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Title page for Master's Thesis
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Investigating phosphate release from EBPR sludge and associated possibility of controlled struvite precipitation at SNJ Wastewater Treatment Plant.

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Abstract

The main treatment stage at SNJ Wastewater Treatment Plant was recently upgraded from chemical treatment to mechanical-biological treatment with Enhanced Biological Phosphorous Removal (EBPR). The implementation of EBPR leads to higher levels of free phosphate within the reject streams from sludge thickening and dewatering, increasing the risk of operational problems due to uncontrolled struvite ($MgNH_4PO_4 \bullet 6H_2O$) precipitation. However, controlled struvite precipitation provides a way of mitigating these problems as well as a possibility for nutrient recovery.

The primary aim of this project was to investigate the phosphate release from the EBPR sludge at SNJ with respect to the possibility of controlled struvite precipitation associated with the waste sludge thickening process. The potential of struvite precipitation in the reject stream from the sludge thickener depends on the amount of phosphate released from the sludge during storage. Batch tests were performed to assess the endogenous and stimulated phosphate release from the EBPR sludge, with addition of VFA-rich filtrate from fermented primary sludge to stimulate excess release. In addition, sampling in the treatment plant was conducted to investigate the EBPR performance and phosphate flow through the bioreactor.

The average endogenous and stimulated phosphate release rates in the batch tests were 1.22 g P/kg TSS d^{-1} and 2.90 g P/kg TSS d^{-1} respectively. The stimulated release rate and the overall phosphate release during the tests were lower than expected due to low poly-P reserves within the EBPR sludge. Sampling in the treatment plant revealed low EBPR activity due to oxygen entrainment in the anaerobic zone of the bioreactor, and significant secondary phosphate release in the secondary settler. The consequence of these observations is less phosphate available for controlled struvite precipitation, thus lower phosphorous recovery. Based on the stimulated release rate, the theoretical struvite yield from the sludge thickener reject stream was 13.7 kg/d, while the struvite precipitation potential calculated from the phosphate load on the bioreactors was 474 kg struvite/d. The discrepancy was attributed to the low EBPR activity in the treatment plant. If phosphate is to be recovered as struvite at SNJ, the EBPR process needs to be optimized, and the operational problems associated with oxygen entrainment and secondary release must be mitigated.

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To my friends and family: thank you for your support and encouragement during this time. And to my husband, Anders, I am very grateful. I know that you have been taking more than your share at home these past two years. Without your support, it would have been much more difficult to go through with this degree. Thank you.

Mari Egeland

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Abbreviations

BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
EBRP	Enhanced Biological Phosphorous Removal
DO	Dissolved Oxygen
GAO	Glycogen Accumulating Organisms
HAc	Acetic acid
HRT	Hydraulic Retention Time
HPr	Propionic acid
IVAR	Interkommunalt Vann, Avløp og Renovasjon
LCFA	Long Chain Fatty Acids
MLSS	Mixed Liquor Suspended Solids
OHO	Ordinary Heterotrophic Organisms
P	Phosphorous
PAO	Polyphosphate Accumulating Organisms
PHB	Poly-β-hydroxyburate
RAS	Return Activated Sludge
RBCOD	Readily Biodegradable COD
SCFA	Short Chain Fatty Acids
SRB	Sulphate Reducing Bacteria
SNJ	Sentralrenseanlegg Nord-Jæren
SVI	Sludge Volume Index
TDS	Total Dissolved Solids
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

1 Introduction

Phosphorus is an essential nutrient to all organisms and is involved in a wide array of biochemical reactions within the cells. It is also a vital component of commercial fertilizers. Phosphorous for fertilizer production is derived from mining of phosphorous rock. However, the phosphorous resources are limited, and reserves are being depleted (Britton & Baur, 2010). The majority of the mined phosphorus will eventually reach the aquatic environment, either by natural run-off or with domestic and industrial wastewater discharge. In aquatic systems, increasing levels of phosphorous will enhance algal growth, which can lead to eutrophication (Tchobanoglous, Tsuchihashi, Burton, & Stensel, 2014). To prevent this, several wastewater treatment plants have strict limitations for phosphorous discharge. Phosphorus is commonly removed from wastewater by either chemical precipitation, Enhanced Biological Phosphorous Removal (EBPR) or a combination.

IVAR recently reconstructed their municipal wastewater treatment plant Sentralrenseanlegg Nord-Jæren (SNJ), implementing biological treatment with EBPR instead of chemical treatment. The implementation of EBPR ensures compliance with future discharge restrictions, and provides a possibility for phosphorous recovery in form of controlled struvite precipitation. However, uncontrolled struvite precipitation in piping, valves, heat exchangers and pumps can cause operational problems and lead to high maintenance costs.

Biological wastewater treatment plants with an EBPR process are subject to increased struvite precipitation due to increased amounts of PO_4^{3-} present in reject streams from sludge treatment (Baur, Benisch, Clark, & Sprick, 2002). The struvite formation potential in a wastewater treatment plant is dependent on the availability of PO_4^{3-}, NH_4^+ and Mg^{2+} ions. Conductivity testing and ion identification analyses at SNJ has confirmed that the wastewater is subject to seawater inflow when the tide is high (Leif Ydstebø, *pers. com*.). Because of this, the water at SNJ have higher Mg^{2+} concentrations than average wastewater in this region. Combined with the implementation of biological treatment and EBPR, SNJ is expecting higher levels of uncontrolled struvite precipitation. As PO_4^{3-} is released from the EBPR sludge under anaerobic conditions, the potential for struvite precipitation is highest downstream of the anaerobic phases of sludge treatment. SNJ is experiencing both higher concentrations of PO_4^{3-} in the anaerobic digesters, and decreasing dewaterability of the

digested sludge after converting form chemical to biological treatment. In addition, the implementation of EBPR could lead to nutrient recycling with the sludge treatment reject streams, and increased phosphorous load.

Although the EBPR process increases the risk of operational problems related to struvite, its implementation also provides an opportunity for phosphorous recovery through struvite crystallization. This project has been initiated in order to investigate the phosphorous release from the EBPR sludge at SNJ, and the associated possibility of controlled struvite precipitation.

2 Theory and Background

2.1 Biological Wastewater Treatment and Enhanced Biological Phosphorous Removal

The essence of biological wastewater treatment is to utilize microorganisms´ metabolic processes to remove organic substrates, particles and nutrients from the wastewater before it is released to the recipient waters (Tchobanoglous et al., 2014). The bacteria convert dissolved and particulate organic matter into biomass, CO_2 and H_2O, following the general formula below:

$$\text{Organic substrate} + O_2 + NH_3 + PO_4^{3-} \rightarrow \text{new cells} + CO_2 + H_2O$$

As the bacteria grow, nutrients and biodegradable organic matter, usually measured as BOD or COD, is removed from the wastewater. The bacteria in the wastewater grows in flocs, which will capture colloidal particles as they settle in the settling tanks. This results in removal of suspended solids in addition to the COD.

As described by Tchobanoglus et al. (2014), wastewater contains a wide array of indigenous microorganisms. In conventional aerobic biological wastewater treatment, ordinary heterotrophic organisms (OHOs) are dominant. They utilize organic molecules as a source of carbon and are dependent on a continuous supply of oxygen, as it is the terminal electron acceptor in their metabolic reactions. By altering the environmental conditions in the wastewater treatment plant, it is however possible to promote the growth of other types of bacteria.

Under anaerobic conditions, the aerobic OHOs will no longer be able to carry out their metabolic process as described above. However, a number of the OHOs are facultative, and will carry out fermentation when no oxygen is present (Tchobanoglous et al., 2014). During fermentation, the organic molecules are utilized as both electron donors and electron acceptors. This type of metabolic pathway yields low-molecular-weight fermentation products such as acetate, propionate and other volatile fatty acids (VFAs). The process of fermentation is further described in chapter 2.2. The net energy output from fermentation is low (Madigan, Brock, Martinko, & Clark, 2009), as are the growth yields for the organisms utilizing this pathway. While the typical biomass yield is 0.45 g VSS/g COD under aerobic conditions, it is 0.15 g VSS/g COD during fermentation (Tchobanoglous et al., 2014; van Lier, Mahmoud, & Zeeman, 2008). However, the suppression of OHOs growth under anaerobic conditions gives way to organisms with other metabolic strategies, such as the polyphosphate accumulating organisms (PAOs).

2.1.1 Metabolism of Polyphosphate Accumulating Organisms (PAOs)

The PAOs are a group of facultative heterotrophic organisms that have the ability to store phosphate as polyphosphate chains (poly-P chains) within their cells (Mino, van Loosdrecht, & Heijnen, 1998; Wentzel, Comeau, Ekama, van Loosdrecht, & Brdjanovic, 2008). The biochemical process is illustrated in figure 2-1.

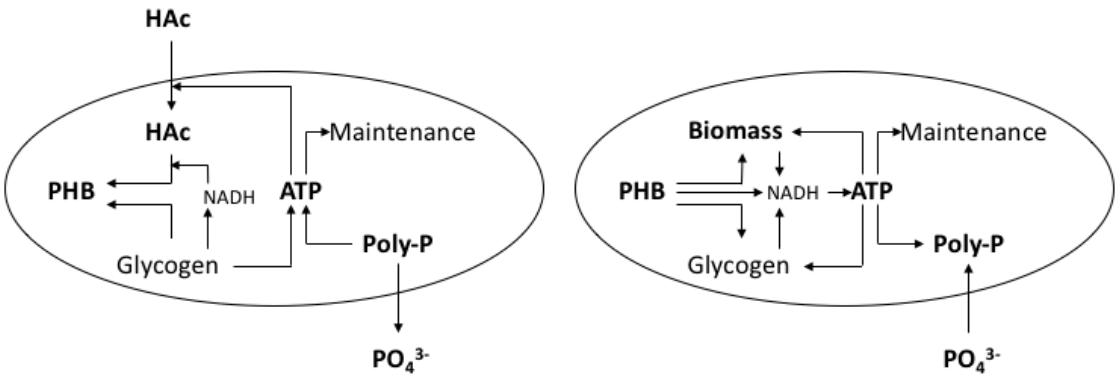

Figure 2-1: PAO metabolism under anaerobic conditions (left) and aerobic conditions. Adapted from Smolders et al. (1995).

In an anaerobic environment, no external electron acceptors such as O_2 or NO_3^- are present. Under such conditions, the PAOs utilize the poly-P within their cells as an energy source for

assimilation of acetate (HAc) and other VFAs present in the wastewater. The carbon is stored as intracellular solids, such as poly-β-hydroxyburate (PHB) (Rittmann & McCarty, 2001). This biochemical process consumes energy, and ATP required for HAc uptake and PHB formation is obtained by hydrolysis of the poly-P chains. This results in release of inorganic phosphorous as PO_4^{3-} into the wastewater. Utilisation of glycogen stored within the cells provides additional ATP as well as reducing power in form of NADH (Smolders, van der Meij, van Loosdrecht, & Heijnen, 1994).

Growth of PAOs will occur under aerobic conditions: the PHB stored in the cells is hydrolysed and oxidized, and the energy from these processes is used for biomass production (Wentzel et al., 2008). In addition, some of the energy will be used for incorporation of PO_4^{3-} from the environment into the cells, regenerating the poly-P chains. Glycogen is also produced from PHB during the aerobic phase and stored within the cell (Smolders et al., 1995). The total amount of phosphorous incorporated into the biomass by the PAOs during this process will exceed the initial release in the anaerobic phase due to the biomass growth (Tchobanoglous et al., 2014), resulting in a net phosphorous removal.

2.1.2 The EBPR process

The promotion of PAO-growth is the essential concept of the EBPR process. In a conventional biological treatment plant the biomass incorporates about 0.015 – 0.020 g P/g VSS, which covers the metabolic requirement of the cells (Tchobanoglous et al., 2014; Wentzel et al., 2008). The P uptake of OHOs results in 15 – 25 % overall phosphorous removal in the treatment process. In an EBPR plant, the PAOs can incorporate up to 0.38 g P/g VSS (Wentzel et al., 2008). The overall poly-P accumulation by the PAOs depend on the COD/P ratio in the wastewater, as the aerobic phosphate incorporation depends on the amount of available VFAs in the anaerobic zone.

In an EBPR plant, the OHOs and the PAOs coexist, and the relative proportion of the two is one of the factors determining the extent of phosphorous removal (Wentzel et al., 2008). The fractionation of PAOs and OHOs greatly depends on the amount of readily biodegradable COD (RBCOD) available to each group. When most of the RBCOD is fermented by the OHOs in the anaerobic zone, a high level of VFAs will be available to the PAOs, resulting in a high PAO fraction of the biomass. The combined biomass can incorporate 0.06

– 0.15 g P/g VSS (Wentzel et al., 2008), providing a total phosphorous removal exceeding 80 % (Tchobanoglous et al., 2014).

EBPR is a process of alternating anaerobic and aerobic conditions. The influent wastewater and the return activated sludge are mixed in the anaerobic zone of the bioreactor. Here, the facultative OHOs will ferment RBCOD to VFAs. The PAOs take up and store VFAs as PHB, releasing PO_4^{3-} into the water. In the aerobic zone, OHOs will utilize the remaining COD for energy and growth. In addition, the PAOs will grow as they use the PHB stored within their cells and restore their poly-P chains. The mixed liquor is then settled, and most of it is recycled back to the anaerobic reactor. The waste sludge in the EBPR plant contains a high level of phosphorous within the biomass, and results in a net removal of phosphorous (Rittmann & McCarty, 2001). A schematic of the process is shown in figure 2-2.

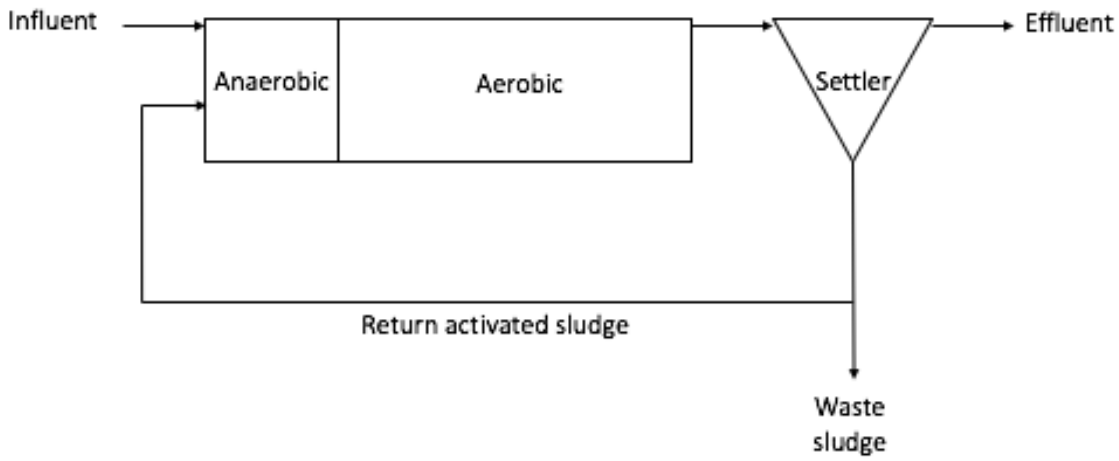

Figure 2-2: EBPR process schematic.

For further details on EBPR process configurations, it is referred to wastewater treatment textbooks such as Tchobanoglus et al.(2014) or Rittmann and McCarty (2001).

2.1.3 Parameters affecting the EBPR process

Extensive research has been done on the factors affecting the establishment of a PAO biomass and the success of EBPR. This includes the effects of available substrate, environmental conditions and operational parameters. In addition, the presence of competitive species has been investigated. An overview of these parameters is presented in this section.

Availability of VFA

The success of EBPR is dependent on VFA uptake by the PAOs, and VFAs should be readily available in the anaerobic zone. As cited by Mulkerrins et. al (2004), Barnard (1993) reported that the removal of 1 mg of phosphorous from wastewater requires 7-9 mg of VFA. The anaerobic zone in an EBPR plant should therefore have sufficient retention time to allow for the RBCOD to be converted to VFAs (Wentzel et al., 2008). In addition, the concentration of RBCOD in the inlet wastewater must be sufficient to provide the required amount of VFAs.

An alternative to fermentation in the anaerobic zone, is prefermentation of primary sludge for substrate generation (Barnard, 1984). The successful application of fermented primary sludge to enhance the phosphorous removal by PAOs has been demonstrated by many, including Rabinowitz and Oldham (1986) and Christensson et. al (1998), and will be further discussed in section 2.2.

Anaerobic phosphate release and energy requirement

The amount of phosphate released under anaerobic conditions indicates the amount of energy required for PHB storage. In their extensive review of the microbiology and biochemistry of the EBPR process, Mino et. al (1998) reports P release/acetate uptake ratios varying from 0.15 – 1.52 mol P/mol C. They state that the energy requirement for PHB formation depends on the balance between energy production and consumption within the cell, the metabolic pathway of the PAOs and the pH of the wastewater. Smolders et al. (1994) found that at pH 6 the overall energy requirement was lower than at pH 8. The additional energy required was obtained through poly-P hydrolysis, highlighted in table 2-1.

Table 2-1: ATP requirement and production by PAOs in the anaerobic phase at pH 6 and 8. Table modified from Smolders et al. (1994).

		ATP required (mmol/l)			ATP produced (mmol/l)		
pH	HAc uptake	Acetyl CoA	Transport	*Total*	**Poly-P**	Glycogen	*Total*
6	6.04	3.02	-	*3.02*	**1.45**	1.51	*2.96*
8	6.04	3.02	3.02	*6.04*	**4.68**	1.51	*6.19*

Consequently, the increase in energy requirement leads to higher phosphate release. From their findings, Smolders et al. (1994) concluded that the effect of pH on anaerobic P-release was due to the change in electrical potential across the cell membrane: uptake of a

negatively charged ion like acetate require more energy in an alkaline environment. Wen-Tso et al. (1996) confirmed this in their study, and suggested an optimum pH of 6.8 $\pm$ 0.7 based on the combined phosphate release/acetate uptake rates. The anaerobic phosphate release associated with PHB storage within the cells is known as *primary release*. In addition, phosphate can be released from the PAO biomass without carbon assimilation; this is known as *secondary release*.

Secondary phosphate release occurs if VFA availability under anaerobic conditions is insufficient. The poly-P chains are then utilized for endogenous respiration. This release does not contribute to PHB storage, thus less energy is available for poly-P regeneration in the aerobic zone (Barnard, 1984). If significant levels of secondary release occurs within the EBPR process, the potential for phosphate removal will decrease. A PAO biomass that exhibits a high level of secondary release will have a lower phosphate uptake in the aerobic zone, as illustrated in figure 2-3. Consequently, the overall phosphorous removal within the treatment plant will be less efficient.

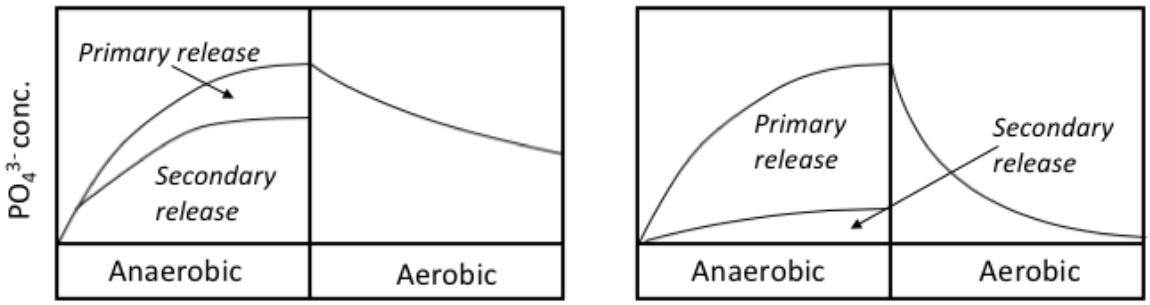

Figure 2-3: Illustration of primary (right) and secondary phosphate release and associated aerobic uptake. Adapted from Barnard (1984).

Too long hydraulic retention time (HRT) in the anaerobic zone will lead to depletion of RBCOD and VFA before the wastewater reaches the aerobic zone. VFA concentration will also be affected if the HRT is too short for the OHOs to effectively ferment the RBCOD within the anaerobic zone. These factors can lead to increasing levels of secondary phosphorous release and deterioration of the EBPR process (Barnard, 1984; Danesh & Oleszkiewicz, 1997). Secondary release can also occur from the sludge in the settling tanks if the HRT is too long.

In addition to exhausting the poly-P reserves without energy storage in form of PHB, this could lead to increasing phosphate concentrations in the effluent.

Dissolved oxygen and nitrate

Keeping the anaerobic zone devoid of oxygen is crucial to the success of EBPR, and upstream processes that mix air into the wastewater (vortexes, cascades, air lift pumps etc) should be minimized (Wentzel et al., 2008). Nitrate may be produced in the aerobic zone due to oxidation of ammonia (nitrification), and could be recycled with the sludge return from the settling tank.

Both oxygen and nitrate will interfere with the anaerobic metabolism of PAOs, as they both serve as external electron acceptors (Tchobanoglous et al., 2014; Wentzel et al., 2008). If one or both are present, the OHOs will be able to utilize the RBCOD for energy and growth, rather than fermentation. This leads to a lower VFA level in the wastewater, and subsequently less carbon available for PHB storage. If VFAs are already present in the influent wastewater, and oxygen and/or nitrate are being recycled, the OHOs will grow aerobically and outcompete the PAOs for the substrate. Either way, the phosphorous uptake, release and net removal will be adversely affected (Wentzel et al., 2008).

The dissolved oxygen (DO) concentration in the aerobic zone of an EBPR plant must be sufficient for the OHO metabolism and PAO growth. Shehab et al. (1996) recommended DO concentrations within the range of 2 – 4 mg/l, and excessive aeration is both energy consuming and can lead to oxygen recycling to the anaerobic reactor.

Environmental parameters

As previously stated, pH can influence the EBPR process due to changes in the energy requirement for VFA incorporation into the biomass. In addition, the pH of the wastewater will affect the overall microbial growth. A pH between 6.5 and 7.5 is generally considered optimal for growth in biological wastewater treatment (Tchobanoglous et al., 2014). The bacteria can tolerate pH levels outside this range, but the growth rates will then be affected. When studying the microbial population change of an EBPR system when the pH was altered from 7.0 to 6.5, Zhang et al. (2005) observed a clear shift in the community structure combined with deterioration of P removal. This indicates that the PAOs were outcompeted by bacteria better adapted to the more acidic conditions.

The temperature affect biological reaction rates, and thus the overall efficiency of a biological wastewater treatment plant (Mulkerrins et al., 2004). According to Tchobanoglous et al. (2014), the microbial growth rate doubles with approxematly every 10°C until reaching the organisms optimum growth temperature. Above optimum, the temperature effect is less significant. Growth rates increasing with temperature can be explained by the increasing enzymatic activity within the bacterial cell, which accelerates growth (Madigan et al., 2009). With regards to EBPR, elevated temperatures will lead to higher phosphate uptake- and release rates due to an overall increase in metabolic activity. This may lead to higher levels of secondary release in settling tanks and increasing phosphate concentrations in the effluent. Low temperatures will cause less phosphate to be released into the wastewater in the anaerobic zone, and the EBPR will be less efficient. In addition, low temperatures in EBPR plants increase the risk of oxygen recycling to the anaerobic zone due to increased solubility of oxygen in the cold wastewater. However, the most significant temperature influence is on the biological processes. A study conducted by Brdjanovic et al. (1998) concluded that metabolic processes of EBPR in the aerobic zone, such as PHB consumption and growth, was strongly affected by temperature changes. The poly-P regeneration was only moderately affected. In addition, they observed that the structure of the microbial population changed with temperature. Panswad et al. (2003) reported that PAOs were the main microbial group in an EBPR system at 20°C, while the fraction of glycogen accumulating organisms (GAOs) increased and became dominating as the temperature was elevated to 25°C and 30°C.

Competition between PAOs and GAOs

To achieve EBPR in a biological wastewater treatment plant, the establishment of the desired type of microorganisms – the PAOs – is crucial (Wentzel et al., 2008) . The GAOs are a microbial group that are metabolically similar to the PAOs, and could compete for VFAs under anaerobic conditions (Mino et al., 1998; Tchobanoglous et al., 2014). Table 2-2 compares the metabolic characteristics of PAOs and GAOs. As indicated in the table, the main difference between the GAOs and the PAOs is the latter's ability to incorporate and release phosphate.

Table 2-2: Comparison of the PAO and GAO metabolism in the anaerobic and aerobic zones. Adapted from Mino et al. (1998).

Metabolism	PAOs	GAOs
Anaerobic		
External organic substrate uptake	+	+
Consumption of intracellular glycogen	+	+
Accumulation of intracellular PHB	+	+
Consumption of intracellular poly-P and consequent release of phosphate	+	–
Aerobic		
Recovery of intracellular glycogen	+	+
Consumption of stored PHB	+	+
Biomass production – growth	+	+
Recovery of intracellular poly-P	+	–

There are several factors affecting the competition between GAOs and PAOs, among these are feed composition, temperature and pH (Tchobanoglous et al., 2014). Oehmen et al. (2007) states that a COD/P ratio above 50 mg COD/mg P in the wastewater feed tends to favour GAO growth, and it follows that a lower COD/P ratio should favour the PAOs. This was confirmed by Gu et al. (2008). When investigating several municipal wastewater treatment plants in the U.S., they observed stable and efficient EBPR performance at ratios between 25 and 38 mg COD/mg P.

The VFA composition in the feed may also influence the dominance of one organism over the other. Lu et al. (2006) found that alternating acetate and propionate as the carbon source affected the presence of GAO species, increasing the amounts of PAOs due to the competitive advantage of the latter under these conditions. In addition, Oehmen et al (2007) concludes in their review that the competitive advantage of GAOs become stronger at higher temperatures. Lopez-Vasques et al. (2009) found that PAOs were the dominating species at 10°C and below, due to inhibition of GAO metabolism at low temperatures. The relationship between GAOs and PAOs is also influenced by pH (Oehmen et al., 2007).

2.2 VFA generation through primary sludge fermentation

As the PAOs depend on availability of VFAs in the anaerobic zone, sufficient fermentation of RBCOD is essential to achieve an efficient EBPR process. If the retention time in the anaerobic zone is insufficient or the level of RBCOD is low, additional VFAs may be added. A common strategy for increasing VFAs is addition of supernatant from fermented primary sludge to the anaerobic zone. In this chapter the process of primary sludge fermentation will be presented.

2.2.1 Anaerobic degradation of organic matter – overview

Fermentation is part of the anaerobic degradation of organic matter. In the first step, particulate organic matter is disintegrated to soluble compounds which can be further hydrolysed to monomers. Carbohydrates are converted to monosaccharides, proteins to amino acids, and lipids to long chain fatty acids (LCFA). The hydrolysis reactions are catalysed by the extracellular enzymes of a variety of microorganisms, both facultative and obligate anaerobes. After hydrolysis, the monomers serves as substrate for the acidogenic bacteria. The substrates are used both as an electron donor and an electron acceptor in the process of fermentation or acidogenesis. The fermentation products acetate and hydrogen are used directly in methanogenesis to produce biogas (CH_4 and CO_2). Other fermentation products such as propionate, butyrate and valerate produced in acidogenesis are converted to acetate and hydrogen in acetogenesis before they are utilized by the methanogens (Tchobanoglous et al., 2014). An overview of the anaerobic digestion process is presented in figure 2-4.

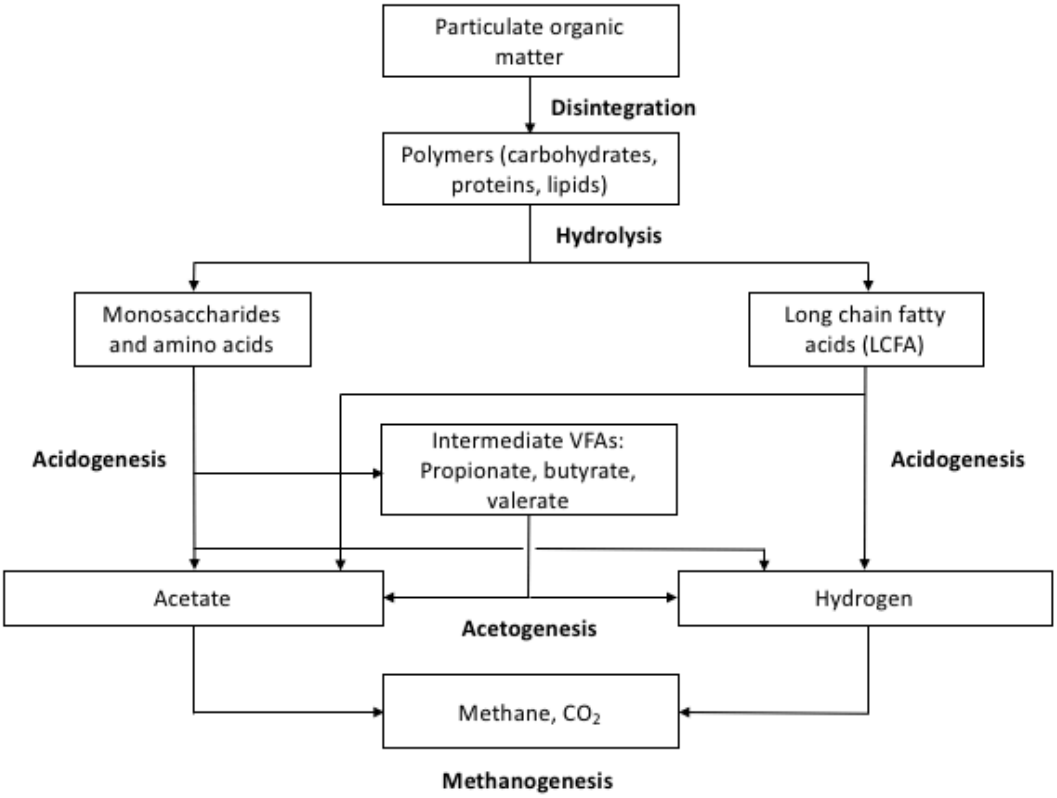

Figure 2-4: Overview of the anaerobic oxidation of organic matter. Adapted from Tchobanoglous et al. (2014) and Safitri (2016).

When generating substrate for EBPR using prefermentation of primary sludge, the anaerobic degradation process is deliberately stopped before the methanogenesis step to avoid VFA consumption by methanogens. The methanogens are inhibited by low pH values caused by high levels of free acids (Batstone et al., 2002). The VFA production in acidogenesis is rapid and the process require short HRT, while the methanogens have significantly lower substrate conversion rates and require much higher HRT (van Lier et al., 2008). When the aim of the anaerobic digestion is biogas production, the VFA levels and alkalinity must be carefully monitored to avoid pH decreasing due to VFA build-up. During primary sludge fermentation however, this is used to inhibit methanogenesis. Low sludge retention time (SRT) will also prevent VFA consumption by methanogens, as they have significantly lower growth rates than the acidogenic bacteria. Table 2-3 shows the average kinetic properties of acidogenesis and methanogenesis.

Table 2-3: Average kinetic properties of acidogenesis and methanogenesis. Adapted from van Lier et al. (2008).

Process	Conversion rate (g COD/g VSS d)	Yield (g VSS/g COD)	Max growth rate - μ_{max} (1/d)
Acidogenesis	13	0.15	2.00
Methanogenesis	3	0.03	0.12

2.2.2 Primary sludge fermentation

With regards to EBPR, the fermentation products of interest are VFAs that can be utilized by the PAOs. The VFA distribution in table 2-4 show that primary sludge fermentation generally yields acetate as the dominating VFA, followed by propionate (HPr) and other VFAs (e.g. butyrate and valerate).

Table 2-4: VFA distribution during fermentation of primary sludge. All results are from batch tests performed without pH control at approx. 20°C.

Time (d)	HAc (%)	HPr (%)	Other (%)	Reference
3 – 7	50	33	>20	Cokgor et al. (2005)
5	36-54	29-47	>20	Ucisik and Henze (2008)
5	39	31	30	Wu et al. (2009)
4	40 - 42	13-14	>50	Yuan et al. (2010)

However, there are other fermentation products possible besides VFAs. The overall composition of fermentation products depends on the composition of the substrate, and environmental and operational factors such as temperature, pH and retention time (Banister & Pretorius, 2016; Cokgor, Oktay, Tas, Zengin, & Orhon, 2009; Madigan et al., 2009). Ethanol and lactate are produced from carbohydrates in lactic and mixed acid fermentations (Madigan et al., 2009), and will contribute to the soluble COD. Lactate and ethanol can be further utilized by some of the acidogenic bacteria to produce acetate and propionate. The conversion of lactate and ethanol to VFAs is rapid and high levels are generally not observed during anaerobic digestion, since methanogenic reactors are operated as low loaded systems (Batstone et al., 2002). However, Ydstebø (2005) summarized that lactate and ethanol was generally observed at high organic loads (e.g. in batch fermentations) and low pH. Due to a pK_a of 3.86, the presence of lactic acid in a fermenter will affect pH, while

ethanol is a neutral product. For further details on fermentation reactions and their products, it is referred to microbiology textbooks such as Madigan et al. (2009).

The presence of nuisance organisms will affect the amount of VFA generated during prefermentation of primary sludge. If sulphate is present in anaerobic processes, sulphate reducing bacteria (SRB) are able to reduce the sulphate to sulphide (Tchobanoglous et al., 2014). High concentrations of sulphide can be toxic to methanogens, and the presence of SRB can be a problem in anaerobic digesters. In addition, some of the SRB can utilize acetate and propionate as electron donors and carbon sources in the sulphate reduction process (Liamleam & Annachhatre, 2007). These reactions produce alkalinity in addition to sulphide. During primary sludge fermentation, the presence of SRB can reduce the overall yield by consuming a portion of the VFAs produced. Furthermore, the H_2S produced by the SRBs is toxic and will inhibit biological processes (Batstone et al., 2002), and addition of H_2S-rich substrate could affect EBPR activity.

2.3 Struvite formation in wastewater treatment plants

In most wastewater treatment plants, the waste sludge is subject to further treatment. Common processes involved are thickening, anaerobic digestion and dewatering. In an EBPR plant, phosphate will be released from the PAOs when the waste sludge is kept under anaerobic conditions. When anaerobic holding tanks are located upstream of a sludge thickener or dewatering unit, the released phosphate will enter the reject streams and be recycled back into the treatment plant (Münch & Barr, 2001). Besides increasing the nutrient load on the treatment plant, phosphate may react with ammonium and magnesium present in the wastewater and precipitate as magnesium ammonium phosphate in accordance with the general formula presented by Tchobanoglous et al. (2014):

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightleftharpoons MgNH_4PO_4 \bullet 6H_2O$$

The resulting mineral is commonly known as struvite. The molar ratio of the ions is 1:1:1, and struvite formation is possible when the combined concentrations of magnesium, ammonium and phosphate exceeds the struvite solubility limit. Uncontrolled struvite precipitation is common in EBPR plants and may cause reduction in pipe diameters, clogging of small-bore pipes, pumps, and valves, and other operational problems (Tchobanoglous et al., 2014).

For uncontrolled struvite crystallisation to occur at a significant rate, a concentration between 100 and 200 mg/l of PO_4^{3-} is required (Melia, Cundy, Sohi, Hooda, & Busquets, 2017). As the total dissolved concentrations of Mg^{2+}, NH_4^+ and PO_4^{3-} are dependent on the system pH, so is struvite precipitation (Ohlinger, Young, & Schroeder, 1998). Within the expected pH range in a wastewater treatment plant, the solubility of struvite will decrease as the pH increases. Struvite precipitation is expected in treatment processes where sudden pressure drops cause pH to increase due to lower levels of dissolved CO_2 (Fattah, 2012).

During post-digestion storage, the pH of digested sludge and centrate from the dewatering centrifuge increase. The anaerobic digestion results in release of phosphate and magnesium from the biomass, and combined with elevated pH, the struvite formation potential increases (Baur et al., 2002) . The maximum struvite formation potential under typical wastewater treatment plant conditions is approximately at pH 9 (Münch & Barr, 2001). Wastewater suspended solids will serve as nuclei for crystal growth during the struvite formation. If little or no suspended solids are present, such as in dewatering filtrate, roughness present in the surface of piping will act as a nucleus (Baur et al., 2002).

Although uncontrolled struvite precipitation is associated with operational problems and high maintenance costs, high struvite formation potential may also be an asset for the wastewater treatment plant. As struvite contains both nitrogen and phosphorous, its formation provides a possibility for nutrient recovery and fertilizer production (Bhuiyan, Mavinic, & Koch, 2008; Melia et al., 2017).

2.3.1 Controlled struvite formation for nutrient recovery

A high struvite formation potential allows for the possibility of phosphorous recovery from the wastewater. Crystallization of struvite results in a solid with few impurities, that will release nutrients more slowly than commercial fertilizers if applied to soil (Melia et al., 2017; Münch & Barr, 2001). This makes struvite a desirable substance for nutrient enrichment in soils, either for direct application or incorporated as part of a fertilizer production. As described by Tchobanoglous et al. (2014) and Melia et. al (2017), there have been developed several technologies for phosphorous recovery by precipitation of struvite, some of which are briefly presented below:

AirPrex® process

Struvite is crystallized directly within the sludge stream from an anaerobic digester. As opposed to using a side stream, this method prevents struvite precipitation in the dewatering facilities. It consists of two tanks where air is used to strip CO_2 to elevate pH, and to keep sludge in suspension. Magnesium ($MgCl_2$) is added in the process, and struvite precipitates. The heavier struvite settles and is collected at the bottom of the reactor, while the effluent at the top carries the digested sludge further on to the dewatering process.

Cone-shaped fluidized bed crystallizer

This technology utilizes a sludge side stream from the anaerobic digester, which is pumped in through the bottom of a conical reactor. The dimensions of the reactor are designed to uphold the selected upflow velocities. $MgCl_2$ and sodium hydroxide (NaOH) is added, the latter to elevate pH. The effluent is at the top of the cone, while the struvite crystals are collected at the bottom for further processing.

Ostara Pearl® process

This is a fluidized bed crystallizer with a segmented construction, where the zone diameters increase from the bottom to the top. This reduces the upflow velocity and retains struvite crystals of different sizes in each zone. The effluent is recirculated from the top to the bottom of the reactor and mixed with the inflowing high nutrient side stream. As the struvite crystals grow, they will sink to the next zone, and are eventually collected from the bottom zone. As with the cone-shaped fluidized bed crystallizer, $MgCl_2$ and NaOH is added. This technology is commonly used on reject streams from dewatering units.

These processes have been shown efficient at full-scale facilities, and the AirPrex® process is applied at several plants in Germany and the Netherlands (Melia et al., 2017). All the methods described depend on magnesium addition as a solid, as magnesium concentration often is the limiting parameter of struvite formation in wastewater (Münch & Barr, 2001).

2.4 EBPR and dewatering

Aside from struvite deposits in pipelines and valves, an additional operational problem that may occur when EBPR is a part of the treatment process is poor sludge dewatering after anaerobic digestion (Tchobanoglous et al., 2014). Higgins and Novak (1997) found that a monovalent to divalent cation ratio higher than two resulted in deterioration of dewatering

properties in activated sludge systems. The divalent cations are presumed to influence dewaterability by bridging the negatively charged groups on the extracellular polymeric substances secreted by the microorganisms in the sludge. This helps aggregation and provide stabilization of the bioflocs, thus improving dewaterability (Bergmans, Veltman, van Loosdrecht, van Lier, & Rietveld, 2014). The removal of Mg^{2+} due to struvite formation will increase the monovalent/divalent ratio. In addition, the presence of free PO_4^{3-} in the digested sludge may also affect the dewatering properties. Bergmans et al. (2014) investigated the effects of struvite formation on the dewaterability of activated sludge, and found that addition of Mg^{2+} for struvite precipitation had a positive effect. However, when exceeding the 1:1 molar ratio between Mg^{2+} and PO_4^{3-}, no further improvement was found. This indicated that the positive effect of struvite formation on sludge dewaterability may have been caused by the removal of PO_4^{3-} rather than the addition of Mg^{2+}, or a combination of both.

2.5 Objectives of this thesis

The start-up of the biological treatment and EBPR at SNJ was during the summer of 2017, and this project includes investigations of the EBPR performance and phosphate flow through the new wastewater treatment plant. Mapping of the phosphate release will provide information on the potential of controlled struvite precipitation at SNJ. Phosphate may be recovered as struvite in the reject stream from the waste sludge thickener, and addition of VFAs from fermented primary sludge has been proposed as a method of stimulating phosphate release in the waste sludge storage tank upstream of the thickener. Most of the free phosphate will follow the reject water, and this provides a possibility for controlled struvite formation. If significant phosphate release can be achieved this early in the sludge treatment process, the level of uncontrolled struvite precipitation in subsequent treatment stages could be reduced.

The objectives of this research project was to investigate:

- The activity of the EBPR biomass in the bioreactors. This was done through a series of sampling campaigns in the wastewater treatment plant, focusing on phosphate concentrations at the inlet, through the bioreactors and effluent.

- The endogenous phosphate release from the EBPR sludge in anaerobic batch tests, and the possibility of increasing phosphate release by addition of VFA-rich filtrate from fermented primary sludge.
- The fermentation potential of the primary sludge at SNJ.

Together, these investigations will provide an answer to the question: is it possible to release sufficient amounts of phosphate during secondary sludge storage to implement controlled struvite precipitation on the reject stream from the sludge thickener?

3 Materials and Methods

This section gives an overview of the wastewater treatment plant where this research was conducted, as well as details on sampling, experimental setups and analytical procedures.

3.1 Overview of SNJ Wastewater Treatment Plant

The SNJ wastewater treatment plant is the largest plant in the Rogaland region, and receives mainly domestic wastewater from Stavanger, Sandnes, Randaberg, Sola and Gjesdal municipalities (IVAR, 2017). The original plant from 1992 was built as a chemical treatment plant dimensioned for 240 000 pe. To accommodate both higher loads due to population growth in the region, and more strict discharge limits, the plant has been expanded and rebuilt as a mechanical-biological treatment plant. The plant is being rebuilt in two stages: stage 1 is dimensioned for 400 000 pe, which is the expected load in 2035. In stage 2, the capacity of the plant will be further expanded to 500 000 pe, corresponding to the loads expected in 2050. A schematic of the wastewater treatment process is shown in figure 3-1.

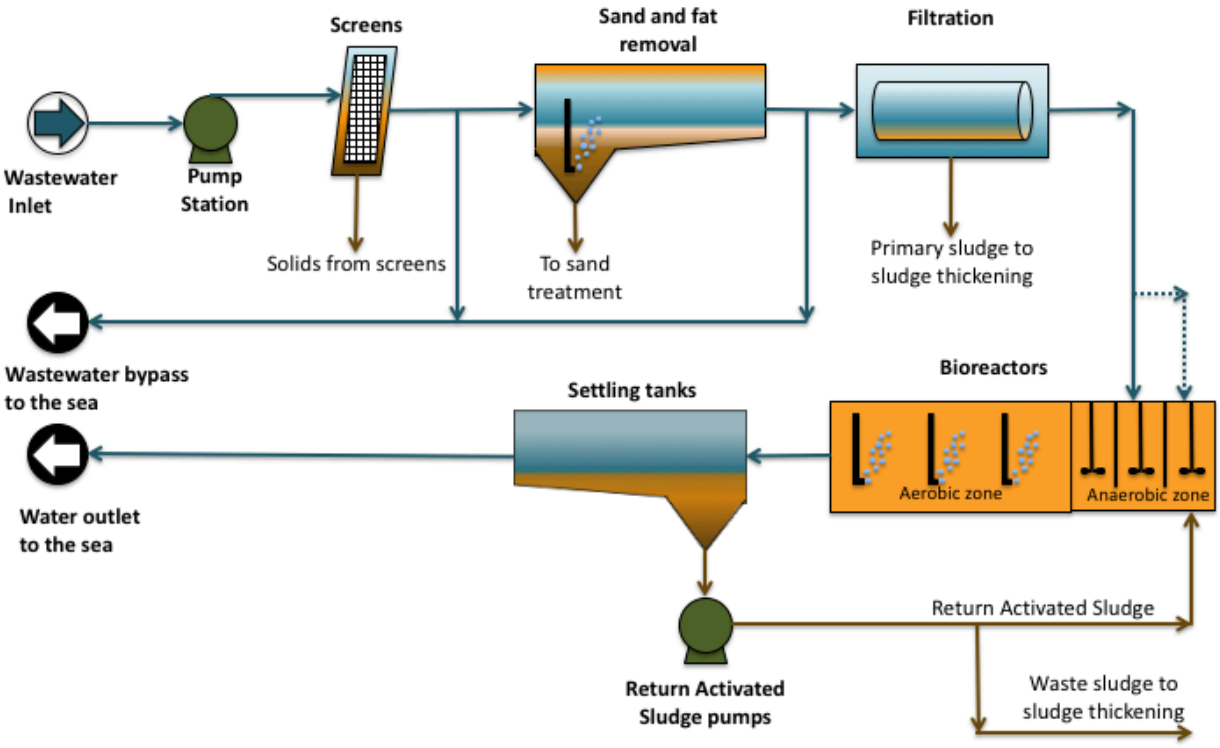

Figure 3-1: Schematic of the wastewater treatment process at SNJ.

The following process description is based on the feasibility study conducted by Norconsult (2013) and the SNJ process operation manual (Norconsult, 2018). Primary treatment at SNJ

consists of screens (Escamax 6mm), grit and fat removal and 20 Hydrotech drum filters with 100 µm pores. The drum filters remove particulate matter as primary sludge, and this reduces the suspended solids content of the wastewater with approximately 60%. The primary sludge is removed and thickened to 5-6 % TS in a drum thickener with addition of polymer.

From the drum filters, the wastewater flows to the bioreactors, which is the main treatment step. Upon completion of stage 2, the flow will be split between four treatment lines with one bioreactor each. In stage 1 treatment lines L1, L2 and L3 are operative. The bioreactors consist of an anaerobic zone and an aerobic zone. The anaerobic zone is divided into three chambers, An 1 (500 m^3), An 2 (950 m^3) and An 3 (950 m^3). The aerobic zone (5500 m^3) is sectioned so that the aeration is highest at the inlet of the zone, gradually decreasing towards the effluent. The main inlet to the bioreactor is to the second anaerobic chamber, while the first anaerobic chamber receives return sludge from the settling tanks. It is possible to direct a wastewater side stream into the first anaerobic zone to provide substrate for denitrification in case of nitrate being present in the return sludge.

The wastewater flows from the bioreactor to the settling tanks. There are four settling tanks for each bioreactor. Here, the sludge settles while the clarified water overflows to the effluent. Sludge is recycled back to the anaerobic zone of the bioreactor to ensure sufficient microbial population, while a portion of the sludge is wasted. The waste sludge is thickened in the same manner as the primary sludge, and all sludge is collected and treated in anaerobic digesters for stabilization and biogas production. The sludge from the anaerobic digester is then dewatered and dried, before it is used for fertilizer production. Reject water produced during thickening and dewatering is recycled back to the treatment plant.

Table 3-1 presents the average operating conditions in the bioreactors at SNJ during the first quarter of 2018.

Table 3-1: Average operating conditions in the bioreactors during the first quarter of 2018.

Q_{in}	MLSS	MVSS	SVI	Q_{RAS}		SRT	F/M-ratio
(m^3/h)	(mg/l)	(mg/l)	(ml/g)	(m^3/h)	(%)	(d)	(kg COD/kg MLSS d)
1800	2070	1550	68	455	25	6.3	0.21

3.2 Sampling in the wastewater treatment plant

All samples used in this research were collected at SNJ wastewater treatment plant. As the tanks in the bioreactors were considered completely mixed, the activated sludge samples from the anaerobic (An 1, An 2, An 3) and aerobic (Aer 1, Aer 2) zones were collected as grab samples. Samples from the treatment plant inlet, bioreactor inlet and plant effluent were collected by mixing three grab samples of equal volumes to reduce the risk of random errors due to uneven distribution of solids. The return activated sludge (RAS) was collected as grab samples in the return sludge pipeline, as the return flow was considered continuous, turbulent and uniform.

Mapping of the phosphate flow through the wastewater treatment plant was done through a series of sampling campaigns carried out during normal operating conditions at SNJ. All samples were collected at the same positions in bioreactor L3, see Appendix A. Table 3-2 shows the dates of and conditions during sampling.

Table 3-2: Weather conditions, process temperature, inlet flow rate (Q), pH, conductivity and DO during sampling campaigns carried out at SNJ.

Date	Q (m^3/h)	pH	Conductivity (mS/cm)	DO (mg/l)	Temperature (°C)	Weather conditions
06.02.18	4360	7.79	1.59	n.a.	8.5	Heavy rainfall 4-5 days prior to sampling.
21.02.18	4884	7.17	2.52	7.6	8.0	Some rainfall in the days prior to sampling.
07.03.18	4026	6.92	5.98	2.2	8.5	Following a long period of sub-zero temperatures and dry weather.

n.a.: not available.

Samples were immediately brought to the process lab at SNJ for further analysis or conservation with 4M H_2SO_4. In samples where the filtrate was of interest, a Thermo Scientific Megafuge 8 centrifuge was utilized for solids separation prior to filtration through a 47 mm Whatman GF 6 filter. Samples collected for total COD (COD_{tot}) and total phosphorous (P_{tot}) were homogenized with a Ultra-Turrax T25 (Janke & Kunkel IKA®-Labortechnik) at 8000 rpm.

Special consideration was taken when collecting activated sludge samples for PO_4-P analysis. To ensure minimal PO_4-P release from the activated sludge between sampling and analysis/conservation, these samples were allowed to settle and the supernatant was filtered immediately upon arrival in the lab. Samples were collected from the aerobic zone first, so that the anaerobic samples had the shortest timespan between sampling and filtration and analysis or conservation.

3.3 Experimental setups

Both primary sludge fermentation and phosphate release tests were conducted in anaerobic batch reactors with plastic or styrofoam beads on the surface to minimize gas exchange through the air-water interface. The concentration of DO in the bulk liquid was measured at appropriate intervals during the tests.

Primary sludge fermentation

Primary sludge was collected from the filter units and concentrated by settling and decanting to achieve approximately 1 – 1.5 % TS. The concentrated sludge was homogenized, transferred to a flask and placed on a magnetic stirrer. Samples were collected with a syringe at appropriate intervals. VFA-concentration, alkalinity, filtered COD, PO_4-P and NH_4-N were analysed on the samples, as well as TS, TSS, VSS, TDS, conductivity, DO and pH. Total COD was measured at the start and end of test 4. All four fermentation tests were performed without temperature control, starting at 8-10°C, gradually increasing to ambient temperature (approx. 20°C). Table 3-3 gives an overview of the durations and experimental conditions of the tests.

Table 3-3: Durations and initial experimental conditions of the fermentation tests.

			Initial experimental conditions					
Test no.	Date	Duration (d)	Volume (l)	TS (%)	pH	T (°C)	DO (mg/l)	Conductivity (mS/cm)
1	29.01.18	2	2 x 0.25	0.95	7.30	9	n.a.	n.a.
2	06.02.18	10	1.1	0.97	7.38	9	5.15	1.83
3	19.02.18	7	1.1	0.94	7.27	10	1.6	2.20
4	27.02.18	13	2.1	1.46	7.51	11	0.6	2.21

Phosphate release tests

Activated sludge was collected from the L3 bioreactor effluent and concentrated to 4000 – 7000 mg/l TSS to achieve higher PO_4-P concentrations during the tests. Samples were collected with a syringe at appropriate intervals, and immediately centrifuged and filtered prior to conservation. The experimental conditions of the tests are presented in table 3-4.

Table 3-4: Durations and experimental conditions of the phosphate release test.

				Initial experimental conditions					
Test no.	Date	Time (hrs)	VFA addition	Volume (l)	TSS (mg/l)	DO (mg/l)	T (°C)	pH	Conductivity (mS/cm)
1	24.01.18	23.7	no	2 x 1.0	1643/6835*	n.a.	8.5**	6.7	n.a.
2	31.01.18	2.8	yes	2 x 0.9	6803	0.79	11.2**	6.7	n.a.
3	13.02.18	72	no	2 x 0.9	1709/4636*	2.57	8.2	7.5	4.59
4	22.03.18	4.5	yes	4 x 0.8	6971	0.55	8.5	6.7	2.54

*TSS of activated sludge/concentrated activated sludge; ** carried out without temperature control.

Tests 1 and 3 were comparative tests with regards to endogenous phosphate release from activated sludge directly from the bioreactor, and concentrated activated sludge. The tests were carried out in 1 litre flasks on magnetic stirrers. Test 1 was performed with no temperature control and a gradual increase from 9°C to 23°C, while test 3 was carried out at approx. 9°C (process temperature).

Tests 2 and 4 compared the endogenous release and the release stimulated by substrate addition (VFA) in the concentrated activated sludge. In these tests, filtrate of fermented primary sludge (from fermentation tests 1 and 4, respectively) was added to stimulate excess phosphate release. The pH of the filtrate was adjusted to approx. 7 with 1 M NaOH prior to addition. Test 2 was conducted without temperature control. One flask was kept as an endogenous control, while the other received 150 ml fermented substrate. Test 4 was carried out using a jar tester, and temperature control at 9°C by water baths. The jars were added 0, 15, 40 and 70 ml of fermented substrate, respectively.

3.4 Analytical procedures

Solids analyses

TSS was determined by filtrating samples through a 47 mm Whatman GF/C filter with 1 μm pores and drying at 105 °C in a Fermaks TS9053 drying oven. TS was determined by drying a known amount of sample in an aluminium dish over night. For determination of TDS, the filtrate from the TSS analysis was treated in the same manner as the TS samples. VSS was determined after combustion at 550 °C for 20-30 minutes in a Carbolite Furnaces CSF 1100 muffle oven. All samples were weighed on a Satorious Basic B 120 S scale. Duplicate analyses were conducted, providing there was space available in the drying oven. The solids analyses procedures were adapted from standardized methods for wastewater analysis (Clesceri, Greenberg, & Eaton, 1998) and IVAR internal procedures.

The filter loss of the GF/C filters during drying and combustion were determined for each new batch of filters, and all results were adjusted accordingly. See Appendix B for details.

VFA and alkalinity

VFA and alkalinity was determined by a 5 point titration procedure with HCl (0.05 M) to pH 6.7 ± 0.1, 5.9 ± 0.1, 5.2 ± 0.1, and 4.3 ± 0.1, in accordance with Moosbrugger et al. (1993). If the initial pH of the sample was lower than 6.6, it was adjusted to 6.7 ± 0.1 with NaOH (0.05 M). Prior to titration, the samples were centrifuged and filtered, and if necessary diluted with distilled water. A total volume of 50 ml was used for each titration. The titration data was entered into the computer programme TITRA 5, yielding the VFA concentration as HAc, and the $CaCO_3$ alkalinity. The titration to different end points considered the buffer capacities of carbonates and VFAs. In addition, the TITRA 5 programme included the weak acid/base interactions from phosphate, ammonium and sulphate, if present. The method was developed by Moosbrugger et. al (1993), and a verification of the method is included in Appendix C.

During titration, pH was measured with a VWR pHenomenal 1100 L pH meter with a VWR pHenomenal LS221 probe. The instrument was calibrated weekly with pH 4, 7 and 10 buffers.

Total and filtered COD, NH4-N, PO4-P and total P

All the following analyses were in accordance with the internal procedures used by SNJ laboratory staff, which are in compliance with Norwegian standards for wastewater analysis.

COD, NH_4-N, PO_4-P and P_{tot} were determined spectrophotometrically with a Spectroquant Prove 300 spectrophotometer. The spectrophotometer was zero adjusted with Merck Spectroquant Zero Cell prior to each analysis series, as well as a reagent blank configuration against a test cell with deionized water and reagent added. Prior to COD_{tot} and P_{tot}, the samples were homogenized as described in section 3.2.

Depending on the presumed COD concentration of the test series, either Merck Spectroquant COD Cell Test kit with a range of 10 – 150 mg/l COD (product no. 114540) or 25 – 1500 mg/l COD (product no. 114541) were used. As described in the COD test kit procedures, the test cells contained a sulphuric solution of potassium dichromate ($K_2Cr_2O_7$) with silver sulphate as a catalyst, that oxidized the sample during digestion at 148°C for 120 minutes. A sample volume of 3.0 ml was added to each cell, mixed and placed in a Spectroquant TR420 Thermoreactor for digestion. After digestion the samples were cooled for 10 minutes in the reactor, then mixed before cooling to room temperature. The 10 – 150 mg/l test cells were read at 445 nm wavelength, determining the amount of unconsumed yellow $Cr_2O_7^{2-}$ ions. The concentration of green Cr^{3+} ions was determined at 605 nm for the 25 – 1500 mg/l cells. The spectrophotometer yielded the COD concentration based on the amount of residual reagent, as 1 mol of $K_2Cr_2O_7$ was equivalent to 1.5 mol O_2.

NH_4-N was determined using a Merck Spectroquant Ammonium Cell Test with a range of 4.0 – 80.0 mg/l NH_4-N (product no. 114559). Samples were filtered and diluted if necessary, before 0.1 ml of the sample was added to the alkaline test cell solution together with one dose of the enclosed NH_4-1K reagent. After 15 minutes, the concentration of blue indophenol derivate formed between the reagent and the ammonium in the sample was determined spectrophotometrically.

PO_4-P was analysed by adding 10 ml filtered sample (diluted or undiluted) to an empty Spectroquant 16 mm test cell, adding 400 µl of ascorbic acid and 400 µl of molybdate reagent, and mixing. The acidic molybdate reagent, containing ammonium molybdate and antimonyl tartrate formed phosphomolybdic acid with PO_4-P in the sample. This was

reduced by ascorbic acid to molybdenum blue, and the absorbance was measured at 880 wavelength nm 10 minutes after reagent addition.

To determine P_{tot}, the particulate phosphorus was converted to phosphate by oxidation in a pressure cooker with the addition of an oxidant solution. The oxidant solution was prepared by dissolving 5 g of potassium persulfate ($K_2S_2O_8$) in 100 ml of distilled water. 2 ml of the oxidant was added to 10 ml of homogenized sample in a plastic scintillation tube, and cooked for 30 minutes in a pressure cooker. The sample was then cooled to room temperature prior to addition of 400 µl of ascorbic acid and 400 µl of molybdate reagent. 30 minutes after reagent addition, the sample was transferred to an empty Spectroquant 16 mm test cell, and the phosphate concentration was measured photometrically at 880 nm.

The ascorbic acid and the molybdate reagents used in the PO_4-P and P_{tot} analyses were prepared by the laboratory staff at SNJ.

pH, Conductivity, DO

A portable WTW Multi 340i pH/Conductivity/O_2 meter was used for determination of pH, DO and conductivity. An overview of probes and calibration intervals can be found in table 3-5.

Table 3-5: Overview of probes used with WTW 340i pH/conductivity/O_2 meter.

Analysis	Probe used	Calibration interval
pH	WTW SenTix 41	Weekly with pH 4 and 7 buffer solutions
Conductivity	WTW CellOx 325	Weekly with a standard KCl solution
DO	WTW TetraCon 325	Calibrated prior to each use

As a part of the results verification, the accuracy of the WTW TetraCon 325 membrane oxygen probe was controlled with an Oxi 3315 oxygen meter with an optical WTW FDO 925 probe. The results are presented in Appendix D.

4 Results and discussion

In this chapter, the results of the experimental work conducted during this project are presented and discussed. Section 4.1 concerns the results of the sampling campaigns in the wastewater treatment plant, 4.2 deals with the primary sludge fermentations, and the results from the lab scale PO_4-P release tests are presented and discussed in section 4.3. In section 4.4, the possibility of controlled struvite precipitation at SNJ is assessed theoretically. Section 4.5 presents the mass balances over the compartments of the bioreactor, while error analysis is included in section 4.6.

4.1 Mapping of phosphate release in the bioreactor

Wastewater characteristics

Table 4-1 shows the average values and standard deviations of the main parameters analysed during the sampling campaigns at SNJ.

Table 4-1: Average and standard deviations of flow rates (Q), TSS, P_{tot}, PO_4-P, filtered COD, DO and pH in treatment line L3.

Position	Q (m^3/h)	TSS (mg/l)	DO (mg/l)	pH	P_{tot} (mg/l)	PO_4-P (mg/l)	COD_{filt} (mg/l)
Inlet L3	2069 ± 320	122 ± 41	6.6 ± 1.4	7.4 ± 0.4	2.92 ± 0.7	1.51 ± 0.5	56 ± 12
An 1	552 ± 89	5528 ± 944	0.6 ± 0.2	6.9 ± 0	97.2 ± 9.4	3.90 ± 1.4	48 ± 11
An 2	2621 ± 409	898 ± 140	2.5 ± 1.2	7.1 ± 0.1	15.4 ± 0.7	1.51 ± 0.3	55 ± 34**
An 3	2621 ± 409	2036 ± 218	0.7 ± 0.1	7.0 ± 0.1	35.4 ± 3.6	1.92 ± 0.5	46 ± 13
Aer 1	2621 ± 409	1913 ± 192	2.4 ± 1.1	7.0 ± 0.1	31.4 ± 3.4	0.97 ± 0.5	49*
Aer 2	2621 ± 409	1885 ± 231	2.3 ± 1.4	7.0 ± 0	32.0 ± 1.8	0.78 ± 0.4	42 ± 10
Effluent	2069 ± 320	30.5 ± 11.4	n.a.	7.1 ± 0.2	3.24*	1.57 ± 0.8	43*
RAS	552 ± 89	3877 ± 886	n.a	7.1 ± 0.1	69.1 ± 9.8	2.78 ± 1.4	63*

* parameter only measured 07.03; **average of 21.02. and 07.03; n.a.: not available

Two treatment lines (L2 and L3) were operative during sampling, and the inlet flow was split between them. The flow data was collected for treatment line L3 only, as all sampling was carried out in L3. The average flow data on each sampling date was obtained from SNJs digital process control system AIM. The flow through the bioreactor was equal in all positions except An 1, which received the RAS flow only. The subsequent sections in the bioreactor received the combined flow of the RAS and the L3 inlet.

The data in table 4-1 generally display high standard deviations. This may be explained by sampling being carried out at different weather conditions. The inlet flow rate at SNJ is regulated by the amount of wastewater received at the treatment plant, and is higher during rainfall. Besides contributing to larger volumes of water, heavy rainfall will dilute the wastewater. The two sampling campaigns in February were carried out at dry weather conditions, but were influenced by rainfall prior to sampling. Sampling in March was carried out after a long period of sub-zero temperatures and dry weather. Consequently, the inlet wastewater was less dilute in March compared to February. The conductivity of the inlet wastewater indicates the level of dilution, and table 3-2 shows increasing conductivity consistent with the weather conditions in the sampling period. Parameters such as pH, inlet TSS, PO_4-P and filtered COD are influenced by dilution.

It is likely that more frequent sampling over a longer period of time would have yielded more even results with lower standard deviations. However, unstable operational conditions in the treatment plant could also have affected the results of the sampling campaigns. As the biological treatment commenced only six months prior to this project, the overall operation of the plant was periodically influenced by finalization of the construction work and testing of process units. Even though sampling was carried out at times when the operation of the plant was stable, there were several periods in February and March where the inlet water was held back. This caused instability in the overall process, an may be reflected in the results of the sampling campaigns. The results are considered representative for the period of February and March, but not necessarily for the WWTP as it is today.

Table 4.2 presents detailed data of the flow, PO_4-P and TSS in each compartment of the L3 bioreactor during sampling.

Table 4-2: Flow, PO_4-P and TSS of each compartment of the L3 bioreactor during sampling.

Position	V	06.02.2018			21.02.2018			07.03.2018		
		Q	PO_4-P	TSS	Q	PO_4-P	TSS	Q	PO_4-P	TSS
	(m^3)	(m^3/h)	(mg/l)	(mg/l)	(m^3/h)	(mg/l)	(mg/l)	(m^3/h)	(mg/l)	(mg/l)
Inlet	-	1702	2,08	173	2212	1,06	94	2292	1,40	100
An 1	500	449	2,90	5287	603	3,30	4615	604	5,50	6682
An 2	950	2151	1,34	897	2815	1,30	743	2896	1,90	1055
An 3	950	2151	1,58	1948	2815	1,68	1856	2896	2,50	2304
Aer 1*	2750	2151	0,58	1734	2815	0,80	1857	2896	1,52	2148
Aer 2*	2750	2151	0,50	1683	2815	0,60	1798	2896	1,25	2174
RAS	-	449	1,90	3574	603	2,03	3087	604	4,40	4982

*The total volume of the aerobic zone is 5500 m^3

Not all the data in table 4-2 were as expected. The TSS of the RAS and in An 1 should be within the same range, since An 1 receives RAS only. However, the TSS measured in An 1 was approximately 30 % higher. In addition, the TSS measured in An 2 was between 50 % and 70 % lower than the TSS measured in An 3. The TSS of An 2 and An 3 should also have been similar. Although An 2 receives the inlet wastewater and some dilution is expected, the deviations are too high to be explained by dilution alone. These discrepancies are further discussed in chapter 4.5.

The calculated COD/P ratios of the inlet to the L3 bioreactor during sampling are presented in table 4-3. The samples were collected at the effluent of the Hydrotech drum filters, which is the inlet water to the bioreactors.

Table 4-3: COD/P ratio of the L3 bioreactor inlet wastewater.

Date	COD_{filt} (mg/l)	PO_4-P (mg/l)	COD/P (mg/mg)
06.02.18	63	2,08	30
21.02.18	37	1,06	35
07.03.18	59	1,40	42

The filtered COD and PO_4-P was highest on 06.02.18, even though the inlet to the wastewater treatment plant was most dilute at that time. A possible explanation for this is that the sampling on this date was carried out around mid-day, while sampling on the other dates was carried out in the morning. The wastewater at SNJ is more dilute in the morning,

and concentrations of both filtered COD and PO_4-P are expected to increase during the day. The COD/P ratio of the L3 bioreactor inlet varied between 30 and 42 mg COD/mg P during February and March, and increased as the inlet wastewater became more concentrated. This indicates that the COD/P ratio was affected by dilution of the inlet wastewater due to rainfall. For the purpose of calculating the COD/P ratio, the fractions of biodegradable and inert filtered COD were not specified. However, the filtered COD available for EBPR will be lower than the inlet filtered COD due to the inert fraction.

As described in section 2.1.3, Oehmen et al. (2007) states that the COD/P ratio should be lower than 50 mg/mg to favor PAO growth over GAOs. The ratios measured at SNJ are below this value, and within the range reported by Gu et al. (2008). They observed stable and efficient EBPR performance at ratios between 25 and 38 mg COD/mg P when investigating the PAO/GAO population fractions at several wastewater treatment plants. At these ratios, GAOs were observed, but PAOs were dominating. The combination of a COD/P ratio which favors PAOs, the low temperature of the wastewater and an average pH of 7 makes it unlikely that a significant GAO population has established at SNJ.

Table 4-4 shows the HRT calculated for each of the anaerobic zones and the aerobic zone of the L3 bioreactor. The calculation of HRT was based on the respective flow rates and volumes of the anaerobic and aerobic sections (table 4-2). The HRTs of all compartments except An 1 were calculated using the combined flow of the inlet and RAS. The HRT in An 1 depends on the RAS flow rate, as this compartment receives return sludge only.

Table 4-4: HRT in the zones of the L3 bioreactor during sampling.

Date	An 1 (h)	An 2 (h)	An 3 (h)	Aerobic (h)	Total (h)
06.02.18	1.13	0.44	0.44	2.56	4.57
21.02.18	0.83	0.34	0.34	1.95	3.46
07.03.18	0.83	0.33	0.33	1.90	3.39

An 1 has the highest HRT of the anaerobic reactors, and it is likely that COD in form of VFA is produced here as a result of fermentative activity. As shown in table 4-1 , the average filtered COD measured in An 1 was 48 mg/l, while the average concentration measured at the bioreactor effluent was 42 mg /l. As An 1 does not receive COD from an external source,

this indicates COD production in either An 1, the return sludge, or both. An average filtered COD of 63 mg/l was measured in the RAS on 07.03.18, while the An 1 concentration was 53 mg/l on the same date. At the same time, PO_4-P concentration was 5.50 mg/l in An 1 and 4.40 mg/l in the RAS, indicating COD consumption and phosphate release consistent with PAOs.

An 2 and An 3 have equal HRTs, and receives the same flow. The combined retention time of the two is slightly lower than the retention time in An 1. The total anaerobic retention time in the bioreactor is 1.5 – 2 hrs, while the aerobic HRT is 2 – 2.5 hrs. Based on typical HRT values for EBPR (Tchobanoglous et al., 2014), the overall retention time is regarded as sufficient for EBPR at SNJ. However, the long retention time in An 1 compared to An 2 and An 3 may lead to secondary phosphate release due to lack of external COD.

EBPR activity in the bioreactor

Figure 4-1 shows the PO_4-P concentrations at the different locations during the sampling campaigns in February and March 2018.

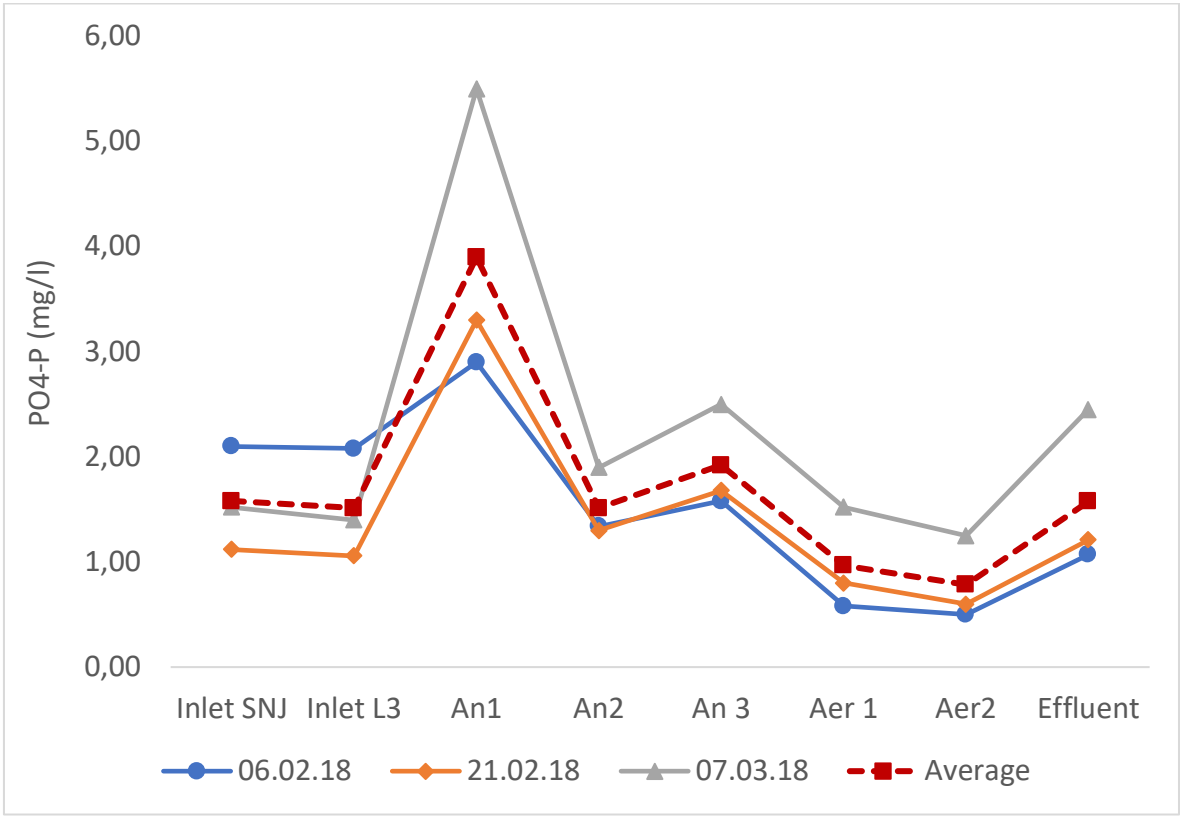

Figure 4-1: PO4-P concentrations through SNJ.

The PO_4-P concentration decreased from the anaerobic zone to the aerobic zone and bioreactor effluent (Aer 2), which indicates PO_4-P uptake by PAOs. All concentrations measured in Aer 2 were lower than the concentrations at the bioreactor inlet (Inlet L3). However, the PO_4-P concentration increased in the effluent, and the average effluent concentration 1.58 mg/l was equal to the average concentration measured at the WWTP inlet. This indicates that phosphate is released from the sludge in the settling tanks due to long retention time and ineffective sludge return and/or wasting. Historical data obtained from SNJ show that the sludge blankets in the settling tanks were high during February and March. Table 3-1 show that the SVI at SNJ was 68 ml/g during the sampling period, and the settling properties of the sludge was good. Thus, the high sludge blankets indicate accumulation of sludge rather than poor sludge settling. Although some fermentation will occur within the sludge in the settling tanks, the RBCOD will have been consumed in the bioreactor. The VFA yield would be low, and the PO_4-P release in the settling tanks was secondary. Significant secondary release in the settling tanks would reduce the poly-P reserves available for carbon storage in An 2 and An 3.

The PO_4-P concentrations measured in the bioreactor in March were significantly higher than the values measured in February, even though the inlet concentration was within the same range. The effluent concentrations from the bioreactor and the wastewater treatment plant effluent were 1.25 mg/l and 2.45 mg/l, respectively. Considering an inlet concentration of 1.52 mg/l, this indicates lower EBPR performance in March compared to February.

Table 4-5 shows the P/VSS ratio in the L3 bioreactor during sampling. P_{tot} was analysed in the samples collected at Aer 2.

Table 4-5: P/VSS ratio in L3 bioreactor during sampling.

Date	P_{tot} (mg/l)	VSS (mg/l)	P/VSS (mg/mg)
06.02.18	30.0	1279	0.023
21.02.18	32.4	1330	0.024
07.03.18	33.6	1730	0.019

Wentzel et al. states that the P/VSS ratio in an EBPR plant can increase to values between 0.06 and 0.15 mg/mg, while the P/VSS ratios measured at SNJ were between 0.019 and

0.024 mg/mg. These values are consistent with the metabolic requirement of the biomass, and may indicate low EBPR activity. The value decreases from February to March, possibly as a result of less phosphorous retention within the biomass. As shown in figure 4-1, the amount of phosphate released in the settling tanks increased from February to March. This would lead to lower poly-P reserves within the biomass, and it is likely that the reduction in P/VSS ratio was due to increasing secondary phosphate release in the settling tank.

The PO_4-P release and uptake rates per kg TSS per day through the bioreactor are presented in table 4-6 and figure 4-2. The positive values represent phosphate release to the wastewater, while the negative values represent phosphate uptake from the wastewater.

Table 4-6: PO_4-P release and uptake rates through the bioreactor.

	PO_4-P release/uptake rate (g P/kg TSS d^{-1})			
Position	06.02.18	21.02.18	07.03.18	Average
An 1	4,08	7,96	4,77	5,60
An 2	-55,20	-22,94	-24,60	-34,24
An 3	6,69	14,56	19,05	13,44
Aer 1	-10,82	-11,64	-11,53	-11,33
Aer 2	-0,89	-2,73	-3,14	-2,25

The rates are based on the data given in table 4-2, and the equations used for the calculation of PO_4-P release and uptake rates are included in Appendix E. As the total volume of the aerobic zone was 5500 m^3, it was assumed that the volumes of Aer 1 and Aer 2 were 2750 m^3 each.

The rates calculated for An 1 and An 3 were positive, while for An 2, Aer 1 and Aer 2 they were negative. The rates in all anaerobic sections were expected to be positive, and the negative values of An 2 indicate that the PAOs take up phosphate here rather than releasing it. At all sampling days the DO concentration in An 2 was above 1 mg/l, and table 4.1 shows an average of 2.5 mg DO/l in that position. This oxygen intrusion makes An 2 an aerobic rather than anaerobic reactor. Under such conditions the OHOs will not convert the fermentable COD to VFAs, but utilize it for energy and growth with oxygen as the external electron acceptor. Consequently, less VFAs will be available for the PAOs, which in turn will

affect the overall P-removal. As long as the DO concentration in An 2 remains at this level, it is likely that most of the inlet RBCOD at SNJ is consumed aerobically by OHOs.

The phosphate release in An 1 is most likely a combination of secondary release, and release related to the VFA production in the return sludge. The phosphate release in An 3 is higher than in An 1 due to substrate supplied from the inlet in An 2. Even though it is likely that most of the substrate is consumed aerobically in An 2, the phosphate release in An 3 indicate some VFA production due to fermentation of inlet COD. The relative uptake rates of Aer 1 and Aer 2 show that most of the phosphate is taken up in the beginning of the aerobic section. The high uptake rate indicates that the PHB utilization and PAO growth is at a maximum in Aer 1. In Aer 2, the uptake rate is significantly lower. This indicates that phosphate uptake in the last section of the bioreactor is limited due to exhaustion of intracellular PHB through the aerobic zone. From this one can assume that the overall phosphorous removal is limited by the level of VFAs available in the anaerobic zone, rather than HRT of the aerobic zone.

Figure 4-2 shows the trends of the phosphate release and uptake rates. It was observed that the rates follow the same patterns on all three sampling dates, with the highest uptake rate in An 2 and the highest release rate in An 3.

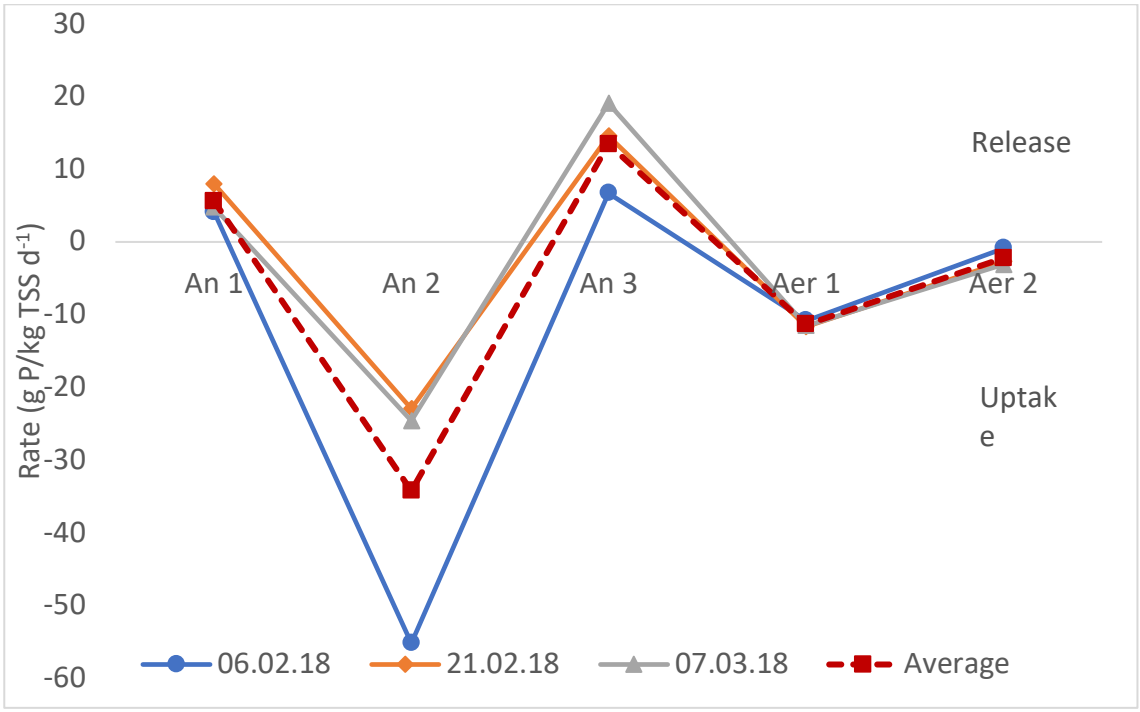

Figure 4-2: PO_4-P release and uptake rates through the bioreactor.

The rates in An 1, Aer 1 and Aer 2 are very similar, while there is more variation in the rates of An 2 and An 3. This variation could be explained by variation of DO and RBCOD in An 2. It was observed that a high uptake rate in An 2 was followed by a lower release rate in An 3. When DO concentrations were high, the phosphate uptake rates in An 2 were high as well. Under such conditions, the PAOs will regenerate the poly-P chains in An 2, while the OHOs will oxidize rather than ferment the RBCOD. Consequently, there will be less VFA available in An 3, and the phosphate release rates will be lower.

4.2 Primary sludge fermentation

This chapter presents the results of the four fermentation tests, with main focus on test 4 as this test was subject to the most detailed analysis. The main results of test 1 and 3 are briefly discussed, while some of the data from test 2 are used for comparison with test 4 .

VFA, COD, alkalinity and pH

Figure 4-3 shows the VFA production, alkalinity and pH during fermentation test 1.

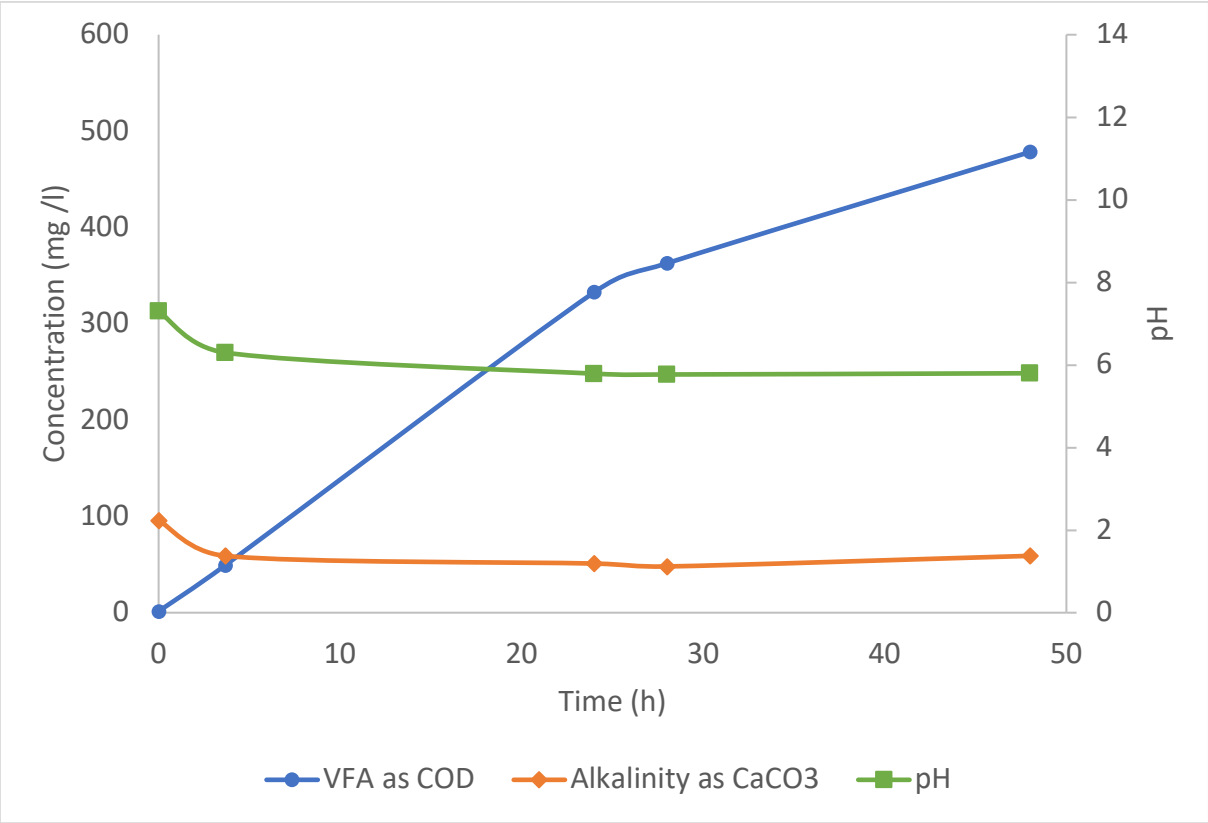

Figure 4-3: VFA, alkalinity and pH during fermentation test 1.

Filtered COD, NH_4-N and PO_4-P was not measured during this test. Filtrate from fermentation test 1 was used as the substrate in phosphate release test 2. The VFA yield after 48 hours was approx. 480 mg COD/l. It seemed that there was a slight increase in alkalinity towards the last sampling point. As this was the first fermentation test conducted, it is however likely that this increase was due to a random error during titration rather than an actual increase in alkalinity.

The VFA production, alkalinity and pH measured during fermentation test 2 are presented in figure 4-4.

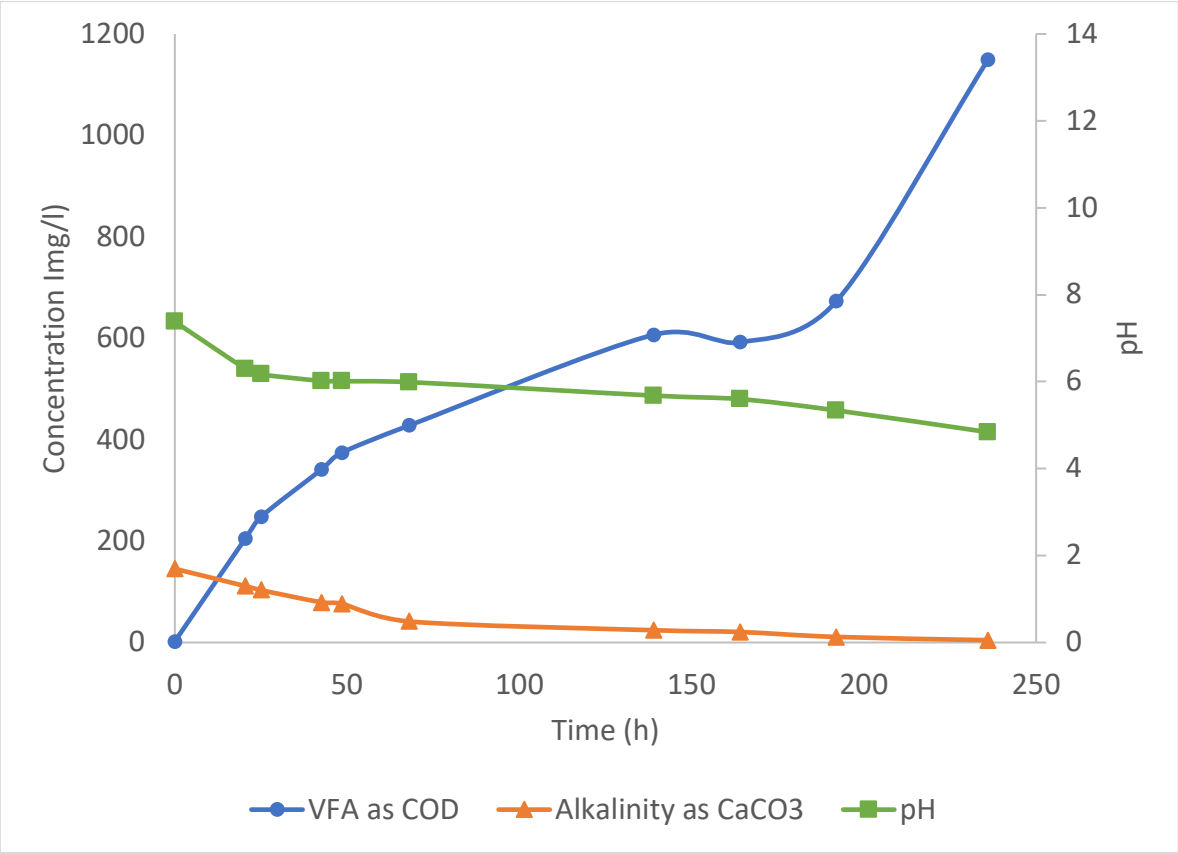

Figure 4-4: VFA, alkalinity and pH during fermentation test 2.

During fermentation test 2, it was observed that the VFA production increased towards the end of the test. This phenomena was observed in test 4 as well, and will be discussed later in this chapter. As expected, the pH and alkalinity was decreasing throughout the test.

Filtered COD, NH_4-N and PO_4-P was measured at the last four sampling intervals in test 2. The results are given in table 4-7.

Table 4-7: Filtered COD, PO4-P and NH4-N measured during fermentation test 2.

Time (h)	COD_{filt} (mg/l)	PO_4-P (mg/l)	NH_4-N (mg/l)
139	1298	21.8	55
164	1426	21.8	58
192	1620	20.6	60.5
236	2360	19.6	46

Both PO_4-P and NH_4-N concentrations were decreasing towards the end of this test, and might indicate precipitation of a substance containing both P and N. This is however discussed later in this chapter.

Figure 4-5 shows the VFA production, alkalinity and pH during fermentation test 3.

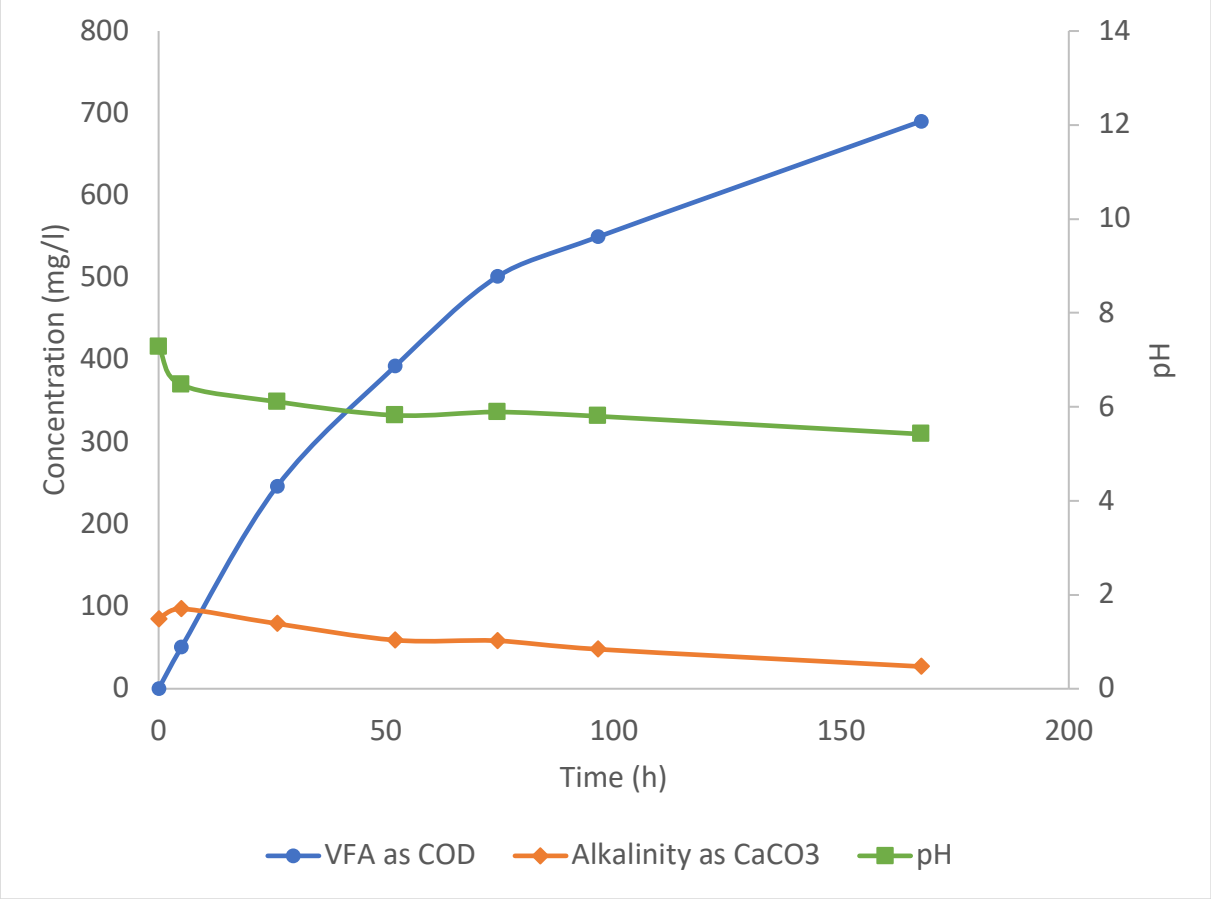

Figure 4-5: VFA, alkalinity and pH during fermentation test 3.

The analysis of fermentation test 3 was not completed due to problems with the magnetic stirrer. The stirrer stopped several times, causing the sludge to settle. As this happened

repeatedly, the samples collected during the test were considered not representative and no further analyses were conducted.

Even though the results of fermentation tests 1, 2 and 3 were not as thoroughly analysed as fermentation test 4, they all display similar rates of VFA production, alkalinity consumption and pH reduction.

Figures 4-6 show the VFA production and filtered COD during primary sludge fermentation test 4, and figure 4-7 show alkalinity and pH during the same test.

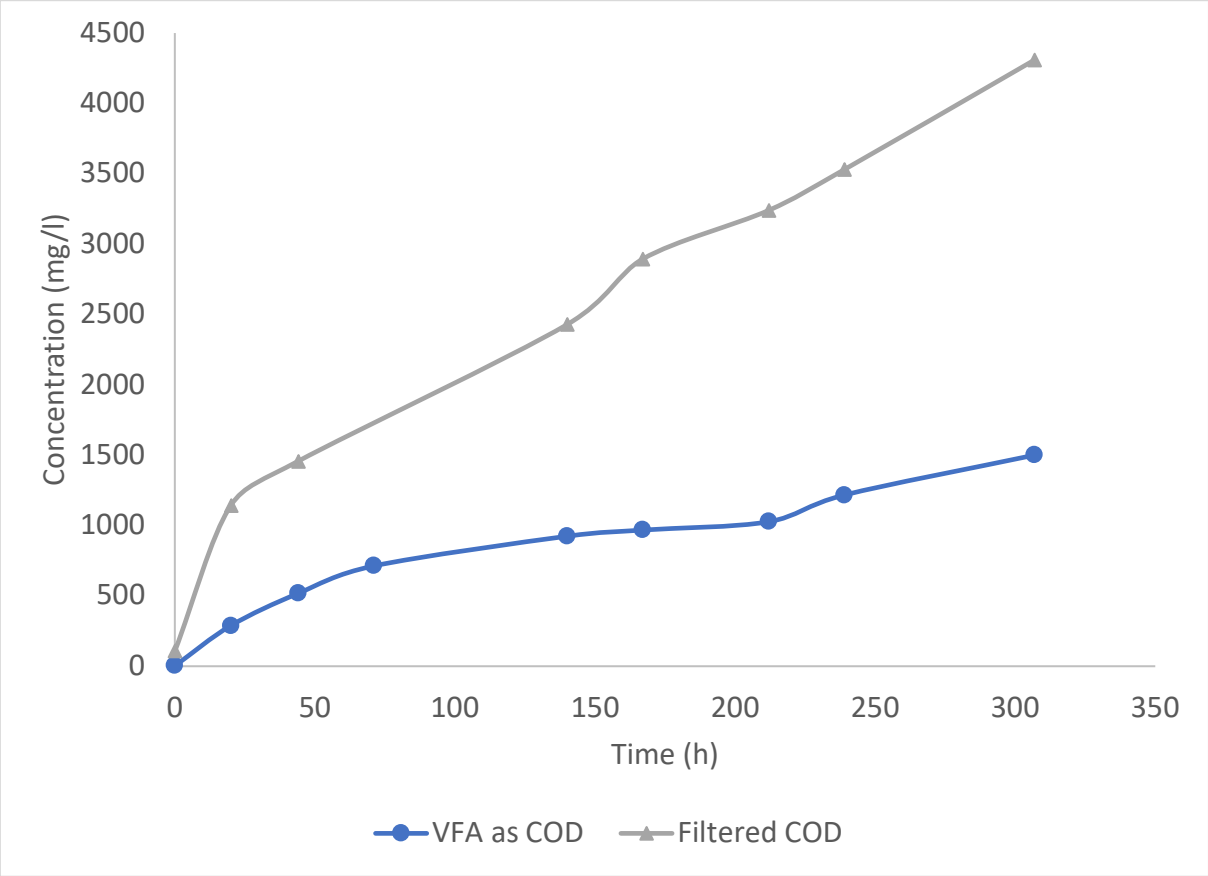

Figure 4-6: VFA and filtered COD during fermentation test no. 4.

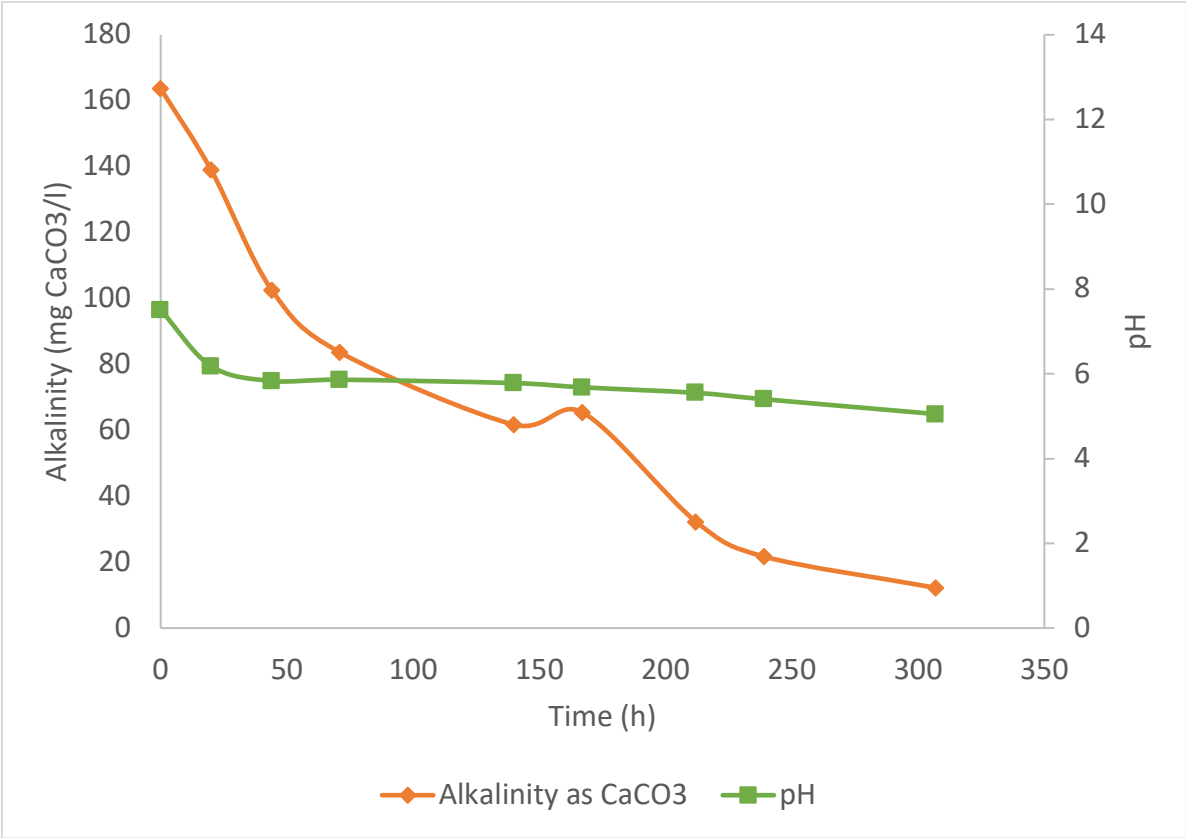

Figure 4-7: Alkalinity and pH during fermentation test 4.

In addition to the parameters shown in figures 4-6 and 4-7, TS, TSS, VSS, TDS, conductivity and DO were measured at each sampling interval. The degradation of organic matter releases ions into the liquid, and it was observed that the conductivity increased with VFA production during the fermentation. DO was measured between 0.4 mg/l and 0.7 mg/l at each sampling interval during the test, initially indicating some oxygen intrusion into the batch reactor. Similar DO concentrations were measured in all anaerobic tests. However, at the end of the experimental period of this project, the accuracy of the membrane oxygen probe used in all measurements was compared to an optical oxygen probe. The optical probe has a higher accuracy at lower DO concentrations, and it was confirmed that the membrane probe generally displayed higher and more unstable values than the optical probe at low concentrations. It is therefore likely that the values measured during anaerobic tests were lower than the actual reading on the oxygen meter. The findings in the accuracy test of the membrane probe are included in Appendix D.

A discrepancy between the COD contribution of VFA produced and the filtered COD measured during primary sludge fermentation was observed in both fermentation test 2 and 4. The filtered COD/VFA (mg COD/mg COD) ratios were approx. 2.3 and 2.9, respectively. The total VFA concentration was determined by titration, and the calculation yielded the result as mg HAc/l. Even though the composition of the VFAs was not identified during this project, it is assumed that other acids besides HAc were produced during fermentation. Considering the VFA fractions found in other studies (table 2-4), batch fermentation of primary sludge generally yields an average of 30 % propionic acid. The COD equivalent of HAc is 1.07 mg COD/mg HAc, while the COD equivalent of HPr is 1.53. Thus, if a significant fraction of HPr was produced, the COD contribution of the VFAs would be higher. In table 4-8, the results of test 2 and 4 were adjusted for 30 % HPr and the unidentified filtered COD fraction determined.

Table 4-8: The COD equivalents of VFA production in fermentation test 2 and 4 and corresponding unidentified COD.

	Measured		Adjusted			
Test no.	VFA (mg HAc/l)	COD_{filt} (mg/l)	HAc (mg/l)	HPr (mg/l)	COD_{VFA} (mg/l)	Unidentified COD (%)
2	1074	2360	752	322	1297	45
4	1403	4310	982	421	1695	61

After adjusting for 30 % HPr, there was still a considerable amount of the filtered COD that was not VFAs. It is likely that the unidentified filtered COD fraction was fermentation products other than VFAs, such as ethanol and lactate.

In figure 4-8 the VFA production during fermentation tests 2 and 4 is compared. The initial TS of the sludge used in the tests were 0.97 % and 1.46 %, respectively. The TS indicates the level of substrate available for hydrolysis, and may explain why the overall VFA production is lower in test 2 than test 4.

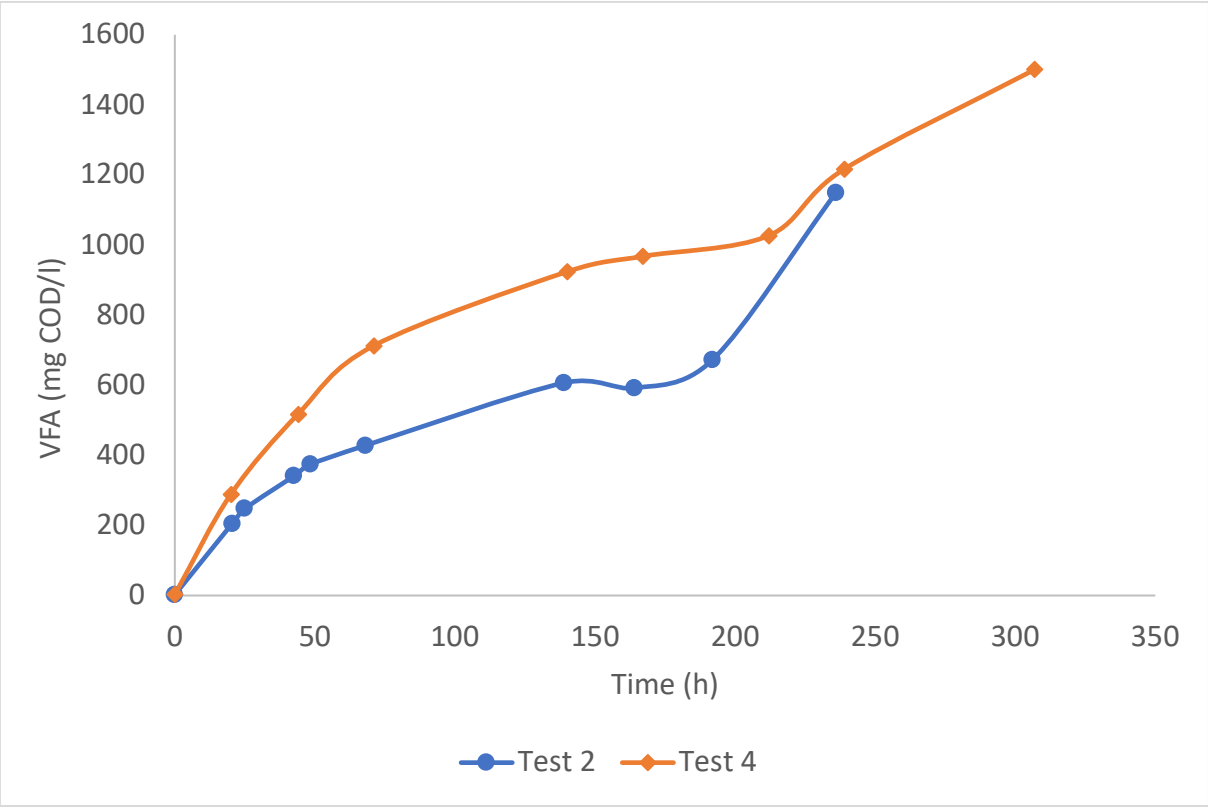

Figure 4-8: VFA production as mg COD/l in fermentation tests 2 and 4.

During both tests the VFA production started to level off after approx. 140 hrs (6 days). This initial decrease in VFA generation rate was then followed by an acceleration towards the end of the tests. This sudden increase in VFA production was not expected.

If DO was present in the sludge during the tests, the observation could have been explained by aerobic organisms initially consuming the VFA produced. As pH decreased, the aerobes may have been inhibited, and the fermentative bacteria would have become more dominant. As previously discussed, some DO was measured during the test, but the actual concentration was probably significantly lower than the measured concentration. It is therefore unlikely that the sudden increase in VFA production was due to aerobic consumption of VFA at the start of the test.

Another possible explanation for this phenomena is that methanogens were present in the primary sludge, and initially consumed some of the acetate produced. As pH declined, the methanogenic activity would have stopped due to inhibition by more acidic conditions, resulting in accelerated VFA production. However, total COD was measured at 19.1 g/l both

at the start and the end of fermentation test 4, indicating that no COD was removed due to methane gas escaping the liquid. It is assumed that the COD was conserved in test 2 as well. The indigenous population of methanogens in municipal wastewater is low, and the establishment of an active methanogenic biomass requires suitable conditions and time. Considering the that the pH was at approx. 6.2 after 24 hrs in both tests (figure 4-9), and an initial temperature of approx. 10°C, it was considered unlikely that there was any methanogenic activity during the fermentation tests.

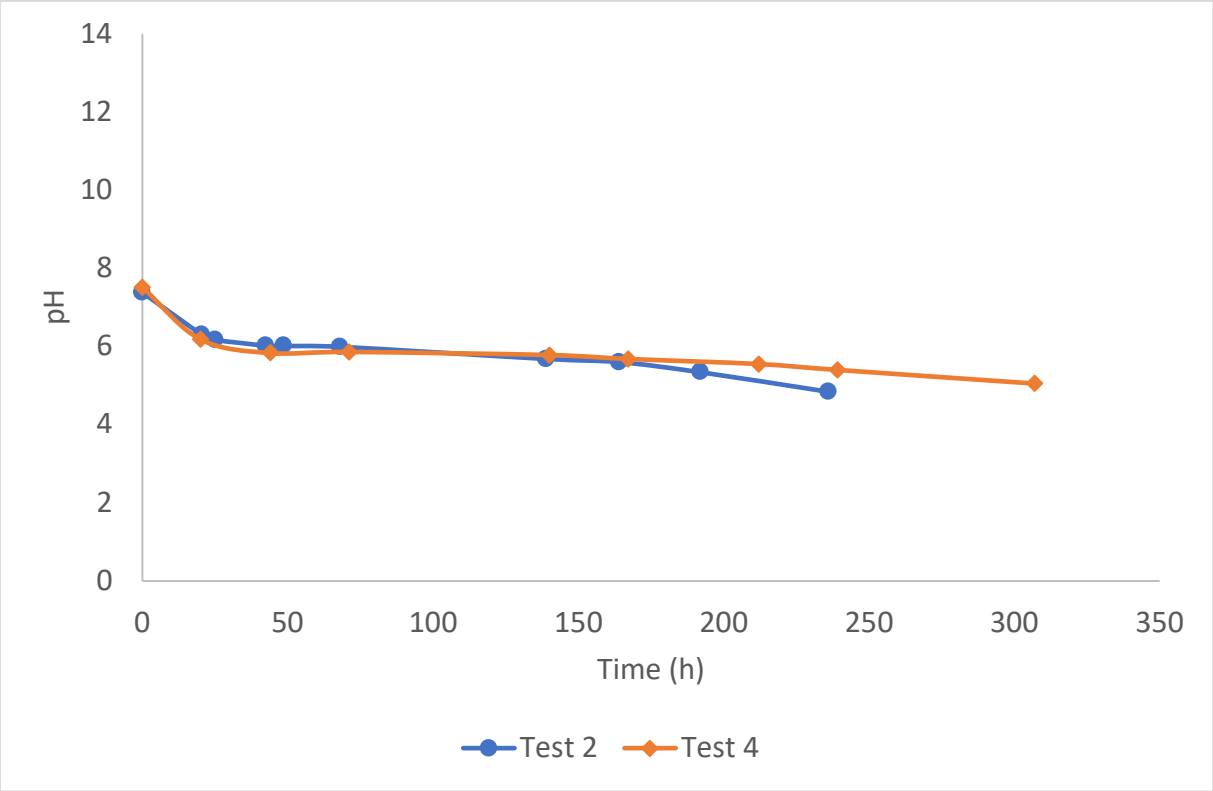

Figure 4-9: pH measured during fermentation tests 2 and 4.

It is more likely that the sudden increase in VFA production was due to the conversion of non-VFA fermentation products. As stated by Madigan et al. (2009), there are several fermentative bacteria that can convert lactate and ethanol to VFAs. Lactic acid is a stronger acid than both acetate and propionate, with a pK_a of 3.86 versus 4.76 and 4.86, respectively. Consequently, the conversion of lactate to VFA would not cause the pH to decrease further. The conversion of ethanol to VFAs would however lower the pH, as ethanol is neutral while the product would be acidic. As shown in figure 4-9 the pH continues to decrease throughout both test 2 and 4. At the same time the filtered COD generation curve in test 4

was nearly linear (figure 4-6). This indicates conversion of existing COD rather than an increasing filtered COD production rate, and the increasing VFA generation is considered a result of conversion of ethanol to VFA.

Nitrogen and phosphorous

Figure 4-10 shows NH_4-N and PO_4-P during primary sludge fermentation test 4.

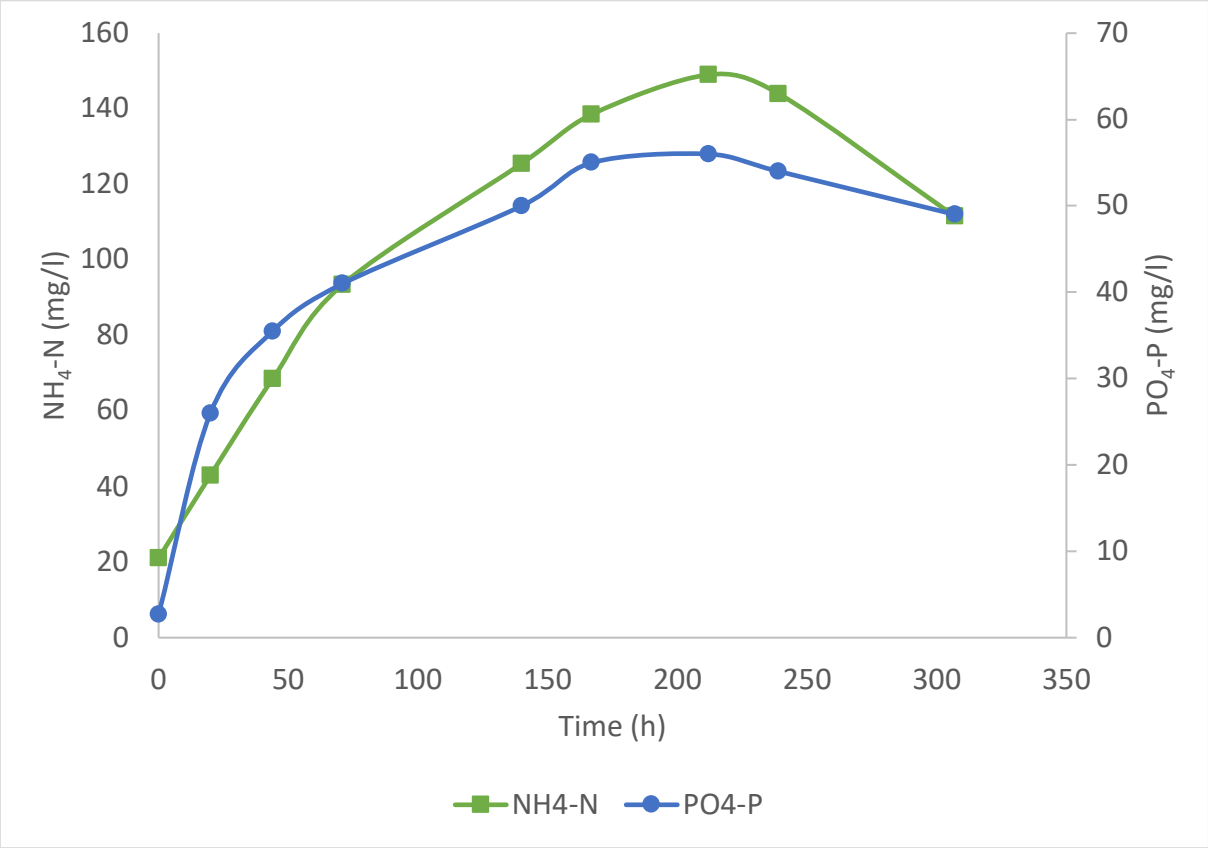

Figure 4-10: NH_4-N and PO_4-P during fermentation test 4.

Nitrogen and phosphorous were released due to disintegration of proteins and cellular compounds during the fermentation tests. However, at 212 hrs and pH 5.55 the concentration of both started declining. In test 2, NH_4-N and PO_4-P were analysed between 139 and 236 hrs (pH 5.68 to 4.84). During this time, the concentrations of both levelled off and started declining. Some of the nitrogen may have been stripped from the solution as gaseous ammonia. However, the pH in both tests was approximately 5.5 at the point when concentrations started declining. Thus the ammonium/ammonia ratio would have been high, and it is unlikely that considerable amounts of nitrogen was lost as NH_3 gas. The

declining concentrations indicate precipitation of a compound containing P and N. Due to the low pH, it is unlikely that the decreasing concentrations of NH_4-N and PO_4-P was due to struvite precipitation. It was however considered beyond the scope of this project to identify the precipitant.

Solids analyses

TS, TSS, TDS and VSS during fermentation test no. 4 are presented in figure 4-11.

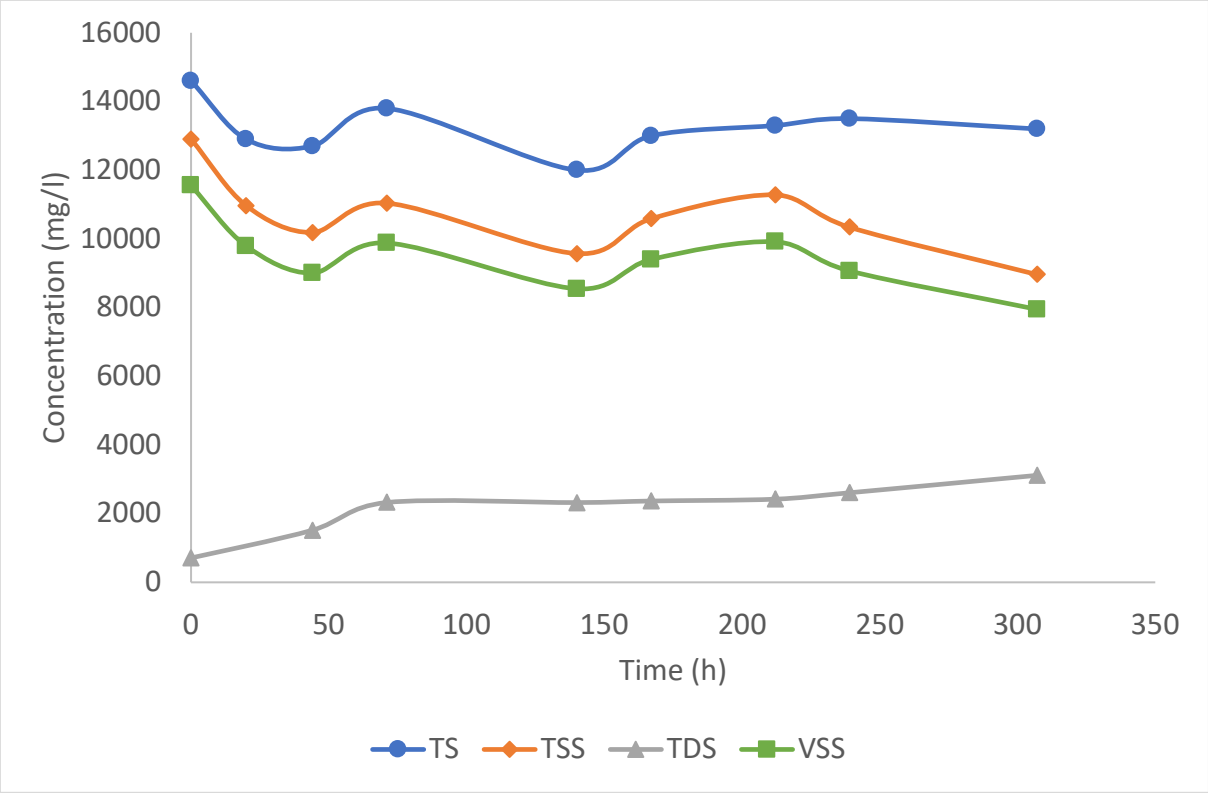

Figure 4-11: TS, TSS, VSS and TDS in fermentation test 4.

The sample collected at each interval was used for TS, TSS, VSS and TDS, and all curves except TDS display the same fluctuations. The bulk liquid was considered completely stirred during the test, but the size and composition of the particles in the sludge was diverse. Sampling was carried out using a syringe, and this may have contributed to the variations in the measurements. The vacuum created when the piston of the syringe was pulled back may have caused more of the smaller particles to be collected, leaving the TS, TSS and VSS analyses less representative. TDS and other analyses on dissolved constituents would not have been affected by variations in the particle composition of the samples. The fluctuations

between TS and TSS follow the same pattern, and are too consistent to have been caused by random errors during analytical work such as filtration, weighing and drying.

Even though the measurements fluctuate, the general trend is consistent with fermentation reactions. The overall TS, TSS and VSS were decreasing, and TDS was increasing. This was expected as the TSS is hydrolysed to soluble constituents. TS does not decrease as much as TSS and VSS towards the end, possibly due to the precipitation of the un-identified substance previously discussed.

Modelling of hydrolysis and COD production

An attempt to determine the hydrolysis rate constant (k_{hyd}) from the VSS reduction during fermentation test 4 is shown in figure 4-12. Based on the shape of the VFA and COD generation curves in figure 4-6, it was assumed that the hydrolysis during fermentation followed first order kinetics. The following rate expression was used to determine the rate constant:

$$\text{rate}_{\text{hyd}} = \frac{d\text{VSS}}{d\text{t}} = -\text{k}_{\text{hyd}} \cdot \text{VSS}_{\text{t}} \quad \rightarrow \quad \ln\left[\frac{\text{VSS}_{\text{t}}}{\text{VSS}_{0}}\right] = -\text{k}_{\text{hyd}} \cdot \text{t}$$

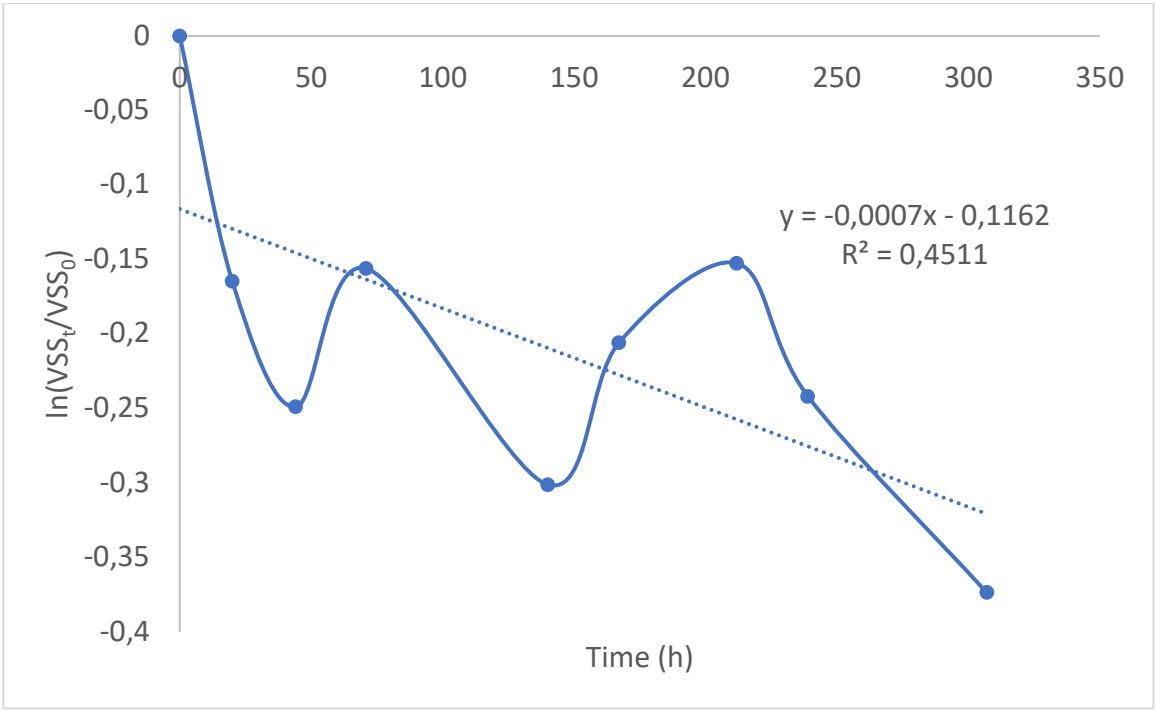

Figure 4-12: Linearization of VSS reduction during fermentation test no. 4.

Although the measurements display an overall reduction in VSS, the linearization of the data does not provide a representative hydrolysis rate constant. The level of fluctuation in VSS concentration was too high to be used for modelling purposes. The fluctuations in VSS was caused by the instability of the TSS measurements shown in figure 4-11.

The increase in filtered COD during the fermentation test 4 was a result of VSS hydrolysis. The overall VSS reduction was approx. 3500 mg/l. Assuming 1.4 mg COD/mg VSS, this equals about 4900 mg COD/l. The production of filtered COD during the test was approx. 4300 mg/l, which corresponds well to the VSS reduction. Consequently, the following estimation could be made:

$$1.4\ \mathrm{VSS} = \mathrm{COD_{VSS}} \approx \mathrm{COD_{filt}}$$

Assuming the above, the filtered COD production rate could be used to estimate the hydrolysis rate constant as follows:

$$\mathrm{rate_{hyd}} = \frac{d\mathrm{COD_{filt}}}{d\mathrm{t}} = \mathrm{k_{hyd}} \cdot \mathrm{COD_{filt(t)}} \rightarrow \ln\left[\frac{\mathrm{COD_{filt(t)}}}{\mathrm{COD_{filt(0)}}}\right] = \mathrm{k_{hyd}} \cdot \mathrm{t}$$

The linearization of the filtered COD production is shown in figure 4-13.

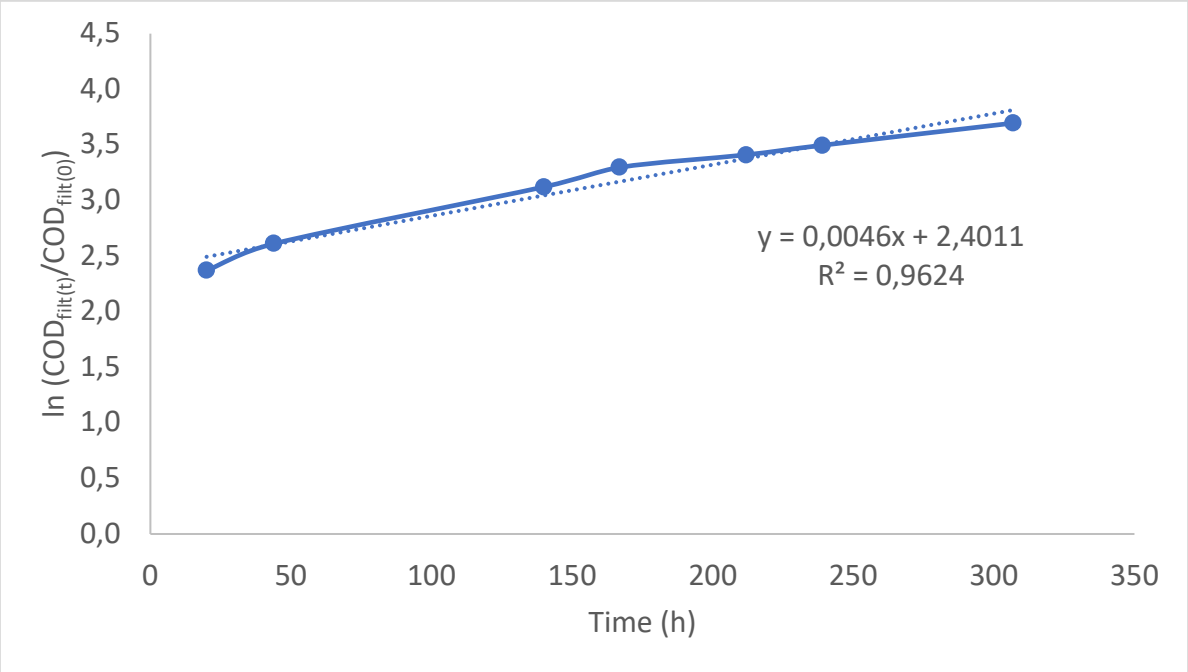

Figure 4-13: Linearization of filtered COD production during fermentation test 4.

The modelling of the filtered COD production rate during test 4 resulted in a rate constant of 0.0046 h^{-1}, or 0.11 d^{-1}. This constant should be similar to the hydrolysis rate constant. The hydrolysis rate constants in primary sludge presented in ADM 1 (Batstone et al., 2002) vary from 0.21 – 1.94 d^{-1} in mesophilic primary sludge digestion, depending on the type of polymer hydrolyzed. Tchobanoglous et al. (2014) suggests using k_{hyd} = 0.33 d^{-1} during mesophilic digestion of combined primary and activated waste sludge. As temperature is an important factor when considering biological rates, it is reasonable that the hydrolysis rate during fermentation at 20°C will be lower than at 35°C. The rates obtained from other studies are all associated with mesophilic anaerobic digesters where an active biomass is well established. During the fermentations in this project, no pre-established biomass was introduced. The primary sludge was collected directly from the sludge lines, and the indigenous biomass was responsible for the hydrolysis. Consequently, the hydrolysis rate would be lower due to the need for establishment of an active biomass at the start of each test.

4.3 PO_4-P release from activated sludge in batch tests

The results from the phosphate release tests carried out in the laboratory are presented and discussed in this section.

Endogenous release – tests 1 and 3

PO_4-P release tests 1 and 3 were carried out to investigate the endogenous phosphate release from the activated sludge. The PO_4-P release from activated sludge and concentrated activated sludge was measured. The PO_4-P release relative to TSS during tests 1 and 3 are presented in figures 4-14 and 4-15, respectively.

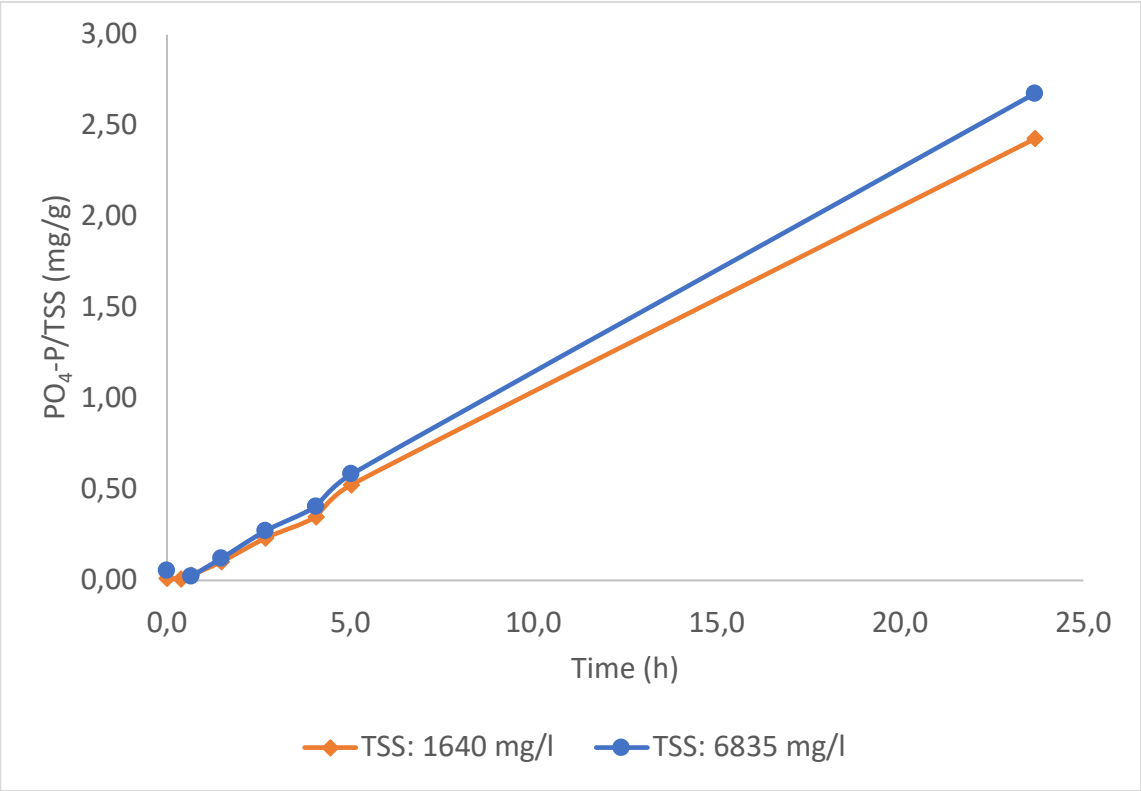

Figure 4-14: PO_4-P release vs time in test 1, carried out without temperature control.

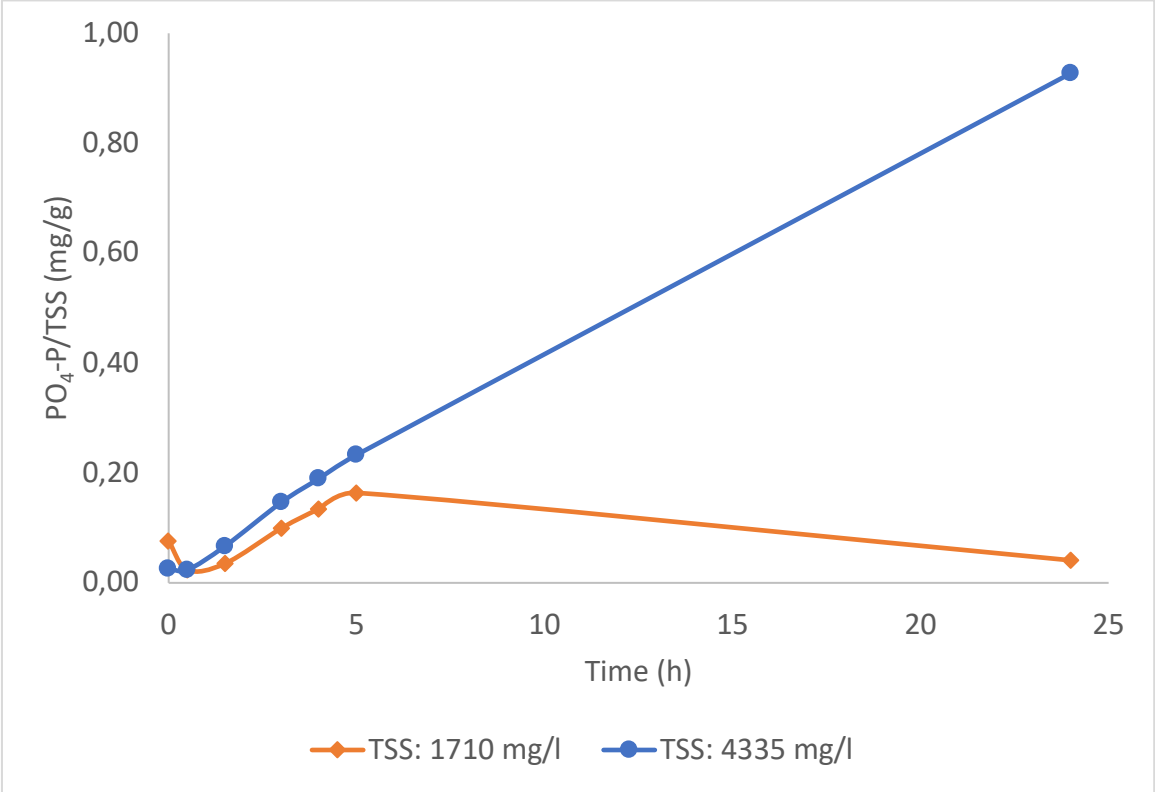

Figure 4-15: PO_4-P release vs time in test 3, carried out at 9°C.

During both tests 1 and 3, DO was measured between 0.17 mg/l and 0.36 mg/l. As previously discussed, the probe used for DO measurements during all anaerobic tests was inaccurate at low concentrations. Hence, it is likely that DO during the tests was lower than the measured values.

Due to a problem with the magnetic stirrer, air got mixed into the activated sludge batch during test 3. This caused the phosphate released during the first five hours of the test to be re-incorporated into the biomass. Therefore, only the first five hours of test 3 are used for comparison between the two batches.

The overall relative PO_4-P release from the sludge was higher in test 1 than in test 3. As temperature has a significant effect on biological processes, the difference in is most likely due to the tests being conducted at 20°C and 9°C, respectively.

During test 1 the concentrated sludge displayed a slightly higher relative release than the activated sludge, especially towards the end of the test. The same observations were made during the first five hours of test 3. In test 1, the pH in the activated sludge collected from the bioreactor was 6.9 at the start of the test. At the end of the test, the pH in the activated sludge had not changed, while the pH in the concentrated activated sludge was 6.7. The pH reduction in test 1 may indicate some VFA production due to fermentation. pH was not measured after five hours in test 3, and it cannot be confirmed that the pH was lower in the concentrated batch at that time. As the tests were kept anaerobic, there could have been some conversion of VSS to VFAs in all batches. The concentrated batches had a significantly higher TSS, and would produce higher concentrations of VFA which could affect pH. However, the VFAs produced would have been consumed rapidly, and it is not likely that there were sufficient amounts of VFAs present to affect pH. It is more likely that the difference in pH was due to the intrinsic inaccuracy of the pH meter. Still, the relative phosphate release in the concentrated batches was consistently higher than the activated sludge batches. It is therefore considered likely that there was a higher net VFA production due to fermentation in the concentrated batches of tests 1 and 3. The VFAs produced would have been rapidly consumed by PAOs, leading to a slightly higher phosphate release in the concentrated activated sludge.

Substrate stimulated release – tests 2 and 4

The relative PO_4-P release during tests 2 and 4 are presented in figures 4-16 and 4-17, respectively. The filtrate from fermented primary sludge was added approx. 10 minutes after the test started to allow for any residual oxygen within the sludge to be consumed prior to substrate addition.

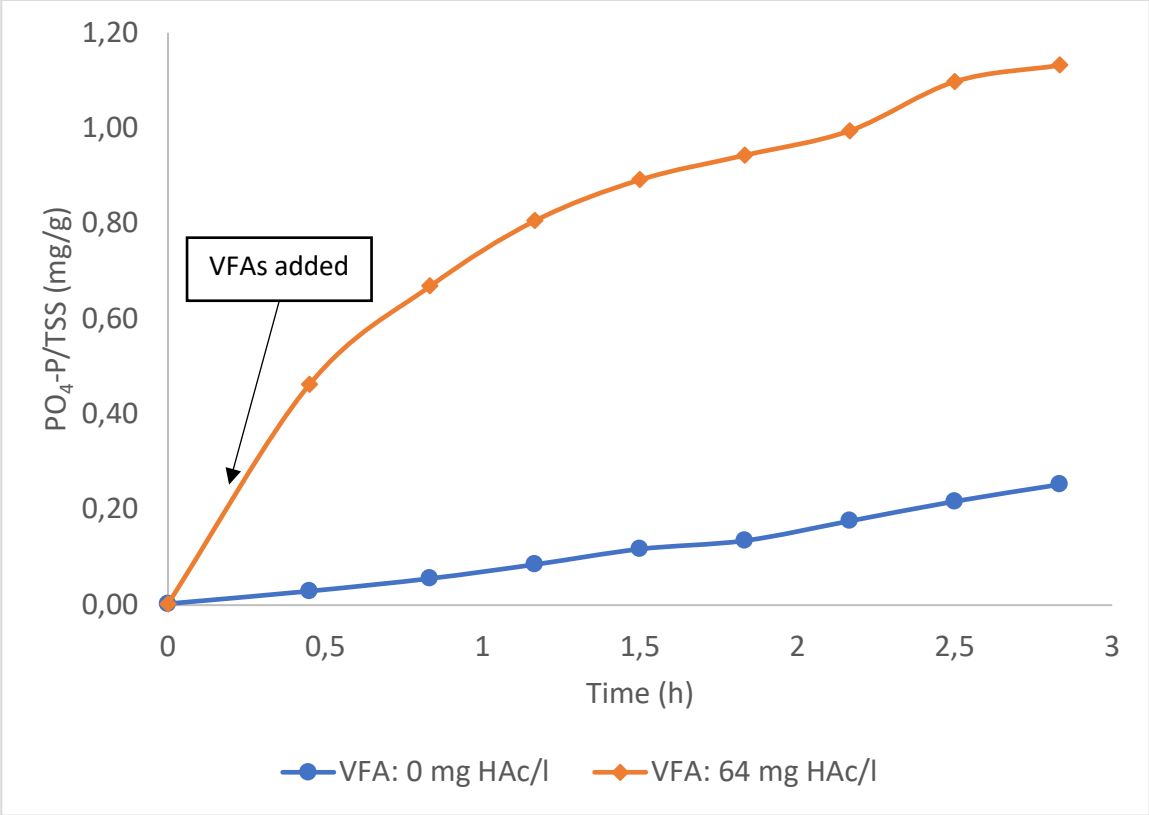

Figure 4-16: Relative PO_4-P release during test 2.

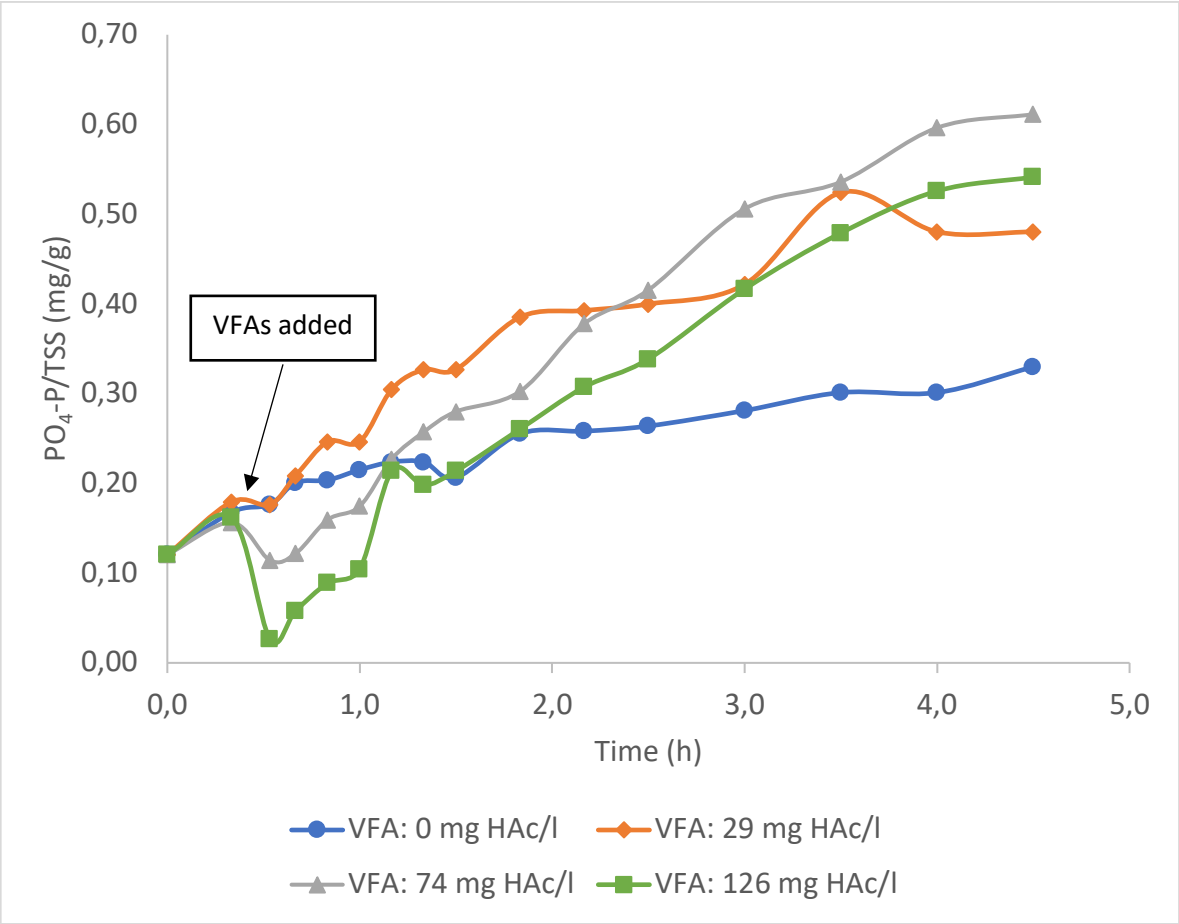

Figure 4-17: Relative PO_4-P release during test 4.

In the stimulated phosphate release tests, VFA-rich filtrate from fermented primary sludge was added. This caused the activated sludge to become diluted, and all results of the analyses during these tests has been adjusted in accordance with the dilution. In addition, the results of test 4 were adjusted to compensate for the PO_4-P concentration measured in the filtrate. Phosphate concentration was not measured in the filtrate used in test 2, and the results could not be adjusted. It is however likely that some PO_4-P was added with the substrate in test 2 as well.

The curve of the stimulated batch in test 2 developed as expected with respect to the endogenous control. However, as the phosphate concentration in the added substrate was not measured, it is likely that the actual release was lower. When the results of test 4 were adjusted for the PO4-P concentration of the primary sludge filtrate used in this test, the resulting curves were not as expected. It was expected that the jars that received the highest VFA dose would display the highest initial PO_4-P release. However, figure 4-17 shows that

the phosphate concentrations in these two jars dropped immediately after VFA addition. This indicates phosphate uptake, which may be due to oxygen being mixed in when the substrate was added. DO concentrations in the jars were measured with the membrane probe at 0.28 – 0.55 mg/l. With only one oxygen probe available, it was not possible to measure DO in all four jars at each sampling interval during test 4. It is possible that DO was higher when the substrate was added, however there are no measurements confirming this. The initial drop in PO_4-P was then followed by a close to linear release in all jars. The linearity indicates that even though there was an initial oxygen intrusion when the substrate was added, the rest of the test was anaerobic.

The jar added 74 mg HAc/l displayed the highest overall PO_4-P release. It seems that substrate addition above this concentration has little effect on the amount of phosphate released. This is consistent with the work of Carlsson et al. (1996), who found that the phosphate release did not increase when exceeding VFA addition corresponding to 70 mg COD/l. Figure 4-18 shows the filtered COD during the test. For the test added 29 mg/l, the COD was exhausted after approx. 2 hrs, and the subsequent phosphate release was mainly endogenous. The COD was consumed at approximately the same rate in the jars receiving the highest VFA doses. If the phosphate release during the test was substrate limited, the filtered COD removal rate would be higher in the jar receiving the highest substrate dose. Thus it can be assumed that phosphate release was limited by the exhaustion of the poly-P reserves within the PAOs.

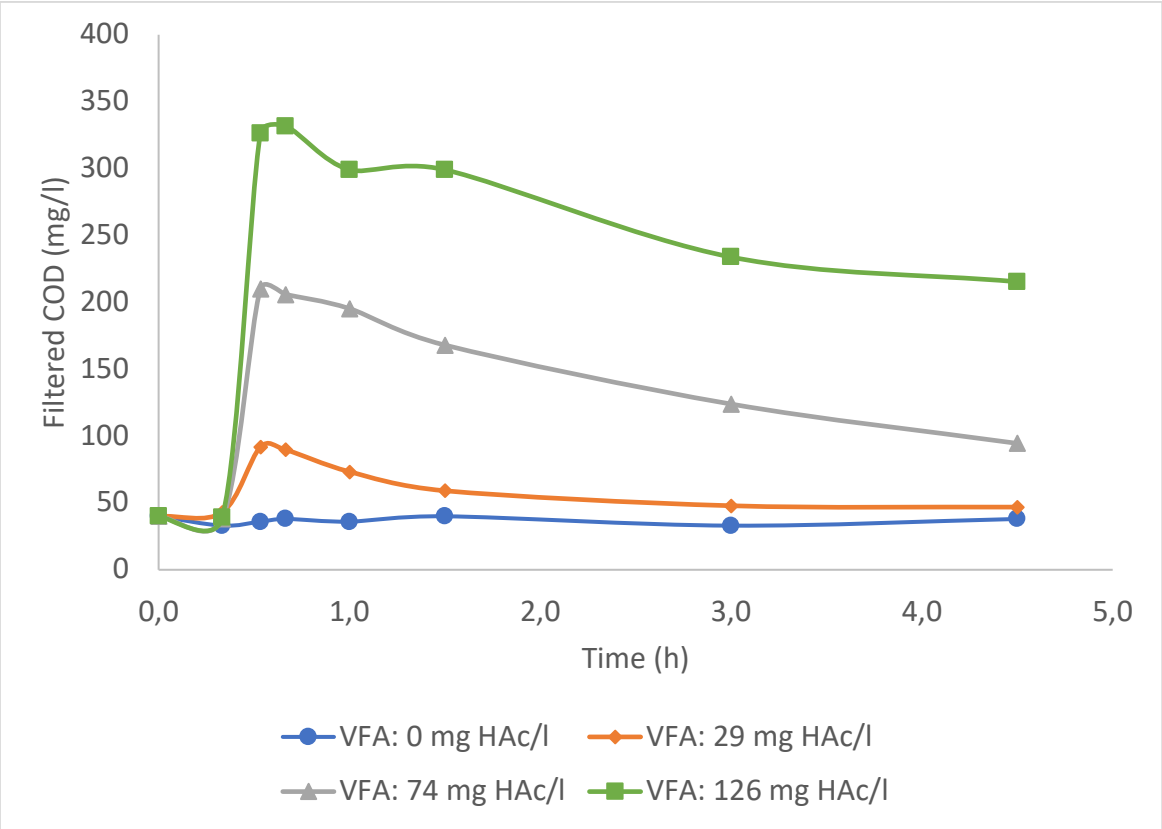

Figure 4-18: Filtered COD consumption during test 4

The PO_4-P/VFA and P/C ratios of test 2 and test 4 are presented in table 4-9. When calculating the ratios, the initial phosphate concentration in the sludge was subtracted. In test 4, the phosphate contribution from substrate addition was subtracted as well.

Table 4-9: PO_4-P/VFA and P/C ratios in tests 2 and 4.

	Test 2	Test 4		
VFA added (mg HAc/l)	64	29	74	126
VFA remaining (mg HAc/l)	15	0	0	41
PO_4-P/VFA (mg/mg)	0.13	0.09	0.05	0.04
P/C (mol/mol)	0.15	0.08	0.04	0.03

Comparing the values presented in table 4-9 with the ratios presented in other studies, the ratios in test 4 are unexpectedly low at high substrate doses. Mino et al. (1998) listed PO_4-P/VFA ratios within the range of 0.15 – 1.52 mol P/mol C upon VFA addition to EBPR sludge. If oxygen was mixed in when the substrate was added, some of the VFAs would have been

consumed aerobically by OHOs. Consequently, the actual VFA consumption by PAOs would be lower than the total consumption during the test. As previously discussed, the data in figure 4-17 indicate that this may have happened. However, the low PO_4-P/VFA ratios may also be the result of low poly-P reserves within the biomass.

The PO_4-P/VFA ratio calculated for test 2 is within the range presented by Mino et al. (1998). It is however assumed that an unknown fraction of the phosphate was added with the filtrate of fermented primary sludge. Consequently, the actual PO_4-P/VFA ratio in test 2 was probably lower than the value presented in table 4-9.

Phosphate release rates in batch tests

Table 4-10 and 4-11 summarizes the endogenous and stimulated PO_4-P release rates calculated based on the results from the batch tests. The release rates of the endogenous controls used in the substrate stimulated tests are also included in table 4-10.

Table 4-10: Endogenous PO_4-P release rates.

PO_4-P release rate (g P/kg TSS d^{-1})							
Test 1		Test 2	Test 3		Test 4	Average (20°C)	Average (9°C)
2.46*	2.71	2.15	0.79*	1.10	1.76	2.44	1.22

*activated sludge directly from the bioreactor

The endogenous release rates of all tests are within the same range at 20°C, and at 9°C. The rates at 9°C are lower than at 20°C, which is expected due to the influence of temperature on biological reaction rates. The endogenous release rates of the activated sludge were lower than the concentrated activated sludge. It is likely that some VFAs were produced due to fermentation during the tests, and more VFAs would have been produced in the concentrated sludge due to higher TSS. The presence of VFAs would stimulate phosphate release, and this would lead to a higher release rate. The highest endogenous release rate at low temperature was measured in test 4.

Table 4-11: Substrate stimulated PO_4-P release rates.

PO_4-P release rate (g P/kg TSS d^{-1})					
Test 2 (20°C)		Test 4 (9°C)			
64 mg HAc/l	Corrected	29 mg HAc/l	74 mg HAc/l	126 mg HAc/l	Average
9.58	7.00	2.56	3.25	2.88	2.90

The difference between the release rates in test 2 and test 4 is too high to be satisfyingly explained by the difference in temperature. As previously discussed, the PO_4-P concentrations in test 2 are considered elevated due to an unknown contribution from the fermented primary sludge filtrate. This would also affect the calculation of the phosphate release rate. Considering the phosphate concentrations measured in fermentation test 4, and the duration and TS of fermentation test 1, it is likely that the PO_4-P concentration of the filtrate was between 10 and 20 mg/l. Assuming 15 mg/l, addition of 150 ml of substrate would lead to the addition of 2.25 mg PO_4-P. Adjusting the calculations accordingly, this gives a PO_4-P release rate of approx. 7 g P/kg TSS d^{-1}. This rate is 2.4 times the average of 2.9 g P/kg TSS d^{-1} at 9°C, and the relative relationship between the rates at different temperatures is roughly the same as for the endogenous release.

The relative relationship between the rate in the endogenous control and the stimulated batches is significantly lower in test 4 compared to test 2. If oxygen was present in test 4, it may have affected the rates slightly by decreasing the overall release. However, the PO_4-P concentration increased almost linearly after the initial decrease. In addition, the COD consumption was more or less constant throughout the test. This indicates that there was sufficient substrate available to stimulate phosphate release. It is therefore assumed that the small difference between the endogenous and the stimulated batches in test 4 was a result of low levels of poly-P available within the biomass.

4.4 Estimation of the potential for controlled struvite precipitation

The primary aim of this project was to investigate the phosphate release from the EBPR sludge at SNJ with respect to the possibility of controlled struvite precipitation on the reject stream form the sludge thickener. As the waste sludge from the biological treatment is contained in an un-aerated storage chamber prior to thickening, phosphate will be released from the sludge during storage.

In table 4-12, the theoretical struvite yield from the reject stream is estimated using the experimental phosphate release rates determined in the batch tests. It was assumed that the endogenous release rate in the EBPR waste sludge was equal to the average release rate of 1.22 g P/kg TSS d^{-1} in the concentrated sludge at process temperature. Addition of VFAs from fermented primary sludge yielded an average release rate of 2.90 g P/kg TSS d^{-1} at

process temperature. Assuming this release rate would be achieved by adding substrate to the waste sludge holding tank, the struvite yield was estimated as follows:

Table 4-12: Estimated struvite yield in the reject stream.

	Endogenous	Stimulated	Unit
Waste sludge	600	600	m^3/d
	6000	6000	kg TSS/d
PO_4-P release rate	1.22	2.90	g P/kg TSS d^{-1}
HRT in the waste sludge tank	3	3	h
Total PO_4-P release during storage	0.92	2.18	kg P/d
PO_4-P in waste sludge	1.53	3.63	mg/l
Reject (thickening to 5 % TS)	480	480	m^3/d
Total PO_4-P in the reject	0.73	1.74	kg P/d
Struvite yield	5.9	13.7	kg/d

The average volume of waste sludge per day at SNJ during February and March was obtained from historical data. Assuming an average fill level of 50 % in the waste sludge tank, which corresponds to 75 m^3, and an average flow of 25 m^3/h to the sludge thickener, the HRT was 3 hrs. The reject flow was calculated based on the sludge being thickened from 1 % TS to 5 % TS. This would lead to removal of 80 % of the water within the waste sludge. Assuming even distribution of phosphate in the sludge prior to thickening, 80 % of the phosphate will enter the reject stream. The struvite yield was calculated assuming that all the phosphate released into the reject stream precipitated as struvite, i.e. 1 mole of phosphate resulted in 1 mole of struvite.

The waste sludge had an average TSS of 10 000 mg/l, which was higher than the approximate TSS of 7000 mg/l in the concentrated sludge used in the tests. As the endogenous tests showed that the release rates increased with increasing TSS, it is possible that the rates were higher than the values rate used in table 4-12. However, the sludge used in the tests were collected at the bioreactor effluent (Aer 2), while the waste sludge is removed from the settling tank. During this project it was discovered that phosphate was released from the sludge in the settling tank (figure 4-1). The average inlet PO_4-P concentration of 1.58 mg PO_4-P/l was equal to the average PO_4-P concentration in the

effluent. This indicates that a significant amount of the phosphate accumulated by the PAOs is released before the sludge is wasted or returned to the bioreactor, and would affect the phosphate release from the waste sludge. Historical data at SNJ show that the sludge blankets in the settling tanks were high in February and March. At the same time, the average SVI was 68 ml/g, indicating effective sludge settling. Thus, the high sludge blankets were caused by accumulation of sludge in the settling tanks. Poor performance of the bottom scrapers in the settling tanks, and unfavourable hydraulic conditions caused by an uneven inlet flow pattern are most likely the explainations for this accumulation.

After the sampling period of this project, adjustments were made to the settling tank inlets to improve the hydraulics. Some improvements have also been made to the scrapers, but the process of sludge removal from the settling tanks has not been optimized. Increasing the RAS flow may improve the overall conditions in the settling tank by shortening the retention time of the sludge, leaving less time for PO_4-P release. However, higher sludge return rates will increase the overall solids load on the settler, and may lead to problems with thickening in the sludge treatment process. It is evident that there is need for optimization of the sludge return- and wasting processes at SNJ. Before this is completed, it is likely that SNJ will continue to experience elevated PO_4-P concentrations in the effluent due to release from the sludge in the settling tanks. Most of this release is considered secondary, although the results of this project indicate some fermentative activity and VFA production within the return sludge. The amount of phosphate released in the settling tanks is related to the release potential in the waste sludge holding tank. High effluent PO_4-P concentrations as a result of secondary release within the settling tanks is consistent with exhaustion of the poly-P reserves within the biomass. Thus, the potential for phosphate release during waste sludge storage decreases.

The results of this research demonstrated that addition of fermented primary sludge to the concentrated EBPR sludge would lead to increasing phosphate release rates. Modelling of the filtered COD production during fermentation yielded $k_{hyd} = 0.11\ d^{-1}$. Comparing this value to the hydrolysis constants from other studies and factoring in the temperature, k_{hyd} was considered within the expected range. Even though the fermentation potential of the primary sludge at SNJ is adequate, the effects of addition is however likely to be limited due to low poly-P reserves as a result of the secondary phosphate release in the settling tank.

Another parameter that will affect the possibility of controlled struvite precipitation is the DO concentration in the anaerobic zone of the bioreactor. The results from the sampling campaigns revealed an average DO concentration of 2.5 mg/l in An 2. Based on the calculated PO_4-P uptake- and release rates through the bioreactor it is evident that the DO entrainment effectively short-circuits the EBPR process. The overall effect of this disruption is that the VFA assimilation of the PAOs decreases, and consequently the potential for PO_4-P uptake in the aerobic zone decreases. The poly-P reserves within the biomass diminishes, and the potential for phosphate release in the waste sludge holding tank is reduced.

When estimating the potential for controlled struvite precipitation based on the phosphorous load at SNJ, the overall struvite yield is significantly higher than 13.7 kg/d (table 4-12). The average phosphorous load on the bioreactors during February and March was 2.92 mg P_{tot}/l, with a dissolved fraction of 1.51 mg PO_4-P/l (table 4-1). The dissolved fraction is readily available for uptake by PAOs, and some of the particulate phosphorous will also be released into the wastewater due to disintegration of particles. However, for the purpose of estimating the potential phosphate release from the EBPR sludge, it is assumed that the amount of phosphate incorporated into the biomass is similar to the PO_4-P load on the bioreactors. This equals 150 kg PO_4-P/d for the two bioreactors combined. By stimulating phosphate release in the waste sludge holding tank, it is assumed that 50 % of the incorporated phosphate could be released from the EBPR waste sludge. If the sludge is thickened from 1 % to 5 %, the total amount of phosphate in the reject stream is 60 kg PO_4-P/d. Assuming that all the phosphate in the reject stream could be recovered as struvite, the overall potential for struvite precipitation at SNJ is 474 kg/d. The details of this estimation are included in Appendix F. The struvite yield calculated from the experimental phosphate release rate was 13.7 kg/d, which is 3 % of the calculated potential based on phosphorous load. It is evident that low EBPR activity affects the potential of phosphate release from the sludge, thus affecting the overall possibility of controlled struvite precipitation. If phosphate is to be recovered as struvite, the EBPR process at SNJ needs to be optimized. The operational problems associated with secondary release in the settling tank and oxygen entrainment into the anaerobic zone must be mitigated.

4.5 Mass balances in the bioreactor

Based on the measurements presented in table 4-2, the mass flow through the bioreactor was calculated. The mass flow at the outlet of each compartment in the L3 bioreactor is presented in table 4-13.

Table 4-13: Mass flow through the bioreactor

	Mass flow (kg TSS/h)							
Date	RAS	An 1	In	An 1 + In	An 2	An 3	Aer 1	Aer 2
06.02	1604	2374	294	2668	1929	4190	3729	3620
21.02	1861	2782	208	2990	2092	5225	5228	5061
07.03	3009	4036	229	4265	3055	6672	6220	6295

The mass balances of the compartments in the bioreactor are in accordance with the following equations:

An 1: $Q_{RAS} \cdot TSS_{RAS} = Q_{RAS} \cdot TSS_{An\ 1}$

An 2: $Q_{RAS} \cdot TSS_{An\ 1} + Q_{In} \cdot TSS_{In} = (Q_{RAS} + Q_{In}) \cdot TSS_{An\ 2}$

An 3: $(Q_{RAS} + Q_{In}) \cdot TSS_{An\ 2} = (Q_{RAS} + Q_{In}) \cdot TSS_{An\ 3}$

Aer 1: $(Q_{RAS} + Q_{In}) \cdot TSS_{An\ 2} = (Q_{RAS} + Q_{In}) \cdot TSS_{Aer\ 1}$

Aer 2: $(Q_{RAS} + Q_{In}) \cdot TSS_{Aer\ 1} = (Q_{RAS} + Q_{In}) \cdot TSS_{Aer\ 2}$

Considering the values in table 4-12, the mass flows between An 3 and Aer 1, and Aer 1 and Aer 2 are well balanced. There are slight deviations between the calculated mass flows in these compartments, however no more than 11 %.

The mass flow between An 2 and An 3 is however not balanced. Based on the measurements in the bioreactor, the mass flow out of An 2 is approximately 55 % lower than the mass flow out of An 3. As this was the trend on all three sampling dates, the results indicate that there was a disturbance in the flow between An 2 and An 3, such as short-circuiting. On a regular basis it has been observed on the surface that wastewater flows from Aer 1 to An 3, that is from the aerobic to the anaerobic zone of the bioreactor. This backflow of could have led to accumulation of MLSS in An 3, which would have yielded elevated TSS in this compartment.

The magnitude and effect of the backflow is however unknown, and is it considered unlikely that this alone is responsible for the discrepancy between the mass flow in An 2 and An 3. It is possible that some of the MLSS in An 2 settles due to inadequate mixing. There are two 2.5 kW mechanical mixers installed in each of the anaerobic sections. Considering the volumes of An 1, An 2 and An 3, this gives a maximum volumetric power input of 10 kW/1000 m^3 in An 1, and 5.2 kW/1000 m^3 in An 2 and An 3. Grady et al. (2011) states that the required volumetric power input to keep the settleable solids in suspension during domestic wastewater treatment in lagoons is at least 6 kW/1000 m^3, while the design mixing energy of the anaerobic zone at Groos WWTP in Grimstad was 5 kW/1000 m^3 (Reid Crowther & Partners Ltd., 1995). A volumetric power input of 5.2 kW/1000 m^3 may then be considered barely sufficient to keep the sludge completely mixed. The average SVI of the MLSS at SNJ is 68 ml/g, and the sludge would settle fairly quickly if mixing was inadequate. Upon visual inspection of the mixing in the anaerobic zones, all three zones seemed to be completely mixed due to observed movement on the surface. However, it was discovered that one of the mixers in An 2 was out of operation, and probably had been so during the sampling campaigns in February and March. If this was the case, the volumetric power input in An 2 was 2.6 kW/1000 m^3, which is significantly lower than the required values stated above. Furthermore, gas bubbles breaking the surface was observed in An 2 during inspection, but not in the other anaerobic compartments. This could indicate gas production within settled sludge in An 2, and that the compartment was not completely mixed.

The discrepancy between the sum of the mass flow from An 1 and the inlet to the bioreactor, and An 2 is between 25 and 30 %. This difference could also be explained by sludge settling in An 2, as discussed above. In addition, some deviations are expected when calculating mass flow from samples rather than theoretical values. The values obtained from samples are subject to errors associated with sampling methods and analytical procedures. There is also a time delay through the bioreactor, which also could affect the overall results of the analyses.

The mass flow of the RAS and out of An 1 should have been similar. The difference between them could be explained by the RAS samples not being representative. When the RAS samples were collected from the return pipeline, the positions of the bottom scrapers in the settling tanks were not considered. The scrapers move back and forward along the length of

the settling tank at specific time intervals. The exact effect of the scrapers on the TSS of the RAS is unclear, but significant variations in the samples collected in the return pipeline has been observed both during this project and by SNJ staff. The most likely explanation for these variations is that the scraper positions differed when the grab samples were collected. An 1 receives RAS from the four settling tanks, and the retention time here is approximately one hour. As the tank is considered completely mixed, the TSS concentrations measured in An 1 is regarded as more representative for the RAS concentration than the grab samples collected in the return pipeline. This is confirmed when considering the relationship between TSS of the RAS, the MLSS and the flow in accordance with the mass balance of the settling tanks:

$$(Q_{RAS} + Q_{In}) \cdot TSS_{Aer\ 2} = Q_{RAS} \cdot TSS_{RAS} + Q_{In} \cdot TSS_{Effluent}$$

The effluent mass flow was considered negligible, as the effluen TSS was approximately 1 % of the MLSS ($TSS_{Aer\ 2}$). Thus the expression was simplified as follows:

$$\frac{Q_{RAS}}{Q_{RAS} + Q_{in}} = \frac{TSS_{Aer\ 2}}{TSS_{RAS}}$$

Table 4-14 shows the results of calculating the left side and the right side of the equation separately, with TSS from both the RAS pipeline, and An 1.

Table 4-14: Balance of flow, MLSS and RASSS calculated using TSS of RAS and An 1.

Date	$\frac{Q_{RAS}}{Q_{RAS} + Q_{in}}$	$\frac{TSS_{Aer\ 2}}{TSS_{RAS}}$	$\frac{TSS_{Aer\ 2}}{TSS_{An\ 1}}$
06.02	0.21	0.47	0.32
21.02	0.21	0.58	0.40
07.03	0.21	0.44	0.32

The calculation show less deviation between the values when using the TSS measured in An 1 compared to TSS in the RAS. The values calculated for the left and the right side of the equation should have been equal. However, it is expected that there are some deviations when using experimental values. It is clear that the TSS measured in An 1 is more representative for the RAS than the TSS measured in the return pipeline.

4.6 Error analysis

In this section the accuracy during the practical work, and the sources of errors in the methods and analyses are presented and discussed.

Sampling in the bioreactors

The different compartments of the bioreactor were considered completely mixed in all sampling points at the time of sampling. It is however possible that one of the mixers in An 2 were out of order during sampling, which would have led to less volumetric power input than required to keep all solids in suspension. This may have caused sludge to settle in An 2, yielding lower TSS values. The mass balances calculated in chapter 4.5 indicate this. It was also observed backflow from Aer 1 to An 3, which could have affected the overall TSS analyses in An 3. The magnitude of the backflow was however not determined, and the effect on the samples collected in An 3 is not clear. It is likely that the samples collected in the return pipeline was affected by the position of the scrapers in the settling tanks. Thus, the TSS measurements in An 1 was more representative for RAS TSS than the samples collected in the return pipeline.

Solids analyses and titration

Collection of representative samples from the wastewater is a source of errors during solids analyses. Especially with regards to the dilute wastewater samples, homogenous particle distribution could have been an issue. In addition, the intrinsic error associated with the scale could also have affected the solids analyses.

The accuracy related to solids analysis and titration was calculated, and is presented in table 4-15. During these analyses two parallels were analysed on the same sample. The deviation between the parallels indicates how accurately the analyses were conducted. The calculation is based a selection of results considered representative for all similar analyses.

Table 4-15: Average deviations between parallel analyses.

Analysis	Average deviation between parallels
TS	3 %
TDS	6 %
TSS (dilute)	12 %
TSS (concentrated)	2 %
VSS	3 %
VFA	4 %
Alkalinity	14 %

Most of the deviations are below 5 %, and indicate accurate analyses. The TSS analysis was more inaccurate in the dilute samples, such as the inlet samples (both to the WWTP and L3) and the effluent samples. The error in the concentrated samples from the bioreactor and return sludge were significantly lower. If the samples were not completely homogenized prior to analysis, this would have a higher impact on the dilute TSS samples. It is also possible that the analyses of the dilute samples would have been more accurate if sample volumes larger than 100 ml had been filtrated. The difference between the parallels of the VSS analyses in both the dilute and concentrated sample was low, and was therefore presented as one average.

The accuracy during titration was higher for VFA than for alkalinity. Alkalinity was present in significantly lower concentrations than VFA, especially towards the end of the fermentation tests. Low concentrations and small sample volumes may have caused the titrations to be less accurate. It is also possible that some of the alkalinity was lost due to stripping of CO_2 from the liquid during stirring.

The general trend of the titration data and solids analyses was increasing accuracy as the project progressed. This is consistent with increasing practical experience in the lab.

Spectrophotometrical analyses

The potential errors during spectrophotometrical analyses are associated with pipetting. COD and NH_4-N were analyzed using test kits, and the main source of error during these

tests is both the indigenous uncertainty within the pipettes, and inaccuracy during sample collection. Due to several pipetting operations per sample in P_{tot} and PO_4-P analyses, the inaccuracy of these tests is regarded as slightly higher than for the test kits. As the P_{tot} and PO_4-P analyses were performed reusing empty glass cuvettes, the results may have been affected if the cuvettes were not thoroughly rinsed prior to each test series.

The accuracy of the spectrophotometer itself was controlled by analysing standard solutions as part of each test series. It was generally observed that the measured values corresponded well with the concentrations of the standard solutions. From these controls the error of the spectrophotometer can be estimated to < 10 %.

pH and DO

The accuracy of the pH measurements carried out with both pH meters was $\pm$ 1 %, which corresponds to approximately 0.1 pH units. Both pH meters had temperature sensors, which corrected the values measured at low temperature.

DO measurements were, as previously discussed, inaccurate at low concentrations. Consequently, there is some degree of uncertainty associated with the DO concentrations presented in this project. An accuracy control of the DO probe was carried out in order to determine the magnitude of the uncertainty associated with the measurements. Details of the accuracy control of the DO probe used during this project can be found in Appendix D. It is likely that when DO concentrations of 0.4 – 0.5 mg/l were measured, the actual concentrations were less than 0.2 – 0.3 mg/l. It was however found that the oxygen measurements were representative at higher concentrations, and measurements above 1 mg/l are considered representative.

Anaerobic batch tests

The greatest uncertainty associated with the batch tests is whether they were kept anaerobic. The use of plastic beads or styrofoam beads on the surface appeared to work well against oxygen entrainment, even though DO was measured at low concentrations. These DO measurements were however regarded as not representative due to the inaccuracy of the oxygen probe.

There is some uncertainty linked to the accuracy of the samples collected from the anaerobic batch tests. These were collected with a syringe connected to a narrow plastic tube. When pulling the piston, more of the smaller particles may have been collected due to a high vacuum and narrow passage. This would affect the TS and TSS/VSS of the anaerobic tests, but not the analyses of the dissolved constituents.

5 Conclusions

The average endogenous phosphate release rate in the EBPR sludge at SNJ was 1.22 g P/kg TSS d^{-1} at process temperature (9°C). Substrate addition in form of VFA-rich filtrate from fermented primary sludge caused the release rate to increase to an average of 2.90 g P/kg TSS d^{-1} at the same temperature. The optimal VFA dose with respect to phosphate release was approximately 70 mg HAc/l, as additional substrate did not yield higher phosphate release. Based on the hydrolysis rate constant calculated, the primary sludge at SNJ was found to have a good fermentation potential and is considered suitable for stimulation of phosphate release from the EBPR sludge.

Based on the phosphorus load at SNJ, the potential for controlled struvite precipitation in the sludge thickening reject stream was estimated to 474 kg struvite/d. However, the experimental phosphate release rates produced significantly lower struvite yields. A theoretical yield of 13.7 kg struvite/d was determined based on phosphate release from substrate stimulated waste sludge, while the endogenous equivalent was 5.9 kg/d. The discrepancy between the potential calculated based on phosphorous load and experimental release rates is explained by the low EBPR activity in the bioreactors.

The EBPR at SNJ is low mainly due to two operational problems identified; oxygen entrainment in the anaerobic zone of the bioreactor, and secondary phosphate release in the settling tanks. Both of these issues have a negative effect on the poly-P storage within the PAO biomass, consequently affecting the overall potential for controlled struvite precipitation and phosphorous recovery from the reject of the waste sludge thickening. If phosphorous is to be recovered as struvite at SNJ the overall EBPR process needs to be optimized, and the issues with oxygen entrainment and secondary release needs to be mitigated.

6 Suggestions for improvements and future work

Based on the results of this project, it is evident that there is need for process optimization at SNJ. The following actions are suggested for further and more accurate assessment of the struvite precipitation potential associated with waste sludge thickening:

- The oxygen entrainment in the anaerobic reactor needs to be minimized. The possibility of avoiding dissolving oxygen in the effluent from the Hydrotech filters should be investigated. Now, the effluent water from the filters cascades into a basin, resulting in mixing of air into the water.
- The hydraulic conditions in the bioreactor needs to be investigated and improved, especially with regards to possible sludge settling in An 2 and backflow from Aer 1 to An 3.
- The sludge removal form the settling tanks should be improved to avoid secondary release and elevated phosphate concentrations in the effluent.
- When the overall conditions in the wastewater treatment plant are stabilized after the completion of the reconstruction, the phosphate concentrations and EBPR activity in the wastewater treatment plant should be re-assessed.
- When the EBPR activity is considered stable, new phosphate release tests should be performed to investigate the phosphate release from the sludge and the associated struvite formation potential. The release tests may be performed on both the waste sludge and the activated sludge to determine if there is a difference between them.

7 References

Banister, S. S., & Pretorius, W. A. (2016). Optimisation of primary sludge acidogenic fermentation for biological nutrient removal. Retrieved from http://nrfnexus.nrf.ac.za/handle/20.500.11892/171766

Barnard, J. L. (1984). Activated primary tanks for phosphate removal. *Water SA*, *10*(3), 121–126.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhniy, S. V., Pavlostathis, S. G., Rozzi, A., … Vavilin, V. A. (2002). *Anaerobic digestion model no. 1: (ADM1)*. London: IWA Publ.

Baur, R., Benisch, M., Clark, D., & Sprick, R. G. (2002). Struvite Control - A Common and Nuisance. *Proceedings of the Water Environment Federation*, *2002*(14), 480–495.

Bergmans, B. J. C., Veltman, A. M., van Loosdrecht, M. C. M., van Lier, J. B., & Rietveld, L. C. (2014). Struvite formation for enhanced dewaterability of digested wastewater sludge. *Environmental Technology*, *35*(5), 549–555.

Bhuiyan, M. I. H., Mavinic, D. S., & Koch, F. A. (2008). Phosphorus recovery from wastewater through struvite formation in fluidized bed reactors: a sustainable approach. *Water Science & Technology*, *57*(2), 175.

Brdjanovic, D., Logemann, S., van Loosdrecht, M. C. M., Hooijmans, C. M., J. Alaerts, G., & Heijnen, J. J. (1998). Influence of temperature on biological phosphorus removal: process and molecular ecological studies. *Water Research*, *32*(4), 1035–1048.

Britton, A., & Baur, R. (2010). Field Report - Phosphorus: A Resource in Decline. *American Water Works Association*, *102*(9), 117–118.

Carlsson, H., Aspegren, H., & Hilmer, A. (1996). Interactions between wastewater quality and phosphorus release in the anaerobic reactor of the EBPR process. *Water Research*, *30*(6), 1517–1527.

Christensson, M., Lie, E., Jönsson, K., Johansson, P., & Welander, T. (1998). Increasing substrate for polyphosphate-accumulating bacteria in municipal wastewater through hydrolysis and fermentation of sludge in primary clarifiers. *Water Environment Research*, *70*(2), 138–145.

Clesceri, L. S., Greenberg, A. E., & Eaton, A. D. (Eds.). (1998). *Standard methods: for the examination of water and wastewater* (20. ed). Washington: American Public Health Association.

Cokgor, E. U., Oktay, S., Tas, D. O., Zengin, G. E., & Orhon, D. (2009). Influence of pH and temperature on soluble substrate generation with primary sludge fermentation. *Bioresource Technology*, *100*(1), 380–386.

Cokgor, E. U., Oktay, S., Zengin, G. E., Artan, N., & Orhon, D. (2005). Effect of primary sludge fermentation products on mass balance for biological treatment. *Water Science and Technology*, *51*(11), 105–114.

Danesh, S., & Oleszkiewicz, J. A. (1997). Use of a new anaerobic-aerobic sequencing batch reactor system to enhance biological phosphorus removal. *Water Science and Technology*, *35*(1), 137–144.

Fattah, K. (2012). Assessing Struvite Formation Potential at Wastewater Treatment Plants. *International Journal of Environmental Science and Development*, *3*, 548–552.

Grady, C. P. L., Daigger, G. T., Love, N. G., & Filipe, C. D. M. (2011). *Biological Wastewater Treatment.* IWA Publishing.

Gu, A. Z., Saunders, A., Neethling, J. B., Stensel, H. D., & Blackall, L. L. (2008). Functionally Relevant Microorganisms to Enhanced Biological Phosphorus Removal Performance at Full-Scale Wastewater Treatment Plants in the United States. *Water Environment Research*, *80*(8), 688–698.

Higgins, M. J., & Novak, J. T. (1997). The effect of cations on the settling and dewatering of activated sludges: Laboratory results. *Water Environment Research*, *69*(2), 215–224.

IVAR. (2017). Sentralrenseanlegg Nord-Jæren. Retrieved November 9, 2017, from http://www.ivar.no/sentralrenseanlegg-nord-jaren/category618.html

Liamleam, W., & Annachhatre, A. P. (2007). Electron donors for biological sulfate reduction. *Biotechnology Advances*, *25*(5), 452–463.

Lopez-Vazquez, C. M., Oehmen, A., Hooijmans, C. M., Brdjanovic, D., Gijzen, H. J., Yuan, Z., &

van Loosdrecht, M. C. M. (2009). Modeling the PAO–GAO competition: Effects of carbon source, pH and temperature. *Water Research*, *43*(2), 450–462.

Lu, H., Oehmen, A., Virdis, B., Keller, J., & Yuan, Z. (2006). Obtaining highly enriched cultures of Candidatus Accumulibacter phosphates through alternating carbon sources. *Water Research*, *40*(20), 3838–3848.

Madigan, M. T., Brock, T. D., Martinko, J. M., & Clark, D. P. (2009). *Brock biology of microorganisms* (12. ed., internat. ed). San Francisco, Calif.: Pearson/Benjamin Cummings.

Melia, P. M., Cundy, A. B., Sohi, S. P., Hooda, P. S., & Busquets, R. (2017). Trends in the recovery of phosphorus in bioavailable forms from wastewater. *Chemosphere*, *186*(Supplement C), 381–395.

Mino, T., van Loosdrecht, M. C. M., & Heijnen, J. J. (1998). Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research*, *32*(11), 3193–3207.

Moosbrugger, R. E., Wentzel, M. C., Ekama, G. A., & Marais, G. v R. (1993). A 5 pH Point Titration Method for Determining the Carbonate and SCFA Weak Acid/Bases in Anaerobic Systems. *Water Science and Technology*, *28*(2), 237–245.

Mulkerrins, D., Dobson, A. D. ., & Colleran, E. (2004). Parameters affecting biological phosphate removal from wastewaters. *Environment International*, *30*(2), 249–259.

Münch, E. V., & Barr, K. (2001). Controlled struvite crystallisation for removing phosphorus from anaerobic digester sidestreams. *Water Research*, *35*(1), 151–159.

Norconsult. (2013). *Utvidelse Sentralrenseanlegg Nord-Jæren. Forprosjekt: Aktiv slamanlegg.* (No. 5120011–2).

Norconsult. (2018). *Utvidelse Sentralrenseanlegg Nord-Jæren. Driftsinstruks Prosess* (No. 5120011).

Oehmen, A., Lemos, P., Carvalho, G., Yuan, Z., Keller, J., Blackall, L., & Reis, M. (2007). Advances in enhanced biological phosphorus removal: From micro to macro scale.

Water Research, *41*(11), 2271–2300.

Ohlinger, K. N., Young, T. M., & Schroeder, E. D. (1998). Predicting struvite formation in digestion. *Water Research*, *32*(12), 3607–3614.

Panswad, T., Doungchai, A., & Anotai, J. (2003). Temperature effect on microbial community of enhanced biological phosphorus removal system. *Water Research*, *37*(2), 409–415.

Rabinowitz, B., & Oldham, W. K. (1986). Excess biological phosphorus removal in the activated sludge process using primary sludge fermentation. *Canadian Journal of Civil Engineering*, *13*(3), 345–351.

Reid Crowther & Partners Ltd. (1995). Grimstad BNR Prototype Wastewater Treatment Plant. Operating Manual.

Rittmann, B. E., & McCarty, P. L. (2001). *Environmental biotechnology: principles and applications*. Boston, Mass.: McGraw-Hill.

Safitri, A. S. (2016). *Biogas potential of high strength municipal wastewater treatment in laboratory scale up-flow anaerobic sludge blanket (UASB) reactors* (Master thesis). University of Stavanger.

Shehab, O., Deininger, R., Porta, F., & Wojewski, T. (1996). Optimizing phosphorus removal at the Ann Arbor Wastewater Treatment Plant. *Water Science and Technology*, *34*(1–2), 493–499.

Smolders, G. J. F., van der Meij, J., van Loosdrecht, M. C. M., & Heijnen, J. J. (1994). Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence. *Biotechnology and Bioengineering*, *43*(6), 461–470.

Smolders, G. J. F., van der Meij, J., van Loosdrecht, M. C. M., & Heijnen, J. J. (1995). A structured metabolic model for anaerobic and aerobic stoichiometry and kinetics of the biological phosphorus removal process. *Biotechnology and Bioengineering*, *47*(3),

Tchobanoglous, G., Tsuchihashi, R., Burton, F., & Stensel, D. H. (Eds.). (2014). *Wastewater engineering: treatment and resource recovery* (5. ed., internat. student ed). New York, NY: McGraw-Hill.

Ucisik, A. S., & Henze, M. (2008). Biological hydrolysis and acidification of sludge under anaerobic conditions: The effect of sludge type and origin on the production and composition of volatile fatty acids. *Water Research*, *42*(14), 3729–3738.

van Lier, J. B., Mahmoud, N., & Zeeman, G. (2008). Anaerobic Wastewater Treatment. In M. Henze, M. C. M. van Loosdrecht, G. A. Ekama, & D. Brdjanovic (Eds.), *Biological Wastewater Treatment: Principles, Modelling and Design* (pp. 415–454). London: IWA Publishing.

Wen-Tso, L., Mino, T., Matsuo, T., & Nakamura, K. (1996). Biological phosphorus removal processes - effect of pH on anaerobic substrate metabolism. *Water Science and Technology*, *34*(1–2), 25–32.

Wentzel, M. C., Comeau, Y., Ekama, G. A., van Loosdrecht, M. C. M., & Brdjanovic, D. (2008). Enhanced Biological Phosphorus Removal. In M. Henze, M. C. M. van Loosdrecht, G. A. Ekama, & D. Brdjanovic (Eds.), *Biological Wastewater Treatment: Principles, Modelling and Design* (pp. 154–220). London: IWA Publishing.

Wu, H., Yang, D., Zhou, Q., & Song, Z. (2009). The effect of pH on anaerobic fermentation of primary sludge at room temperature. *Journal of Hazardous Materials*, *172*(1), 196–

Ydstebø, L. (2005). *Substrate generation for enhanced biological phosphorus removal between 5 and 20°C*. University of Stavanger, Faculty of Science and Technology, Department of Mathematics and Science, Stavanger.

Yuan, Q., Baranowski, M., & Oleszkiewicz, J. A. (2010). Effect of sludge type on the fermentation products. *Chemosphere*, *80*(4), 445–449.

Zhang, T., Liu, Y., & Fang, H. H. P. (2005). Effect of pH change on the performance and microbial community of enhanced biological phosphate removal process. *Biotechnology and Bioengineering*, *92*(2), 173–182.

Appendix

A. Sampling positions at SNJ

Figure A and table A presents the positions where samples were collected at SNJ.

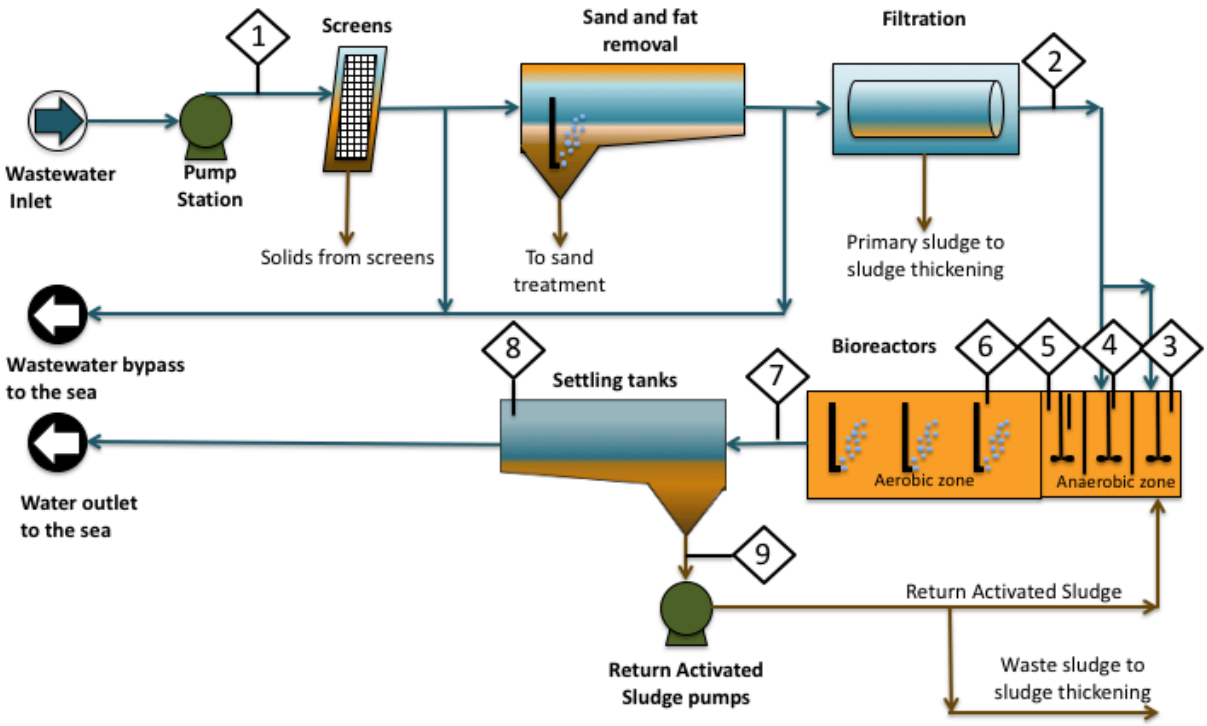

Figure A: Sample points at SNJ.

Table A: SNJ sample point description.

Position no.	Description	Stream type
1	Inlet wastewater treatment plant	Wastewater
2	Inlet bioreactor	Wastewater
3	Anaerobic chamber 1 (An 1)	Activated sludge
4	Anaerobic chamber 2 (An 2)	MLSS
5	Anaerobic chamber 3 (An 3)	MLSS
6	Aerobic zone 1 (Aer 1)	MLSS
7	Aerobic zone 2 (Aer 2)	MLSS
8	Treatment effluent	Treated wastewater
9	Return Activated Sludge (RAS)	Activated sludge

B. Determination of filter loss

Three filters were dried at 105°C, cooled in a desiccator and weighed before 100 ml of distilled water was filtrated. The filters were then dried and weighed before combustion in the muffle oven at 550°C. The intrinsic loss of the filters during drying and combustion was calculated and presented in table B. It was assumed that the average loss applied to all filters in one batch. The results of all TSS and VSS measurements were corrected by subtracting the average filter loss of the corresponding filter batch.

Table B: Average filter loss during drying and combustion.

Filter batch no.	Average loss during drying (g)	Average loss during combustion (g)
10153046	0.0003	0.0100
12786044	0.0006	0.0100

C. Verification of 5-point titration for determination of VFA

The accuracy of the titration method used to determine VFA concentrations was tested by titrating set of acetic acid standard solutions, and the results are presented in figure C.

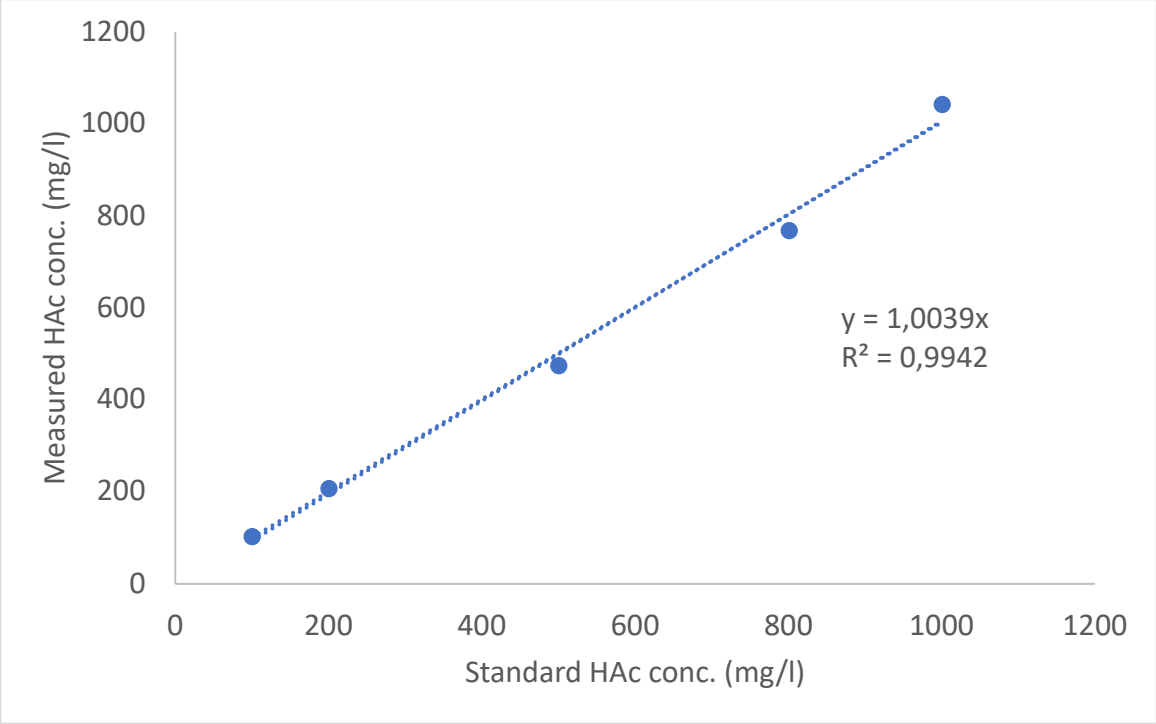

Figure C: Average measured HAc during titration method verification.

Sodium bicarbonate was added to the solutions to elevate pH and provide buffer capacity. Standard solutions of 100, 200, 500, 800 and 1000 mg/l HAc were prepared, and two parallels of each titrated with 0.05 M HCl at the same mixing rate as used in the experiments.

The difference between the parallels was less than 5 % at all concentrations except 100 mg/l, where it was 10 %. As the titration process requires a high level of accuracy, especially at low concentrations, the difference between the parallels at 100 mg/l is most likely due to inaccuracy at the hand of the titrator, not the method itself. The general accuracy of the test was found to be high, both at low and high concentrations.

D. Accuracy control of WTW CellOx 325 oxygen probe

Due to continued measurements of 0.3 – 0.4 mg/l dissolved oxygen (DO) in reactions that ought to be anaerobic, the accuracy of the WTW CellOx 325 oxygen probe was controlled with an Oxi 3315 oxygen meter with an optical WTW FDO 925 probe. The optical oxygen meter has a high accuracy at low DO concentrations.

Two tests were carried out; one with sludge collected from the anaerobic section (AN 3) of the L3 bioreactor (test 1), and one with primary sludge (test 2). A 250 ml Erlenmeyer flask was filled with sludge and sealed with a rubber stopper. The WTW FDO 925 optical probe was inserted though the stopper and oxygen level monitored. When the DO concentration reached 0.0 mg/l, the WTW CellOx 325 probe was inserted, and oxygen level monitored. In both tests, it was observed that the CellOx 325 failed to reach 0.0 mg/l. In test 1 the measurement stabilized at 0.22 mg/l after 20 minutes, while it stabilized at 0.20 mg/l in test 2. In both tests the optical probe displayed more stable readings at low values than the membrane probe. It can therefore be concluded that the CellOx 325 membrane probe has low sensitivity at low DO levels, thus yielding too high concentrations in anaerobic tests.

E. Calculation of phosphate release and uptake rates

The rates were calculated by balancing the PO_4-P flow through each of the reactors. For simplification, PO_4-P was expressed as P, and the rate expression for each section is

presented below. These expressions yielded the rates as g P/g TSS h^{-1}, which were converted to g P/kg TSS d^{-1}.

$$\text{rate}_{\text{An 1}} = \frac{Q_{RAS}(P_{\text{An 1}} - P_{RAS})}{V_{\text{An 1}}TSS_{\text{An 1}}}$$

$$\text{rate}_{\text{An 2}} = \frac{[(Q_{RAS} + Q_{Inlet})P_{\text{An 2}}] - [(Q_{RAS}P_{RAS}) + (Q_{Inlet}P_{Inlet})]}{V_{\text{An 2}}TSS_{\text{An 2}}}$$

$$\text{rate}_{\text{An 3}} = \frac{(Q_{RAS} + Q_{Inlet})(P_{\text{An 3}} - P_{\text{An 2}})}{V_{\text{An 3}}TSS_{\text{An 3}}}$$

$$\text{rate}_{\text{Aer 1}} = \frac{(Q_{RAS} + Q_{Inlet})(P_{\text{Aer 1}} - P_{\text{An 3}})}{V_{\text{Aer 1}}TSS_{\text{Aer 1}}}$$

$$\text{rate}_{\text{An 3}} = \frac{(Q_{RAS} + Q_{Inlet})(P_{\text{Aer 2}} - P_{\text{Aer 1}})}{V_{\text{Aer 2}}TSS_{\text{Aer 2}}}$$

F. Calculation of struvite formation potential from PO_4-P load

The following assumptions and calculations were carried out to estimate the struvite formation potential based on the PO_4-P load at SNJ:

$$PO_4\text{-P load} = \frac{1.51\ \frac{g}{m^3} \cdot 2069\ \frac{m^3}{h} \cdot 24\ \frac{h}{d}}{1000\ \frac{g}{kg}} = 75\ \frac{\text{kg } PO_4\text{-P}}{d}$$

Assuming the loads on bioreactors L2 and L3 are equal, the overall load is 150 kg PO_4-P/d. Further, it was assumed that the amount phosphate available for uptake by PAOs was equal to the load. By assuming that 50 % of the phosphate within the biomass could be released in the waste sludge holding tank, and that 80 % of this phosphate would enter the reject stream, the potential for controlled struvite precipitation was calculated as follows:

$$\frac{150\ \frac{\text{kg } PO_4\text{-P}}{d} \cdot 0.5 \cdot 0.8}{31\ \frac{\text{kg P}}{\text{kmol}}} = 1.93\ \frac{\text{kmol P}}{d}$$

Assuming 1 mole of phosphate yields one mole of struvite:

$$1.93\ \frac{\text{kmol struvite}}{d} \cdot 245.4\ \frac{\text{kg struvite}}{\text{kmol}} = 474\ \frac{\text{kg struvite}}{d}$$