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Simultaneous nitrification and denitrifying phosphate uptake in a continuous moving bed biofilm reactor (The HIAS-process)

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Abstract

Nutrients such as nitrogen (N) and phosphorus (P) can cause eutrophication and harmful algal blooms in waterbodies. Removal of these nutrients from wastewater discharges are therefore necessary to insure healthy waters. The increased focus on the world's limited phosphate reserves have led to an increased interest for biological phosphorus removal enhancing P-recovery from wastewater. A newly developed biological phosphorus removal process, the HIAS-process, has shown good removal for phosphorus, but have also detected some nitrogen removal. By enhancing removal of nitrogen in this process, the process could be a more compact and cost-effective treatment option compared to today's treatment processes for both phosphorus and nitrogen.

In this study, three main parameters influencing the HIAS-process were investigated to get further knowledge about the process, so that an optimization of N-removal in the process could be done. The parameters investigated were anaerobically carbon consumption, oxygen concentrations and temperature, and were investigated through batch experiments at the laboratory and grab sample experiments in the pilot plant at HIAS Wastewater Treatment Plant.

Results show that high oxygen concentration gives high nitrification rates, which results in a high production of nitrite/nitrate. It is also seen that higher denitrification rates are present when the nitrite/nitrate concentrations are initially higher. However, high oxygen concentration over time does indicate that lack of an anoxic layer in the biofilm could occur when oxygen consumption by nitrifyers and phosphate accumulating organisms (PAOs) decreases. This further decreases the denitrification, due to the need for an anoxic layer to be present for denitrification to occur. Experiments conducted with initially high oxygen concentration, followed by low oxygen concentration, showed that better N removal could occur due to maintenance of the anoxic layer.

Results from the batch experiments show that higher anaerobically carbon consumption gives higher denitrification rates and that carbon taken up in the anaerobic period can effectively be utilized for N removal, with a potential C/N-ratio found in the batch experiments to be approximately 5 mg sCOD/mg N. Results have also shown higher N removal efficiencies under higher temperatures.

The results from the experiments performed in this thesis give reasons to believe that optimization of the process could be done by controlling the DO concentration in the aerobic period of the reactor, to have high oxygen concentrations in the first chambers, and lower oxygen concentrations in the last chambers. Optimization of the process can also be done by adding an external carbon source if the organic carbon load into the reactor has too low capacity to remove the desired amount of ammonium. The aerobic retention time can also be extended to improve the nitrogen removal.

Sammendrag

Næringsstoffer som nitrogen (N) og fosfor (P) kan forårsake eutrofiering og skadelig algeoppblomstring i vann. Fjerning av disse næringsstoffene fra avløpsvann er derfor nødvendig for å sikre god vannkvalitet. Det økte fokuset på verdens begrensede fosfatreserver har ført til økt interesse for biologisk fjerning av fosfor, noe som øker P-utvinning fra avløpsvann. En nyutviklet biologisk renseprosess, HIAS-prosessen, har vist god fjerning av fosfor, men noe nitrogenfjerning har også blitt observert. Ved å forbedre fjerningen av nitrogen i denne prosessen, kan prosessen være et mer kompakt og kostnadseffektivt behandlingsalternativ i forhold til dagens renseprosesser for fosfor og nitrogen.

I denne studien ble tre hovedparametere som kan påvirke HIAS-prosessen undersøkt, for å få ytterligere kunnskap om prosessen, slik at prosessen kunne optimaliseres for N-fjerning. Parameterne som ble undersøkt var anaerobt karbonforbruk, oksygenkonsentrasjoner og temperatur, og de ble undersøkt gjennom batchforsøk på laboratoriet og ved forsøk i pilotanlegget ved HIAS renseanlegg.

Resultatene viser at høy oksygenkonsentrasjon gir høye nitrifiseringshastigheter, noe som resulterer i en høy produksjon av nitritt/nitrat. Det er også funnet at høyere nitritt/nitrat-konsentrasjoner gir høyere denitrifiseringshastigheter. Høy oksygenkonsentrasjon over tid viser imidlertid at mangel på et anoksisk lag i biofilmen kan oppstå når oksygenforbruket av nitrifiserere og fosfatakkumulerende organismer (PAO) reduseres. Dette reduserer denitrifikasjonen ytterligere, grunnet behovet for et anoksisk lag for at denitrifikasjon skal kunne forekomme. Eksperimenter utført med høy oksygenkonsentrasjon i starten, etterfulgt av lav oksygenkonsentrasjon senere i prosessen, viste at bedre N-fjerning kunne oppnås på grunn av opprettholdelse av det anoksisk lag.

Resultater fra batchforsøkene viser at høyere anaerobt karbonforbruk gir høyere denitrifiseringshastigheter og at karbon tatt opp i den anaerobe perioden effektivt kan utnyttes for N-fjerning, med et potensielt C/N-forhold funnet i batcheksperimentene til å være ca. 5 mg sCOD/mg N. Resultatene har også vist at høyere nitrogenfjerning kan oppnås ved høyere temperaturer.

Resultatene fra forsøkene utført i denne oppgaven gir grunner til å tro at optimalisering av prosessen kan gjøres ved å kontrollere DO-konsentrasjonen i den aerobe perioden i reaktoren. Dette kan gjøres ved å ha høye oksygenkonsentrasjoner i de første kamrene og lavere

oksygenkonsentrasjoner i siste kamrene. Optimalisering av prosessen kan også gjøres ved å legge til en ekstern karbonkilde, hvis karbonbelastningen inn til reaktoren ikke har nok kapasitet til å fjerne ammonium-mengden som ønskes fjernet. Den aerobe oppholdstiden kan også forlenges for å forbedre nitrogenfjerningen.

Preface

This master thesis has been completed at the Department of Civil and Environmental Engineering at The Norwegian University of Science and Technology (NTNU).

The laboratory work has been carried out at Hias Wastewater Treatment Plant in Stange municipality, Norway, during spring semester 2018. I am grateful for all help and guidance related to the laboratory work, as well as all discussions with my co-supervisor at the treatment plant, Dr.ing. Torgeir Saltnes. I am also thankful for the inspiration and discussions at the laboratory with several of Hias' employees.

I would also thank my supervisor Professor Stein Wold Østerhus and my co-supervisor Postdoc Blanca Magdalena Gonzalez Silva at NTNU for discussions and help with my thesis.

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List of abbreviations

COD – chemical oxygen demand

DNGAO – denitrifying glycogen accumulating organism

DNPAO – denitrifying phosphate accumulating organism

DO – dissolved oxygen

GAO – glycogen accumulating organism

HRT – hydraulic retention time

N – nitrogen

NH_4-N – Ammonium

NO_2-N – nitrite

NO_3-N – nitrate

P – phosphorus

PAO – phosphate accumulating organism

PO_4-P – phosphate

sCOD – soluble chemical oxygen demand

SWB – synthetic water batch

WWB – wastewater batch

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1. Introduction

Nutrients such as nitrogen (N) and phosphorus (P) can cause eutrophication and harmful algal blooms in waterbodies. Removal of these nutrients from wastewater discharges are therefore necessary to insure healthy waters. Different guidelines and directives (EU-directive, 2000) are setting effluent limits for these nutrients. Efficient and cost saving removal mechanisms in wastewater treatment plants are therefore important.

The main part of the nitrogen in wastewater is organically bound to particles or present as the inorganic ammonium-ion, NH_4^+ (Ødegaard et al., 2014). The most common inorganic nitrogen removal process is nitrification followed by denitrification, which both are natural biological processes participatory in the nitrogen cycle. Today's most common systems for nitrification and denitrification are activated sludge systems (AS) and moving bed biofilm reactors (MBBR). An amount of the nitrogen is also removed by assimilation due to bacteria growth as well as by sludge separation of particulate matter containing N.

Traditionally, phosphorus is removed by chemical precipitation, which gives a good removal efficiency. Unfortunately, the process has several disadvantages, where the main issue is related to costs of the chemicals, and the costs related to treatment of a large sludge production. Low plant availability of chemically bound phosphorus is also a disadvantage when the sludge is used as a fertilizer. This can lead to runoff of excess phosphorus to receiving waters and decomposition of phosphorus in waterbodies over time may in next turn lead to eutrophication.

Phosphorus can also be removed biologically, and processes for biological P-removal have existed for a long time. Due to the costs and environmental issues related to chemical treatment of phosphorus, biological phosphorus removal has been more highlighted in recent years. In addition, the increased focus on the limited phosphate reserves for extraction have led to an interest in P-recovery from nutritious wastewaters. Biological P-removal makes this possible.

The processes for biological phosphorus removal are based on activated sludge systems, where the bacteria are present in suspended flocs in the reactor. The P removal systems are most commonly referred to as enhanced biological phosphorus removal (EBPR) systems. The EBPR process is performed by phosphate accumulating organisms (PAOs), which have the ability to accumulate more phosphate than required for growth when exposed to sequencing anaerobic and aerobic conditions.

Previously, all full-scale processes that perform biologically P and N removal in the same process have been AS systems that include both EBPR and biological N-removal. This is systems that rely on highly concentrated return sludge. Problems may occur in these systems when excess sludge leaves the system and thus wash off the necessary bacteria. Deterioration of the P removal activity has also been seen due to transfer of oxidized ammonium (nitrate/nitrite) through the return sludge from aerobic to anaerobic conditions.

To avoid the problems related to excess sludge, and also deterioration of PAO-activity, a biofilm system can be introduced, by use of carriers. Here, the biomass including microorganisms are immobilized within a biofilm layer attached to the carriers' surface. Until now, the challenges with biofilm processes have been to find effective solutions for transporting the carriers between anaerobic and aerobic conditions. Sequencing batch reactors (SBR) are an example on such a system that allow alternating conditions for biofilm on carriers (Helness, 2007, Rahimi et al., 2011).

Another biofilm process, which is newly developed at Hias WWTP in Hamar, Norway (Saltnes et al., 2017), allows carriers to be transported between anaerobic and aerobic conditions. This is done by a design that transports the carriers along with the water through different zones or chambers. By the outlet, a conveyor belt is transporting the carriers back to the inlet zone, while the water leaves the reactor. The process is called the HIAS-process.

The HIAS-process has shown good removal rates for phosphorus and in addition, some nitrogen removal has been observed (Saltnes et al., 2014, Saltnes et al., 2017). The removal of nitrogen in this process is found to occur due to denitrification done by PAOs (Saltnes et al., 2017).

The scope of this study was to optimize N-removal in the HIAS-process by investigating conditions that facilitates simultaneous nitrification and denitrifying P-uptake (SNDP). Three main parameters were investigated to obtain knowledge about how this could be done: anaerobically carbon consumption, oxygen concentration and temperature.

2. Theory and literature review

Biological processes and removal mechanisms are complex and rely on operating conditions that facilitate growth of the necessary microorganisms responsible for removal.

The literature review has mostly been related to EBPR processes for P-removal and MBBR processes for N-removal when looking at microbiology related to the HIAS-process. Some experience from P- and N-removal in sequencing batch reactors are also included.

2.1. Nitrogen removal by nitrification and denitrification

When nitrogen enters a biological treatment step, the nitrogen is mainly organically bound in particulate matter or found as ammonium ions. Organically bound nitrogen is either removed by sludge separation or converted to ammonium by heterotrophic bacteria under aerobe conditions.

2.1.1. Nitrification

Biological nitrogen removal is mainly done by nitrification and denitrification. The nitrification process proceeds in two stages as shown in Table 1. Ammonium (NH_4^+) is first oxidized to nitrite (NO_2^-) by ammonium oxidizing bacteria (AOB), mainly the bacteria genus *Nitrosomonas*. Thereafter, the nitrite will be oxidized to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB), mainly the bacteria genus *Nitrobacter* (Ødegaard et al., 2014).

Table 1: The nitrification process, reactions and most common bacteria (Ødegaard et al., 2014).

Reaction	***Common bacteria***
$NH_4^+ + 3/2O_2 = NO_2^- + 2H^+ + H_2O$	*Nitrosomonas*
$NO_2^- + 1/2O_2 = NO_3^-$	*Nitrobacter*
$\boldsymbol{NH_4^+ + 2O_2 = NO_3^- + 2H^+ + H_2O}$	**Total reaction**

As seen from the equations in Table 1, nitrification occurs under aerobic conditions. The oxygen needed is measured in terms of dissolved oxygen (DO) [g O_2/l] and the consumption is approximately 4,5 g O_2/g NH_4-$N_{oxidized}$ (Ødegaard et al., 2014). The nitrification rate is strongly dependent on the DO concentration, with optimal ranges depending on the reactor/treatment process (Cao et al., 2017).

All microorganisms are dependent on both an energy source and a carbon source. NH_4^+ is mainly used as the energy source within the cells of the nitrifiers, but parts of the ammonium is also used as nitrogen source for cell growth (Ge et al., 2015). The nitrifiers, both AOB and NOB are autotrophic bacteria, which means that they use CO_2 as carbon source instead of organic matter as the heterotrophs.

The nitrifying bacteria are slow growing compared to heterotroph bacteria, and an even more reduced growth rate can occur in competitive situations (Gray, 2017). The heterotroph bacteria have the ability to operate under less oxygen surplus and will compete against nitrifiers in biofilm systems where a limited growth area is available (Ødegaard et al., 2014, Henze et al., 2008). Figure 1 shows qualitative profiles for how BOD (biological oxygen demand), oxygen and ammonium limit the removal of organic matter and ammonium. Results from a MBBR

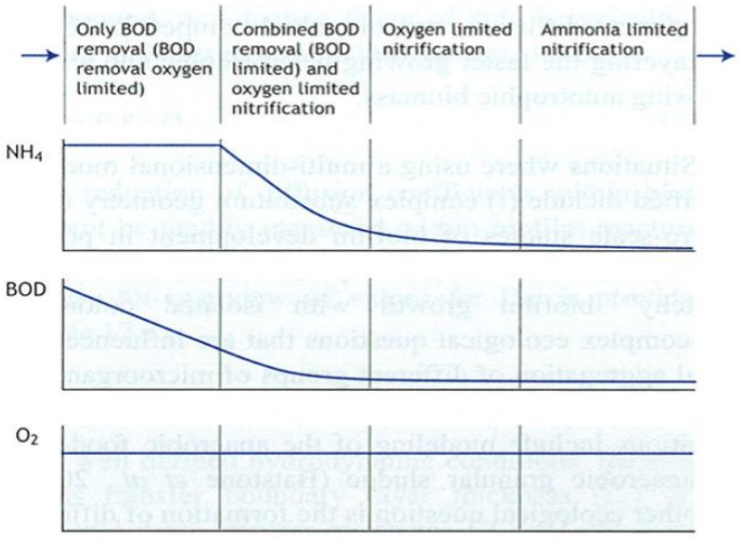

Figure 1: Qualitative profiles for bulk phase ammonia, COD and oxygen concentration. (Henze et al.,

experiment are shown in Figure 2, which shows the nitrification rates at different organic loading rates and oxygen conditions (Figure 2a). Figure 2b shows nitrification rates limited by ammonium concentration at different DO concentrations at an organic loading rate of 0.4 gBOD $m^{-2}d^{-1}$. Both Figure 1 and Figure 2 implies that nitrification can only occur in less loaded plants, where the organic matter already is degraded by heterotroph organisms (Ødegaard et al., 2014).

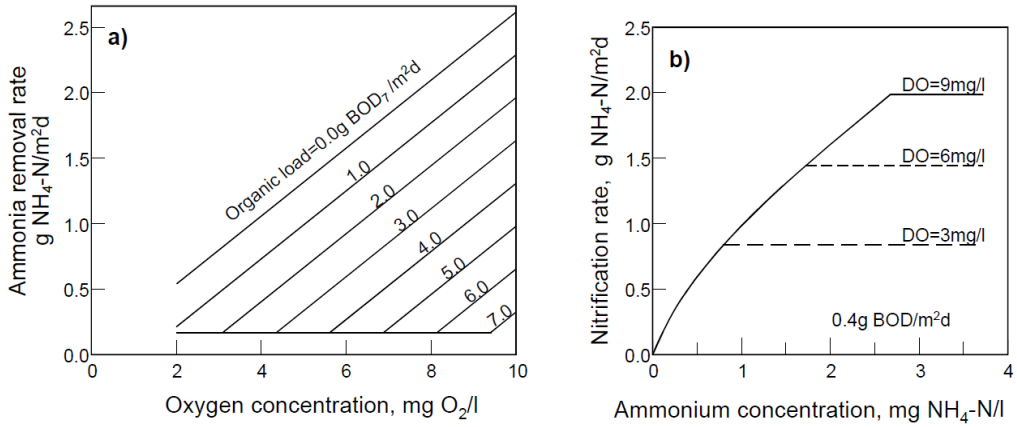

Figure 2: Nitrification rate as function of organic load and DO concentration (a) and as function of ammonia concentration and DO concentration (b) found in an MBBR experiment. (Ødegaard, 2006).

The nitrifying bacteria are mesophilic, which means that they flourish in moderate temperatures. They have an increasing growth rate up to about 35-40°C, where the growth rate rapidly declines (Helness, 2007). The nitrification rate dependent on the temperature can be described approximately by a simplified Arrhenius equation (1)(Ødegaard et al., 2014).

$$r_{N-T_2} = r_{N-T1}\theta^{T_2-T_1} \quad (1)$$

T_1 and T_2 are the temperatures [°C], r_{N-T_1} and r_{N-T_2} are the corresponding rates and θ is the temperature coefficient.

The pH in the liquid phase is important for two reasons. The first is that the nitrifying bacteria are very sensitive to pH, with different optimum ranges depending on the bacteria type. *Nitrosomonas* has an optimal pH range of approximately 7.0 – 8.0, and the corresponding range for *Nitrobacter* is approximately 7.5 – 8.0 (USEPA, 2002). However, several factors are affecting the nitrifying bacteria, which have led to occurrence of nitrification in the pH level ranging from 6.6 to 9.7 (Odell et al., 1996). The second reason is that the nitrification consumes alkalinity due to the consumption of bicarbonate (HCO_3^-), which in next term can reduce the nitrification rate due to a corresponding drop in pH (Helness, 2007).

2.1.2. Denitrification

Denitrification is the process where nitrate/nitrite is reduced, producing nitrogen gas (N_2), which rises and leaves the water phase through the water surface (Ødegaard et al., 2014). To induce this, a reactor or part of a reactor is made anaerobic, where NO_x can be used as an

electron acceptor (oxidant) instead of O_2. Due to the presence of nitrite/nitrate, the conditions are said to be anoxic. Several denitrifiers are in fact facultative bacteria, i.e. they can live under both aerobic and anoxic conditions, and can therefore also use oxygen as an electron acceptor, but will not denitrify in this case (Ødegaard et al., 2014).

Denitrifiers are heterotroph microorganisms and relies on organic matter as both energy and carbon source. The denitrifiers can be divided into groups, where some reduce nitrate to nitrite, while others reduce nitrite to molecular nitrogen, and others again perform the entire process from nitrate to nitrogen gas (Ødegaard et al., 2014). An example of the total reaction (without biomass growth) for denitrification by use of acetic acid as the carbon source is seen in equation 2 (Helness, 2007).

$$0.2\ NO_3^- + 0.2\ H^+ + 0.125\ CH_3COO^- \rightarrow \quad (2)$$
$$0.1\ N^2 + 0.225\ H_2O + 0.125\ CO_2 + 0.125\ HCO_3^-$$

One of the parameters referred to regarding denitrification, is the relationship between the amount of organic matter present in the water and the nitrite/nitrate removed. This is called the C/N-ratio and should not be confused with the ratio of organic matter added compared to the nitrogen added. The C/N-ratio is commonly referred to as mg $COD_{available}$ /mg NO_x-$N_{removed}$. It is therefore a parameter that shows the amount of organic matter needed to remove a certain amount of nitrogen. The theoretical value for COD consumption when acetate is used for denitrification is 2.87 mg COD_{HAc}/mg NO_x-$N_{removed}$ (Helness, 2007). Janssen et al. (2002) have reported the more general value 4 mg COD/mg NO_x-N.

As reviewed in section 2.1.1, alkalinity is consumed during nitrification. In denitrification, however, the pH increases, which leads to a replacement of the alkalinity consumed in the nitrification (Gray, 2017). Denitrifiers have an optimal pH range between 6 and 8 (Elefsiniotis and Li, 2006).

An average denitrification rate is reported to be 2.2 g NO_x-N/m^2/d, where acetic acid is used as the carbon source and the average temperature was 20.1°C (Pastorelli et al., 1997).

2.1.3. Partial nitrification

Investigations have been done during the last years, trying to reduce the energy consumption in nitrogen removal processes. This has led to a number of different processes, the most important

ones being partial nitrification, simultaneous nitrification and denitrification, and anaerobic ammonium oxidation (Anammox) (Ge et al., 2015).

Partial nitrification is defined as the biological oxidation from ammonium to nitrite. It differs from complete nitrification by not including further oxidation to nitrate. By creating optimal conditions for the microorganisms, the nitrite can be directly denitrified to N_2(g) (Jaramillo et al., 2018), see Figure 3. This phenomenon is of interest due to cost savings related to less need of oxygen, and also due to lower carbon need in the denitrification step.

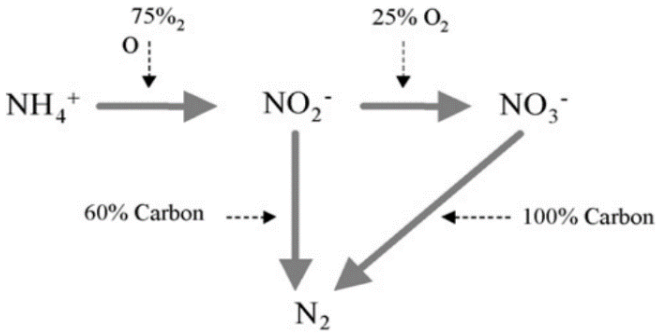

Figure 3: Partial nitrification. Nitrite (NO_2^-) is directly denitrified to N_2. (Pambrun et al., 2008).

The most powerful tool to suppress nitrite oxidation without excessively retarding the ammonium oxidation rate, is biochemical selection by inhibition of nitrite oxidation. One effective way of doing this is to lower the DO concentration since AOB has a lower oxygen saturation coefficient of Monod kinetics than NOB (Jianlong and Ning, 2004). Due to several other factors determining the nitrate accumulation, such as temperature and pH, only DO adjustment may not contribute to an effective partial nitrification (Ge et al., 2015).

2.1.4. Simultaneous nitrification and denitrification

Simultaneous nitrification and denitrification occurs within microbial flocs and biofilm, when DO concentration gradients is present due to diffusional limitations (see section 2.3.1). This means that the centre of the floc or the inner layer of a biofilm have anoxic micro-zones, which will allow occurrence of denitrification (Cao et al., 2017), while nitrification continues in the outer layer where aerobic conditions are still present. A relatively thick biofilm is essential for maintaining anoxic conditions in the inner layers of the biofilm.

2.2. Phosphorus removal by PAOs

2.2.1. Overview and metabolism of PAO

Phosphate accumulating organisms (PAOs) are the main driver for enhanced biological phosphorus removal (EBPR) (Henze et al., 2008). These organisms develop an ability to store large quantities of phosphate when they are exposed for alternating anaerobic and aerobic conditions.

The most abundant PAO is believed to be *Candidatus* Accumulibacter (hereafter Accumulibacter) (Marques et al., 2017). Under anaerobic conditions, this organism store poly-β-hydroxyalkanoate (PHA) within the cell by conversion of volatile fatty acids (VFA), such as acetate or propionate. VFA are easily biodegradable soluble organic matter (BSCOD), and is present in the inlet water or can be produced under anaerobic conditions by fermentation (Henze et al., 2008).

PHA is the organic acid storage molecule that eventually is needed for phosphate uptake and growth of PAO (Grady et al., 2011). The conversion of VFA to PHA relies on getting energy by degrading previously stored poly-P, resulting in a release of PO_4. The phosphate is transferred out of the cell accompanied with a release of a light metal cation. The release of the cation helps maintaining the charge balance and is typically a potassium or magnesium cation (Grady et al., 2011). The production of PHA also requires a reducing power, nicotinamide adenine dinucleotide (NAD), which is described in the Mino Model (Arun et al., 1988) to be produced by the metabolism of previously stored glycogen and possibly the TCA cycle (Grady et al., 2011). A schematic diagram of the cell under anaerobic conditions is shown in Figure 4.

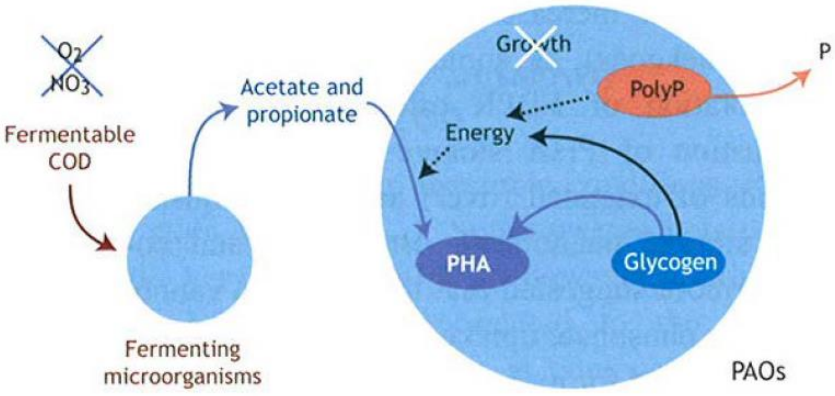

Figure 4: PAO under anaerobic conditions. Uptake and conversion of VFA to PHA by using previously stored poly-P and glycogen, resulting in phosphate release. (Henze et al., 2008).

Under aerobic conditions, oxygen is used as electron acceptor for normal aerobic metabolism for growth, by utilization of the stored PHA as energy and carbon source (Grady et al., 2011). Polyphosphate synthesis replenish the polyphosphate storage in the cell by removing ortho-P and associated light metal cations from the liquid phase. PHA is also utilized for gluconeogenesis, regenerating stored glycogen. All utilization of PHA produces carbon

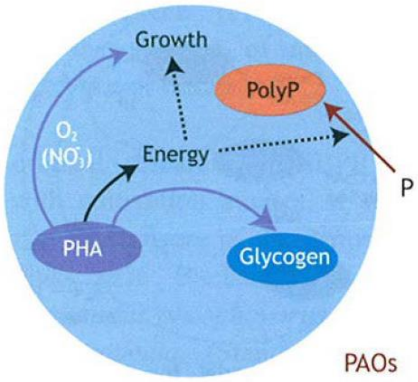

Figure 5: PAO under aerobic conditions. PHA and oxygen used for growth. Phosphate uptake. (Henze

dioxide, which is removed from the cell. Due to a large amount of energy produced through the aerobic metabolism, growth of PAO leads to an increased capacity for phosphate uptake. The additional phosphate uptake is often called a luxury uptake, since the organisms have the ability to accumulate larger amounts of P than required for growth. This makes it possible to remove P from the wastewater (Henze et al., 2008). An illustration of the cell under aerobic conditions is shown in Figure 5.

Figure 6 illustrates how the biochemical processes affect the concentrations of ortho-P, poly-P, PHA, glycogen and VFA in anaerobic and aerobic zones within the biomass and the liquid phase.

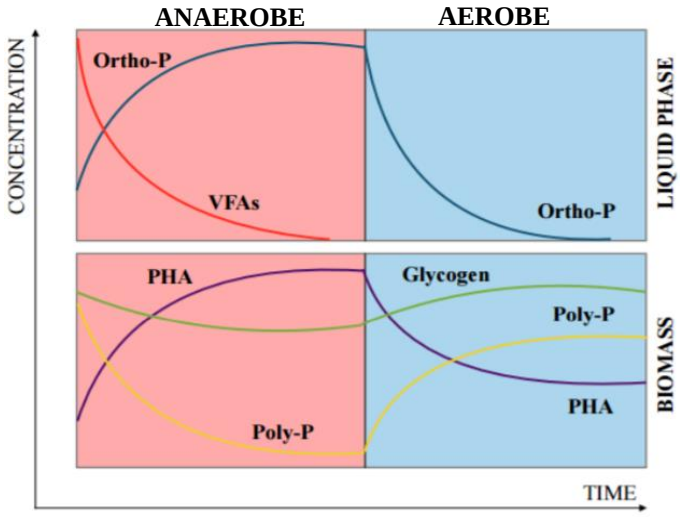

Figure 6: Concentration of ortho-P, poly-P, PHA, VFA and glycogen changing over time under anaerobe/aerobe conditions in the liquid phase and within the biomass. (Richardsen, 2017).

2.2.2. Denitrifying PAO (DNPAO)

It is found that some clades of PAOs can use nitrate and nitrite as electron acceptor for phosphorus uptake instead of oxygen (Figure 5) (Flowers et al., 2009, Kerrn-Jespersen and Henze, 1993). The groups of PAOs that can utilize the oxygen atoms in the nitrate/nitrite ion are called denitrifying PAOs (DNPAOs). The DNPAOs are believed to be ordinary PAOs that is able to use both nitrate/nitrite and dissolved oxygen (Kerrn-Jespersen and Henze, 1993). Other clades of PAOs are only able to utilize dissolved oxygen as the electron acceptor.

Researchers have investigated the use of nitrate and nitrite as electron acceptors and differences in the phosphate uptake between the two have been reported. Ahn et al. (2001) found that both nitrate and nitrite could be utilized, but the report did also conclude that nitrite as electron acceptor gave a smaller amount of phosphate uptake per nitrogen denitrified compared to nitrate. The same study also investigated how the initial loading of the two components affected the $P_{uptake}/N_{denitrified}$ rate, where the results showed an increasing rate when nitrate was used, and a decreasing rate for nitrite. The initial nitrite concentrations investigated in this study was approximately 20-50 mg NO_x-N/L.

Inhibiting effects of the present amount of nitrite in the liquid phase have also been found during some studies. A sequencing batch reactor performed on abattoir wastewater showed inhibition on the P uptake on nitrite levels higher than 40 mg NO_2-N/L (Pijuan and Yuan, 2010).

Another research, Meinhold et al. (1999), found that exposure of higher nitrite concentration than 8 mg/L was inhibiting the anoxic phosphate uptake completely and the aerobic phosphate uptake severely. It was also found that the inhibiting effect lasted for several hours after the nitrite exposure.

Saito et al. (2004) did also report that the P uptake rate decreased with an increasing initial nitrite concentration and the maximum P uptake rate was found at nitrite concentration of 2.5 mg NO_2-N/L. The study found a decreasing P uptake rate when having higher concentrations than this value. The study also found that denitrification was not inhibited at values below 12 mg N/L, and the study suggests that nitrite does not have negative impact on the enzyme system related to denitrification, but rather on the enzyme system related to phosphate uptake.

Saito et al. (2004) was also investigating the effect of nitrite on the aerobic phosphate uptake and the aerobic phosphate uptake was more inhibiting than the anoxic phosphate uptake.

All the previous studies conclude that both nitrate and nitrite is able to be used as electron acceptor. The different studies do not agree on the optimal concentration of nitrite for anoxic P uptake, but they all agree on its inhibiting effect in high concentrations. In addition, Saito et al. (2004) speculated in whether the presence of nitrite could be one of the factors enhancing the presence of a competitor to PAO, the glycogen accumulating organism (GAO – section 2.2.4).

2.2.3. Ordinary heterotroph organisms (OHO)

Ordinary heterotroph organisms (OHOs) may grow in the aerobic period of an EBPR process if the anaerobic period is operated shorter than necessary for removal of all COD from the inlet. This may decrease the population of PAOs, and thereby decrease the phosphate removal efficiency (Helness, 2007). The anaerobic retention time is therefore an important factor in the operation of a P removal process dependent on PAO activity.

2.2.4. Glycogen accumulating organisms (GAOs)

Glycogen accumulating organisms have been presented in literature to be responsible for suboptimal operation and deterioration of the EBPR process (Cech and Hartman, 1993, Saunders et al., 2003). This is due to their ability to have anaerobically carbon uptake, which

they utilize in the aerobic phase without any P uptake. Presence of GAOs may therefore lead to reduced performance of P-removal.

Several researchers have done investigations to find the optimal conditions that facilitates the presence of PAOs over GAOs in activated sludge systems and a summary of the findings is presented in Table 2.

Filipe et al. (2001b) found that PAOs' acetate uptake rate in anaerobic phase are independent on external pH, while the acetate uptake rate for GAOs is higher at lower pH. This means that increasing the pH will make it less favourable for GAOs, but it makes no difference for PAOs (within the pH range studied: 6.5-8.0). Filipe et al. (2001a) found that PAOs metabolism had a competitive advantage over GAOs metabolism at pH > 7.5. Values where PAOs is the most competitive compared to GAOs is also found at pH > 7 (Schuler and Jenkins, 2002). This implies that pH control can be used as a strategy for minimizing the growth of GAOs.

Research done on the temperature effect on PAO-GAO competition show that a higher temperature (>25-30°C) is favourable for growth of GAOs (Lopez-Vazquez et al., 2009, Panswad et al., 2003, Lopez-Vazquez et al., 2007). The competition between the two have not been investigated in lower temperatures than 10°C.

PAOs are found to have a higher survival capacity than GAOs in periods with low carbon availability when operated in a low pH range (pH = 6.4-7.0) (Tu and Schuler, 2013). Tu and Schuler (2013) are also indicating that the competition between GAO and PAO is not caused by competition of the available VFA in the anaerobic phase. This is due to an observed deterioration of the PAO population in periods when plenty of acetate were available, but accumulation of GAO was not seen until there were a non-detectable value of the PAO population.

Some researchers have investigated the PAO-GAO competition with respect to anaerobic carbon source. Several have found that propionate as anaerobically carbon source is facilitating enrichment of PAO against GAO (Oehmen et al., 2006, Pijuan et al., 2004, Chen et al., 2005). However, this could be influenced dependent on the most abundant PAO in the system (Accumulibacter vs. Tetrasphaera) (Marques et al., 2017). Others have found that the abundance of PAO was not influenced by the carbon source under the given conditions investigated (Lopez-Vazquez et al., 2009).

Table 2: Summary of favourable conditions for PAO versus GAO found in literature based on EBPR systems based on activated sludge.

Beneficial for PAO	**Beneficial for GAO**	**Literature**
High pH (> 7)	Low pH	Filipe et al. (2001a), Schuler and Jenkins (2002)
Low temperature	High temperature (>25-30°C)	Lopez-Vazquez et al. (2009), Lopez-Vazquez et al. (2007), Panswad et al. (2003)
Low DO	High DO	Carvalheira et al. (2014)
Low aerobic HRT	High aerobic HRT	Carvalheira et al. (2014)
Periods with low carbon availability		Tu and Schuler (2013)

Carvalheira et al. (2014) investigated the DO concentrations effect on the domination of PAOs vs GAOs in the aerobic phase of an EBPR. It was found that low aeration (2 mg O_2/L) was beneficial for PAO over GAO, and the opposite under high aeration (8 mg O_2/L). The same study also found that the aerobic HRT was of importance for the PAO-GAO competition. Too long aerobic phase was not beneficial for PAOs, leading to an increase in the population of GAOs. Even after only one day, the study showed a secondary P release at the end of the aerobic phase, leading to less phosphate inside the cell for VFA uptake in the subsequent anaerobic phase, which again makes it possible for GAOs to make use of the extra carbon available.

2.3. Biofilm systems

Some activated sludge systems for simultaneous P- and N-removal have shown inhibition of P release due to presence of nitrate in the anaerobic phase (Patel and Nakhla, 2006, Javier et al., 2012). This is caused by the return of biomass, which transports nitrate produced in the aerated phase, back to the anaerobic phase. Inhibitory effect of denitrification intermediates, such as nitric oxide, has also been reported (van Niel et al., 1998). Heterotroph activity may also be triggered by presence of nitrate and nitrite, where COD is used as the electron donor for NO_x reduction. This results in less available carbon for PAO growth.

Another issue related to the AS system is flushing of the necessary biomass. This can be avoided by introducing a biofilm system where biomass stays on the carriers' surface. The carriers are more easily separable from the liquid, and invention of some mechanisms for transporting the carriers between the anaerobic and aerobic liquid phase makes this possible.

2.3.1. Diffusion

A carrier containing biofilm is shown in Figure 7. The biofilm is mainly built up by bacteria, that grows until pieces of the biofilm loosens and falls off. The microbial metabolism takes place within the biofilm and the substances, which participates within the reaction, are initially present within the water flow outside the biofilm. This means that the reactants (organic matter, ammonium, oxygen etc.) need to diffuse into the biofilm where the reaction can take place. The products of the reaction, such as CO_2, nitrate, etc., need to diffuse out of the biofilm to the water phase (Ødegaard et al., 2014). Diffusion is therefore a fundamental process in biofilm processes (Figure 9).

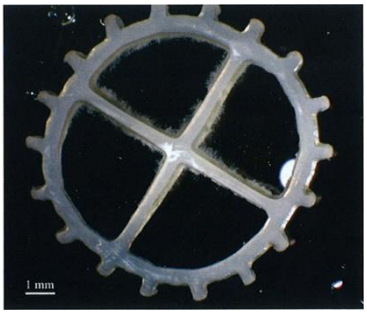

Figure 7: A layer of biofilm has grown on a suspended biofilm carrier.

Diffusion determines the mass transport of substrates and electron acceptors, which usually is slower than the reactions including these substrates. This gives substrate gradients within the biofilm, leading to even slower growth rates of the substrates. On the other hand, this leads to the opportunity to develop layers within the biofilm, including certain ecological niches (Henze et al., 2008). Due to diffusional limitations, oxygen may be inhibited to reach into the inner layer of the biofilm. Simultaneous nitrification and denitrification may therefore occur, due to the ability to create an anoxic layer inside the biofilm.

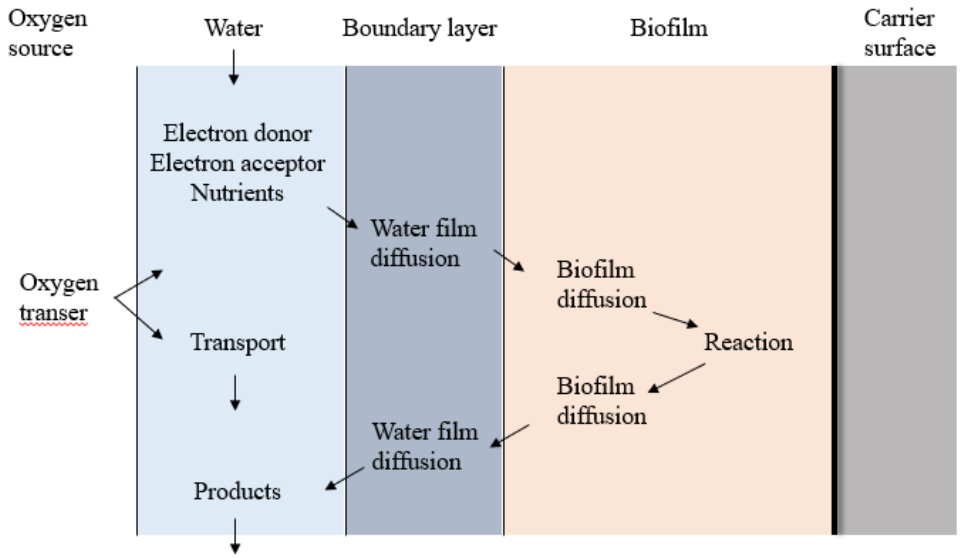

Figure 9: Diffusion of reactants and products in to and out from the biofilm. Microbial metabolism takes place inside the biofilm. (Modified from Ødegaard et al. (2014)).

Diffusion limitations occur when two or more substrates/electron acceptors are included in a reaction, and the substrate gradient for one of the substrates have a more rapid decrease than the other, limiting the reaction (Ødegaard et al., 2014). Figure 8 shows four examples of diffusional limitations, where either the substrates limit the reactions (a, b and c) or the thickness of the biofilm limits the reaction (d). In the case where both the substrates are limiting, a zone of inactive biomass will be present in the biofilm. Diffusional limitations become more

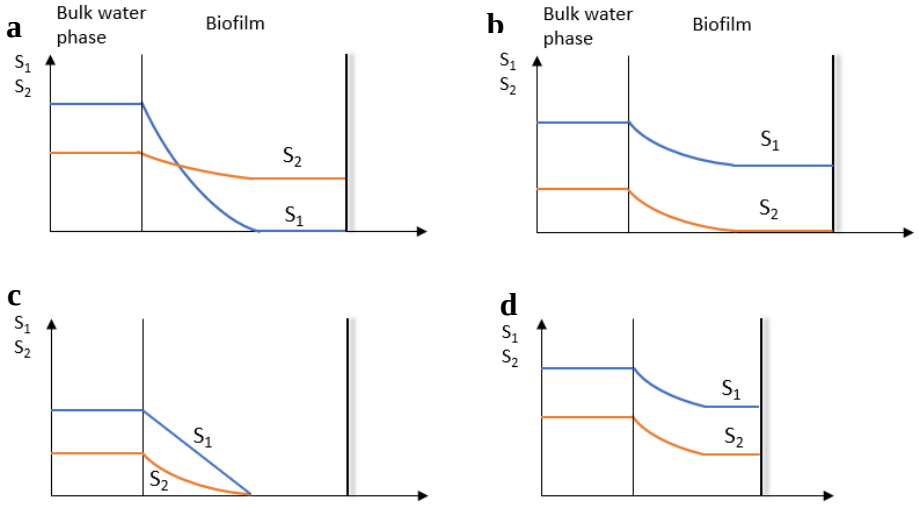

Figure 8: Substrate concentrations of substrate S_1 and S_2 in a biofilm layer. a) S_1 limiting, b) S_2 limiting, c) both S_1 and S_2 limiting, leading to inactive biomass, d) Biofilm thickness limiting. (Modified by Ødegaard et al. (2014)).

complex when two (or more) substrates are used in more than one process within the biofilm (Rauch et al., 1999).

2.3.2. "Active" biomass and type of carrier

As seen in the previous section, diffusion plays an important role in transferring substrates and electron acceptors, and the diffusion determines if the biomass can be "active" or not (Figure 8c). A large surface area or large amount of biomass is therefore not beneficial if the microorganisms' nutrient and/or oxygen supply is too low to maintain growth of the organisms (Geiger and Rauch, 2017).

Too thick biomass may lead to scaling during movement processes, and slow growing bacteria, like nitrifying bacteria, do not have a chance to survive in biomass where this is prone to occur. It is therefore crucial how much "active" biomass that can be permanently established on the carrier media, which is mainly related to the design of the carrier (Geiger and Rauch, 2017).

Previously, investigations of different types of carriers have been done, and it is found that the shape and size of the carrier does not seem to be significant on the removal rates as long as the effective surface area is the same (Ødegaard et al., 2000). However, in later studies, it is found that the shape and geometry of the carrier is playing an important role in terms of how much of the biofilm that is "active" in the specific reactor with a certain wastewater (Geiger and Rauch, 2017).

Figure 7 and Figure 10 shows different carrier media, which have different features regarding the biomass formation on the carrier. Figure 7 shows the more traditional Kaldnes carrier, while Figure 10a) shows the newly developed Z-MBBR carrier designed to control excessive biofilm thickness (VEOLIA, 2017) and Figure 10b)Figure 10 shows a porous polyvinyl alcohol gel carrier effective for retention of autotrophic and heterotrophic biomass (Levstek et al., 2010).

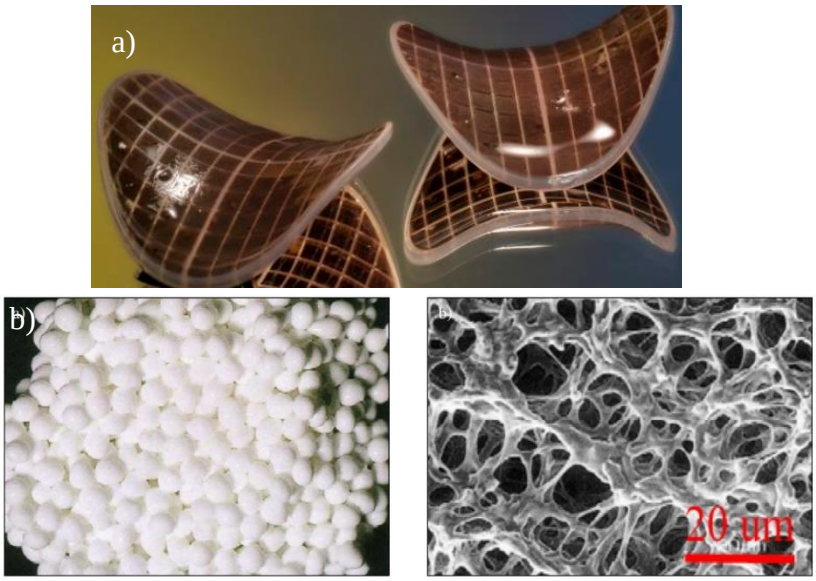

Figure 10: a) ANOXKALDNES Z-MBBR carrier (VEOLIA, 2017). B) Porous carrier media (Levstek et al., 2010).

2.4. The HIAS-process

The HIAS-process is based on completely mixed flow reactors (CMFR) in series, which is a combination of ideal plug-flow and completely mixed batch reactors (CMBR) in series. Every chamber in the reactor (Figure 11) has a constant in- and outflow of both water and carriers.

Alternating anaerobic and aerobic conditions is possible due to the conveyor belt at the end of the last aerobic chamber, which transports the carriers, including the associated biomass, back to the first anaerobic chamber.

Growth of PAOs are facilitated in this type of reactor, due to the alternating anaerobic and aerobic conditions. Easily biodegradable organic matter is taken up while phosphate is released in the anaerobic chambers, followed by utilization of the stored carbon and luxury P uptake in the aerobic chambers.

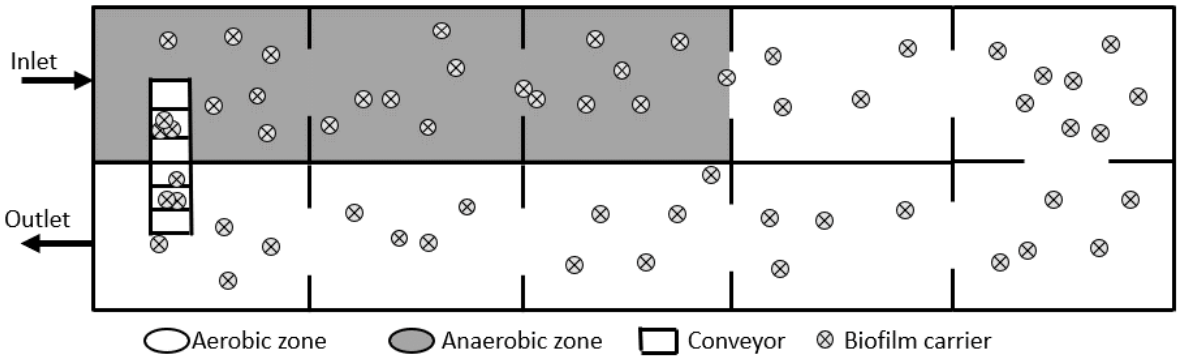

Figure 11: Sketch of the HIAS-process. Carriers with biofilm are transferred between anaerobic and aerobic conditions by a conveyor belt. (Modified from Saltnes et al. (2017)).

The formation of an anoxic layer may occur inside the biofilm under the aerobic period (Figure 12), due to diffusional limitations under low dissolved oxygen concentrations. PAOs that are able to utilize nitrate/nitrite as electron acceptor could then denitrify available NO_x to nitrogen gas. Promising results have been found for simultaneous nitrification and denitrifying phosphate uptake in this process (Saltnes et al., 2017).

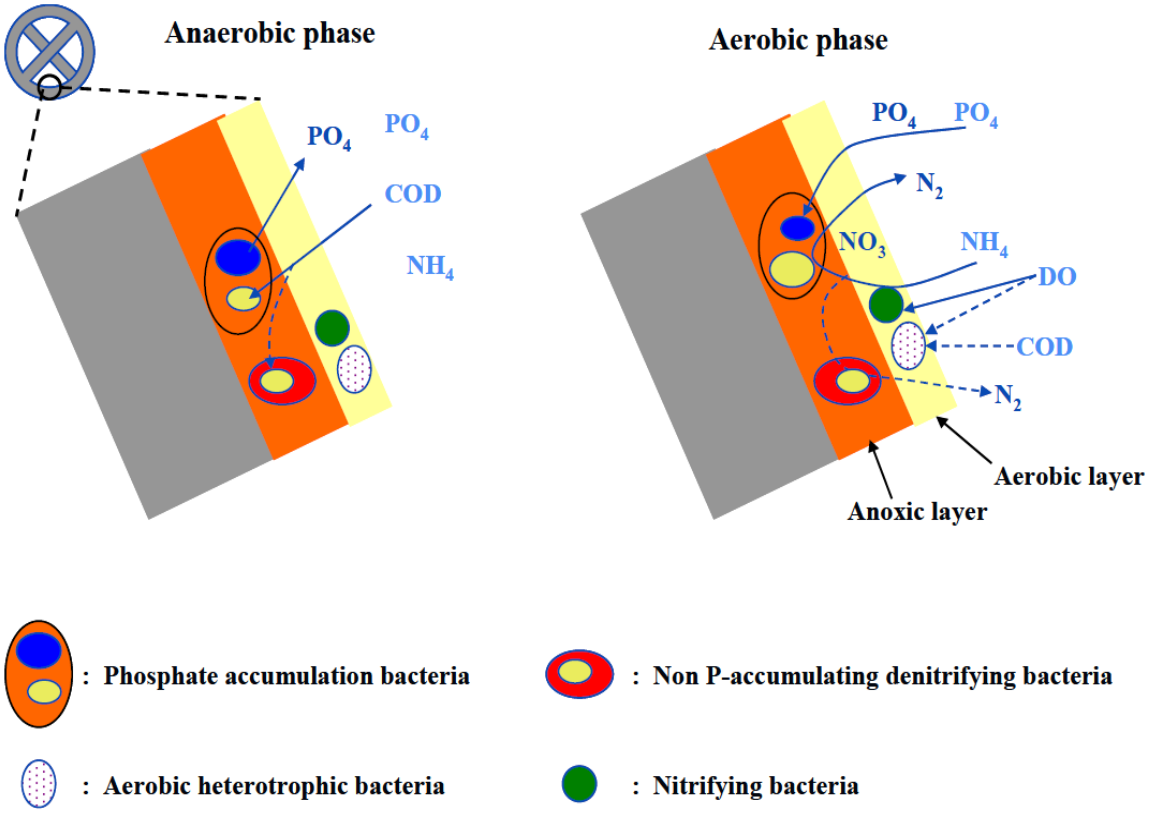

Figure 12: Illustration of biofilm growing on carrier, performing simultaneous nitrification and denitrifying phosphate uptake. (Helness, 2007).

2.5. Objective

The objective of this study was to optimize the HIAS-process for N-removal. To obtain this, three different parameters were investigated to get further understanding of the process:

- Anaerobically carbon consumption
- Dissolved oxygen concentrations
- Temperature

The parameters were examined through batch experiments at the laboratory and by grab sampling in the pilot plant at Hias WWTP. The results from the experiments were used to recommend operational improvements of the process to enhance N-removal.

3. Materials and methods

Materials and methods used in the experiments performed in this master thesis are presented in this chapter.

3.1. Experiments at Hias WWTP

All investigations were conducted at Hias WWTP through batch experiments at the laboratory or by grab sampling in the pilot plant.

3.1.1. Wastewater characteristics

Hias WWTP receives municipal wastewater from 60 000 persons living in 4 neighbouring municipalities in Norway (Stange, Hamar, Ringsaker and Løten). Industrial wastewater, mostly related to food processing industry, is also entering the WWTP, creating seasonal and weekly variations in the inlet concentrations. The wastewater is treated by a grid, sand- and fat trap and primary sedimentation before entering the HIAS-process' pilot plant. Average inlet concentrations after primary sedimentation at the WWTP is reported from 3 years of daily grab samples, presented in Saltnes et al. (2017): 3.65 mg PO_4-P/L, 60.8 mg NH_4-N/L and 312 mg sCOD/L.

3.1.2. The pilot plant

The pilot plant at Hias WWTP has a depth of 0.8 m and a total volume of 7 m^3. Each chamber has a surface area of 0.88 m^2. The reactor is filled with 4 m^3 AnoxKaldnes K1 carriers which gives a filling degree of 57%. A volume displacement test showed that the corresponding water volume in the reactor would be 77% of the total volume at this filling degree ($V_{water,\ reactor}$ = 5.39 m^3). The execution of the displacement test and the related results are presented in Appendix A2.

3.2. Batch experiments in the laboratory

Batch experiments were conducted to investigate the different parameters in the HIAS-process. The experiments were performed under anaerobic, aerobic and anoxic conditions using K1 biofilm carriers collected from the conveyor belt in the pilot plant. All batch experiments were conducted in parallel with synthetic water or wastewater.

Operational conditions and starting concentrations for all experiments are presented in tables within the section where the experiments have been discussed in the Result and Discussion

chapter. Every single experiment has been named based on its conditions. Batch-names starting with SWB refers to batches containing synthetic water, WWB refers to experiments conducted with real wastewater and GRAB refers to the experiments conducted as grab samples in the pilot plant.

3.2.1. Synthetic water

Experiments were performed on synthetic water to exclude influencing factors in the wastewater that is hard to detect and may influence the results.

The synthetic water was made based on tap water by addition of different substances. Depending on the experiments conducted, the synthetic water was made to contain ammonium, nitrate or nitrite, and phosphate and acetate as VFA source. The substances were added as different solutions made by ammonium chloride (NH_4Cl), sodium nitrate (NO_3^-), sodium nitrite (NO_2^-), di-sodium hydrogen phosphate (Na_2HPO_4) and sodium acetate trihydrate ($CH_3COONa*3H_2O$). The acetate solution was made not more than half an hour prior to the start-up of the experiment.

All solutions of ammonium, nitrite, nitrate, phosphate and acetate was made as described in Appendix 1.

3.2.2. Experimental setup

3.2.2.1. Carbon experiments

Six batch experiments were performed to obtain information of the nitrogen removal influenced by the inlet concentrations of carbon (hereafter referred to as the carbon experiments). Four batches were conducted with synthetic water, and two batches were conducted with wastewater.

Synthetic water was made for the parallel experiments by adding 18 ml of 4 mg/ml ammonium solution and 18 ml of 0.5 mg/ml phosphate solution into 1.8 L of tap water.

The experiments started by filling two beakers with 0.6 L of biofilm carriers. Thereafter, 750 ml of the synthetic water was filled in each beaker together with 5 ml and 20 ml of 10 mg/ml acetate solution in the two batches (68 mg Ac/L and 267 mg Ac/L, respectively). This gives initial filling degrees of 60% and 59%, respectively (see appendix A2 for displacement test and calculations for filling degrees). Immediately after addition of acetate, the first sample extraction was done. Samples were extracted with 10-30 minutes time intervals.

The same procedure was used for the second round of parallel experiments, except that the acetate additions were 10 ml and 40 ml of the 10 mg/ml acetate solution (132 mg Ac/L and 526 mg Ac/L, respectively), resulting in initial filling degrees of 59% and 58%, respectively.

Parallel wastewater experiments were performed using the same procedure, where wastewater was collected from the inlet of the pilot plant. The wastewater was completely stirred to get a similar wastewater in the two batches. One of the batches was added 10 ml of 10 mg/ml acetate solution.

The batches were initially exposed to anaerobic conditions, where efficient stirring was obtained by using a 4 Station Portable Jar Test Apparatus. Diffusers for aeration were inserted to the batches immediately after the last sample of the anaerobic period were extracted. The anaerobic retention time was 165 minutes for all batches. The experiments were conducted under aerobic conditions for as long as necessary to see constant behaviour or trends in the parameters. The experimental setup is shown in Figure 13.

Figure 13: Experimental setup for anaerobic period of the batch experiments. Air pumps were aerating the batches through golf ball air stone diffusors in the aerobic period.

The samples were analysed for sCOD, ammonium, nitrite, nitrate and phosphate. Temperature and dissolved oxygen concentration was measured at the beginning and towards the end of the aerobic periods by use of HQ30D Portable Multi Meter by Hach.

Summary of the conditions and initial measured concentrations in the carbon experiments are presented in table 3.

3.2.2.2. Anoxic experiments

Four batch experiments were conducted under anaerobic – anoxic conditions to obtain information about the anoxic phosphate uptake and the corresponding denitrification, where two batches were performed with nitrite (SWB NO2a, SWB NO2b), and two batches were performed with nitrate (SWB NO3a, SWB NO3b). An anaerobic – aerobic batch experiment (SWB O2) and an anaerobic – anoxic (NO_3) – aerobic batch experiment (SWB NO3 → O2) was conducted for comparison purposes with the anoxic nitrate and nitrite tests. All these experiments are referred to as the anoxic experiments.

All six experiments were performed using synthetic water containing the same phosphate concentration as in the carbon experiments and each batch was added 20 ml of 10 mg/ml acetate solution. The anaerobic retention time was chosen to 100 minutes based on the carbon experiments showing a clear decrease in the phosphate release rate at this time when using the chosen initial acetate concentration.

The experiments were performed using the same procedure as in the carbon experiments, except that the anoxic experiments were held in the jar test apparatus for stirring when nitrite/nitrate dosages were added. To minimize the errors in the batch reactor volume related to sample extraction, the amount of synthetic water added to each batch were 850 ml, giving initial filling degree of 55%.

Since several researchers have found that a large amount of nitrite could inhibit the anoxic phosphate uptake (section 2.2.2), both nitrite and nitrate was added in doses of 5 ml of 4.8 mg/ml nitrite or nitrate solution.

Samples were extracted both before and immediately after addition of the dosages to confirm the added concentrations. A new dosage was added when the expected concentration in the batch were between 5 and 10 mg NO_x/L.

Summary of the conditions and initial measured concentrations in the anoxic experiments are presented in table 5.

3.2.2.3. DO experiments

Batch experiments were performed to obtain information of the dissolved oxygen influence on the nitrogen removal (hereafter referred to as the DO experiments).

3 litre homemade containers were used in these experiments to reach a lower aeration, due to no aeration adjustment possibilities in the aeration pump. The containers were filled with 1.7 L of K1 biofilm carriers from the pilot plant, and the added water had a volume of 2.35 L, giving an initial filling degree of 56%.

Four batch experiments were conducted, where the aeration was constant during the entire aerobic period of the experiment. The experiments were performed in parallel, where the first two experiments were based on synthetic water with low and high aeration (SWB DO(L), SWB DO(H)), and the other two experiments were conducted under the same aeration levels, but by use of wastewater instead of synthetic water (WWB DO(L), WWB DO(H)).

Synthetic water was made by addition of 50 ml of 5 mg/ml ammonium solution, 50 ml of 0.5 mg/ml phosphate solution to 5L of tap water. 60 ml of 10 mg/ml acetate solution was added to each of the 3L containers containing 2.35L of the synthetic water during start-up of the experiment.

High aeration was obtained by using three diffusers, while low aeration was obtained by using one diffuser. The experimental setup for parallel batches are shown in Figure 14. The jar test apparatus was used for stirring in the aerobic period of the batches having only one diffuser (low aeration) to obtain effective stirring of the carriers.

Another four batches were performed, where the aeration in the aerobic period was initially high, and after a given time, the aeration was lowered by removing two of the diffusers. The first two experiments were based on synthetic water (SWB DO(HL)1, SWB DO(HL)2) having different ammonium concentrations. Two similar synthetic waters were made by adding 25 ml of 0.5 mg/ml phosphate solution in two containers consisting of 2.5L of tap water each. One of the containers containing synthetic water was added 15 ml of 5 mg/ml ammonium solution, while the other container was added 25 ml of 5 mg/ml ammonium solution. 2.35 L of each of the synthetic waters were filled in the 3L homemade containers together with 60 ml of the 10 mg/ml acetate solution during start-up of the experiments.

The other two experiments were performed on wastewater (WWB DO(HL)3, WWB DO(HL)4), where one of the batches were added 10 ml of the 10 mg/ml acetate solution.

The anaerobic retention time in all eight experiments was 100 minutes and the experiments were conducted under aerobic conditions until desired behaviour were seen.

Summary of the conditions and initial measured concentrations in the DO experiments are presented in table 6.

Figure 14: Experimental setup for the DO experiments by use of 3 L homemade containers. Low aeration was obtained by using one diffuser. High aeration was obtained using three diffusers.

3.2.2.4. Temperature experiments

Four batches were operated to evaluate the temperature as an influencing factor in the process (hereafter referred to as the temperature experiments). Two batches were performed in parallel, where one of the batches was held in room temperature, and the other batch was placed within a refrigerator room. Due to the differences in location, a one-beaker portable flocculator apparatus was used for stirring in the anaerobic period of the experiments.

Two of the experiments were conducted with synthetic water (SWB LOW, SWB HIGH). The tap water, that was used to make this synthetic water, was tapped the day prior to the experiment to obtain the desired start-up temperatures. 0.9L of tap water was placed in a container in the refrigerator room during the night, while another 0.9L of tap water was placed in a container in room temperature. At the day when the experiments were performed, 9 ml of 0.5 mg/ml phosphate solution, 9 ml of 6 mg/ml ammonium solution and 22.5 ml of 10 mg/ml acetate solution was added to each container.

Each of the one litre beakers were filled with 0.6 L of biofilm carrier. The first sample was extracted from each or the synthetic waters prior to pouring 0.8L of each of them into the beakers.

Two experiments performed on wastewater were also done, using the same procedure, having one beaker in the refrigerator room and one beaker in room temperature. The wastewater was collected from the inlet of the pilot plant, having a temperature of approximately 8°C. 0.8 L of the wastewater was poured into a container which was placed in the refrigerator room, while 0.8 L of the wastewater was poured into a container which was placed in a bucket containing warm water. The HQ30D Portable Multi Meter by Hach was used to detect the temperature in the wastewater while the water was heated, and when the temperature reached 14°C, the container was removed from the bucket and the first sample was extracted. The first sample of the container in the refrigerator room was also extracted at this time. The wastewater from both containers were poured into two beakers containing 0.6L of biofilm carriers each, which had been picked up at the conveyor belt of the pilot plant just before the wastewater was extracted from the inlet of the plant.

Temperature and dissolved oxygen concentrations were detected by using the HQ30D Portable Multi Meter by Hach throughout both the synthetic water experiments and wastewater experiments.

Summary of the conditions and initial measured concentrations in the temperature experiments are presented in table 7.

3.3. Experiments in the pilot plant

Experiments were conducted to evaluate the performance of the pilot plant related to the removal of phosphorus and nitrogen (hereafter referred to as the pilot experiments).

Four experiments were carried out in the pilot plant by taking grab samples from each chamber and from the inlet of the reactor. The samples were taken out with a time delay, so that the sample extraction corresponded with the hydraulic retention time. HRT in each chamber was found by the equation 3.

$$HRT_{chamber} = \frac{V_{water}}{Q}/10, \quad (3)$$

where V_{water} is the total actual water volume in the reactor (not empty bed volume) and Q is the waterflow throughout the reactor. The water volume in the reactor is 5.39 m^3 (section 3.1.2) and the water flow was held constant at 0.65 m^3/h, giving retention time of 48 minutes in each chamber (time between each sample extraction).

Two of the experiments were conducted under high DO concentration throughout the entire aerobic period (named GRAB H1 and GRAB H2). Another two experiments were conducted with high DO concentration in the four first aerobic chambers and low DO concentration in the three last chambers (named GRAB L1 and GRAB L2).

The pilot plant had been running under high DO concentrations for a few weeks prior to the experiments operated with high DO concentrations in all chambers. The pilot plant had been running for one day with the experimental condition prior to the experiments operated with lowered aeration in the three last chambers.

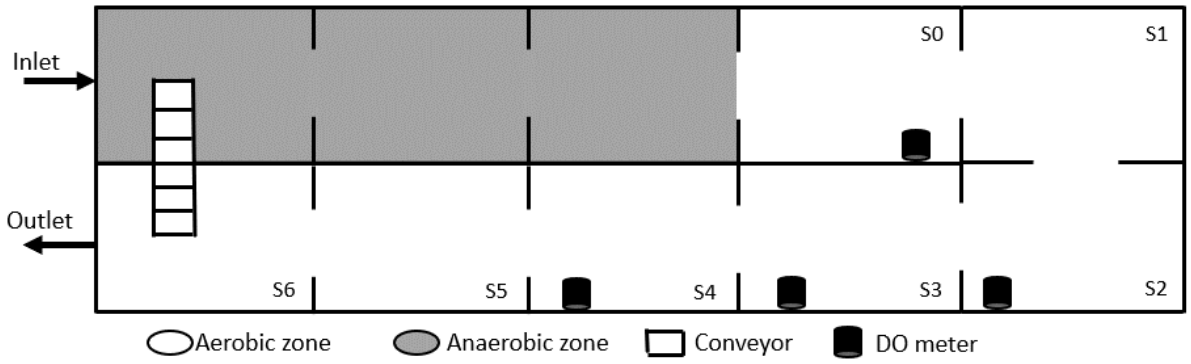

Figure 15: Sketch of the pilot plant including names of each chamber and placement of the installed DO meters.

The pilot plant was installed with four DO meters, that were placed in chamber S0, S2, S3 and S4 during the experiments (Figure 15). The DO concentration in the rest of the aerobic chambers were measured with the handheld HQ30D Portable Multi Meter by Hach. A simple test showed that the handheld DO meter and the installed DO meters corresponded well. The DO was measured in all chambers prior to the outtake of the grab samples.

Grab samples extracted from the pilot plant are analysed for ammonium, nitrite, nitrate, sCOD and phosphate. In addition, analysis of VFA and pH was performed on the inlet water and the anaerobic chambers.

Summary of the conditions and initial measured concentrations in the pilot plant experiments are presented in table 8.

3.4. Analysing the samples

All samples were filtrated through 1.2 μm glass microfibres filters (VWR European Cat. No. 516-0869) immediately after the samples were taken out. The concentrations of ammonium-N,

nitrite-N, nitrate-N, phosphate-P and sCOD were found by analyses using Spectroquant® test kits by Merck. The analyses were determined photometrically by insertion of the cells into the photometer Spectroquant® NOVA 60. The enclosed procedure sheet for analysis by use of the test kit was followed.

Depending on which analyses that should be performed on the sample, the volume of the sample extraction was between 15 and 25ml. The excess sample volume after the performance of the analyses was poured back into the beakers.

VFA analyses were performed by using the titration method by Moosbrugger et al. (1993). The sample were stirred with a magnetic stirrer and pH was measured with 827 pH lab by Metrohm. Due to time limitation between each sample, a correlation was found between sCOD and measured VFA: VFA as acetate = 1.0218*sCOD + 41.721. The test and calculations performed to find this correlation is presented in Appendix A3.

3.5. Sources of error related to analysis method

Due to measuring ranges in the sampling equipment, dilution of some samples was necessary. Errors related to dilution might be present due to extraction of a potential inhomogeneous sample. However, the samples were filtrated before they were diluted, and the parameters that were analysed for were all dissolved in the water and could be expected to be quite homogenous. The filter size is also a potential source of error, due to larger pore opening than the defined size for soluble matter (0.45 μm).

Duplicates of samples should always be done to verify the results and reveal deviations.

The test performed to find the correlation between sCOD and VFA values, revealed that some errors in the calibration of the photometric reading of the sCOD analysis were present. It was seen that the values had larger deviation in the smallest values, and that measured values of sCOD below 45 mg sCOD/L could seem to actually be zero. However, duplicates of both VFA measurement and sCOD analysis was not done and the correlation is therefore not verified.

4. Results and discussion

4.1. Laboratory batch experiments

Laboratory batch experiments were conducted to investigate the biological activity within the biofilm.

Typical nutrient and carbon profiles during one cycle are shown in Figure 16. The profiles show the measured values from a batch experiment containing wastewater with an addition of acetate. The oxygen supply was constant during the aerobic period, giving DO concentrations starting from approximately 7.5 mg/L to 9.0 mg/L at the end. The duration of the anaerobic and aerobic periods were 165 min and 345 min, respectively. During the anaerobic period, sCOD was consumed while phosphate was released. In the subsequent aerobic period, ammonium was nitrified with an accumulation of NO_x. The total nitrogen removal in this batch experiment was 63.3%, meaning that some denitrification did occur simultaneously in the aerobic stage. Some ammonium was probably also used for biological growth. A small amount of sCOD was consumed during the aerobic period, indicating some ordinary heterotrophic activity. Uptake of PO_4 was good, giving a total P removal efficiency of 99.1% under these conditions.

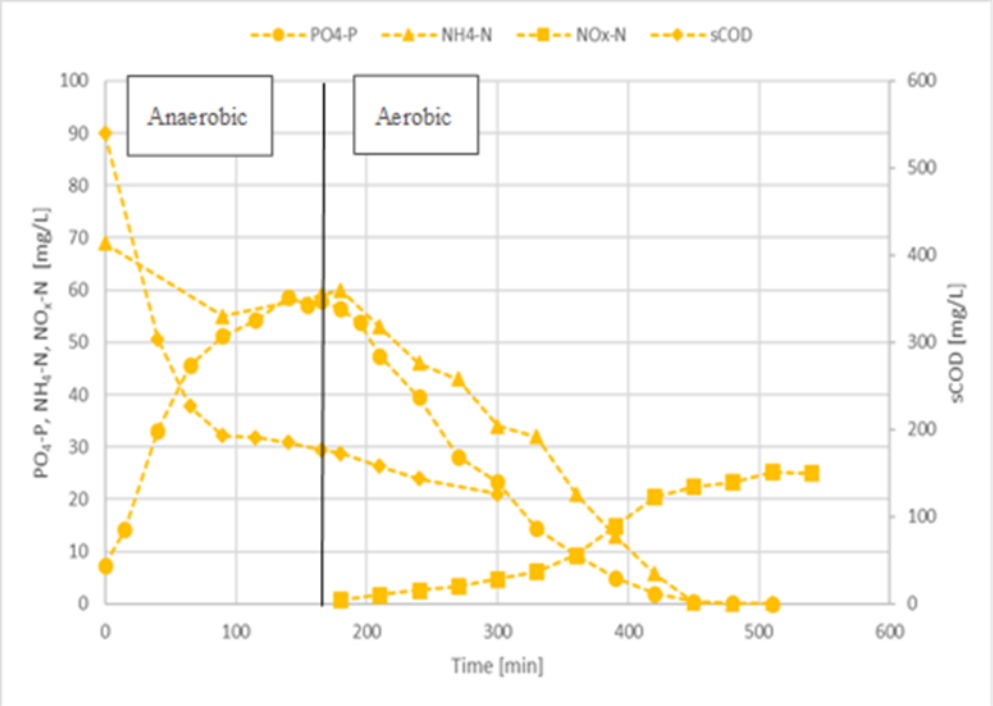

Figure 16: Typical nutrient and carbon profiles during one reactor cycle performed as a batch experiment containing wastewater. The wastewater contained 90 mg VFA as acetate/L and an extra amount of 133 mg acetate/L was added at the start of the experiment.

4.1.1. Influence of carbon concentration on SND

An overview of the batch experiments performed to see the influence of carbon on the biological activity related to nitrogen removal is shown in Table 3. The start concentration of VFA in the synthetic water is calculated from the measured sCOD analysis. The two wastewater batches were performed in parallel, where the only difference was an addition of acetate in WWB 223. The VFA concentration in WWB 90 was measured using the titration method. VFA concentration in WWB 223 was calculated by adding up the measured value (90 mg VFA/L) and a known amount of added acetate solution (133 mg Ac^-/L). The numbers behind SWB and WWB represent the inlet VFA concentration.

Table 3: Influence of carbon on the nitrogen removal – batch experiments, overview and operational conditions. All values are measured unless marked. Calculated VFA concentration from sCOD analysis (*). Measured VFA in inlet water + a known amount of acetate added during the first 30 seconds of anaerobic operation time (**).

Batch	Type of water	HRT anaerobic [min]	HRT aerobic [min]	VFA/sCOD (start)	NH4-N start	PO4-P start	DO [mg/L]	Volume container	Volume water	Temp
SWB 66	synthetic	173	375	66*/109	40	4.70	ca. 8-9	1 L	0.75 L	ca.17°C
SWB 105	synthetic	165	305	105*/149	42	4.56	ca. 8-9	1 L	0.75 L	ca.17°C
SWB 252	synthetic	173	375	252*/299	42	4.56	ca. 8-9	1 L	0.75 L	ca.17°C
SWB 429	synthetic	165	305	429*/480	39	4.42	ca. 8-9	1 L	0.75 L	ca.17°C
WWB 90	wastewater	165	315	90/425	67	7.50	ca. 7.5-9	1 L	0.75 L	ca.17°C
WWB 223	wastewater	165	327	223**/540	69	7.40	ca. 7.5-9	1 L	0.75 L	ca.17°C

4.1.1.1. Nitrification

Ammonium concentration profiles for the six batches performed as described in Table 3 are shown in Figure 17. The profiles show that all the ammonium in four of the batches have been nitrified. The two synthetic water batches containing the least VFA (SWB 66 and SWB 105) have a decrease in the nitrification rate and is not able to remove all the ammonium present. These batches have not been investigated with respect to nitrogen removal, due to their behaviour (see Appendix B1 for further discussion). However, they have been used in discussions about P release and GAO competition.

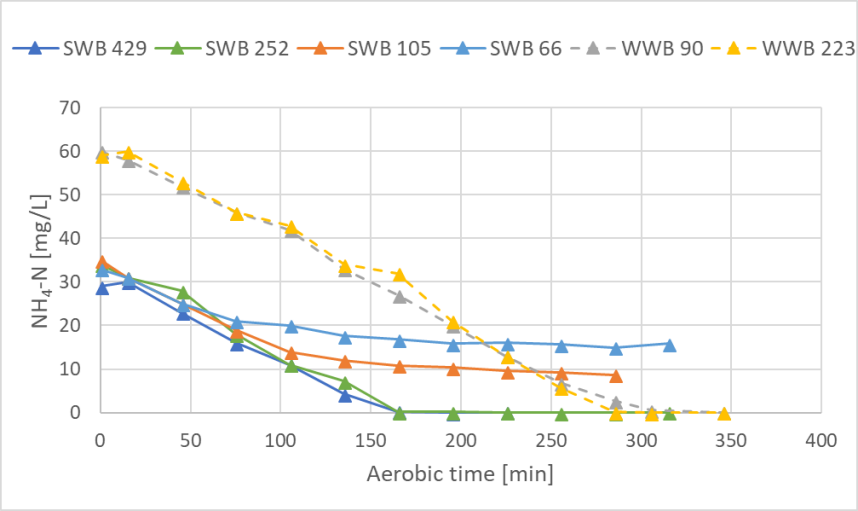

Figure 17: Ammonium profiles. Measured NH_4-N concentrations (triangle) in all 6 batches. Aerobic period starts at time zero.

Nitrification rates were found for the four other batches (SWB 252, SWB 429, WWB 90 and WWB 223). A plot of the ammonium concentration versus the N-rate for the two wastewater batches and the two synthetic water batches are shown in Figure 18a and Figure 18b, respectively. Below an ammonium concentration of approximately 15 mg/L, the nitrification rate seemed to be concentration limited. Other researchers have suggested concentration limited nitrification rates below 3 mg/L in MBBR systems (Figure 2, at 9 mg DO/L, Ødegaard, 2006) and SBR systems (Helness, 2007, Ødegaard, 2006). The difference between the experimental result and literature is not known, but a possible explanation could be related to diffusion and

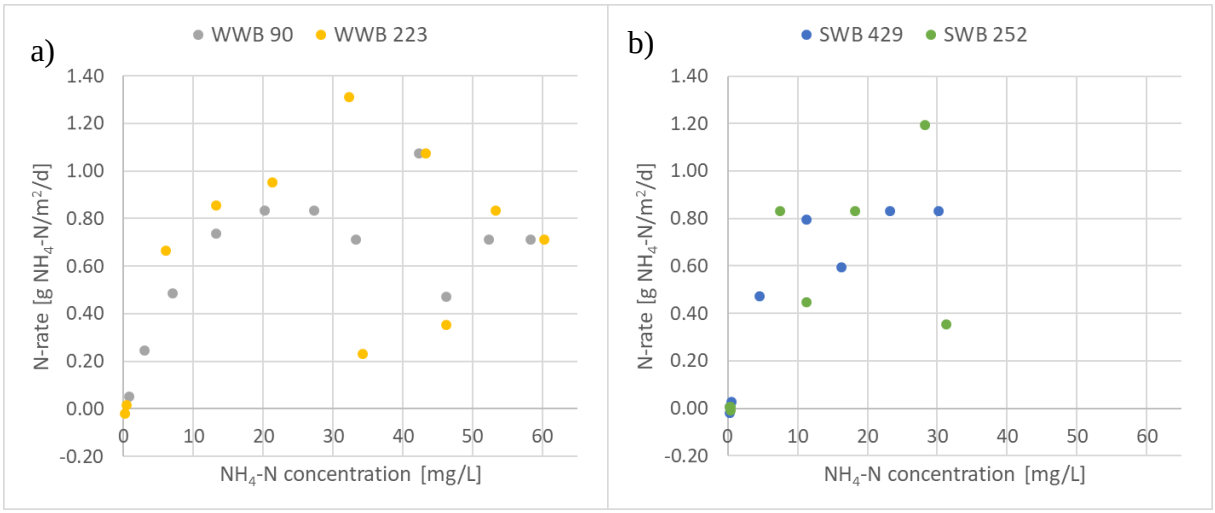

Figure 18: Effect of ammonium concentration on the nitrification rate. Measured in wastewater batch experiments (a) and synthetic batch experiments (b).

the thickness of the biofilm. There is a large biodiversity within the biofilm compared to biofilm systems where only nitrification is meant to occur. If the nitrifyers are placed among other bacteria, such as PAOs and ordinary heterotrophs, diffusion could create a lower ammonium concentration locally than what is seen in the liquid phase. This might have been the reason why the results from Figure 18 indicates a higher value for concentration limited N-rate than what is found in literature. Another reason could simply be errors in the measurement. If this is the case, it seems like there is a systematic measurement error, since the results from both batches show smooth curves in the lower ammonium concentration regions.

At the highest ammonium concentrations, which occurs in the start of the aerobic period, the N-rate was oxygen concentration limited. In the following, the N-rates have been investigated in this region, i.e. not the region where the N-rate seem to be concentration limited by ammonium.

The results from the batch experiments containing wastewater showed an increase in the nitrification rate during the sampling period (Figure 19). This can most likely be described by the amount of organic matter entering the aerobic phase. It can also be seen from Figure 19 that

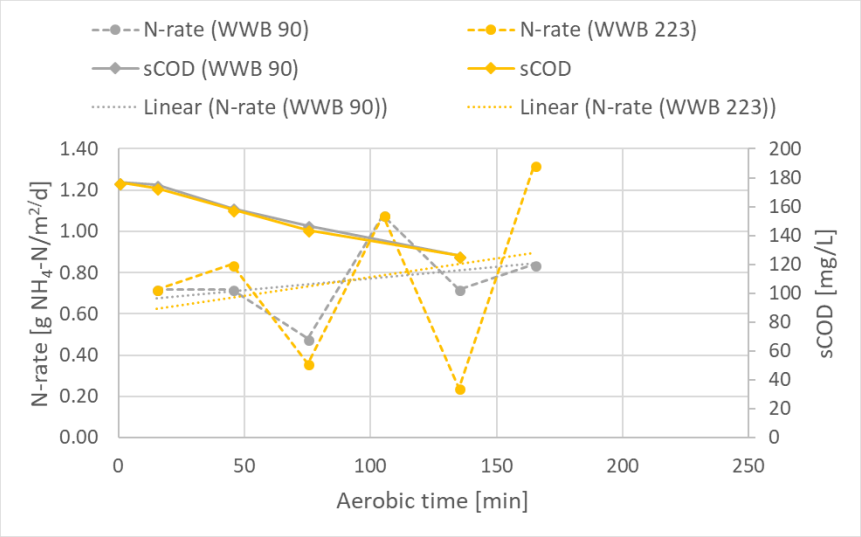

Figure 19: Nitrification rate and sCOD concentration in batch experiments containing wastewater. DO between 7.5 and 9 mg/L. Time zero: changing from anaerobic to aerobic conditions.

the sCOD values decrease, which means that there is some heterotrophic activity in the biofilm. It is well known that nitrifying bacteria have slower growth rates than heterotroph bacteria and therefore have disadvantages when competing for oxygen compared to the heterotrophs (Ødegaard, 2006). Thus, the nitrification rate is believed to be influenced by the carbon content in the aerobic phase. Since the aeration is constant in the batches, the oxygen competition by

some organisms, such as PAOs and heterotrophs, results in an increasing DO concentration throughout the aerobic period when the oxygen consumption by these organisms are decreasing. The increasing oxygen concentrations could be the reason for the increasing N-rate.

The synthetic water batches did not have many data points in the oxygen limited N-rate region, due to a much lower ammonium concentration into the aerobic period than the wastewater batches. The tendency for these N-rates throughout the aerobic period were therefore quite hard to detect (data not shown).

Average nitrification rates in the oxygen limited region (NH_4-N > 10 mg/L) for all batches are presented in Figure 20, which shows quite similar results for both wastewater and synthetic water. It is reasonable to believe that the N-rates for the wastewater batches could have been somewhat higher if they not had been influenced by oxygen competition due to carbon in the beginning of the aerobic period. N-rates are plotted against oxygen concentration in Figure 35, section 4.1.3.1.

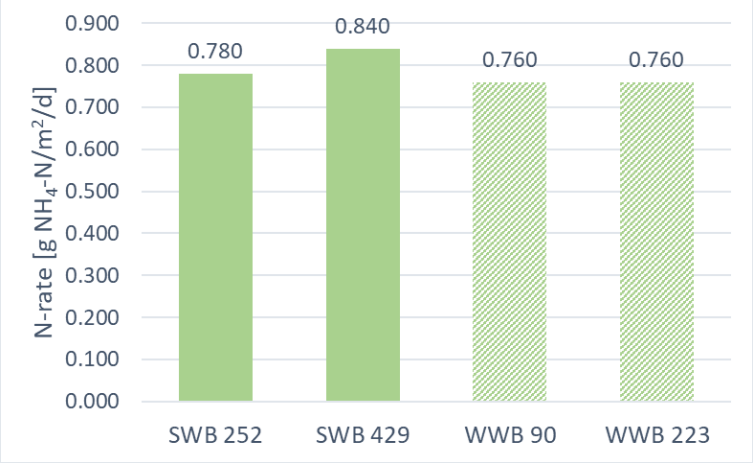

Figure 20: Nitrification rates in batch experiments containing different initial VFA concentration. The overall average nitrification rate was found to be 0.785 g NH_4-N/m^2/d.

The average nitrification rate in all batches is 0.785 g NH_4-N/m^2/d for DO concentrations around 8 mg/L. This rate is low compared to nitrification rates found in MBBR reactors specialized for nitrification at this DO concentration (Ødegaard, 2006). This could be expected since an oxygen competition by PAOs and other heterotrophs in the outer layer of the biofilm is present in the HIAS-process. However, the rate is higher than what was found in the biofilm sequencing batch reactor performed by Helness (2007).

4.1.1.2. Phosphate release and anaerobic carbon consumption

Before looking further into nitrogen removal, the P-release in the anaerobic period needs to be investigated. The P-release indicates the amount of carbon taken up by PAOs, which in next turn can be utilized for denitrification. Phosphate and sCOD concentration profiles for SWB 252, SWB 429, WWB 90 and WWB 429 are shown in Figure 21. By comparing the two wastewater batches, and also by comparison of the two synthetic water batches, it can be seen directly from the figure that an initially higher sCOD concentration gives a higher PO_4 release.

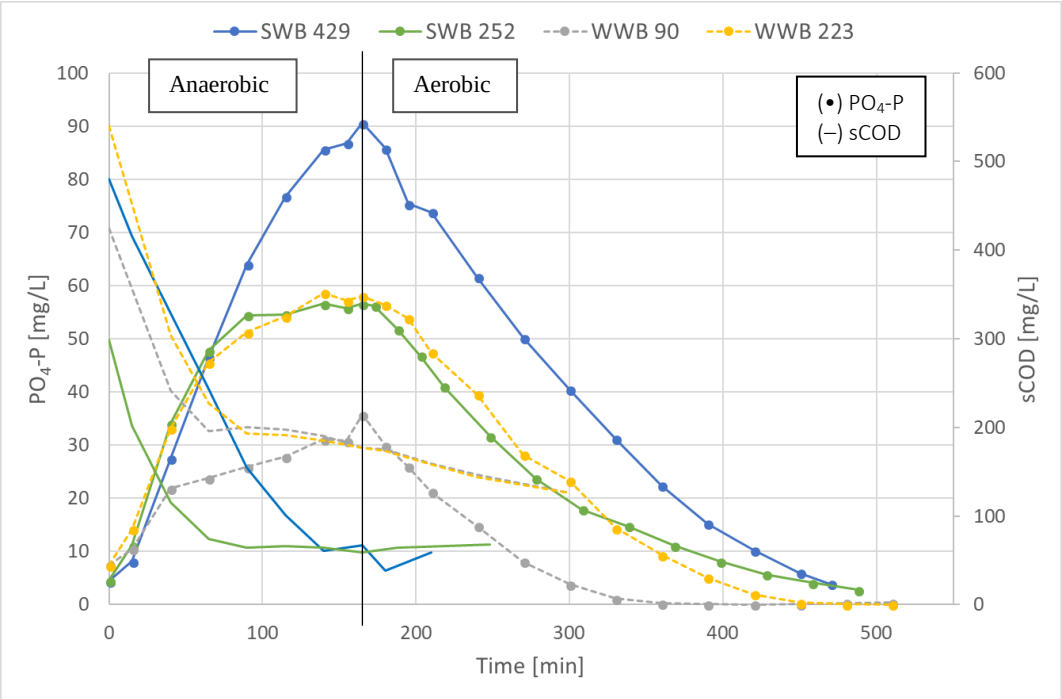

Figure 21: Phosphate (•) and sCOD (–) concentration profiles in SWB 429 (blue), SWB 252 (green), WWB 90 (dotted grey) and WWB 223 (dotted yellow).

The sCOD concentration only indicates the oxygen needed to decompose the carbon compounds and no information of the actual carbon source is therefore possible to find through this analysis. It is seen from Figure 21 that there are large differences between the sCOD concentrations within the synthetic water and the wastewater. All sCOD in the synthetic water batches are acetate, which can be used directly for P release. The sCOD in the wastewater consist of both VFA and more complex and longer carbon chains, which have to be degraded before it can be utilized by PAOs. It can also be seen that some of the carbon in the wastewater cannot be degraded during the anaerobic period, resulting in a higher sCOD loading into the aerobic period, some of which is able to be decomposed there.

The relationship between P-release and sCOD consumed in the anaerobic period of each batch is shown in Figure 22. This rate indicates how much of the consumed carbon that is used by PAOs. WWB 223 and SWB 252 does have approximately the same amount of phosphate released during the anaerobic period (Figure 21), but the rates in Figure 22 indicates a higher carbon consumption in WWB 223 to obtain this. This is the result of the different carbon compounds in the synthetic water and the wastewater. The most likely reason for the high carbon consumption in the wastewater batches, is that the hydrolytic and fermentative bacteria utilize some of the carbon for cell growth and to be able to degrade the more complex carbon chains into shorter carbon chains and VFAs.

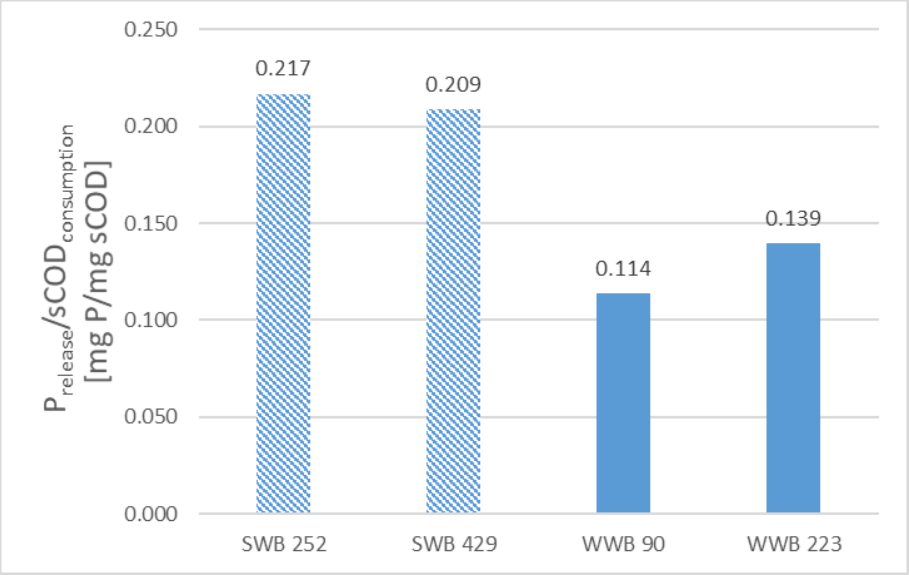

Figure 22: Phosphate released per carbon consumed. The values are calculated from the first and last values in the anaerobic zone.

$P_{released}/sCOD_{consumed}$ could also say something about the competition between PAO and GAO. Both organisms are consuming carbon anaerobically, but only PAOs can release phosphate. Literature have previously evaluated the domination of PAOs by finding the $P_{release}/VFA_{uptake}$ (Smolders et al., 1994, Filipe et al., 2001b, Schuler and Jenkins, 2002). This could be done in the synthetic water batches, since all sCOD consumed is known to be acetate. Figure 23 shows this ratio in the unity of mol P/mol C for the four synthetic batches (SWB 66, SWB 105, SWB 252 and SWB 429). Schuler and Jenkins (2002) found that rates below 0.25 mol P/mol C indicates GAO domination over PAO. The average value for the synthetic water batches (0.203 mol P/mol C) indicates that this might be the case in the pilot plant at Hias WWTP. However, the results show good PAO performance, and it is therefore not reasonable to conclude that the

biomass is dominated by GAOs.

Figure 23: The rate of phosphate released per VFA consumed, given in mol P/mol C.

Table 4: Results from amplicon sequencing[1] performed on the carriers' biomass collected in the pilot plant (January 2018).

Group of organisms	Bacterial genera	% abundance of total sequences, **sum**		Group of organisms	Bacterial genera	% abundance of total sequences, **total**	
PAO	Tetrasphaera Accumulibacter	1.7 24.7	**26.3**	Denitrifiers	Hydrogenophaga Acidovorax Comamonas Rhodoferax Simplicispira Pseudorhodobacter Leucobacter Zoogloea Flavobacterium Dechloromonas	1.6 0.0 0.0 2.6 5.3 0.1 0.4 0.0 2.2 0.3	**12.5**
Putitative PAO	*Rhodocyclaceae 12up*	*0.1* *0.3*	***0.4***				
GAO	Competibacter Propionivibrio Micropruina Spb280	0.2 9.6 0.1 0.2	**10.1**				
Nitrifiers – AOB	Nitrosomonas	0.44	**0.6**	Fermenters	Clostridia Paludibacter Tetrasphaera Trichococcus Streptococcus Vagococcus Lactococcus Rhodoferax Propionivibrio Flavebacterium	4.8 0.3 1.7 0.2 0.0 0.0 0.0 2.6 9.6 2.2	**21.4**
Nitrifiers-AOB+NOB	Nitrospira	0.00					
Nitrifiers-NOB	Nitrotoga	0.20					
Possible Anammox	*Planctomyces*	*0.0*	***0.0***				
Filamentous	Chloroflexi Candidatus Microthrix Trichoccus	1.4 0.0 0.2	**1.7**				

[1] Samples were sent to Veolia Waters' laboratory (Denmark), where DNA was extracted and processed for amplicon sequencing right after receiving the sample. The analysed sample was taken in January 2018, within the same period as the experiments in this thesis were performed. Hias IKS is responsible for the collection of these results and all rights regarding the use of these results are reserved for Hias IKS.

DNA-sequencing of the carriers' biomass (Table 4) is regularly carried out for the pilot plant at Hias (the results in Table 4 are reproduced with permission from Hias IKS). The results from the characterization of the biomass show that the abundance of PAO is 26.3%, while the abundance of GAO is 10.1% in the carrier's biomass. This underlines that there have been conditions facilitating both organisms, and that some GAO activity most likely is present. However, the abundance of the bacteria group could not determine to which degree GAOs are competing against PAOs.

Several factors could facilitate growth of GAOs, as could be seen in section 2.2.4. However, it could be difficult to determine the reason for the presence of GAO in the pilot plant at HIAS WWTP, since this is a result of long term conditions. Temperature and pH in the pilot plant's inlet water (found in pilot plant experiment, section 4.2.1) is indicating favourable conditions for PAO (section 2.2.4). This means that it may be other conditions facilitating GAOs, for example operating the plant under high oxygen levels. It might also be conditions facilitating GAOs that are not discussed in section 2.2.4, if the reason for the presence of GAOs in this plant are not detected in other plants or reactors.

4.1.1.3. Denitrification related to carbon consumption

The total amount of nitrogen remaining in the batches are found by adding all the inorganic nitrogen concentrations (NH_4-N, NO_2-N, NO_3-N) at each timestep. Figure 24 shows a decrease in the total nitrogen concentration throughout the aerobic period of the cycle, which means that

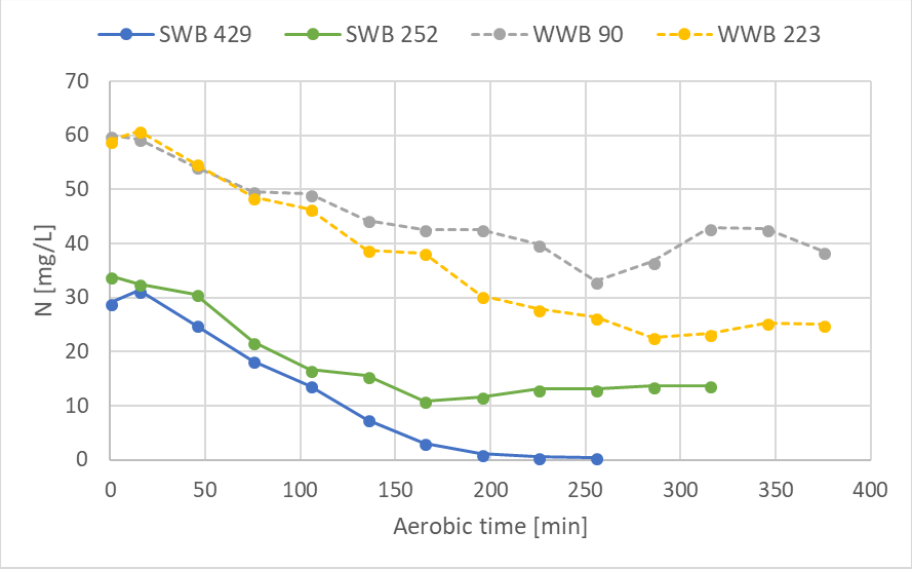

Figure 24: Total nitrogen concentration (NH_4-N + NO_2-N + NO_3-N) measured in each batch throughout the experiments.

SND has occurred. Denitrification has most likely occurred in an anoxic layer deep into the biofilm. It is clear from the figure that a higher concentration of VFA or sCOD denitrifies more, and thereby have a better nitrogen removal efficiency.

Two of the batches, SWB 252 and WWB 223, stopped denitrifying when the ammonium concentration reached approximately zero ($Time_{SWB267} \approx 165$ min, $Time_{WWB223} \approx 285$ min) (Figure 17 and Figure 24). Since an amount of NO_x is still present, this indicates that the denitrification was limited by lack of an efficient anoxic layer. It seems like when the oxygen consumption decreases in the outer layers, the oxygen is penetrating deeper into the biofilm, making the entire biofilm aerobic. Meyer et al. (2005) investigated SND and P removal in microbial aggregates and did conclude that denitrification could not occur without simultaneous nitrification towards the end of the reactor cycle. This statement was based on oxygen microsensor profiles revealing that the oxygen consumption without nitrifying activity was too low to maintain anoxic zones within the centre of the aggregates. Gibbs et al. (2004) did also discuss the importance of having ammonium present in a SND process. However, for WWB 223, both phosphate and ammonium concentration reached zero approximately at the same time. A conclusion can therefore not be drawn, on whether it was lack of an anoxic layer or lack of phosphate that is limiting the denitrification in this batch.

Figure 25 shows the denitrification rates (DN-rates) in the beginning (blue columns) and after some time (orange columns), as well as the amount of sCOD consumed through the anaerobic period (grey line). The DN-rate could be used as a measure for the removal efficiency of nitrogen when considering a given retention time. It can be seen from the figure, that the DN-

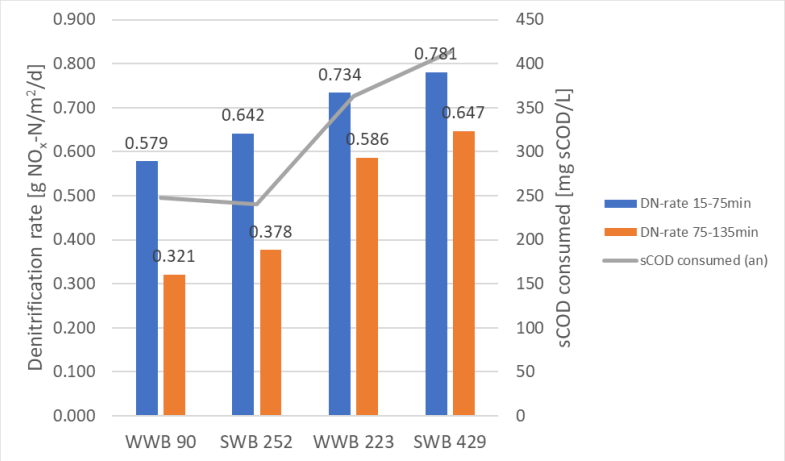

Figure 25: Average denitrification rates on time interval 15-75 min (blue) and on time interval 75-135 min (orange) in aerobic phase. sCOD consumed in the anaerobic phase are also shown (grey line).

rates are dependent on the carbon consumption. As indicated in the previous, more available carbon gives a higher DN-rate.

When evaluating the DN-rates related to the carbon consumption, it is important to remember that all measured sCOD in the synthetic water batches are acetate, while sCOD in wastewater also contains carbon in other forms.

An amount of the consumed sCOD in the anaerobic period must have been VFA that is used for denitrification in the aerobic period. However, this is based on the assumption that no carbon is consumed by ordinary denitrifiers in the aerobic period. A small amount of sCOD is actually consumed in the aerobic period in WWB 223 (15-75min: 29 mg sCOD/L, 75-135min: 18 mg sCOD/L), which may have had a slight impact on the DN-rate. On the other side, this carbon could also have been consumed by other heterotroph bacteria, making the first assumption correct. There was no carbon consumption in the aerobic period in SWB 252, which means that all denitrification occurs by use of carbon stored in the anaerobic period.

Due to carbon consumed by fermenters, not all sCOD consumed in the anaerobic period is taken up by PAOs and can thereby not be used for denitrification in the aerobic period. This could probably explain why there is a difference in DN-rates in the wastewater batch (WWB 90) and the synthetic water batch (SWB 252) where the sCOD consumption are approximately the same.

Figure 25 also shows that there is a decrease in the denitrification rate after some time. This could be explained by several factors. Depletion of ammonium could decrease the DN-rate, even though large amounts of NO_x are still present, as discussed in the previous. Two of the DN-rates found between aerobic time 75-135min in Figure 25 (SWB 252 and SWB 429) may have had some effect of this since the ammonium concentration at time 135min was 7.2 and 4.3 mg NH_4-N/L for SWB 252 and SWB 429, respectively. Regarding literature, these values should not influence the nitrification and thereby not the oxygen consumption, but since results shown in Figure 18 indicates concentration limited nitrification below ammonium concentrations of approximately 10-15 mg/L, this should be considered as a possible explanation.

Depletion of other oxygen consuming parameters, such as sCOD, could also be decreasing the DN-rate. Ordinary heterotroph activity could create oxygen competition and to some extent influence the P-uptake rate and N-rate. After some time, when sCOD decreases or is depleted,

more oxygen is free to be transferred deeper into the biofilm, resulting in less anoxic layers for denitrification.

If all denitrification is done by PAOs, depletion of phosphate will decrease the DN-rate. None of the batches in Figure 25 had full depletion of phosphate in the examined time intervals, but WWB 90 did only have 3.86 mg PO_4-P/L at aerobic time 135 min. In this batch, it could be reasonable to believe that the DN-rate after some time was somewhat affected by the low phosphate concentration.

Since denitrification is a result of phosphate uptake by PAOs, the DN-rate is related to the phosphate uptake rate. Figure 26 shows the P-uptake rates for the same time intervals as the

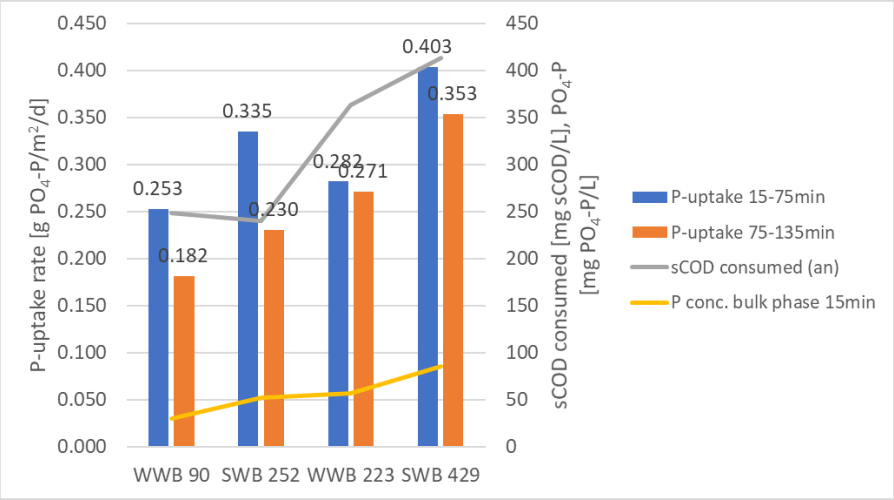

Figure 26: Phosphate uptake rate on time interval 15-75 min (blue) and on time interval 75-135 min (orange) in aerobic phase. sCOD consumed in the anaerobic phase are also shown (grey line). PO_4-P concentration after 15 min in aerobic zone (yellow line).

calculated DN-rates in Figure 25. The P-uptake rate shows the similar trend as the DN-rate, with high P-uptake rate at high carbon consumption in the anaerobic period, and opposite for low carbon consumption. It is also seen that the P-uptake rates decreases after some time. It is reasonable to believe that all the batches are affected by this in addition to an eventual influence by depletion of ammonium, phosphate or sCOD.

The DN-rates in WWB 223 (Figure 25) is not affected by too low NH_4 or PO_4 concentrations (>34 mg NH_4-N/L, >23.2 mg PO_4-P/L). Based on the other batches, the P-uptake rate could have affected the DN-rate, but this is not what is shown for WWB 223 in Figure 26. It can be seen from the phosphate concentration profiles in Figure 27 that the PAO activity in WWB 223

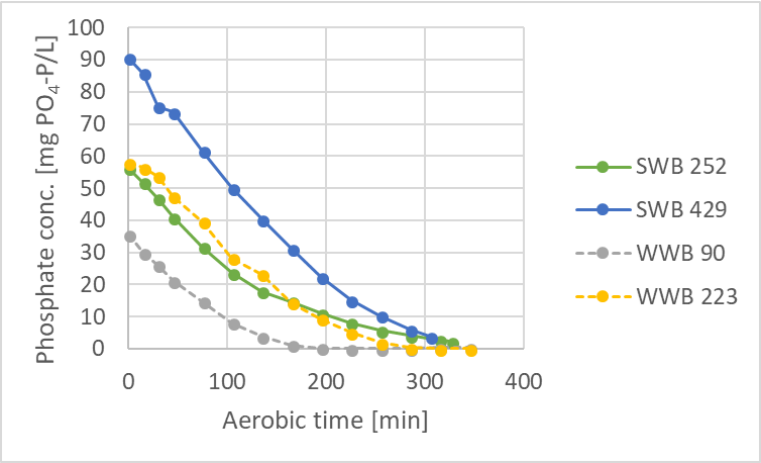

Figure 27: Phosphate concentration profiles in aerobic period.

is inhibited by something in the beginning of the aerobic period. However, the DN-rate for the first time interval in Figure 25 for the same batch does not show any inhibition. When looking at WWB 223 independently, the graphs could for example indicate some ordinary denitrifying activity due to an amount of sCOD entering the aerobic period. It could also be speculated whether denitrifying GAOs are responsible for some of the initial denitrification. However, if some of these theories are correct, it could be expected a similar behaviour in the other batches since the bacteria culture and operating conditions are the same. The only difference between WWB 223 and WWB 90 is the amount of carbon consumed in the anaerobic period, which creates a higher PO_4 concentration in the beginning of the aerobic period. The amount of sCOD entering the aerobic phase is the same in the two batches and a potential influence of the factors discussed above should have been the same in both batches. Due to this inconsistency, a conclusion should not be drawn for WWB 223 alone. However, since a strong relationship between the P-uptake rate and the DN-rate is seen for the three other batches, DN-PAOs are believed to be responsible for the denitrification.

Based on the previous findings (section 4.1.1.2), GAO is believed to be responsible for some of the carbon consumption in the anaerobic period. Therefore, considerations should be done whether DN-GAOs are participating in some of the denitrification.

4.1.1.4. Additional comments related to assimilation of ammonium

When analysing the results, it was seen that an amount of ammonium was removed during the anaerobic period. Several theories could be describing the phenomenon, where one of them could be oxygen transfer within the biofilm from the aerobic phase to the anaerobic phase.

Thereby could some ordinary heterotroph activity occur and a need for ammonium assimilation is therefore present. Due to competition between heterotrophs and nitrifyers (section 4.1.1.2), nitrifying activity is not very likely. Growth of fermenters and other organisms operating anaerobically would also require some ammonium.

Another option is the occurrence of anammox, where ammonium and nitrite are used to produce N_2-gas. However, nitrite is not present in the inlet water and this have to be transferred in the biofilm from the aerobic phase. In addition, higher temperature than what is measured within the batches are required for this to occur, which makes this suggestion not very likely.

The last possibility is anaerobic ammonium uptake and storage by PAOs (or GAOs), which then would be used for growth of biomass in the aerobic phase. Literature is not found whether ammonium for growth of PAOs are taken up under anaerobic or aerobic conditions. Based on the results, questions are raised whether anaerobically ammonium uptake by PAOs could occur due to the relatively large amount of ammonium removed in the anaerobic phase. The anaerobic removal has been observed to be between 5 and 10 mg NH_4-N/L for an anaerobic retention time of 100 minutes.

Since heterotroph activity are assumed to be minimal in the aerobic phase, at least in the synthetic water batches, where no carbon is consumed aerobically, the ammonium assimilation by OHOs would therefore be minimal. However, ammonium assimilation by PAOs could occur if this is where PAOs consume ammonium for growth.

As stated above, many uncertainties are present regarding the ammonium assimilation, making the calculation of ammonium used for growth quite difficult. All ammonium removed in the aerobic period in all experiments within this thesis has therefore been treated as it was nitrified, meaning that it is considered as nitrate/nitrite. However, this is probably not correct, since some of the ammonium would have been used for growth either by heterotrophs or PAOs (GAOs), and could be responsible for some errors in the results.

4.1.2. Anoxic P-uptake and denitrification

An overview of the batch experiments conducted to investigate the anoxic phosphate uptake and the corresponding denitrification is shown in Table 5.

Table 5: NO_x as electron acceptor for P-uptake – batch experiments, overview and operational conditions. All values are measured unless marked. Calculated VFA concentration from sCOD analysis (*).

Batch	Type of water	HRT anaerobic [min]	HRT anoxic/ HRT aerobic [min]	VFA/sCOD (start)	NH4-N start	PO4-P start	DO [mg/L]	Volume container	Volume water	NOx addition
SWB NO2a	synthetic	100	295/	218*/259	0	4.36	0	1 L	0.85 L	NO2
SWB NO3a	synthetic	100	295/	221*/262	0	4.40	0	1 L	0.85 L	NO3
SWB NO2b	synthetic	100	493/	221*/262	0	4.06	0	1 L	0.85 L	NO2
SWB NO3b	synthetic	100	493/	231*/272	0	4.20	0	1 L	0.85 L	NO3
SWB O2	synthetic	100	/255	215*/257	0	4.12	ca. 8-9	1 L	0.85 L	-
SWB NO3 → O2	synthetic	100	90/165	220*/261	0	4.18	0 → ca.8-9	1 L	0.85 L	NO3

The difference between nitrate and nitrite as electron acceptor could be examined when the conditions for all the batches were similar and thereby had a similar phosphate concentration entering the anoxic phase.

Nitrite analysis were taken at some points during the anoxic period in SWB NO3b, since nitrate could be denitrified to nitrite before it is completely denitrified to nitrogen gas. The analysis showed that the amounts of nitrite were very small (< 0.17 mg NO_2-N/L) compared to the amounts of nitrate (5 - 34.4 mg NO_3-N/L). The nitrate batches were therefore treated as no nitrite accumulation were present.

4.1.2.1. Denitrifying phosphate uptake capacity

Figure 28 shows that both nitrite and nitrate as well as oxygen could be used as electron acceptor to some extent. The phosphate uptake stops in the nitrite batches (Figure 28d and Figure 28f) after some time, while phosphate uptake in the nitrate batches (Figure 28c and Figure 28e) are still present. However, the phosphate uptake in the nitrate batches are much lower than the phosphate uptake in the aerobic batch (Figure 28a) using only oxygen as electron acceptor.

Neither of the nitrite/nitrate batches (SWB NO2a, SWB NO3a, SWB NO2b, SWB NO3b) were able to reach a luxury uptake of P within the given anoxic retention time. However, when the nitrate batch in Figure 28b gets aerated, the phosphate is fully depleted. It is seen that the phosphate uptake increases after aeration compared to the batches where the only electron acceptor is nitrate. Kerrn-Jespersen and Henze (1993) indicates that such behaviour may be due to different types of PAO responsible for the uptake. PAOs that can only use nitrate as electron

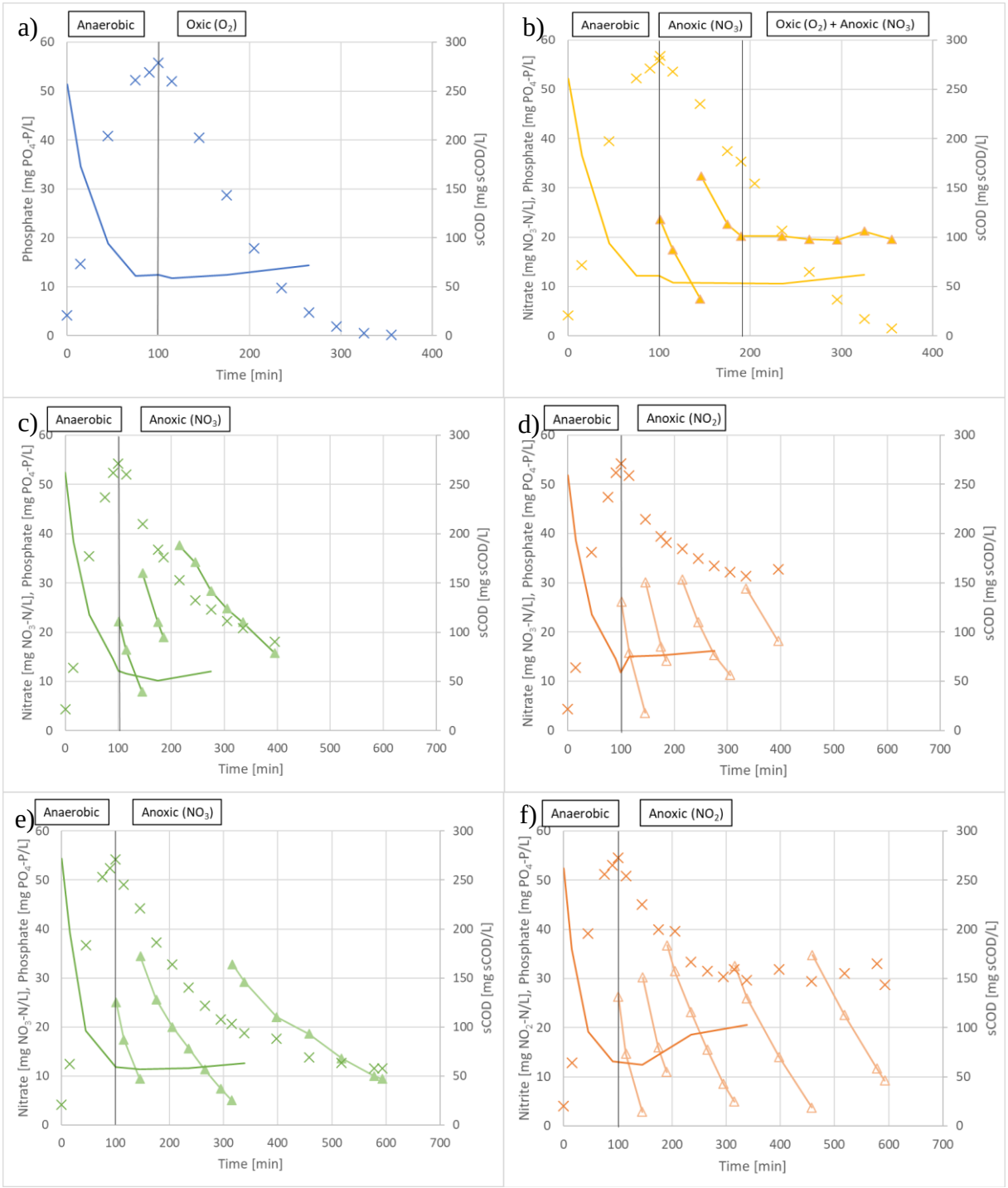

Figure 28: Phosphate concentration profiles (cross), nitrite concentration profiles (unfilled triangles), nitrate concentration profiles (filled triangles) and sCOD concentration profiles (line). a) SWB O2, b) SWB NO3 → O2, c) SWB NO3a, d) SWB NO2a, e) SWB NO3b, f) SWB NO2b.

acceptor is starting to empty their storage of PHB, so that when oxygen is present, another group of PAO that can use DO in addition to nitrate does still have PHB reserves and may therefore be responsible for the increased P uptake rate. Figure 28b does also show that the denitrification stops when the container is getting aerated, which may indicate that PAOs able

to denitrify switches from using nitrate as electron acceptor to using oxygen, which is more available for the organisms, due to the need for reduction of the nitrate.

The phosphate uptake rate in the beginning of the aerobic period (first 75 minutes) are shown in Figure 29. The figure shows that the phosphate uptake rate was higher for the aerobic batch (SWB O2) than for all the other batches containing nitrate/nitrite. Similar results are also found in several studies, for example Kerrn-Jespersen and Henze (1993) and Zhou et al. (2010). The phosphate uptake rate is also slightly higher when nitrate is used compared to nitrite, which corresponds to previous findings (Ahn et al., 2001, Zhou et al., 2010).

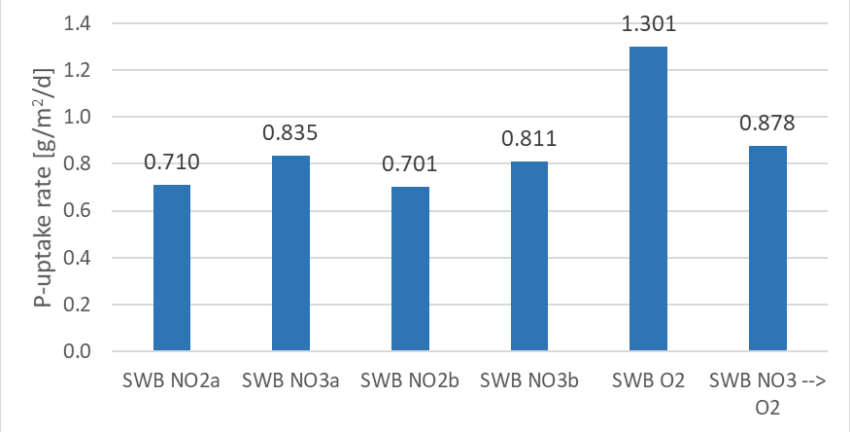

Figure 29: The average P uptake rate of the first 75 minutes of the anoxic/aerobic period.

Since more oxygen can be utilized by reduction of nitrate than reduction of nitrite, a nitrate equivalent may be used to compare the two electron acceptors. The conversion factor is found from Rusten et al. (1995a): 1 mg NO_2-N/L = 0.60 mg NO_3-N/L.

The average ratio of phosphate uptake per nitrate equivalent removed (PO_4-P /NO_3-N_{eq}) are found for three different anoxic retention times (Figure 30). It is seen from Figure 30 that this ratio is quite similar for all the nitrate/nitrite batches within the first 75 minutes of the anoxic period. This shows a similar denitrifying capacity when nitrite is used as electron acceptor compared to nitrate, even though the phosphate uptake is lower for the batches utilizing nitrite, seen in Figure 29. This shows that more nitrogen can be removed per phosphate uptake when nitrite is used as electron acceptor, indicating that conditions facilitating partial nitrification (section 2.1.3) is preferable with respect to nitrogen removal.

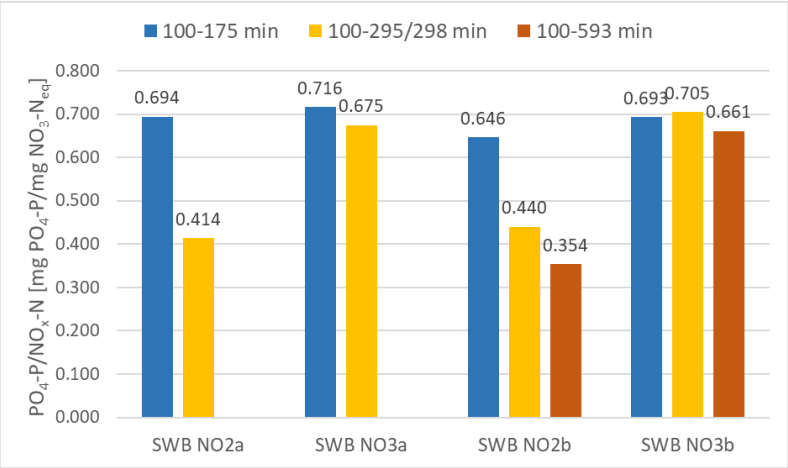

Figure 30: Average ratios of phosphate uptake per nitrate equivalent removed (PO_4-P/NO_3-N_{eq}) for three different time intervals.

The PO_4-P/NO_3-N_{eq} ratio for the nitrate batches shows that the relationship between the denitrification and the phosphate uptake does not have any rapid changes through the anoxic period. The ratio does not say anything about the actual P uptake or N removal rate, and it is seen in Figure 31 that both P uptake and N removal are decreasing for the nitrate batches, resulting in a similar PO_4-P/NO_3-N_{eq} rate throughout the experiment. However, a small decrease in the P/N ratio is seen, indicating that more nitrate is removed per phosphate uptake.

After some time, the nitrite batches showed decreasing PO_4-P /NO_3-N_{eq} ratio (Figure 30), which means that a higher nitrite removal occurs per phosphate removed. This could also be seen in Figure 31, where the accumulated amount of nitrite increases, and the phosphate uptake have stopped. This means that some other bacteria which does not take up phosphate are responsible for the excess amount of nitrite. The carbon content into the anoxic zone are very small (< 21 mg VFA/L), and ordinary denitrifyers could not have denitrified all the excess nitrite by this amount of carbon. The behaviour might be explained by denitrification by GAOs, since the results indicates that carbon for denitrification has to be stored from uptake in the anaerobic period. However, when looking further into the results, it seems more likely that some degradation of the biomass has occurred. It is seen that the carbon content is increasing through the anoxic period (Figure 28f) and in addition, a more turbid water was visually observed for the nitrite batch (SWB NO2b) compared to the nitrate batch (SWB NO3b) (Figure 32). This could indicate that the added doses of nitrite were too high, resulting in toxicity issues, which is also found in literature (section 2.2.2).

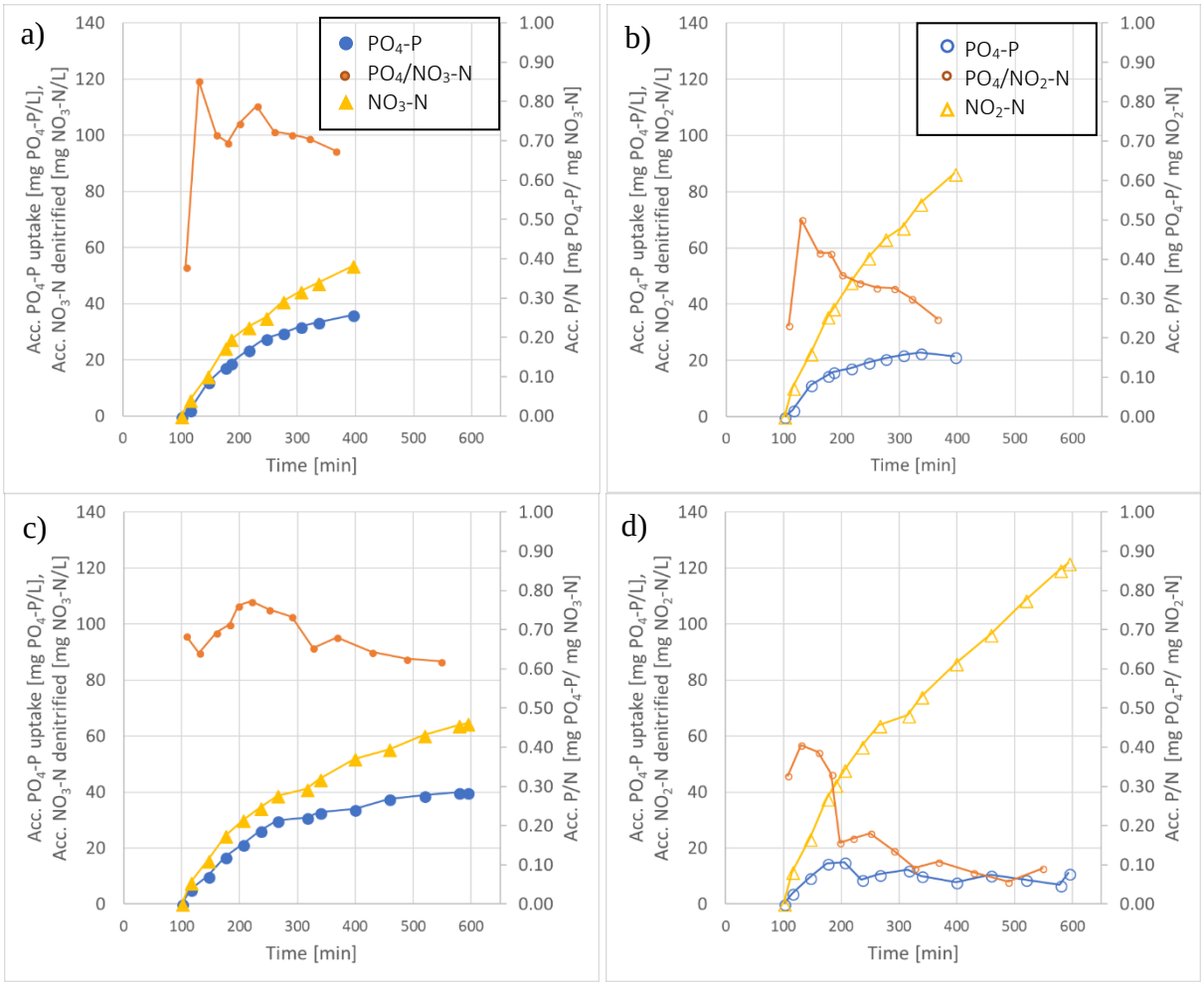

Figure 31: Sum of the amount of nitrate (a and c) and nitrite (b and d) denitrified. Sum of the amount of phosphate taken up. PO_4-P/NO_x-N rate of the accumulated values of phosphate and nitrite/nitrate. a = SWB NO3a, b = SWB NO2a, c = SWB NO3b, d = SWB NO2b.

It is also seen from Figure 31 that there were some differences in the phosphate uptake between the two nitrite batches (SWB NO2a and SWB NO2b). The reason for this might be explained by the specific nitrite dosage that were added at time 190 minutes in SWB NO2b, which gave a nitrite concentration in the liquid phase of 36.8 mg NO_2-N/L and at the same time the phosphate uptake stopped completely. This is underlining the theory about toxic nitrite concentrations which inhibits further phosphate uptake.

If carbon could be present in the anoxic period due to degradation of the biomass, ordinary denitrification could be the reason for the high denitrification rate after phosphate uptake has stopped. It is therefore difficult to examine whether DN-GAO had some contribution to the denitrification.

Figure 32: Difference in turbidity in the liquid phase after nitrite (left) and nitrate (right) batch experiments.

The phosphate uptake in SWB NO2b stops around 175 minutes (Figure 31d), meaning that the excess amount of nitrite after this time could be done by ordinary denitrifyers. The amount of COD needed to denitrify this amount of nitrite (84 mg NO_2-N/L) is estimated to be 263 mg rbCOD/L (readily biodegradable COD) by using equation (7-128) in Burton et al. (2013) and a synthesis yield of 0.32 g VSS/g COD (Muller et al., 2003, Burton et al., 2013)[2]. This is a relatively large amount of carbon compared to the amount of carbon transferred into the anoxic zone. However, the increasing sCOD concentration profile shown in Figure 28f) indicates that the required amount of carbon could have been produced by degradation of biomass in this phase.

4.1.2.2. Limitations

It could be speculated in how representative these experiments are compared to the actual HIAS-reactor. In the reactor, nitrate and nitrite is produced within the biofilm, while in these batch experiments, NO_x needs to diffuse into the biofilm. This could most likely create a NO_x profile in the biofilm, which may have the smallest amount of NO_x in the inner layers, while the largest amount is in the outer layers. This could be different of what occurs in the reactor, where nitrite and nitrate are produced within the biofilm and the concentration might be more constant or largest in the area where it is produced.

[2] This synthesis yield is found for nitrate (not nitrite) as electron acceptor. This could make an error in the calculation. However, the yield is believed to give a better estimate than using the yield for oxygen as electron acceptor.

The concentrations added was most likely much higher than what is reasonable to be accumulated in the liquid phase in a real wastewater reactor. At least, accumulation of nitrite happens over time, not added in shock concentrations.

4.1.3. SND influenced by dissolved oxygen concentration

An overview of the batch experiments performed to investigate the effect of dissolved oxygen on the nitrogen removal (hereafter referred to as the DO experiments) is shown in Table 6.

Table 6: Nitrogen removal influenced by DO – batch experiments, overview and operational conditions. All values are measured unless marked. Calculated VFA concentration from sCOD analysis (*). Measured VFA in inlet water + a known amount of acetate added during the first 30 seconds of anaerobic operation time (**).

Batch	Type of water	HRT anaerobic [min]	HRT low DO/ HRT high DO [min]	VFA/sCOD (start)	NH4-N start	PO4-P start	DO [mg/L]	Volume container	Volume water	Temp
SWB DO(L)	synthetic	100	428/	217*/261	44	4.78	3.5-5.4	3 L	2.35 L	ca.18°C
SWB DO(H)	synthetic	100	/428	213*/257	49	5.20	7.4-8.8	3 L	2.35 L	ca.17°C
WWB DO(L)	wastewater	100	415/	/406	71	7.72	2.1-5.2	3 L	2.35 L	ca.15°C
WWB DO(H)	wastewater	100	/345	/413	73	7.34	6.9-8.3	3 L	2.35 L	ca.15°C
SWB DO(HL) 1	synthetic	100	195/180	248*/293	30	4.44	8.1-8.5/ 6.2-6.5	3 L	2.35 L	ca.17°C
SWB DO(HL) 2	synthetic	100	335/105	227*/271	45	4.46	8.0-8.6/ 3.9-6.3	3 L	2.35 L	ca.17°C
WWB DO(HL) 3	wastewater	100	285/120	111/472	64	7.70	7.3-8.2/ 5.4-3.6	3 L	2.35 L	ca.16°C
WWB DO(HL) 4	wastewater	100	240/165	174**/537	70	7.70	7.4-8.4/ 4.3-4.9	3 L	2.35 L	ca.16°C

4.1.3.1. Nitrification influenced by DO

Ammonium concentration profiles for all DO experiments are shown in Figure 33. Both the synthetic water experiments and the wastewater experiments in Figure 33a) shows that a larger amount of ammonium is nitrified under a higher DO level.

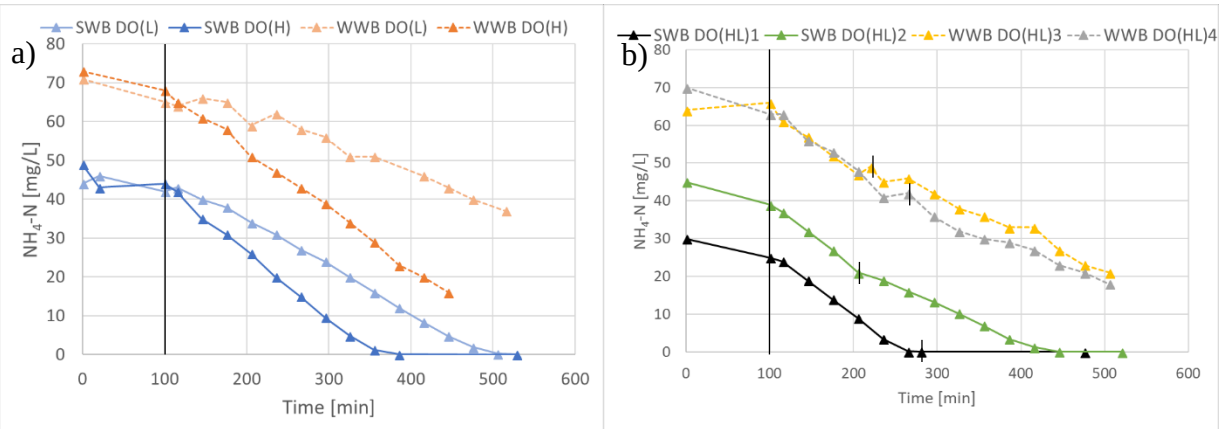

Figure 33: Ammonium concentration profiles for all DO experiments. Anaerobic conditions until time 100min (first vertical line). a) constant aeration through the whole aerobic period. b) aeration is lowered at the short vertical lines.

Figure 34 shows ammonium concentration limited nitrification below approximately 12 mg NH_4-N/L for SWB DO(H) and below approximately 8 mg NH_4-N/L for SWB DO(L). This means that lower oxygen concentration has a shorter concentration limited region, which corresponds well to literature (Ødegaard, 2006). The corresponding trend for the wastewater batches are not included since these batches are not operated until depletion of ammonium.

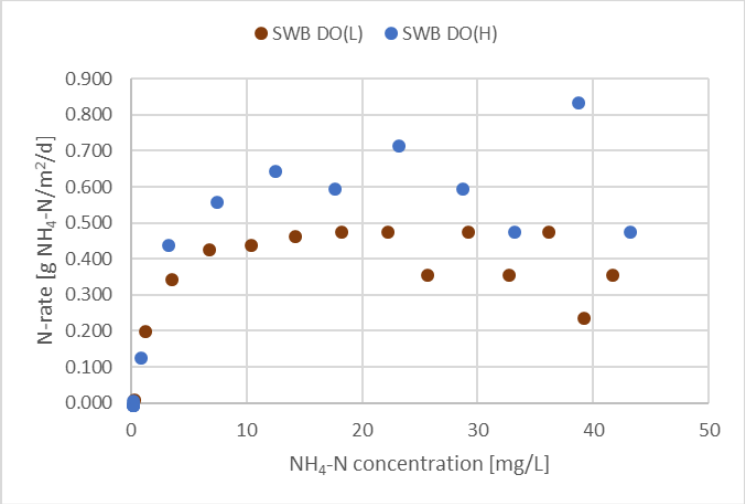

Figure 34: Ammonium concentration vs. nitrification rate for the two synthetic batches having constant aeration.

The dependency between the oxygen concentration and the nitrification rate is shown in Figure 35. The graph shows that the N-rate increases with DO concentration, which corresponds well with literature (Figure 2a presented in section 2.1.1 (Ødegaard, 2006)). Figure 2a) does also

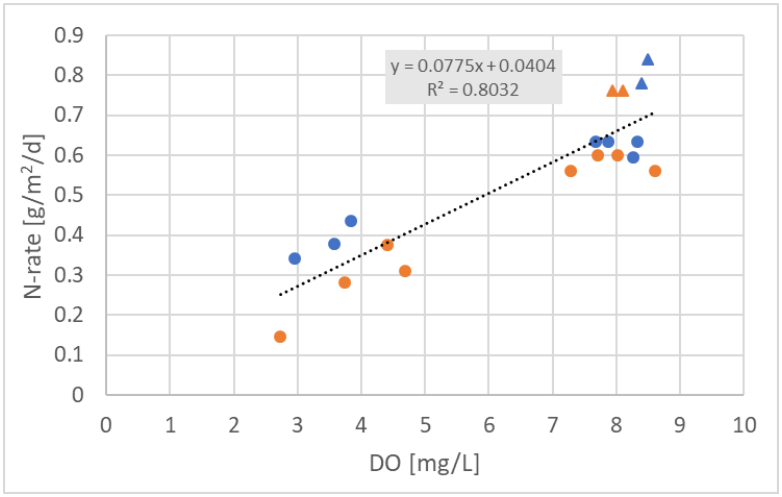

Figure 35: Nitrification rate dependent on the dissolved oxygen concentration for the DO experiments and the carbon experiments (discussed in section 4.1.1). Wastewater batches – red, synthetic water batches – blue, DO experiments – circles, carbon experiments – triangles.

show that higher contents of BOD gives lower nitrification rates. This might be the explanation to the differences between the synthetic water experiments (blue points) and the wastewater experiments (red points) since a small amount of carbon is seen removed in the aerobic phase in all the batch experiments performed with wastewater.

The temperature varied between 14.8°C and 18.0°C in the experiments plotted in Figure 35, which most likely may have influenced the N-rate and the spread in the results may be explained by this. Temperature is found to have large influence on the biological processes in the system in section 4.1.4.

The four points with the highest nitrification rates (triangles in Figure 35) are found for the four carbon experiments (SWB 252, SWB 429, WWB 90 and WWB 223). The high N-rate may indicate that some conditions are somewhat different between the DO experiments and the carbon experiments.

4.1.3.2. Denitrification influenced by DO

Constant aeration level throughout the aerobic phase

The four batch experiments conducted with a constant high or low aeration (SWB DO(L), SWB DO(H), WWB DO(L), WWB DO(H)) show that the oxygen concentration (Figure 40) is influencing the total nitrogen removal (Figure 36). It is seen from the experiments that accumulation of NO_x is occurring when phosphate is depleting (Figure 36a, b and d). This is especially seen in SWB DO(H), where NO_x begins to accumulate at the time when the phosphate uptake starts to decline, seen from the NO_x-N and PO_4-P concentration curves in Figure 36b. Since the NO_x concentration stabilizes around 12 mg/L in this experiment, it is seen that a higher total nitrogen removal could occur under lower aeration when comparing SWB DO(L) and SWB DO(H).

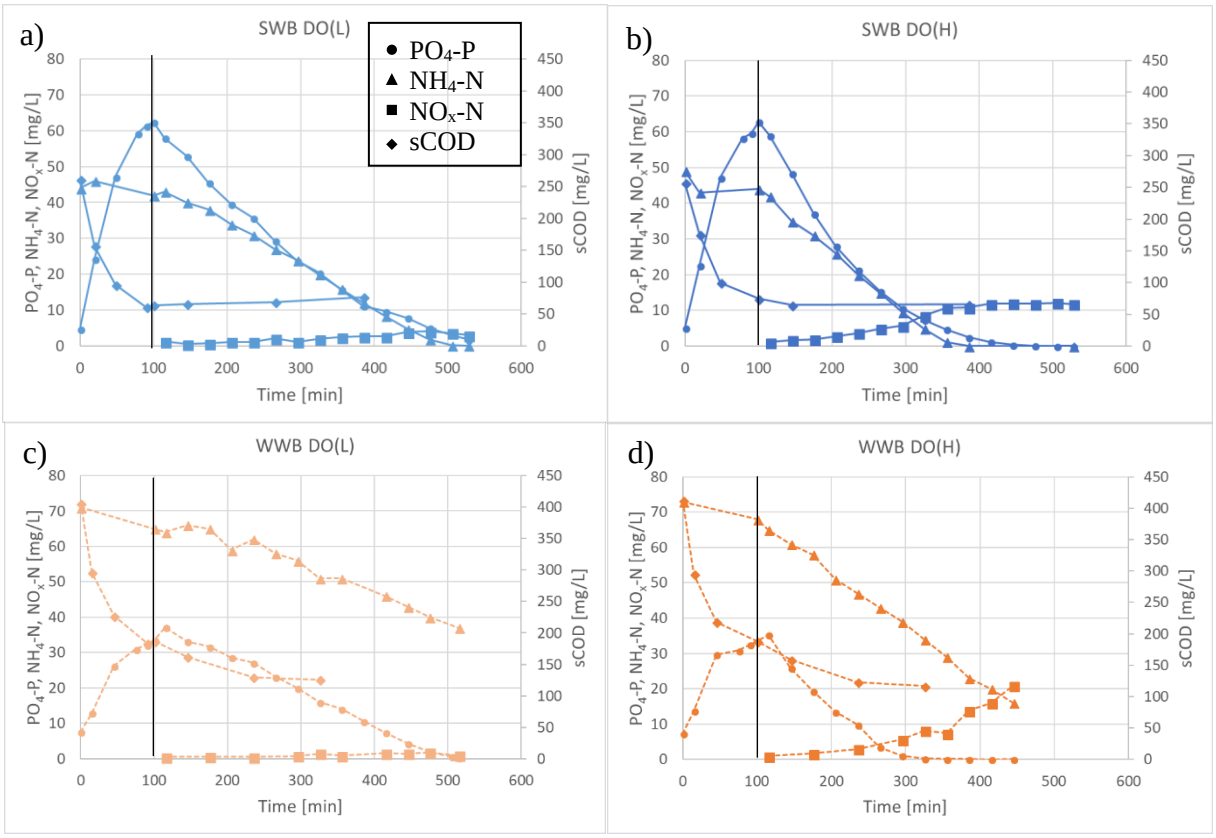

Figure 36: Ammonium, NO_x, sCOD and phosphate concentration profiles for the batches having constant low (a and c) and high aeration (b and d).

When no carbon is left in the aerobic phase, denitrification by ordinary denitrifyers could not occur, meaning that the remaining amount of NO_x after depletion of phosphate could be difficult to remove since the only responsible denitrifyer is PAO, unless some GAO that are able to denitrify is present in the system.

The wastewater batches (WWB DO(L), WWB DO(H)) show approximately the same total nitrogen removal under high and low DO concentrations. However, it is seen that the aerobic retention time determines whether the excess nitrogen is present as ammonium or nitrate/nitrite.

Both batches operated with low aeration (SWB DO(L) and WWB DO(L)) showed lower phosphate uptake rates than the high aerated batches (Figure 37). The difference in P uptake rate between the synthetic water batches and the wastewater bathes could be explained by the carbon consumption during the anaerobic period. The P uptake rate is previously found to be lower for lower carbon consumption during the anaerobic phase (section 4.1.1.3). The amount of P released gives an indication on the amount of VFA consumed, which can be seen to be higher in the synthetic water batches (SWB DO(L), SWB DO(H)) than the wastewater batches

(WWB DO(L), WWB DO(H)). Figure 37 does underline the previous findings, by showing lower P uptake rates for the wastewater batches, which have lower carbon consumption than the synthetic water batches.

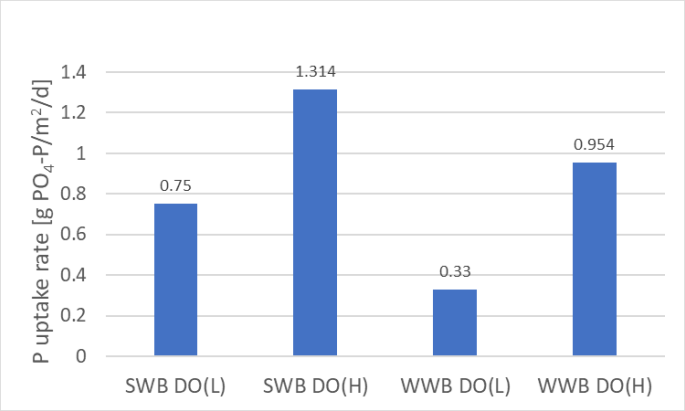

Figure 37: Average phosphate uptake rate for aerobic retention time 15 – 75 min (time 115-175min).

The total removal of nitrogen is also highly related to the amount of nitrified ammonium, which was seen in the previous subchapter to be higher under higher DO concentration. Figure 38 shows a clear difference in the denitrification rates under high and low aeration. It is seen that when higher amounts of NO_x are produced due to higher nitrification rate in the beginning of the aerobic period, the denitrification is also higher (SWB DO(H)). However, after a while in the high aerated zone, the denitrification rate decreases. This might be explained by PAOs' phosphate uptake capacity, since the P uptake rate decreases through the aerated period. It could also be explained by the high aeration, which might decrease the anoxic layer in the biofilm after some time due to less oxygen consumption by PAOs. However, Figure 39 shows that the relationship between the phosphate uptake and denitrification does not change in the last time

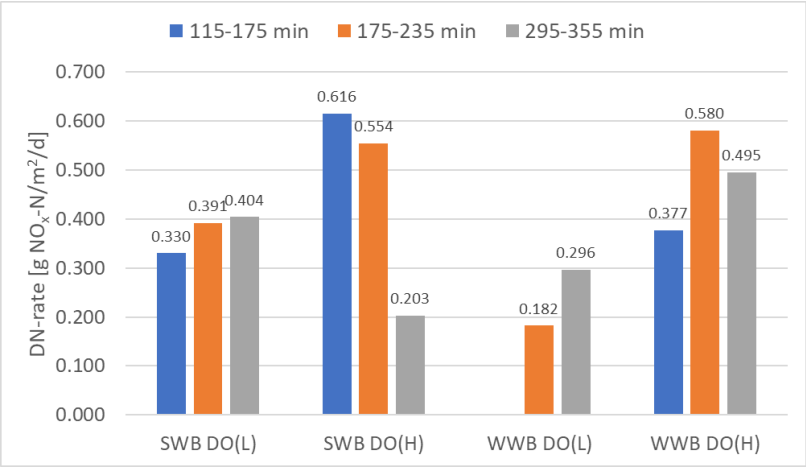

Figure 38: Denitrification rates in aerobic time intervals.

intervals calculated in SWB DO(H), indicating that this is not what occurs in the last part of the period.

The denitrification in the first time interval of WWB DO(H) shows a lower value than expected compared to SWB DO(H) (Figure 38), based on the trend in denitrification rates in SWB DO(H). This might be explained by lower nitrification rate do to a slightly lower DO concentration in this batch. It might also be due to some measurement uncertainties in the measured ammonium value at time 175 min, which seems to be slightly too high (Figure 36d).

It is seen that the denitrification rate increases in the low aerated batches (SWB DO(L), WWB DO(L))(Figure 38). This may be mostly related to the low nitrification rate under low aeration. WWB DO(L) has no nitrification in the time interval 115-175 min, resulting in no denitrification in the same period. The low nitrification rates result in low concentrations of nitrate/nitrite and Figure 39 shows that more phosphate is taken up by use of oxygen as electron acceptor rather than nitrate/nitrite in the beginning of the aerated period compared to later in the period.

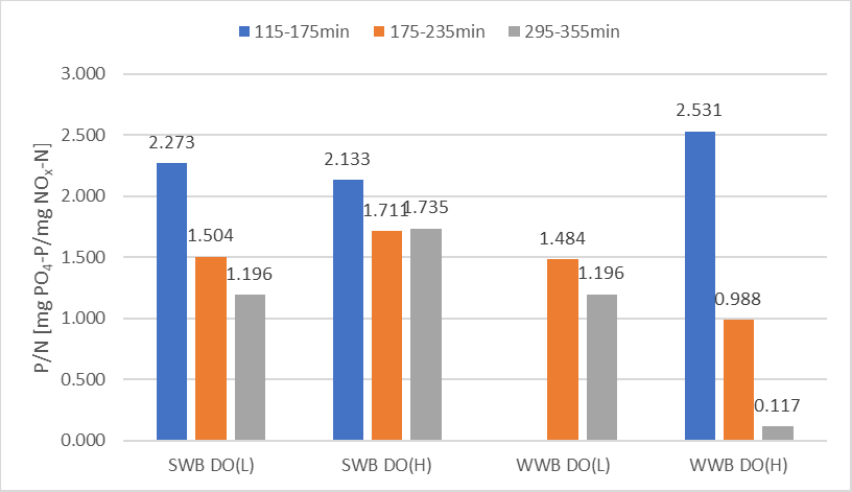

Figure 39: Amount of phosphate uptake per nitrogen removed in different time intervals.

Figure 39 does also indicate that the anoxic layer is thicker in the low aerated batches, which corresponds well with literature (Helness, 2007), since the phosphate uptake per nitrogen removed ratio is lower after some time in SWB DO(L) than SWB DO(H). The value for the P/N-ratio for time interval 295-355min is highly affected by the depletion of phosphate. It is seen from Figure 38 that the DN-rate in the same interval is quite high even though the phosphate is depleting around this time, indicating that it might be some delay in the transport of substances in the biofilm due to diffusion, or a result of some denitrification done by GAOs.

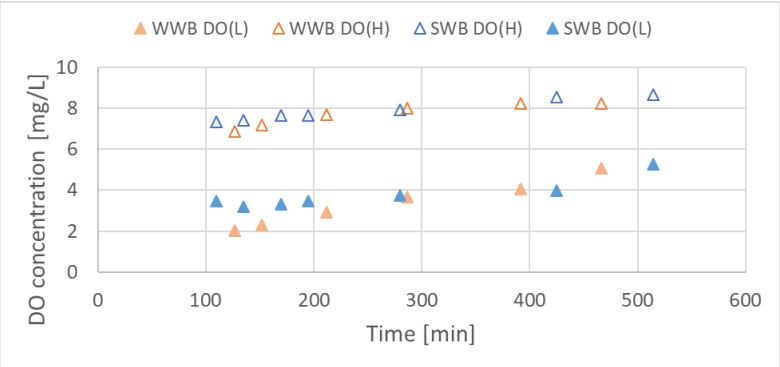

Figure 40: Dissolved oxygen concentration profiles for the constant aerated batch experiments.

Lowered aeration level after some time

It is seen in the previous that higher nitrification rates give initially higher denitrification rates (Figure 38), which indicates that the denitrification capacity is dependent on the production of NO_x by nitrification. However, the denitrification rate decreases quite fast under these conditions (high aeration), indicating a decreasing anoxic layer in the biofilm. This gives reasons to believe that having initially high aeration that facilitates high nitrification, which gives high production of NO_x, and thereafter lowering the aeration to maintain an anoxic layer, could give better nitrogen removal.

The synthetic water experiment shown in Figure 41b, shows that when the aeration is lowered, both phosphate uptake rate and nitrification rate decreases, which could be expected (Ødegaard, 2006). It is also seen that the NO_x concentration is low during the entire aerobic period, and when ammonium is depleted, the remaining NO_x concentration is removed. Comparison with the synthetic batch experiment having high aeration through the entire aerobic period (Figure 42a) shows that lowering the aeration gives better nitrogen removal. This effect occurs most likely due to maintenance of an anoxic layer, which could be expected, since lower oxygen diffusion is controlled by the oxygen concentration in the liquid phase (oxygen concentration profiles in Figure 45).

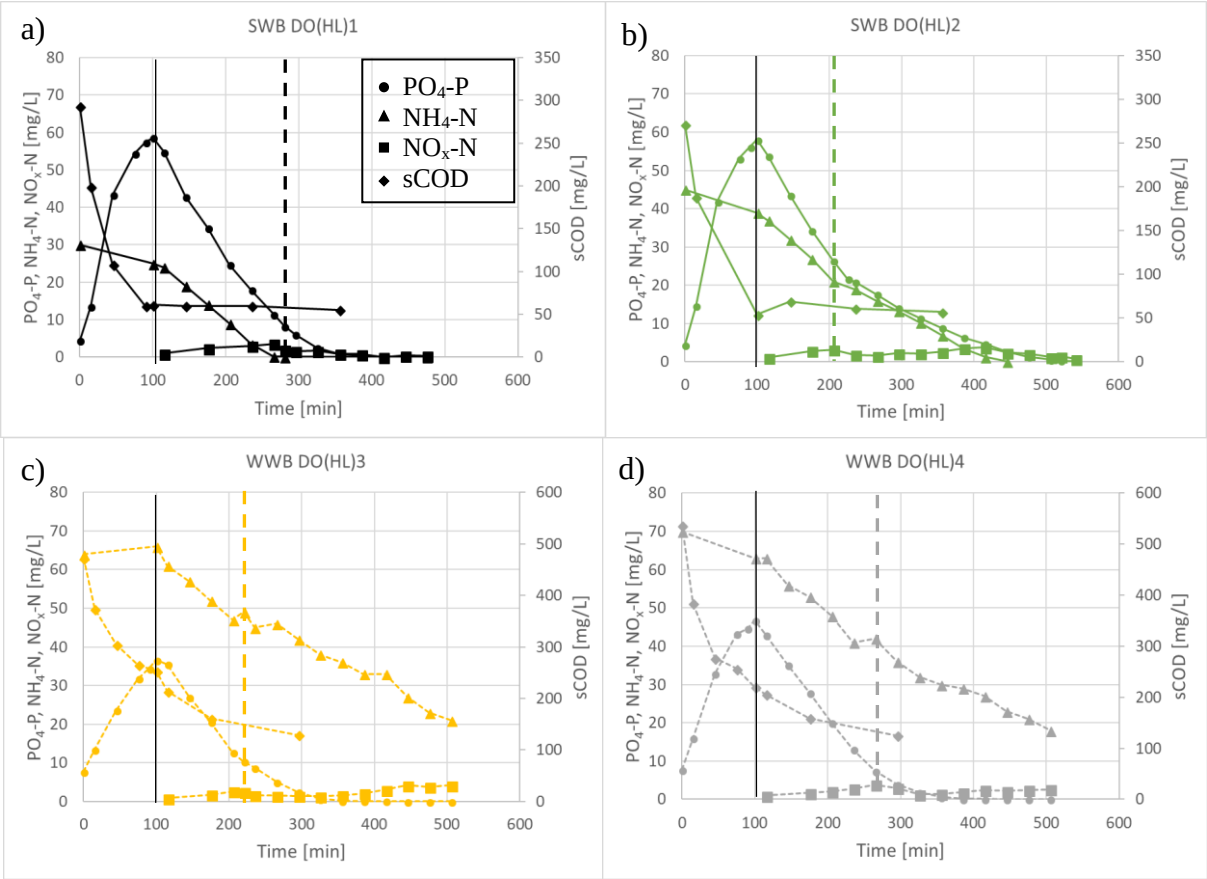

Figure 41: Ammonium, NO_x, sCOD and phosphate concentration profiles for all the batches having lowered aeration. Anaerobic conditions until black vertical line at 100 minutes. Lowered aeration after dotted coloured line at 280 minutes (a), 205 minutes (b), 220 minutes (c) and 265 minutes (d).

The wastewater batches with lowered aeration (Figure 41c and Figure 41d) show that denitrification is still occurring after depletion of phosphate, since ammonium is nitrified without accumulation of NO_x. This behaviour might be explained by some activity by ordinary denitrifyers, since an amount of carbon is still present and slightly decreasing. However, it was seen in section 4.1.1.2, that GAOs are present in the biomass, which means that the denitrification also could have been performed by GAOs that are able to denitrify. If this is the case, it could be beneficial to have GAOs in the system, since this can contribute to better nitrogen removal when the phosphate uptake is completed. However, this should be further investigated to determine if the long-term effects of such behaviour by GAOs is influencing PAO activity, so that P removal efficiency decreases.

Regardless of which organism that is responsible for the denitrification, the anoxic layer is seen to be very important, and controlling the aeration is therefore of great importance.

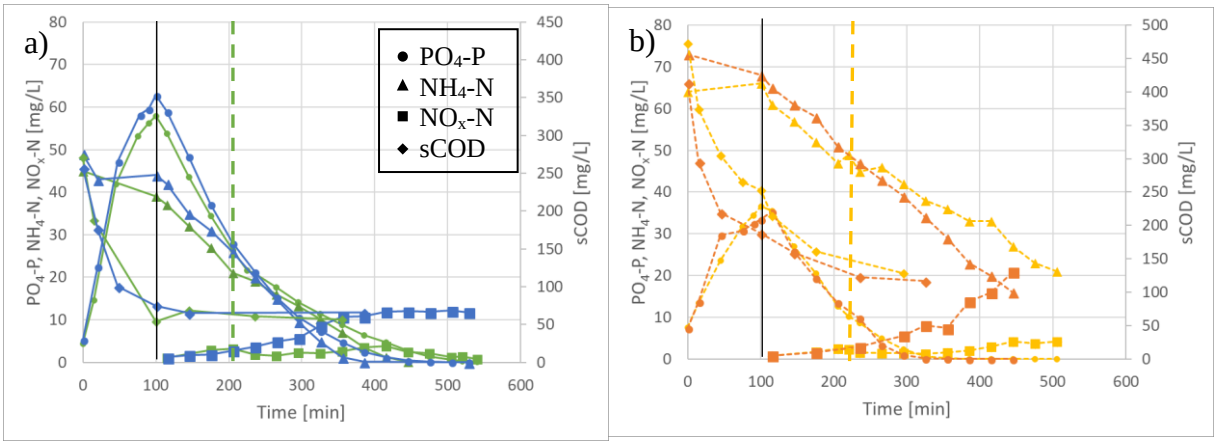

Figure 42: Comparison of experiments operated under constant aeration the entire period (blue and orange graphs) and lowered aeration after some time (green and yellow graphs). Anaerobic conditions until black vertical line. Lowered aeration after green and yellow line.

Figure 41a shows that almost all nitrogen could be removed under high aerated conditions. This is linked to the amount of ammonium compared to the phosphate concentration. Also, other batch experiments, for example WWB DO(H), show that about 20 mg NH_4-N/L is removed before increased amounts of NO_x are present. This indicates that it takes some time to make the entire biofilm aerobic, and that denitrification are able to occur in the meantime.

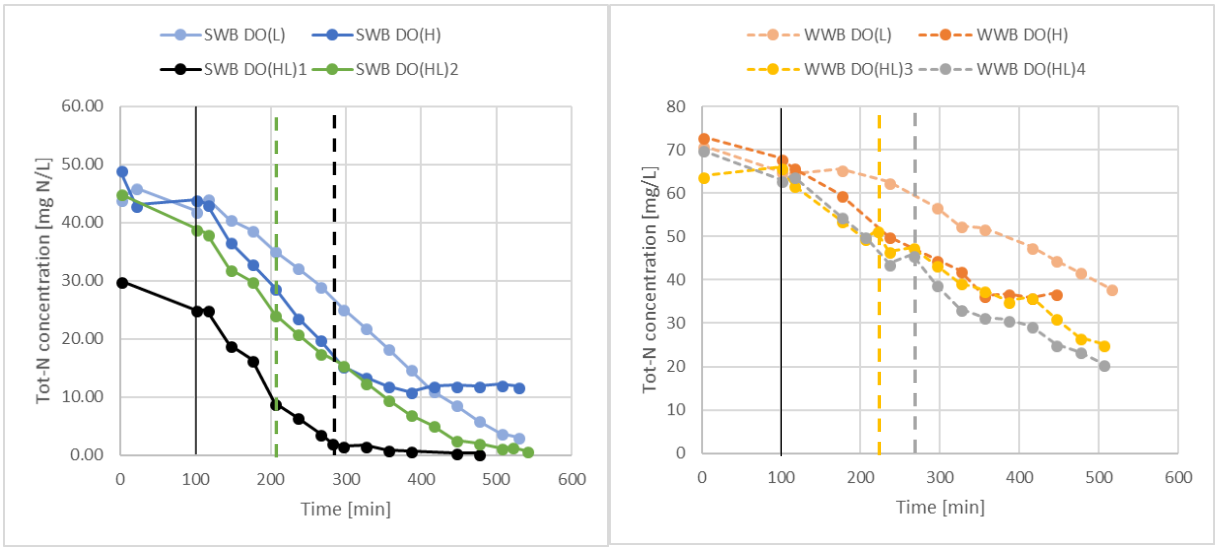

Figure 43: Total nitrogen removal profiles for all DO experiments. Experiments conducted with synthetic water (left) and experiments conducted with wastewater (right).

The total nitrogen concentration profiles for all DO experiments are shown in Figure 43. The two wastewater batches conducted with initially high aeration, followed by low aeration (WWB DO(HL)3, WWB DO(HL)4) seem to be able to remove even more nitrogen, if the experiment were conducted for a longer aerobic retention time. The ratio of carbon consumed per nitrogen removed (C/N-ratio) are found for the batch experiments with initially high aeration, followed by low aeration over time (Figure 44). All batches show similar trends, where the batches having the lowest anaerobically carbon consumption are converging towards 5 mg sCOD/mg N. This ratio is close to the theoretical C/N ratio for ordinary denitrification, 4 mg COD/mg N (Janssen et al., 2002), indicating that the process are able to have an efficient utilization of the carbon stored in the anaerobic period. WWB DO(HL)4 shows a higher ratio due to a higher anaerobically carbon consumption. It might seem like an even lower rate could be present in the wastewater batches (yellow and grey lines in Figure 43, Figure 44), due to still decreasing N concentration. This underlines the importance of the aerobic retention time, which could give higher N removal when the retention time is longer.

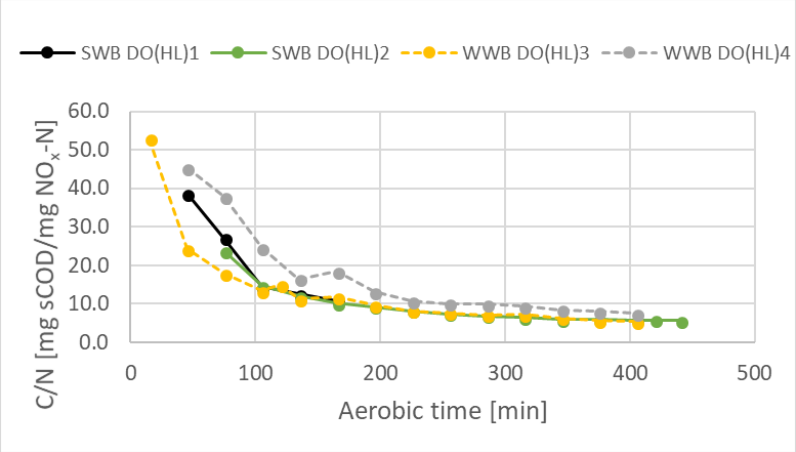

Figure 44: Ratio between anaerobically consumed carbon and nitrogen removed dependent on retention time for synthetic water (green) and wastewater (yellow). Anaerobically carbon consumption for DO(HL)1-3 are between 217 and 232 mg SCOD/L, while DO(HL)4 have carbon consumption 317 mg SCOD/L.

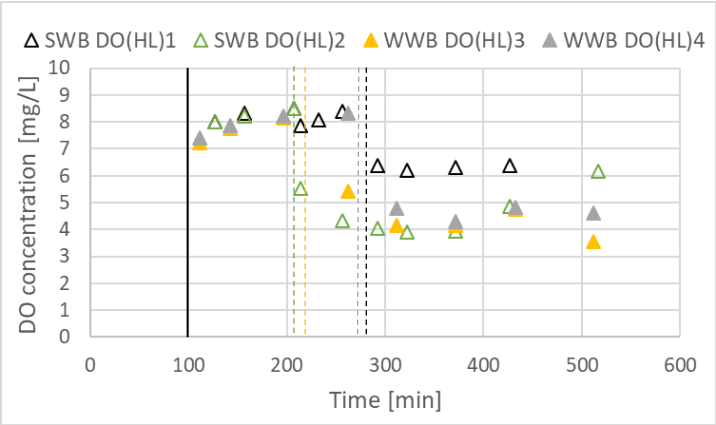

Figure 45: Measured dissolved oxygen concentrations during the aerobic period of the experiments having lowered aeration.

4.1.4. Temperature effects on nitrogen removal

An overview of the batch experiments conducted to examine the temperature effects on the nitrogen removal is shown in Table 7 (hereafter called temperature experiments).

Table 7: Temperature effects on the nitrogen removal – batch experiments, overview and operational conditions. All values are measured unless marked. Calculated VFA concentration from sCOD analysis (*). Measured VFA in inlet water + a known amount of acetate added during the first 30 seconds of anaerobic operation time (**).

Batch	Type of water	HRT anaerobic [min]	HRT aerobic [min]	VFA/sCOD (start)	NH4-N start	PO4-P start	DO [mg/L]	Volume container	Volume water	Temp
SWB LOW	synthetic	135	420	200*/243	60	4.90	ca. 10-12	1 L	0.8 L	3.3-7.0°C
SWB HIGH	synthetic	105	315	200*/243	60	4.78	ca. 8-9	1 L	0.8 L	14.1-16.6°C
WWB LOW	wastewater	165	195	/190	29	2.05	ca. 11-12.5	1 L	0.8 L	3.9-7.7°C
WWB HIGH	wastewater	165	195	/190	29	1.99	ca. 9-10	1 L	0.8 L	13.9-15.3°C

The aeration was constant and equal in all four batches. It can be seen in Table 7 that the DO concentration differs between the batches. The large difference between the cold and warm batches are related to the solubility of oxygen under different temperatures. 100% oxygen saturation for high temperature have a lower value than for lower temperatures. For temperatures between 3 and 17°C, the solubility of oxygen varies between 13.46 and 9.67 mg/L, respectively (Burton et al., 2013).

4.1.4.1. Nitrification

The nitrification rates were found in the time intervals where the temperatures were approximately constant. For the synthetic water batches, these temperatures were 3.7±0.3°C and 14.3±0.1°C for the cold water batch and the warm water batch, respectively. For the wastewater batches, these temperatures were 5.4±1.1°C and 14.4±0.4°C for the cold and warm water batches, respectively.

The SWBs showed constant nitrification rates, while the WWBs showed a slight increase in the N-rate throughout the experiment (data not shown), which is similar to what occurs in Figure 19. The nitrification rates for both synthetic water and wastewater operated under cold and warm temperatures are shown in Figure 46.

The nitrification rates are well known to be temperature dependent (Helness, 2007)and values for the temperature coefficients in the Arrhenius' equation (1) have been reported for some MBBR systems; 1.09 (Rusten et al., 1995b) and 1.124 (Pastorelli et al., 1997). The temperature coefficient for the synthetic water experiment was found to be 1.074, by calculation as described

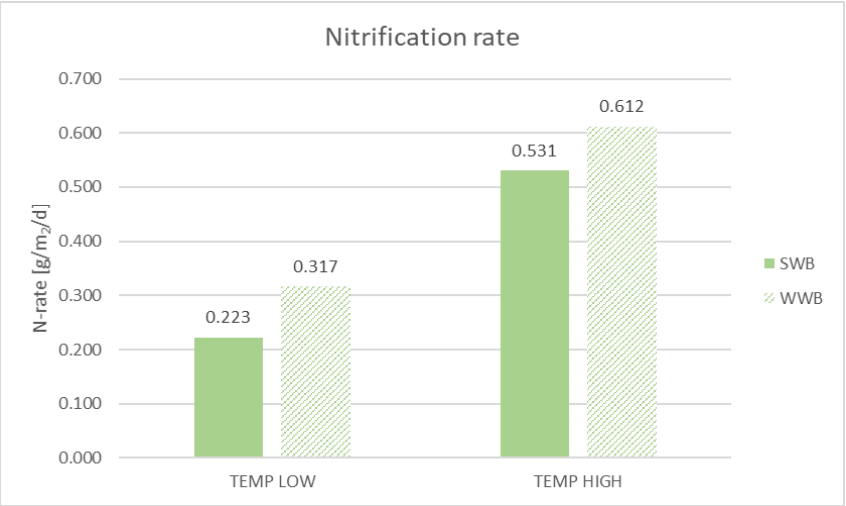

Figure 46: Nitrification rates for synthetic water batches and wastewater batches operated under low and high temperatures.

in section 3.2.2.4. This value is slightly lower than the previously reported values, which means that the difference between the N-rates are smaller in this experiment than in the reported experiments. The same coefficient for the wastewater batches was found to be 1.085.

The nitrification rates for both SWB HIGH and WWB HIGH are lower than what was found for the batches reported for the carbon experiments (Figure 20). This might be explained by the average temperature differences between the batches, which was found to be 2.7°C higher for the batches in Figure 20 than for SWB HIGH and WWB HIGH. By using the Arrhenius equation with the calculated temperature coefficient for synthetic water (1.085) and the N-rate for WWB HIGH (0.612), a theoretical N-rate for the batches in Figure 20 is found to be 0.763 $g/m^2/d$. This value deviates by only 0.022 $g/m^2/d$ from the average N-rate given in Figure 20. By using the temperature coefficient found for wastewater (1.074), the N-rate was found to be 0.742 $g/m^2/d$, which deviates from the average N-rate by only 0.041 $g/m^2/d$. Thus, the temperature could be the reason for the difference in N-rates.

It is seen from Figure 46 that the nitrification rate was higher for the wastewater batches than for the synthetic water batches. Table 7 shows that the wastewater batches have slightly higher DO concentrations than the synthetic water batches, which may be the explanation. The differences in the batches having low water temperature (SWB LOW and WWB LOW) could also be explained by the temperature difference, which was 1.7°C between the average temperature values of the two.

4.1.4.2. Denitrification influenced by temperature

The batches were held under anaerobic conditions until approximately all carbon was consumed or no more carbon could be consumed anaerobically (Figure 47). It is seen from the results, that not all carbon has been stored by PAOs, since the amount of phosphate released in the low

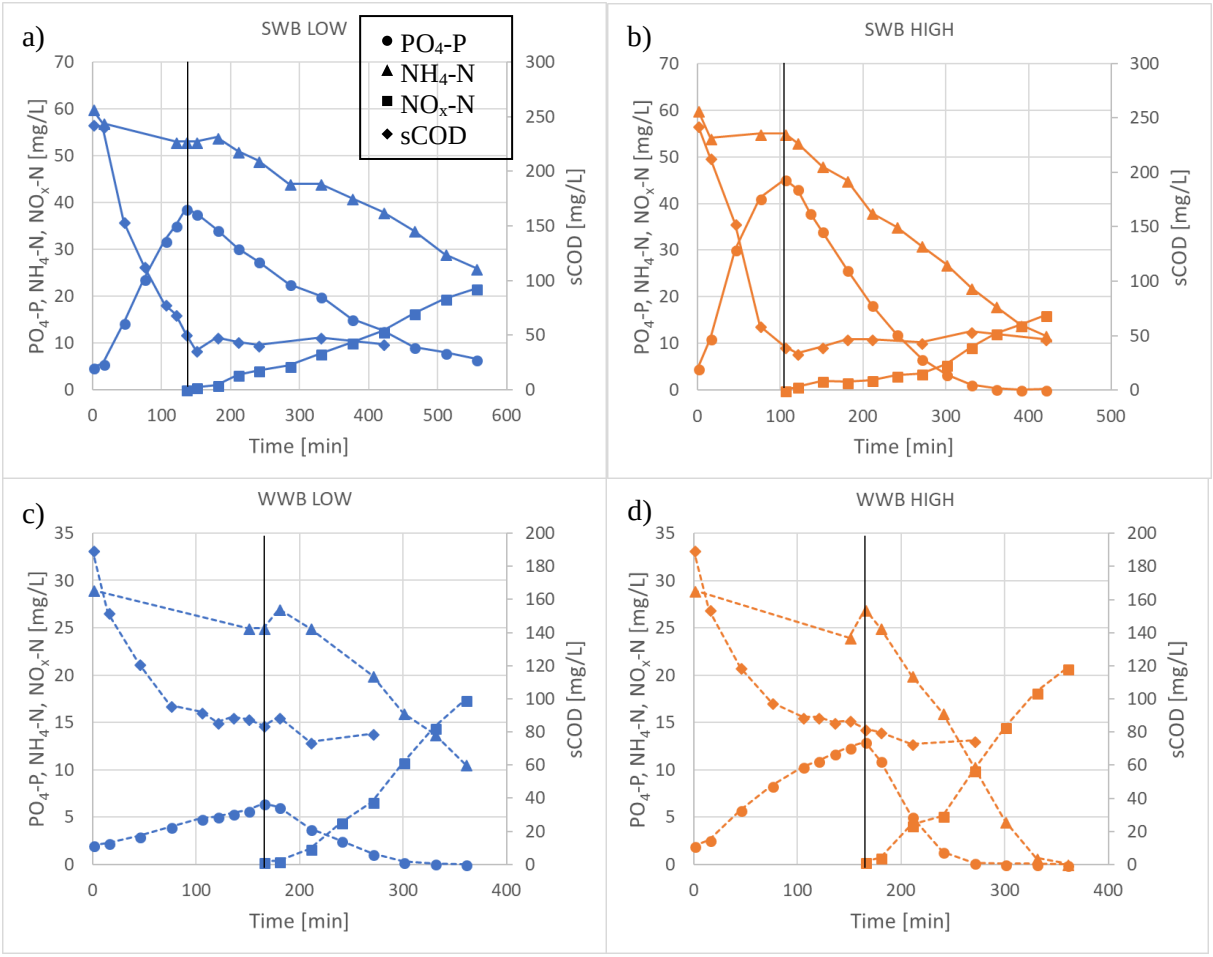

Figure 47: Ammonium, NO_x, sCOD and phosphate concentration profiles for the temperature experiments conducted in synthetic water (a and b) and in wastewater (c and d).

temperature batches (SWB LOW, WWB LOW) did not reach the amount of released phosphate that were detected in the high temperature batches (SWB HIGH, WWB HIGH), even though the carbon consumption was approximately the same. This means that the temperature does not only slow down the PAO activity, but it also seems like it gives room for other organisms to utilize the available carbon in the anaerobic period. This could be GAOs. However, this is opposite of what is found in literature, where low temperatures are beneficial for PAOs (Lopez-Vazquez et al., 2009). On the other side, the literature reported have not considered as low temperatures as were used in these experiments. The long-term effect of low temperatures may also be quite different from what was found in this experiment.

The total nitrogen concentration profiles (Figure 48) show no nitrogen removal in WWB LOW and a very small removal in SWB LOW. Compared to the batches operated with higher temperatures, it is clear that the temperature influences the nitrogen removal efficiency.

The biological activity related to denitrification is shown in literature to be a function of the Arrhenius equation. However, due to the differences in DO concentrations related to oxygen

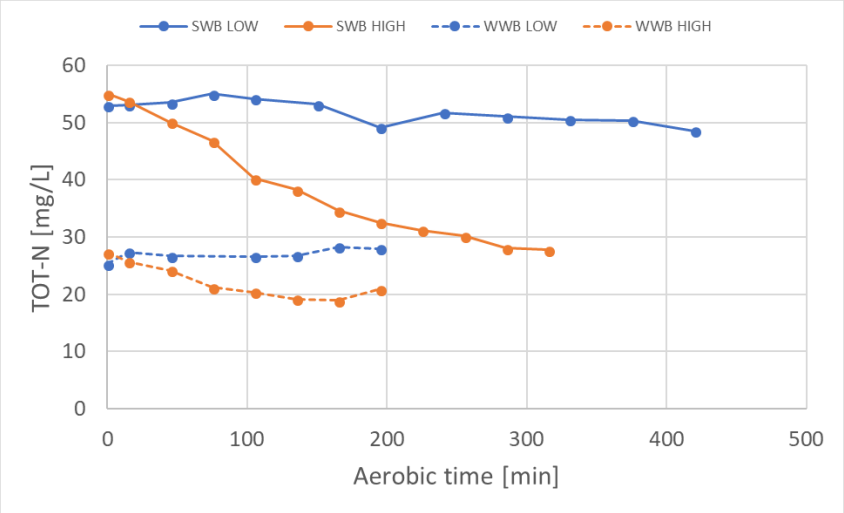

Figure 48: The total amount of nitrogen (NH_4-N + NO_2-N + NO_3-N) present in the liquid phase in SWB LOW (blue), SWB HIGH (red), WWB LOW (dotted blue) and WWB HIGH (dotted red).

solubility at different temperatures (temperature profiles in Figure 49a, DO concentration profiles in Figure 49b) higher oxygen diffusion into the biofilm at low temperatures may have occurred, resulting in a thin or no anoxic layer. This is most likely inhibiting the denitrification, since PAOs are using oxygen as electron acceptor instead of NO_x in this case. Due to the very low N removal at low temperatures, DN-rates are zero or close to zero.

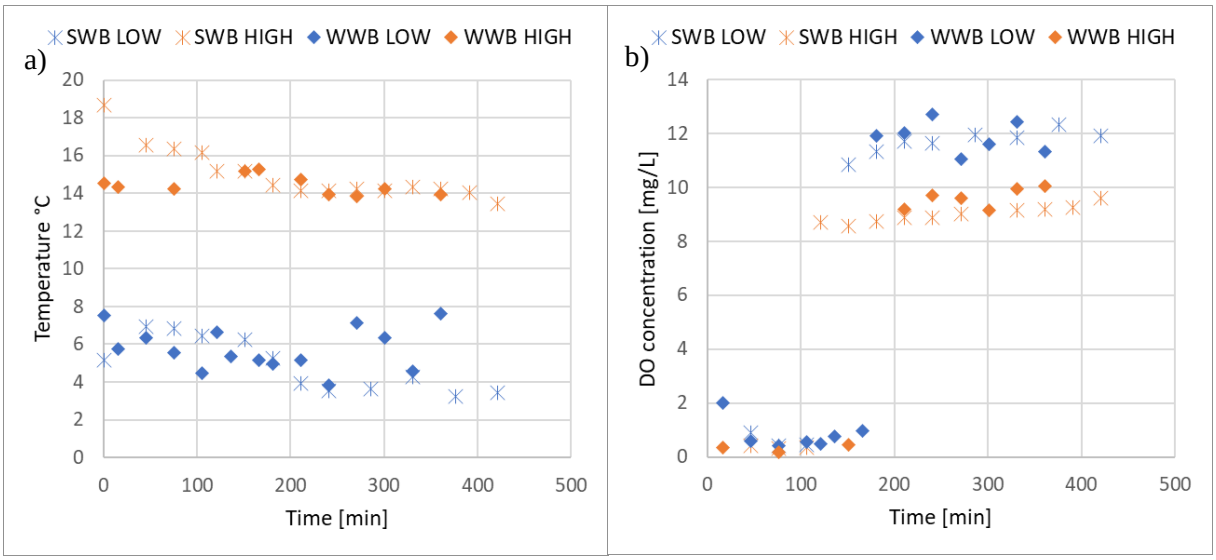

Figure 49: a) Temperature profiles throughout the experiments. b) Dissolved oxygen concentrations throughout the experiments.

4.1.4.3. Limitations and sources of error

The temperature experiments examined on wastewater, were conducted during the snow melting period, which made the wastewater quite diluted. Since this only occurs in relatively short periods during the year, the inlet concentrations were probably not very representative for the situation throughout the rest of the year. However, the process should be effective all year around, and these experiments showed the behaviour of the process under diluted situations.

Due to the diluted wastewater, the sCOD values in the inlet water were very low, resulting in low anaerobically carbon uptake, and thereby low phosphate release. Since the denitrifying capacity is highly related to the carbon uptake, this could show trends that are less representative for the performance of the process.

The temperatures were quite difficult to keep at a constant level during the experiments (temperature profiles in Figure 49). Due to the difficulties regarding the adjustment of the temperature, SWB LOW is not very representative for a real temperature situation. However, temperatures around 6°C may occur in Norwegian wastewater treatment plants due to infiltration into wastewater pipes during the snow melting period (Saltnes et al. (2017) reports temperatures for Hias WWTP between 6 and 14°C). At the same time, this does not occur for long periods during a year, and temperatures higher than 8°C are more common for the WWTPs.

The bacteria culture was not acclimatized prior to the batch experiments regarding temperature. Bacteria were therefore exposed to a rapid change in temperature and other behaviour may have been observed if the culture were acclimatized to the temperatures before the experiments were conducted. However, the water temperature was around 8°C in the pilot plant reactor at the time when these temperature experiments were conducted, meaning that the temperature was decreased by maximum 5°C from the reactor temperature.

4.1.5. Sources of errors in the experimental methods

The conversion factor for conversion between the unities mg/L/min and $g/m^2/d$ have been calculated for some filling degrees in Appendix A4. Differences in initial filling degrees, and also changing filling degree throughout the execution of the experiment, due to sample extraction made correct calculation of the rates difficult. Rates in the batch experiments was calculated with conversion factor 3.60, which is evaluated to be in a respectable area for calculation of all rates. Conversion factor 3.89 was used for calculation of rates in the pilot plant, which corresponds to the correct filling degree.

Since the first sample was taken out from the batch experiments after the addition of acetate solution (within 30 seconds), the actual initial concentrations may not have been detected, making some errors in the calculations. This might be the reason for the difference in the theoretical concentration presented in the method section, compared to the starting values presented in the tables for each experiment. However, it could also be some errors related to the amount of added acetate solution. The measured values are always used in the calculations.

The batch experiments were conducted without a lid and to avoid interference with the atmosphere. However, the good results indicate that this might not have been of large importance.

4.2. Pilot plant experiments and further discussion

4.2.1. Comparison of batch experiments and pilot plant experiments

The batch experiments show trends of what occurs when the conditions are easily controlled, for example by use of synthetic water. More realistic values are found by using wastewater in the experiments. However, the performance of the process is only seen by investigating the actual reactor through experiments. Results from the DO experiments indicates that lowering the DO concentration after some time is efficient to give initially high nitrification, but still maintaining an anoxic layer in the biofilm for denitrification to occur. This DO regime was implemented in the pilot plant by operating the first four chambers in the reactor with high aeration, resulting in high DO concentration, while the three last chambers were operated under aeration that gave lower DO concentration.

An overview of the pilot plant experiments is shown in Table 8.

Table 8: Pilot plant experiments – overview and operational conditions. All values are measured. DO concentrations for the first four chambers are presented before the slash, while the concentration for the three last chambers are shown after the slash.

Experiment	HRT anaerobic [min]	HRT aerobic [min]	HRT chamber [min]	VFA/sCOD (start)	NH4-N start	PO4-P start	DO [mg/L]	Temp	pH
GRAB H1	144	336	48	-/532	56	6.56	5.9-8.6	10.2°C	-
GRAB H2	144	336	48	93/581	68	6.86	6.5-8.2	11.5°C	7.59
GRAB L1	144	336	48	101/501	74	7.84	7.0-8.3/3.2-4.5	11.0°C	7.84
GRAB L2	144	336	48	81/493	58	7.52	7.0-8.2/3.0-4.8	10.7°C	7.61

It is seen from the batch experiments that temperature and DO concentration are important factors influencing the nitrification. Nitrification rates found in the pilot plant seem to be lower than what was found for the batch experiments, when plotting nitrification rates for GRAB L1 and GRAB L2 against DO concentration (Figure 51). However, this plot was found for temperatures between 14.8 and 18.0°C. The temperature experiments (section 4.1.4.1) showed that lower temperatures give lower N-rates, which could be one of the reasons for the low nitrification rate at high DO concentration in the pilot plant, since the temperatures in the pilot plant were between 10.2 and 11.5°C. It is also seen from the pilot experiments that higher nitrification rates are present later in the reactor (Figure 50), even though the DO concentration have approximately the same values (DO concentration profiles in Figure 52). This indicates that the nitrification rates in the first chambers of the reactor are influenced by competition with heterotrophs that are consuming sCOD, which could also be a factor influencing the highest N-

rate values in the DO vs N-rate plot. sCOD values entering the aerobic period is higher in the pilot plant experiments, than in the batch experiments.

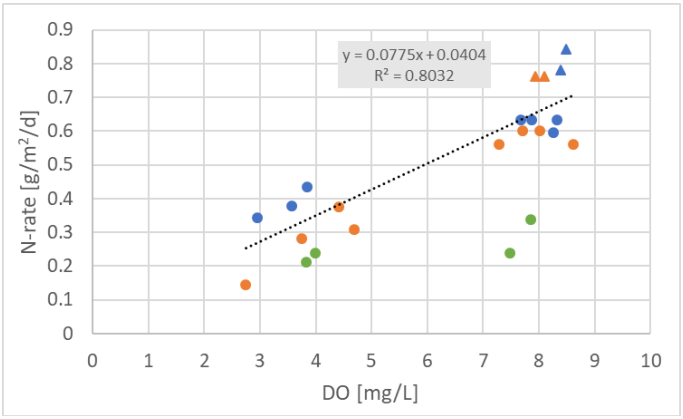

Figure 51: Nitrification rates for the pilot plant (green dots) incorporated in the finding from the batch experiments. Temperatures in pilot plant between 10.2 and 11.5°C. Temperature in batch experiments (red and blue dots) between 14.8 and 18.0°C.

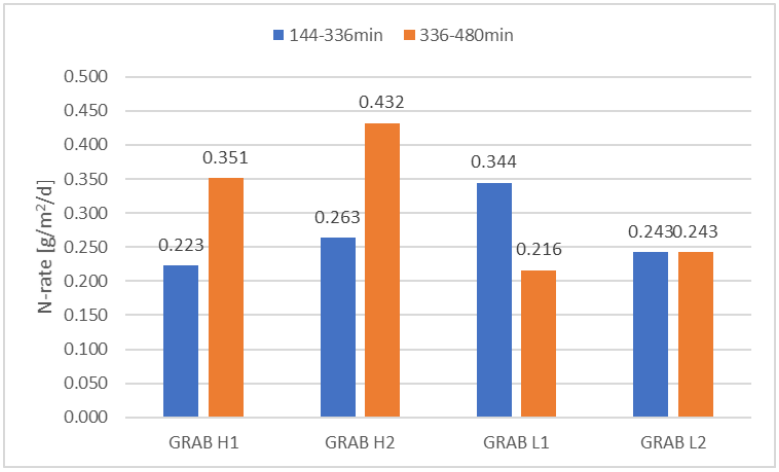

Figure 50: Nitrification rates calculated for the 4 first aerobic chamber (blue), and for the three last aerobic chambers (red).

All calculated N-rates are prone to error due to some random scatter in the ammonium concentrations. In the pilot plant, this could be related to the constant mixing of the water in each chamber, while more concentrated water always is entering the chamber. This could result in inhomogeneous distribution of the substances. However, both the batch experiments and the pilot plant experiments show some scatter in the ammonium concentration, and the phosphate concentration shows smooth curves in the pilot plant. It may therefore be more likely that the scatter is due to some uncertainties in the measuring method, for example due to dilution of the samples prior to analysis.

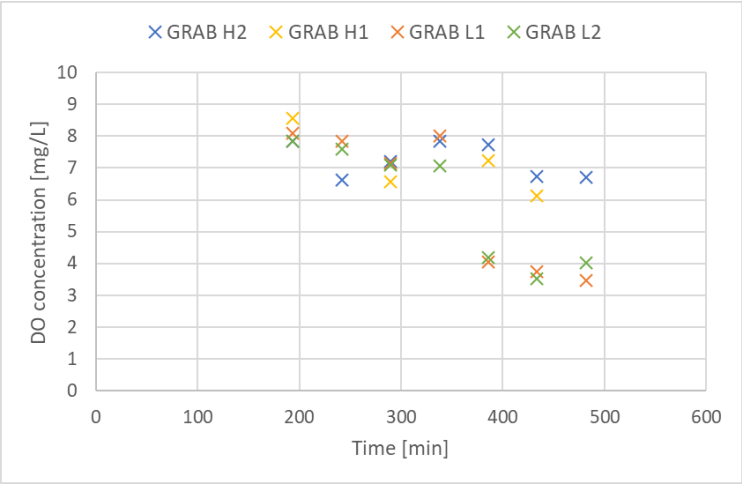

Figure 52: Dissolved oxygen concentration measured in the aerobic chambers of the pilot plant.

Figure 53 shows that only small amounts of NO_x accumulates in GRAB H2, GRAB L1 and GRAB L2. These experiments underline that the anaerobically carbon consumption is very important for the denitrification. It is seen that NO_x is accumulated in GRAB H1 where the anaerobically carbon consumption is lower than in the other three experiments. Based on the batch experiments investigating DO concentration (section 4.1.3.2), some more nitrogen was expected to be removed in the experiments were the DO concentration was lowered in the three last chambers (GRAB L1, GRAB L2). However, it is seen that since the anaerobically carbon consumption is higher in GRAB H2 than in both GRAB L1 and GRAB L2, more nitrogen is able to be denitrified, giving a higher total nitrogen removal efficiency (Table 9).

Table 9: Removal efficiencies and anaerobically carbon consumption in the pilot plant experiments. N removal are calculated for the aerobic period. C/N rates are sCOD consumed anaerobically per nitrogen removed in the aerobic period.

	GRAB H1	GRAB H2	GRAB L1	GRAB L2
P removal [%]	64	95.6	92.9	97
N removal [%]	27.1	34.9	31.1	27.4
sCOD consumed [mg/L]	254	333	289	287
C/N [mg sCOD/mg N]	15.7	13.3	13.5	17.5

Table 9 does also show values for the C/N ratio at the end of the reactor, which is found to be higher for the pilot plant experiments, than the comparable batch experiments at the same retention time (Figure 54), and approximately same anaerobically carbon consumption. This could be related to the low nitrification rates in the first aerobic chambers of the reactor, discussed above.

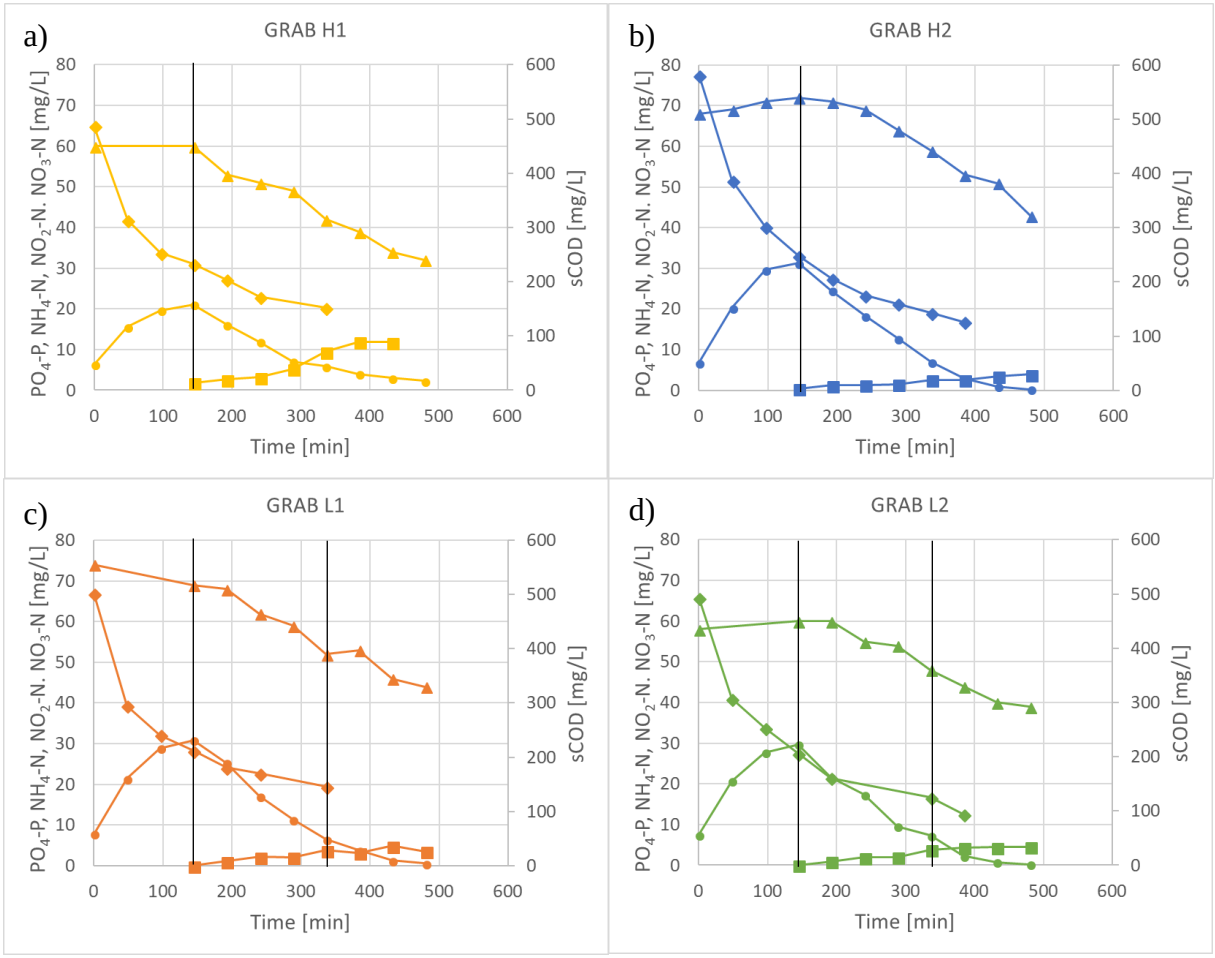

Figure 53: Ammonium, NO_x, sCOD and phosphate concentration profiles for the pilot plant experiments. Anaerobic conditions until first vertical line at 144 minutes. Lowered aeration after second vertical line at 336 minutes (c and d).

Conducting experiments in pilot plants (compared to full-scale plants) are always prone to some uncertainties, since these plants may be hard to operate under the desired to conditions, due to limitations in smaller equipment, but also due to non-optimal hydraulic, such as mixing issues and short-cuts. However, the grab samples show that each chamber seems to have good mixing and short-cuts are not seen, since the curves for the parameters are quite smooth.

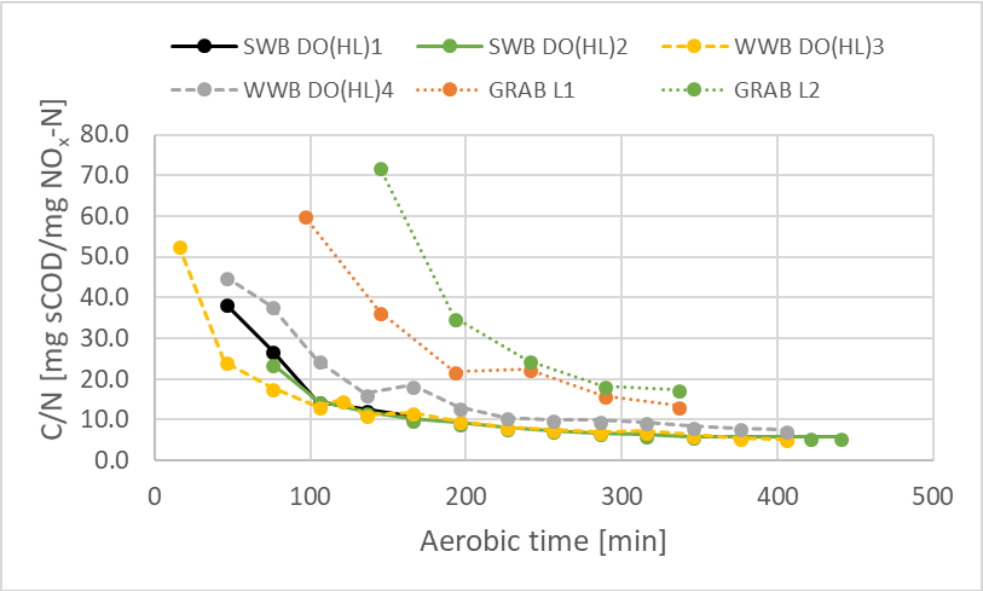

Figure 54: Ratio between anaerobically consumed carbon and nitrogen removed dependent on aerobic retention time.

4.2.2. Optimization of the HIAS-process for N-removal

It is seen that the removal efficiencies for nitrogen were higher in some of the batch experiments, than what was obtained in the pilot plant experiments. The reasons for this may be explained by anaerobically carbon consumption, aerobic retention time and temperature.

Optimization of the HIAS-process could be done by operating the first chambers of the reactor with a high DO concentration, and the last chambers with lower DO concentration. This enhances nitrification in the beginning of the reactor, producing nitrite/nitrate. Some of the conducted experiments in this thesis have shown that high DO concentration during the entire aerobic period decreases the anoxic layer in the biofilm, which reduces the ability to denitrify. The reactor could therefore be operated with lower DO concentrations in the last chambers to maintain the anoxic layer in the biofilm, and thereby enhancing denitrification.

However, the results do also show that higher anaerobically carbon consumption increases the denitrification rate, and results from the pilot plant show that better N removal could occur under high DO concentration in all chambers, than for those with low oxygen concentration in the last chambers, when the anaerobically carbon consumption is higher. These results indicate that if the wastewater contains large amount of ammonium compared to carbon and high N removal is desired, addition of an external carbon source could be implemented. Most of today's treatment processes for N-removal is adding an external carbon source, and by implementing

this in the HIAS-process, the process could remove both P and N using the same carbon source. This makes the process cost-effective compared to today's treatment processes based on two different reactors (or processes) to obtain both P and N removal.

The C/N ratio found in the experiments conducted in this thesis, show that PAOs (or PAOs in combination with GAOs) are able to effectively utilize the carbon taken up in the anaerobic phase. An external carbon source might not be needed if the retention time is long enough. The C/N ratio curve could be used to determine the necessary retention time in the aerobic phase. Longer retention time in the reactor increases the needed reactor volume, which also could be a result of making these adjustments.

The temperature of the wastewater cannot be adjusted. However, it is important to have information about how the temperature influences the bacterial processes, to be able to operate the reactor as best as possible. An operational adjustment that could be implemented is to increase the retention time, due to decreased rates in colder water.

5. Conclusion

Investigations of the HIAS-process, through batch experiments, has shown very good removal rates for both phosphorus and nitrogen, done by simultaneous nitrification and denitrifying phosphate uptake. Results have shown that enhancing conditions that gives high nitrification rates, as well as high denitrification rates are important to obtain this.

Through the conducted experiments, higher anaerobically carbon consumption is found to give higher denitrification rates, which could be expected from literature (Janssen et al., 2002)

Low oxygen concentrations are shown to give lower nitrification rates, which gives lower production of NO_x. However, lowered oxygen concentrations are seen to give better conditions for denitrification, due to the need for an anoxic layer in the biofilm for denitrification to occur. The results showed that high DO concentration in the entire aerobic period decreased or entirely stopped the denitrification after some time, often when ammonium or phosphate depleted. This indicates that the anoxic layer in the biofilm decreased, which were underlined in the experiments conducted with initially high oxygen concentration, followed by low oxygen concentration, where denitrification was maintained when the DO concentration was lowered.

It is also found that the nitrification rates are ammonium concentration limited below approximately 15 mg NH_4-N/L at DO concentrations around 8 mg/L, which is a quite high value compared to literature.

Experiments conducted with only nitrate and nitrite as electron acceptor, show that nitrite as electron acceptor gives higher nitrogen removal compared to nitrate. However, the PO_4-P_{uptake}/NO_3-$N_{eq,\ rem}$ ratio is equal for both nitrite and nitrate, which means that the P-uptake rate is actually lower for the nitrite batches, indicating that nitrite as electron acceptor is desired for nitrogen removal. This further indicates that conditions facilitating partial nitrification could increase the efficiency of the nitrogen removal in the process. However, how this can be implemented requires further investigation.

Higher wastewater temperatures are shown to give higher biological activity, resulting in higher removal rates for both P and N. The temperature experiments conducted in this thesis, are also indicating that higher anaerobically carbon consumption is done by other organisms than PAOs under low temperatures.

The results from the experiments performed in this thesis give reasons to believe that optimization of the process could be done by controlling the DO concentration in the aerobic period of the reactor, to have high oxygen concentrations in the first chambers, and lower concentrations in the last chambers. Optimization of the process could also be done by adding an external carbon source or increasing the retention time.

6. Further research

Further research may focus on finding more exact oxygen values and the most optimal time where the aeration could be lowered to obtain the fastest desired N removal. Finding the C/N-ratios for different DO concentrations at different anaerobically carbon consumption may be a tool to do this. Investigations related to the aerobic carbon consumption should also be done, to examine this effect on the removal, since competition for oxygen between PAOs and ordinary heterotrophs are seen to influence the N removal. Further work could focus on the effect of implementing an external carbon source.

In all scenarios, both DO adjustments, longer retention time and addition of an external carbon source, the long-term effects of the adjustments would be important to examine.

Further work could also focus on the type of biofilm carrier that is used in the process, to see if other types of carriers could be better at maintaining an anoxic layer, which is found to be a very important parameter for nitrogen removal to occur.

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Appendix A1 – Preparation of solutions

All solutions are made from crystallized/salt compounds (EMSURE® products by Merck) and distilled water (EMSURE® by Merck).

Molecular weights were used to find the weight% of each compound (equation 4), which was used for the calculation of the needed amount of compound to obtain the desired concentration of the solution.

N = 26 weight% of NH_4Cl

P = 22 weight% of Na_2HPO_4

Ac^- = 43 weight% of $CH_3COONa*3H_2O$

N = 17 weight% of $NaNO_3$

N = 20 weight% of $NaNO_2$

$$\frac{M_{N,P\ or\ Ac}}{M_{compound}} \cdot 100\% = Weight\% \quad (4)$$

$M_{N,P\ or\ Ac}$ represent the molecular weight of nitrogen, phosphorus or acetate and $M_{compound}$ represent the molecular weight of ammonium chloride, di-sodium hydrogen phosphate, sodium acetate trihydrate, sodium nitrite or sodium nitrate.

Equation 5 was followed to determine the amount of compound needed to make solutions containing the desired concentrations.

$$\frac{C \cdot V}{Weight\%} = m \quad (5)$$

m is the needed mass of the respective compound, which need to be added in the water volume, V, to obtain desired concentration, C, of nitrogen, phosphorus or acetate.

Preparation of ammonium solution

Carbon experiments

769 mg of ammonium chloride was dissolved in 50 ml of distilled water to obtain an ammonium solution of 4 mg NH_4-N/ml.

DO experiments

1923 mg of ammonium chloride was dissolved in 100 ml of distilled water to obtain an ammonium solution of 5 mg NH_4-N/ml.

Temperature experiments

1153 mg of ammonium chloride was dissolved in 50 ml of distilled water to obtain an ammonium solution of 6 mg NH_4-N/ml.

Preparation of phosphate solution

Carbon experiments, anoxic experiments, temperature experiments

114 mg of di-sodium hydrogen phosphate was dissolved in 50 ml of distilled water to obtain a phosphate solution of 0.5 mg PO_4-P/ml.

DO experiments

318 mg of di-sodium hydrogen phosphate was dissolved in 140 ml of distilled water to obtain a phosphate solution of 0.5 mg PO_4-P/ml.

Preparation of acetate solution

Carbon experiment

2326 mg of sodium acetate trihydrate was dissolved in 100 ml of distilled water to obtain an acetate solution of 10 mg Ac^-/ml.

DO experiment

3489 mg of sodium acetate trihydrate was dissolved in 150 ml of distilled water to obtain an acetate solution of 10 mg Ac^-/ml.

Temperature experiments

3489 mg of sodium acetate trihydrate was dissolved in 150 ml of distilled water to obtain an acetate solution of 10 mg Ac^-/ml.

Preparation of nitrite solution

Anoxic experiments

1182 mg of sodium nitrite was dissolved in 50 ml of distilled water to obtain a nitrite solution of 4.8 mg NO_2-N/ml.

Preparation of nitrate solution

Anoxic experiments

1455 mg of sodium nitrate was dissolved in 50 ml of distilled water to obtain a nitrate solution of 4.8 mg NO_3-N/ml.

Appendix A2 – Volume displacement test

A volume displacement test was performed to find the percentage of water volume of the total volume at some filling degrees of carriers including biomass.

The test was performed by filling a volume of carriers into a measuring cylinder, for example 570ml. The measuring cylinder was thereafter filled with water until the total volume in the measuring cylinder was 1000ml. The volume of carriers divided by the total volume represents the filling degree.

The water was poured into another measuring cylinder, while the carriers where retained in a sieve. The water volume could thereby by found and the water volume percentage was found by dividing the water volume by the total volume.

All tests were performed with a total volume of 1000ml.

Duplicate tests were done, and average values from the test is given in Table A 1.

Table A 1: Results from displacement test.

$V_{carrier}$ [ml]	V_{water} [ml]	Filling degree [%]	Water percentage [%]
570	770	57	77
580	760	58	76
590	750	59	75
600	740	60	74

Appendix A3 – Correlation between VFA and sCOD

5 pH-point titration method (Moosbrugger et al., 1993) was used for determination of VFA concentrations by implementation of the method in the data program TITRA5.

Several acetate solutions were made (with the method described in Appendix A1) and measured with the 5 pH-point titration method. The measured values showed good correlation with the concentration of the solution (Figure A 1). However, duplicates of all measured values should have been done to verify this correlation.

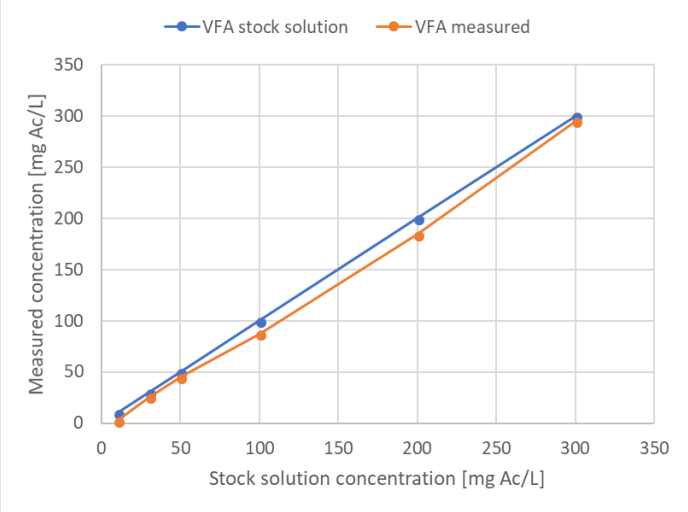

Figure A 1: Correlation between made stock solution and measured values of VFA found by the 5 pH point titration method.

Due to the time-consuming titration method for determination of VFA, a relationship between sCOD and VFA (as acetate) was found by measuring sCOD on the same acetate solutions that were measured with the titration method. The theoretical relationship between sCOD and HAc (acetic acid) is 1.07 mg sCOD/mg HAc, and a plot between the theoretical and measured values were made (Figure A 2). The measured values showed a linear deviation from the theoretical values, which might indicate some errors in the calibration of the photometer for sCOD cell tests.

The trend line from the plot can be used, but it is only valid for measurements on synthetic water where all contribution to sCOD is an added amount of acetate.

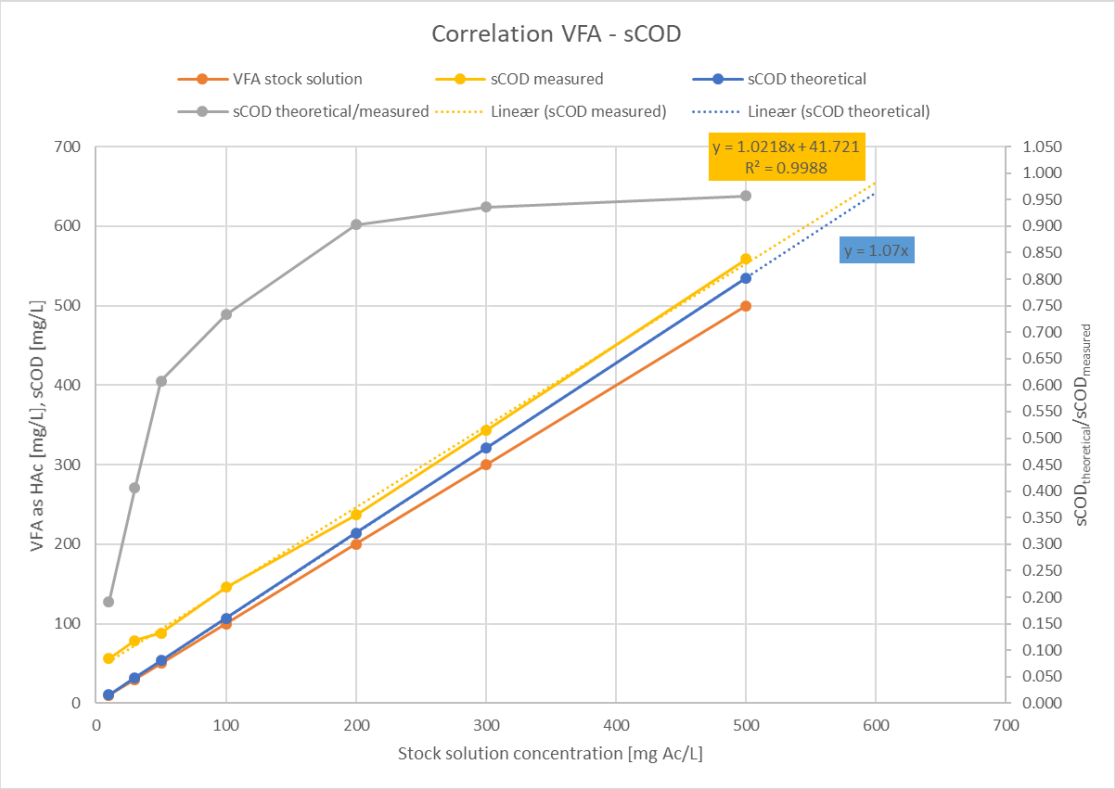

Figure A 2: Relationship between VFA and sCOD. Blue line and equation are theoretical values of sCOD. Yellow line and equation are the relationship between measured values of sCOD and actual VFA concentrations, where y is the measured sCOD, and x is the actual VFA concentration.

There is low accuracy in the smallest values. From the equation, this gives VFA concentration of 0 mg/L. However, measurements have given values below 45 mg sCOD/L, which indicates some uncertainties in the measurements, which could be expected, since the lowest sCOD value that can be found by the sCOD cell test is 25 mg/L.

For simplicity and due to inaccuracy, the sCOD values below 45 mg/L has been set to 0 mg VFA/L instead of negative values in the calculations.

Appendix A4 – Calculations

Calculation of rates

All rates are calculated by equation 6.

$$r = \frac{C_1 - C_2}{t_1 - t_2} \cdot f_c, \tag{6}$$

where $C_1 - C_2$ is the difference in concentration, while $t_1 - t_2$ is the time used to make the concentration change. f_c is a conversion factor to give rates in the unity of g/m^2/d instead of mg/L/min.

The conversion factor is calculated by equation 7.

$$f_c = 0.001 \frac{g}{mg} \cdot \frac{1}{\frac{a_c}{V_w}} \cdot 60 \cdot 24 \frac{min}{d} \tag{7}$$

a_c is the surface area of carriers in one litre of reactor volume, and V_w is the volume of water in the same reactor volume. K1 carriers have bulk specific surface area of 500 m^2/m^3, giving surface area in the reactor by multiplying with the filling degree of carriers.

By using results from the volume displacement test in Appendix A2, the following $\frac{a_c}{V_w}$ values are found, which results in the following conversion factors.

Table A 2: Conversion factors for different filling degrees.

Filling degree [%]	**Water percentage [%]**	$\frac{a_c}{V_w}$ **[m^2/L]**	f_c
57	77	0.37	3.89
58	76	0.38	3.79
59	75	0.39	3.69
60	74	0.41	3.51

Temperature coefficient in Arrhenius' equation

T_1 represented the temperature in the coldest batch and T_2 represented the temperature in the warmest batch. r_1 and r_2 was the corresponding nitrification rates in the two batches. The Arrhenius coefficient was thereby found.

Standard deviation is calculated for the temperatures, using the STDEV.P function in excel.

Calculation of $P_{released}/VFA_{uptake}$ [mol P/mol C]

The calculation of the $P_{released}/VFA_{uptake}$ ratio is calculated over the entire anaerobic period.

The conversion factor between $\frac{g\ P}{g\ C}$ to $\frac{mol\ P}{mol\ C}$ is calculated by the molar weight of carbon, phosphorus and acetate.

$$\frac{1\ gP}{1\ gC} = \frac{\frac{1}{30.97\ {}^{gP}/_{mol\ P}}}{\frac{1}{12.01\ {}^{gC}/_{mol\ C}}} = 0.388\,\frac{{}^{mol\ P}/_{mg\ P}}{{}^{mol\ C}/_{mg\ C}}$$

The $P_{released}/C_{uptake}$ ratio can then be calculated by incorporating a conversion factor between acetate and carbon (molecular weights):

$$X\,\frac{mg\ P}{mg\ Ac} \cdot \frac{1}{(\frac{24.02 mg\ C}{59.04\ mg\ Ac})} \cdot 0.388\ \frac{{}^{mol\ P}/_{mg\ P}}{{}^{mol\ C}/_{mg\ C}}$$

where $X = P_{released}/VFA_{uptake} = (P_{end,\ anaerobic} - P_{inlet})/(VFA_{inlet} - VFA_{end,\ anaerobic})$. The $P_{released}/C_{uptake}$ ratio is then in the unity of mol P/mol C.

Appendix B1 – Additional comments on the use of synthetic water

Due to the low carbon content that could be consumed anaerobically in SWB 66 and SWB 105, a possible explanation of the decreased nitrification in these two batches could be lack of CO_2 for autotrophic nitrifying activity. Wett et al. (1998) calculated that rising air bubbles from aeration could strip more CO_2 than what is transferred from them. The effect of this is determined by the aeration system and the relationship between the surface area and the depth of the reactor. This means that the amount of CO_2 needed for nitrification have to be produced within the reactor. It is seen from the results that the P-uptake stopped at aerobic time 105 min for both SWB 66 and SWB 105, which is approximately the time where the nitrification slows down. This is underlining the assumption of a lack of CO_2, since the CO_2 production by PAOs stopped approximately at the same time as the nitrification stopped. CO_2 issues would not be an issue in municipal wastewater, and the two batches (SWB 66 and SWB 105) have therefore not been investigated with regards to nitrogen.