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Nitrogen removal in the Hias Process with a side-stream nitrification reactor

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#### Abstract

The Hias Process, developed by Hias IKS and Hias How2O, is an enhanced biological phosphorus removal process based on the moving bed biofilm reactor technology. The Hias Process has already shown results for efficient phosphorus removal, but to widen this process' application, both within Norway and internationally, it is also desired to increase the nitrogen removal efficiency. Currently the nitrogen removal of the Hias process happens through simultaneous nitrification and denitrification, but nitrogen removal efficiencies within the requirements of the Norwegian or European regulations have not yet been reached. Through this Master's thesis a pilot scale side-stream two-reactor setup for nitrification was implemented to the Hias process. To achieve Norwegian and European removal requirements of at least 70% total nitrogen removal, an initial goal of 60% removal of the sum of ammonia, nitrite and nitrate through the Hias Process was proposed. The experiments were conducted from January through April at Hias wastewater treatment plant. The results show a tendency of increasing nitrogen removal though the experiments, and an average reduction of 57% for ammonia and 54% for the sum of ammonia, nitrite and nitrate was achieved for the last four weeks of the analysis. This was a significant increase relative to the previous test that have been done for nitrogen removal with the Hias Process. Although the 60% mark was not achieved, this side-stream setup shows great promise regarding nitrogen removal in the Hias Process, while not significantly impacting the phosphorus removal.

#### Norsk sammendrag

Hias-prosessen, utviklet av Hias IKS og Hias How2O AS, er en biologisk renseprosess for fosforfjerning fra avløpsvann basert på MBBR-teknologi. Hias-prosessen har vist gode resultater for effektiv fosforfjerning, men for å utvide prosessens målgruppe, både nasjonalt og internasjonalt, er det et ønske om økt renseeffekt for nitrogen. Hittil har Hias-prosessen oppnådd nitrogenfjerning gjennom simultan nitrifisering og denitrifisering, men uten å oppnå renseeffekt innenfor norske og europeiske rensekrav. Gjennom denne masteroppgaven ble et sidestrøms system for nitrifisering med to reaktorer implementert i pilotskala for Hias-prosessen. Et mål om 60% fjerning av ammonium, nitritt og nitrat ble forslått for å oppnå norske og europeiske rensekrav for nitrogen på minimum 70% fjerning av total nitrogen. Eksperimentene ble utført fra januar til og med april ved Hias avløpsrenseanlegg. Resultatene viser en tendens til økende nitrifisering, og en rensegrad på 54% løst nitrogen og 57% ammonium ble oppnådd i løpet av de fire siste ukene. Dette representerer en stor økning av rensegrad i forhold til tidligere tester for nitrogenfjerning for Hias-prosessen. Selvom 60% fjerning av løst nitrogen ikke ble oppnådd, viser resultatene at Hias-prosessen har et stort potensial for nitrogenfjerning med sidestrøms reaktorer, samtidig som rensegraden for fosfor ikke blir betydelig påvirket.

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## Acronyms

(aq) - Solved in water (g) - Gas phase (1) - Liquid phase **AOB** - Ammonia oxidizing bacteria **AS** - Activated sludge **Bio-P** - Biological treatment of phosphorus **BOD** - Biological oxygen demand **bsCOD** - Biodegradable soluble chemical oxygen demand CH<sub>3</sub>OH - Ethanol  $\mathbf{CO}_2$  - Carbon dioxide **DNPAO** - Denitrifying phosphate accumulating organisms **EBPR** - Enhanced biological phosphorus removal  $\mathbf{H}^+$  - Hydrogen ion, representing the Hydonium ion:  $\mathbf{H}_3\mathbf{O}^+$  $H_2O$  - Water **IFAS** - Integrated fixed-film activated sludge **MBBR** - Moving Bed Biofilm Reactor  $N_2$  - Nitrogen gas  $\mathbf{NH}_{4}^{+}$  - Ammonia  $\mathbf{NH}_{4}^{+}$ -**N** - Nitrogen portion of ammonia  $NO_2^-$  - Nitrite  $NO_2^--N$  - Nitrogen portion of nitrite  $NO_3^-$  - Nitrate  $NO_3^-$ -N - Nitrogen portion of nitrate **NOB** - Nitrite oxidizing bacteria  $O_2$  - Oxygen gas OH<sup>-</sup> - Hydroxide **P** - Phosphorus PHA - Polyhydroxyalkanoates  $\mathbf{PO}_4^{3-}$  - Phosphate  $\mathbf{PO}_{4}^{3-}$ -**P** - Phosphorus portion of phosphate **PAO** - Phosphate accumulating organisms sCOD - Soluble chemical oxygen demand **SND** - Simultanous Nitrification and Denitrification **SNR** - Sidestream Nitrification Reactor Tot-P - Total phosphorus  $\mathbf{TN}$  - Total nitrogen

**VFA** - Volatile fatty acids

# Terms

 $\mathbf{Anoxic}$  - Conditions without oxygen, but with nitrite, nitrate or sulphate as an electron acceptor

**Anaerobic** - Conditions without oxygen, nitrite, nitrate or sulphate as electron acceptor, but organic matter might serve as both electron acceptor and donor

Aerobic - Conditions with oxygen, could be used together with

 $\mathbf{Oxic}$  - Conditions with oxygen

## 1. Introduction

Wastewater contains organic material and nutrients such as phosphorus (P) and nitrogen (N) compounds, like phosphate ( $PO_4^{3-}$ ), ammonia ( $NH_4^+$ ), nitrite ( $NO_2^-$ ) and nitrate ( $NO_3^-$ ). Discharge of these phosphate and nitrogen compounds to recipients can cause eutrophication (Schindler, 1974). For freshwater recipients the main limiting nutrient for eutrophication is P (Schindler, 1974), while N has a greater limiting effect in estuaries and coastal recipients (Howarth and Marino, 2006). Estuaries and coastal recipients can also be impacted by local N loads in the watersheds, and therefore N removal before discharge to freshwater recipients can reduce eutrophication in estuaries and coastal recipients downstream (Ocean Studies Board and National Reasearch Council, 2000). Eutrophication can alter the conditions of a recipient, thus cause habitat degradation, change in species composition and reduced water quality (e.g. toxins) (de Jonge et al., 2002). These issues, while negative for the recipients, can also cause health and recreational issues for humans. Therefore, to protect recipients and human interests, discharge limits have been placed on wastewater treatment plants (WWTPs) to reduce nutrient loadings to recipients.

Total nitrogen (TN) and total phosphorus (Tot-P) removal requirements for EU member states is described by 'Council Directive 91/271/EEC' (European Economic Community, 1991), the TN requirement is minimum 70-80% reduction and Tot-P the requirement is minimum 80%. Norwegian removal requirements, covered by 'forskrift om begrensning av forurensing' (2004) part 4 regarding wastewater treatment, are minimum 70% reduction of TN, but only WWTPs with specified requirement for N removal are covered by the removal requirements.

Hias WWTP located near the city of Hamar, Innlandet in Norway receive wastewater from the four municipalities Hamar, Løten, Stange and Ringsaker. The WWTP receive both domestic and industrial wastewater, causing big differences in organic and nutrient loadings throughout the week. Hias inter-municipal company, Hias IKS, who is in charge of operating Hias WWTP, have together with its subsidiary, Hias How2O AS, invented the Hias process. The Hias process is a MBBR process for P-removal in wastewater by utilization of PAO in oscillating anaerobic and aerobic conditions (Saltnes et al., 2017). PAO release  $PO_4^{3-}$  when exposed to anaerobic conditions and accumulate more  $PO_4^{3-}$ in oxic conditions, resulting in a net accumulation of  $PO_4^{3-}$  (Saltnes et al., 2017). The process uses 3 anaerobic zones followed by 7 oxic zones with biofilm carriers continuously flowing with the wastewater from zone 1 to 10. The Hias process removes some nitrogen in the oxic zones through simultaneous nitrification and denitrification (SND), with nitrification at oxic conditions in the outer biofilm and denitrification at anoxic conditions in the inner biofilm (Saltnes et al., 2017). According to Sørensen (2021), the SND can achieve up to 40-50% NH\_4^+-removal during warm weather in the summer months. Currently the nitrite oxidizing bacteria (NOB) are inhibited for the SND, and thus NO<sub>2</sub><sup>-</sup> is the main end product of nitrification (Saltnes, 2021). Although the explanation for this is unknown, Saltnes (2021) believes that this is caused by competition between denitirifying phosphate accumulating organisms (DNPAO) and NOB.

Currently, Hias WWTP have no nitrogen removal requirements. However, the relevance of the Hias process is dependent on its capability to fulfill such removal requirements. Therefore, an increase of the N removal is necessary for broadening the Hias process' target group. Although, the increase in N-removal should not heavily impact the Premoval.

To achieve higher N removal than what is reached by SND, additional measures must be introduced to the process. A side-stream nitrification reactor (SNR) was implemented to increase nitrification, while additional anoxic zones, in the Hias process, following the SNR was introduced to increase denitrification. Later an additional reactor was introduced in the side-stream setup, to increase nitrification. SND will contribute to N removal in the last oxic zones. An estimated 60% removal of the sum of ammonia, nitrite and nitrate was suggested to achieve Norwegian and European nitrogen removal requirements of at least 70% reduction of total nitrogen. This proposition was based on an assumption by Hias that the remaining portion of total nitrogen removal to achieve 70% reduction was through particle removal in the clarifying steps preceding and following the Hias process. The setup with a SNR combined with the Hias process with anoxic zones will likely increase N removal of the Hias process, although high bsCOD concentrations could pose a problem to the nitrification rate. Other research questions that will be looked at are what nitrification product that will be dominant in the SNR effluent, what impacts this will have on the Hias process and whether the inhibitory effect on NOB are reduced.

## 2. Background

## 2.1 VEAS pilot

The VEAS pilot have formerly achieved 40-50% NH<sup>+</sup><sub>4</sub> removal during summer months (Sørensen, 2021), with high wastewater temperatures. The experimental part of this thesis happened during January to April, with low wastewater temperatures due to the season and meltwater intrusion. Therefore lower nitrification rates than during summer months was expected.

## 2.2 Biological wastewater treatment

Biological methods for wastewater treatment use microorganisms and their respective metabolism and growth processes for treatment of wastewater. Activated sludge (AS) systems treat wastewater by utilization of microorganisms that are suspended in the wastewater, and therefore the activated sludge systems relies on sedimentation and sludge recirculation to maintain microorganisms in the reactor (Jeppsson, 1996). Integrated fixed-film activated sludge (IFAS) reactors are an adaptation of activated sludge systems, using biofilm media to increase microorganism concentrations and treatment efficiencies, especially for nitrification, but still requiring sludge recirculation (Randall and Sen, 1996).

### 2.2.1 Moving bed biofilm reactor

A moving bed biofilm reactor (MBBR) is a type of biological wastewater treatment process utilizing non-clogging biofilm-covered carriers with high specific surface area, resulting in compact reactors with low head loss through the process (Ødegaard et al., 1994). MBBR carriers have free movement within the reactor, initiated by aeration or mechanical stirring to achieve aerobic or anaerobic/anoxic conditions respectively (Ødegaard et al., 1994). To prevent non-uniform distribution of carriers in the MBBR, degrees of filling are advised not to exceed 70% (Rusten et al., 2006; Ødegaard et al., 1994). Both MBBR and IFAS systems use some form of biofilm growth media, but in contrast to IFAS and AS, MBBR require no sludge recirculation.

#### 2.2.2 Biofilm diffusion

Diffusion is a central part of the nutrient transport in and out of the biofilm. Diffusion is based on transport by concentration gradients between the wastewater and biofilm. Li et al. (2016) produced concentration profiles of DO,  $NH_4^+$  and  $NO_3^-$  for biofilm in an IFAS system, as seen in Figure 2.1a), showing a concentration reduction of DO and  $NH_4^+$ inwards and a concentration reduction of  $NO_3^-$  outwards as DO and  $NH_4^+$  are consumed, while  $NO_3^-$  is made. As shown in Figure 2.1b), Li et al. (2016) also found that a reduction of bulk DO concentration can result in a relatively higher concentration drop deep inside the biofilm. The layer between the bulk and the biofilm is called the boundary layer. According to Lewandowski and Beyenal (2014), the boundary layer can be divided into the hydrodynamic boundary layer, characterized by decreasing flow velocities caused by viscous forces near the biofilm surface, and the diffusion boundary layer, similar to the hydrodynamic boundary layer, but also characterized by concentration gradients caused by concentration differences between the bulk and the biofilm. For Figure 2.1 the boundary layer refers to the diffusion boundary layer. Because of such gradients, low DO concentrations can cause little to no DO diffusion into the inner biofilm.



Figure 2.1: Concentration profiles for a) DO,  $NH_4^+$ -N and  $NO_3^-$ -N, and b) for different bulk DO concentrations, as functions of biofilm depth for an IFAS system. Acquired from Li et al. (2016).

## 2.3 Nitrification

Nitrification is a process where  $NH_4^+$  is oxidized to  $NO_2^-$  and  $NO_3^-$  by chemoautotrophic microorganisms, classified into ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) respectively (Pepper et al., 2015). According to Hem (2021), a common experience for new nitrification reactors is elevated  $NO_2^-$  concentrations and low  $NO_3^-$  concentrations during the first period after startup, however over time the  $NO_3^-$  will

be the main nitrification end product. Nitrification can be summarized in the reaction equations below:

Ammonia oxidation:  $NH_4^+(aq) + 1, 5O_2(aq) \rightarrow NO_2^-(aq) + 2H^+(aq) + H_2O(l)$ Nitrite oxidation:  $NO_2^-(aq) + 0, 5O_2(aq) \rightarrow NO_3^-(aq)$ Total nitrification reaction:  $NH_4^+(aq) + 2O_2(aq) \rightarrow NO_3^-(aq) + 2H^+(aq) + H_2O(l)$ 

According to the equations above, nitrification requires 2 moles of  $O_2$  per mole ammonia, but if nitrite is the main end product, only 1.5 moles of  $O_2$  is required. Considering the molecular mass of  $O_2$  is 32.00 g/mole and  $NH_4^+$  is 18.042 g/mole, 3.55 mg of  $O_2$  is required per 1 mg of  $NH_4^+$ . Since  $NH_4^+$  often is measured as  $NH_4^+$ -N, and 18.042 g  $NH_4^$ equals 14.01 of  $NH_4^+$ -N the required ratio is 4.57 mg of  $O_2$  per 1 mg of  $NH_4^+$ -N. If nitrite is the main end product the ratios are 2.66 mg of  $O_2$  per 1 mg of  $NH_4^+$  and 3.43 mg of  $O_2$  per 1 mg of  $NH_4^-$ -N. It is important to note that the diffusion coefficient of  $NH_4^+$  is a bit higher than for  $O_2$ , as this will affect the ratio in-situ (Hem, 2021).

When the DO levels are below the required  $O_2$  to  $NH_4^+$ -N ratio,  $O_2$  is the limiting parameter, while for low  $NH_4^+$ -N concentrations,  $NH_4^+$  is the limiting parameter. This relationship between  $NH_4^+$  and  $O_2$  affects the nitrification rate. When there is a very low concentration, in the wastewater, of the limiting parameter for nitrification (e.g.  $NH_4^+$  or  $O_2$ ) a 1<sup>st</sup> order dependency will occur, causing a linear relationship between the nitrification rate and the concentration of the limiting parameter. Half order reactions are dependent on the square root of the concentration of the limiting parameter. For  $NH_4^+$ -N concentrations up to 0.5-1.0 mg/L or for  $O_2$  concentrations up to 2 mg/L, a 1<sup>st</sup> order reactions will occur, while concentrations above this will cause half order reactions (Hem, 2021). Hem et al. (1994) studied nitrification of artificial wastewater for MBBR reactors and found a linear relationship between nitrification rate and DO concentration for different loads of BOD, as shown in Figure 2.2. Ødegaard (2006) presented a relationship between nitrification and  $NH_4^+$ -N concentrations for  $NH_4^+$  limited nitrification, as shown in Figure 2.3.

The slow growth rate of nitrifying bacteria compared to heterotrophic microorganisms can cause DO-competition in the biofilm. Researchers have found by simulation and experimentally that heterotrophic bacteria outcompete nitrifying bacteria in the outer biofilm, causing a layering with nitrifying bacteria in the deeper biofilm and heterotrophic bacteria in the outer biofilm (Wanner and Gujer, 1985; Rittmann and Manem, 1992; Okabe et al., 1995; Okabe et al., 1996). Additionally Ohashi et al. (1995) and Okabe et al. (1996) found that increasing C:N ratios reduce the portion of nitrifying bacteria in the biofilm, although at a C/N ratio of 0 the nitrifying bacteria were present also in the outer biofilm. This is in accordance with the results of Hem et al. (1994), showing lower nitrification rates when higher BOD loads are supplied.



Figure 2.2: Curves for nitrification rates plotted against DO concentration for different organic loads for temperature 15°C. Acquired from Hem et al. (1994).



Figure 2.3: Curve of nitrification rate plotted against ammonia concentration, with different DO concentrations marked on the plot. Acquired from Ødegaard (2006).

## 2.4 Denitrification

Denitrification is an anoxic process where heterotrphic facultative microorganisms, in the absence of  $O_2$ , use  $NO_3^-$  or  $NO_2^-$  as an electron acceptor, thus reducing  $NO_3^-$  or  $NO_2^$ to  $N_2$ -gas (Ødegaard et al., 2014). The microorganisms oxidize organic matter with  $NO_3^$ and  $NO_2^-$  as oxidizing agents when  $O_2$  is unavailable (Pepper et al., 2015). The organic matter needed for denitrification could be an internal or an external carbon source, where the internal carbon source is the organic matter already in the wastewater, while external carbon could be methanol, ethanol or other easily degradable carbon sources supplied to the wastewater (Ødegaard et al., 2014). According to Ødegaard et al. (2014), some microorganisms can reduce  $NO_3^-$  via  $NO_2^-$  to  $N_2$ , while most will only perform one of these reactions. The sub reactions of denitrifications are as follows:

Sub reaction 1:  $3NO_{3}^{-}(aq) + CH_{3}OH(aq) \rightarrow 3NO_{2}^{-}(aq) + CO_{2}(aq) + H_{2}O(l)$ Sub reaction 2:  $2NO_{2}^{-}(aq) + CH_{3}OH(aq) \rightarrow N_{2}(g) + CO_{2}(aq) + H_{2}O(l) + 2OH^{-}(aq)$ Total denitrification reaction:  $6NO_{3}^{-}(aq) + 5CH_{3}OH(aq) \rightarrow 3N_{2}(g) + 5CO_{2}(aq) + 7H_{2}O(l) + 2OH^{-}(aq)$ 

## 2.5 Effect of alkalinity on nitrification and denitrification

The pH of the wastewater is affected by nitrification and denitrification, reducing and increasing the pH respectively. From the chemical equations provided in section 2.3 and section 2.4, nitrification of 1 mole  $NH_4^-$  produces 2 moles  $H^+$ , while denitrification of 1 mole of  $NO_3^-$  produces 0.33 moles of  $OH^-$ . As seen in these equations, in section 2.3 and section 2.4, no  $H^+$  or  $OH^-$  produced when  $NO_2^-$  is oxidized to  $NO_3^-$  or  $NO_3^-$  is reduced to  $NO_2^-$ . Thus pH change of the wastewater is unaffected by nitrite or nitrate being the end product of nitrification. Totally nitrification and denitrification of 1 mole  $NH_4^-$  produces a net increase of  $H^+$  ions of 1.67 moles. To prevent the pH from decreasing rapidly by this process, the alkalinity, buffer capacity, of the wastewater must be sufficient, or else the pH will drop when the alkalinity is consumed.

According to Park et al. (2007), the optimal pH for ammonia oxidation is  $8.2 \pm 0.3$  and nitrite oxidation is  $7.9 \pm 0.4$ , while the same study showed that the maximum pH range for achieving half of optimal nitrification rates is 6.05-10.35 and 6.2-9.6 for ammonia and nitrite oxidation respectively. Thus half of optimal nitrification rates are mostly dependent on the pH range for nitrite oxidation, pH 6.2-9.6. The optimal pH range for denitrification was found by Beaubien et al. (1995) to be within pH 6.5-8.5, although short term changes of more than  $\pm 0.5$  within this range affect denitrification negatively. Biesterfeld et al. (2003) found that carbonate,  $CO_3^{2-}$ , is required for nitrification, since  $CO_3^{2-}$  act as an inorganic carbon source for nitrifying bacteria. Therefore, alkalinity, which for wastewater is often represented by the carbonate system (Shanahan and Semmens, 2015), is important both for cell growth and pH neutralization.

## 2.6 Enhanced biological phosphorus removal

Enhanced biological phosphorus removal (EBPR) is a biological wastewater treatment technology for removal of phosphorus compounds, like phosphates, in the wastewater (Comeau et al., 1986). In anaerobic conditions phosphate accumulating organisms (PAO) release  $PO_4^{3-}$  to take up easily biodegradable carbon sources, like volatile fatty acids (VFA), from the wastewater and store it as Polyhydroxyalkanoates (PHA) (Mino et al., 1998; van Loosdrecht et al., 1997). When exposed to oxic conditions the PAO use the stored PHA and  $O_2$  as electron acceptor to take up a higher amount of  $PO_4^{3-}$ , thereby accumulating  $PO_4^{3-}$  in the cell (Mino et al., 1998; van Loosdrecht et al., 1997). The EBPR process utilizes these traits of the PAO for phosphate removal, by introducing the PAO to a cycle of anaerobic conditions and oxic conditions. For AS systems this is achieved by use of non-aerated reactors followed by aerated reactors and sludge recycling from the aerated to the non-aerated reactors (Blackall et al., 2002). For the Hias Process, as described in the introduction, section 1, this is achieved in a similar fashion by transporting the biofilm carriers from the last oxic zone to the first anaerobic zone.

## 2.7 Denitrifying phosphate accumulating organisms

In anoxic conditions some PAO, called denitrifying phosphate accumulating organisms (DNPAO), can use  $NO_3^-$  as oxidizing agent for  $PO_4^{3-}$  accumulation (Vlekke et al., 1988; Kerrn-Jespersen and Henze, 1993). According to Saltnes (2021), DNPAO in the Hias process can utilize both nitrate and nitrite for anoxic  $PO_4^{3-}$  accumulation. Furthermore Kuba et al. (1993) found that DNPAO can achieve similar phosphorus removals as PAO. Ahn et al. (2001) found that DNPAO are able to utilize both  $NO_3^-$  and  $NO_2^-$  for denitrification, however some differences in denitrification rate and efficiency was found, indicating that  $NO_3^-$  was a more efficient electron acceptor than  $NO_2^-$  with respect to  $PO_4^{3-}$  uptake.

However some researchers have reported inhibition of PAO's  $PO_4^{3-}$  uptake when exposed to high NO<sub>2</sub><sup>-</sup>-N concentrations of 10 mg/L (Comeau et al., 1987), between 5 to 10 mg/L (Kuba et al., 1996) and between 5 to 8 mg/L (Meinhold et al., 1999). DNPAO have been found as more resistant to nitrite inhibition than non-denitrifying PAO (Saito et al., 2004; Yoshida et al., 2006; Saito et al., 2008). Additionally, Zhou et al. (2007) and Pijuan et al. (2010) discovered that free nitrious acid (HNO<sub>2</sub>) is likely the reason for the inhibition rather than nitrite, inhibiting phosphate uptake at concentrations of 0.002 mg HNO<sub>2</sub>-N /L. If DNPAO are responsible for some of the denitrification seen in the Hias process, the stored carbon can be utilized for denitrification without bsCOD in the wastewater. According to Saltnes (2021) there are not sufficient bsCOD concentrations in the oxic zones of the Hias process to justify the achieved denitrification, and thus DNPAO must be contributing to the denitrification.

### 2.8 Simultaneous nitrification and denitrification

Nitrification and denitrification can occur simultaneously in oxic conditions, as observed by Kokufuta et al. (1988), when local anoxic zones are available inside the biofilm. According to Saltnes (2021), NOB are inhibited in the SND of the Hias process, providing a "short-cut" SND, with denitrification directly from  $NO_2^-$ . The reason for the inhibition of NOB is not known. However, it is believed to be caused by competition for  $NO_2^-$  between DNPAO and NOB, and possibly helped by the presence of  $NH_4^+$ , providing continuous ammonia oxidation (Saltnes, 2021). When removing nitrite oxidation, denitrification is achieved with lower COD demand. Theoretically, reduction of  $NO_2^$ represents 60% of the COD demand of denitrification, resulting in a theoretical COD demand of 60% compared with denitrification from  $NO_3^-$ . If the inhibition of NOB carries over to the SNR, mostly  $NO_2^-$  will be produced and the COD requirement kept low, leading to lower consumption of internal carbon storage of the DNPAO. Combining denitrification with phosphate uptake in the Hias process could result in a lower bsCOD demand for denitrification, since the DNPAO use internally stored PAH and  $NO_3^-$  or  $NO_2^-$  as electron acceptor for phosphate uptake in anoxic conditions. Thus, both  $PO_4^{3-}$ and nitrogen removal could occur in the Hias process simultaneously by DNPAO, both in the anoxic zones when  $NO_3^-$  or  $NO_2^-$  are available and in the oxic zones within local anoxic zones in the inner biofilm.

## 3. Methods

## 3.1 Facility setup

The influent wastewater has been treated mechanically by inlet screens, sand and grease removal and primary sedimentation before entering the pilot plant.

The experimental part of this thesis is performed with a pilot scale Bio-P MBBR, hereby called the VEAS pilot, with a SNR. The VEAS pilot is owned by Hias How2O AS, but is called the VEAS pilot, since VEAS, a wastewater treatment company based in Asker, Norway, are part of an ongoing project with Hias How2O AS involving the pilot plant. The reactor volume is  $11 \text{ m}^3$  and it is equally compartmentalized into 10 zones with free flow of biofilm carriers from zone 1 to 10. A conveyor belt transports the biofilm carriers from zone 10 to zone 1. The carriers, pictured in Figure 4.35, have a hexagonal honeycomb shape with a thickness of approximately 5 mm, maximum diameter of 18 mm and specific surface area of  $800 \text{ m}^2/\text{m}^3$ . The carriers used in the VEAS pilot and the side-stream reactors were part of a trial batch, produced by a prototype tool, showing some material weakness when exposed to the propel stirrers of the VEAS pilot (Saltnes, 2021). Therefore, the material quality was not similar to carriers produced in ordinary production. Although, as I have later been told, this issue with the manufacturing of the carriers have later been improved by the supplier (Saltnes, 2021), although this does not affect this thesis. These carriers were used primarily so that the experiments could start.

The conditions of the first 3 zones are anaerobic, with  $PO_4^{3-}$  release and carbon accumulation, mainly easily biodegradable organic compounds like VFA, by PAO and DNPAO. In the 4<sup>th</sup> zone only the wastewater was transferred in and out of the SNR, therefore zone 4 was a mixed zone and seen as a part of the side-stream setup. A pump was installed to transfer wastewater from zone 4 to side-stream reactors, thus enabling control of wastewater flow and retention times in the initial SNR, later BOD-R and SNR. The pump was coupled with the control box of the VEAS pilot, with a possibility to control the wastewater flow through the pump by adjusting the frequency the pump operated on. The goal with zone 4 was to achieve some denitrification under anoxic



Figure 3.1: The carrier used in the VEAS pilot and the sidestream reactors.

conditions to utilize bsCOD of the wastewater, thereby saving internally stored carbon of the DNPAO. DO rich wastewater from the side-stream reactor/-s could have an effect on the DO concentration in zone 4. The initial SNR volume was  $0.768 \text{ m}^3$ , with a 60% degree of filling. The carriers with biofilm (0.461 m<sup>3</sup>) were moved from the VEAS pilot to the initial SNR and were confined within the initial SNR. The biofilm was expected to adapt to the oxic conditons in the initial SNR during a transition phase.

The last six zones were distributed between zones with anoxic and oxic conditions. The first zones following the SNR, zone 4 and 5, were anoxic with denitrification, preferably by heterotrophic bacteria utilizing the remaining bsCOD of the wastewater. The remaining bsCOD after phosphate release by PAO and DNPAO are mainly heavily biodegradable organic compounds which are difficult for the PAO and DNPAO to utilize. Although some denitrification by DNPAO should also be expected in anoxic conditions. The final zones, zone 6 through zone 10, were oxic zones with the main purpose of phosphate accumulation. In the oxic zones SND occurred, thus participating in the nitrogen removal. There had to be a sufficient amount of oxic zones to achieve the necessary P-removal in the wastewater. The air supply in the different zones were controlled by DO set points to optimize the SND.

The experimental part of the thesis was divided into a single reactor setup and a tworeactor setup. For the single reactor setup there was only one side-stream reactor, but as time went by it began to be clear that there was need for an additional reactor to achieve sufficient nitrification.

### 3.1.1 Single reactor setup

During the single reactor setup the biofilm was exposed to wastewater with an average retention time of 2 hours and 25 minutes and average wastewater flow through the side-

stream reactor of 316L/h. This was expected to, under the assumption that the bsCOD in the wastewater was low, give nitrifiers sufficient time to adapt to its new conditions, while tolerating some bsCOD removal by heterotrophic organisms.



**Figure 3.2:** An overview of the MBBR with the initial SNR (single reactor setup) is shown.

### 3.1.2 Two-reactor setup

With constantly low nitrification rates over the first 3 weeks of the project it was believed that the presence of bsCOD in the wastewater entering the initial SNR inhibited growth of AOB and NOB due to competition for  $O_2$ . Therefore, the setup was changed to achieve higher nitrification rates. A two reactor set up was implemented, with two reactors connected in series. The initial SNR was renamed BOD-R and used for removal of bsCOD, to stabilize the conditions for nitrification in the following SNR. The SNR contained a wastewater volume of 0.90 m<sup>3</sup> and was filled with carriers from the VEAS pilot to a degree of filling of 60%. While the VEAS pilot was refilled with equivalent amount of carriers from line 1 of the Hias WWTP full-scale Hias process MBBR, biofilm and carriers of the same type and with the same function as that of the VEAS pilot. Although these carriers were produced during ordinary production and thus did not show any material weakness such as the original carriers in the VEAS pilot. The new setup is displayed in Figure 3.3. The wastewater volume of the BOD-R was increased to 0.826 m<sup>3</sup> and filled with carriers from the VEAS pilot to maintain the degree of filling at 60%. The implementation of the two reactor setup also influenced the pump, decreasing the flow to about 225L/h, from 316L/h during the single reactor setup.

During the initial adaptation of the SNR, snow melting led to increased hydraulic loads to the facility, with equivalent reductions in organic and nitrogen loads. To navigate decreased nitrogen loads the sidestream wastewater flow was increased to 330L/h (at 22.02.2021 11.15 AM). It was striven to keep NH<sub>4</sub><sup>-</sup>-N concentrations in the discharge from the SNR between 3 and 10 mg/L, to secure constant nitrification, but also increase the retention time in the BOD-R to remove more bsCOD.



Figure 3.3: The two reactor setup with a BOD-R preceding the SNR.

## 3.2 Pilot tests

#### 3.2.1 Sampling

During the single reactor setup, samples were taken from each indicated zone in the VEAS pilot and from the SNR. Each sample was analyzed according to the zone it was sampled from, as shown in Table 3.1. The samples were analyzed with a spectrophotometer, Spectroquant NOVA 60.

sCOD is analyzed instead of BOD<sub>5</sub>, since it is cheaper and faster. It is assumed near 100% reduction of bsCOD at the end of the VEAS pilot. bsCOD in zone *i* is therefore approximated as  $bsCOD = sCOD_{zi} - sCOD_{z10}$ .

Tests		Zones		
	Day 1-5	Day 6-14	Day 15-34	
$\rm NH_4^+$ -N	3, 4, SNR	3, 4, SNR, 5	3, 4, SNR, 5	
$NO_3^N$	3, 4, SNR	3, 4, SNR, 5	3, 4, SNR, 5	
$NO_2^N$	3, 4, SNR	3, 4, SNR, 5	3, 4, SNR, $5$	
$sCOD^{(1)}$	-	-	3, SNR	
$\overline{{\rm PO}_4^{3-} - {\rm P}^{(2)}}$	-	-	3, 4, SNR, 5	

Table 3.1: Overview of tests for each zone. Zone numbering refers to the numbering of Figure 3.2.

(1): The COD tests were analyzed 3 hours prior to the other samples.

(2): The  $PO_4^{3-}$ -P tests were analyzed 3 hours prior to the other samples.

The numbers 3, 4 and 5 represents the corresponding zones Z3, Z4 and Z5.

After changing the facility setup, to the two-reactor setup with both a BOD-R and a SNR, the sampling and analysis was changed based on the new setup, as shown in Table 3.2. During the first 25 days a smaller amount of samples were analyzed and fewer tests were conducted for each sample, since the biofilm in the SNR was adapting to their new oxic conditions. From day 26 the amount of samples and tests were increased.

Table 3.2: New overview of analysis after implementation of BOD-R + SNR facility setup. Zone numbering refers to the numbering of Figure 3.3.

Tests		Zones	
	Day 4-13	Day 14-25	Day 26-68
$\rm NH_4^+$ -N	3, 4, BOD-R, SNR	3, 4, BOD-R, SNR, 5	In, 3, 4, BOD-R, SNR, 5, 10
$NO_3^N$	3, 4, BOD-R, SNR	3, 4, BOD-R, SNR, 5	In, 3, 4, BOD-R, SNR, 5, 10
$NO_2^N$	3, 4, BOD-R, SNR	3, 4, BOD-R, SNR, 5	In, 3, 4, BOD-R, SNR, 5, 10
$sCOD^{(1)}$	In*, 3, BOD-R, SNR, $10^*$	In*, 3, 4, BOD-R, SNR, $10^*$	In, 3, 4, BOD-R, SNR, 5, 10
$PO_4^{3-}-P^{(2)}$	In*, 3, 4, BOD-R, SNR, 10*	In, 3, BOD-R, SNR, 7, 10	In, 3, BOD-R, SNR, 7, 10
pH	3, 4, BOD-R, SNR	3, 4, BOD-R, SNR, 5	In, 3, 4, BOD-R, SNR, 5, 10
DO	BOD-R, SNR	BOD-R, SNR	BOD-R, SNR

\*: The indicated samples were analyzed 3 hours prior to the other samples.

The numbers 3, 4, 5 and 10 represents the corresponding zones Z3, Z4, Z5 and Z10.

"In" represents the inlet wastewater to the pilot plant.

### 3.2.2 Preparation and analysis of samples

The preparation and analysis of the samples are explained below. All samples were first filtrated with VWR Glass Fibre Filters, Grade 693. The filters have a particle retention of 1.2  $\mu$ m.

#### $NH_4^+-N$

For  $NH_4^+$ -N the Spectroquant Ammonium Cell Test provided by Supelco was used for analyzing. 1.00 mL of sample was diluted with 3 mL of water (diluted by a factor of

4 (1:3)). 500.0  $\mu$ L of diluted sample and 1 dose of reagent NH<sub>4</sub>-1K were added to the provided cell test tube. Spectrophotemeter signal was read after 15 minutes.

#### $NO_3^--N$

For  $NO_3^--N$  the Spectroquant Nitrate Cell Test provided by Supelco was used for analyzing. 1 provided "microspoon" of reagent  $NO_3-1K$  was added to the cell test tubes and shaken to dissolve. 5.00 mL of sample was mixed with 1 measuring spoon, approximately 50 mg, of amidosulfuric acid provided by Nanocolor. After a few minutes waiting for the amidosulfuric acid to dissolve, 1.50 mL was added to the cell test tube and shaken. Spectrophotemeter signal was read after 10 minutes.

#### $NO_2^--N$

For  $NO_2^--N$  the Spectroquant Nitrite Cell Test provided by Supelco was used for analyzing. Dependent on the day of the week the sample was diluted by a factor of 4 to 50 to achieve a concentration within the measuring range of the spectrophotometer. The outlet of the SNR required most dilution, while zones before the SNR and zones after the SNR during adaptation phase required no dilution. 5.00 mL of sample was added to the cell test tubes, shaken vigorously and spectrophotemeter signal was read after 10 minutes.

#### sCOD

For sCOD the Spectroquant COD Cell Test provided by Supelco was used for analyzing. 3.00 mL of sample was added to cell test tube, shaken vigorously and heated to 148 °C for 120 minutes in a thermoreactor, Spectroquant TR 620. Spectrophotemeter signal was read the cells were cooled down to room temperature, approximately 30 minutes.

### $PO_4^{3-}$

For  $PO_4^{3-}$  the Spectroquant Phosphate Test for the determination of orthophosphate provided by Supelco was used for analyzing. Dependent on what zone the sample is taken from the samlpe was diluted with water or not. The wastewater entering the VEAS pilot was diluted by a factor of 2. The wastewater from zone 1 to the last anoxic zone was diluted by a factor of 10, while samples from the oxic zones required no dilution. 5.00 mL solutions were made from each zone except zone 10 where 10.00 mL was required. The samples were added 5 drops of reagent PO<sub>4</sub>-1 per 5.000 mL of diluted sample and 1 dose of reagent PO<sub>4</sub>-2 per 5.00 mL of diluted sample. The samples were shaken and spectrophotemeter signal was read after 5 minutes.
#### Tot-P

For Tot-P the Spectroquant Phosphate Cell Test for the determination of orthophosphate and total phosphorus provided by Supelco was used for analyzing. 5.00 mL of diluted sample and 1 dose of reagent P-1K was added to the cell test tube, mixed and heated to 120 °C for 30 minutes in a thermoreactor, Spectroquant TR 620. The cell test tube was cooled down, shaken, added 5 drops of reagent P-2K and 1 dose of reagent P-3K and then was shaken vigorously again. Spectrophotemeter signal was read after 5 minutes.

#### Total alkalinity

The total alkalinity tests was not done by me, but by the staff of Hias WWTP after I had left the facility, although the reactor setup continued after my departure, as a part of their own testing. I was then given data for 10 days of testing for total alkalinity.

For total alkalinity the Spectroquant Acid Capacity Cell Test to pH 4.3 (total alkalinity) provided by Supelco was used for analyzing. 1.00 mL of sample and 4.00 mL of Reagent AC-1 was added to a clean cell and mixed. 0.50 mL of Reagent AC-2 was then added to the solution. The solution was shaken and spectrophotemeter signal was read. The analysis result was given as "acid capacity to pH 4.3,  $K_{S4.3}$ " in mmol/L. This was converted to mmol CaCO<sub>3</sub>/L (as 1 mmol/L of  $K_{S4.3}$  corresponded to 0.5mmol CaCO<sub>3</sub>/L).

### 3.3 Laboratory batch test

During the experimental stage laboratory batch tests were utilized for assessment of nitrification and denitrification rates of the biofilm carriers.

# 3.3.1 Nitrification rate of the SNR compared with the VEAS pilot

For evaluation of the progress of the biofilm adaptation in the SNR, a laboratory batch test was performed. Wastewater with low bsCOD content and relatively high  $NH_4^+$ content from zone 8 in the VEAS pilot was used in both batch reactors. The nitrification rate of biofilm carriers from the SNR and the VEAS pilot were compared during the experiment, testing for  $NH_4^+$ -N and  $PO_4^{3-}$ -P every 30 minutes and COD in the initial wastewater and in each batch reactor after 200 minutes. DO concentration in each batch reactor was measured every 30 minutes, simultaneously with the sampling.

The second batch test was performed similar to the first, but by accident the stirrers

were turned off. After 150 minutes the stirrers were turned on.

A different approach towards the laboratory batch test was conducted due to the results of the first and the second laboratory test.

#### 3.3.2 Laboratory batch test comparing stirring to no stirring

For evaluation of the hydraulic conditions in the SNR two laboratory batch experiments comparing stirring to no stirring was preformed. Wastewater from zone 7 with high ammonia and low bsCOD was used in both jars with 60% degree of filling of carriers from the SNR. One jar was mixed by stirring and aeration, while the second jar was aerated only. A grab sample of the starting wastewater was taken before startup. Samples were taken every 30 minutes. For the first test slow stirring was compared to no stirring, while the second test compared rapid stirring to no stirring.

# 3.3.3 Laboratory batch test comparing inlet wastewater to the SNR with artificial wastewater

To investigate if the reason for low nitrification rates in the SNR are caused by the composition of the wastewater, a laboratory batch test was conducted comparing prepared ammonia rich raw water with the outlet wastewater of the BOD-R. The ammonia rich raw water was prepared by addition of NH4<sup>+</sup>Cl and NaHCO<sub>3</sub> to lake water from Mjøsa to increase  $NH_4^+$ -N concentrations and have sufficient alkalinity for nitrification of the added  $NH_4^+$ . 160 mg NH4<sup>+</sup>Cl and 2g NaHCO<sub>3</sub> were added per liter lake water. The jars were mixed by aeration only, and held under similar conditions. Sampling was done every 30 minutes. This was done two times, as addition of alkalinity was forgotten for the first try.

# **3.4** Other tests

#### 3.4.1 Testing biomass on the carriers

For a quantitative test of biological growth on the carriers in the BOD-R and the SNR a test was conducted comparing them with the carriers from the VEAS pilot. 20 carriers with biofilm was dried at 110 °C for 22 hours and weighed. Then the carriers were shaken in 50 mL 0.05 mol/L NaOH solution for 2.5 hours to separate the carriers and biofilm. The carriers were then washed with 100 mL water and dried at 110°C for 22 hours and weighed. The biomass was then calculated. The washing liquid, 50 mL NaOH solution, was added the wash water and 50 mL 0.05 mol/L HCl solution. 2 mL of this liquid was added 18 mL of water and analyzed for Tot-P.

# 4. Results and discussion

# 4.1 General comments on the wastewater characteristics

#### 4.1.1 Load differences on the VEAS pilot

The VEAS pilot received relatively stable hydraulic loads during the experiments, however two incidents during the time period led to an increase of the hydraulic load. The cause of the higher hydraulic loads, shown in Figure 4.1 for the first 8 days and after 34 to 42 days was snow melting, leading to cold, diluted wastewater.



Figure 4.1: The average daily hydraulic loads on the VEAS pilot, for the 68 days of operation of the two-reactor setup.

The wastewater temperatures for the VEAS pilot and sidestream reactors are shown in Figure 4.2. The low temperatures during the first week and from day 34 to 44 corresponds to the periods with higher wastewater flow, caused by snow melting.



Figure 4.2: Wastewater temperatures for the VEAS pilot and side-stream reactors, for the 68 days of operation of the two-reactor setup.

The organic loads, given as nutrient concentrations, for the 68 days of operation of the two-reactor setup are shown in Figure 4.3 and 4.4. The concentration of all nutrients were not measured in the influent to the VEAS pilot before day 26, and thus the figures start at this day. The hydraulic load during the period is relatively stable, except for the snow melting periods during the first 8 days and from day 34 to 42 of the operation of the two-reactor setup, Figure 4.1. Thus, the fluctuations of the influent concentrations to the VEAS pilot are representative of the loads to the VEAS pilot.



Figure 4.3: Ammonia and sCOD loads, given as nutrient concentrations, for the influent of the VEAS pilot, for day 26-68 of operation of the two-reactor setup.



Figure 4.4: Nitrite, nitrate and phosphate loads, given as nutrient concentrations, for the influent of the VEAS pilot, for day 26-68 of operation of the two-reactor setup.

#### 4.1.2 Wastewater quality in and out of the BOD-R and SNR

The sCOD load in the BOD-R was normally much higher than for the SNR, as seen in Figure 4.5. This is as expected, since the primary goal of the BOD-R is bsCOD removal, and thus the sCOD load to the SNR will decrease. Most of the bsCOD in the side stream wastewater was consumed in the BOD-R, while only a little bsCOD was consumed in the SNR, as seen in Figure 4.6. In some cases the bsCOD in the effluent of the SNR was higher than in the influent, shown by negative values in Figure 4.6, which is either caused by bsCOD release in the SNR, from the biofilm, by uncertainty of the cell test analysis or by small load differences, due to the side stream reactors having an average retention time of a little more than 5 hours.



Figure 4.5: Comparison of the sCOD loads to the BOD-R and the SNR from day 26 to 68 of the two-reactor setup.



Figure 4.6: Comparison of the bsCOD consumption in the BOD-R and the SNR from day 26 to 68 of the two-reactor setup.

Figure 4.7 clearly depicts the daily fluctuation in  $PO_4^{3-}$ -P load to the side-stream setup throughout the week - with an increase from Monday trough Tuesday and stabilizing on Wednesday. To further show this relationship clearer, Figure 4.8 show the change of load in a typical week. The low loads during the first week are caused by high hydraulic load, from Figure 4.1. These  $PO_4^{3-}$ -P loads are a result of  $PO_4^{3-}$  release in zone 1 through 3 (the anaerobic zones). Thus, the loads and variation in loads shown in Figure 4.7 are higher than for the influent to the VEAS pilot.



**Figure 4.7:** Comparison of the  $PO_4^{3-}$ -P loads to the BOD-R and the SNR from day 5 to 68 of the two-reactor setup.



**Figure 4.8:** Comparison of the  $PO_4^{3-}$ -P loads to the BOD-R and the SNR from day 32 to 36 (one week) of the two-reactor setup.

## 4.2 Pilot plant performance

#### 4.2.1 Nitrification rates of the SNR

#### Single reactor setup (initial SNR)

The term "initial SNR" refers to the "single reactor setup". Figure 4.9 shows nitrification rates of the initial SNR. The results show a rapid decrease of nitrification rate during the first days, but a slow and steady increase during the following weeks. The DO concentrations in the wastewater in the initial SNR were not recorded during the first two weeks of operation, however it was kept between 7-8 mg  $O_2/L$  during this time. After 15 days the DO-concentrations were noted, as could be seen in the appendix, and during this time period the DO-concentrations were kept between 7.4 and 8.5 mg  $O_2/L$ . There is therefore no dramatic decrease of DO concentrations in the wastewater during the operation of the initial SNR. However the drop in nitrification rate after 4 days indicates a change of conditions for nitrification.

High sCOD loads was supplied to the initial SNR from the wastewater in zone 4 of the VEAS pilot, shown in Figure 4.10. Primarily easily biodegradable organic matter (e.g. VFA) is consumed by PAO during the first three anaerobic zones of the VEAS pilot, and thus it is to be expected that more heavy biodegradable organic matter was supplied to the initial SNR. From theory it is known that an abundant supply of bsCOD and  $O_2$  favors growth of heterotrophic bacteria. It is therefore likely that the conditions in the initial SNR was favoring heterotrophic growth, causing  $O_2$  consumption by heterotrophic bacteria. A rapid increase of biofilm thickness on the carriers in the initial SNR was observed during the operation, backing up this hypothesis.

This could indicate that the diffusion of  $O_2$  into the biofilm, and to the nitrifying bacteria, was reduced, causing lower  $O_2$  concentrations in the inner biofilm. This could lead to very low  $O_2$  concentration, limiting the nitrification, and thus cause a 1<sup>st</sup> order dependency between nitrification and DO concentration. It is likely that before this happened, from day 1 to 3, there was higher  $O_2$  diffusion to the inner biofilm, causing higher  $O_2$  concentrations and a half order dependency between nitrification and  $O_2$ .

The weekly differences could be explained by the weekly differences in organic load, increasing from Monday through Friday. It is very likely that the low nitrification rate is caused by high competition and growth of heterotrophic bacteria, inhibiting the nitrifying bacteria, due to the conditions in the initial SNR with abundant bsCOD and  $O_2$  supply. The heterotrophic bacteria consume  $O_2$ , thus reducing the diffusion of  $O_2$ into the inner biofilm. The relationship between DO-concentration, organic load and



Figure 4.9: Nitrification rate in the initial SNR (single reactor setup). The DO concentrations were kept between 7.4 to 8.5 mg/L during the 35 days of operation.



Figure 4.10: The sCOD loads to the initial SNR from day 15 to 35 of the single-reactor setup.

nitrification rates found by Hem et al. (1994), shown in Figure 2.2, show that an increase of organic load in the wastewater has a big impact on the achievable nitrification rates. This support that the high bsCOD loads could have an effect on the nitrification in the initial SNR. A growth of heterotrophic bacteria on the carriers is also indicated in the results from Figure 4.11b), indicating that there was a significant growth of heterotrophic bacteria on the biofilm.

Two laboratory batch reactor tests were performed to compare the nitrification of the carriers in the VEAS pilot and the carriers in the initial SNR. The first laboratory batch test, Figure 4.11a), shows no substantial difference between the carriers in the initial SNR and the VEAS pilot. The second laboratory batch test, Figure 4.11b), shows lower nitrification rate of the initial SNR carriers than the VEAS pilot carriers. However, when the stirrers were turned on after 150 minutes, both SS in the wastewater and nitrification increased rapidly during the last 30 minutes of the test.

During the experiment shown in Figure 4.11a) the batch reactor containing the initial SNR carriers had significantly higher SS in the wastewater than the other batch reactor. It was believed that this was either due to dissimilar SS content of the initial wastewater or higher bacterial growth on the initial SNR carriers. However, for the second laboratory batch test, Figure 4.11b), this did not occur until after 150 minutes, when the stirrers were turned on.



Figure 4.11: Nitrification rate of carriers from single reactor setup initial SNR compared with carriers from the VEAS pilot after a) 2 weeks operation and b) 4 weeks of operation. For b), the stirrers were turned on after 150 minutes.

That could indicate higher bacterial growth on the initial SNR carriers, consisting primarily of heterotrophic bacteria, leading to lower DO diffusion for the initial SNR carriers than the VEAS pilot carriers, as discussed previously in this section. If the nitrifiers are located deeper in the biofilm than the heterotrophic bacteria, which is likely due to the heterotrophic bacteria's competition advantage with high sCOD load to the initial SNR. This would explain the initial low nitrification rate. When the stirrer were turned on the SS increased, probably due to excess biofilm releasing from the initial SNR carriers, leading to easier DO diffusion to nitrifiers and therefore a higher nitrification rate.

Therefore, maintaining a slimmer biofilm could lead to higher nitrification rates. However, according to Saltnes (2021), Hias IKS has seen this in relation to  $PO_4^{3-}$  removal in laboratory scale batch experiments, without being able to obtain the same conditions in pilot or full scale. Nevertheless, this backs up the hypothesis that the low nitrification rates are induced by heterotrophic growth on the carriers in the initial SNR, leading to low  $O_2$  supply to the nitrifiers within the biofilm.

#### Two reactor setup (BOD-R and SNR)

The nitrification rates of the SNR usually out-compete the nitrification rates of the BOD-R, as shown in Figure 4.12, except for a few incidents where the SNR nitrification rates were reduced due to DO reduction. Overall, the nitrification rates of the BOD-R are lower than for the SNR, due to higher bsCOD concentrations fed to the BOD-R, continuing the trend seen in Figure 4.9 from the single reactor setup, averaging at 0.11 g/m<sup>2</sup>d, compared to the SNR which achieved an average nitrification rate of 0.33 g/m<sup>2</sup>d. The effect of bsCOD and  $NH_4^+$  loads to the SNR is further assessed later in the following sub-sections. The nitrification rates in the SNR are more variable due to variations in DO concentrations in the SNR, as discussed in the following sub-section and shown in 4.13, and therefore cannot be directly compared with those for the BOD-R, but it is clear that the nitrification rates are much higher. This corresponds with what is expected from the literature, since the BOD-R was fed higher bsCOD loads than the SNR, thus influencing the nitrification rates.



Figure 4.12: The nitrification rates of the BOD-R and the SNR during the operation of the two-reactor setup.

# 4.2.2 Effect of DO concentrations and temperature on nitrification rates

Figure 4.13 shows a clear connection between DO and ammonia removal in the SNR. The air supply to the SNR is connected to the same fans as the VEAS pilot aerated zones. The SNR can experience small DO fluctuation due to aeration changes in the VEAS pilot.

After 32 days DO was changed from 8 to 4 mg/L, with a clear drop in the nitrification rate. This shows that the biofilm is showing expected behavior to changes in its environment. The DO concentration in the wastewater was reduced to a half while the nitrification rate was reduced to approximately a quarter. This indicates that the reduction in  $O_2$  diffusion into the biofilm is reduced more than expected for a thin biofilm. This supports that the biofilm has an outer layer of heterotrophic bacteria reducing  $O_2$  diffusion by its thickness and consumption of  $O_2$ . From the way the nitrification rates changes with the DO concentration change, it is clear that the nitrification rate is dependent on the DO.

During the same time frame, from day 34 to 42, the period with snow melting resulting lower wastewater temperatures and higher hydraulic load was occurring, as seen when comparing the results from 4.14, 4.1 and 4.2. Although, there is a temperature reduction shortly after the DO concentration change, the effect of the reduction of DO seems to be more significant. However, according to theory nitrification is also temperature dependent, and an effect of the temperature change should be expected on the nitrification rate. It is however, with the current data, difficult to separate the effects from DO and temperature and likely a combination of DO and temperature reductions could be the cause of the significant reduction of nitrification rate, both for the initial reduction and the continued low nitrification rates during the snow melting period.



Figure 4.13: Relation between DO concentration and nitrification rate in the SNR during the two-reactor setup.

#### 4.2.3 Effect of ammonia and sCOD loads on nitrification rates

Figure 4.14 shows a comparison between the  $NH_4^+$ -N loads on the SNR and the nitrification rates observed. As we can see from these results the nitrification rates for some days are optimal compared with the ammonia load, while for most days the ammonia load is higher than the nitrification rate, resulting in incomplete nitrification. This indicates that the nitrification rate, at least for some days, are dependent on the ammonia load. Although, as seen in Figure 4.13, the nitrification rate shows a higher dependency of the DO concentration and temperature.

The same comparison is made between the nitrification rates and the sCOD loads to the SNR, shown in Figure 4.15. If the sCOD load had a significant impact on the nitrification rates, the nitrification rates would reduce with higher sCOD load, but according to these results the nitrification rate does not seem to be much affected by this. This could indicate that the bsCOD fraction of the sCOD load is small, and that there is little competition of  $O_2$  between heterotrophic microorganisms and the nitrifiers. Thus, the BOD-R seems to be performing as intended, reducing the bsCOD load to the SNR.



Figure 4.14: Relation between  $NH_4^+$ -N loads and nitrification rates in the SNR for the two-reactor setup.



Figure 4.15: Relation between sCOD loads and nitrification rates in the SNR for the two-reactor setup.

#### 4.2.4 Denitrification

After the implementation of the two reactor setup, the denitrification relative to nitrification in the SNR was reduced compared to the initial SNR, as seen when comparing the results from Figure 4.16 and Figure 4.17. The main reason for this is likely caused by a reduced  $O_2$  competition in the SNR, caused by the bsCOD removal prior to the SNR, resulting in higher nitrification and which was not the case for the initial SNR.



Figure 4.16: Nitrification given in terms of  $NH_4^+$ -N nitrified and  $NO_x^-$ -N denitrified by the SNR for the two-reactor setup. The denitrification in zone 4 and 5 is not accounted for in this plot.

As shown in figure 4.17, both nitrification and denitrification occurred in the initial SNR. Between 50-90% of the nitrified  $NH_4^+$  is denitrified in the initial SNR. This indicates that the carriers already had a significant amount of heterotrophic denitrifying bacteria, which should be expected when having heterotrophic bacteria and nitrification (Hem, 2021). Wastewater treatment plants have observed that denitrification will occur in wastewater sludge under anoxic conditions when nitrification has previously occurred (Hem, 2021). Having SND is advantageous for multiple reasons. First, denitrification in the initial SNR lowers the demand for anoxic zones following the SNR, while simultaneously clearing up space for oxic zones for  $PO_4^{3-}$  uptake and more SND. Second, denitrification by heterotrophic bacteria will utilize some of the less biodegradable bsCOD, reducing the demand for internal carbon usage by DNPAO, which is assumed to be a limiting parameter for the overall denitrification of the plant. And third, utilization of bsCOD for denitrification will reduce the demand for addition of external carbon sources if the internally stored carbon by DNPAO is insufficient.

Figure 4.18 show a comparison of nitrification and denitrification in the BOD-R. When comparing these results with the results from the initial SNR, we can see that the trend of high ratio of denitrification to nitrification is continued. This shows that the SND in the BOD-R continued after the implementation of the two-reactor setup, with the advantages this involves. The period from day 36 to 48 corresponds with the snow-melting period, causing high hydraulic loads and lower wastewater temperatures. This is likely the reason for the low denitrification during this period.



Figure 4.17: Nitrification given in terms of  $NH_4^+$ -N nitrified and  $NO_x^-$ -N denitrified by the initial SNR (single reactor setup) during the time frame of 15 to 35 days of operation. The denitrification in zone 4 and 5 is not accounted for in this plot.



**Figure 4.18:** Nitrification given in terms of  $NH_4^+$ -N nitrified and  $NO_x^-$ -N denitrified by the BOD-R for the two-reactor setup.

#### 4.2.5 Overall nitrogen removal

The relative  $NH_4^+$ -N removal of the pilot during the last five weeks of operation, as seen in Figure 4.19, averaged at 54%  $NH_4^+$ -N removal. However the average was increased to 57% for the last four weeks of operation. When considering soluble nitrogen removal, as  $NH_4^+$ -N,  $NO_2^-$ -N and  $NO_3^-$ -N, in the wastewater, from influent to effluent, the removal efficiency is as described in Figure 4.19. The relative nitrogen removal, when considering ammonia, nitrite and nitrate is a bit lower than the relative removal of ammonia, and averages at 54% for the last four weeks. The average removal from day 26 to 68 is 52%. This shows the impact of there being some nitrite and nitrate left in the effluent, however the two plots for ammonia and nitrogen removal follow each other closely.



**Figure 4.19:** Ammonia and nitrogen (as ammonia, nitrate and nitrite) removal given in percentages for the VEAS pilot with side-stream reactors during the last six weeks of operation of the two-reactor setup.

#### 4.2.6 DO and denitrification - zone 4

The DO rich effluent from the SNR is discharged into zone 4. Zone 4 is operated as an anoxic zone, and it is therefore preferred to keep the DO concentration as low as possible. By measuring the DO concentration in zone 4, it was found that the DO concentration did not rise above 0.33 mg/L. When blending wastewater from zone 3 and the SNR at the ratio relative to the flow, it would be expected to see DO concentrations of approximately 2.75 mg/L, assumed there is no DO in zone 3, 7mg O<sub>2</sub>/L in the SNR effluent and no O<sub>2</sub> consumption in zone 4. A significantly lower DO concentration is seen, which could be partly caused by O<sub>2</sub> consumption by the biofilm formed in the connecting pipe, but mainly by continuous O<sub>2</sub> consumption in zone 4, thereby preventing the DO content to rise.

Figure 4.20 shows that the measured  $NO_2^--N$  concentrations for the first 20 days are much lower than the calculated concentrations of the combined influent from zone 3 and SNR. The same applies for the measured  $NO_3^--N$  concentrations from day 10, as seen Figure 4.21. The calculated concentrations are estimated by using a weighted arithmetic mean for the concentrations of zone 3 and the SNR, weighted on the volume flux contributed to zone 4. The volume fluxes from the SNR were measured each day, while the volume fluxes from zone 3 were approximated as the average volume flux through the VEAS pilot, thus giving an underestimation of the calculated concentration, since the out-flux to the BOD-R was not considered.

For the parts where there is no significant difference between measured and calculated concentrations, the last 50 days and the first 10 days for  $NO_2^-$ -N and  $NO_3^-$  respectively, the most probable explanation is that the influent concentrations in these instances are so low that they are limiting for denitrification of this part of the nitrate and nitrite respectively. From Figure 4.21 one can observe that on most days there is nitrate left in zone 4, which according to Figure 4.23 is mostly denitrified in zone 5, indicating that the denitrification in zone 4 is not complete. As seen in Figure 4.22 and 4.23 there is a reduction of  $NO_2^-$  and  $NO_3^-$  from zone 4 to zone 5, confirming that denitrification occurs in both of the anoxic zones. For both zone 4 and zone 5 the  $NO_2^-$  and  $NO_3^-$  concentrations are very low, causing anaerobic conditions. Although, as should be expected, this happens more often in zone 5.

The  $O_2$  supply to zone 4 will cause some  $O_2$  consumption in zone 4 either by heterotrophic organisms or by PAO/DNPAO. The  $O_2$  supply of approximately 3 mg  $O_2/L$ to zone 4 will correspond to an equivalent sCOD consumption of approximately 3mg  $O_2/L$ , however the carbon source could be both the sCOD in the wastewater and internally stored PAH in PAO/DNPAO. From theory it is known that oxic conditions will impact denitrification, since  $O_2$  is the preferred electron acceptor when both  $O_2$ ,  $NO_2^$ and  $NO_3^-$  are available. Oxic conditions in zone 4 will therefore impact the denitrifying bacteria. This should mainly affect the outer biofilm, due to  $O_2$  diffusion and consumption. Although denitrification is observed in zone 4 it is not complete. Due to the factors addressed above and the presence of nitrate in the effluent from zone 4, denitrification might be affected by the  $O_2$  supply to zone 4, however to what extent denitrification is affected is difficult to say with the data available.



Figure 4.20: The calculated and measured  $NO_2^--N$  concentrations in zone 4, during the two-reactor setup. The calculated concentrations are given no denitrification in zone 4.



Figure 4.21: The calculated and measured  $NO_3^--N$  concentrations in zone 4, during the two-reactor setup. The calculated concentrations are given no denitrification in zone 4.



Figure 4.22: Comparison of  $NO_2^-$ -N concentrations in zone 4 and 5, during the two-reactor setup.



**Figure 4.23:** Comparison of  $NO_3^-$ -N concentrations in zone 4 and 5, during the two-reactor setup.

Figure 4.24 and 4.25 show the relationship between nitrate and bsCOD concentration change and between phosphate and bsCOD concentration change in zone 4 compared to the calculated influent from zone 3 and the SNR combined. The bsCOD concentration change is very low relative to  $NO_3^-$ -N concentration change, less than 1 mg sCOD/mg  $NO_3^-$ -N, for a quarter of the days studied. Thus, bsCOD concentration change during these days are not sufficient to explain all of the denitrification, as according to Saltnes (2021) a rato of about 4 mg sCOD/mg  $NO_3^-$ -N is theoretically necessary for denitrification. During the other days this concentration change ratio is above 4 mg sCOD/mg  $NO_3^-$ -N. As discussed earlier in this section, the DO of the influent to zone 4 is expected to cause some bsCOD consumption by heterotrophes with  $O_2$  as electron acceptor, Although, this should not be expected to cause a significant sCOD concentration difference, as the DO load supplied to zone 4 is less than 3 mg  $O_2/L$ . Additionally from Figure 4.25  $PO_4^{3-}$  release is observed in zone 4, and thus some bsCOD consumption should be expected to occur by  $PO_4^{3-}$ -P release.

From Figure 4.25 it is observed that for the majority of days studied (approximately 2/3 of the days) there is a  $PO_4^{3-}$  uptake in zone 4. The  $PO_4^{3-}$  uptake could be related to both the  $O_2$  load supplied from the SNR and denitrification by DNPAO. Due to the relatively low  $O_2$  supply from zone 4, it is likely that the majority of  $O_2$  is consumed by heterotrophic microorganisms, however oxic  $PO_4^{3-}$ -P uptake cannot be disregarded based on the results available. The results from figures 4.24 and 4.25 show a tendency of DNPAO contributing to denitrification in zone 4, however the results are not unambiguous.



Figure 4.24: Comparison of  $NO_3^-$ -N and bsCOD concentrations differences between wastewater in zone 4 compared to the calculated influent from zone 3 and the SNR combined, during the two-reactor setup.



Figure 4.25: Comparison of  $PO_4^{3-}$ -P and bsCOD concentrations differences between wastewater in zone 4 compared to the calculated influent from zone 3 and the SNR combined, during the two-reactor setup.

Differences in bsCOD and  $PO_4^{3-}$ -P concentrations between zone 4 and 5 can give some insight to what happens during the denitrification in zone 5. Figure 4.26 shows variations between bsCOD concentration differences between zone 4 and 5, with both increasing and decreasing bsCOD concentrations. The same is seen for the  $PO_4^{3-}$ -P concentration differences, which vary between release and uptake between the zones. On average there is a slight reduction of 6.8 mg/L in bsCOD concentration, while the average  $PO_4^{3-}$ -P concentration change is an insignificant 0.3 mg/L.

The reason for the bsCOD concentration differences could be a combination between uncertainty related to sCOD measurements, but also could be caused by denitrification, as there is a tendency of more reduction of bsCOD than increase, especially for the last three to four weeks of the experiment. The reason for the fluctuations in  $PO_4^{3-}$ -P concentration could also be influenced by denitrification, namely denitrification by DNPAO which will cause an uptake of  $PO_4^{3-}$ . This  $PO_4^{3-}$  could then be released into the wastewater by uptake of easily biodegradable bsCOD by DNPAO, thus resulting in little difference of  $PO_4^{3-}$ -P concentrations. The results shown in figure 4.22 and 4.23, show that the  $NO_2^{-}$ -N and  $NO_3^{-}$ -N concentrations are low, causing anaerobic conditions which is favoring phosphate release, but sometimes the concentrations are higher, causing anoxic conditions which enables phosphate uptake by DNPAO. However, since the concentration difference is varying between uptake and release from zone 4 to 5 it is difficult to verify this, but there could be a tendency of this occurring which could be studied further.



**Figure 4.26:** Difference between bsCOD (left) and  $PO_4^{3-}$  (right) concentrations in zone 4 and zone 5 during the two-reactor setup. A negative value represents a concentration decline.

#### 4.2.7 Overall phosphate removal

The  $PO_4^{3-}$ -P concentrations of the effluent of the VEAS pilot from day 26 to 68 of operation with the two-reactor setup is shown in Figure 4.27. The  $PO_4^{3-}$ -P concentrations in the effluent of the VEAS pilot was on most days below 0.3 mg/L and above 0.5 mg/L only one day out of the 24 days when analysis of the effluent was performed. This day was the first day of the snow melting period, when the water temperature was low and the hydraulic load high. The relative removal of  $PO_4^{3-}$ -P from influent to effluent was in this period above 95%, except for the first day of snow melting where the removal was 85%. This show that during the operation the  $PO_4^{3-}$ -P removal was not significantly negatively impacted.



Figure 4.27: Phosphate concentrations of the effluent of the VEAS pilot during day 26 to 68 of the operation of the two-reactor setup.

# 4.3 Biofilm adaptation

#### 4.3.1 Carrier condition

Many of the carriers in the SNR are misshaped caused by damage in the VEAS pilot before introduction to the SNR, as seen in Figure 4.28. As described during the method description, section 3, the carriers are damaged in the VEAS pilot due to low material quality caused by production with a prototype tool. The misshaped forms result in lower area to volume ratios for the carriers, however the average area to volume ratio of the carriers are not known and likely it would not be very far below  $800 \text{ m}^2/\text{m}^3$ , and thus result in a very little influence on the results. If the average specific surface area of the carriers were known, this would affect the calculated nitrification rates, resulting in a slight increase. How big this influence is on the results is difficult to estimate, but when considering the representative selection in Figure 4.28 one can see that the influence of the misshaping is not very big. The observed nitrification rates are therefore likely somewhat lower than the actual nitrification rates due to lower effective biofilm area in the SNR, than what has been used during the calculations.

For a quantitative assessment of the reduction in specific surface area of the carriers the following criteria were used: Each cell in each carrier was given a status of "not damaged" or "damaged", where the specific surface area of a "damaged" cell was counted as 0. A cell was counted as "damaged" when; (1) the outer cell wall was missing, (2) the cell was squeezed to half the size or less. When multiple cells were squeezed to



Figure 4.28: A representative selection of carriers from the SNR (two-reactor setup), showing some deformation.

approximately half of the original size on the same carrier, not all of the cells were counted. Additionally, when only a small piece of the outer cell wall was missing the cell was counted as "not damaged". Each carrier has 37 cells where the biofilm can grow. For the 64 carriers pictured in 4.28 this equals a total of 2368 cells. Among the 64 carriers a total of 269 were considered "damaged". Thus, a reduction of specific area of approximately 11% was observed for this assessment, corresponding to a specific surface area of 710 m<sup>2</sup>/m<sup>3</sup> (instead of 800 m<sup>2</sup>/m<sup>3</sup>).

Although this is not a perfect method for assessing the reduction of specific surface area, being somewhat subject to what is counted as a damaged cell and how a damaged cell should be counted, it is a good approximation. There are also uncertainty regarding how much influence a deformation in a cell has on the specific surface are of the cell. If the reduction of specific surface area is 11%, as was approximated, the nitrification rates should be expected to be influenced correspondingly. This is however not considered during the other analysis and a surface area of 800 m<sup>2</sup>/m<sup>3</sup> have been used.

#### 4.3.2 Comparison of SNR and VEAS characteristics

Figure 4.29 shows about similar nitrification rates for the carriers from the VEAS pilot and the SNR. The fast declination for the SNR carriers can be explained by dissimilar startup concentrations, thus the grab sample not being representative for the wastewater in both batch reactors. Such a difference is seen in later laboratory tests and it is likely the case for this test as well. It must be assumed that the temperature of the wastewater in the two batch reactors are similar, as the same wastewater has been used. The DO concentration were kept around 8 mg  $O_2/L$ , although the average DO concentration in the batch reactor with the SNR carriers were approximately  $0.1 \text{ mg O}_2/\text{L}$  lower than for the batch reactor with VEAS carriers. Disregarding the sample taken after 0 minutes, the average nitrification rates are 0.46 and 0.40 g  $NH_4^+$ -N/m<sup>2</sup>d for the VEAS pilot and the SNR respectively. Not much improvement in regards to the nitrification rate for the carriers is therefore seen 5 weeks after start up of the SNR. One reason for this could be that even with an increase of nitrifiers in the biofilm, an increase of heterotrophic growth on the biofilm, possibly caused by different hydraulic conditions in the SNR, have canceled out the positive effects of more nitrifiers. Without stirring in the SNR the biofilm has been allowed to grow visibly thicker, primarily by heterotrophic growth induced by aeration and some bsCOD from the inlet wastewater.



Figure 4.29: Nitrification by carriers from SNR compared with carriers from the VEAS pilot after 5 weeks of operation of the two-reactor setup.

#### 4.3.3 Nitrification adaptions in the SNR

During the first week after starting the SNR, the nitrification by NOB was inhibited, but as seen in Figure 4.30 the  $NO_2^-$ -N concentration in the SNR effluent decreases after the first week, gradually being replaced by higher  $NO_3^-$ -N concentrations. This change in the biofilm, from short-cut nitrification to regular nitrification results in a higher bsCOD requirement for denitrification. This development is normal for startup of nitrification reactors, as commented earlier.



**Figure 4.30:** Development of  $NO_2^-$ -N and  $NO_3^-$ -N concentrations in SNR effluent during the first 68 days of operation for the two-reactor setup.

#### 4.3.4 Quantitative biomass test

As seen in Figure 4.31 the appearance of the biofilm between the reactors are different in color and volume. The carriers from the VEAS pilot appears to have the least biomass, followed by the SNR and the BOD-R with most biomass. However, according to the results from the quantitative test, Figure 4.32, the biomass of the VEAS pilot carriers are equal to the BOD-R carriers, while the carriers from the SNR had lowest biomass. The qualitative and quantitative results contradict each other, and it seems that the color difference between the reactors could have an impact on the qualitative assessment of biomass amount. However, the color differences also indicate that the biofilm adaptation, as expected, has led to a different bacterial composition which could cause a different biofilm density, but also different diffusion rates. Voluminous biofilms could be caused by an increased presence of filaments in the biofilm, resulting in more internal water storage (Hem, 2021).



Figure 4.31: Wet, dried and cleaned carriers from a) the VEAS pilot, b) the BOD-R and c) the SNR, for the two-reactor setup.



Figure 4.32: The biomass content relative to carrier mass for carriers the VEAS pilot, the BOD-R and the SNR, for the two-reactor setup.

## 4.4 Laboratory batch tests

#### 4.4.1 Effects of stirring on nitrification rates

Figure 4.33 shows no significant difference between stirring and no stirring, with nitrification rates of 0.66 and 0.69 respectively. The laboratory batch tests, show higher nitrification rates than what is achieved in the SNR, which is likely caused by higher wastewater temperatures, as the wastewater is warmed up when exposed to the warmer air temperatures in the laboratory. Some time during the last 30 minutes of the test, the nitrification conditions for slow stirring changed from oxygen dependent to ammonia dependent, and the sample taken after 150 minutes is therefore disregarded for the calculation. Figure 4.34 shows slightly higher nitrification rates for rapid stirring than for no stirring, with values of 0.65 and 0.59 (alternatively 0.93 and 0.79) respectively.



Figure 4.33: Nitrification by carriers from SNR with and without slow stirring after 8 weeks of operation of the two-reactor setup.



Figure 4.34: Nitrification by carriers from SNR with and without rapid stirring after 9 weeks of operation of the two-reactor setup.

The slowly stirred jar had visibly more SS than the one without stirring. That was also the case fore the rapidly stirred jar, but with even more SS. That can be explained by release of biofilm from the carriers due to different hydraulic conditions, mainly higher velocities. Although there is a decrease of biofilm thickness for the slowly and rapidly stirred carriers, the effect on nitrification rate is minimal. There might be multiple explanations for why this is happening. First, the density/amount of nitrifiers in the biofilm could be so small that sufficient amounts of  $O_2$  is diffused into the biofilm even with a thicker layer. Second, the aeration might be to aggressive, supplying more  $O_2$ to the biofilm. Clearer results could have been seen by keeping lower DO levels, like 4 or 6 mg/L. However, the DO supply to the jars was difficult to operate and to achieve low DO concentrations. And third, the decrease of biofilm thickness could be smaller than anticipated, leading to only small differentiation in  $O_2$  diffusion conditions between carriers in stirred and non-stirred jars.



Figure 4.35: 1 L wastewater from laboratory batch tests after a) no stirring and b) stirring.

#### 4.4.2 Investigation of wastewater composition

In figure 4.36 shows clear inhibition of nitrification in the artificial wastewater. For this test the artificial wastewater was not added alkalinity, and therefore the lack of sufficient alkalinity for nitrification resulted in a rapid alkalinity decrease, causing a rapid decrease of the pH to a level which did not support nitrification. The pH of the artificial wastewater started at 7.1. and ended up at pH 5.79. The wastewater however, started at pH 7.55 and ended at pH 7.04. The average nitrification rate for this test was  $0.58 \text{ g } \text{NH}_4^-\text{-N/m}^2\text{d}.$ 

For the second lab test, the artificial wastewater was added sufficient alkalinity. In figure 4.37 the nitrification in the jar with artificial wastewater appeared to be faster during



Figure 4.36: Comparison of nitrification of inlet wastewater to the SNR and lake water added  $NH_4^+$ , by carriers from SNR after 8 weeks of operation for the two-reactor setup. The average DO of the two batch reactors were 8.06 and 8.84 mg/L for the wastewater and the artificial wastewater respectively. The artificial wastewater had no to little alkalinity.

the first 30 minutes, however it slowed after 30 minutes and kept going at a steady rate. There was a tendency after 30 minutes, that the artificial wastewater nitrification rate, 0.45 g/m<sup>2</sup>d, was slightly lower than the nitrification rate for the wastewater, 0.54 g/m<sup>2</sup>d. However, if the whole time frame from start to finish was considered, there was no significant difference between the nitrification rates, averaging at 0.58 and 0.54 for the lake water jar and the wastewater jar respectively. There was no significant difference between the two batches for NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N, but the pH changes showed some difference. The pH for the wastewater was reduced from 7.73 to 7.39, while the pH of the artificial wastewater increased from 8.35 to 8.61. The latter could be explained by removal of  $CO_2^-$  from the water.

This test shows that there is no evidence for direct inhibition of nitrification due to components in the influent wastewater to the SNR. However, some components of the influent, e.g. bsCOD, could have a long term effect on the growth of nitrifying bacteria, which this test does not consider. Based on these results and the extra growth on the carriers it is likely that heterotrophic growth has, to some extent, inhibited the long term growth of AOB and NOB and prevents  $O_2$  diffusion to the inner biofilm.



Figure 4.37: Comparison of nitrification of inlet wastewater to the SNR and lake water added  $NH_4^+$  and alkalinity, by carriers from SNR after 9 weeks of operation for the two-reactor setup. The average DO of the two batch reactors were 8.22 and 8.11 mg/L for the wastewater and the artificial wastewater respectively.

### 4.5 Alkalinity analysis for the side-stream setup

As described in section 3, the alkalinity tests were conducted by Hias WWTP staff and started one week after my last tests where conducted.

As seen in Figure 4.38, the total alkalinity in the wastewater in the SNR is reduced to almost 0 mmol  $CaCO_3/L$  for several of the 9 days where testing was done. When comparing the alkalinity of Zone 3 and the SNR we can see that there is a substantial decrease of total alkalinity in the SNR compared with the wastewater in the VEAS pilot. Although, these tests were conducted after the other testing in the previous subsections, the conditions of the SNR and the process setup was similar for day 74-89 of the two-reactor setup as it was up until day 68. Therefore, these results indicate that the total alkalinity of the wastewater in the SNR showed similar patterns for the days and weeks before day 68 of the two-reactor setup. Therefore, the low alkalinity in the SNR could be a reason for the low nitrification rates observed in the SNR. Since the alkalinity in the wastewater is already very low in the SNR, it would be expected that the alkalinity within the biofilm is lower still. This due to the diffusion of alkalinity into the biofilm, which must be assumed causes a concentration decline further into the biofilm, and the production of  $H^+$  ions during nitrification within the biofilm. The combination of low alkalinity and nitrification within the biofilm could therefore lead to lack of sufficient alkalinity and rapid pH decrease within the biofilm, which in turn would result in inhibition of NOB and AOB causing lower nitrification rates. Although this can not be verified for the results analysed before day 68, it shows a tendency which can likely have affected the nitrification in the SNR during the experiments. From day 4 and until day 68 only the pH was tested, to monitor whether there was a significant pH change during the nitrification in the SNR. A significant pH change was not measured and the lowest pH recorded was 6.77 in the SNR effluent. It was therefore believed that the alkalinity was sufficient. However, in retrospect it would have been preferable to do alkalinity analysis also then.



Figure 4.38: Total alkalinity for Zone 3 and the SNR from day 75 to 89 of the two-reactor setup, done by Hias WWTP staff.

# 4.6 General discussion - Consequences/Implications for pilot design

Although the average ammonia removal throughout the plant is below the initial nitrogen removal goal indicated by Hias How2O (60% reduction of ammonia), the side-stream setup with a BOD-R and a SNR shows some good tendencies. The average removal rates are 54% with an increasing tendency. However, some alterations must be introduced to ensure higher removal rates for the side-stream setup. Some bsCOD is fed to the reactor, possibly resulting in a long-term growth of heterotrophic bacteria on the biofilm surface and suppression of nitrification. To evade this consequence a few possible solutions will be discussed. A larger BOD-R would increase hydraulic retention time, resulting in a higher bsCOD removal and thus could reduced the bsCOD of the influent to the SNR. Another measure that should be evaluated is segregation of the SNR. Division of the SNR into three to four separate reactors connected in series will cause decrease of loads from the first reactor to the last reactor. This could increase the nitrification efficiencies of the last reactors, getting lower bsCOD loads, while still being supplied with sufficient ammonia concentrations most days. Implementation of a larger reactor volume could increase nitrification capacity of the SNR by increasing the retention time. However, such modifications should be carefully evaluated regarding areal requirements of bigger reactors, especially for full scale systems. Although for some of the days, the results showed that the ammonia load to the SNR was only slightly higher than the nitrification rate, and thus an increase of the wastewater flow through the side-stream setup could also be considered.

The denitrification in zone 4 and zone 5 can utilize some of the remaining bsCOD content of these zones. However, the results regarding this is not clear to what extent heterotrophic microorganisms and DNPAO contribute to the denitrification. It is however a clear tendency when studying the  $PO_4^{3-}$ -P accumulation, denitrification and bsCOD consumption in these zones which supports contribution of DNPAO for denitrification. It is also likely that other denitrifying bacteria are contributing to denitrification by consumption of bsCOD in zone 4 and 5. Thus this bsCOD is used for denitrification rather than for heterotrophic respiration in the oxic zones. In zone 5 there are on most occasions anoxic conditions, which favor  $PO_4^{3-}$ -P release and bsCOD consumption by the DNPAO, if easily biodegradable organic matter like VFA are still available. The average reduction in bsCOD concentrations between zone 3 and 4 and 5 show that some anoxic bsCOD consumption happens in the zones and thus supports this and denitrification by heterotrophic microorganisms. Thus, the carbon source available for SND in the oxic zones will not be significantly reduced, due to heterotrophic microorganisms utilizing the bsCOD in zone 4 and 5 for denitrification.

Another somewhat contributing reason for the observed low nitrification rate in the SNR is the condition of the carriers. Replacing the carriers and waiting for new biofilm to form would likely increase the nitrification rate to some extent, however this would only explain a small part of the reason for the observed low nitrification rates.

The observed low alkalinity in the SNR between day 75-89 is a more likely contributor to the observed low nitrification rates in the SNR. From theory it is known that lack of alkalinity will result in pH reduction during nitrification which has an inhibiting effect to nitrification. Although these results were gotten after the analysis of the main experiment was finished this show that lack of alkalinity could pose a problem for the nitrification in the SNR. However, this issue could be solved by either pH-adjustment or addition of alkalinity prior to the nitrification in the SNR. An addition of a small stirred reactor after the SNR, with the purpose of reducing the DO concentration of the wastewater, could have an effect on the denitrification in zone 4. If less  $O_2$  is supplied to zone 4, less aerobic consumption of bsCOD should be expected, and thus cause more efficient denitrification in zone 4. This would in turn result in more available bsCOD for denitrification by heterotrophic organisms and some potential  $PO_4^{3-}$  uptake after denitrification in anaerobic conditions.
## 5. Conclusion

Side-stream nitrification for the Hias Process with the two-reactor setup has shown promising results for  $NH_4^+$ ,  $NO_3^-$  and  $NO_2^-$  removal. Although the goal of 60% reduction of soluble nitrogen (as the total fraction of  $NH_4^+$ ,  $NO_3^-$  and  $NO_2^-$ ) was not reached, an average reduction of 54% and additionally an average reduction of 57% of  $NH_4^+$ was achieved during the last four weeks of operation. The results show a tendency of increasing removal during the last weeks of operation and thus the systems shows a good potential for achieving the removal goal of 60%. The main factors that are likely the cause of the lower than expected nitrification rates in the SNR are temperature, possible impact by small bsCOD loads, occasional low NH<sub>4</sub><sup>+</sup>-N loads and occasional lack of alkalinity. To further assess what can be done to increase the nitrification rates of the side-stream setup, further studying is necessary. Some changes could be applied to the side-stream setup for increased nitrification capacity, like implementation of a reactor for DO consumption following the SNR or compartmentalization of the SNR for increased nitrification rates. Occasional low alkalinity could pose a problem towards optimal nitrification in the SNR, however this could be solved by pH-adjustments or addition of alkalinity preceding the SNR. The VEAS pilot achieved 95% reduction of  $PO_4^{3-}$  and effluent concentrations below 0.5 mg  $PO_4^{3-}$ -P/L on all days analyzed, except the first day of the snow melting period where the reduction was 85% and the effluent concentration was  $0.7 \text{ mg PO}_4^{3-}$ -P/L. This indicates that the increased nitrification by the side-stream setup did not significantly reduce the  $PO_4^{3-}$  removal.

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# 6. Appendix

Day		NH4-N	NO2-N	NO3-N		Z3		NH4-N	NO2-N	NO3-N	sCOD	PO4-P	DO
	Z3	50	1.25	0.1			Z3	52.4	0.023	0.3		38.4	
1.00	SNR	27	12.55	0.2		14.00	SNR	46.8	0.418	0.5		38.2	
	Z4	48		0.2		14.00	Z4	46.4	0.02	0.1		38.6	
	Z3	45	0.029	0.1			Z5	46.8	0.015	0.3		38.9	
4.00	SNR	26	9.8	0.2			Z3	54.5	0.019	0.1	140	34.8	
	Z5	32	6.89	0.2		15.00	SNR	49.2	0.627	0.1	90	32.4	0.00
	Z3	44	0.05	0.1		15.00	Z4	53.6	0.019	0.1		33.7	0.20
5.00	SNR	36	0.55	0.2			Z5	51	0.014	0.1		35.4	
	Z5	38	0.15	0.2			Z3	35.2	0.12	0.2	92	11.5	
	Z3	60	0.034	0.3		18.00	SNR	29.2	2.44	0.1	67	10	0.00
6.00	SNR	57	0.35	0.4		18.00	Z4	34.8	0.052	0.1		12.4	0.20
0.00	Z4	57	0.07	0.5		2	Z5	32.8	0.015	0.1		12.7	
	Z5	56	0.07	0.2			Z3	48	0.022	0.1	156	34.7	
	Z3	50				10.00	SNR	37.6	1.956	0.2	69	25	7.00
7.00	SNR	50				19.00	Z4	45.2	0.35	0.2		32.1	7.60
7.00	Z4	52					Z5	42.4	0.016	0.1		31.3	
	Z5	45					Z3	50.8	0.022			42	
	Z3	36	0.07			20.00	SNR	47.2	1.052	0.6	83	46.2	0.00
8.00	SNR	44	0.21			20.00	Z4	51.6	0.017	0.5		44.6	8.00
0.00	Z4	40					Z5	50.4	0.016	0.5		47.1	
	Z5	44					Z3	53.2	0.02	0.2	148	40.7	
	Z3	41	0.016	0.2		21.00	SNR	50.8	0.864	0.2	82	42.5	7.50
11.00	SNR	39	1	0.2		21.00 Z	Z4	57.2	0.018	0.2		42.3	7.50
11.00	Z4	41	0.1	0.1	Z:	Z5	54.4	0.016	0.4		42.6		
	Z5	41	0.35	0.1	Z3	Z3	54.8	0.018	0.2	133	39.5	.5	
	Z3	40	0.06	0.3		SNR	49.2	1.078	0.3	76	43.2		
12.00	SNR	36	0.9	0.2		22.00 Z4	Z4	52	0.018	0.4		41.8	7.98
12.00	Z4	41	0.05	0.4			Z5	53.2	0.014	0.1		41.9	
1	-		0.07										

Figure 6.1: Rawata for the one-reactor setup for day 1-22. Concentrations are given in mg/L for each nutrient specified. The zones are referred to as specified in Figure 3.2.

Day		NH4-N	NO2-N	NO3-N	sCOD	PO4-P	DO
	Z3	43.2	0.014	0.4	124	15.2	
25.00	SNR	34	1.904	0.5	76	16.2	0.41
25.00	Z4	42	0.036	0.4		16.2	0.41
	Z5	38	0.013	0.3		17	
	Z3	51.6	0.021	0.2	117	26.6	
26.00	SNR	42.8	1.724	0.3	67	22.1	7 50
20.00	Z4	47.6	0.028	0.2		26.4	7.50
	Z5	48	0.014	0.5		26.9	
	Z3	59.2	0.022	0.8	157	40.2	
27.00	SNR	50.8	1.376	0.5	80	40	7 50
27.00	Z4	56.8	0.022	0.3		42.7	7.50
	Z5	53.6	0.016	0.3		40.2	
	Z3	53.6	0.028	0.6	230	43.7	
28.00	SNR	44.8	1.256	0.5	130	42.4	0 1 2
20.00	Z4	52	0.023	0.4		46.2	0.12
	Z5	53.6	0.014	0.5		46.9	
	Z3	54.4	0.023	0.3	160	40.4	
20.00	SNR	50.8	1.044	0.3	95	43.2	7.01
29.00	Z4	54	0.014	0.5		42.4	7.91
	Z5	56.8	0.016	0.2		44.8	
	Z3	38.4	0.015	0.3	99	15.6	
22.00	SNR	32.4	2.372	0.5	67	17.9	0.45
52.00	Z4	37.6	0.048	0.3		18.4	0.45
	Z5	36	0.013	0.3		19.4	1
	Z3	58.4	0.022	0.3	135	40.8	
22.00	SNR	44.8	1.832	0.5	76	35.5	7.26
55.00	Z4	54	0.037	0.2		41	7.50
	Z5	54	0.023	0.4		41.5	1
	Z3	59.6	0.027	0.5	173	46.8	
34.00	SNR	52.4	1.496	0.7	90	59.2	7 37
34.00	Z4	56.4	0.034	0.4		50.7	1.57
	Z5	58	0.026	0.5		47.5	

Figure 6.2: Rawata for the one-reactor setup for day 25-34. Concentrations are given in mg/L for each nutrient specified. The zones are referred to as specified in Figure 3.2.

		(	Q.	NH4-N [mg/L]						NO2-N [mg/L]							
day		VEAS	LOOP	NH4-I	NH4-3	NH4-B	NH4-S	NH4-4	NH4-5	NH4-10	NO2-I	NO2-3	NO2-B	NO2-S	NO2-4	NO2-5	NO2-10
	4.0	27.00	0.225	25.0	17.6	11.6	0.50	17.2		10.0							
	5.0	30.00	0.330	26.0	28.8	21.6	1.60	24.0		20.0		0.032	1.588	16.40	0.830		
	6.0	29.00	0.330	40.0	35.2	23.2	1.50	28.0		17.0		0.026	1.680	23.35	0.786		
	7.0	32.00	0.330	34.0	32.4	19.6	1.00	27.2		12.0		0.019	1.708	14.90	0.700		
	8.0	29.00	0.330		39.2	26.0	4.10	32.0				0.021	1.392	21.35	0.814		
	11	20.00	0.300	39.0	38.8	23.2	1.10	31.8		17.0		0.021	1.372	23.85	1.069		
	12	21.00	0.336	51.5	44.0	29.2	4.80	35.6		21.0		0.032	0.812	13.70	0.708		
	13	21.00	0.417	48.0	41.6	32.4	15.0	36.0		22.0		0.024	0.592	7.600	0.540		
	14	21.00	0.313	61.0	51.2	36.8	14.5	43.2	40.0	25.0		0.031	0.620	9.500	0.520	0.028	
	15	20.00	0.309		48.8	37.2	21.0	43.6	41.6			0.038	0.552	7.550	0.208	0.028	
	18	18.00	0.240		45.2	27.2	8.20	36.8	36.4			0.027	0.736	3.850	0.144	0.034	
	19	20.00	0.537		52.4	39.2	32.8	46.0	44.8			0.033	0.452	0.400	0.080	0.021	
	20	19.00	0.558	58.0	58.4	42.4	30.2	45.2	44.8	36.0		0.030	0.480	0.950	0.122	0.043	
	21	19.00	0.409	45.0	55.6	44.8	29.6	48.0	45.0	26.0		0.036	0.352	1.350	0.106	0.015	
	22	19.00	0.340	48.0	50.4	41.6	37.2	47.6	47.2	33.0		0.039	0.452	0.690	0.108	0.033	
	25	19.00	0.333	47.0	42.8	32.0	8.40	36.4	37.2	18.0	_	0.036	0.472	1.090	0.126	0.064	
	26	20.00	0.327	51.6	48.8	33.2	8.40	39.2	38.8	22.8	1.398	0.025	0.388	0.860	0.122	0.027	0.934
	27	20.00	0.327	52.0	54.4	46.4	23.2	52.4	50.4	31.6	1.216	0.025	0.368	0.610	0.146	0.025	0.814
	32	18.00	0.327	41.6	37.6	24.0	6.40	31.2	30.8	16.0	0.021	0.017	0.472	0.580	0.096	0.051	1.828
	33	21.00	0.327	45.2	42.8	32.8	16.4	38.8	37.2	22.0	0.900	0.023	0.416	0.460	0.154	0.020	1.294
	34	23.00	0.336	54.0	51.2	45.6	38.8	49.6	50.8	32.8	1.040	0.034	0.332	0.320	0.070	0.024	0.636
	35	26.00	0.333	51.2	46.0	36.4	29.6	42.0	40.8	21.6	0.748	0.022	0.344	0.360	0.072	0.023	0.822
	36	26.00	0.327	40.8	40.4	36.0	30.0	40.4	40.0	24.0	1.378	0.024	0.340	0.390	0.054	0.240	0.670
	40	27.00	0.327	36.0	30.8	23.2	13.6	28.8	29.2	19.6	0.900	0.017	0.404	0.460	0.048	0.030	0.914
	42	25.00	0.333	36.4	34.0	24.0	14.4	30.4	29.6	18.4	0.482	0.022	0.408	0.470	0.051	0.027	1.770
	43	22.00	0.336	29.2	28.4	16.0	0.80	21.6	21.6	9.20	0.580	0.014	0.532	0.350	0.066	0.042	3.860
	47	21.00	0.327	36.0	31.6	18.8	0.80	26.0	24.8	11.6	0.890	0.033	0.380	0.340	0.078	0.054	6.100
	48	22.00	0.327	41.6	38.0	22.4	5.60	32.0	28.4	14.4	0.596	0.029	0.244	0.440	0.093	0.029	0.570
	49	21.00	0.327	50.4	44.8	34.0	16.8	40.4	38.8	23.6	1.748	0.027	0.248	0.340	0.107	0.025	0.072
	50	21.00	0.333	51.8	52.8	44.8	26.4	49.6	49.2	30.8	1.040	0.030	0.244	0.450	0.116	0.080	0.077
	53	19.00	0.327	49.2	44.4	27.2	3.20	34.0	34.0	19.6	1.117	0.019	0.412	0.520	0.077	0.044	1.250
	54	21.00	0.333	56.0	50.8	25.2	6.40	39.6	39.6	23.6	0.490	0.026	0.372	0.560	0.157	0.031	0.510
	55	21.00	0.333	62.8	56.0	43.2	20.4	47.6	48.8	28.4	0.800	0.030	0.268	0.430	0.228	0.048	1.700
	56	21.00	0.333	59.6	54.4	42.0	24.4	46.8	46.0	25.6	0.450	0.031	0.036	0.410	0.108	0.030	1.570
	57	21.00	0.343	52.8	50.0	40.4	22.8	44.0	44.8	21.6	1.040	0.027	0.228	0.380	0.207	0.019	1.670
	61	21.00	0.343	58.8	55.6	39.6	13.2	46.8	46.4	27.2	1.770	0.030	0.252	0.550	0.185	0.029	0.630
	62	21.00	0.343	60.8	58.8	49.2	26.0	53.6	50.8	27.2	0.590	0.030	0.220	0.400	0.187	0.029	1.740
	63	21.00	0.343	57.6	57.6	48.8	28.8	46.0	51.6	29.2	0.680	0.031	0.240	0.530	0.204	0.024	2.020
	64	21.00	0.343	55.2	55.6	47.2	27.2	49.2	48.0	22.8	1.050	0.039	0.260	0.640	0.291	0.027	2.600
	67	18.00	0.343	52.8	46.8	30.8	7.20	37.2	36.0	20.8	0.620	0.030	0.320	0.690	0.073	0.066	1.880
1	68	20.00	0.343	51.2	48.4	33.6	10.0	37.2	37.2	22.4	0.860	0.040	0.228	0.540	0.178	0.025	0.680

Figure 6.3: Rawdata for wastewater flow through BOD-R and SNR (Q),  $NH_4^+$ -N and  $NO_2^-$ -N concentrations for the two-reactor setup. . The zones are referred to as specified in Figure 3.3. The numbering/letters behind the type of nutrient indicates the zone number (e.g. NH4-3 represents the  $NH_4^+$ -N concentration in zone 3) (I=influent to VEAS pilot, B=BOD-R and S=SNR). The same system applies for Figure 6.4, 6.5 and 6.6.

			NO	03-N [mg	[/L]					PC	04-P [mg	/L]		
day	NO3-I	NO3-3	NO3-B	NO3-S	NO3-4	NO3-5	NO3-10	PO4-I	PO4-3	PO4-B	PO4-S	PO4-4	PO4-5	PO4-10
4.0														
5.0		0.30	0.60	0.60	0.30				18.4	15.9	16.9	18.7		
6.0		0.30	0.70	0.30	0.30				21.0	22.1	23.3	21.4		
7.0		0.30	1.10	0.60	0.20				18.4	17.1	17.9	17.7		
8.0		0.10	0.80	0.80	0.30				23.9	21.9	24.0	23.0		
11		0.50	1.50	3.30	0.30				8.2	7.80	10.2	8.2		
12		0.30	0.70	2.50	0.10				34.0	35.6	33.4	35.4		
13		0.40	0.70	2.70	0.40				37.8	41.5	42.4	40.1		
14		0.60	0.70	6.30	0.50	0.40			52.4	50.4	43.7	49.5	53.9	
15		0.50	1.10	7.90	0.70	0.50			33.5	31.3	36.6	34.6	36.1	
18		0.80	1.70	17.1	1.10	0.50			14.6	10.1	13.3	13.6	14.2	
19		0.70	1.20	5.30	0.50	0.60			43.9	33.8	22.4	32.6	36.1	
20		0.70	1.30	8.80	0.90	0.40			37.1	32.4	31.2	34.6	33.4	
21		0.30	1.00	12.0	0.90	0.40			44.8	42.7	39.6	42.9	44.4	
22		0.20	0.90	8.70	0.70	0.40			37.7	39.5	39.2	38.8	40.3	
25		0.10	1.60	16.6	1.90	0.40			13.4	10.7	12.0	10.5	10.0	
26	1.50	0.50	2.00	24.8	1.40	0.40	0.20	5.60	37.2	28.5	20.8	32.4	29.1	0.20
27	0.80	0.30	1.20	22.0	0.90	0.40	0.30	5.90	41.8	45.1	45.4	44.1	44.9	0.32
32	0.40	0.30	2.60	25.4	1.50	0.30	0.10	4.20	15.4	11.1	13.1	12.0	11.8	0.10
33	0.80	0.30	1.60	18.0	1.10	0.30	0.30	4.10	31.3	26.3	24.1	28.5	27.7	0.15
34	1.10	0.50	1.00	7.60	0.60	0.30	0.30	6.10	36.9	42.0	41.5	41.2	42.6	0.24
35	1.10	0.30	1.00	8.20	0.30	0.80	0.30	4.40	34.2	33.5	37.5	35.4	36.0	0.70
36	4.30	0.30	1.20	6.00	0.40	0.80	0.50	5.20	30.6	34.1	35.8	32.4	33.4	0.26
40	2.80	0.60	3.80	12.6	0.80	0.60	0.50	2.40	13.1	12.1	11.2	12.4	13.3	0.11
42	0.80	0.10	3.50	13.0	0.40	0.10	0.30	4.40	23.1	19.6	18.4	21.3	21.0	0.18
43	0.80	0.30	5.70	19.2	1.80	0.50	0.20	2.36	7.90	5.30	6.70	5.40	5.10	0.18
47	1.60	0.50	6.20	13.0	2.40	1.30	0.20	4.20	5.80	6.20	6.60	5.60	5.30	0.47
48	0.40	0.50	2.20	18.2	0.70	0.10	0.40	4.80	33.1	21.7	16.4	28.5	26.3	0.21
49	2.00	0.20	1.40	17.2	0.80	0.60	0.40	6.50	33.1	32.6	32.5	32.8	33.5	0.23
50	0.80	0.20	1.00	16.6	0.70	0.30	0.20	5.70	37.4	39.9	38.3	39.5	40.3	0.31
53	0.60	0.20	3.60	26.8	2.00	0.30	0.40	4.00	12.9	9.40	10.0	10.2	9.80	0.08
54	0.70	0.30	2.90	20.6	1.40	0.50	0.50	5.40	30.3	25.8	22.3	30.6	29.9	0.13
55	0.60	0.40	1.60	21.6	0.70	0.30	0.40	0.00	45.0	41.9	40.5	41.2	42.8	0.18
50	0.80	0.60	1.70	21.6	1.00	0.60	0.30	6.00	20 7	20.6	20.4	20.2	40.9	0.24
57	1.10	0.50	1.50	17.2	1.10	0.50	0.70	6.00	30.7	39.0	39.4	39.2	40.0	0.24
61	1.50	0.50	1.00	20.0	1.10	0.60	0.50	5.60	40.0	30.7	40.2	35.7	42.1	0.24
62	1.00	0.40	1.00	19.8	0.80	0.40	0.30	6.00	40.5	40.5	40.5	40.9	42.1	0.18
64	1 10	0.30	0.90	19.2	0.80	0.40	0.40	7.40	42.0	41.2	41.5	42.1	44.1	0.25
67	0.40	0.40	2.40	24.6	1.80	0.20	0.00	4 30	13.0	7.40	7.00	8 80	8 10	0.20
68	2.60	0.50	1.50	24.0	1.30	0.60	0.60	6.60	26 7	20.4	18 1	22.3	22.0	0.14

Figure 6.4: Rawdata for  $NO_3^-$ -N and  $PO_4^{3+}$ -P concentrations for the two-reactor setup.

			sCO	D [mg 0	5/L]						pН				DO [r	ng/L]
day	COD-I	COD-3	COD-B	COD-S	COD-4	COD-5	COD-10	pH-I	pH-3	pH-B	pH-S	pH-4	pH-5	pH-10	DO-B	DO-S
4.0		101	89.0	104											8.52	9.72
5.0		103	58.0	75.0						7.75	7.01				8.33	7.65
6.0		131	81.0	101						7.75	7.01				7.28	7.19
7.0		88.0	50.0	69.0						7.64	6.95				7.62	7.98
8.0		101	54.0	70.0					7.82	7.69	7.03	7.51			7.67	7.27
11		91.0	53.0	72.0					7.57	7.71	6.96	7.6			7.32	7.72
12		156	80.0	85.0					7.49	7.69	7.2	7.54			6.64	6.73
13		160	84.0	81.0	114				7.49	7.66	7.37	7.51			6.75	6.6
14		158	84.0	87.0	112	112			7.50	7.68	7.25	7.52	7.49		6.47	6.96
15		140	109	81.0	105	94.0			7.61	7.80	7.62	7.74	7.70		6.66	6.6
18		147	65.0	55.0	91.0	73.0			7.64	7.88	7.42	7.72	7.77	1	7.02	7.19
19		94.0	48.0	51.0	80.0	69.0			7.80	8.09	8.08	7.89	7.94	ŀ	5.33	6.56
20		201	72.0	86.0	110	119			7.37	7.59	7.54				5.58	6.64
21		147	68.0	63.0	99.0	116			7.47	7.62	7.46	7.55	7.44		6.11	5.64
22									7.20	7.50	7.38	7.25	7.23		6.01	7.67
25		192	122	98.0	160	152			7.26	7.44	6.96	7.45	7.34		5.99	7.91
26	356	136	63.0	46.0	80.0	78.0	46.0	7.85	7.30	7.52	7.03	7.47	7.33	7.16	5.87	8.05
27	396	156	76.0	49.0	120	98.0	55.0	7.86	7.39	7.51	7.20	7.42	7.40	7.28	6.04	8.06
32	116	99.0	51.0	61.0	70.0	92.0	69.0	7.63	7.28	7.44	6.93	7.30	7.28	7.05	6.45	8.66
33															6.16	6.97
34	404	154	/2.0	54.0	98.0	/4.0	42.0	7.60	7.28	7.49	7.37	7.30	7.32	7.23	6.09	4.06
35			48.0	56.0				8.26	7.36	7.52	7.33	7.32	7.31	. 7.21	6.69	4.32
50	047	07.0	46.0	60.0	67.0		42.0	8.25	7.54	7.50	7.52	7.50	7.28	7.19	6.47	4.45
40	21/	97.0	46.0	69.0	67.0	81.0	43.0	7.83	7.43	7.46	7.21	7.41	7.35	7.18	6.28	4.69
42	260	100	48.0	51.0	64.U	67.0	48.0	8.20	7.60	7.55	7.25	7.48	7.45	7.24	6.40	4.74
43	142	77.0	32.0	40.0	33.0	56.0	42.0	7.65	7.44	7.40	7.05	7.05	7.37	7.00	0.09	7.01
47	220	92.0	47.0	45.0	/5.0	79.0	40.0	7.90	7.77	7.78	7.22	7.81	7.83	7.44	5.92	0.03
40	340	100	55.0	24.0	87.0	/6.0	49.0	7.85	7.44	7.51	7.10	7.45	7.43	7.52	4.94	4.00
49	400	107	82.0	75.0	125	107	50.0	8.04	7.01	7.59	7.20	7.50	7.50	7.30	4.07	5.41
50	100	140	/0.0	45.0	70.0	62.0	41.0	8.07	7.40	7.33	6.77	7.40	7.42	7.00	5.07	5.37
54	199	150	49.0	43.0	113	95.0	41.0	7.80	7.35	7.49	6.03	7.35	7.33	7.09	5.57	6.57
55	424	105	72.0	50.0	113	107	68.0	7.00	7.41	7.49	7.16	7.43	7.35	7.13	6.08	6.61
56	-102	1//	72.0	50.0	120	107	00.0	7.50	7.38	7.51	7.10	7.44	7.40	7.23	5.88	6.75
57	438	161	74.0	55.0	114	113	93.0	7.68	7.44	7.55	7.31	7,48	7.45	7.30	5.22	6.41
61	461	169	64.0	46.0	128	100	54.0	8.00	7.51	7.64	7.21	7.53	7.53	7 30	5,83	6.99
62	523	105	73.0	53.0	136	118	92.0	8.05	7.01	7.67	7.34	7.49	7.52	7.50	5.87	6.64
63	545	208	82.0	57.0	123	111	59.0	7 74	7.50	7.62	7.39	7.59	7.54	7 44	5.62	6.92
64	556	191	85.0	68.0	123	129	65.0	7.66	7.53	7,76	7.46	7,56	7.57	7,45	5,49	7.04
67	215	109	81.0	61.0	95.0	83.0	57.0	7.85	7.49	7.63	7.11	7.55	7.52	7.30	6.30	7.19
68	436	164	65.0	56.0	126	111	50.0	8.13	7.54	7.71	7.28	7.52	7.48	7.35	6.23	7.04

Figure 6.5: Rawdata for sCOD, pH and DO concentrations for the two-reactor setup.

	(	Q		NH4-N [mg/L]						Alkalinity [mmol CaCO <sub>3</sub> /L]					
day	VEAS	LOOP	NH4-I	NH4-3	NH4-B	NH4-S	NH4-4	NH4-10	Alk-I	Alk-3	Alk-B	Alk-S	Alk-4	Alk-10	
74												0.18			
75	19.00			51.5	35.0	1.4	41.6	27.4		3.64	2.63	0.25	3.23	2.53	
76	19.00		69.0	60.0	50.0	6.0		30.0	3.19	3.67	2.95	0.42		2.46	
77	19.00		70.0	63.5	53.0	20.0	54.0	31.0	3.52	3.62	3.19	1.04	3.07	2.39	
78	19.00		72.0	71.0	55.0	22.0		36.0	3.11	3.83	3.52	1.12		2.64	
80		0.216		50.0		3.5	43.5			2.95		0.08	2.77		
81	18.00	0.325	53.0	49.0	35.0	9.0		23.0	3.05	2.98	2.72	0.93		2.14	
82	18.00	0.290	56.0	44.0	30.0	3.00	34.0	24.0	3.11	2.84	2.20	0.46	2.44	1.98	
83	22.00		70.0	62.0	48.0	13.00	57.0	35.0	3.27	4.11	2.92	0.81	3.48	2.58	
89	29.00	0.6	25.0	25.0	15.0	5.00	20.0	12.0	1.96	1.91	1.47	0.79	1.64	1.27	

Figure 6.6: Rawdata for alkalinity tested by the Hias WWTP staff from day 74-89 for the two-reactor setup. The wastewater flow through the VEAS pilot is measured in  $m^3/d$ , while the wastewater flow through the side-steam setup (LOOP) is measured in  $m^3/h$ .

Tests	NH4-N	[mg/L]	PO4-P	[mg/L]	sCOD	[mg/L]	DO [1	mg/L]
Time [min]	iSNR	VEAS	iSNR	VEAS	iSNR	VEAS	iSNR	VEAS
0	42	42.4		42	7	1		
30	33.6	35.2	3.1	0.56			7.61	8.56
60	31.6	32.8	1.24	0.23			7.51	8.56
90	23.6	27.2	0.62	0.193			7.41	8.47
120	20	23.6	0.59	1.1			7.35	8.43
150	12.4	16.8	0.27	0.25			7.27	8.35
180	12	11.6	0.29	0.15			7.23	8.46
200	11.6	9.6	0.4	0.16	83	78	7.32	8.34
					Ave	erage DO:	7.39	8.45

initial SNR carriers (ISNR) vs VEAS carriers (VEAS)

Figure 6.7: Rawdata for the first laboratory batch test comparing carriers from the initial SNR and the VEAS pilot during the single reactor setup.

#### initial SNR carriers (ISNR) vs VEAS carriers (VEAS)

Tests	NH4-N	[mg/L]
Time [min]	iSNR	VEAS
0	49	.6
30	46.8	40.8
60	42	36
90	42	34.4
120	39.6	27.2
150	35.2	23.2
180	22	18.8

**Figure 6.8:** Rawdata for the second laboratory batch test comparing carriers from the initial SNR and the VEAS pilot during the single reactor setup.

Tests	NH4-N	[mg/L]	PO4-P	[mg/L]	DO [I	mg/L]
time [min]	NH4-A	NH4-B	PO4-A	PO4-B	DO-A	DO-B
0.000	46.8	43.6	7.10	6.36		
30.00	32.8	26.0	11.55	11.15	7.83	8.32
60.00	25.7	20.0	11.20	10.05	7.86	7.97
90.00	20.0	13.2	11.40	12.00	7.87	7.90
120.0	15.2	6.40	12.10	9.05	7.79	7.90
			Ave	erage DO:	7.84	8.02

### No stirring (A) vs rapid stirring (B)

Figure 6.9: Rawdata for the laboratory batch test comparing rapid stirring to no stirring during the two-reactor setup.

Tests	NH4-N	[mg/L]	PO4-P	[mg/L]	DO [mg/L]		
time [min]	NH4-A NH4-B		PO4-A	PO4-B	DO-A	DO-B	
0.000	36.8	32.0	13.75	13.45			
30.00	28.8	23.6	13.95	14.25	8.17	7.88	
60.00	22.0	16.1	13.50	13.80	8.07	7.78	
90.00	16.8	12.8	13.80	14.10	8.08	7.93	
120.0	10.8	4.40	14.10	14.20	8.01	8.81	
150.0	150.0 3.90 0.90		13.40	14.85	8.12	8.66	
			Ave	erage DO:	8.09	8.21	

No stirring (A) vs slow stirring (B)

Figure 6.10: Rawdata for the laboratory batch test comparing slow stirring to no stirring during the two-reactor setup.

Wastewater (A) vs	lake water (B) -	failure edition
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Tests	NH	4-N	PO	PO4-P		DO		2-N	NO3-N		sCOD		p	Н
time [min]	NH4-A	NH4-B	PO4-A	PO4-B	DO-A	DO-B	NO2-A	NO2-B	NO3-A	NO3-B	COD-A	COD-B	pH-A	pH-B
0.000	35.2	34.4	30.4	7.00			0.256	0.084	4.7	3.8	59	13	7.55	7.1
30.00	27.6	30.8	35.8	7.70	8.01	8.78								
60.00	21.6	28.4	32.9	7.80	8.00	8.91								
90.00	18.0	27.2	37.3	8.56	8.08	8.88								
120.0	10.4	27.2	34.8	10.6	8.01	8.81								
150.0	6.40	28.4	37.3	10.2	8.19	8.81	0.400	0.001	38	15	50	15	7.04	5.79
			Ave	erage DO:	8.06	8.84								

Figure 6.11: Rawdata for the laboratory batch test comparing wastewater to lake water without sufficient alkalinity during the two-reactor setup.

Wastewater (A) vs lake water (B)														
Tests	NH4-N		PO4-P		DO		NO2-N		NO3-N		sCOD		pH	
time [min]	NH4-A	NH4-B	PO4-A	PO4-B	DO-A	DO-B	NO2-A	NO2-B	NO3-A	NO3-B	COD-A	COD-B	pH-A	pH-B
0.000	42.4	46.8	43.6	6.20			0.27	0.14	4.5	4.7	68	18	7.73	8.35
30.00	36.4	35.2	40.4	7.00	8.69	8.33								
60.00	30.4	31.2	40.4	7.60	8.13	8.3								
90.00	24.4	25.6	39.9	7.3	8.25	8.13								
120.0	20.4	24.4	41.3	8.7	8.17	7.94								
150.0	15.20	17.6	43	9.3	7.87	7.87	0.880	0.92	37	34	56	34	7.39	8.61
			Average DO:		8.22	8.11								

Figure 6.12: Rawdata for the laboratory batch test comparing wastewater to lake water with added alkalinity during the two-reactor setup.



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