature coefficient [ºC^{-1}]

In biofilm reactors, the temperature coefficient is 0.01-0.05 ºC^{-1} (Hem et al., 1994). The expression applies in the temperature range 10-22 ºC. At higher temperatures around 30-35 ºC the growth rate is constant and between 35-40 ºC it starts to decline towards zero (Henze et al., 2002). In municipal wastewaters the temperatures are almost always under 15-20 ºC and the optimum in temperature lies between 35-40 ºC for nitrification, see figure 3.3. This means that the temperatures in the wastewaters are almost always under the optimal temperatures. This does not matter if the load of ammonium to a trickling filter for instance, is limiting the nitrification rate, but if the load is high the temperature is lowering the nitrification rate in the trickling filter (Svenska kommunförbundet, 1978).

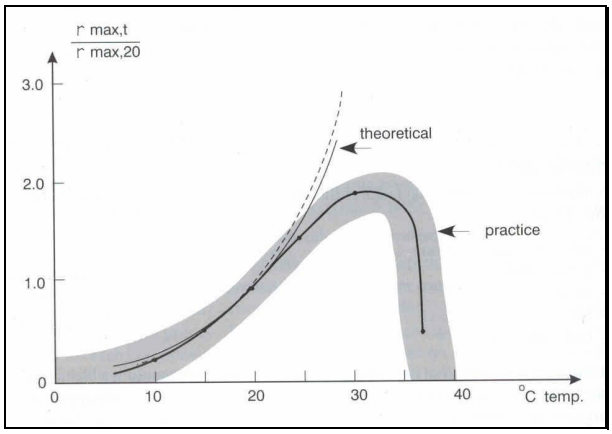

Diagram 3.3 The nitrification rate's dependency of the temperature, T_0 = 20 ºC (Henze et al., 2002).

Nitrifying bacteria are sensitive to sudden variations in temperature. When the temperature rise is fast the increase in growth rate is lower than expected and a sudden temperature drop gives a much higher decline in activity than could be expected (Henze et al., 2002).

The temperature will affect the growth rate of ammonium and nitrite oxidizing bacteria in different ways, e.g. ammonia oxidizing bacteria have higher growth rates than nitrite oxidizing bacteria at high temperatures (Ruiz et al., 2003).

3.4 NITRIFICATION KINETICS

Biofilms have a low efficiency of the biomass due to the fact that the substances must be transported through the biofilm to reach the bacteria. Not all substances reach the deepest parts of the biofilm (Henze et al., 2002). For example, the oxygen penetration depth in biofilms generally ranges from 100-200 µm and the biofilm can sometimes be even thicker (Lazarova et al., 1998). The nitrification process is a redox process, which

mainly requires two substances, an oxidant and a reductant i.e. oxygen and ammonium (Henze et al., 2002). Most of the time either oxygen or ammonium is limiting for the nitrification to occur if the alkalinity is in excess and there is no organic load. If there is organic matter in the water, heterotrophic bacteria will dilute the density of nitrifiers in the aerobic part of the biofilm and at high organic loads no nitrification of importance is likely to occur, since the heterotrophic bacteria will outrival the nitrifying bacteria (Hem et al., 1994).

The rate of reaction is often found to be proportional to the molar concentrations of the reactants raised to a simple power. An experimentally determined equation of this kind is called the rate law of the reaction. A rate law provides a basis for the classification of reactions according to their kinetics. The classification of reactions is based on their order, the power to which the concentration of species is raised in the rate law. For a first order of reaction the concentration of species is raised to the power of one, for a half order of reaction the concentration of species is raised to the power of one half and for a zero order reaction the concentration of species is raised to the power of zero. A zero order reaction is independent of the concentration of the species raised to the power of zero provided that some amount of the species is present (Atkins, 2001).

When the oxygen to ammonium mass concentration ratio is less than two, oxygen is rate limiting for the nitrification. When the oxygen to ammonium ratio is larger than five, ammonium is rate limiting for the nitrification. The shift from ammonium being rate limiting to oxygen being rate limiting occurs at different oxygen to ammonium ratios depending on the oxygen concentration in the water. For example this happens approximately at an oxygen to ammonium ratio of 2.7 when the oxygen concentration is 9-10 mg/l and approximately at an oxygen to ammonium ratio of 3.2 when the oxygen concentration is 6 mg/l. When the oxygen is rate limiting, the oxygen concentration has a great influence on the nitrification rate. The nitrification rate will have half order reaction rates with respect to oxygen for purely nitrifying biofilms, however if liquid film diffusion through stagnant liquid layer becomes dominating, the reaction will approach first order. Diffusion through the boundary layer and/or the biofilm is an important rate limiting mechanism when there is a shortage of oxygen, resulting in a nitrification rate that is a first order function of the oxygen concentration. When ammonium is rate limiting the nitrification rate is between a half order and a first order function of the ammonium concentration (Hem et al., 1994). Experiments carried out by Lazarova et al. (1998) show almost the same results. Their experiments indicate a gradual transition from a first order reaction at lower oxygen concentrations to a half order reaction at medium oxygen concentrations to a zero order reaction at higher oxygen concentrations. According to the data reported by Lazarova et al. (1998) the transition from ammonium to oxygen limiting conditions occurs for oxygen to ammonium concentration ratios between 1.2-2. These values are lower than the values reported by Hem et al. (1994). According to Lazarova et al. (1998) this may be due to the high turbulence in the MBBR used for the experiments, resulting in a thinner biofilm, thus minimizing the external and internal resistances to oxygen mass transfer.

Hem et al. (1994) found that when oxygen is rate limiting for the nitrification reaction, the reaction rate for nitrification was found to be a first order of reaction:

$$r = 0.28 \cdot c_{b,O_2}{}^{1} \qquad [3.4]$$

r = reaction rate per area and time [g NH_4^+-N/(m^2·d)]
c_{b,O_2} = bulk oxygen concentration [g/m^3]
0.28 = k [m/d]

The coefficient k, which is characteristic of the reaction being studied, is called the rate constant. The rate constant is independent of the concentrations of the species taking part in the reaction but depends on the temperature. The units of k are always such as to convert the product of concentrations into a rate expressed as a change in concentration divided by time (Atkins, 2001). Hem et al. (1994) found that when ammonium is rate limiting for the nitrifying reaction, the reaction rate for nitrification was found to be between a first and a half order of reaction:

$$r = 1.1 \cdot c_{b,NH_4^+-N}^{0.7} \qquad [3.5]$$

c_{b,NH_4^+-N} = bulk ammonium concentration [g/m^3]
1.1 = k [$g^{1/0.7}$/(d·$m^{2/2.1)}$]

If the transport of a substance, needed in the nitrification process, to the nitrifying bacteria is faster than the consumption of the substance, then the consuming reaction rate is limiting the bacterial growth. If on the other hand the substance is consumed instantaneously when it reaches the nitrifying bacteria, the transport of the substance is limiting the bacteria growth. This might be due to slow transport through the biofilm and the surrounding boundary layers or low concentrations of the substance in the liquid bulk or both (Fogler, 2001).

4. MATERIALS AND METHODS

4.1 THE LABORATORY-SCALE PLANT

Before deciding whether or not to build the additional nitrification step at the Rya WWTP the capacity for nitrification in water with such low oxygen concentrations as will be required to maximize the denitrification efficiency and to minimize the dosage of carbon, had to be investigated. Experiments made by Hem et al. (1994) in a similar system as the one used in this study have shown that it is possible to have some nitrification even at low oxygen concentrations. What had to be investigated was how the wastewater at the Rya WWTP behaves under similar conditions, since the compositions of different wastewaters never are exactly the same. Another aspect to be investigated was the effect of the ammonium concentration on the nitrification rate. These investigations were to be made to find out whether or not it is profitable to build the additional nitrification step, and if profitable, how to build it in the best way concerning carrier element volume and sludge liquor flow (Mattsson, 2004).

For these investigations a laboratory-scale plant was built mainly consisting of two feed water containers and two containers for nitrification rate measurements. The laboratory-scale plant was built with the containers placed in parallel lines, so that two different experiments could be carried out simultaneously. Before the start of each experiment, the water needed for that experiment was mixed in one of the two 60 l water containers. At the Rya WWTP there is a nitrification pilot MBBR where nitrification is studied. This MBBR consists of four compartments in which two different kinds of carrier elements are moving along with the water in the reactor. The movement is produced by coarse-bubble aeration. In compartment one and three the carrier elements consists of so called Kaldnes chips and in compartment two and four the carrier elements consists of Kaldnes carrier elements K1. It is on these different kinds of carrier elements the nitrifying bacteria are attached in a biofilm covering the carrier elements. It was from the fourth compartment of this reactor water was taken and mixed in different proportions with sludge liquor. The addition of sludge liquor served the purpose of adding ammonium to the water, since the sludge liquor is very ammonium rich. This mixture of sludge liquor and pilot plant effluent water constituted the feed water mixture to the water containers. The full-scale additional nitrification step will be fed with effluent water from the trickling filters. For practical reasons, the effluent water from the full-scale trickling filters was not used for these experiments. For the experimental results it would not matter whether the feed water for the experiments was taken from the trickling filters or the pilot plant since the influent water to both of these is the same and the treatment of the water within each of them is similar. The water containers were aerated to keep a saturated oxygen concentration in the water mixtures. From each of these containers the water mixtures were pumped with a peristaltic pump with a flow slightly below 20 l/h to two 20 l nitrification reactors. In the nitrification reactors carrier elements, Kaldnes biofilm carrier elements K1, from the fourth compartment of the pilot MBBR were put for the experiments. These carrier elements, made of polyethylene, have a high specific surface area of 500 m^2/m^3. The nitrification reactors kept a constant total volume of water and carrier elements of 4.2 l, although the ratio between the two varied for the different experiments. After the completion of each test, the carrier elements were put back in the pilot reactor.

Figure 4.1 Kaldnes biofilm carrier elements K1 in varying stages of maturity (Koi ponds, 2004).

The carrier elements and the water mixtures in the nitrification reactors were stirred with pitched blade stirrers. The stirrer impellers had a diameter of 100 mm. The nitrification reactors were covered with lids and inert nitrogen gas was constantly spurted into the head spaces over the water mixture surfaces to prevent oxygen from the air to be transported down in the water mixtures. To avoid the build up of a high overpressure of gas in the head spaces, a small hole in the lids to each of the containers was made. The hole was covered with a soft piece of plastic material that could flap up to let out gas from the containers. It was through this hole that samples of the water were taken and the oxygen concentration and temperature were measured. From the bottom of the nitrification reactors the water mixtures were led out with a flow equal to the inlet flow. The outgoing water mixtures went through tubes bent to form siphons. The upper parts of the tubes were in the same heights as the water surfaces in the nitrification reactors, when they were filled with water mixture and carrier elements and the stirring was on. The effluent water from the nitrification reactors was led to the drains. To prevent the effluent tubes from being filled with water leading to draining of the water in the nitrification reactors, smaller tubes were inserted into the main tubes. These smaller tubes were placed vertical and had one end open into the air. A flowchart of the laboratory-scale set up is shown in figure 4.2 and a picture of the entire laboratory-scale plant in figure 4.3.

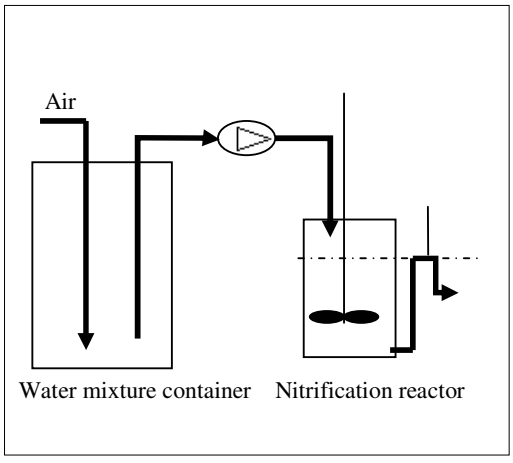

Figure 4.2 Flowchart of one line in the laboratory-scale plant.

Figure 4.3 The laboratory-scale plant with the water containers in the back, the nitrification reactors in the front and the pump in the upper right corner.

The nitrification capacity was investigated during different concentrations of oxygen and ammonium in the water in the nitrification reactors. To vary the ammonium concentration in the nitrification reactors, the sludge liquor addition to the water mixture was varied. The more sludge liquor added the higher the ammonium concentration. The water mixtures in the water containers were aerated, resulting in a saturation of oxygen

in the water mixtures. By adding different amounts of carrier elements to the nitrification reactors, the oxygen concentration in these reactors varied. The more carrier elements added to the nitrification reactors, the more biofilm containing nitrifying bacteria was added. Hence, the more nitrifying bacteria added, the higher the oxygen consumption and the lower the concentration of oxygen in the water.

4.2 FEED WATER TEST

4.2.1 Performance

Before the laboratory-scale plant was built, a test was made to see if anything happens in time with the composition of the nitrogen compounds ammonium, nitrite and nitrate in a water mixture with the same proportions of sludge liquor and water from the pilot moving bed biofilm reactor's fourth compartment, as the feed water mixture that would be used in the laboratory-scale plant. Two different test mixtures were prepared and the mixtures were left to stand for five hours. To find out in what proportions the water and the sludge liquor were to be mixed for the test, a rough estimation was done over in what ammonium concentration range the laboratory-scale plant experiments would be carried out. In the laboratory-scale nitrification reactors the oxygen in the influent water mixture would be the only oxygen accessible for the nitrifying bacteria. Therefore the maximal ammonium consumption possible in the nitrification reactors would be proportional to the amount of oxygen in the water mixtures. The dissolved oxygen concentration in the water mixtures entering the nitrification reactors could be estimated from tables on oxygen solubility at different temperatures (Unisens, 2004). The oxygen concentration in the water mixtures was estimated to be around 8.5 mg/l considering the water temperature being somewhere near 15 ºC. This gives an oxygen mole concentration of about 266 µmoles/l. One mole of ammonium nitrogen consumes two moles of oxygen when nitrate is formed (equation 3.1 and 3.2). This gives that about 133 µmoles/l or 1.86 mg/l of ammonium nitrogen can be nitrified with the given oxygen concentration. According to laboratory data at the Rya WWTP the average ammonium nitrogen concentration in the sludge liquor the latest year is 820 mg/l. Thus, to add 1.86 mg of ammonium nitrogen to one liter of water, 2.27 ml of sludge liquor must be added. In these calculations the ammonium content in the water from the pilot plant MBBR was assumed to be zero, although in reality there is always some ammonium in that water.

For the test of the feed water mixture, two 3 l containers were filled with 2 l effluent water from the pilot plant MBBR and 4.5 ml of water was removed from each of the containers. The water in one of the containers was aerated and stirred for 15 min before about 4.5 ml of sludge liquor was added to the water. For the other container the water was not aerated but stirred for 15 min before about 4.5 ml of sludge liquor was added to the water. The aeration and stirring went on throughout the entire experiment. The water mixture that was aerated and stirred simulated the situation in the aerated water mixture containers in the laboratory-scale plant experiments, where the water mixtures would be aerated thoroughly, which would have a mixing effect. The test with the non-aerated water mixture was done as a check as to whether anything happens with a less aerated water mixture that is left to stand. Samples from each of the containers were taken every 15 min during the first hour and then once every hour until 5 h had passed. At each sampling occasion, the oxygen concentration, temperature, alkalinity, ammonium nitrogen, nitrite nitrogen and nitrate nitrogen concentrations were measured and

analyzed as described in section 4.7. The results from the analyses can be seen in appendix 2.

4.2.2 Result

From the tests it was found that some changes in the composition of nitrogen compounds occurred as can be seen in the tables of the test results in appendix 2. The analysis results for the aerated water mixture show that the ammonium concentration did not change very much. In the aerated water mixture, 0.069 mg/l of ammonium nitrogen i.e. 4.93 µmoles/l of ammonium were consumed during the 5 h the test went on. This corresponds to 3 % of the total ammonium. Simultaneously 0.037 mg/l of nitrite nitrogen i.e. 2.64 µmoles/l of nitrite and 0.510 mg/l of nitrate nitrogen i.e 36.40 µmoles/l of nitrate were formed. The consumption of ammonium and the formation of nitrite and nitrate are due to nitrification in the water mixture. This must be caused by some detached biofilm with nitrifying bacteria from the pilot plant floating around in the water used for the test.

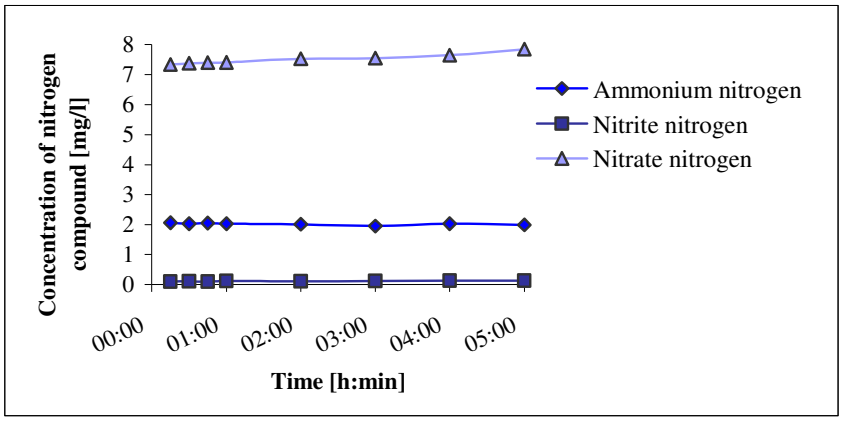

Diagram 4.1 The nitrogen compounds' concentrations over time for the aerated water mixture test.

In the non-aerated water mixture, 0.251 mg/l of ammonium nitrogen i.e. 17.92 µmoles/l of ammonium were consumed during the 5 h the test went on. This molar consumption gives that 13 % of the total ammonium was consumed during the test. During the same period of time 0.070 mg/l of nitrite nitrogen i.e. 5.00 µmoles/l of nitrite and 0.231 mg/l of nitrate nitrogen i.e. 16.49 µmoles/l of nitrate were formed.

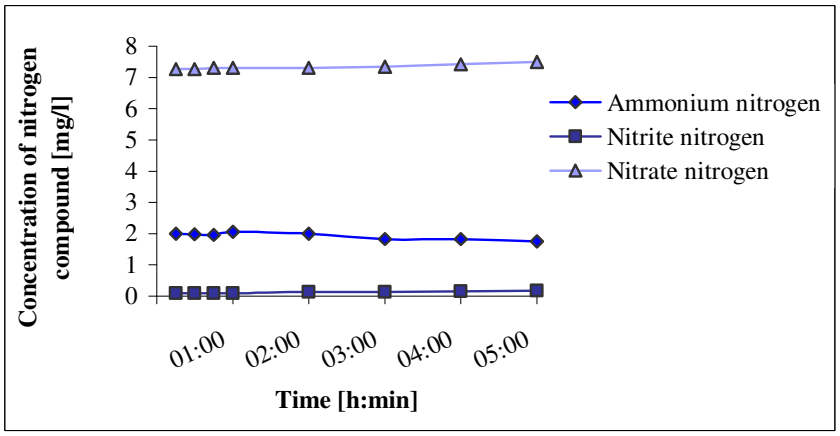

Diagram 4.2 The nitrogen compounds' concentrations over time for the non-aerated water mixture test.

According to the test results for the aerated water mixture, 34.11 µmoles/l or 0.48 mg/l more nitrite and nitrate nitrogen were formed than should be formed based on the consumption of ammonium. For the non-aerated water mixture the corresponding amount of surplus nitrite and nitrate nitrogen formed was 3.5 µmoles/l or 0.05 mg/l, respectively. This cannot be true, since the amount of ammonium consumed is supposed to be equal to the amount of nitrite and nitrate formed. When analyzing ammonium with the FIAstar-instrument, as described in section 4.7, a deviation of ± 0.025 mg/l of ammonium nitrogen is to be counted on according to Foss Tecator (2000a). The deviation when analyzing nitrite is ± 0.15 mg/l of nitrite nitrogen according to Foss Tecator (2000b) and the deviation when analyzing the sum of nitrite and nitrate is ± 0.01 mg/l of nitrite and/or nitrate nitrogen according to Foss Tecator (2000c). With these deviations included in the test results for the aerated and non-aerated water mixtures the extra moles of formed nitrite and nitrate can partly be explained as a consequence of the margin of error for the analyses. The extra moles of formed nitrite and nitrate may also be due to sources of error related to the sampling procedure. However these deviations of reacted and formed nitrogen compounds are very small.

Even though the composition of the nitrogen compounds varied during the test, the change in composition was considered low enough to make it acceptable to build the laboratory-scale plant like planned with the water mixtures needed for the entire experiment in question prepared in the feed water containers before the start of each test. Since samples of the water mixtures were to be taken at each sampling occasion, the current composition in the water mixture would always be measured. Too high nitrite concentrations may inhibit the nitrification, but the nitrite concentrations in the water mixtures were low and they gave no indications that nitrite would occur in high enough concentrations in the water mixtures in the laboratory-scale plant to affect the nitrification process.

As can be seen in appendix 2 the alkalinity was never close to zero for the water mixtures. It was around 1.4-1.6 meqv CO_3^{2-}/l, which indicates that if the water mixtures would be added to the nitrification reactors no drastic pH drop followed by inhibition of the nitrification would occur. In appendix 2 it can also be seen that the temperature decreased for the aerated water mixture as a consequence of the aeration with cold air. With decreasing water temperatures increasing dissolved oxygen concentrations followed as can be seen from the test results. The temperature increased for the non-aerated water mixture as it was heated by air in the room in which the experiment was carried out (air used for aeration was not taken from the same room as the experiments were performed in). With the increasing water temperatures followed decreasing dissolved oxygen concentrations as can be seen from the test results. None of these test results concerning dissolved oxygen, temperature or alkalinity indicated that these factors should cause any problems in the laboratory-scale plant experiments. From this test it was concluded that the laboratory-scale plant could be built in the way it was planned to.

4.3 PRELIMINARY TEST OF THE LABORATORY-SCALE PLANT

4.3.1 Performance

A test was made to find out how the laboratory-scale plant behaves when it is running. One of the nitrification reactors was tested. To simplify the test, water from the first instead of the fourth compartment of the pilot plant MBBR was used. Since this water is

ammonium rich no sludge liquor was added to it. For this test carrier elements were taken from the second compartment of the pilot MBBR. An earlier test had shown that 1 l of carrier elements from the second compartment of the pilot moving bed reactor weigh around 300 g and that when 1 l of these carrier elements was filled up with water to the surface of the carrier elements, 0.65 l of water was roomed. This gives the formula:

$$V_{total} = V_{water} - 0.65 \cdot V_{carrier\ elements} + V_{carrier\ elements} \qquad [4.1]$$

V_{water} = water volume [m^3]
$V_{carrier\ elements}$ = carrier element volume [m^3]
V_{total} = obtained volume when water and carrier elements are mixed [m^3]

From this formula the carrier element volume was decided for 50 % filling of the total volume. With a water mixture volume of 10 l in the nitrification reactor, just over 6 l or 1820 g carrier elements give a 50 % filling of the total volume, which was 12 l. To measure up the carrier elements they were weighed instead of measured volumetrically, since weighing is a faster and more precise method. The impeller that stirred the water mixture and the carrier elements in the nitrification reactor was set at a speed of about 268 rounds per minute (rpm). The test went on for 3.5 h and samples were taken every 30 min.

4.3.2 Result

The results from the test of the laboratory-scale plant showed that it worked as planned. The analytical data from the test are shown in appendix 3. As shown in diagram 4.3 the composition of nitrogen compounds in the feed water mixture remained relatively constant throughout the experiment, except for a small jump in concentration of the nitrogen compounds after 3 h. This was due to a refill of the water mixture after the 2.5 h sampling. When the ammonium, nitrite and nitrate concentrations in the nitrification reactors started to level out steady state had been reached (diagram 4.4). It took approximately three to four hydraulic retention times, i.e. 1.5-2 h of running of the plant, before this started to happen. A change in concentration of ammonium, nitrite and nitrate is noticed after 3 h and this change is due to the feed water mixture. Other small variations in concentrations according to laboratory data could be due to analytical errors and/or heterogenic samples taken at the different occasions of sampling.

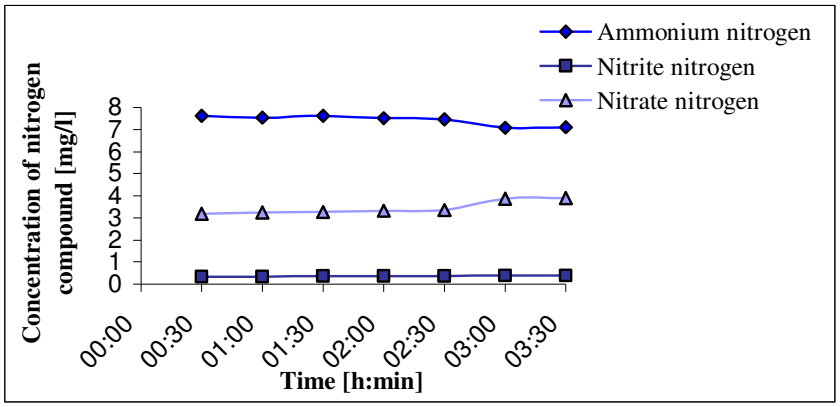

Diagram 4.3 The nitrogen compounds' concentrations over time for the water mixture in the water container.

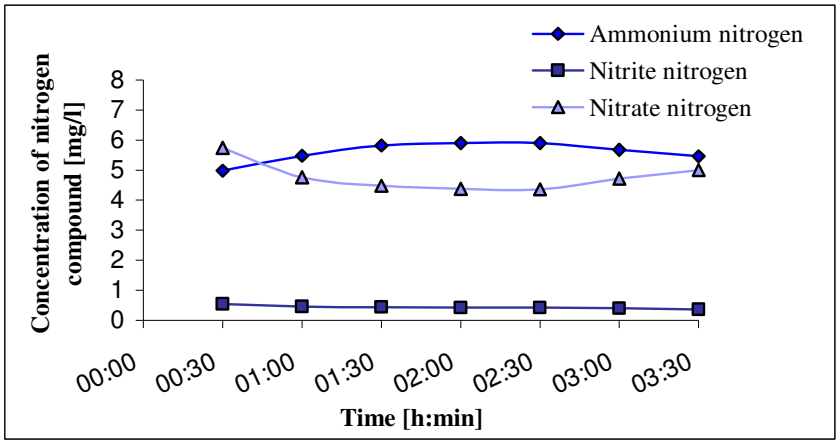

Diagram 4.4 The nitrogen compounds' concentrations over time for the water mixture in the nitrification reactor.

The pump flow capacity was adjusted before the test of the laboratory-scale plant to be somewhere around 20 l/h but the several tests made to make the adjustments showed that it was difficult to sustain a constant flow. From these tests an average flow of around 19.5 l/h was obtained. The flow changed with time even though all the adjustable parameters such as choice of tubes and pump speed were kept constant. The change in flow might have been due to the fact that the water mixture was aerated and the pump may have pumped water with air bubbles causing different flows depending on the amount of bubbles within the water. Another reason to the uneven pumping might be the pump itself, which might not be able to keep a constant flow. The changes of the flow were not very big, but wrong pump flow used in calculations give an unnecessary calculation error of the nitrification capacity. Therefore it was decided to always measure the pump flow after each finished experiment to make sure the flow obtained for each experiment was used in the calculations made for the experiment in question.

The total volume of the water and the carrier elements in the nitrification reactors was kept constant during the test and the water flow was measured to be 20.2 l/h, giving a nitrification rate around 0.26 g NH_4^+-N/(m^2·d). In the beginning of the experiment the nitrification rate was larger than 0.26 g NH_4^+-N/(m^2·d), which must be due to the higher oxygen concentration in the water before steady state was reached. The oxygen to ammonium nitrogen ratio in the nitrification reactor was calculated. It was found to be less than two during the entire experiment. According to Hem et al. (1994) the nitrification rate is equal to the oxygen concentration multiplied by 0.28 when the oxygen to ammonium nitrogen ratio is less than two. The calculated nitrification rate according to Hem et al.'s formula was around 0.19 g NH_4^+-N/(m^2·d), which is lower than the obtained nitrification rate in this study. One reason to the difference could be that the mass transport through the biofilm in this experiment was larger than in Hem et al.'s experiments. It is also possible that the biofilm area on the carrier elements is larger than 500 m^2/m^3, which is the reported efficient biofilm surface area for Kaldnes K1 carrier elements and also the area used for the calculations in this experiment. However, the difference between the obtained nitrification rate in the test of the laboratory-scale plant and the one according to Hem et al.'s formula (1994) is very small and could be within the margin of error for the formula. The obtained nitrification rate decreased a little during the experiment even after steady state was reached, this

could be due to some detachment of biofilm from the carrier elements caused by the impeller's rotation in the mixture of water and carrier elements in the nitrification reactor. From this it was decided to lower the rotational speed of the impeller for the upcoming experiments to about 155 rpm.

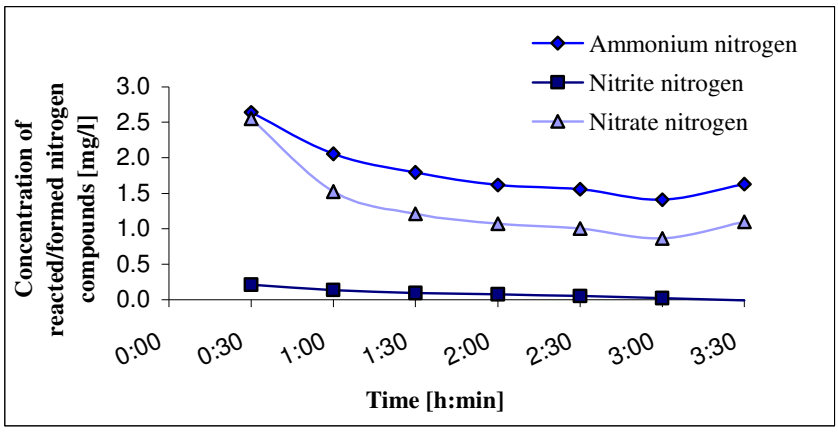

Diagram 4.5 Reacted ammonium nitrogen and formed nitrite and nitrate nitrogen in the nitrification reactor.

4.4 CHOICE OF THE LEVELS FOR THE FACTORIAL DESIGN

Experiments have been carried out by Hem et al. (1994) where nitrification rates were studied in different environments in which either oxygen or ammonium or both were equally limiting the nitrification process. In their paper (Hem et al., 1994) a diagram was presented in which the nitrification rate to ammonium nitrogen concentration ratio (r/NH_4^+-N) was plotted as a function of the nitrification rate to oxygen concentration ratio (r/O_2). This diagram is presented below.

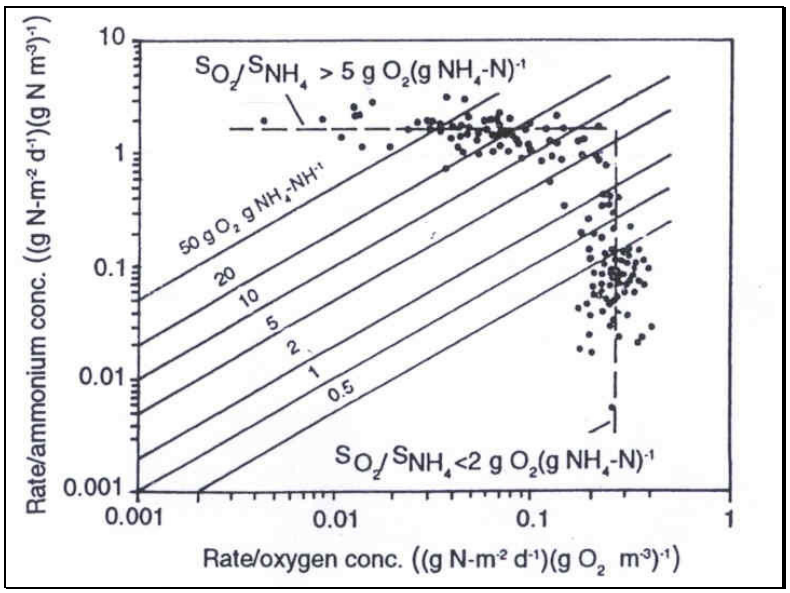

Diagram 4.6 The nitrification rate to ammonium nitrogen concentration ratio as a function of the nitrification rate to oxygen concentration ratio (Hem et al., 1994).

In order to get an estimation of what nitrification rates that can be obtained for different combinations of ammonium nitrogen and oxygen concentrations, the different dots in

diagram 4.1 were read off carefully. For the reading of each dot one r/NH_4^+-N value and one r/O_2 value was given. The r/NH_4^+-N values were plotted as a function of the r/O_2 values in a non-logarithmic diagram. Lines for some different ratios of oxygen to ammonium nitrogen concentrations were plotted as well.

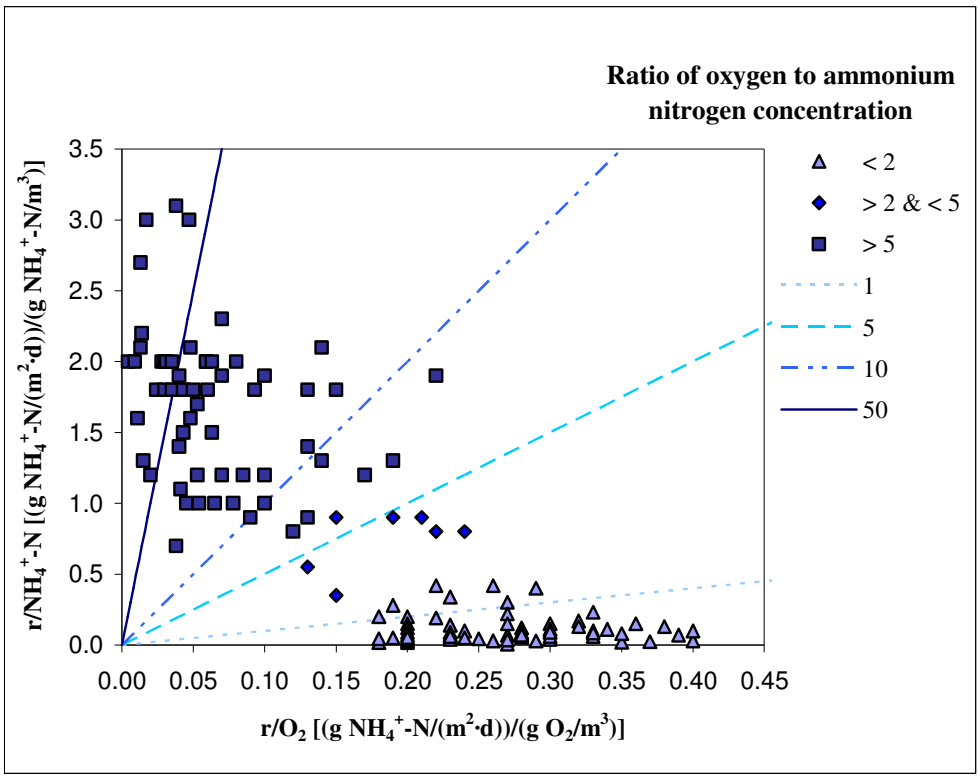

Diagram 4.7 The nitrification rate to ammonium nitrogen concentration ratio as a function of the nitrification rate to oxygen concentration ratio.

From this diagram further readings were done. A table over different combinations of ammonium nitrogen and oxygen concentrations was made and for the different combinations the oxygen to ammonium nitrogen concentrations ratios were calculated. For the obtained oxygen to ammonium nitrogen ratios the nitrification rates were estimated by diagram 4.7 trying to find the average value of r/NH_4^+-N and r/O_2 at the oxygen to ammonium nitrogen ratio in question. Since this was done for known values of the oxygen and ammonium concentrations, two nitrification rates could be calculated for each combination of oxygen and ammonium nitrogen concentration by multiplying the read average values of the r/NH_4^+-N and r/O_2 ratios with the ammonium nitrogen and the oxygen concentration, respectively. An average nitrification rate was calculated for each combination of oxygen and ammonium nitrogen concentrations. These two nitrification rates should be the same but because of the sources of error in trying to read off the correct values in the diagrams 4.6 and 4.7, they were not always equal. However the difference between the two nitrification rates for each oxygen and ammonium nitrogen concentration was never very large. As an example of the process of finding the nitrification rates the following example can be given. For the ammonium concentration 0.25 mg/l and the oxygen concentration 0.25 mg/l the oxygen to ammonium nitrogen ratio is 1. In diagram 4.7 the average r/O_2-value at the line for oxygen to ammonium nitrogen ratio equal to 1 is estimated to be 0.25 (g NH_4^+-N/(m^2·d))/(g O_2/m^3) and the average r/NH_4^+-N-value is estimated to be

0.25 (g NH_4^+-N/(m^2·d))/(g O_2/m^3). By multiplying the r/O_2 value with the oxygen concentration i.e. 0.25 mg/l a nitrification rate of 0.063 g NH_4^+-N/(m^2·d) is obtained. Another nitrification rate of 0.063 g NH_4^+-N/(m^2·d) is obtained if the same thing is done for the r/NH_4^+-N value and the ammonium nitrogen concentration i.e. 0.25 mg/l. In this case these two nitrification rates were the same. For the combinations of oxygen and ammonium nitrogen concentrations where this was not the case, the average value of the two nitrification rates was calculated. In the table below are the obtained nitrification rates for different oxygen and ammonium nitrogen concentrations showed.

Table 4.1 Nitrification rates [g NH_4^+-N/(m^2·d)] for different combinations of ammonium nitrogen and oxygen concentrations [mg/l].

O_2 \ N	**0.25**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**5.00**	**7.00**	**9.00**
0.25	0.06	0.07	0.07	0.08	0.08	0.07	0.08	0.08	0.11	0.14	0.17
0.50	0.11	0.13	0.14	0.13	0.14	0.16	0.15	0.17	0.15	0.18	0.21
0.75	0.15	0.17	0.19	0.21	0.20	0.22	0.22	0.24	0.26	0.22	0.25
1.00	0.18	0.22	0.25	0.25	0.28	0.27	0.28	0.28	0.31	0.30	0.29
1.50	0.24	0.29	0.33	0.35	0.38	0.42	0.40	0.40	0.44	0.45	0.51
2.00	0.28	0.35	0.41	0.44	0.50	0.50	0.54	0.55	0.56	0.61	0.58
2.50	0.30	0.40	0.47	0.51	0.56	0.58	0.63	0.66	0.66	0.74	0.77
3.00	0.34	0.48	0.53	0.58	0.66	0.70	0.71	0.75	0.80	0.81	0.84
5.00	0.36	0.60	0.79	0.80	0.94	1.03	1.10	1.12	1.25	1.33	1.39
7.00	0.40	0.68	0.89	1.06	1.17	1.29	1.40	1.47	1.69	1.75	1.90
9.00	0.44	0.71	1.03	1.28	1.43	1.53	1.66	1.74	1.97	2.27	2.25

From these values a surface plot over the nitrification rate as a function of the ammonium nitrogen and the oxygen concentrations was made. This diagram is shown in appendix 4. The diagram in appendix 4 was used to get an estimation of what nitrification rates that will be obtained in the additional nitrification step as at total when the influent water, from the trickling filters, contains about 8 mg/l of oxygen and the influent water flow is about 2.5 m^3/s. Calculations were made for four different percentages of filling with carrier elements; 25, 50, 75 and 100 %, giving an efficient biofilm surface area of 40 000, 80 000, 120 000 and 160 000 m^2 respectively for each reactor i.e. 240 000, 480 000, 720 000 and 960 000 m^2 for the additional nitrification step as a total. However, more than 60 % carrier element filling is not to be recommended, but the 75 % and 100 % carrier element filling examples can be used to simulate a larger additional nitrification step that with around 60 % carrier element filling obtains a biofilm area of 720 000 m^2 or 960 000 m^2 respectively. Likewise 25 % carrier element filling can be used to simulate a smaller nitrification step that with 60 % carrier element filling obtains a biofilm area of around 240 000 m^2. For each area the nitrification rates were calculated for ammonium concentrations of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/l with help of the diagram in appendix 4. The ammonium nitrogen and oxygen concentrations in the additional nitrification reactors, which are assumed to be equal to the effluent concentrations in a completely mixed reactor, can be calculated if the ammonium concentration in the influent, the biofilm area and the nitrification rate are known. By guessing a nitrification rate ammonium nitrogen and oxygen concentrations in the effluent were calculated.

$$c_{NH_4-N,out} = c_{NH_4-N,in} - \frac{r \cdot A}{\dot{V}} \qquad [4.2]$$

$c_{NH_4-N,out}$ = ammonium nitrogen concentration in the effluent [g/m^3] = [mg/l]
$c_{NH_4-N,in}$ = ammonium nitrogen concentration in the influent [g/m^3] = [mg/l]
r = nitrification rate [g NH_4^+-N/(m^2·d)]
A = efficient biofilm surface area [m^2]
$\dot{V}$ = water flow [m^3/d]

$$c_{O_2,out} = c_{O_2,in} - \frac{r \cdot A \cdot 64.00/14.01}{\dot{V}} \qquad [4.3]$$

$c_{O_2,in}$ = oxygen concentration in the influent [g/m^3] = [mg/l]
$c_{O_2,out}$ = oxygen concentration in the effluent [g/m^3] = [mg/l]

By looking at the diagram in appendix 4, it could be checked if the ammonium nitrogen and oxygen concentration in question gave the guessed nitrification rate. If so the occurring nitrification rate for the influent ammonium concentration and the biofilm area in question had been found, if not a new nitrification rate was guessed until the guessed nitrification rate gave the same effluent ammonium nitrogen and oxygen concentrations through calculations as could be found in the diagram in appendix 4. The obtained nitrification rates, oxygen and ammonium nitrogen effluent concentrations for the different influent ammonium nitrogen concentrations and the different biofilm areas are presented in table 4.2-4.5.

Table 4.2 Biofilm area of 240 000 m^2.

Ammonium-N conc., in [mg/l]	**Nitrification rate [g NH_4^+-N/(m^2·d)]**	**Ammonium-N conc., out [mg/l]**	**Oxygen conc., out [mg/l]**
0.5	0.15	0.33	7.24
1	0.55	0.39	5.21
2	0.75	1.17	4.19
3	0.85	2.06	3.68
4	0.87	3.03	3.58
5	0.90	4.00	3.43
6	0.90	5.00	3.43
7	0.90	6.00	3.43
8	0.91	6.99	3.38
9	0.92	7.98	3.33
10	0.93	8.97	3.28

Table 4.3 Biofilm area of 480 000 m^2.

Ammonium-N conc., in [mg/l]	Nitrification rate [g NH_4^+-N/(m^2·d)]	Ammonium-N conc., out [mg/l]	Oxygen conc., out [mg/l]
0.5	0.15	0.17	6.48
1	0.35	0.22	4.44
2	0.52	0.84	2.72
3	0.55	1.78	2.41
4	0.57	2.73	2.21
5	0.58	3.71	2.11
6	0.58	4.71	2.11
7	0.59	5.69	2.01
8	0.59	6.69	2.01
9	0.59	7.69	2.01
10	0.59	8.69	2.01

Table 4.4 Biofilm area of 720 000 m^2.

Ammonium-N conc., in [mg/l]	Nitrification rate [g NH_4^+-N/(m^2·d)]	Ammonium-N conc., out [mg/l]	Oxygen conc., out [mg/l]
0.5	0.11	0.13	6.32
1	0.25	0.17	4.19
2	0.40	0.67	1.90
3	0.42	1.60	1.60
4	0.43	2.57	1.45
5	0.43	3.57	1.45
6	0.43	4.57	1.45
7	0.43	5.57	1.45
8	0.43	6.57	1.45
9	0.44	7.53	1.30
10	0.44	8.53	1.30

Table 4.5 Biofilm area of 960 000 m^2.

Ammonium-N conc., in [mg/l]	Nitrification rate [g NH_4^+-N/(m^2·d)]	Ammonium-N conc., out [mg/l]	Oxygen conc., out [mg/l]
0.5	0.11	0.01	5.77
1	0.22	0.02	3.53
2	0.32	0.58	1.50
3	0.33	1.53	1.30
4	0.34	2.49	1.09
5	0.34	3.49	1.09
6	0.34	4.49	1.09
7	0.34	5.49	1.09
8	0.34	6.49	1.09
9	0.34	7.49	1.09
10	0.34	8.49	1.09

For every kilo of free oxygen consumed in the additional nitrification step about 1.17 l or 0.92 kg of methanol, with a COD-value of 1.2 kg COD/l, is saved in the subsequent denitrification step (Mattsson, 1997). In diagram 4.8-4.11 are the total effluent flows of ammonium nitrogen and oxygen from the additional nitrification step and the

accompanying methanol saving for the four different biofilm areas presented as a function of the influent ammonium nitrogen concentration.

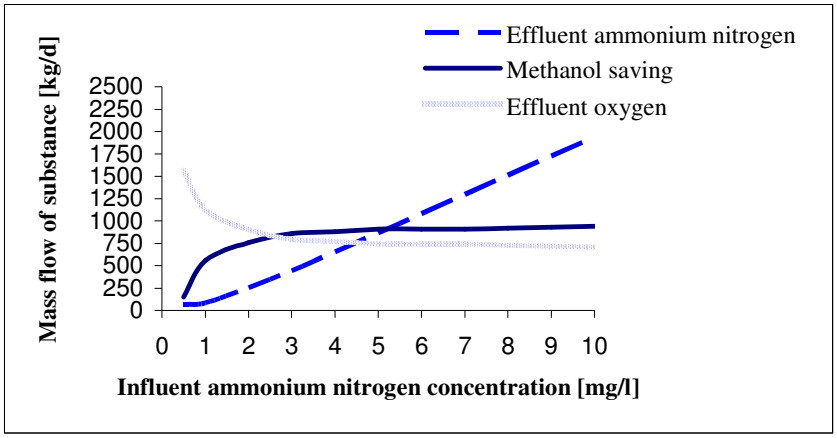

Diagram 4.8 Biofilm area of 240 000 m^2.

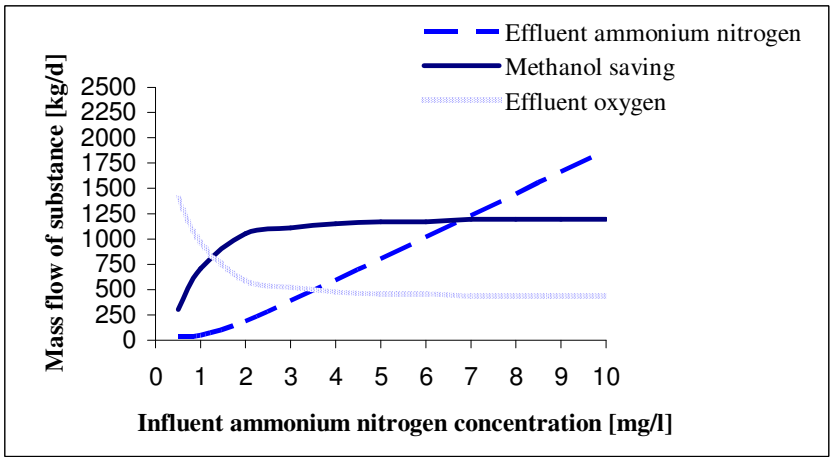

Diagram 4.9 Biofilm area of 480 000 m^2.

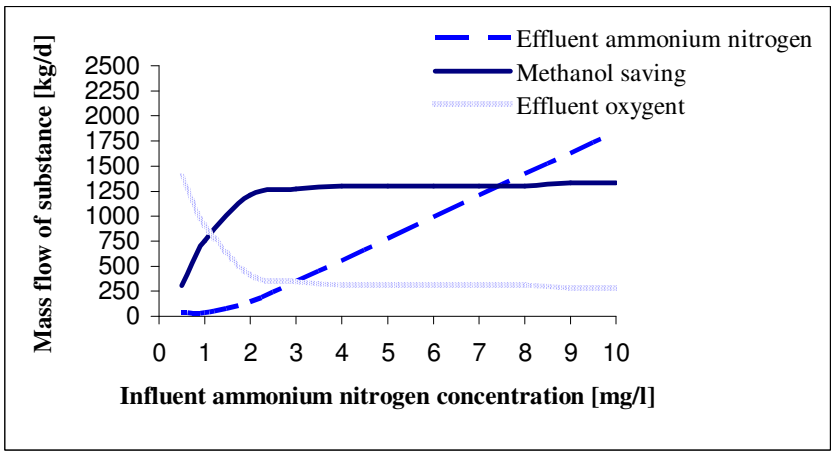

Diagram 4.10 Biofilm area of 720 000 m^2.

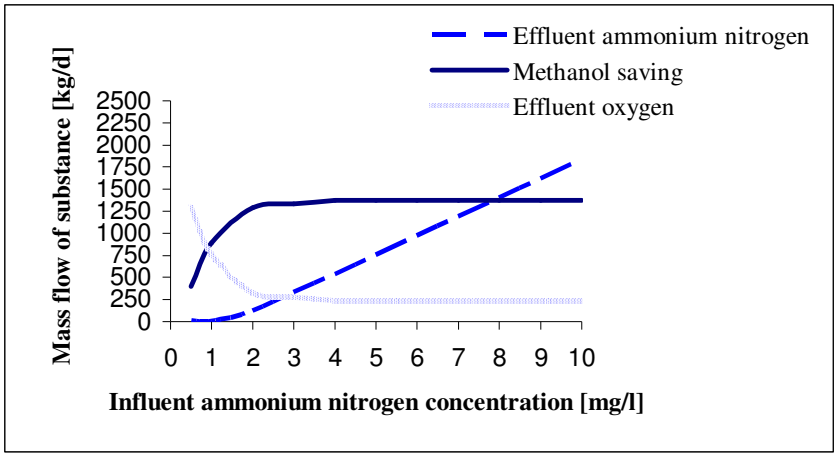

Diagram 4.11 Biofilm area of 960 000 m^2.

In these diagrams it can be seen that for all four biofilm areas a relatively high nitrification rate is obtained at an ammonium nitrogen concentration in the influent of around 2 mg/l. For higher ammonium nitrogen concentrations almost the same nitrification rates are obtained. However, the higher the ammonium nitrogen concentration in to the additional nitrification step, the higher the discharge of ammonium. The additional nitrification and denitrification steps are supposed to lower the nitrogen discharge and therefore the ammonium discharge from the additional nitrification step cannot be very large. From this it can be concluded that the ammonium nitrogen concentration in the influent to the additional nitrification step should be between 1-2 mg/l. By comparing the methanol savings for the different carrier element filling volumes it can be seen that for 25 % filling, the methanol saving reaches a maximum around 900 kg/d and for the other filling volumes this saving lies around 1200-1400 kg/d. From this it can be concluded that the carrier element filling volume for the additional nitrification step should be around 50 %. For less filling, less methanol is saved, for more filling the increase in methanol saving is quite little per increase in biofilm area. Having made these two conclusions it was of interest to test the nitrification capacity for these conditions, i.e. 50 % filling and 2 mg/l of ammonium nitrogen in the influent, in the laboratory-scale plant.

For 50 % carrier element filling volume and with 2.0 mg/l of ammonium nitrogen and 8.0 mg/l of oxygen in the influent to the additional nitrification step, the nitrification rate can be estimated to 0.52 g NH_4^+-N/(m^2·d), the ammonium nitrogen concentration and the oxygen concentration in the effluent somewhere around 0.84 mg/l and 2.7 mg/l, respectively. Factorial design was to be used to asses the influence of the two factors ammonium nitrogen and oxygen concentration on the nitrification rate. Two factors at two levels, i.e. 2^2-factorial design was applied. The two variables used to change the conditions in the nitrification reactors were ammonium nitrogen concentration in the inlet flow and carrier element filling volume in the nitrification reactors. By varying the carrier element filling volume the oxygen concentration in the nitrification reactors was varied, the more carrier elements the lower the oxygen concentration. The two levels were supposed to be one at high and one at low concentrations of ammonium nitrogen and two different carrier element filling volumes. The four different combinations of ammonium nitrogen concentrations and carrier element filling volumes were to be experimentally tested in the laboratory-scale plant. Factorial design has many benefits, such as obtaining desired information with a decreased number of experiments.

Table 4.6 Factorial design groups.

[NH4+-N] \ [O_2]	*low*	*high*
low	1	a
high	b	ab

When deciding the ammonium nitrogen and carrier element filling volume levels for the experiments, 50 % filling and 0.84 mg/l of ammonium nitrogen in the effluent were the levels that the experiments started from. The carrier element filling volume was 50 % of a total volume of 4.16 l, i.e. 2.08 l. This total volume gives the same hydraulic retention time for the laboratory-scale plant as for the planned full-scale nitrification step (equation 4.1), i.e. 11 min for a feed flow of around 19.5 l/h and 50 % filling. By knowing the ammonium nitrogen concentration in the effluent water, the carrier element volume, the water flow and the nitrification rate, the required ammonium nitrogen concentration in the influent water could be calculated from equation 4.2. The biofilm area for the carrier element volume was calculated to be 1.04 m^2 for an efficient surface area for the carrier elements of 500 m^2/m^3. The water flow into the nitrification reactors was about 19.5 l/h. This gave an ammonium nitrogen concentration of 2.00 mg/l in the influent to the laboratory-scale plant's nitrification reactor, which is the same as the estimated optimal influent ammonium nitrogen concentration in the additional nitrification step. The effluent oxygen concentration from the laboratory-scale plant was calculated to be 2.72 mg/l from equation 4.3, when the influent oxygen concentration was assumed to be 8 mg/l. This is the same as the effluent oxygen concentration at estimated influent optimal conditions for the additional nitrification step. Thus, the estimated optimal conditions in the additional nitrification step for 50 % carrier element filling and 0.84 mg/l of ammonium nitrogen in the effluent was chosen as the initial laboratory conditions for the laboratory-scale plant.

The other ammonium nitrogen level was chosen as the double influent concentration and the other carrier element filling volume was chosen as half the carrier element filling i.e. 25 % filling. Four different experiments were carried out with the two factors at two different levels (table 4.6). To estimate the nitrification rates and the ammonium nitrogen and oxygen concentrations in the effluent, the diagram in appendix 4 was used. A nitrification rate was guessed and the outgoing ammonium nitrogen and oxygen concentrations were calculated. For the obtained concentrations the nitrification rate was read off in the diagram in appendix 4 and if it was the same as the guessed nitrification rate in the calculations, the actual nitrification rate for the influent ammonium nitrogen concentration in question had been found, if not a new nitrification rate was guessed until the guessed nitrification rate gave the same effluent ammonium nitrogen and oxygen concentrations as could be found in the diagram in appendix 4. The results from these calculations are presented in table 4.7.

Table 4.7 The different combinations of levels for the factorial design.

Carrier filling [%]	Ammonium-N conc., in [mg/l]	Nitrification rate [g/(m^2·d)]	Biofilm area [m^2]	Oxygen conc., out [mg/l]	Ammonium-N conc., out [mg/l]
25	2.00	0.76	0.52	4.14	1.15
25	3.99	0.87	0.52	3.58	3.02
50	2.00	0.52	1.04	2.72	0.84
50	3.99	0.57	1.04	2.21	2.72

4.5 THE START-UP OF THE LABORATORY-SCALE PLANT

4.5.1 The preparing of the water mixtures

To obtain different ammonium concentrations in the feed water, different amounts of sludge liquor were added to the water taken from the pilot plant. The ammonium addition required is the difference between the ammonium nitrogen concentration needed and the ammonium nitrogen concentration already in the water leaving the pilot MBBR's fourth compartment, which is about 0.6 mg/l according to laboratory data from the personnel at the Rya WWTP. The ammonium nitrogen concentration in the sludge liquor has been estimated to be around 820 mg/l also according to laboratory data. The required sludge liquor addition to the four different combinations of filling and ammonium nitrogen influent concentrations and the result is presented in table 4.8.

Table 4.8 The needed sludge liquor addition for the different combinations of levels.

Carrier filling [%]	Ammonium-N conc., in [mg/l]	Sludge liquor addition per liter water [ml]	Total sludge liquor addition to 60 l of water [ml]
25	2.00	1.70	102
25	3.99	4.14	248
50	2.00	1.70	102
50	3.99	4.14	248

The water mixtures were prepared by adding 60 l of water from the pilot plant to each of the water containers. After that the sludge liquor was added and the two liquids were mixed by compressed air. The increased total volume due to the added sludge liquor was neglected.

In the future additional nitrification step Kaldnes carrier elements K1 will be used. Since these are only used in the second and fourth compartment of the nitrification pilot MBBR, the carrier elements for the experiments had to be taken from one of these compartments. The reason carrier elements from the fourth compartment were used for the factorial design experiments was that the ammonium concentration in the fourth compartment (around 0.6 mg/l) was closest to the ammonium concentration that was estimated to be optimal for the additional nitrification step (0.84 mg/l). Therefor the biomass growing on the carrier elements from the pilot reactor's fourth compartment was assumed to be more similar to the one that will be obtained in the additional nitrification step, than the biomass on the carrier elements from the second compartment. The impact of the biofilm's growing environment on the nitrification capacity was studied as well by comparing the nitrification capacity for carrier elements from the second and the fourth compartment of the pilot moving bed reactor. The carrier

elements from the second compartment are used to higher ammonium concentrations than the carrier elements from the fourth compartment. The comparison was done for an ammonium concentration around 2 mg/l and 4 mg/l in the influent and 50 % carrier element filling of the total volume in the nitrification reactors. For all these experiments the carrier elements were weighed to measure up the amount needed for the experiments. One liter of carrier elements from the second compartment in the pilot MBBR weigh about 300 g and the same amount of carrier elements from the fourth compartment in the pilot MBBR weigh about 295 g.

4.5.2 The preparing of the laboratory-scale plant

Before the experiments started the carrier elements were put in the nitrification reactor and water from the last compartment of the pilot moving bed reactor was added until a total volume of 4.2 l was reached. The water mixtures were prepared and the aeration was started. The stirring of the mixture of water and carrier elements in the nitrification reactor was started and the nitrogen gas flow was turned on. Finally the pump was turned on and the experiments were started.

4.6 SAMPLING PROCEDURE

It took about three hydraulic retention times for a 50 % carrier element filling i.e. approximately 30 min according to the test of the laboratory-scale plant to reach steady state. The time to reach steady state for 25 % carrier element filling was assumed to be approximately the same. With the flows and water volumes used in the factorial design experiments this would mean that it would take somewhere between 30-60 min to reach steady state for those experiments. The first time each kind of experiment was run it went on for a total of 2.5 h during which samples were taken with 30 min intervals from the upstart until the end of the experiment. When a certain experiment was repeated, sampling was only done once the steady state had been reached. The time to reach steady state was found from the results from the first run of the experiment in question. To make sure steady state was reached when sampling was done for repeated experiments the oxygen concentration in the water in the nitrification reactors was measured three times with 15 min intervals before the samples of the water in the different containers were taken. At steady state the oxygen concentration in the water in the nitrification reactors remain stable. Some fluctuations may occur even though steady state is reached due to variations in water temperature and oxygen concentrations in the influent water.

For each experiment sampling was done in the nitrification reactors and of the feed water mixture containers in use. At each sampling a 100 ml water sample was taken from each water mixture container and two samples each of 100 ml were taken from each nitrification reactor. Immediately after sampling, the samples from the water mixture containers were placed in a freezer. The samples were stored there until they were analyzed for ammonium, nitrite and nitrate nitrogen. For the nitrification reactors double samples were taken and one of these samples from each container and sampling was put in freezer and stored there until analyzed for ammonium, nitrite and nitrate nitrogen just like the samples from the water containers. The samples from the nitrification reactors that were not frozen were analyzed for pH and alkalinity. When taking the samples from the nitrification and water containers the temperatures and oxygen concentrations were measured directly in the containers.

4.7 ANALYTICAL METHODS

4.7.1 Temperature and oxygen concentration

The nitrification rate is dependent on temperature and oxygen concentration in the water. Temperature and oxygen concentration were measured with an oximeter equipped with a built in thermometer.

4.7.2 pH and alkalinity

The nitrification rate is dependent on the pH in the water and therefore also on the alkalinity which helps to prevent a drastic pH drop when hydrogen ions are released during nitrification. The pH need to be in the range where it does not inhibit the nitrification and the higher the alkalinity the smaller the risk of a pH drop to below the inhibition limit. pH of the water in each sample from the experiments performed was measured with a pH-meter.

Alkalinity is normally analyzed through titration with sulfuric acid to a pH of 4.5 (Olsson & Newell, 1999). However, to determine the total alkalinity in the water i.e. the total contribution to the alkalinity from all the buffering compounds in the water in each sample from the experiments performed, a very simple and fast method was used. 10 ml of a solution called Orion Total Alkalinity Reagent, from Thermo Electron Corporation, was added to 100 ml of the water sample. The pH was then measured. From this pH the alkalinity in the water, expressed as calcium carbonate ($CaCO_3$), was calculated. For a more detailed description of the analysis method for alkalinity see appendix 5. When measuring up 100 ml of the water the temperature of the water was lower than 20 °C, which is the minimum temperature at which volumetric cylinders are made for to measure at. Therefore the alkalinity measurements made on the water in the experiments are not completely accurate. The real alkalinity might be slightly lower than the measured one. However, the alkalinity was only measured to make sure it was not zero and the exact value was not important to find, therefore the volumetric error of the water cannot be considered to be a problem.

4.7.3 Ammonium, nitrite and nitrate nitrogen concentration

The concentrations of ammonium, nitrite and nitrate nitrogen in the water in the experiments performed were measured to find the nitrification capacity. For this an analyzing instrument called FIAstar, from Foss Tecator, was used. FIAstar analyzes the different compositions of nitrogen compounds by photometric methods. The amount of ammonium, nitrite and sum of nitrite and nitrate nitrogen can be measured. For a more detailed description of the analysis methods for ammonium, nitrite and the sum of nitrite and nitrate nitrogen see appendix 5. The difference between the amount of the sum of nitrite and nitrate nitrogen and the amount of nitrite nitrogen gives the amount of nitrate nitrogen in a water sample.

5. RESULTS

5.1 EXPERIMENTAL RUNNING TIME

The first time each of the four different factorial design experiments were carried out, they were run for 2.5 h and sampling was done each 30 min to find the time necessary for each type of experiment to reach steady state, i.e. the time for which repeated factorial design experiments had to be run before a representative sampling could be done. The time to reach steady state can be read from the oxygen and ammonium nitrogen concentrations in the water leaving the nitrification reactors, which is the same as the water in the nitrification reactors. In the diagrams 5.1-5.4 the normalized oxygen and ammonium nitrogen concentrations are plotted as a function of the running time for each of the factorial design experiments. When the oxygen and ammonium nitrogen concentrations started to level out, steady state must have been reached. From these diagrams it can be seen that steady state must have been reached after 1.5 h of experimental running for all the factorial design experiments. The oxygen and ammonium nitrogen concentrations were a bit unstable throughout the entire experiment, but this was all in the margin of error of the performance of the experiments and the analyses. 1.5 h of running equals about seven to eight hydraulic retention times and during that time steady state should have been reached. It could therefore be decided that repeated factorial design experiments should be kept running for 1.5 h and after that time representative sampling could be done.

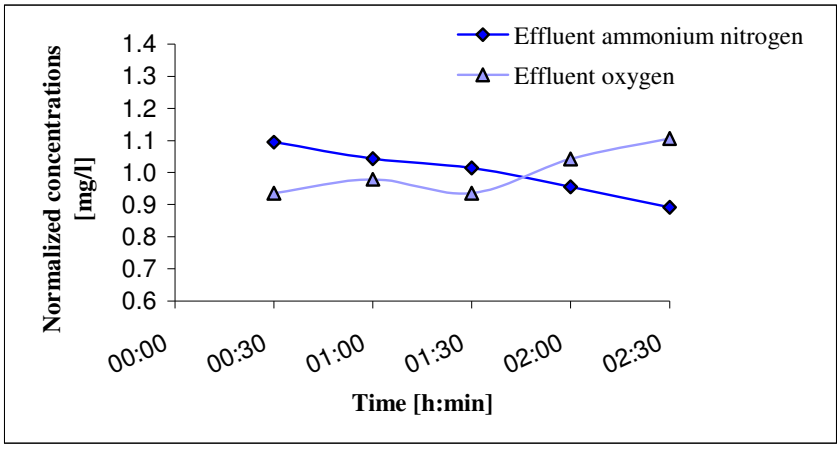

Diagram 5.1 Effluent ammonium nitrogen and oxygen concentrations for 25 % carrier element filling and low influent ammonium nitrogen concentration.

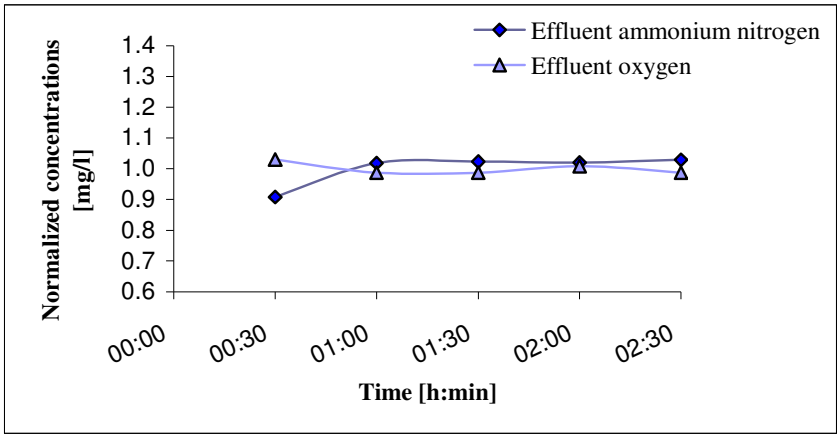

Diagram 5.2 Effluent ammonium nitrogen and oxygen concentrations for 25 % carrier element filling and high influent ammonium nitrogen concentration.

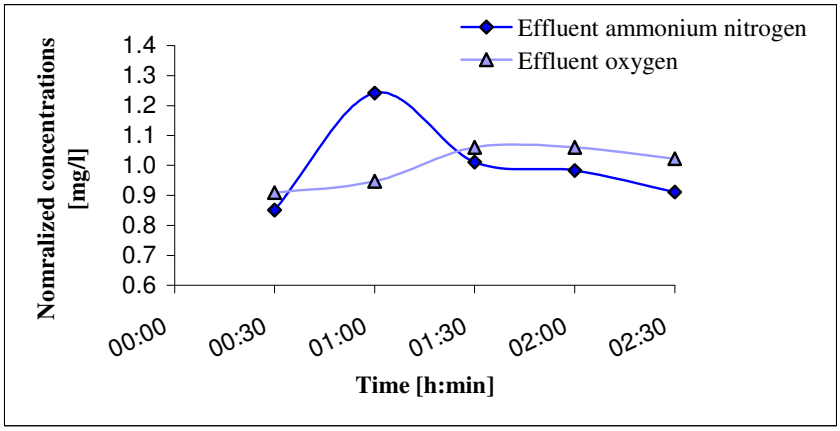

Diagram 5.3 Effluent ammonium nitrogen and oxygen concentrations for 50 % carrier element filling and low influent ammonium nitrogen concentration.

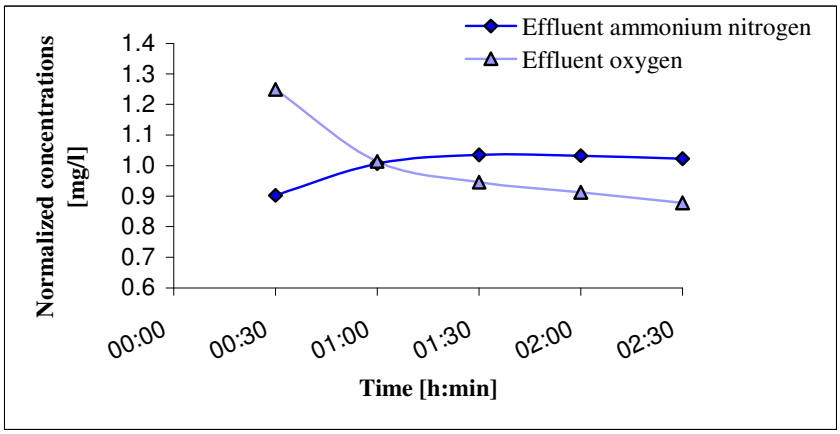

Diagram 5.4 Effluent ammonium nitrogen and oxygen concentrations for 50 % carrier element filling and high influent ammonium nitrogen concentration.

5.2 THE EXPERIMENTAL RESULTS

All the analytical results from the factorial design experiments are shown in appendix 6 and in table 5.2 are all the analytical results for samples taken in the nitrification reactors 1.5 h after the start of each experiment shown. The results are presented in the order the experiments were carried out. Since the laboratory-scale plant had two lines two different experiments were run at the same time. The two different nitrification reactors were marked A and B. The experiments performed at the same time have the same experiment number and the experiments performed in the same nitrification reactor have the same experiment letter, i.e. A or B. Experiment is denoted "Exp." in the table. The table also reveals the date and at what time during the day each experiment was carried out. In the column "Filling" the amount of carrier element filling can be seen and in the column "MBBR-comp." it can be seen from which compartment of the pilot MBBR the carrier elements were taken. In the rest of the columns the ammonium, nitrite, nitrate nitrogen and oxygen concentrations, oxygen to ammonium nitrogen mass concentration ratio, water temperature, pH, alkalinity, influent flow to the nitrification reactor in question and nitrification rate are shown.

When the factorial design experiments were planned it was decided to carry out the experiments at certain concentration levels of ammonium nitrogen in the nitrification reactors. Henze, the needed influent ammonium nitrogen concentrations were estimated. To obtain these ammonium nitrogen concentrations in the water mixtures used for the experiments, the needed amount of sludge liquor to add was estimated from the average ammonium nitrogen concentration in the sludge liquor the latest year. As expected, the actual ammonium nitrogen concentrations that were obtained were not exactly equal to the estimated ones. However it was not important to obtain these exact ammonium nitrogen concentrations in the water mixture, as long as the obtained ammonium nitrogen concentrations were in the same magnitude as the planned ones, which they were.

The factorial design experiments were planned to consist of four different groups with respect to ammonium and oxygen concentrations in the nitrification reactors; low oxygen and low ammonium concentrations, low oxygen and high ammonium concentrations, high oxygen and low ammonium concentrations and high oxygen and high ammonium concentrations. The actual average oxygen and ammonium concentrations in the nitrification reactors from the experiments can be seen in table 5.1.

Table 5.1 The average oxygen and ammonium nitrogen concentrations in the nitrification reactors from the factorial design experiments.

	Low [O_2] Low [NH_4^+-N]	**Low [O_2] High [NH_4^+-N]**	**High [O_2] Low [NH_4^+-N]**	**High [O_2] High [NH_4^+-N]**
[O_2] [mg/l]	2.7	2.8	4.4	4.5
[NH_4^+-N] [mg/l]	0.5	2.7	0.8	2.9

However, because of the varying ammonium nitrogen concentration in the sludge liquor, for one days experiments the ammonium nitrogen concentrations in the nitrification reactors were so high that what was meant to be the low ammonium nitrogen concentrations ended up being categorized into the group of high ammonium nitrogen concentrations and what was meant to be the high ammonium nitrogen

Table 5.2 Analytical results from the factorial design experiments, for samples taken from the nitrification reactors 1.5 h after the upstart of each experiment.

Exp.	Date	Time	Filling [%]	MBBR-comp.	$[NH_4^+-N]$ [mg/l]	$[NO_2^--N]$ [mg/l]	$[NO_3^--N]$ [mg/l]	$[O_2]$ [mg/l]	$[O_2]/[NH_4^+]$	Temperature [°C]	pH	Alkalinity [meqv/l]	Flow [l/h]	Nit. rate [g N/(m^2·d)]
1 A	2004-12-01	morning	25	4	2.701	0.247	8.255	4.2	1.55	15.4	7.3	1.2	17.4	0.703
1 B	2004-12-01	morning	50	4	2.425	0.194	8.572	2.8	1.15	15.3	7.3	1.4	18.9	0.412
2 A	2004-12-01	afternoon	25	4	0.819	0.185	7.765	4.4	5.37	13.9	7.3	1.1	17.4	0.644
2 B	2004-12-01	afternoon	50	4	0.542	0.177	8.116	2.8	5.17	15.0	7.3	1.3	18.1	0.447
3 A	2004-12-07	morning	50	2	0.405	0.146	7.335	2.2	5.43	14.3	7.2	0.6	19.2	0.437
3 B	2004-12-07	morning	50	4	0.398	0.133	7.440	2.8	7.04	15.7	7.3	0.8	18.4	0.440
4 A	2004-12-07	noon	25	4	0.832	0.175	6.722	4.4	5.29	14.3	7.3	0.7	18.8	0.621
4 B	2004-12-07	noon	50	4	0.588	0.170	7.146	2.6	4.42	15.7	7.3	0.8	18.4	0.473
5 A	2004-12-07	afternoon	25	4	2.936	0.202	7.231	4.5	1.53	13.8	7.3	0.7	18.5	0.725
5 B	2004-12-07	afternoon	50	4	2.722	0.185	7.613	2.6	0.96	15.0	7.3	0.9	18.2	0.450
6 A	2004-12-08	morning	25	4	3.043	0.326	9.635	4.8	1.58	13.7	7.3	1.3	18.2	0.638
6 B	2004-12-08	morning	50	4	2.601	0.291	10.190	3.1	1.19	14.7	7.3	1.5	18.4	0.480
7 A	2004-12-08	afternoon	25	4	5.968	0.293	7.864	4.8	0.80	13.5	7.3	1.8	18.4	0.592
7 B	2004-12-08	afternoon	50	4	5.953	0.247	8.308	2.9	0.49	14.5	7.3	1.9	18.5	0.364
8 A	2004-12-15	morning	50	2	2.860	0.647	8.994	2.9	1.01	13.6	7.2	1.1	19.5	0.629
8 B	2004-12-15	morning	50	4	3.021	0.222	9.258	2.9	0.96	15.6	7.3	1.3	18.4	0.423

concentrations ended up being so high in ammonium nitrogen concentration that they were categorized as an own "group" of very high ammonium nitrogen concentrations. This happed for experiment 6 and 7 in table 5.2. Thus the experiments performed with carrier elements from compartment four in the pilot MBBR ended up existing of six different groups concerning ammonium and oxygen concentrations in the nitrification reactors.

For the higher ammonium nitrogen concentrations, the oxygen to ammonium nitrogen concentration ratio was less than two, which means that oxygen was limiting the nitrification reaction. For the lower ammonium nitrogen concentrations the oxygen to ammonium nitrogen concentration ratio was larger than five, which means that ammonium nitrogen was limiting the nitrification reaction.

In experiment 1A, the nitrogen gas flow to one of the nitrification reactors did not reach the nitrification reactor because the tube leading the gas was squeezed. This was not discovered until the experiment had been run for 1 h and 50 min. Right away when the squeezed tube was discovered it was fixed and the experiment went on for another hour and a half with nitrogen gas spurted into the head space of the nitrification reactor. The high oxygen concentration in the water in the nitrification reactor from the upstart was reduced almost right away as the nitrogen gas was allowed to reach the nitrification reactor. This proved that the nitrogen gas flow into the nitrification reactors played an important role in preventing oxygen from the air to be transported down into the water. In the additional nitrification step the surface area will be small in relation to the volume of the water and the transportation of oxygen into the water will not be of large signification.

The pH for all the experiments was almost constant at 7.2-7.3. The alkalinity varied between 0.6-1.9 meqv CO_3^{2-}/l. The feed water test had shown that the alkalinity in the feed water to the nitrification reactors was about 1.4-1.7 meqv CO_3^{2-}/l but for the experiments the alkalinity was measured to be up to 1.9 meqv CO_3^{2-}/l in the nitrification reactors, which indicates that the alkalinity in the feed water for some experiments was a bit higher than 1.7 meqv CO_3^{2-}/l. Anyhow, with a minimum alkalinity of 1.4 meqv CO_3^{2-}/l in the influent water to the nitrification reactors, which corresponds to an alkalinity of 2.8 meqv HCO_3^-/l, the ratio between bicarbonate and ammonium in the influent water was never less than 2.5 eqv HCO_3^-/mole of ammonium. It could therefore be concluded that the alkalinity in the water in the nitrification reactors in the experiments was high enough to not have an inhibiting effect on the nitrification. The flow varied from experiment to experiment, but it was always measured in the end of each experiment so that the accurate flow could be used for the nitrification rate calculations.

In Hem et al.'s experiments (1994) the nitrification rates are given for a temperature of 15 °C. The factorial design experiments were carried out at temperatures between 13-16 °C and since this is very close to 15 °C the nitrification rates have not been recalculated to the corresponding value at 15 °C. It was assumed that the temperature effect for these small variations was insignificant.

The nitrification rate to ammonium nitrogen concentration (in the nitrification reactor) ratio was plotted as a function of the nitrification rate to oxygen concentration (in the nitrification reactor) ratio for the analytic results from the factorial design experiments.

The plot is shown in diagram 5.5. The six different groups from the factorial design experiments, performed with carrier elements from compartment four (denoted C4 in the diagram) of the pilot MBBR, are shown in one series each. In the plot there are also two series with experimental results from experiments carried out with carrier elements from compartment two (denoted C2 in the diagram) instead of four of the pilot MBBR. These were made to investigate the effect of the biofilm on the nitrification capacity and will be described later on in section 5.2. The series are named after the amount of carrier element filling used for the experiment and the average ammonium nitrogen concentration obtained for that type of factorial design experiment in the nitrification reactors. The results from Hem et al.'s experiments (1994) are also shown in the same plot.

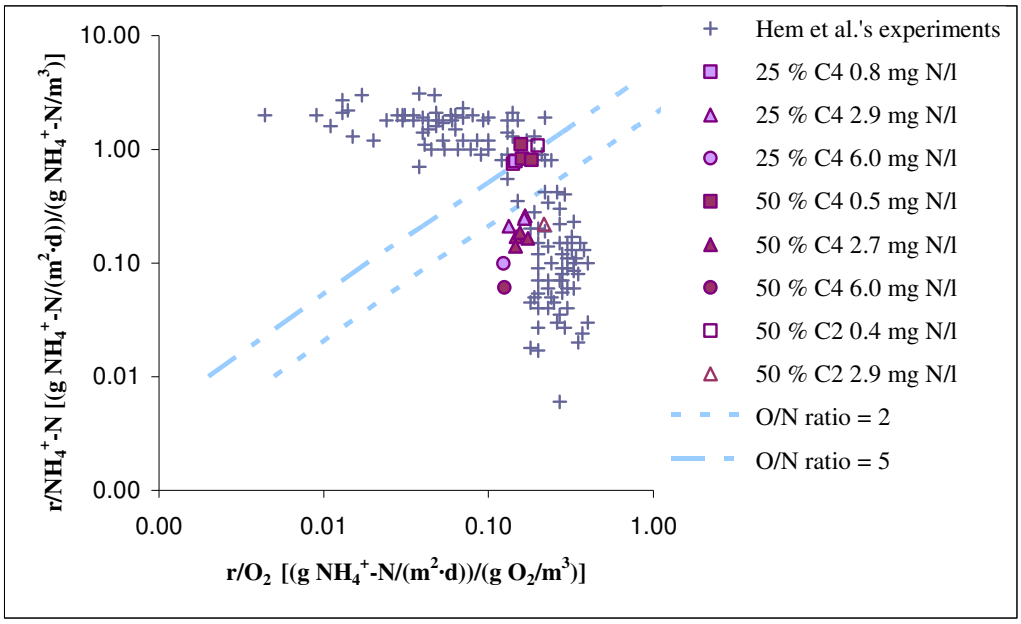

Diagram 5.5 The nitrification rate to ammonium nitrogen concentration ratio as a function of the nitrification rate to oxygen concentration ratio.

The biofilm grown on the carrier elements from compartment four in the pilot MBBR is acclimatized to an ammonium limiting environment for the nitrification. The biofilm grown on carrier elements from compartment two in the pilot MBBR is acclimatized to an oxygen limiting environment for the nitrification.

Table 5.3 Environment to which the bacteria were acclimatized and environment in which the experiments were performed for the different series.

Bacteria acclimatized to: / **Experiments performed in:**	*Oxygen limiting environment*	*Ammonium limiting environment*
Oxygen limiting environment	50 % C2 2.9 mg N/l	25 % C4 2.9 mg N/l 25 % C4 6.0 mg N/l 50 % C4 2.7 mg N/l 50 % C4 6.0 mg N/l
Ammonium limiting environment	50 % C2 0.4 mg N/l	25 % C4 0.8 mg N/l 50 % C4 0.5 mg N/l

When experiments with biofilm acclimatized to ammonium limiting conditions were performed in an ammonium limiting environment (series 25 % C4 0.8 mg N/l and 50 % C4 0.5 mg N/l) the experimental results agreed well with Hem et al.'s experimental results (1994) in diagram 5.5. When the experiment with biofilm acclimatized to oxygen limiting conditions was performed in an oxygen limiting environment (series 50 % C2 2.9 mg N/l) the experimental result agreed well with Hem et al.'s experimental results (1994) in diagram 5.5. When experiments with biofilm acclimatized to ammonium limiting conditions were performed in an oxygen limiting environment (series 25 % C4 2.9 mg N/l, 25 % C4 6.0 mg N/l, 50 % C4 2.7 mg N/l and 50 % C4 6.0 mg N/l) the experimental results did not agree so well with Hem et al.'s experimental results (1994) in diagram 5.5. When the experiment with biofilm acclimatized to oxygen limiting conditions was performed in an ammonium limiting environment (series 50 % C2 0.4 mg N/l) the experimental result did not agree completely with Hem et al.'s experimental results (1994) in diagram 5.5, but it agreed pretty well. However, for the experiment performed with biofilm acclimatized to oxygen limiting conditions in an ammonium limiting environment, the conditions were close to being in the concentration area where the switch from ammonium limiting to oxygen limiting environment occurs. This might be the reason why this experimental result agrees as well as it does with Hem et al.'s experimental results (1994).

For the different ammonium nitrogen and oxygen concentrations obtained in the nitrification reactors during the experiments, Hem et al.'s nitrification rates according to the surface diagram in appendix 4 were read off. In diagram 5.6 are the actual obtained nitrification rates from the experiments plotted against these nitrification rates from the diagram in appendix 4.

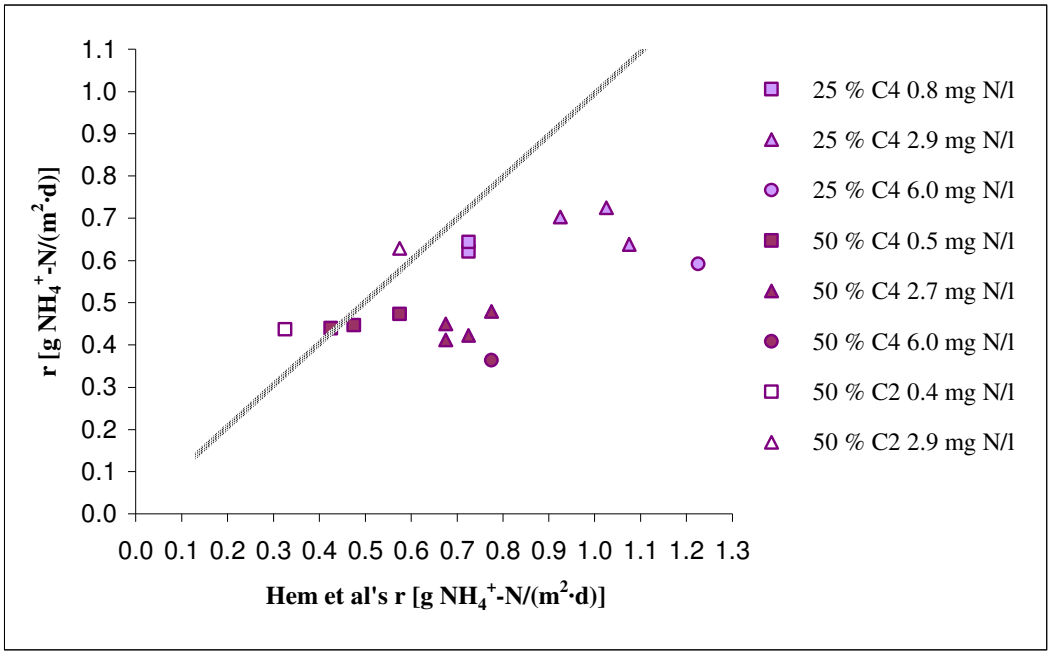

Diagram 5.6 The obtained nitrification rates from the experiments plotted against Hem et al.'s corresponding nitrification rates (1994).

As can be seen clearly from this plot, the nitrification rate at each of the two oxygen concentration levels for carrier elements from compartment four where the biofilm is

acclimatized to ammonium limiting conditions, did not increase very much with increasing ammonium nitrogen concentration. For experiments performed with these carrier elements at low ammonium nitrogen concentrations, where the ammonium nitrogen was limiting for the nitrification, a good agreement with Hem et al.'s experiments (1994) was obtained, but as the ammonium nitrogen concentration increases and oxygen becomes limiting for the nitrification, the agreement decreases. However, from the experimental results from the experiments performed with carrier elements from compartment two, that have a biofilm that is acclimatized to oxygen limiting conditions, it can be seen in diagram 5.6 that for the experiment performed at the higher ammonium nitrogen concentration, where oxygen was limiting for the nitrification during the experiment, has a good agreement with Hem et al.'s experimental results (1994). For decreased ammonium nitrogen concentrations, resulting in an experimental environment that is just ammonium limiting, the agreement is decreased.

From these experiments it was concluded that as long as the biofilm was acclimatized to the conditions that the experiments were carried out at, the agreement with Hem et al.'s experimental results (1994) was good. When biofilm acclimatized to ammonium limiting conditions was used for experiments carried out in an oxygen limiting environment and vice versa, the agreement with Hem et al.'s experimental results (1994) was not so good. This means that for the additional nitrification step, which will have a biofilm acclimatized to the environment that will be obtained in the nitrification step, the nitrification rates will be approximately the same as the nitrification rates obtained for Hem et al. (1994) at the corresponding ammonium nitrogen and oxygen concentrations that the additional nitrification step will be working at.

5.3 STATISTICAL ANALYSIS

A statistical analysis was made to find out whether or not the experiments carried out with carrier elements from compartment four at the two oxygen concentration levels could be considered to have obtained the same nitrification rate at each of the oxygen concentration levels, respectively, regardless of increasing ammonium nitrogen concentration. To check this, the results from the factorial design were divided into four groups; low ammonium nitrogen concentration and low oxygen concentration (group 1), low ammonium nitrogen concentration and high oxygen concentration (group a), high ammonium nitrogen concentration and low oxygen concentration (group b), high ammonium nitrogen concentration and high oxygen concentration (group ab). In table 4.6 this classification is clarified. The low ammonium nitrogen concentrations were represented by influent ammonium nitrogen concentrations to the nitrification reactors around 1.4-1.7 mg N/l and the high ammonium nitrogen concentrations were represented by influent ammonium nitrogen concentrations to the nitrification reactors around 3.4-4.0 mg N/l. The low oxygen concentrations were represented by 50 % carrier element filling and the high oxygen concentrations were represented by 25 % carrier element filling. The experimental results for ammonium nitrogen concentrations around 6.7-6.8 mg N/l were not used for this statistical check because there was only one experiment carried out at this ammonium nitrogen concentration level for each of the oxygen levels and to be able to perform a statistical check several values belonging to the same group are needed.

Four different hypotheses were tested statistically; *1* and *b* belong to the same group, i.e. these two groups have the same nitrification rate, *a* and *ab* belong to the same group, *1*

and *a* belong to the same group, *b* and *ab* belong to the same group. Equations 5.1 and 5.2 were used to calculate a t-value that together with the degrees of freedom give the probability of the, for each hypothesis, two tested groups belonging to the same group.

$$S_p^{\,2} = \frac{(n-1)\cdot S_1^{\,2} + (m-1)\cdot S_2^{\,2}}{n+m-2} \qquad [5.1]$$

n = number of samples in group 1
m = number of samples in group 2
S_1^2 = variance of the nitrification rates for the different samples in group 1
S_2^2 = variance of the nitrification rates for the different samples in group 2

$$t = \frac{r_{average,1} - r_{average,2}}{S_p \cdot \sqrt{\frac{1}{n} + \frac{1}{m}}} \qquad [5.2]$$

$r_{average,\,1}$ = average nitrification rate for group 1
$r_{average,\,2}$ = average nitrification rate for group 2

The degree of freedom for each hypothesis is calculated from n + m – 2. For the combination *1* and *b* the degree of freedom is 5 and the t-value 0.61, for the combination *a* and *ab* the degree of freedom is 3 and the t-value 1.61, for the combination *1* and *a* the degree of freedom is 3 and the t-value 11.53 and for the combination *b* and *ab* the degree of freedom is 5 and the t-value 8.75. This gives the statistical result that *1* and *b* are the same group with a probability of more than 50 %, *a* and *ab* are the same group with a probability of more than 20 %, *1* and *a* are the same group with a probability of less than 10 % and *b* and *ab* are the same group with a probability of less than 1% (Schöön, 1989). From this it can be concluded that the nitrification rate for a nitrifying biofilm acclimatized to ammonium limiting conditions is mostly affected by the oxygen concentration but also to some extent of the ammonium nitrogen concentration when working in an oxygen limiting environment. The higher the oxygen concentration the more the ammonium nitrogen concentration affects the nitrification rate. Thus, for a biofilm acclimatized to ammonium limiting conditions the nitrification rate can be increased some with increasing ammonium nitrogen concentration, but not very much. This is due to the biofilm's incapability to handle ammonium nitrogen concentrations larger than the concentrations the biofilm is acclimatized to.

5.4 ESTIMATED CONDITIONS IN THE ADDITIONAL NITRIFICATION STEP

From the experiments it was concluded that Hem et al.'s experimental results (1994) and thus the surface diagram in appendix 4 could be used to make good estimations over the conditions that will be obtained in the additional nitrification step for different carrier element fillings and influent ammonium nitrogen concentrations. Thus the diagrams 4.8-4.11 could be used as a good approximation to describe the effluent flows of ammonium nitrogen and oxygen from the planned additional nitrification step and the accompanying methanol savings obtained for different carrier element fillings and influent ammonium nitrogen concentrations. As described earlier a biofilm area of

240 000 m^2 represents 25 % carrier element filling of all the six reactors in the additional nitrification step, a biofilm area of 480 000 m^2 represents 50 % carrier element filling of all the six reactors in the additional nitrification step, a biofilm area of 720 000 m^2 represents 75 % carrier element filling of all the six reactors in the additional nitrification step and a biofilm area of 960 000 m^2 represents 100 % carrier element filling of all the six reactors in the additional nitrification step. Also like descried earlier, more than 60 % carrier element filling is not to be recommended, but the 75 % and 100 % carrier element filling examples can be used to simulate a larger additional nitrification step that with around 60 % carrier element filling obtains a biofilm area of 720 000 m^2 or 960 000 m^2 respectively. Likewise 25 % carrier element filling can be used to simulate a smaller nitrification step that with 60 % carrier element filling obtains a biofilm area of around 240 000 m^2. The nitrification rates for the different biofilm areas and influent ammonium nitrogen concentrations to the additional nitrification step can be seen in table 4.2-4.5.

As can be seen in the diagrams 4.8-4.11 the amount of removed oxygen and therefore also saved methanol per day increases as the biofilm area increases. For a biofilm area of 240 000 m^2 a maximal methanol saving of about 900 kg/d is obtained. For a biofilm area of 480 000 m^2 a maximal methanol saving of about 1200 kg/d is obtained, for biofilm areas of 720 000 m^2 and 960 000 m^2 the obtained maximal methanol savings are 1300 kg/d and 1400 kg/d respectively. The increase in saved methanol for an increase in biofilm area between 240 000 to 480 000 m^2 is the largest, for biofilm areas larger than 480 000 m^2 the increase in methanol saving is not that big. However, the larger the biofilm area the less sensitive is the system to temporarily increased influent ammonium nitrogen concentrations. The larger the biofilm area, the better the capacity to reduce higher influent ammonium nitrogen concentrations to acceptable concentrations in the effluent water. Therefore it might be a good thing to have a biofilm area a bit larger than 480 000 m^2. It can therefore be concluded that the biofilm area in the additional nitrification step should be somewhere between 500 000-600 000 m^2 to both lower the oxygen concentration optimally and also to have a more robust system to variations in influent ammonium nitrogen concentration. For all the biofilm areas the methanol savings are rapidly increased for increasing influent ammonium nitrogen concentrations up to 2 mg/l, but for none of the biofilm areas the methanol savings increased very much for influent ammonium nitrogen concentrations larger that 2 mg/l. Thus to have an optimal methanol saving that does not result in too high effluent ammonium nitrogen concentrations, the influent ammonium nitrogen concentration should be somewhere around 2 mg/l. In diagram 5.7-5.10 the ammonium nitrogen effluent concentration and the effluent oxygen concentration are plotted as a function of the influent ammonium nitrogen concentration. As can be seen from these plots the oxygen concentration in the water passing the additional nitrification step can be lowered from about 8 mg/l to somewhere around 2-3 mg/l with an influent ammonium nitrogen concentration somewhere around 2 mg/l and a biofilm area around 500 000-600 000 m^2. The effluent ammonium nitrogen concentration from the additional nitrification step should not exceed 1 mg N/l. This demand can be met for influent ammonium nitrogen concentrations up to 1.5 mg/l for a biofilm area of 240 000 m^2. For a biofilm area of 480 000 m^2 this demand can be met for influent ammonium nitrogen concentrations up to 2 mg/l and for biofilm areas of 720 000-960 000 m^2 this demand can be met for influent ammonium nitrogen concentrations up to around 2.5 mg/l. Thus the influent ammonium nitrogen concentration in the additional nitrification step should be somewhere around 2 mg/l. For an influent ammonium nitrogen concentration around

2 mg/l about 4.5-5.0 l/s of sludge liquor must be added to the influent water if the influent water stream is about 2.5 m^3/s. For a biofilm area around 500 000-600 000 m^2 and an influent ammonium nitrogen concentration around 2 mg/l a nitrification rate around 0.4-0.5 g NH_4^+-N/(m^2·d) will be obtained in the additional nitrification step. The ratio between the oxygen and the ammonium nitrogen concentration for such a step will be between two and five. This means that either oxygen or ammonium may be inhibiting for the nitrification in the additional nitrification step. For oxygen to ammonium nitrogen ratios less than two oxygen is inhibiting for the nitrification and for ratios larger than five ammonium nitrogen is inhibiting for the nitrification. In the interval between two and five a shift takes place from one of these substances being limiting for the nitrification to the other one being limiting. When this shift takes place varies and it is therefore difficult to predict which substance that will be inhibiting the nitrification in the additional nitrification step when run as suggested.

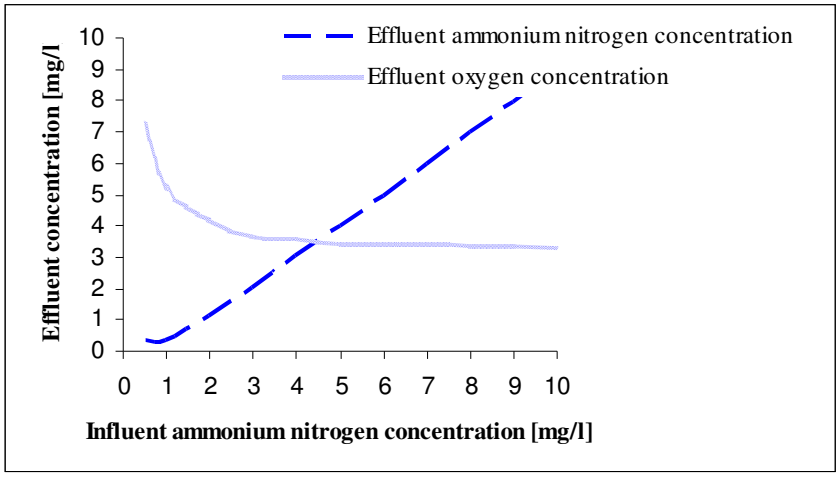

Diagram 5.7 Biofilm area of 240 000 m^2.

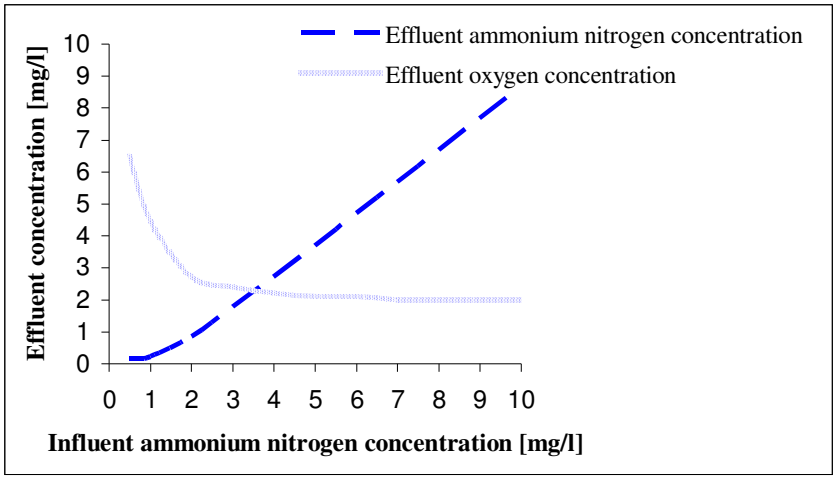

Diagram 5.8 Biofilm area of 480 000 m^2.

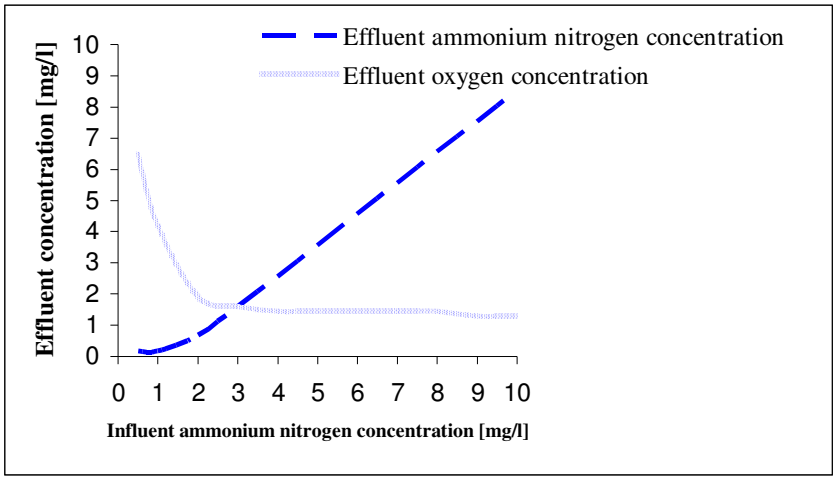

Diagram 5.9 Biofilm area of 720 000 m^2.

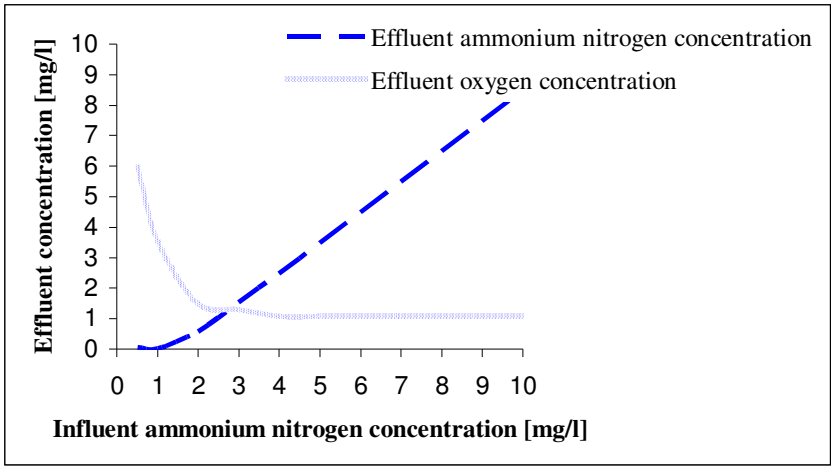

Diagram 5.10 Biofilm area of 960 000 m^2.

6. ECONOMICAL ESTIMATION

For the building of the additional nitrification step to be profitable the capital costs of the investment must be covered by the economic savings related to decreased methanol consumption in the additional post-denitrification step. For a biofilm area of around 500 000-600 000 m^2 in the additional nitrification step about 1000-1200 kg of methanol is saved per day, when the additional nitrification step is working at maximal load, which is at an influent water flow of 2.5 m^3/s. This means that, with a methanol cost of about 3 SEK/l, around 1.1-1.3 MSEK are saved from decreased methanol consumption every year. However, most of the time the additional nitrification step will not be working at maximal load and therefore the economical savings will be less, maybe around half of the maximal savings. If the additional nitrification step is built with a larger biofilm area than 500 000-600 000 m^2 the oxygen concentration in the effluent water from the additional post-denitrification step can be decreased some more, resulting in larger savings related to the methanol consumption, but at the prize of larger investment costs.

7. DISCUSSION

The results from the experiments indicate very strongly that if the additional nitrification step is built, the biofilm on the carrier elements in the nitrification step will, as it acclimatizes to its growing environment, give the same nitrification rates as the ones that were obtained in Hem et al.'s experiments (1994). All calculations for the additional nitrification step have been made based on that indication. It is most likely that this assumption is correct since Hem et al.'s experiments (1994) have been carried out under similar conditions as the experiments carried out for this project. However, the estimations of the nitrification capacity were made from the diagram over the nitrification rate as a function of the ammonium nitrogen and oxygen concentrations in appendix 4 and this diagram is in turn based on readings of diagram 4.6, a diagram taken from Hem et al.'s paper (1994). The process of taking information from diagram 4.6 and creating the diagram in appendix 4 meant many sources of uncertainty considering the preciseness of the estimated nitrification rates. However, the estimated nitrification rates must be considered to be reliable and if not completely accurate, then almost so.

Based on the estimations made for the additional nitrification step concerning nitrification rates, the optimal solution for the nitrification step seams to be an influent ammonium nitrogen concentration around 2 mg/l and a biofilm area around 500 000-600 000 m^2, i.e. 50-60 % carrier element filling. This will result in a nitrification rate around 0.4-0.5 g NH_4^+-N/(m^2·d). For larger biofilm areas lower nitrification rates will be obtained but the larger the biofilm area the higher influent ammonium nitrogen concentrations can be handled without exceeding the maximal accepted ammonium nitrogen concentration in the effluent from the additional nitrification step, which is 1 mg/l. Increased biofilm area brings robustness to the system and decreases the sensitivity to variations in ammonium nitrogen concentration in the sludge liquor. If such robustness is desired it will result in a larger investment cost, therefore building a nitrification step that is too robust will not be profitable. A balance has to be struck between needed robustness and investment costs for the additional nitrification.

8. CONCLUSIONS

The additional nitrification step should have a biofilm area around 500 000-600 000 m^2, i.e. 50-60 % carrier element filling of the planned total nitrification step volume of 1920 m^3. For smaller biofilm areas the methanol savings are rapidly decreased and for larger biofilm areas the methanol savings are not increased that much. However, the larger the biofilm area the larger is the tolerance to variations in influent ammonium nitrogen concentration, i.e. the nitrification step can handle higher influent ammonium nitrogen concentrations without exceeding acceptable effluent ammonium nitrogen concentrations. For influent ammonium nitrogen concentrations larger than 2 mg/l the increase in methanol saving is insignificant for all the biofilm areas investigated and for ammonium nitrogen concentrations less than 2 mg/l the methanol saving decreases rapidly. The effluent ammonium nitrogen concentration should not exceed 1 mg/l. This demand can be met for influent ammonium nitrogen concentrations up to 1.5 mg/l for a biofilm area of 240 000 m^2. For a biofilm area of 480 000 m^2 this demand can be met for influent ammonium nitrogen concentrations up to 2 mg/l and for biofilm areas of 720 000-960 000 m^2 this demand can be met for influent ammonium nitrogen concentrations up to around 2.5 mg/l. Thus the influent ammonium nitrogen concentration in the additional nitrification step should be around 2 mg/l.

With the additional nitrification step built with a biofilm area of 500 000-600 000 m^2 and an influent ammonium nitrogen concentration around 2 mg/l at a maximal influent water load to the step i.e an influent water flow of 2.5 m^3/h, a nitrification rate around 0.4-0.5 g NH_4^+-N/(m^2·d) can be expected. The ratio between the oxygen and the ammonium nitrogen concentration for such a step will be between two and five. This means that either oxygen or ammonium may be inhibiting for the nitrification in the additional nitrification step. For oxygen to ammonium nitrogen ratios less than two oxygen is inhibiting for the nitrification and for ratios larger than five ammonium nitrogen is inhibiting for the nitrification. In the interval between two and five a shift takes place from one of these substances being limiting for the nitrification to the other one being limiting. When this shift takes place varies and it is therefore difficult to predict which substance that will be inhibiting the nitrification in the additional nitrification step when run as suggested.

For a biofilm area of 500 000-600 000 m^2 and an influent ammonium nitrogen concentration around 2 mg/l at a maximal load of the additional nitrification step, the methanol consumption in the additional post-denitrificaiton step can be reduced with about 1000-1200 kg of methanol can be saved per day in the additional post-denitrification step, resulting in an economical saving of about 1.1-1.3 MSEK/year. Most of the time the influent water flow to the additional nitrification step will be less than 2.5 m^3/s, resulting in a reduced need for methanol in the additional post-denitrification step and therefore also a reduced methanol saving. This has to be taken into consideration when deciding the economical benefits for building an additional nitrification step. For the additional nitrification step to be profitable to build the economical savings related to decreased methanol consumption in the additional post-denitrification step must exceed the investment costs in the form of amortization and interest costs for the building of it.

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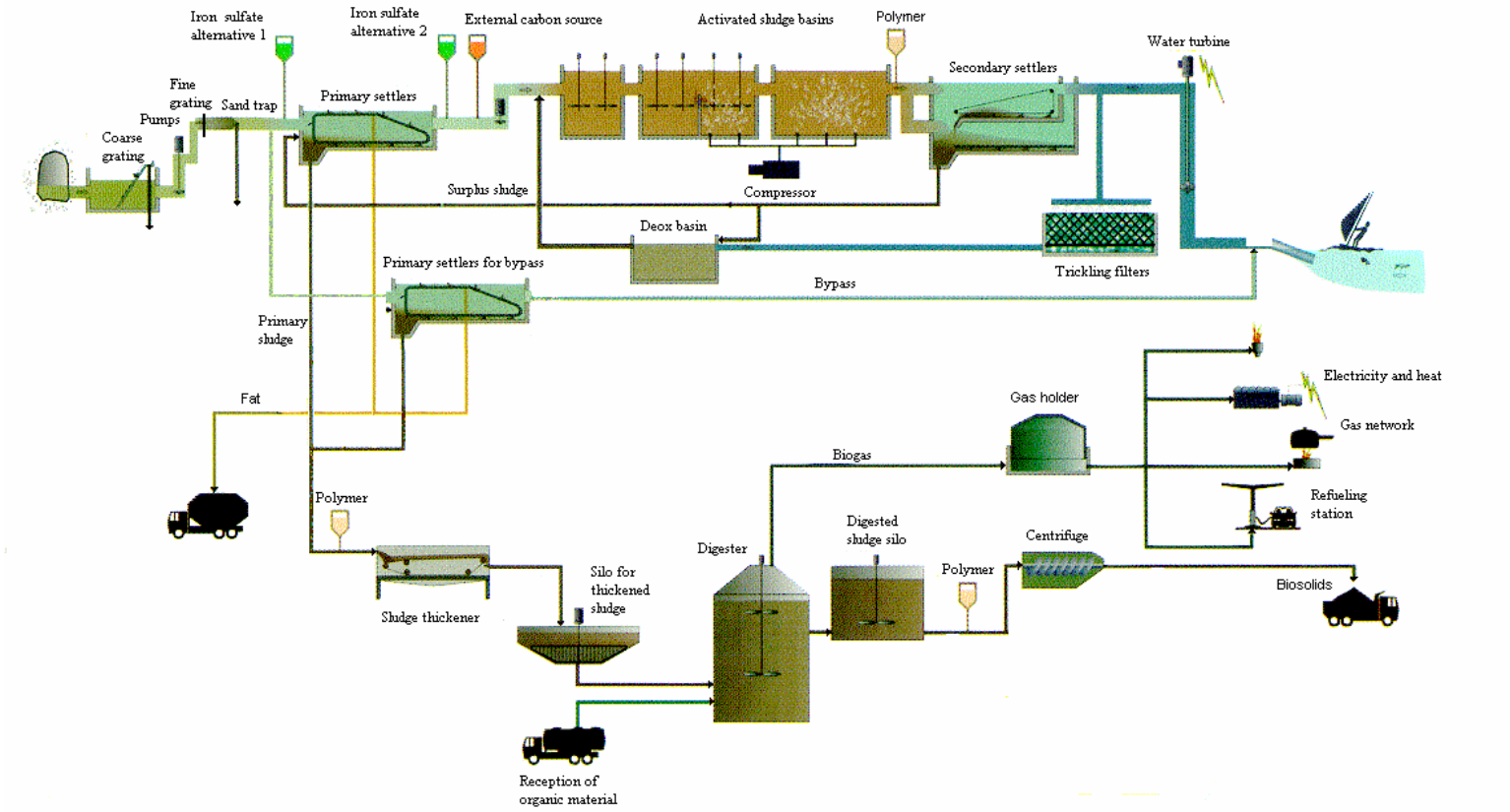


Source: Gryaab (2004) *Miljörapport – enligt miljöskyddsbalken*, Gryaab rapport 2004:2. Gryaab: Göteborg.

AERATED WATER MIXTURE

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]
00:15	2.058	0.098	7.342	9.3	16.7	1.5
00:30	2.031	0.109	7.375	9.4	16.0	1.5
00:45	2.055	0.103	7.402	9.5	15.7	1.5
01:00	2.030	0.118	7.410	9.5	15.5	1.5
02:00	2.008	0.113	7.529	9.7	14.6	1.5
03:00	1.950	0.122	7.549	9.8	14.0	1.6
04:00	2.032	0.128	7.652	10.1	13.6	1.6
05:00	1.989	0.135	7.852	10.2	13.2	1.7

NON-AERATED WATER MIXTURE

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]
00:15	2.000	0.094	7.270	9.1	18.0	1.4
00:30	1.985	0.104	7.269	9.1	18.2	1.5
00:45	1.964	0.100	7.310	9.0	18.6	1.5
01:00	2.062	0.104	7.316	9.0	19.0	1.4
02:00	2.009	0.130	7.313	8.8	20.2	1.4
03:00	1.819	0.133	7.355	8.6	20.8	1.4
04:00	1.822	0.148	7.424	8.7	20.7	1.4
05:00	1.749	0.164	7.501	8.8	20.3	1.4

Date of experiment: 2004-09-23

WATER CONTAINER

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	7.619	0.325	3.190	9.8	15.1
01:00	7.546	0.332	3.226	9.7	15.1
01:30	7.615	0.342	3.267	9.6	15.1
02:00	7.517	0.348	3.307	9.5	15.1
02:30	7.462	0.366	3.351	9.3	15.1
03:00	7.085	0.370	3.854	9.6	15.0
03:30	7.092	0.378	3.895	9.4	14.9

NITRIFICATION REACTOR

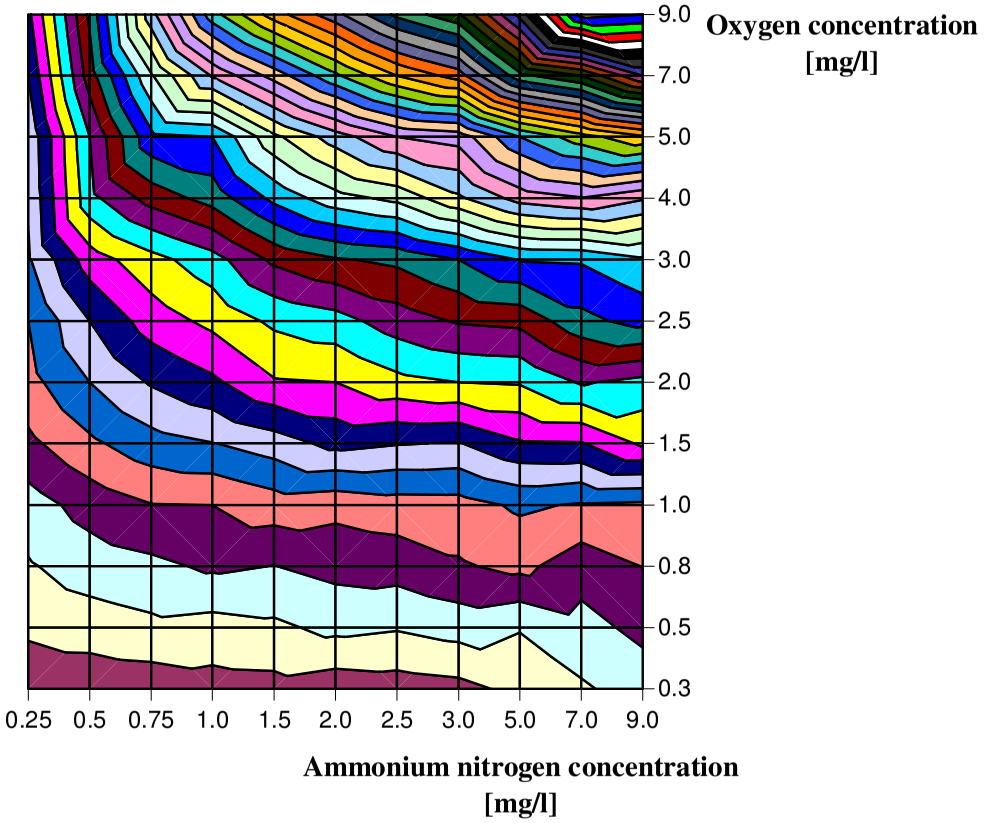
Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	4.980	0.535	5.743	0.8	15.5	1.6	6.9
01:00	5.490	0.469	4.751	0.8	15.4	1.6	7.0
01:30	5.821	0.437	4.476	0.7	15.4	1.7	7.2
02:00	5.901	0.425	4.378	0.7	15.4	1.7	7.1
02:30	5.904	0.415	4.354	0.6	15.4	1.7	7.1
03:00	5.674	0.391	4.719	0.6	15.3	1.7	7.2
03:30	5.462	0.370	4.992	0.6	15.3	1.7	7.2

Date of experiment: 2004-10-29

Water flow = 337 ml/min = 20.2 l/h

REACTED NITROGEN COMPOUNDS

Time [h:min]	Reacted ammonium-N [mg/l]	Formed nitrite-N [mg/l]	Formed nitrate-N [mg/l]	Obtained nit. rate [g NH4-N/(m^2·d)]	O_2/NH_4-N [g O_2/g N]	Calculated nit. rate [g NH4-N/(m^2·d)]
00:30	2.639	0.210	2.553	0.41	0.16	0.23
01:00	2.056	0.137	1.525	0.32	0.14	0.22
01:30	1.794	0.095	1.209	0.28	0.12	0.19
02:00	1.616	0.077	1.071	0.25	0.12	0.19
02:30	1.558	0.049	1.003	0.24	0.11	0.18
03:00	1.411	0.021	0.865	0.22	0.10	0.16
03:30	1.630	-0.008	1.097	0.25	0.11	0.16



Nitrification rate [g NH_4^+-N/(m^2•d)]

0-0.05	0.05-0.1
0.1-0.15	0.15-0.2
0.2-0.25	0.25-0.3
0.3-0.35	0.35-0.4
0.4-0.45	0.45-0.5
0.5-0.55	0.55-0.6
0.6-0.65	0.65-0.7
0.7-0.75	0.75-0.8
0.8-0.85	0.85-0.9
0.9-0.95	0.95-1
1-1.05	1.05-1.1
1.1-1.15	1.15-1.2
1.2-1.25	1.25-1.3
1.3-1.35	1.35-1.4
1.4-1.45	1.45-1.5
1.5-1.55	1.55-1.6
1.6-1.65	1.65-1.7
1.7-1.75	1.75-1.8
1.8-1.85	1.85-1.9
1.9-1.95	1.95-2
2-2.05	2.05-2.1
2.1-2.15	2.15-2.2
2.2-2.25	2.25-2.3

ANALYTICAL METHOD FOR TOTAL ALKALINITY

1. PRINCIPLE

A reagent containing a mixture of several acids reacts with the sample's alkaline compounds. The pH of the sample is lowered and the final pH-value can be related directly to the original alkalinity of the sample.

2. EQUIPMENT

- Beaker; high model; 200 ml.
- Volumetric flask; 100 ml.
- Pipette; 10 ml.
- Magnet stirrer.
- pH-meter.

3. CHEMICALS

- Deionized water.
- pH-buffers with pH 4.00 and 6.88.
- Orion Total Alkalinity Reagent, Orion Cat.nr 700011, from Thermo Electron Corporation.

4. PERFORMANCE

Before taking the samples that are to be measured calibrate the pH-meter with pH 6.88 and pH 4.00 buffers, check the calibration with pH 7.00 buffer. The measuring of alkalinity should be done in room temperature as soon as possible after having taken the sample. Measure up 100 ml of the water sample to be measured in a volumetric flask and pour it over to a 200 ml beaker. Put the beaker on a magnet stirrer. Add 10 ml of Orion Total Alkalinity Reagent to the beaker and stir the sample, wait for 3 min then measure and write down the pH. This pH-value is denoted (pH) in the calculations below.

5. CALCULATIONS

The total alkalinity (TA) expressed as calcium carbonate ($CaCO_3$) is calculated from:

$$TA = \frac{((pH) - 3.66)}{0.0075} \quad \text{[mg /l of calcium carbonate]} \qquad [1]$$

$$TA = \frac{2 \cdot ((pH) - 3.66)}{0.75} \quad \text{[meqv/l of calcium carbonate]} \qquad [2]$$

Equations 1 and 2 are derived from the diagram below:

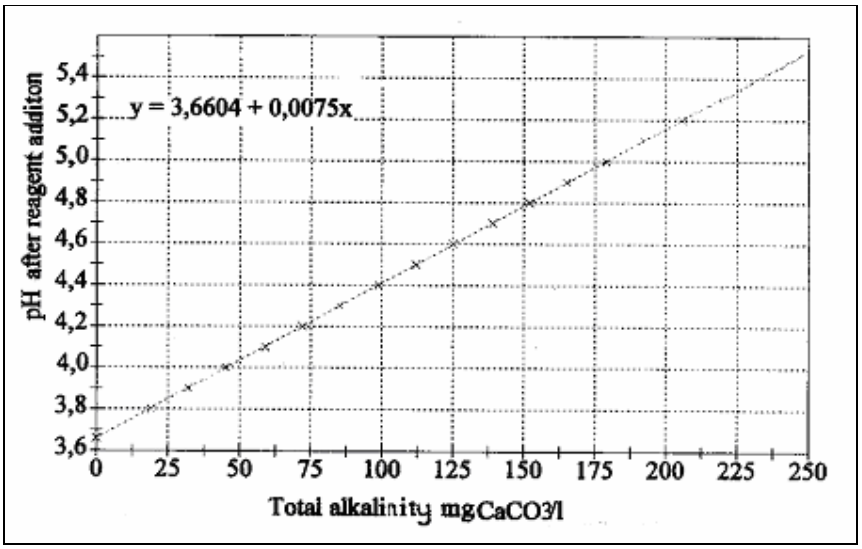


Figure 1. The pH-value after reagent addition plotted against total alkalinity (Robinson, 1994).

6. LITERATURE

Robinson, P. (1994) *Metod: Totalalkalitet, snabbmetod*, code: LAM.0007.02.0, Gryaab: Göteborg.

ANALYTICAL METHOD FOR AMMONIUM NITROGEN

1. PRINCIPLE

The water sample to be analyzed for ammonium is injected in a carrier stream in a flow injection analyzing instrument (FIA). The water stream is mixed with a sodium hydroxide stream and in this alkaline flow ammonia gas is released. The ammonia gas diffuses through a gas diffusion membrane into a receiving stream with an indicator solution. The indicator gives a color shift when it gets in contact with the ammonia. This color shift is measured photometrically. The absorbance of light with wavelength 590 nm corresponds to the ammonium concentration in the water stream injected.

2. EQUIPMENT

- FIAstar 5000 instrument, from Foss Tecator.
- Method cassette NH_4^+ including gas diffusion cell, gas diffusion membrane and interference filter M = 590 nm and R = 720 nm.
- FIAstar 5027 sample exchanger, from Foss Tecator.
- Volumetric flasks; 100ml, 200 ml, 500 ml, 1000 ml.
- Pipettes; 0.5-10 ml.
- Membrane filter unit with membrane filter with pore size 0.45 µm.
- Basic laboratorial equipment for e.g. preparing the reagents and standards.

3. CHEMICALS

- Indicator mixture, article number: 5000 0295.
- Deionized water.
- Sodium hydroxide (NaOH).
- Ammonium chloride (NH_4Cl).
- Ethanol; 95 % (C_2H_5OH).
- Sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$).

Use only chemicals of pro-analysis quality and water of high pureness for the analysis.

4. PERFORMANCE

Prepare the following solutions before starting the analysis:

4.1 Carrier solution

The carrier solution consists of deionized water.

4.2 Reagents

4.2.1 Stock indicator solution

Dissolve 0.2 g sodium hydroxide in 250 ml of deionized water in a 500 ml volumetric flask and dilute to full volume with deionized water. This gives a 0.01 M sodium hydroxide-solution. Weigh out 1 g of ammonium indicator mixture and mix with 10 ml 0.01 M sodium hydroxide and 10 ml 85 % ethanol in a 200 ml volumetric flask and dilute to full volume with deionized water.

4.2.2 Sodium dihydrogen phosphate buffer
Dissolve 13.8 g sodium dihydrogen phosphate in 600 ml deionized water in a 1000 ml volumetric flask and dilute to full volume with deionized water. This gives a 0.1 M sodium dihydrogen phosphate solution.

4.2.3 Sodium hydroxide reagent
Dissolve 10.0 g sodium hydroxide in 250 ml deionized water in a 500 ml volumetric flask and dilute with deionized water to full volume. This gives a 0.01 M sodium hydroxide solution. This reagent should be filled in the bottle marked • on the ammonium analyzing line on the FIAstar instrument.

4.2.4 Indicator solution
Dilute 10 ml of stock indicator solution to 500 ml with deionized water. Prepare this solution one day before use. The absorbance of this solution must be checked before it is being used. To see how the procedure for checking the absorbance goes, see 4.7 (at high ammonium concentrations add 1.5 ml 0.1 M sodium dihydrogen phosphate.). This reagent should be filled in the bottle marked •• on the ammonium analyzing line on the FIAstar instrument.

4.3 Standards

4.3.1 Stock standard
Dry some ammonium chloride for 2 h in an oven at 105 °C. Dissolve 3.819 g of this ammonium chloride in 500 ml deionized water in a 1000 ml volumetric flask and dilute to full volume. This gives a 1000 mg/l ammonium nitrogen solution. The stock standard is stable for several months.

4.3.2 Medium stock standard I
Pour 10 ml stock standard into a 100 ml volumetric flask and dilute to full volume with deionized water. This gives a 100 mg/l ammonium nitrogen solution. The medium stock standard is stable for a week.

4.3.3 Medium stock standard II
Pour 10 ml of stock standard into a 1000 ml volumetric flask and dilute to full volume with deionized water. This gives a 10 mg/l ammonium nitrogen solution. The medium stock standard is stable for a week.

4.3.4 Working standards
The working standards are prepared through dilution of the both medium stock standards. At least five working standards for each analyzing area are recommended. Working standards must be prepared the same day they are to be used.

When working in the range 0.01-1.0 mg/l of ammonium nitrogen the following working standards should be prepared:

Table 1. Preparation of working standards for the range 0.01-1.0 mg/l of ammonium nitrogen (Foss Tecator AB, 2000).

Ammonium nitrogen concentration [µg/l]	Volume medium stock standard II [ml]	Final volume [ml]
0	0	100
10.0	0.1	100
50.0	0.5	100
100.0	1.0	100
200.0	2.0	100
500.0	5.0	100
1000.0	10.0	100

When working in the range 1.0-10.0 mg/l of ammonium nitrogen the following working standards should be prepared:

Table 2. Preparation of working standards for the range 1.0-10.0 mg/l of ammonium nitrogen (Foss Tecator AB, 2000).

Ammonium nitrogen concentration [mg/l]	Volume medium stock standard I [ml]	Final volume [ml]
0	0	100
1.0	1.0	100
2.0	2.0	100
5.0	5.0	100
7.0	7.0	100
10.0	10.0	100

4.4 Storage of the samples

The samples should be analyzed as soon as possible after the sampling or the samples should be preserved. For a maximum 24 h storing the samples can be stored at 4 °C. For longer storing periods 1 ml concentrated sulfuric acid (H_2SO_4) should be added for each 100 ml sample volume and the samples should be stored at 2.5 °C to preserve the samples. Sometimes the samples can be stored in a freezer at –20 °C for 8 days maximum, under the condition that it has been controlled earlier that this method of storing does not give different results for the samples compared to if they are stored according to the other methods mentioned above.

4.5 Starting the FIA-system

- Put fresh deionized water in all bottles and the rinse bowl.
- Let all the solutions that will be used in the analyzing attain room temperature.
- Start the FIAstar instrument and the sample changer 5027. FIAstar needs 15 min of heating up before the start of analyzing samples.
- Make sure that the right method cassette and belonging detector filter are installed.

- Start the pump and pump deionized water through each unit and make sure that the solutions are flowing through the tubes.
- Start the computer and the software, SoFia, used to run the analysis.
- Check the indicator solution's absorbance (see 4.7).
- Start to pump the reagents. The method selections should be in NH_4^+-mode.
- Chose the method in question in the software and make sure that the right sample loop is installed. For concentrations of ammonium nitrogen between 0.01-1.0 mg/l 400 µl sample loop and linear calibration should be used. For concentrations of ammonium nitrogen between 1.0-10 mg/l 40 µl sample loop and non-linear calibration should be used.
- Put the samples in the sample changer and make a sample list in the software.
- Put the working standards in the sample changer and do a few test injections on one of the standards to make sure the system is in equilibrium.
- Do a calibration and check the calibration (see 4.8).
- Start the analyzing of the samples.

4.6 Shutting down

- Move all the pump tubes to the rinse bowl.
- Start the rinsing cycle.
- Remove all tubes when the rinsing cycle is finished and pump air through the units so that all liquid within the system is removed.
- Loosen the pump clips.
- Shut down the FIAstar instrument.

4.7 Checking the absorbance of the ammonium indicator

The ammonium indicator should be adjusted so that its absorbance with water as reference lies in the 450-600 milli absorbance unit (mAU) area. If the absorbance value is lower than 450 mAU it has to be adjusted by adding 0.01 M sodium hydroxide drop by drop. If the absorbance value is higher than 600 mAU it has to be adjusted by adding 0.01 M hydrochloric acid drop by drop. After having checked the absorbance for the ammonium indicator, the absorbance for the sodium hydroxide reagent is checked. There should be an increase in absorbance but this increase must not exceed 50-100 mAU. If so or if there is continues drift, the gas diffusion membrane could be damaged. The indicator should always be checked before use.

4.8 Calibration

Choose which analyzing method the FIA-system should run, the latest method is always loaded automatically. Put the calibration standards in the sample changer. With help from the software a calibration curve is drawn, this curve is non-linear for ammonium. No more than 8 % difference between the old and the new calibration can be accepted.

5. LITERATURE

Foss Tecator AB (2000) *Bestämning av ammonium i vatten med FIAstar 5000*, AN 5220-SE. Foss Tecator AB: Höganäs.

ANALYTICAL METHOD FOR NITRITE NITROGEN

1. PRINCIPLE

The water sample to be analyzed for nitrite is injected in a carrier stream in a flow injection analyzing instrument (FIA). The nitrite in the sample reacts with a sulfanilamide solution and forms a diazo compound. This compound reacts with N-(1-naphtyl)-ethylenediamine dihydrochloride (NED) and forms a purple azo dye. The higher the nitrite concentration the more intense is the color of the sample. This color intensity is measured photometric. The absorbance of light with wavelength 540 nm corresponds to the nitrite concentration in the analyzed sample.

2. EQUIPMENT

- FIAstar 5000 instrument, from Foss Tecator.
- Method cassette NO_2^-/NO_3^- and interference filter M = 540 nm and R = 720 nm.
- FIAstar 5027 sample exchanger, from Foss Tecator.
- Volumetric flasks; 100ml, 200 ml, 500 ml, 1000 ml.
- Pipettes; 0.5-20 ml.
- Basic laboratorial equipment for e.g. preparing the reagents and standards.

3. CHEMICALS

- Sulfanilamide(4-aminobenzenesulfonamide) ($C_6H_8N_2O_2S$).
- N-(1-naphtyl)-ethylenediaminedihydrochloride.
- Hydrochloric acid; 37 % (HCl).
- Sodium nitrite ($NaNO_2$).

Use only chemicals of pro-analysis quality and water of high pureness for the analysis.

4. PERFORMANCE

Prepare the following solutions before starting the analysis:

4.1 Carrier solution

The carrier solution consists of deionized water.

4.2 Reagents

4.2.1 Deionized water

Deionized water should be prepared as one of the reagents. If the samples analyzed have big variations in pH the deionized water can be replaced with ammonium chloride buffer.

4.2.2 Sulfanilamide reagent

Dissolve 5 g of sulfanildiamide in 250 ml deionized water in a volumetric flask with the volume 500 ml. Add 25 ml of concentrated hydrochloric acid and mix thoroughly. Dilute to full volume with deionized water. This reagent is stable for several months and

it should be filled in the bottle marked •• on the nitrite analyzing line on the FIAstar instrument.

4.2.3 NED reagent
Dissolve 0.5 g N-(1-naphtyl)-ethylenediaminedihydrochloride in 250 ml deionized water in a volumetric flask with the volume 500 ml. Dilute to full volume with deionized water. This reagent should be kept from light and is stable for a week. This reagent should be filled in the bottle marked ••• on the nitrite analyzing line on the FIAstar instrument.

4.3 Standards

4.3.1 Stock standard
Dry some sodium nitrite in oven at 105 ºC until it keeps a constant weight. Dissolve 4.928 g dried sodium nitrite in a volumetric flask with the volume 1000 ml and dilute to full volume with deionized water. This gives a 1000 mg/l nitrite nitrogen solution. The stock standard is stable for at least three months.

4.3.2 Medium stock standard I
Add 5 ml of stock standard solution with the help of a pipette into a 500 ml volumetric flask and dilute to full volume with deionized water. This gives a 10 mg/l nitrite nitrogen solution. This solution must be prepared the same day it is to be used.

4.3.3 Medium stock standard II
Add 10 ml of medium stock standard solution with the help of a pipette into a 100 ml volumetric flask and dilute to full volume with deionized water. This gives a 1 mg/l nitrite nitrogen solution. This solution must be prepared the same day it is to be used.

4.3.4 Working standards
The working standards are prepared through dilution of the both medium stock standards. At least five working standards for each analyzing area are recommended. Working standards must be prepared the same day they are to be used.

When working in the range 0.005-0.1 mg/l of nitrite nitrogen the following working standards should be prepared:

Table 1. Preparation of working standards for the range 0.005-0.1 mg/l of nitrite nitrogen (Foss Tecator AB, 2000).

Nitrite nitrogen concentration [µg/l]	Volume medium stock standard II [ml]	Final volume [ml]
0	0	100
5.0	0.5	100
10.0	1.0	100
25.0	2.5	100
50.0	5.0	100
100.0	10.0	100

When working in the range 0.1-2.0 mg/l of nitrite nitrogen the following working standards should be prepared:

Table 2. Preparation of working standards for the range 0.1-2.0 mg/l nitrite nitrogen (Foss Tecator AB, 2000).

Nitrite nitrogen concentration [mg/l]	Volume medium stock standard I [ml]	Final volume [ml]
0	0	100
0.1	1.0	100
0.2	2.0	100
0.5	5.0	100
1.0	10.0	100
2.0	20.0	100

4.4 Storage of the samples

The samples should be analyzed as soon as possible after the sampling or the samples should be preserved. For a maximum 24 h storing the samples can be stored at 4 °C. For longer storing periods 1 ml sulfuric acid (H_2SO_4) should be added for each 100 ml sample volume and the samples should be stored at 2.5 °C to preserve the samples. Sometimes the samples can be stored in a freezer at –20 °C for 8 days maximum, under the condition that it has been controlled earlier that this method of storing does not give different results for the samples compared to if they are stored according to the other methods mentioned above.

4.5 Starting the FIA-system

- Put fresh deionized water in all bottles and the rinse bowl.
- Let all the solutions that will be used in the analyzing attain room temperature.
- Start the FIAstar instrument and the sample changer 5027. FIAstar needs 15 min of heating up before the start of analyzing samples.
- Make sure that the right method cassette and belonging detector filter are installed.
- Start the pump and pump deionized water through each unit and make sure that the solutions are flowing through the tubes.
- Start the computer and the software, SoFia, used to run the analysis.
- Start to pump the reagents. The method selectioner should be in NO_2^--mode.
- Chose the method in question in the software and make sure that the right sample loop is installed. For concentrations of nitrite nitrogen between 0.005-0.1 mg/l, 400 µl sample loop and linear calibration should be used. For concentrations of nitrite nitrogen between 0.1-2 mg/l 40 µl sample loop and linear calibration should be used.
- Put the samples in the sample changer and make a sample list in the software.
- Put the working standards in the sample changer and do a few test injections on one of the standards to make sure the system is in equilibrium.
- Do a calibration and check the calibration (see 4.7).
- Start the analyzing of the samples.

4.6 Shutting down

- Move all the pump tubes to the rinse bowl.
- Start the rinsing cycle.
- Remove all tubes when the rinsing cycle is finished and pump air through the units so that all liquid within the system is removed.
- Loosen the pump clips.
- Shut down the FIAstar instrument.

4.7 Calibration

Choose which analyzing method the FIA-system should run, the latest method is always loaded automatically. Put the calibration standards in the sample changer. With help from the software a calibration curve is drawn, this curve is linear for nitrite. No more than 8 % difference between the old and the new calibration can be accepted.

5. LITERATURE

Foss Tecator AB (2000) *Bestämning av nitrit i vatten med FIAstar 5000*, AN 5200-SE. Foss Tecator AB: Höganäs.

ANALYTICAL METHOD FOR THE SUM OF NITRITE AND NITRATE NITROGEN

1. PRINCIPLE

The water sample to be analyzed for the sum of nitrite and nitrate is injected in a carrier stream in a flow injection analyzing instrument (FIA). There the sample containing nitrite and nitrate is mixed with a buffer solution. Nitrate is reduced to nitrite in a cadmium reductor. The nitrite (both the original nitrite and the nitrite formed from the reduced nitrate) in the sample reacts with a sulfanilamide solution and forms a diazo compound. This compound reacts with N-(1-naphtyl)-ethylenediamine dihydrochloride (NED) and forms a purple azo dye. The higher the nitrite concentration the more intense is the color of the sample. This color intensity is measured photometric. The absorbance of light with wavelength 540 nm corresponds to the nitrite concentration in the analyzed sample.

2. EQUIPMENT

- FIAstar 5000 instrument, from Foss Tecator.
- Method cassette NO_2^-/NO_3^- and interference filter M = 540 nm and R = 720 nm.
- FIAstar 5027 sample exchanger, from Foss Tecator.
- Cadmium reductor.
- Volumetric flasks; 100ml, 200 ml, 500 ml, 1000 ml.
- Pipettes; 0.5-20 ml.
- Basic laboratorial equipment for e.g. preparing the reagents.

3. CHEMICALS

- Sulfanilamide(4-aminobenzenesulfonamide) ($C_6H_8N_2O_2S$).
- N-(1-naphtyl)-ethylenediaminedihydrochloride.
- Hydrochloric acid; 37 % (HCl).
- Sodium nitrate ($NaNO_3$).
- Ammonium chloride (NH_4Cl).
- Ammonia (NH_3OH).

Use only chemicals of pro-analysis quality and water of high pureness for the analysis.

4. PERFORMANCE

Prepare the following solutions before starting the analysis:

4.1 Carrier solution

The carrier solution consists of deionized water.

4.2 Reagents

4.2.1 Ammonium chloride buffer

Dissolve 85 g ammonium chloride in 500 ml deionized water in a 1000 ml volumetric flask. When the solution has reached room temperature add ammonia (around 12 ml) until pH 8.5 is reached. Dilute the solution to full volume with deionized water.

4.2.2 Sulfanilamide reagent

Dissolve 5 g of sulfanildiamide in 250 ml deionized water in a volumetric flask with the volume 500 ml. Add 25 ml of concentrated hydrochloric acid and mix thoroughly. Dilute to full volume with deionized water. This reagent is stable for several months and should be filled in the bottle marked •• on the nitrite/nitrate analyzing line on the FIAstar instrument.

4.2.3 NED reagent

Dissolve 0.5 g N-(1-naphtyl)-ethylenediaminedihydrochloride in 250 ml deionized water in a volumetric flask with the volume 500 ml. Dilute to full volume with deionized water. This reagent should be kept from light and is stable for a week. This reagent should be filled in the bottle marked ••• on the nitrite/nitrate analyzing line on the FIAstar instrument.

4.3 Standards

4.3.1 Stock standard

Dry some sodium nitrate in oven at 105 ºC until it keeps a constant weight. Dissolve 6.068 g dried sodium nitrate in 600 ml deionized water in a volumetric flask with the volume 1000 ml and dilute to full volume with deionized water. This gives a 1000 mg/l nitrate nitrogen solution. The stock standard is stable for at least three months.

4.3.2 Medium stock standard I

Add 10 ml of stock standard solution with the help of a pipette into a 500 ml volumetric flask and dilute to full volume with deionized water. This gives a 20 mg/l nitrate nitrogen solution. This solution must be prepared the same day it is to be used.

4.3.3 Medium stock standard II

Add 5 ml of medium stock standard solution with the help of a pipette into a 100 ml volumetric flask and dilute to full volume with deionized water. This gives a 1 mg/l nitrate nitrogen solution. This solution must be prepared the same day it is to be used.

4.3.4 Working standards

The working standards are prepared through dilution of the both medium stock standards. At least five working standards for each analyzing area are recommended. Working standards must be prepared the same day they are to be used.

When working in the range 0.005-0.25 mg/l of nitrite nitrogen the following working standards should be prepared:

Table 1. Preparation of working standards for the range 0.005-0.25 mg/l of nitrite nitrogen (Foss Tecator AB, 2000).

Nitrate nitrogen concentration [mg/l]	Volume medium stock standard I [ml]	Final volume [ml]
0	0	100
5.0	0.5	100
25.0	2.5	100
50.0	5.0	100
100.0	10.0	100
250.0	25.0	100

When working in the range 0.1-5.0 mg/l of nitrite nitrogen the following working standards should be prepared:

Table 2. Preparation of working standards for the range 0.1-5.0 mg/l of nitrite nitrogen (Foss Tecator AB, 2000).

Nitrate nitrogen concentration [µg/l]	Volume medium stock standard I [ml]	Final volume [ml]
0	0	100
0.1	0.5	100
0.5	2.5	100
1.0	5.0	100
2.0	10.0	100
5.0	25.0	100

4.4 Storage of the samples

The samples should be analyzed as soon as possible after the sampling or the samples should be preserved. For a maximum 24 h storing the samples can be stored at 4 °C. For longer storing periods 1 ml sulfuric acid (H_2SO_4) should be added for each 100 ml sample volume and the samples should be stored at 2.5 °C to preserve the samples. Sometimes the samples can be stored in a freezer at –20 °C for 8 days maximum, under the condition that it has been controlled earlier that this method of storing does not give different results for the samples compared to if they are stored according to the other methods mentioned above

4.5 Starting the system

- Put fresh deionized water in all bottles and the rinse bowl.
- Let all the solutions that will be used in the analyzing attain room temperature.
- Start the FIAstar instrument and the sample changer 5027. FIAstar needs 15 min of heating up before the start of analyzing samples.
- Make sure that the right method cassette and belonging detector filter are installed.

- Start the pump and pump deionized water through each unit and make sure that the solutions are flowing through the tubes.
- Start the computer and the software, SoFia, used to run the analysis.
- Start to pump the reagents. The method selectioner should be in NO_2^--mode.
- When the system is filled with liquid put the method selectioner in NO_2^- + NO_3^--mode. Pump until all the air in the bypass-tube has disappeared. Stop the pump and take away the bypass-tube.
- Put in the cadmium reductor and start the pump. OBS cadmium is poisonous, therefore be careful when handling the reductor.
- Chose the method in question in the software and make sure that the right sample loop is installed. For concentrations of nitrate nitrogen between 0.005-0.25 mg/l 400 µl sample loop and linear calibration should be used. For concentrations of nitrite nitrogen between 0.1-0.25 mg/l 40 µl sample loop and linear calibration should be used.
- Put the samples in the sample changer and make a sample list in the software.
- Put the working standards in the sample changer and do a few test injections on one of the standards to make sure the system is in equilibrium.
- Do a calibration and check the calibration (see 4.7).
- Start the analyzing of the samples.

4.6 Shutting down

- Change back the method selectioner mode to NO_2^- and pump ionized water through the system for a few minutes. Stop the pump. Remove the cadmium reductor and put the bypass-tube back. Start the pump and change the method selectioner mode to NO_2^- + NO_3^-.
- Move all the pump tubes to the rinse bowl.
- Start the rinsing cycle in the software.
- Remove all tubes when the rinsing cycle is finished and pump air through the units so that all liquid within the system is removed.
- Loosen the pump clips.
- Shut down the FIAstar instrument.

4.7 Calibration

Choose which analyzing method the FIA-system should run, the latest method is always loaded automatically. Put the calibration standards in the sample changer. With help from the software a calibration curve is drawn, this curve is linear for nitrite and non-linear for nitrate. No more than 8 % difference between the old and the new calibration can be accepted.

4.8 The cadmium reductor

The efficiency of the reductor must be checked regularly. When doing so inject first a 1 mg/l nitrite nitrogen solution (for description of the preparing of this solution see the "Measuring method for nitrite"-description), then a 1 mg/l nitrate nitrogen solution through the cadmium reductor. The absorbance of the NO_3^- solution should be around 85-100 % of the absorbance of the NO_2^- solution. If the efficiency is lower than that try

to activate the reductor. Inject 1 M hydrochloric acid a couple of times and then inject 200 ppm nitrate nitrogen solution. Thereafter inject a standard a couple of times until the efficiency is stable. The cadmium reductor must always be kept wet.

5. LITERATURE

Foss Tecator AB (2000) *Bestämning av summan av nitrat och nitrit i vatten med FIAstar 5000*, AN 5201-SE. Foss Tecator AB: Höganäs.

WATER CONTAINER: 25 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	1.612	0.155	7.028	10.3	13.4
01:00	1.644	0.158	7.027	10.2	13.6
01:30	1.621	0.166	7.060	10.1	13.9
02:00	1.659	0.173	7.064	10.0	14.0
02:30	1.608	0.184	7.085	9.7	14.4

NITRIFICATION REACTOR: 25 % filling, low ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	0.884	0.182	7.809	4.4	13.9	1.1	7.3
01:00	0.842	0.180	7.759	4.6	13.8	1.1	7.3
01:30	0.819	0.185	7.765	4.4	13.9	1.1	7.3
02:00	0.771	0.185	7.815	4.9	14.1	1.2	7.3
02:30	0.720	0.188	7.825	5.2	14.1	1.2	7.3

Date of experiment: 2004-12-01, afternoon, experiment 2A.

Water flow = 290 ml/min = 17.4 l/h

WATER CONTAINER: 25 % filling, high ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	3.829	0.189	6.508	10.4	13.5
01:00	3.822	0.191	6.526	10.5	13.3
01:30	3.786	0.206	6.537	10.5	13.3
02:00	3.810	0.212	6.502	10.4	13.5
02:30	3.766	0.218	6.576	10.3	14.0

NITRIFICATION REACTOR: 25 % filling, high ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	2.607	0.203	7.183	4.7	13.6	0.8	7.3
01:00	2.924	0.203	7.284	4.5	13.6	0.7	7.3
01:30	2.936	0.202	7.231	4.5	13.8	0.7	7.3
02:00	2.928	0.210	7.293	4.6	13.8	0.7	7.3
02:30	2.953	0.214	7.341	4.5	14.1	0.6	7.3

Date of experiment: 2004-12-07, afternoon, experiment 5A.

Water flow = 308 ml/min = 18.5 l/h

WATER CONTAINER: 50 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	1.642	0.158	6.978	10.2	13.8
01:00	1.626	0.167	6.998	10.1	14.2
01:30	1.568	0.168	7.031	10.1	14.8
02:00	1.543	0.178	7.051	9.9	15.4
02:30	1.540	0.192	7.090	9.7	16.3

NITRIFICATION REACTOR: 50 % filling, low ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	0.456	0.161	8.341	2.4	14.3	1.3	7.3
01:00	0.665	0.164	8.036	2.5	14.5	1.3	7.3
01:30	0.542	0.177	8.116	2.8	15.0	1.3	7.3
02:00	0.527	0.186	8.142	2.8	15.6	1.3	7.3
02:30	0.488	0.192	8.192	2.7	16.4	1.2	7.3

Date of experiment: 2004-12-01, afternoon, experiment 2B.

Water flow = 302 ml/min = 18.1 l/h

WATER CONTAINER: 50 % filling, high ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	3.487	0.203	7.447	10.2	14.1
01:00	3.479	0.210	7.488	10.1	14.5
01:30	3.418	0.216	7.470	10.0	15.2
02:00	3.398	0.229	7.497	9.8	15.9
02:30	3.439	0.230	7.556	9.7	16.6

NITRIFICATION REACTOR: 50 % filling, high ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	2.113	0.204	8.671	3.7	14.4	1.4	7.3
01:00	2.358	0.195	8.549	3.0	14.8	1.5	7.3
01:30	2.425	0.194	8.572	2.8	15.3	1.4	7.3
02:00	2.417	0.187	8.582	2.7	16.0	1.4	7.3
02:30	2.394	0.209	8.637	2.6	16.7	1.4	7.3

Date of experiment: 2004-12-01, morning, experiment 1B.

Water flow = 315 ml/min = 18.9 l/h

WATER CONTAINER: 50 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	1.463	0.126	6.181	10.3	14.1
01:00	1.420	0.141	6.210	10.3	14.2
01:30	1.392	0.142	6.266	10.2	14.4
02:00	1.230	0.144	6.336	10.1	14.6
02:30	1.361	0.166	6.286	10.2	14.4

NITRIFICATION REACTOR: 50 % filling, low ammonium concentration, carrier elements from compartment two

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	0.444	0.138	7.231	3.7	14.1	0.8	7.2
01:00	0.444	0.158	7.276	3.0	14.1	0.7	7.2
01:30	0.405	0.146	7.335	2.2	14.3	0.6	7.2
02:00	0.394	0.164	7.330	1.6	14.4	0.7	7.2
02:30	0.425	0.180	7.207	2.7	14.5	0.6	7.2

Date of experiment: 2004-12-07, morning, experiment 3A.

Water flow = 320 ml/min = 19.2 l/h

WATER CONTAINER: 50 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	1.549	0.133	6.320	10.0	15.4
01:00	1.527	0.158	6.340	10.1	15.4
01:30	1.432	0.157	6.402	9.9	16.2
02:00	1.428	0.183	6.338	9.6	16.7
02:30	1.404	0.194	6.429	9.9	16.4

NITRIFICATION REACTOR: 50 % filling, low ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	0.406	0,114	7.435	3.9	15.1	0.9	7.3
01:00	0.398	0.121	7.446	3.2	15.4	0.9	7.3
01:30	0.398	0.133	7.440	2.8	15.7	0.8	7.3
02:00	0.412	0.160	7.405	2.5	16.6	0.9	7.3
02:30	0.434	0.179	7.442	2.5	17.1	0.8	7.3

Date of experiment: 2004-12-07, morning, experiment 3B.

Water flow = 307 ml/min = 18.4 l/h

WATER CONTAINER: 50 % filling, high ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	4.269	0.257	7.999	10.3	13.2
01:00	4.265	0.262	8.028	10.2	13.4
01:30	4.258	0.263	8.029	10.2	13.5
02:00	4.151	0.279	8.011	10.1	13.9
02:30	4.113	0.282	8.053	9.9	14.1

NITRIFICATION REACTOR: 50 % filling, high ammonium concentration, carrier elements from compartment two

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	2.557	0.350	9.247	3.4	13.2	1.2	7.2
01:00	2.852	0.348	9.083	3.2	13.4	1.1	7.2
01:30	2.860	0.347	8.994	2.9	13.6	1.1	7.2
02:00	2.915	0.350	9.065	2.8	14.1	0.9	7.2
02:30	3.066	0.357	9.032	2.7	14.3	1.2	7.2

Date of experiment: 2004-12-15, morning, experiment 8A.

Water flow = 325 ml/min = 19.5 l/h

WATER CONTAINER: 50 % filling, high ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	4.119	0.251	8.017	9.9	15.0
01:00	4.067	0.247	8.133	9.9	15.0
01:30	4.017	0.265	8.159	9.8	15.6
02:00	4.038	0.282	8.208	9.7	16.2
02:30	3.972	0.322	8.199	9.5	17.3

NITRIFICATION REACTOR: 50 % filling, high ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	2.555	0.222	9.512	4.0	14.3	1.3	7.3
01:00	2.886	0.216	9.379	3.3	14.9	1.2	7.3
01:30	3.021	0.222	9.258	2.9	15.6	1.3	7.3
02:00	3.036	0.225	9.720	2.7	16.4	1.2	7.3
02:30	2.929	0.233	9.291	2.6	17.2	1.3	7.3

Date of experiment: 2004-12-15, morning, experiment 8B.

Water flow = 307 ml/min = 18.4 l/h

WATER CONTAINER: 25 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
01:30	1.546	0.142	6.040	10.2	14.3

NITRIFICATION REACTOR: 25 % filling, low ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
01:00	-	-	-	4.8	14.2	-	-
01:15	-	-	-	4.6	14.2	-	-
01:30	0.832	0.175	6.722	4.4	14.3	0.7	7.3

Date of experiment: 2004-12-07, noon, experiment 4A.

Water flow = 314 ml/min = 18.8 l/h

WATER CONTAINER: 25 % filling, high ammonium concentration, no nitrogen gas the first 1 h and 50 min

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
00:30	3.521	0.192	7.486	10.3	13.6
01:00	3.546	0.197	7.459	10.3	13.6
01:30	3.591	0.205	7.470	10.1	13.9
02:00	3.549	0.214	7.478	10.0	14.2
02:30	3.525	0.227	7.468	9.9	14.3
03:00	3.439	0.237	7.561	10.0	14.9
03:30	3.576	0.251	7.599	9.9	15.0

NITRIFICATION REACTOR: 25 % filling, high ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
00:30	2.190	0.251	8.370	7.2	13.8	1.2	7.3
01:00	2.276	0.260	8.399	6.9	13.8	1.4	7.3
01:30	2.338	0.251	8.402	6.8	13.9	1.2	7.3
02:00	2.401	0.259	8.375	6.3	14.1	1.2	7.3
02:30	2.486	0.240	8.262	5.0	14.2	1.1	7.3
03:00	2.623	0.238	8.239	4.4	14.9	1.0	7.3
03:30	2.701	0.247	8.255	4.2	15.4	1.1	7.3

Date of experiment: 2004-12-01, morning, experiment 1A.

Water flow =290 ml/min = 17.4 l/h

WATER CONTAINER: 50 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
01:30	1.701	0.164	6.033	9.9	15.7

NITRIFICATION REACTOR: 50 % filling, low ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
01:00	-	-	-	2.9	15.2	-	-
01:15	-	-	-	2.7	15.5	-	-
01:30	0.588	0.170	7.146	2.6	15.7	0.8	7.3

Date of experiment: 2004-12-07, noon, experiment 4B.

Water flow = 307 ml/min = 18.4 l/h

WATER CONTAINER: 50 % filling, high ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
01:30	3.795	0.208	6.565	10.3	14.6

NITRIFICATION REACTOR: 50 % filling, high ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
01:00	-	-	-	2.8	14.4	-	-
01:15	-	-	-	2.8	14.7	-	-
01:30	2.722	0.185	7.613	2.6	15.0	0.9	7.3

Date of experiment: 2004-12-07, afternoon, experiment 5B.

Water flow = 303 ml/min = 18.2 l/h

WATER CONTAINER: 25 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
01:30	3.803	0.339	8.894	10.2	13.5

NITRIFICATION REACTOR: 25 % filling, low ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
01:00	-	-	-	5.0	13.7	-	-
01:15	-	-	-	4.9	13.6	-	-
01:30	3.043	0.326	9.635	4.8	13.7	1.3	7.3

Date of experiment: 2004-12-08, morning, experiment 6A.

Water flow = 303 ml/min = 18.2 l/h

WATER CONTAINER: 25 % filling, high ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
01:30	6.664	0.318	7.300	10.3	13.3

NITRIFICATION REACTOR: 25 % filling, high ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
01:00	-	-	-	4.8	13.3	-	-
01:15	-	-	-	4.8	13.3	-	-
01:30	5.968	0.293	7.864	4.8	13.5	1.8	7.3

Date of experiment: 2004-12-08, afternoon, experiment 7A.

Water flow = 307 ml/min = 18.4 l/h

WATER CONTAINER: 50 % filling, low ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
01:30	3.731	0.346	8.973	10.4	14.5

NITRIFICATION REACTOR: 50 % filling, low ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
01:00	-	-	-	3.5	14.5	-	-
01:15	-	-	-	3.3	14.5	-	-
01:30	2.601	0.291	10.190	3.1	14.7	1.5	7.3

Date of experiment: 2004-12-08, morning, experiment 6B.

Water flow = 307 ml/min = 18.4 l/h

WATER CONTAINER: 50 % filling, high ammonium concentration

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]
01:30	6.806	0.306	7.177	10.5	14.1

NITRIFICATION REACTOR: 50 % filling, high ammonium concentration, carrier elements from compartment four

Time [h:min]	Ammonium nitrogen [mg/l]	Nitrite nitrogen [mg/l]	Nitrate nitrogen [mg/l]	Oxygen [mg/l]	Temperature [ºC]	Alkalinity [meqv/l]	pH [pH-units]
01:00	-	-	-	3.1	14.0	-	-
01:15	-	-	-	3.0	14.2	-	-
01:30	5.953	0.247	8.308	2.9	14.5	1.9	7.3

Date of experiment: 2004-12-08, afternoon, experiment 7B.

Water flow = 308 ml/min = 18.5 l/h