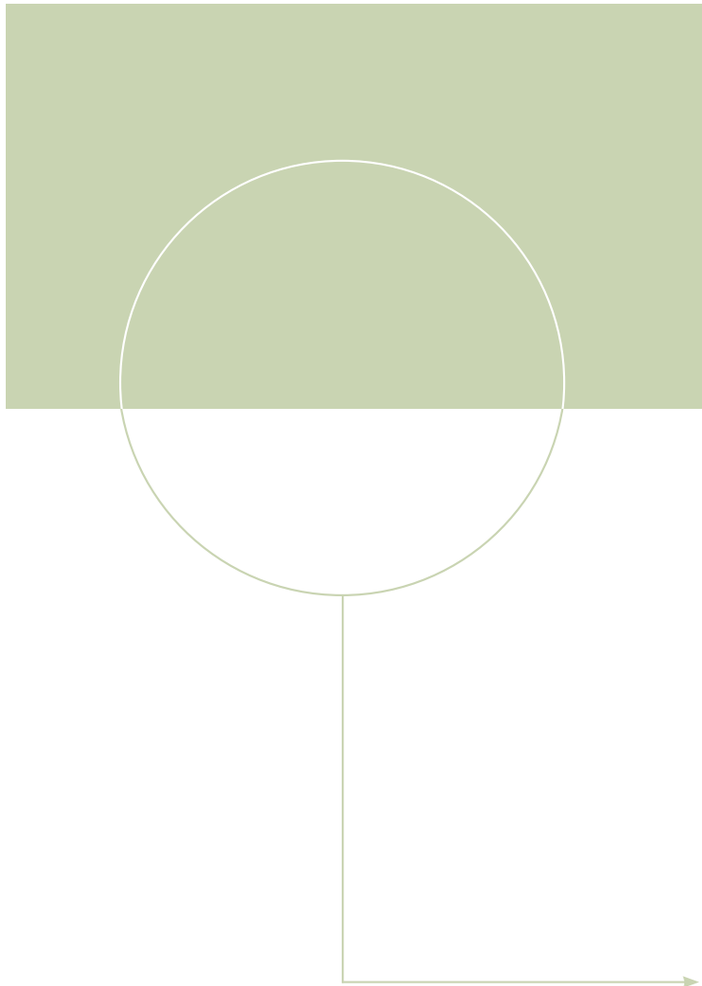

Master's thesis

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Phosphorus stripping of Bio-P sludge in cold conditions

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Submission date: June 2019
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Abstract

Many treatment plants are required to remove phosphorus from the wastewater, since discharge of too much phosphorus can cause eutrophication in aquatic systems. Phosphorus is also a very important nutrient, which all life depends on. However, it is a limited resource, and the world's phosphorus storages are depleting. Finding other ways of retrieving phosphorus is increasingly important. Recovering phosphorus from wastewater is one possibility.

Phosphorus can be removed by both chemical and biological treatment. Using chemicals is expensive and not that environmental. Recovering phosphorus when it is chemically bound is not that easy either. Biological treatment makes phosphorus more accessible for recovery and it is a more sustainable method. This thesis look at Enhanced Biological Phosphorus Removal (EBPR), a process where organisms take up excess phosphate (luxury-P) by moving through alternating anaerobic and aerobic conditions.

The high P-content in the EBPR sludge has caused issues in sludge treatment systems, especially when anaerobic digester has been used. Phosphate and magnesium are released due to hydrolysis and uncontrolled struvite precipitation may be a consequence of this. Struvite clogs the pumps and pipes and increases the operating- and maintenance cost at the treatment plant.

A possible solution that can both prevent uncontrolled struvite precipitation and facilitate P-recovery, is implementing a P-stripping unit before sludge treatment. Good separation of liquid and biomass ensures a sludge with low P-content, and hence decreases the potential for struvite precipitation. The P-concentration in the supernatant will be high and is therefore suited for P-recovery.

This thesis has investigated the potential P-release in a P-stripping unit, using EBPR sludge from both a Sequence Batch Reactor and a Continuous MBBR pilot. The experiments have been conducted in colder temperatures (11-13°C), to see if this method can be used under winter conditions in colder regions. Carbon sources has been added to enhance the P-release.

Acetate, glucose and fermented primary sludge supernatant (FPSS) has been tested as carbon sources. Having a sustainable P-stripping process was a goal, so most experiments were tested with FPSS as carbon source. The FPSS was made by mixing primary sludge and wastewater in a closed anaerobic reactor.

The results has shown that the P-stripping unit is working in colder temperatures. The best results have been 30-40% P-release of the available luxury-P. The best results were obtained when the carbon sources were added evenly throughout the experiments and not when all was added at the beginning.

Using FPSS as carbon source gave just as good results as using acetate, and sometimes even better. Glucose was the carbon source that gave the poorest results. Analyzes of VFA production during fermentation and VFA consumption during P-stripping experiments were also conducted. The analyzes showed that the VFA production was highest within the first 24 hours of the fermentation and that propionic acid and acetic acid represented over 80% of all the VFA produced.

Sammendrag

Fosfor blir på mange renseanlegg fjernet fra avløpsvannet ettersom utslipp av for mye fosfor kan føre til eutrofiering av akvatiske systemer. Fosfor er også et veldig viktig næringsstoff, som alt liv er avhengig av. Fosfor er imidlertid en begrenset ressurs, og dagens fosfor lager tømmes. Å finne andre måter å skaffe fosfor på blir derfor stadig viktigere. Å gjenvinne fosfor fra avløpsvannet er en mulighet.

Fosfor kan fjernes både med kjemisk og biologisk rensing. Å bruke kjemikalier er både kostbart og ikke veldig miljøvennlig. Gjenvinning av fosfor som er kjemisk bundet er heller ikke så lett. Biologisk rensing gjør fosfor lettere tilgjengelig for gjenvinning, samt at det er en mer bærekraftig metode. Denne masteroppgaven ser på EBPR, som er en prosess hvor mikroorganismer tar opp ekstra fosfat (luxury-P) ved å bli flyttet gjennom alternerende anaerobe og aerobe forhold.

Det høye fosfor innholdet i EBPR slam har skapt problemer tilknyttet slambehandling, spesielt hvor anaerob stabilisering blir benyttet. Fosfor og magnesium blir frigjort gjennom hydrolyse og ukontrollert struvitt utfelling kan være en konsekvens. Struvitt kan tette rør og pumper, slik at drift- og vedlikeholds kostnadene på renseanlegget økes.

En mulig løsning som både kan forhindre ukontrollert struvitt utfelling og legge til rette for fosfor gjenvinning, er å implementere en P-stripping enhet før slambehandling. God separering av biomassen og vannet sørger for at slammet har lavt fosfor innhold og det er dermed lavere potensial for ukontrollert struvitt utfelling. Fosfor konsentrasjonen i vannet er derimot høyt, og er dermed egnet for fosfor gjenvinning.

Denne masteroppgaven har sett på potensialet for fosfor frigjøring i en P-stripping enhet, ved å bruke EBPR slam fra både en Sekvensert Batch Reaktor og Kontinuerlig MBBR. Eksperimentene har blitt gjennomført i kaldere temperaturer (11-13°C) for å se om denne metoden vil fungere under vinter forhold i kaldere regioner. Karbon kilder har blitt tilsatt for å forbedre P-strippingen.

Acetat, glukose og fermentert primær slam supernatant (FPSS) har blitt testet som karbon kilder. Hovedfokuset ble å teste FPSS i eksperimentene, for å ha en mest mulig bærekraftig P-stripping. FPSS ble laget ved å blande primær slam og avløpsvann i en lukket, anaerob reaktor.

Resultatene fra eksperimenter har vist at P-stripping er mulig i kaldere temperaturer. De beste resultatene viser at 30-40% av luxury-P ble frigjort fra biomassen. De beste resultatene ble oppnådd når karbonkilden ble fordelt utover eksperimentet og ikke når alt ble tilsatt på begynnelsen.

FPSS gav like gode resultater eller bedre enn acetat. Glukose var den karbonkilden som gav dårligst resultat. Det ble gjennomført analyser for VFA produksjon under fermentering og VFA forbruk under P-stripping eksperimenter. Analysene viste at VFA produksjonen var høyest i de første 24 timene av fermenteringen og at eddiksyre og propionsyre stod for over 80% av all produsert VFA.

Preface

This master thesis has been carried out at the Department of Civil and Environmental Engineering. The thesis has been conducted over a period of 20 weeks, stretching from January to June of 2019. A large part of this thesis has been practical lab work, in NTNUs Wastewater Lab located at Valgrinda. This lab work was conducted in the period of January to April.

This master thesis has been a follow up from a project work completed in the autumn, which was a 7,5 credit subject. This master thesis is worth 30 credits. It is also a follow up from a master thesis from last year, conducted by Dag Birger Fiksdal.

This thesis is a part of the RECOVER project which NTNU is a part of. The main objective for this project is to look at processes for recovery of nutrients and other resources from the wastewater.

I would like to thank my supervisor professor Stein Wold Østerhus for introducing wastewater treatment as a topic and for inspiring me to choose this specific master thesis. I also want to thank for discussions around the obtained results and giving good input for the writing.

A special thanks is needed to my co-supervisor Dr. Blanca Magdalena Gonzalez Silva for great help regarding the practical work in the lab, discussion around results and input in the writing process.

Last, I would like to thank all the other people working in the lab, for answering questions whenever needed. My co-student Kine Hagelund Svendby has been a great support in the lab and made the lab a social place to work. She always helped when an extra pair of hands was needed.

Johanne Prestvik

Trondheim, 10.06.2019

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List of Abbrevations

CMBBR – Continuous Moving Bed Biofilm Reactor

DO – Dissolved Oxygen

DPAO – Denitrifying Polyphosphate Accumulating Organisms

EBPR – Enhanced Biological Phosphorus Removal

FPSS – Fermented Primary Sludge Supernatant

GAO – Glycogen Accumulating Organisms

HRT –Hydraulic Retention Time

MBBR – Moving Bed Biofilm Reactor

OHO – Ordinary Heterotrophic Organisms

PAO – Polyphosphate Accumulating Organisms

PHA – Polyhydroxyalkanoates

TN – Total nitrogen

TCOD – Total Chemical Oxygen Demand

TP – Total phosphorus

TS – Total Solids

VS – Volatile Solids

SBR – Sequence Batch Reactor

sCOD – Soluble Chemical Oxygen Demand

VFA – Volatile Fatty Acids

1. Introduction

1.1. Phosphorus

Phosphorus is a nutrient that is essential for aquatic plant growth. However, if the phosphorus content in the water is too great, this will lead to an overgrowth of algae. This will lead to a high depletion of dissolved oxygen and the result of this can be eutrophication. Many treatment plants therefore have requirements to remove phosphorus before discharging it into the recipient (Zou & Wang, 2016).

The phosphorus that is removed from the wastewater is a valuable resource. Phosphorus is essential for all life on earth and it is a limited resource. The phosphate rock that is used to extract phosphorus is decreasing in amount and it is getting more difficult to access it (Yuan, et al., 2012). Some studies imply that within the next 60 to 70 years about half of today's phosphate resources will be used up (Driver et al., 1999; Pastor, et al., 2008).

Finding other sources for phosphorus is therefore important. There are quite a lot of phosphorus that is produced as human waste. Phosphorus can therefore be recovered from the wastewater (Yuan, et al., 2012).

Phosphorus can be removed from the wastewater with both chemical and biological treatment. P-removal with biological treatment is more economical and environmental, compared to using chemicals for removal. It is also easier to recover the phosphorus when it is biological bound rather than chemical (Zou & Wang, 2016).

In biological phosphorus removal, there are organisms taking up the phosphorus from the wastewater and storing it within their cells (Yuan, et al., 2012). The sludge coming from the biological treatment contains high amounts of particulate phosphorus. If this sludge is stabilized through anaerobic digestion, the phosphorus can cause problems for the treatment plant. High concentrations of phosphorus increases the potential for struvite formation. Struvite is a phosphorus containing mineral which may clog pipes and pumps at the treatment plant (Fattah, 2012).

Implementation of a P-stripping unit prior to the sludge treatment can reduce the potential for struvite formation. This unit would enhance P-release from the biomass, by adding a carbon source. Conducting these experiments in colder temperature will show if the method is feasible during wintertime in colder regions.

In order to have a cheaper carbon source, fermentation of primary sludge can be used as a method to produce sCOD and VFAs internally at the treatment plant. This way more resources in the wastewater is utilized.

The sludge after P-stripping will have a lower phosphorus concentration, while the supernatant will have a high phosphorus concentration. The supernatant can then be used for P-recovery. A product containing phosphorus, such as struvite can then be sold and used as a fertilizer.

A simplified flow chart of wastewater treatment where a P-stripping unit is implemented can be seen in Figure 1.

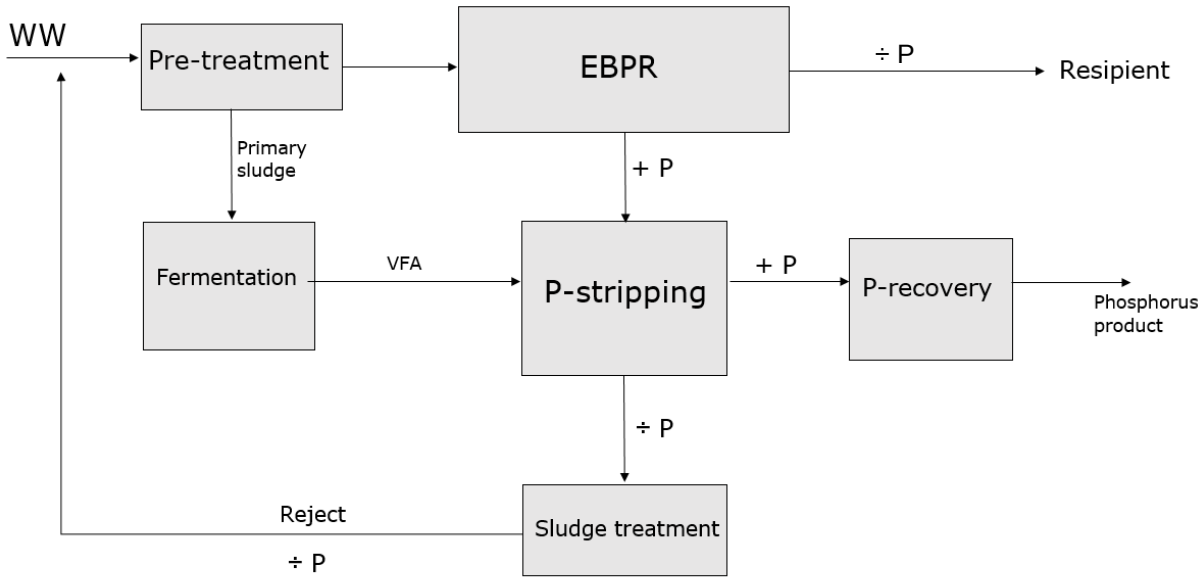

Figure 1: Simplified flow chart of wastewater treatment with implementation of P-stripping unit

1.1. Outline of work

This master thesis is part of the RECCOVER project. NTNU is participating in this project. The aim of RECOVER is to look at solutions for recovery of nutrients and energy from the wastewater. This master thesis is looking at biological phosphorus removal, which provides better opportunities to recover the phosphorus, compared to chemical phosphorus removal.

The scope of this study is to do P-stripping experiments in an anaerobic reactor, using EBPR sludge from both a Sequence Batch reactor and the continuous MBBR pilot in the wastewater lab at NTNU. The experiments are conducted in colder temperatures, to mimic winter conditions in colder regions. In order to enhance the P-stripping, fermentation of primary sludge is conducted, and used as carbon source. In total 15 P-stripping experiments and 4 fermentations is completed.

In addition to the experiments in the lab, this study also includes literature review of relevant topics, which has been used to understand some of the findings in experiments.

This master thesis is a continuation of a project submitted in the autumn. This thesis also builds on a master thesis from last spring by Dag Fiksdal. Some of the results obtained in this study is therefore compared with results from Fiksdal's master.

2. Theory

2.1. EBPR

Enhanced Biological Phosphorus Removal (EBPR) is a process using a special group of microorganisms to remove phosphorus. These organisms are collectively called Polyphosphate Accumulating Organisms (PAO). This process produces a solid stream with a high phosphorus content, which makes it ideal for phosphorus recovery. The PAO take up more phosphate from the wastewater, than is needed for cellular growth. The organisms must be under alternating anaerobic and aerobic conditions to have this excess phosphorus uptake. The alternating conditions can be achieved with a continuous flow through separate chambers with anaerobic and aerobic conditions, or a sequence batch reactor system (Yuan, et al., 2012). Figure 2 shows a simplified overview of the EBPR process.

Ordinary Heterotrophic Organisms (OHO) exists alongside the PAOs. These organisms also take up phosphorus, but only for growth. They do not take up excess phosphorus, also called "luxury-P", like the PAOs. PAOs can incorporate 0,38 mg P/mg VSS or 0,17 mg P/mg TSS. OHOs can in comparison incorporate 0,02 mg P/mg VSS or 0,015 mg P/mg TSS (Wentzel, et al., 2008). To have an effective EBPR process it is important to have a large population of PAOs.

The efficiency of the EBPR-process depends on the concentration of biomass, which is taking up the excess phosphorus, and the separation of biomass. Since the phosphorus is bound in the biomass, the phosphors is removed from the process when the sludge is taken out.

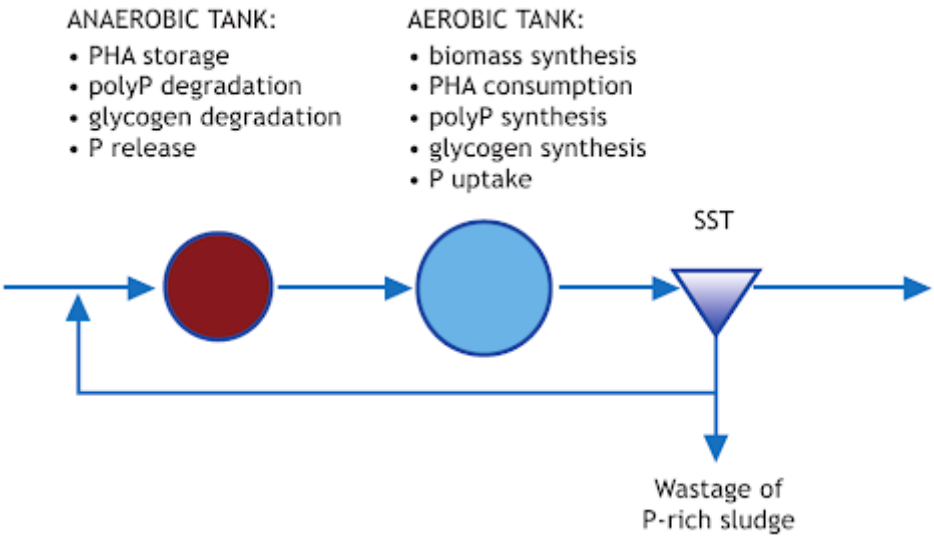

Figure 2: Overview over the EBPR process (Metcalf and Eddy, 2003; Wentzel, et al., 2008)

Prevention of recycling of oxygen and nitrogen is very important in the EBPR process. If oxygen or nitrate is sent back to the anaerobic phase, the phosphorus removal will decrease. With the presence of oxygen or nitrate OHOs will utilize the VFA for growth. This decreases the VFA available for PAOs to use, and thus decreasing the phosphorus release and uptake (Wentzel, et al., 2008).

2.1.1. Polyphosphate Accumulating Organisms

PAOs are responsible for the phosphorus removal from the wastewater. To have a functioning process, the microorganisms needs to be exposed to alternating anaerobic and aerobic conditions. There must be a sufficient amount of organic carbon in the wastewater as an energy source. VFA is the primary carbon source for PAOs (Yuan, et al., 2012). Figure 3 gives an overview of the metabolism for PAOs under anaerobic and aerobic conditions.

Under anaerobic conditions, the PAOs take up VFA. The VFA is converted to polyhydroxyalkanoates (PHA) which is a polymer stored internal in the cells as an energy source. The needed energy to store this polymer is obtained by breaking down glycogen and hydrolysis of polyphosphate bonds (Poly-P). The polyphosphate bonds are broken down to orthophosphate. This orthophosphate is released into the liquid phase (Basu, et al., 2013). The PAOs ability to obtain energy under anaerobic conditions, by taking up VFA and storing it as PHA, gives them an advantage over most organisms in the EBPR system (Yuan, et al., 2012). In this process, counter-ions such as magnesium and potassium are released together with the orthophosphate to balance the electric charge (Wentzel, et al., 2008).

In the following aerobic phase, the stored PHA is used to generate energy for cell growth and refilling the Poly-P and glycogen in the cell. The Poly-P are refilled by uptake of orthophosphate from the liquid phase. To maintain the electric balance, magnesium and potassium are also taken up. It is in the aerobic phase, that phosphorus is removed from the wastewater, creating a biomass rich in phosphorus (Basu, et al., 2013). Figure 4 shows how typical concentration profiles changes in the EBPR system.

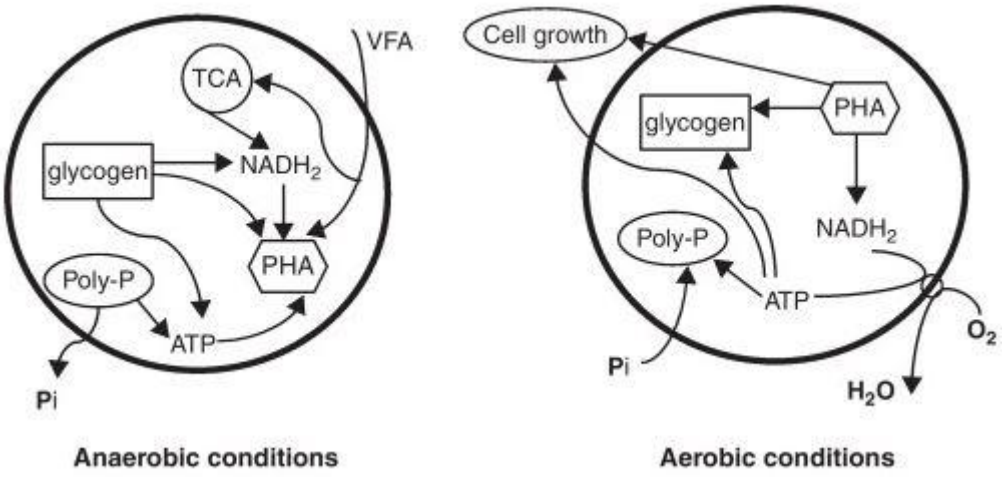

Figure 3: The metabolism for PAOs under anaerobic and aerobic conditions (Yuan, 2012).

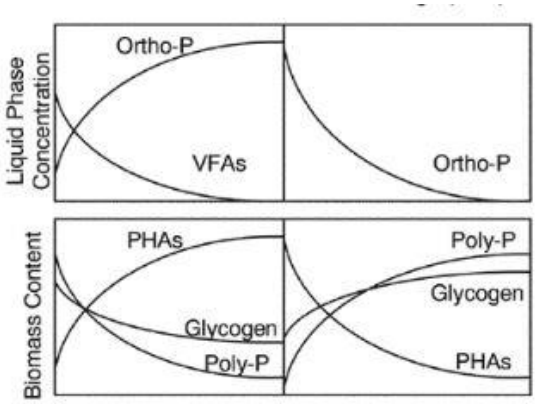

Figure 4: Typical concentration profiles in an EBPR system (Basu, et al., 2013)

Several different bacteria are involved in the process but the most well known group of PAOs is Candidatus Accumulibacter Phosphatis, also called Accumulibacter. These have been found to be representing 5-20% of the bacterial community in full-scale EBPR sludges (Yuan, et al., 2012). Another group of bacteria is Tetrasphaera. Kristiansen et al. (2013), has defined both these groups. Figure 5 illustrates the difference between Accumulibacter and Tetrasphaera.

Accumulibacter is considered to be the organism with most impact on the EBPR process in both laboratory plants and full-scale plants. The Accumulibacter is functioning similar to how the PAOs are explained previously in this chapter. They take up VFA from the liquid and store it as PHA in the cells. Tetrasphaera is functioning somewhat different (Kristiansen, et al., 2013). Both Accumulibacter and Tetrasphaera take up phosphate under aerobic conditions and take up different substrates under anaerobic conditions. However, the Tetrasphaera does not seem to form PHA in the cells. Instead Kristiansen et al. (2013) suggest that glycogen is stored under anaerobic conditions and then used as energy source under aerobic conditions, instead of PHA. Tetrasphaera also seems to be able to ferment the glucose into succinate and other compounds. (Kristiansen, et al., 2013). It also seems like Tetrasphaera is able to take up other carbon substrates than VFA, such as amino acids (Yuan, et al., 2012).

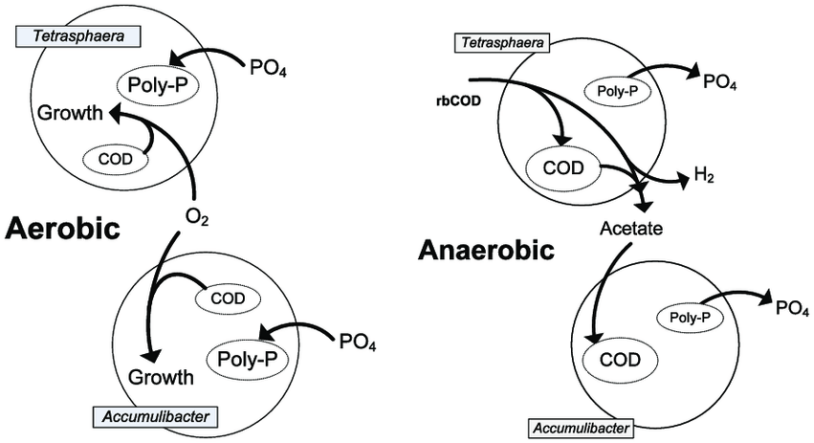

Figure 5: Shows the difference between Tetrasphaera and Accmulibacter under aerobic and anaerobic conditions (Barnard, et al., 2017)

2.1.2. Glycogen Accumulating Organisms

In an EBPR system there can be glycogen accumulating organisms, GAO, present. These bacteria competes with the PAO. GAO can also take up VFA under anaerobic conditions, but unlike PAO they do not store polyphosphate, but get the energy needed for VFA uptake from glycogen hydrolysis. VFA is often limited, so the present of GAOs will decrease the phosphorus removal performance since they take up the VFA without removing excess phosphorus. (Oehmen, et al., 2005)

The competition between PAO and GAO is affected by several parameters. Some of these are temperature, pH, carbon sources, dissolved oxygen and ions.

2.1.3. Denitrifying PAO

Experiments have indicated that two different PAO populations exists in an EBPR system. There are PAOs, which can only use oxygen as electron acceptors and DPAOs, which can use both oxygen and nitrate as electron acceptors. It has been proved that PAOs can accumulate phosphorus under anoxic conditions. This means that EBPR can also take place when there is nitrate instead of oxygen present, as long as there is readily biodegradable substrates available (Basu, et al., 2013). In full-scale treatment plants, the general observation is that the P-uptake rate in anoxic conditions is lower than the P-uptake rate in aerobic conditions. (Zeng, et al., 2003)

An advantage with DPAOs is efficient use of organic substrates. The available organic substrates can limit both biological phosphorus and nitrogen removal. Under anoxic conditions, the DPAOs will take up both phosphorus and nitrate using the same organics. This is an advantage if both nutrients is necessary to remove. It will also decrease the oxygen demand (Basu, et al., 2013).

2.1.4. Parameters effect on the EBPR process

The EBPR process is complex and many parameters affects the efficiency of the process. These are parameters such as temperature, pH, carbon source, dissolved oxygen and ions. They especially affects the competition between PAOs and GAOs.

2.1.4.1. Temperature

The biological reaction rate is affected by temperature. Observations have shown that growth rates doubles with approximately every 10°C temperature increase until optimum temperature is reached. There are however conflicting reports regarding the temperature effects on EBPR. Some reports of high efficiency at higher temperatures (20-37°C), while others reports of better P-removal at lower temperatures (5-15°C) (Mulkerrins, et al., 2004). There has been observations that temperature influences the oxygen consumption rate in EBPR systems. When the temperatures were low (5-10°C) incomplete P-uptake was observed. When the temperature increased, complete P-uptake was observed (Mulkerrins, et al., 2004).

Lopez-Vazquez, et al. (2009) found in a study that PAOs dominate at lower temperatures (10°C) since the metabolism of GAOs is inhibited at these temperatures. This was regardless of pH and carbon source available. The same study showed that GAO dominated at higher temperatures (30°C). With a mixture of acetate and propionate with a ratio of 75:25 and pH greater than 7, the domination of GAOs could however be suppressed at high temperatures.

2.1.4.2. pH

The pH affects the relationship between PAOs and GAOs in EBPR. At high pH, organisms need more energy for substrate uptake. PAOs have more energy sources compared to GAOs, which indicates that higher pH favors PAOs and therefore enhances the phosphorus removal (Ye, et al., 2016).

Wang, et al. (2013) found that phosphorus removal increased when initial pH increased in the range 6,6 to 7,8. Removal decreased when initial pH increased in the range 7,8 to 8,2. This study showed that the biomass at pH 7,8 contained more PAOs than GAOs (Wang, et al., 2013).

Filipe, et al. (2001) suggests that the phosphorus removal is strongly dependent on pH in the aerobic zone. A study was conducted in the pH range of 6,5 to 7,5. This study showed that GAOs was relatively independent of pH in this range, while PAOs showed an increase in phosphorus uptake with increasing pH. The study also showed a decrease in PHA degradation rate in lower pH, causing a decrease in the growth yield. This suggest that a higher pH favors PAOs in the aerobic zone. The same study by Filipe, et al. (2001) shows that pH affects the EBPR process in anaerobic zone as well. PAOs seems to be unaffected by pH in anaerobic zone, but GAOs show a decreasing rate of acetate uptake when pH is increased. Filipe et al. (2001) therefore suggest that pH should be greater than 7 in aerobic zone, since PAOs are sensitive to pH under these conditions.

2.1.4.3. Carbon source

The available carbon sources affects the competition between PAOs and GAOs. According to Oehmen, et al. (2005), PAOs are able to take up propionate when they are enriched using only acetate. GAOs enriched under similar conditions were not taking up propionate as efficiently. The results presented by Oehmen, et al. (2005) suggests that GAOs reacts more slowly to changes in carbon source.

According to Lopez-Vazquez, et al. (2009) the simultaneous presence of both acetate and propionate, in the ratios 75:25 or 50:50, at moderate temperature (20°C), favors the growth of PAO. With the presence of acetate or propionate as the only carbon source, neither PAO nor GAO is favored. If the pH is higher than 7,5 PAOs are favored when acetate or propionate is the sole carbon source (Lopez-Vazquez, et al., 2009).

Glucose cannot be taken up directly by PAOs, but has to be fermented into VFA first. There has been several observation of decreased efficiency in EBPR systems when glucose has been used as sole carbon source (Oehmen, et al., 2007). Mulkerins, et al. (2004) stated that changes in influent organic composition from VFAs to sugars like glucose could enhance growth of GAOs. Experiments using pre-fermented glucose has however shown to improve the P-removal efficiency (Mulkerrins, et al., 2004).

2.1.4.4. Dissolved oxygen

In the anaerobic zone the DO level should not exceed 0,2 mg/L, since the presence of oxygen will lower the efficiency of the process (Mulkerrins, et al., 2004). Mulkerrins, et al. (2004) states that the DO level in the aerobic zone should be around 2 mg/L in order to have a successful process. If nitrification also is required, the DO levels should be between 3 and 4 mg/L. DO concentrations greater than 4 mg/L does not show any improvement to the EBPR process. Maintaining a DO concentration above this level is therefore considered a waste of energy (Mulkerrins, et al., 2004).

The DO level also affects the competition between PAOs and GAOs. According to Ye, et al. (2016) a low DO concentration favors PAOs, since the proliferation of GAOs are reduced at low DO levels. PAOs can utilize more carbon at lower DO levels and consequently take up more phosphate in the aerobic zone (Ye, et al., 2016).

2.1.4.5. Ions

The concentration of cations in the wastewater affects EBPR. Magnesium and potassium is important for the stability of the intracellular Poly-P. These two ions are released and taken up simultaneously with phosphorus, acting like counter-ions in the cell (Aguado, et al., 2006). A study done by Barat, et al. (2005) showed that calcium does not take part in P-release and uptake. It is assumed that ion limitation is not likely to occur in municipal wastewater. However, if a shortage in potassium occurs, it is found that the EBPR process is affected negatively (Mulkerrins, et al., 2004).

The ion concentration is usually not measured, since it can be quite costly. Measuring conductivity variations is relatively simple and less expensive. The changes in conductivity is due to changes in ion concentrations. Aguado, et al. (2006) found that the changes in conductivity for EBPR showed similar trends as the phosphorus concentrations. Measuring conductivity could therefore give information about the EBPR performance (Aguado, et al., 2006).

Aguado, et al. (2006) found a relationship between phosphorus, potassium and magnesium through statistical analysis. 0,28 mol K/ mol P and 0,36 mol Mg/ mol P was found as average molar ratios. Barat, et al. (2005) found the same ratios in another study.

2.2. Struvite

Struvite or magnesium ammonium phosphate is an orthorhombic crystalline mineral with formula $NH_4MgPO_4 \cdot 6H_2O$. Struvite formation occurs when there is a high concentration of ammonia and phosphate. The struvite formation is highly dependent on temperature, pH and the ionic composition (Tansel, et al., 2018). The formation of struvite is determined by its solubility. There will be struvite precipitation when the combined concentrations of magnesium, ammonium and phosphate exceeds struvite's solubility limit (Ohlinger, et al., 1998). This means that the solution is supersaturated (Le Corre, et al., 2009). Struvite will precipitate with an equal molar concentration of magnesium, ammonium and phosphate (minimum 1:1:1) (Booker, et al., 1999).

The general equation for struvite formation is (with n = 0, 1 or 2) (Le Corre, et al., 2009):

$$Mg^{2+} + NH_4^+ + H_nPO_4^{3-n} + 6H_2O \leftrightarrow MgNH_4PO_4 \cdot 6H_2O + nH^+$$

The process of struvite crystallization can be divided in two parts; nucleation and crystal growth. In the nucleation phase, ions combine forming crystal embryos, which are the first state of crystals. There are two types of nucleation, homogeneous and heterogeneous. The homogeneous process is spontaneous crystal occurrences in highly purified or highly supersaturated solutions. The heterogeneous process is the occurrence of crystal formation because of foreign particles or impurities, which function as substrates. In wastewaters, the content of impurities is high. Crystal formation of struvite in wastewater systems is therefor most likely a heterogeneous nucleation process. In the crystal growth phase, crystal embryos increases in size. The final size and structure of the crystals are determined in the crystal growth (Le Corre, et al., 2009).

Struvite may cause problems in the wastewater treatment plants systems. Formation of struvite within the system can cause pipes to clog and pumps to fail, increasing the maintenance costs. Struvite has usually been found in areas of high turbulence, such as pumps or valves, or in areas with high phosphate concentrations, such as anaerobic digesters (Le Corre, et al., 2009).

Struvite could however be beneficial to the treatment plant, if the formation of the crystals are controlled. Struvite crystals could be used as fertilizers, although it is not widely used yet. Studies has however confirmed excellent agronomic properties for struvite (Booker, et al., 1999). Magnesium is usually the limiting compound in struvite formation at treatment plants. To have production of struvite, addition of chemicals containing magnesium may be necessary (Fattah, 2012).

According to Booker, et al. (1999) pH must be greater than 5,5 to have struvite formation since it is readily soluble in water at pH below 5. The optimum pH range for struvite formation varies between studies. Most studies report of struvite crystallization in the pH range from 8 to 9 (Tansel, et al., 2018).

The availability of phosphate, ammonium and magnesium is pH dependent, since the speciation of the components are dependent on pH (Ohlinger, et al., 1998). At higher pH, the ion concentration of phosphate will increase while the ammonium ion concentration will decrease, as free ammonium ions will transform into gaseous ammonia (Booker, et al., 1999). The increase in phosphate or decrease in nitrogen affects the molar ratio which needs to be minimum 1:1:1 to have formation of struvite (Le Corre, et al., 2009).

The solubility of struvite is also pH dependent. The solubility of struvite decreases as pH increases. Ohlinger, et al. (1998) found that the minimum solubility occurred at pH 10,3. The solubility will therefore decrease with increasing pH up to 10,3 and increase when pH is higher than 10,3. When the solubility is low supersaturation can easier be reached (Le Corre, et al., 2009).

Temperature also affects struvite solubility. Aage et al. (1997) did a study looking at the solubility product of struvite at temperatures between 10 and 65°C. The solubility increased as the temperature increased. The maximum solubility was found at 50°C. The results at 65°C was difficult to read. Investigations showed that the structure of struvite changes in the temperature range of 64°C to 67°C, which is likely to affect the solubility (Aage, et al., 1997). Authors therefore usually study struvite precipitation in the temperature range of 25°C to 35°C (Le Corre, et al., 2009).

2.3. Anaerobic digester

In a sludge treatment process, stabilization of sludge is done to prevent unpleasant smell during use and storage of sludge. The stabilization breaks down the organic compounds in the sludge. Stabilization of sludge can be achieved biologically, chemically and thermally. Anaerobic digestion is the most used method of stabilization worldwide (Luduvice, 2007). This is a biological stabilization process using different bacteria to break down the organic matter in the sludge. In addition to stabilizing the sludge, anaerobic digestion gives an opportunity to produce biogas. The method also reduces the amount of sludge, which can lower transportation costs (Ødegaard, 2014).

The anaerobic digestion is happening in three phases. The process takes place in a closed container without oxygen. The first phase is hydrolysis, where the particulate organic matter is solubilized. The next phase is acidogenesis, where a group of bacteria breaks down the organic matter into organic acids. In the third and final phase, methanogensis, a different group of bacteria breaks the organic acids down to methane and carbon dioxide (Luduvice, 2007). Figure 6 gives an overview over the phases of anaerobe stabilization.

The anaerobe digestion is a rather slow process. It is therefore usual that the sludge is heated to increase the degradation rate. The temperature is usually kept in the area of 35-38°C. If the process is run thermophilic, above 55°C, hygenization of the sludge is also achieved (Ødegaard, 2014).

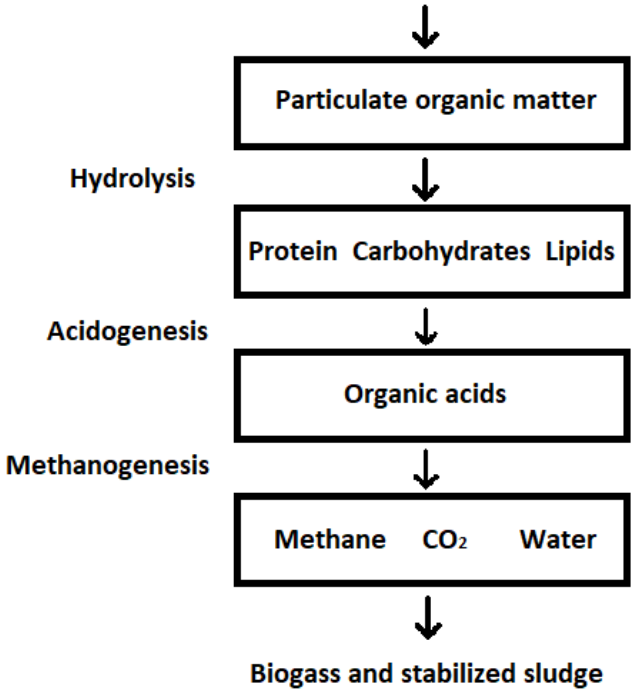

Figure 6: Phases of anaerobe stabilization. Adapted from (Ødegaard, 2014)

2.3.1. Struvite precipitation in anaerobic digester

If the sludge from an EBPR process is stabilized in an anaerobic digester, problems with struvite precipitation might occur. The biomass in the sludge is rich in phosphorus. Much of the phosphorus PAOs removed from the wastewater will be released during anaerobic digestion. Magnesium ions are also released. Ammonium is released due to solid degredation. If the conditions allows it, there could be uncontrolled struvite formation. This struvite formation is highly unwanted, since it increases pumping and maintenance costs. Accumulation of struvite on pipe walls and equipment can cause pipe clogging and foul pumps. The hydraulic capacity of the pipe system and the biological treatment capacity is reduced (Fattah, 2012).

The pH in the anaerobic digester and post-digester processes is usually higher than the preceding processes. The pH is higher due to stripping of CO_2. This means the potential for struvite potential is higher in the digester (Ohlinger, et al., 1998). The temperature in mesophilic operation of anaerobic digestion is around 35°C, giving great temperature conditions for struvite formation.

Many treatment plants experience circulation of nutrients in the treatment system. If phosphate is released in the digester, the reject water coming from the sludge treatment will contain phosphate. When this reject is sent to the head of the treatment plant, phosphate is not removed from the treatment system. This will increase the overall phosphate concentration in the treatment system, increasing the potential for struvite formation (Fattah, 2012).

A possible solution for uncontrolled struvite formation in the digester and treatment system, is to implement a P-stripping unit prior the digester. Under anaerobic conditions, the PAOs will release the phosphate taken up in the EBPR process. Some magnesium will also be released. By separating the solids from the liquid after P-stripping, the biomass going to the digester contains less phosphate and less magnesium. This lowers the potential for struvite formation. The liquid from the mixer will have a high phosphate concentration, making it ideal for phosphorus recovery, for example through struvite precipitation. To make the PAOs release the phosphate, there must be available VFA to take up. Since the carbon in the wastewater is already used in the previous EBPR process, external carbon must be added, which is expensive. One possibility is to ferment primary sludge and wastewater from the treatment plant. Through fermentation, VFA and other sCOD is produced.

2.4. Settling and dewatering properties

Separation of solids is very important in the EBPR process. The amount of phosphorus that is removed though the process is dependent on how much of the biomass is separated from the liquid. It is important with good settling and dewatering properties when the biomass is separated from the liquid. Bioflocculation has great impact on the separation of solids and liquid, since larger particles will settle better. Without aggregation of microorganisms the settling and dewatering properties will be poor (Sobeck & Higgins, 2002).

Divalent Cation Bridging Theory is a theory claiming that divalent cations promotes bioflocculations. Microorganisms produce biopolymers with negatively charged groups. These polymers will attach to the microorganisms. Divalent cations, such as magnesium

and calcium can bridge these negatively charged groups together, creating larger and more stable flocs. This will increase settling properties and the dewatering properties (Sobeck & Higgins, 2002). Figure 7 illustrates how divalent cations can bridge the microorganisms together.

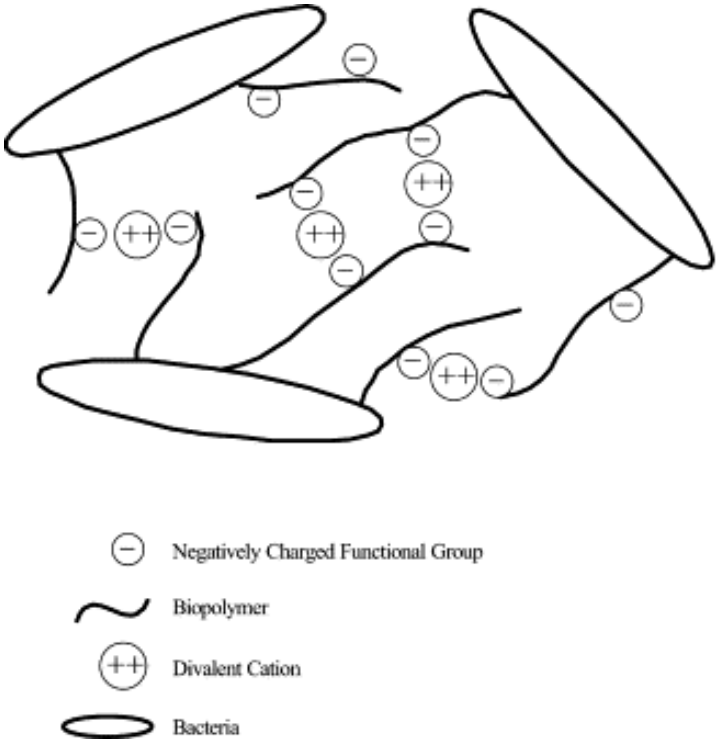

Figure 7: Divalent cation bridging (Soebeck & Higgins, 2002)

Monovalent cations does not have the same bridging effect. If the concentration of monovalent cations is high, ion-exchange processes may occur. The monovalent cations will replace the divalent cations, and thereby weakening the floc structure (Higgins & Novak, 1997).

Higgins & Novak (1997) found in a study that addition of sodium deteriorated settling and dewatering properties. Their study showed that a ratio between monovalent and divalent cations greater than 2 resulted in poorer settling and dewatering properties.

Having pre-dewatering after P-stripping would be beneficial, since magnesium is released in the reactor and hence improving the dewatering properties of the sludge. Pre-dewatering would then separate the solids and liquid better. The liquid would then be rich in phosphate and the biomass moving forwards to anaerobic digestion will not contain phosphate, preventing struvite formation. The reject water from the pre-dewatering will have a high phosphate concentration and could be used for phosphorus recovery.

2.5. Fermentation of Primary Sludge

Primary sludge usually contains large amounts of biodegradable organic compounds, such as proteins, carbohydrates and cellulose. Especially cellulose is highly represented in Western European countries due to flushing of toilet paper into the sewer system. Primary sludge is therefore suited for carbon recovery (Crutchik, et al., 2018). Through fermentation of primary sludge, VFA can be produced and then used as carbon source for biological nutrient removal. Figure 8 shows a schematic diagram of the processes involved in fermentation of sludge and how the carbon changes through the process.

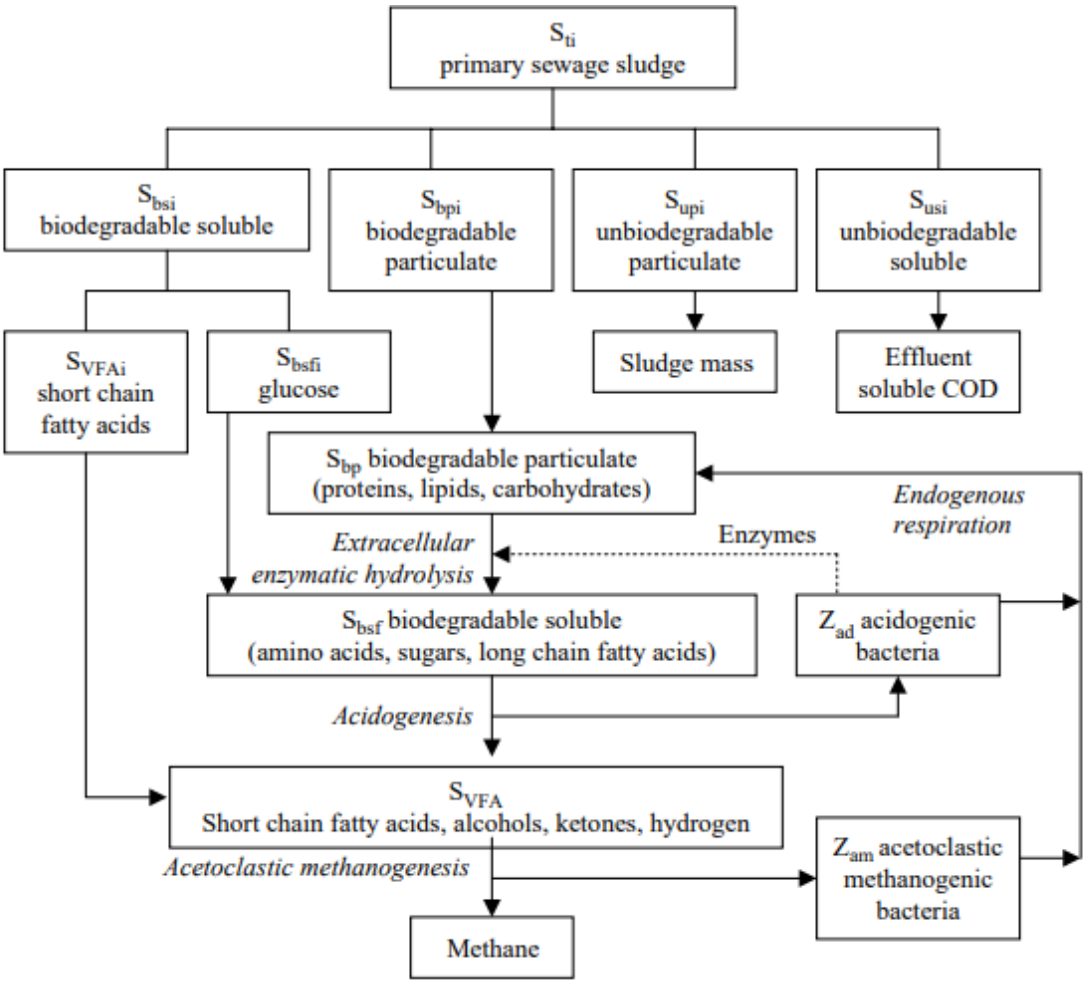

Figure 8: Schematic diagram of processes involved in fermentation of sludge (Ristow, et al., 2006).

Fermentation of primary sludge takes place under anaerobic conditions. Complete degradation of sludge takes place in three steps. First, there is the hydrolysis phase, where enzymes break down particulate organic compounds into soluble organic compounds. These compounds then take part in the subsequent acidogenesis reaction (Ristow, et al., 2006). In this reaction bacteria is generating VFA such as short chained fatty acids and alcohols. The last phase is the methanogenesis, where different bacteria breaks down VFA into methane (Ferreiro & Soto, 2003). If the purpose is to produce carbon sources for nutrient removal, the process needs to end after the acidogenesis, before VFA is broken down to methane gas.

Several operating conditions for fermentation is affecting the VFA production. Temperature, pH, HRT and solid concentration are some.

The biological reaction rate is increasing with higher temperatures, meaning the bacteria will break down the organic matter faster in higher temperatures (Mulkerrins, et al., 2004). Different temperatures has been observed as the optimum temperature for VFA production. Crutchik, et al. (2006) found the highest VFA production at 37◦C. In a study by Maharaj & Elefsiniotis (2001) the highest VFA production was observed at 25◦C.

pH influences the production of VFAs. Zeng, et al. (2006) suggests that the optimal pH for fermenting is between 6 and 7. Crutchik, et al. (2018) found the optimal pH for VFA production to be 8.

The hydraulic retention time also influences the VFA production. Maharaj & Elefsiniotis found highest VFA concentrations with a HRT of 30 hours. They observed a decrease in VFA concentration when the HRT was increased to 48 and 60 hours. Koyunluoglu-Aynur, et al. (2011) observed an increase in VFA concentration when the HRT was increased from 1,5 to 2,5 days. They also observed that the concentration decreased when HRT was increased further to 3,5 days. The primary sludge used in this study was however blended with waste activated sludge.

Zeng, et al. (2006) observed a higher VFA production when the solid concentration was increased. In the same study the solid concentration seemed to affect the type of VFAs that were produced. With low solid concentration, only acetic acid was observed.

3. Materials and methods

Materials and methods used during experiments and tests are described in this chapter. Several types of tests and experiments have been performed. Experiments with fermentations have been conducted and samples has been taken out to see how parameters in the fermenter change over time. The supernatant of the fermented primary sludge has been used in P-stripping experiments as carbon source for PAOs. This product will be called FPSS.

Sludge from both a sequence batch reactor and the lab's CMBBR pilot has been analyzed for TS, VS, TP and PO_4-P before used in P-stripping experiments. These experiments were conducted in cold temperatures (11-13°C). Several parameters were measured regularly during the experiments to see the behavior of the PAOs.

Several parameters has been measured throughout this study. The methods for determining these are also described in this chapter.

All measured parameters in the fermentations can be found in Appendix C, while all data from P-stripping experiments can be found in Appendix D.

3.1. Fermentation

Fermentation of primary sludge and wastewater was done to produce a supernatant with high sCOD concentration for P-stripping experiments. The sludge was collected from the Salsnes filter in the lab. This sludge was mixed with wastewater, that had already been filtered through the Salsnes filter. Picture of the Salsnes filter and the tank holding wastewater is shown in Figure 9.

Figure 9: Salsnes filter and Wastewater tank

The aimed concentration of TS in the fermentation mix was 1%. The composition of primary sludge and wastewater was found based on this percentage. The diagram in Figure 10 was used to find the volume of sludge needed per 1000 mL to have approximately 1% TS in the mix. The volume of wastewater was found by subtracting the needed sludge volume from the total volume in the reactor.

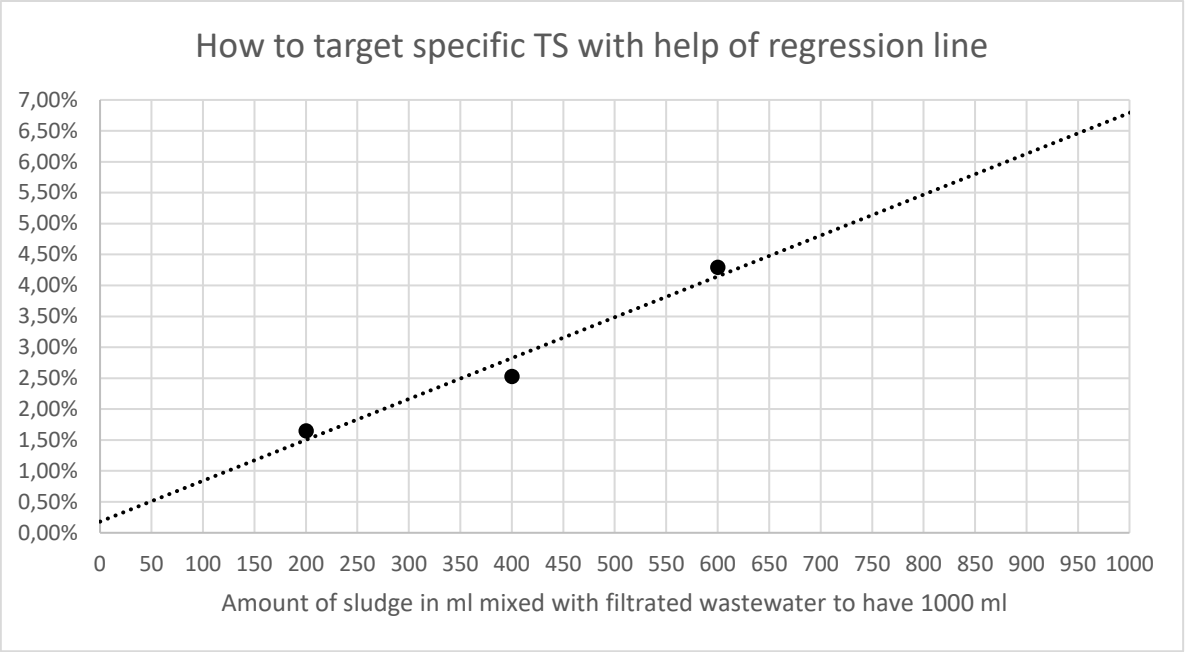

Figure 10: Diagram used to determine the amount of primary sludge and wastewater needed based on TS % (Svendby, 2019)

Two different reactors were used for the fermentation of primary sludge. The first reactor was used for fermentation in room temperature (19-20°C), and the other reactor was used for fermentation in warmer temperatures (25-28°C). Aquarium heaters were used to heat up a water bath, which the reactor was placed in. Figure 11 shows a picture of the reactor in heated water bath. The reactors had a motor with a rod and attached blades to provide mixing. The mixing was set to 100 rpm. The reactors had a lid to avoid oxygen interfering. Four rounds of fermentation were completed.

Figure 11: Fermenter placed in heated water bath

The approach was the same for all different rounds of fermentation. The primary sludge was scooped up with a beaker directly from the Salsnes filter. The wastewater was tapped from the tank holding filtrated wastewater, into a measuring cylinder. The sludge and wastewater was then placed in a reactor. To start the fermentation process, the mixing was turned on.

One sample was taken shortly after mixing started. This sample was analyzed for TS, VS, TP, TN, TCOD, sCOD, PO_4-N and NH_4-N. Samples was then taken regularly to see how parameters changed throughout the fermentation process. Samples were usually taken twice a day; one in the morning and one in the afternoon. These samples were analyzed for sCOD, NH_4-N and PO_4-P. Both pH and temperature were measured whenever a sample was taken.

To have new substrates for the bacteria in the fermenter, new sludge and wastewater needed to be added. Half of the mix in the reactor was taken out using a siphon. A new mix of sludge and wastewater was prepared using half the volume that was used of the original mix. This way the volume in the reactor always stayed the same. The fermentation from the beginning to the time where new mix is added, is referred to as one batch. The duration of the batches depended on the wanted hydraulic retention time. The two first fermentations were running with a retention time of 4 days. One batch therefore lasted for two days. For the last two fermentations one batch lasted four days, to achieve a retention time of 8 days. Table 1 gives an overview over the four fermentations.

The parameters varying between the fermentations were the temperature and the hydraulic retention time. All four rounds of fermentation were planned to last for eight days. The fourth fermentation was however ended after four days. This was because there was no sludge to collect from the Salsnes filter. The lack of sludge was most likely due to dilution of the wastewater coming into the lab. The retention time in the last fermentation was therefore changed to 4 days.

Table 1: Overview of the fermentations

Start date	Fermentation nr.	Duration [Days]	HRT [Days]	Volume	Temp	Nr. of batches
13.02.2019	F1	8	4	450 mL sludge + 2550 mL ww	19-20°C	4
22.02.2019	F2	8	4	225 mL sludge + 1275 mL ww	25-28°C	4
07.03.2019	F3	8	8	300 mL sludge + 1700 mL ww	25-28°C	2
25.03.2019	F4	4	4	300 mL sludge + 1700 mL ww	25-28°C	1

At the end of a batch, the mix that was removed from the reactor was filtrated through a cloth. The filtrated liquid was put in a closed container and stored in the fridge. This was later used in P-stripping experiments. At the end of a fermentation, all the content was filtrated and stored.

3.2. SBR

A Sequence Batch Reactor with Bio-P sludge received from IVAR central treatment plant Nord-Jæren has been running in the lab since the spring of 2018. The SBR was built to provide optimal conditions for PAOs to live and grow. The SBR was set up by a former master student, Dag Fiksdal, and has been running continuously with the same biomass (Fiksdal, 2018). Settled sludge from this reactor has been extracted to use in P-stripping experiments.

The SBR has been operating with a continuous 8-hour cycle. The cycles starts with filling of the reactor, followed by anaerobic and aerobic phases and finishes with settling and draining. The first cycle has an extra 20 minutes at the end to allow for sludge extraction. The time schedule for the SBR can be seen in Table 2.

The SBR system consists of five main elements. A picture of the SBR setup can be seen in Figure 12. There is the actual reactor where the biomass is being kept and where sludge can be extracted. There is wastewater that has been filtrated through a Salsnes filter, being pumped into the reactor. A feeding solution with acetate (VFA) and phosphate is also pumped in the reactor to enhance growth of PAOs. Several pumps are connected, pumping in wastewater and feeding solution and pumping out supernatant. Timers are controlling the process, by starting and stopping the pumps, the mixing and the aeration.

Figure 12: SBR set up

Table 2: Time schedule for the SBR

Phase	Duration [hours : minutes]	Starting time	Ending time
Filling	00:15	00:00	00:15
Anaerobic	02:30	00:15	02:45
Aerobic	03:55	02:45	06:40
Settling	00:40	06:40	07:20
Draining	00:20	07:20	07:40
Sludge extraction	00:20	07:40	08:00
Filling	00:15	08:00	08:15
Anaerobic	02:30	08:15	10:45
Aerobic	04:15	10:45	15:00
Settling	00:40	15:00	15:40
Draining	00:20	15:40	16:00
Filling	00:15	16:00	16:15
Anaerobic	02:30	16:15	18:45
Aerobic	03:55	18:45	23:00
Settling	00:40	23:00	23:40
Draining	00:20	23:40	00:00

3.2.1. SBR Reactor

The reactor keeping the biomass had a working volume of 40 L. The bottom had a frustum-shape, making sedimentation of sludge easier. The frustum height was 20 cm and the sides had slopes around 45°. Sludge was extracted at the bottom. The reactor had sampling ports located at the 20 L, 25 L and 30 L mark, allowing supernatant to be pumped out. A perforated tube was running at the top edge of the frustum to provide aeration in the reactor. Wastewater and the feeding solution was pumped in at the top of the reactor. A motor was also placed at the top to provide mixing (Fiksdal, 2018).

3.2.2. Wastewater

The wastewater is filtrated through the lab's Salsnes filter. This reduces the risk of clogging in the reactor, since the filter removes some of the solids. The wastewater was pumped in at the 30 L mark in the reactor. 10 L of wastewater was pumped in every cycle and 10 L of supernatant was pumped out every cycle (Fiksdal, 2018).

3.2.3. Feeding solution

In order to have good conditions for the PAOs in the reactor, a feeding solution with a high concentration of acetate and phosphate was pumped in at the same time as the wastewater. For the feeding, 150 mL solution with a concentration of 11,1 g sCOD/L and 1,582 g PO_4-P/L was pumped in every cycle. The ratio of mg PO4-P/mg sCOD was therefore 0,14. For lab-systems Schuler & Jenkins (2003) recommended a ratio greater than 0,12 (Fiksdal, 2018).

The sCOD was added as acetate, made by mixing distilled water and sodium acetate trihydrate. The phosphate was added as di-Sodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$).

The solution was pumped in separately from the wastewater. Assuming the feeding solution was pre-mixed with the 10 L of wastewater, the concentration of sCOD would increase with 166,5 mg sCOD/L and 23,73 mg PO_4-P/L. This PO_4-P concentration is not realistic for real wastewaters, but the aim of this SBR reactor was to enhance growth of PAO (Fiksdal, 2018).

3.2.4. Dissolved oxygen

The DO concentration during the aerobic phases was 8 $\pm$ 0,5 mg O_2 /L^{-1} (Fiksdal, 2018).

3.2.5. Mixing

Mixing in the reactor was provided by blades attached to a rod that was connected to the motor at the top of the reactor. Mixing speed was set to have complete mixing in the reactor (Fiksdal, 2018).

3.2.6. Temperature

There was no measures to control the temperature in the reactor (Fiksdal, 2018).

3.3. CMBBR Pilot

At the wastewater laboratory at NTNU, there is a CMBBR pilot doing biological phosphorus removal. This pilot is based on the Hias process. The pilot has a total volume of 1,06 m^3 divided into ten chambers. The first four are anaerobic chambers, while the last six is aerobic chambers. Figure 13 shows pictures of the pilot. Wastewater from the lab's filtrated wastewater tank, is entering the first chamber (Finstad, 2018).

The carriers that is used in the pilot is the K1 Kaldnes. These carriers has a specific area of 500 m^2/m^3 and is produced by Krüger Kaldnes of Veolia Water Solutions & Technologies. The filling degree in the pilot is 60%. There is a conveyor belt in chamber ten, leading carriers back to chamber one, which is the first anaerobic chamber. There is also an outlet in chamber ten, leading the effluent out (Finstad, 2018).

The effluent from the pilot has been collected to use as sludge in P-stripping experiments.

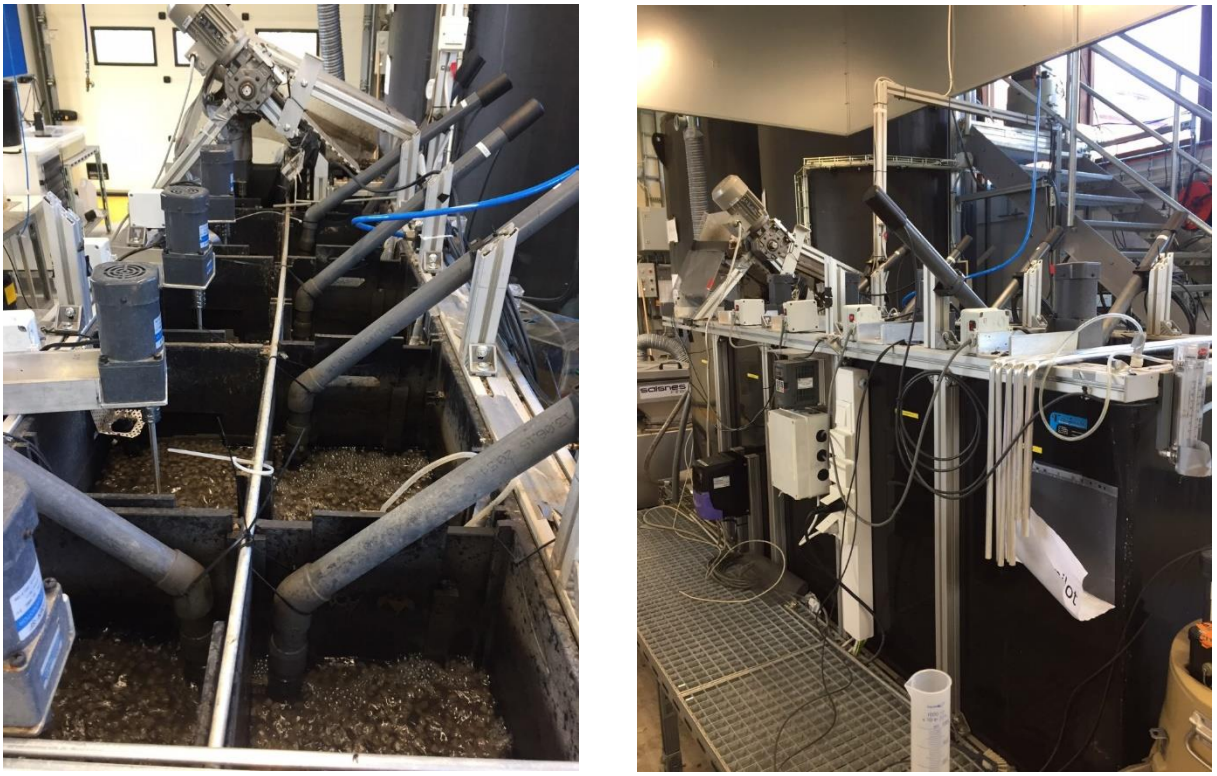

Figure 13: CMBBR Pilot in the wastewater lab

3.4. P-stripping Reactor

An anaerobic reactor was used for P-stripping experiments. The reactor was a cylinder with a lid, containing small holes for sample extraction. A motor was attached at the top with a rod and blades to ensure mixing. Mixing was set to 60 rpm. The working volume of the reactor was 1,6L. Each experiment lasted five hours. Experiments were conducted with sludge from both SBR and CMBBR Pilot. An external carbon source was added in the mixer to promote release of phosphate. The carbon source and dosage varied between the experiments. Two experiments were conducted in room temperature, meaning no temperature control. The rest of the experiments were conducted in colder temperatures, between 11 and 13°C. To achieve colder conditions in the sludge, the reactor was placed in a cold water bath. Snow and ice was added to the water bath in order to keep the temperature down.

Samples were extracted at the beginning of each experiment, after 30 minutes, after one hour and then every hour until five hours had passed. All samples were analyzed for PO_4-P and sCOD. NH_4-N was analyzed for the first and last sample, while Mg^{2+}, K^+ and Ca^{2+} were analyzed for three samples. Temperature and conductivity was also measured every time sample was extracted. All P-stripping experiments is listed in Table 3, with an overview of different carbon sources and dosages used. The P-stripping reactor and the water bath can be seen in Figure 14.

Table 3: Overview of the P-stripping experiments

Kinetics nr.	Date	Temperature	Sludge source	Carbon source	Carbon dosage	Dosing interval
1	25.01.2019	Room	SBR	FPSS	100 mg sCOD/L	Every hour
2	29.01.2019	Cold	SBR	FPSS	100 mg sCOD/L	Every hour
3	15.02.2019	Cold	SBR	FPSS	100 mg sCOD/L	Every hour
4	21.02.2019	Room	SBR	Glucose	100 mg sCOD/L	Every hour
5	27.02.2019	Cold	SBR	FPSS	500 mg sCOD/L	Once at the beginning
6	06.03.2019	Cold	SBR	FPSS	200 mg sCOD/L	Every second hour
7	14.03.2019	Cold	SBR	FPSS	200 mg sCOD/L	Every hour
8	20.03.2019	Cold	Pilot	FPSS	200 mg sCOD/L	Every hour
9	22.03.2019	Cold	Pilot	FPSS	100 mg sCOD/L	Every hour
10	26.03.2019	Cold	SBR	Acetate	100 mg sCOD/L	Every hour
11	29.03.2019	Cold	SBR	Acetate	200 mg sCOD/L	Every hour
12	02.04.2019	Cold	SBR	FPSS	100 mg sCOD/L	Every hour
13	03.04.2019	Cold	Pilot	FPSS	100 mg sCOD/L	Every hour
14	08.04.2019	Cold	Pilot	Glucose	100 mg sCOD/L	Every hour
15	10.04.2019	Cold	Pilot	Acetate	100 mg sCOD/L	Every hour

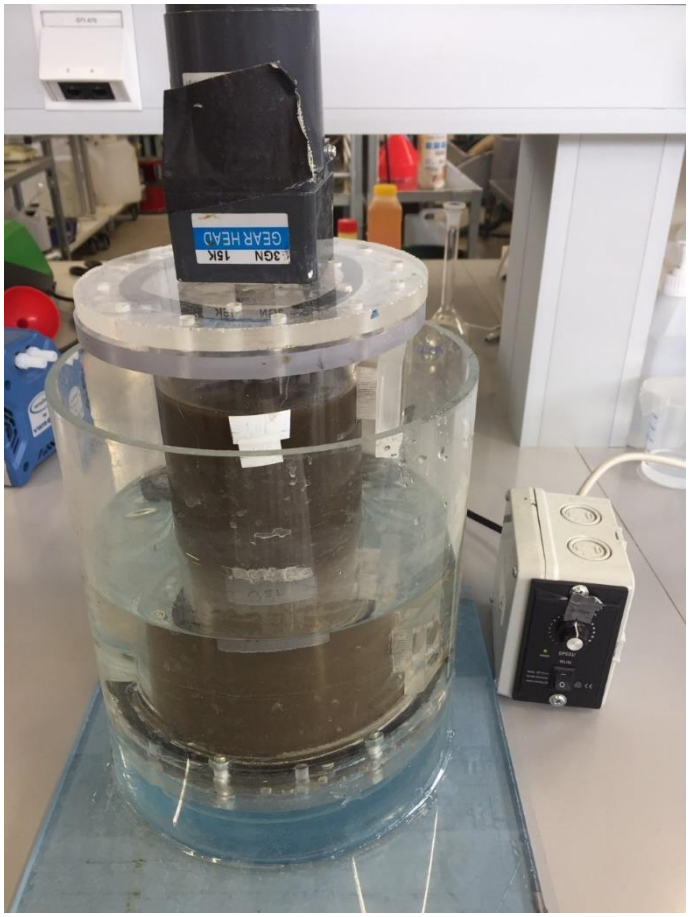

Figure 14: P-stripping reactor in cold water bath

3.4.1. Carbon source

Different carbon sources were tested in the P-stripping experiments, to see what promoted the highest phosphate release. Glucose, acetate and FPSS were tested. FPSS was used in the majority of the experiments. The dose of carbon source also varied.

In all but one experiment, the carbon source was added every hour or every second hour of the experiment. For one experiment, the entire carbon source was added at the beginning of the experiment.

The FPSS was made in the lab using primary sludge from a Salsnes filter and wastewater, as explained previously in this chapter. Acetate and glucose was made by mixing salts and distilled water. Methods of calculating the carbon doses is shown in the following sections.

The calculations of carbon doses for each experiment can be seen in Appendix A.

3.4.1.1. Fermented Primary Sludge Supernatant

The FPSS was extracted at least one day before the P-stripping experiment, because the concentrations of sCOD, PO_4-P and NH_4-N needed to be analyzed. These concentrations needed to be known to calculate the FPSS dose. The FPSS was kept in the fridge in a closed container before being used.

When the sCOD concentration in the FPSS was known, the volume of the dose could be calculated. Equation (1) was used to find the volume of FPSS added in P-stripping experiments.

$$V_1 = \frac{C_2 V_2}{C_1} \quad (1)$$

V_1 = Volume of the FPSS dose

C_2 = sCOD concentration in the reactor

V_2 = Volume in the reactor

C_1 = sCOD concentration in the FPSS

The FPSS with 1% TS did not give high enough sCOD concentrations. Therefore, FPSS, made by another student in the lab was used in experiments. This fermentation was done using 2% TS. This was the only difference between the fermentations, as they started and ended at the same time and samples were drawn simultaneously.

3.4.1.2. Acetate

To make the acetate dose, a mix of distilled water and sodium acetate trihydrate ($C_2H_3O_2Na{\cdot}3H_2O$) was made. Equation (2) shows the relationship between acetate and COD. 1 gram of acetate gives 1,0845 gram COD (Fiksdal, 2018).

$$COD = \frac{2MO_2}{MC_2H_3O_2} = \frac{2 \cdot (2 \cdot 16 \frac{g}{mol})}{(2 \cdot 12 \frac{g}{mol} + 3 \cdot 1 \frac{g}{mol} + 2 \cdot 16 \frac{g}{mol})} = 1{,}0845 \quad (2)$$

The concentration of COD needed to be high enough that a dose of 16 mL would give the desired concentration in the reactor. The volume of the solution needed to be large enough to have all the needed doses for the experiments.

3.4.1.3. Glucose

To make the glucose dose, the salt glucose ($C_6H_{12}O_6$) was mixed with distilled water. The relationship between glucose and COD can be seen in Equation (3). 1 gram of glucose gives 1,07 gram COD.

$$COD = \frac{6MO_2}{MC_6H_{12}O_6} = \frac{6 \cdot (2 \cdot 16 \frac{g}{mol})}{(6 \cdot 12 \frac{g}{mol} + 12 \cdot 1 \frac{g}{mol} + 6 \cdot 16 \frac{g}{mol})} = 1{,}07 \quad (3)$$

The concentration of COD needed to be high enough that a dose of 16 mL would give the desired concentration in the reactor. The volume needed to be large enough to inject all necessary doses through the experiments.

3.4.2. Dilution adjustment

The sCOD concentration in the FPSS was much lower than the concentration that could be achieved by adding glucose or acetate. To have the wanted concentration of sCOD in the P-stripping reactor, a larger volume of FPSS needed to be added. Because of this, the total volume in the reactor would change throughout the experiment. The values of release and uptake therefore needed to be adjusted for dilution. This was done by multiplying the measured concentration with the volume in the reactor at the time of sample extraction. The release and uptake was then represented as a mass.

The sCOD and PO_4-P content in the added FPSS needed to be taken into account. By multiplying the concentration with the volume of the dose, the added mass of sCOD and PO_4-P is found. This mass is used to adjust the release of PO_4-P and uptake of sCOD.

An example of these calculations can be seen in Table 4 and Table 5. This is the results from the P-stripping experiment conducted the 29.01.2019. The samples were taken before the FPSS was added, except for the first sample which were extracted after the addition. The starting volume of SBR sludge was 1070 mL and each dose of FPSS was 86 mL. The sCOD and PO_4-P concentrations in the FPSS were 1860 mg/L and 26,4 mg/L. Equation (4) and (5) shows the calculations for finding the mass of sCOD and PO_4-P added to the sludge through FPSS.

$$0{,}086\ mL\ \cdot 1860\ {}^{mg\ sCOD}/_{L} = 159{,}96\ mg\ sCOD \tag{4}$$

$$0{,}086\ mL\ \cdot 26{,}4\ {}^{mg\ PO4}/_{L} = 2{,}27\ mg\ PO_4 - P \tag{5}$$

Table 4: Corrections for the volume changes

Time [min]	sCOD [mg/L]	Volume correction
0	70	70 mg/L*(1,07L+0,086L) = 80,9 mg
30	41	41 mg/L*(1,07L+0,086L) = 47,4 mg
60	139	139 mg/L*(1,07L+2*0,086L) = 172,6 mg
120	181	181 mg/L*(1,07L+3*0,086L) = 240,4 mg
180	231	231 mg/L*(1,07L+4*0,086L) = 326,6mg
240	279	279 mg/L*(1,07L+5*0,086L) = 418,5 mg
300	283	283 mg/L*(1,07L+5*0,086 L) = 424,5 mg
Time [min]	**PO_4-P [mg/L]**	**Volume correction**
0	13,7	13,7 mg/L*(1,07L+0,086L) = 15,8 mg
30	32,5	32,5 mg/L*(1,07L+0,086L) = 37,6 mg
60	37,7	37,7 mg/L*(1,07L+2*0,086L) = 46,8 mg
120	61,6	61,6 mg/L*(1,07L+3*0,086L) = 81,8 mg
180	87,5	87,5 mg/L*(1,07L+4*0,086L) = 123,7 mg
240	87,2	87,2 mg/L*(1,07L+5*0,086L) = 130,8 mg
300	91,1	91,1 mg/L*(1,07L+5*0,086L) = 136,7 mg

Table 5: sCOD consumed and PO4-P released with corrections for the added sCOD and PO_4-P in the FPSS

Time [min]	sCOD Consumed [mg]
0	0 mg
30	0 mg + (80,9 mg – 47,9 mg)= 33 mg
60	33 mg + (47,9 mg-172,6 mg) + 159,96 mg= 68,3 mg
120	68,3 mg + (172,6 mg -240,4 mg) + 159,96 mg= 160,5 mg
180	160,5 mg + (240,4 mg-326,6 mg) + 159,96 mg = 234,3 mg
240	234,3 mg + (326,6 mg-418,5 mg) + 159,96 mg = 302,4 mg
300	302,4 mg + (418,5 mg-424,5 mg) = 296,4 mg
Time [min]	**PO_4-P Released [mg]**
0	0 mg
30	0 mg + (37,6 mg-15,8 mg) = 21,8 mg
60	21,8 mg + (46,8 mg-37,6 mg) - 2,27 mg = 28,7 mg
120	28,7 mg + (81,8 mg-46,8 mg) - 2,27 mg = 61,4 mg
180	61,4 mg + (123,7 mg-81,8 mg) – 2,27 mg = 101,1 mg
240	101,1 mg + (130,8 mg-123,7 mg) – 2,27 mg = 105,9 mg
300	105,9 mg + (136,7 mg-130,8 mg) mg = 111,8 mg

3.5. Sampling processes

Sampling has been important in all the lab work. Samples has been extracted during experiments and sludge has been extracted from the SBR and pilot for use in kinetics.

Sludge extraction from the SBR was done to test the quality of the sludge and to have biomass for P-stripping experiments. Sludge extractions always took place at the same time in the SBR cycle. The sludge was extracted during the 20 last minutes of the first cycle. By opening a valve at the bottom of the reactor, sludge could be collected in beakers.

Sludge from the CMBBR pilot was collected for use in P-stripping experiments. Two different sampling methods had to be used to collect this sludge, since the first method did not always work. Figure 15 shows both sludge collectors used.

The first method used a cylinder with a filter and a tube leading out of the cylinder at the bottom. The effluent tube from the CMBBR pilot was placed inside the cylinder. An aquarium air pump was also placed in the cylinder to have aeration, avoiding PAOs to release phosphate. The plan was that wastewater would run through the filter, while the solids were kept in the cylinder. It was left like this for one day. Unfortunately, the filter was clogged which resulted in water running over the edges at the top of the cylinder. Some of the solids would also run over. At the time of sludge collection, the effluent tube and the air pump were taken out, and the sludge was left to settle for 15-20 minutes. The water at the top was removed and the more concentrated sludge that was left was taken out for experiments. Unfortunately, this collection only worked a few times. The accumulation of in the cylinder were to low and the sludge was not concentrated enough to be used in experiments. A different method needed to be found.

The second sampling method used a different container to collect the sludge. This was a square container with an outlet in the middle of one side, which allowed supernatant to run out. The effluent tube from the pilot was placed inside the container. An aquarium air pump was also placed in the container to have aeration. The sludge was accumulated over two days. Before the sludge extraction, the aeration was turned off and the sludge was left to settle for 15-20 minutes. Water was then removed from the top, using a siphon tube. The sludge left in the container was then collected with beakers and ready for experiments.

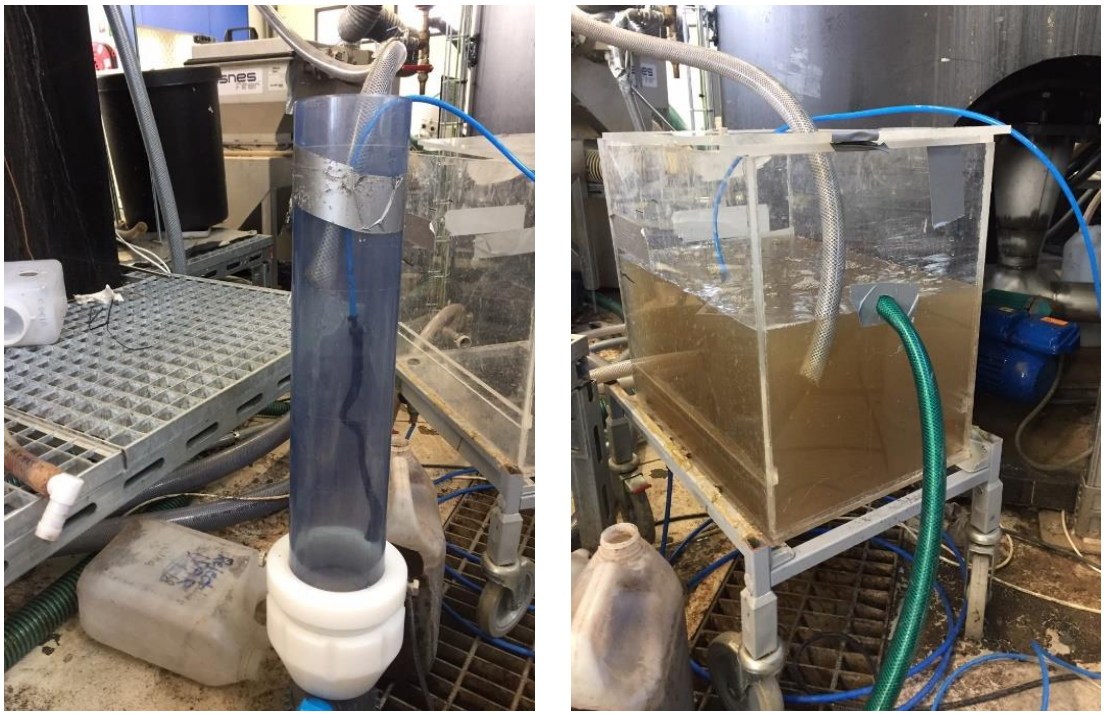

Figure 15: Pilot sludge collectors. a) First method used – cylindrical container b) Second method used – square container

During fermentation and P-stripping experiments, samples were extracted regularly. The methods for these were the same. 20 mL syringes with small tubes were used to extract samples directly form the reactors. From the syringes, the samples were transferred into 15 mL centrifuge tubes. The syringes were rinsed with distilled water in between sampling.

3.6. Parameters

Determination of different parameters have been an important part of this work. The methods for determining the relevant parameters are described in this chapter.

3.6.1. Total Solids

Total Solids was determined using Standard Methods 2540 B (APHA, 2017). Equation (6) was used to calculate the TS. The TS was calculated in mg/L. Two samples were analyzed, and the TS concentration was set as the average of these two values.

$$TS = \frac{W_1 - W_2}{V_{sample}} \cdot 1000\, {}^{mg}/_{g} \tag{6}$$

Where

TS = Total solids [mg/L]

W_1 = Weight of dried residual + dish after 105°C [g]

W_2 = Weight of clean dish [g]

V_{sample} = Volume of sample [L]

Using equation (7), the percentage of TS was also determined.

$$\%\, TS = \frac{W_1 - W_2}{W_{sample}} \cdot 100\% \tag{7}$$

Where

W_1 = Weight of dried residual + dish after 105°C [g]

W_2 = Weight of clean dish [g]

W_{sample} = Weigh of sample [g]

3.6.2. Volatile Solids

Volatile solids were determined using Standard Methods 2540 E (APHA, 2017). The samples used for TS determination was also used for the VS determination. The VS is presented as percentage VS of TS. The average of the two samples were set as the VS percentage. Equation (8) were used for VS calculations.

$$VS = \frac{W_1 - W_3}{W_1 - W_2} \quad (8)$$

Where

VS = Volatile solids [%]

W_1 = Weight of dried residual + dish after 105°C [g]

W_2 = Weight of clean dish [g]

W_3 = Weight of residual + dish after 550°C [g]

3.6.3. Cuvette tests

Cuvette tests from Hach Lange GMBH was used to measure TP, TN, TCOD, sCOD, NH_4-N, PO_4-P, Mg^{2+}, K^+ and Ca^{2+}. The parameters were measured using the manual given with the cuvettes. The totals, TP, TN and TCOD, could be measured directly from the samples. To measure the soluble parameters, NH_4-N, PO_4-P Mg^{2+}, K^+ and Ca^{2+}, the samples had to be filtrated first. A cellulose nitrate filter with a pore size of 0,45 μm was used for this. The samples were centrifuged before filtration, to make the filtration easier and to minimize the use of filters. Some of the samples had to be diluted using distilled water, to get within the ranges of the cuvettes. The samples were stored in 15 mL centrifuge cuvettes. Some of the lab equipment used for samples can be seen in Figure 16.

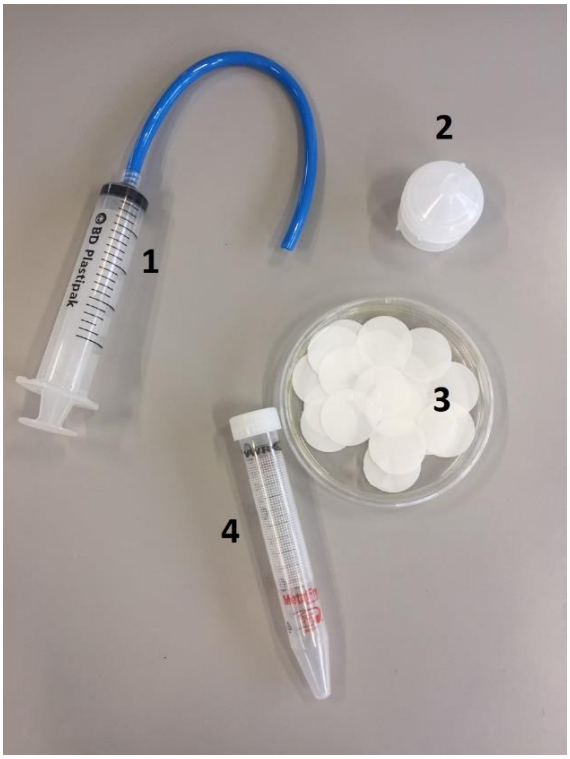

Figure 16: Lab Equipment: 1) Syringe with tube for sampling 2) Filter holder, to attach at syringe 3) 45 µm filters 4) Centrifuge cuvettes for samples

TP, TN, TCOD and sCOD needed to be digested. This was done using a Hach DRB200. The temperature and duration of the digestion was also given in the manuals. Table 6 gives an overview of all methods used for the cuvette tests and the concentration ranges.

To analyze the cuvettes, a Hach DR1900 Portable Spectrophotometer was used. It uses a wavelength range of 340 to 800 nm. The results were given as a concentration.

Table 6: Methods for cuvette tests

Analyze	Method	Range [mg/L]
sCOD	LCI400	1,0 – 1000,0
	LCK314	15,0 -150,0
	LCK614	50,0 – 300,0
TCOD	LCI400	1,0 – 1000,0
PO_4-P	LCK350	2,0 - 20,0
	LCK348	0,5 – 5,0
TP	LCK350	2,0 – 20,0
NH_4-N	LCK303	2,0 – 47,0
	LCK304	0,015 – 2,0
TN	LCK138	1,0 – 16,0
Mg^{2+}	LCK326	0,50 – 50,0
K^+	LCK328	8,0 – 50,0
Ca^{2+}	LCK327	1,0 – 100,0

3.6.4. Conductivity

To measure conductivity, a Hach HQ440d Laboratory Multimeter was used. The conductivity was given in μS/cm.

3.6.5. pH

Portable pH meter was used to measure pH.

3.6.6. Temperature

Temperature was measured using a portable temperature meter.

3.6.7. Weight

The Sartorius Analytic A 210 P Digital Balance was used to weight different substances.

3.6.8. Sludge quality

To see the quality of the sludge, a ratio between non-soluble phosphorus (particulate phosphorus), and total solids was calculated. The ratio is found using equation (9). This ratio tells something about the amount of phosphate the PAOs are taking up in the aerobic phase. If the ratio is high, it indicates that the PAOs are taking up a lot of phosphate. This ratio can be used to compare results from experiments with bio-P sludge.

$$mg\ P_{ns}/_{mg\ TS} = \frac{TP - PO_4^- P}{TS} \quad (9)$$

Where

TP = Total phosphorus concentration in the sludge [mg/L]

PO_4-P = Soluble phosphorus concentration in the sludge [mg/L]

TS = Total solids concentration in the sludge [mg/L]

3.6.9. VFA Analyzes

Samples from the last fermentation, F4, and two p-stripping experiments were sent to NMBU for VFA analyzes. Table 7 lists the VFAs that was measured in the analyzes. Formic acid was added to the samples to keep them stable until the analyzes were run. Acid was added so that 5% of the sample was formic acid.

The VFA analyzes were carried out using gas chromatography with flame ionization detector. The column that was used was 30 m long, 0,25 mm inner diameter and 0,25 µm film thickness. Before analyzing the samples, they were diluted 1:1 with 2 methyl valeric acid.

The results were given in mmol/L. These numbers has been converted to a concentration of sCOD. These conversions can be seen in Appendix B.

Table 7: VFAs analyzed in samples

VFA	Chemical formula
Acetic acid	C_3H_3COOH
Propionic acid	CH_3CH_2COOH
Iso-butyric acid	$C_4H_8O_2$
Butyric acid	$C_4H_8O_2$
Iso-valeric acid	$C_5H_{10}O_2$
Valeric acid	$C_5H_{10}O_2$

4. Results and Discussion

In this chapter the results from tests and experiments will be presented and discussed. All the raw data obtained in the lab can be found in the appendices.

4.1. Fermentation

The aim of the fermentation was to produce sCOD and VFA, to add as carbon source for PAOs in P-stripping experiments. The goal was to achieve high concentrations of sCOD in a short amount of time. Getting high VFA production was highly wanted. Different configurations were tested, to see which gave the best results. Using FPSS from the lab as carbon source would be cheaper than using chemicals.

Table 8 gives an overview of the fermentations that was conducted through the study. The yield is calculated with equation (10) and the rate with equation (11). In equation (10) , the VS is not multiplied with ½ for the first batch. For the other batches, this is needed since only half the volume is added to the mix.

$$Yield = \frac{mg\ sCOD_{end} - mg\ sCOD_{start}}{\frac{1}{2} \cdot g\ VS} \quad (10)$$

$$Rate = \frac{Yield}{Number\ of\ days} \quad (11)$$

Table 8: Overview of fermentations, with calculated yield and rate of fermentation

Fermentation nr.	Start date of fermentation	HRT [days]	Batch	TS [%]	Yield [mg sCOD/g VS]	Rate [mg sCOD/g VS*d]
F1	13.02.19	4	1	0,871	30,770	15,385
			2	0,785	1,160	0,580
			3	0,930	75,863	37,932
			4	0,955	26,620	13,310
F2	22.02.19	4	1	1,163	31,938	15,969
			2	1,311	67,312	33,656
			3	1,214	75,876	37,938
			4	1,144	27,692	13,846
F3	07.03.19	8	1	1,470	61,969	15,492
			2	1,309	147,060	36,765
F4	25.03.19	4	1	0,844	56,243	14,061

4.1.1. VFA Production

Samples from fermentation F4 was sent to NMBU for VFA analyzes. Measured parameters in this fermentation can be found in Table 9. The VFA distribution at the end of fermentation F4 is given in Figure 17. Almost 80 % of the VFA produced is acetic and propionic acid. More than 50 % of the VFA is propionic acid. This is beneficial if the FPSS is used in EBPR, since propionate favors PAOs over GAOs (Oehmen, et al., 2005).

In addition, the mix of acetate and propionate will favor PAO. The biomass in the SBR has been enriched with only acetate. According to Oehmen, et al. (2005) this is beneficial since GAOs seems to have a slower reaction when carbon source is changed. When the PAOs have an advantage, the uptake of VFA and release of phosphate under anaerobic conditions is better. This is therefore beneficial for P-stripping.

Table 9: Measured parameters from Fermentation F4

Fermentation F4		**HRT: 4 days**	**Batches: 1**	**TS: 0,84%**
Day	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	VFA [mg sCOD/L]
0	161	3,3	15,69	75,98
1	452	6,39	21,55	313,99
2	526	6,82	18,94	392,96
3	648	6,48	14,4	464,58
4	584	5,98	7,36	442,27

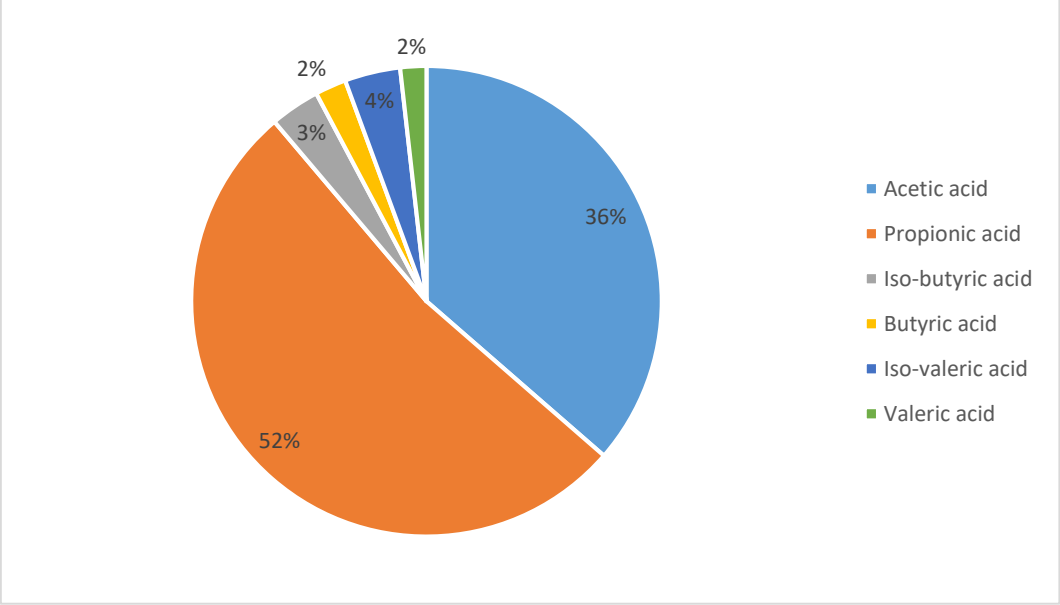

Figure 17: The VFA distribution at the end of fermentation F4 (Day 4) with approximately 1% TS

The production of VFA in percentage of the production of total sCOD is found by using the numbers in Table 9.

$$Produced\ sCOD = 584\ {}^{mg}/_{L} - 161\ {}^{m}/_{L} = 423\ mg/L$$

$$Produced\ acids = 442{,}27\ {}^{mg}/_{L} - 75{,}98\ {}^{mg}/_{L} = 366{,}3\ mg/L$$

$$VFA\ percentage = \frac{366{,}3\ mg/L}{423\ mg/L} \cdot 100\% = 86{,}6\%$$

This means that 86,6% of the total sCOD production in fermentation F4 is in the form of VFA. This is also very beneficial for use in EBPR processes, since PAOs need VFA for P-release.

Figure 18 shows how the production of acetic acid, propionic acid and all acids combined, changes over the timeline of fermentation F4. It illustrates at what times during the fermentation, the production of the VFAs are highest. The highest production of acetic acid is in the first 24 hours. Then the production is decreasing fast, to a point where the production is negative. Production of propionic acid does shows the same trend in the first 48 hours. After a small decrease there is a new increase in production, before dropping in the last 24 hours. The production of the total acids is highly influenced by the production of acetic and propionic acid since it makes up almost 90% of the total VFAs.

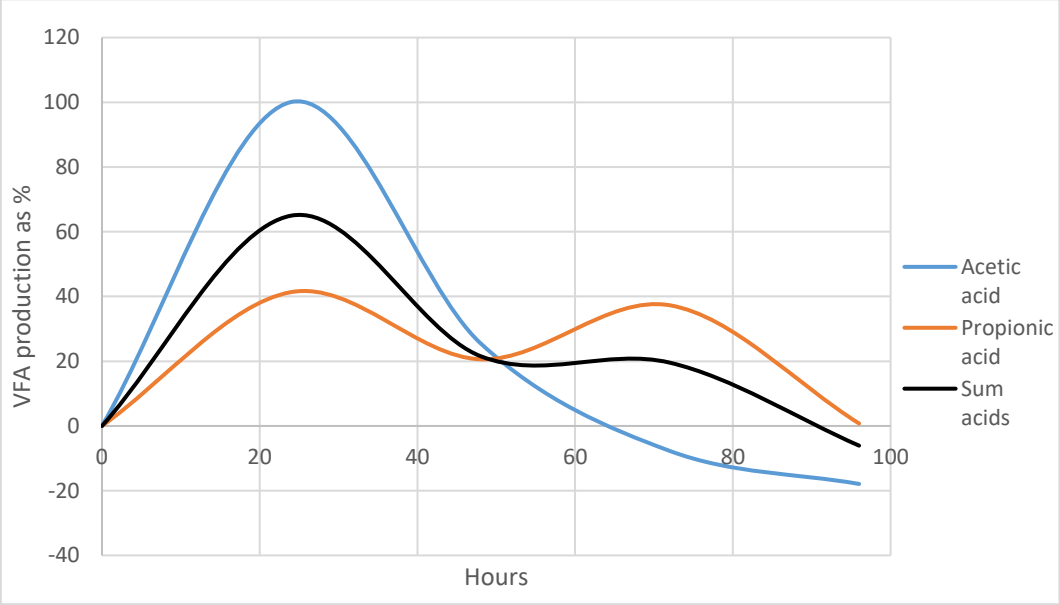

Figure 18: The production of acetic acid, propionic acid and the sum of all acids over the time of fermentation F4, with approximately 1% TS. Given as percentage of the total production of each acid

Calculated ratios between acetic acid and propionic acid are listed in Table 10. According to Lopez-Vazquez, et al. (2009) a ratio between acetate and propionate of 75:25 or 50:50 is favorable for PAOs in EBPR. The ratio in fermentation F4 is close to 50:50, but after 48 hours this ratio is moving more towards 40:60.

The highest mass of VFA occurs after 72 hours. However, the ratio between acetic and propionic acid is worsening after 48 hours, and the production of acetic acid and the total sum of acids decreases after this time. It may therefore be more beneficial to stop the fermentation after 48 hours.

Table 10: Ratio between acetic acid and propionic acid through the fermentation with 1% TS

Time [hours]	Acetic acid [mg]	Propionic acid [mg]	Ratio [acetic acid : propionic acid]
0	59,61	77,60	43:57
24	322,19	237,26	58:42
48	389,21	316,77	55:45
72	368,97	460,71	44:56
96	321,95	463,65	41:59

Figure 19 shows the VFA distribution at the end of the fermentation conducted by Svendby (2019). This fermentation started and ended at the same time as fermentation F4, and the primary sludge and wastewater was collected at the same time. The only difference between the fermentations were the TS percentage. In fermentation F4 the initial TS was around 1%, while the fermentation done by Svendby (2019) was around 2%. The production of acetic acid and propionic acid is similar in these fermentations. The main difference is the production of butyric acid. In fermentation done by Svendby (2019), 11% of the production is butyric acid, while in fermentation F4 the production is 2%. Studies have shown that butyrate is equally good as acetate as a carbon source for P-removal (Rustrian et al., 1996: Mulkerrins, et al., 2004). Zeng, et al. (2006) observed that only acetic acid was produced when the solid concentration was low, during fermentation of primary sludge. The increase in production of butyric acid could therefore be the increase in TS concentration.

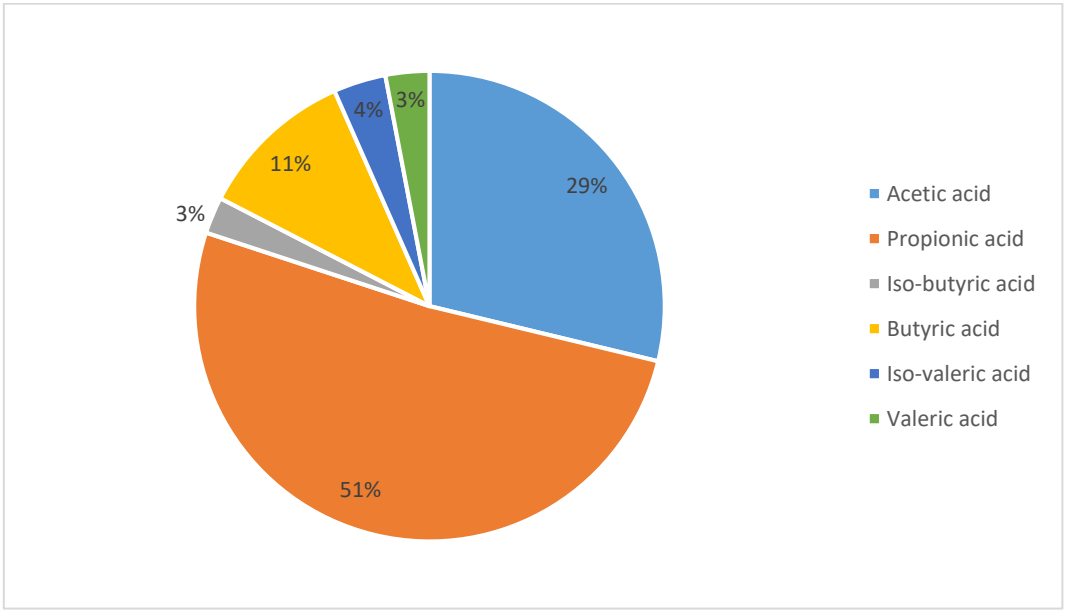

Figure 19: VFA distribution at the end of fermentation, with 2% TS, conducted by Svendby (2019)

Figure 20 shows the production of acetic acid, propionic acid and the combined acids over time for fermentation by Svendby (2019). It shows similar trends as fermentation F4, where most of the production occurs in the first 24 hours of the fermentation.

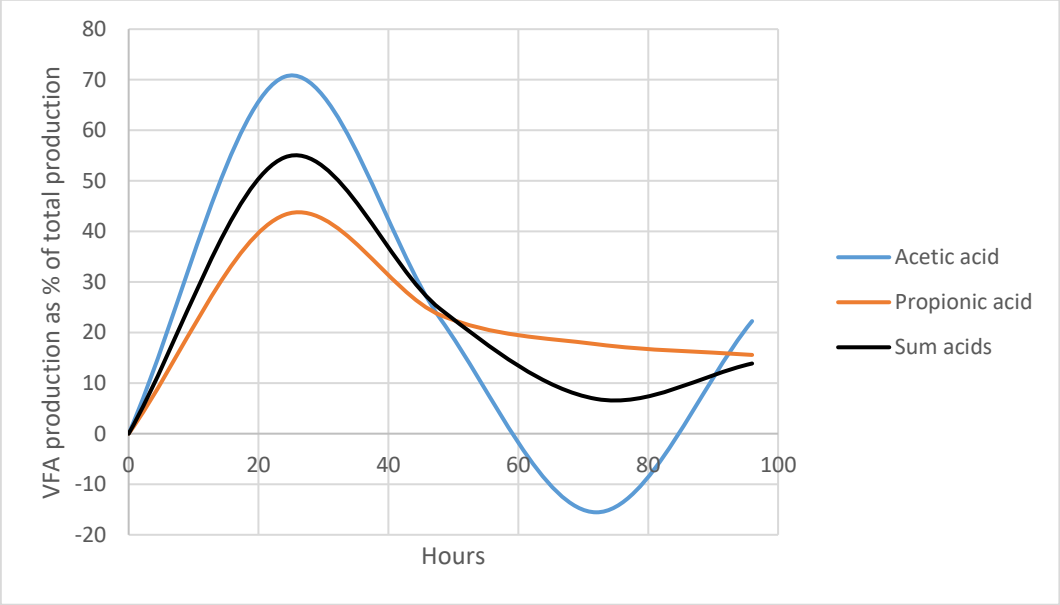

Figure 20: The production of acetic acid, propionic acid and the sum of all acids over the time of fermentation, with 2% TS, by Svendby (2019). Given as percentage of the total production of each acid

The accumulated mass of total VFA in fermentation F4 and fermentation by Svendby (2019) can be seen in Figure 21. The production of VFA in mass is higher in Svendby (2019), where the concentration of TS was around 2%, compared to fermentation 4 with around 1% TS. The rate of production is also higher in the fermentation with the highest TS concentration. This is in accordance with results obtained by Zeng, et al (2006), who found that an increase in solids concentration increased the VFA production rate quite substantially.

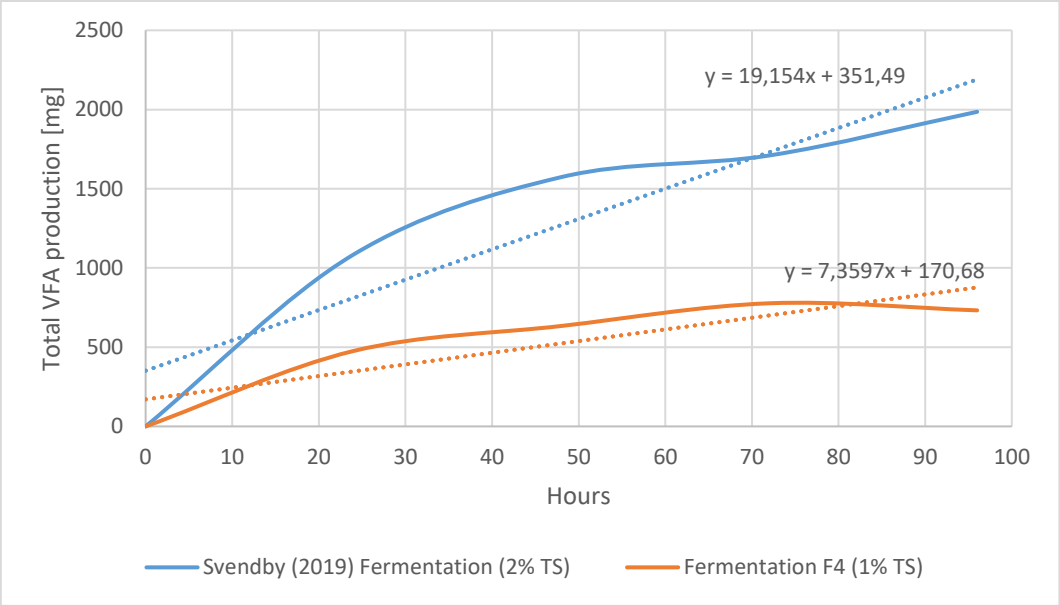

Figure 21: Accumulated VFA production in Fermentation F4 and fermentation by Svendby (2019)

4.1.2. Temperature

The first fermentation was conducted in lower temperatures than the last three fermentations. The first and second fermentation was also run with the same HRT of 4 days. These fermentations can therefore be used to compare the effects of temperature.

The temperature is affecting the rate of the fermentation. The fermentation with the highest temperatures have a faster increase in rate. This is reasonable when we know that biological reaction rate increases with temperature (Mulkerrins, et al., 2004).

A study by Crutchik, et al. (2018) investigated the production of VFA and propionate in fermentation of primary sludge. The fermentations were conducted in temperatures of 37, 55 and 70°C. This study found that the highest propionate production was under mesophilic conditions. This corresponds well to the results from the VFA analyzes for fermentation F4 and fermentation done by Svendby (2019). In these cases the temperatures were between 25 and 28°C and therefore under mesophilic conditions.

Maharaj & Elefiniotis (2001) found that the highest net VFA concentration occurs at a temperature of 25°C.

If the goal is to have high production of propionate, a temperature around 25-28°C could be a good suggestion. If acetate is the preferred VFA, a higher temperature may be a better choice.

4.1.3. HRT

Maharaj & Elefsiniotis, (2001) found in their study that the highest VFA production was obtained at a hydraulic retention time of 30 hours. In this study the HRT tested were 4 and 8 days. Looking at both yield and rate in Table 8, the fermentation with HRT of 8 days have the best results. The yield is the highest and the sCOD concentration reached it's highest value in this fermentation.

Looking at the VFA production in Figure 18, the production is highest in the first day. This result correlates well with the observation done by Maharaj & Elefsiniotis, (2001), where 30 hours retention time gave highest VFA production.

Being able to produce VFA in shorter time is beneficial, as it takes less time to achieve the wanted VFA production, which means more sludge can be fermented.

4.1.4. NH_4-N and PO_4-P Concentrations

In most of the fermentations, there is a trend with decreasing concentrations for both NH_4-N and PO_4-P. The concentrations at the end of the fermentation is lower than the starting concentration. This can be seen in Figure 22 and Figure 23.

The reason behind this is not known. One would expect the concentrations to increase as more organic matter is broken down. A possibility could be that bacteria in the fermenter use it for assimilation. Figure 22 and Figure 23 shows how the concentrations of NH_4-N and PO_4-P develop in the different batches of the fermentations.

The results from Svendby, (2019) does not show this same trend. In these fermentations the ammonium and phosphate concentrations are increasing with time. The only difference between Svendby, (2019) fermentations and the one conducted in this study, is the percentage of TS. Svendby, (2019) used a TS of approximately 2%, while 1% was used here. The reactors were started at the same time, using sludge and wastewater collected at the same time. Samples were always collected at the same time, and the rectors were in the same water bath for the heated fermentations.

Having a fermentation product with less ammonium and phosphate is beneficial if it is used as carbon source for phosphorus removal. Then there won't be that much more phosphorus to remove and the potential for unwanted struvite precipitation is lower when the concentrations of both $NH_{4\text{-}N}$ and PO_4-P is lower (Ohlinger, et al., 1998).

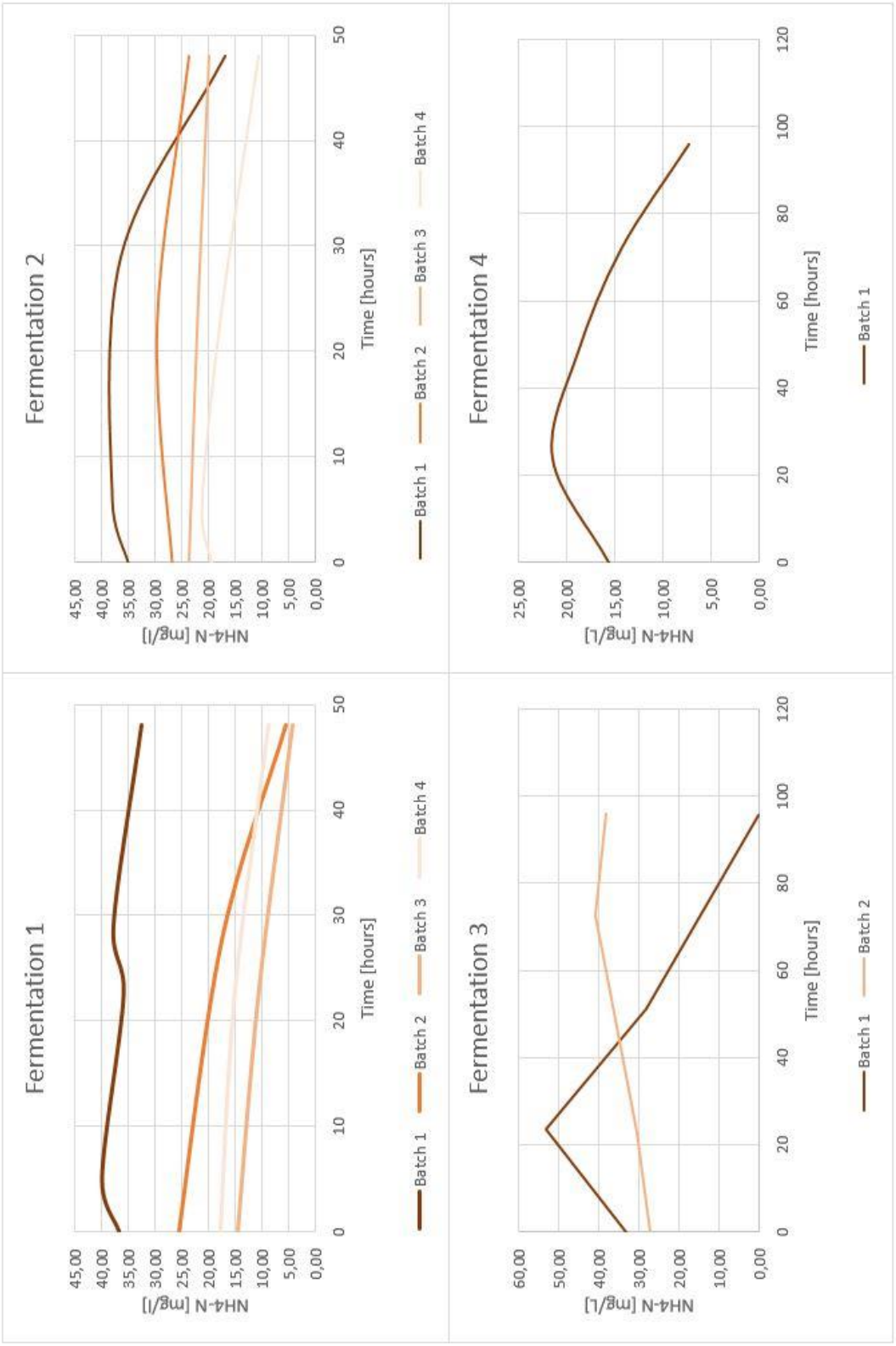

Figure 22: The changes in NH_4-N in fermentation F1, F2, F3 and F4

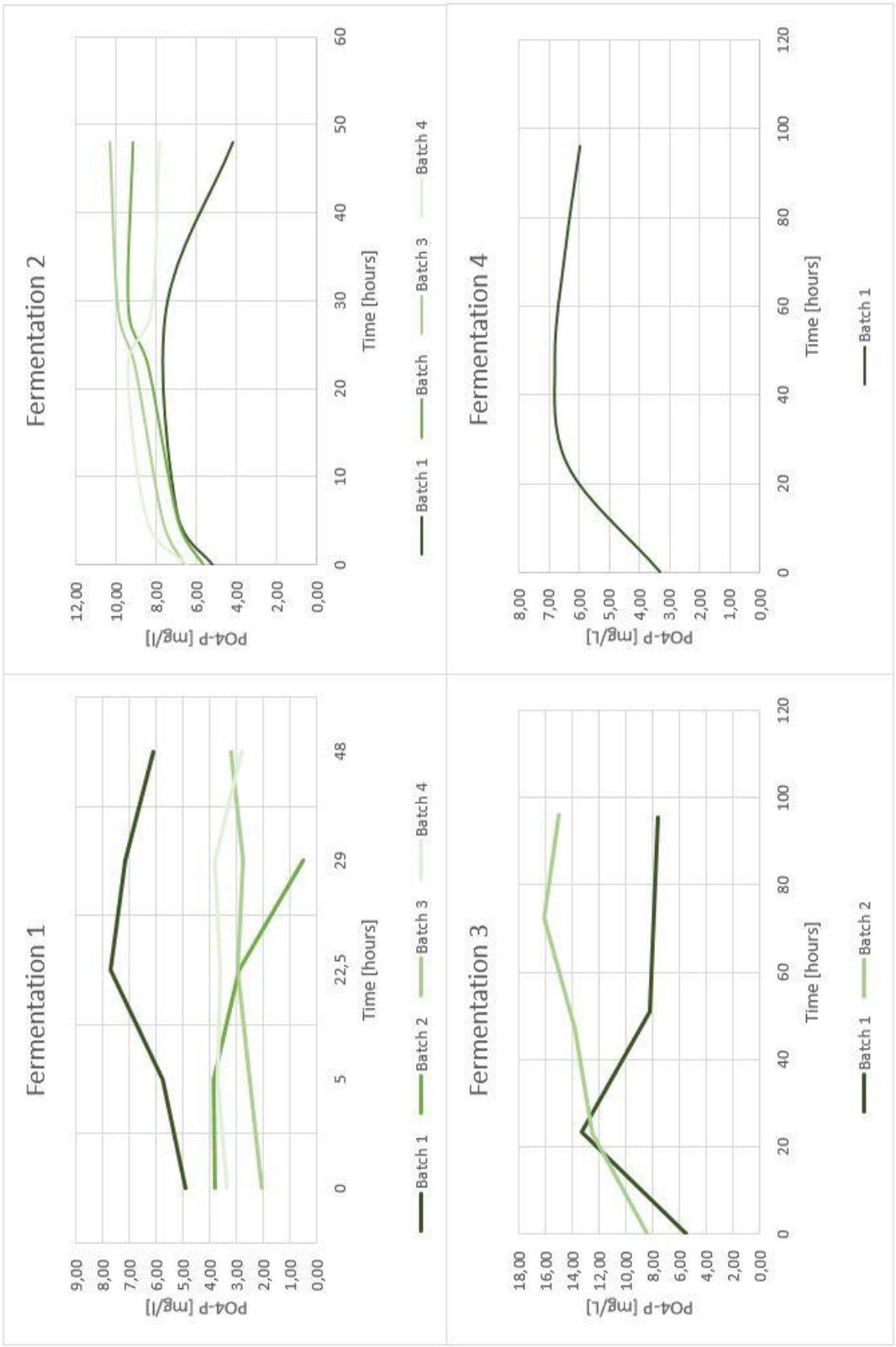

Figure 23: The changes in PO_4-P in fermentation F1, F2, F3 and F4

4.1.5. sCOD produced per TCOD

The production of sCOD per TCOD was calculated for each batch in the fermentations. This was found by dividing the difference between end and start concentration of sCOD by the TCOD at the beginning of a new batch. Table 11 shows the results from these calculations.

Table 11: The sCOD production per TCOD available

Fermentation nr.	Batch	sCOD produced/ TCOD [%]
1	1	2,57
	2	0,06
	3	8,89
	4	2,34
2	1	4,97
	2	-
	3	19,06
	4	2,10
3	1	8,62
	2	6,57
4	1	4,55

According to a study by Hatziconstantinou, et al (1996) a sCOD production in the terms of TCOD could be expected to be around 10% for fermentation when temperature is above 20°C and HRT greater than 2.

The very varying results in Table 11 could be because of the TCOD measurements. There is reason to believe that the TCOD measurements are not very accurate. There was some issues related to larger particles, like undissolved toilet paper, which clogged the pipettes when extracting a sample for the cuvette analyzes. Getting a homogeneous and accurate representation for the whole sample was therefore difficult. This may explain why the TCOD numbers are somewhat odd. The 2% TS fermentation done by Svendby (2019) had in some cases less TCOD than the fermentation done in this study. This strengthens the theory of inaccurate results, since the mix with higher solid concentration should have the higher TCOD concentration.

The large variation of TCOD between batches could be affected by the content of sludge being added for each new batch. The sludge could be diluted if there has been a lot of rain, and thereby give a lower TCOD concentration.

4.2. Sludge Characterization

Sludge characterization was done in connection with P-stripping experiments. For the SBR sludge samples were taken a few more times unrelated to experiments. The characterization consisted of measuring TS, TP and PO_4-P, and then finding the sludge quality ratio (mg P_{ns} /mg TS). These measurements gave an overview of how the sludge changed over the period of time, the sludge was used in experiments.

4.2.1. Sludge from SBR

Figure 24 shows how the TS and TP concentrations in the SBR sludge changes over the time period the sludge was used in experiments. Figure 25 shows how the sludge quality changes over the same time period.

The TS and TP does not change that much in the first half of the time period. The small fluctuations in both TS and TP seems to be following each other. Some clogging issues in the beginning may have affected the TS and TP in the extracted samples. The clogging issue was fixed 18.01.2019, which is between 0 and 6 days. After the clogging issue was fixed the TS and TP stabilized somewhat.

After 57 days the acetate and phosphate addition in the feeding solution was increased with 30%. The TP is increasing after this increase. When the TP is increasing, more Poly-P is stored in the PAOs cell, and thereby the density of PAOs increase (Schuler & Jang, 2007). The increase in density improves the settling properties of the biomass, which can explain the increase in TS after day 63. The reason for the drop in TS between the last two measurements is not known.

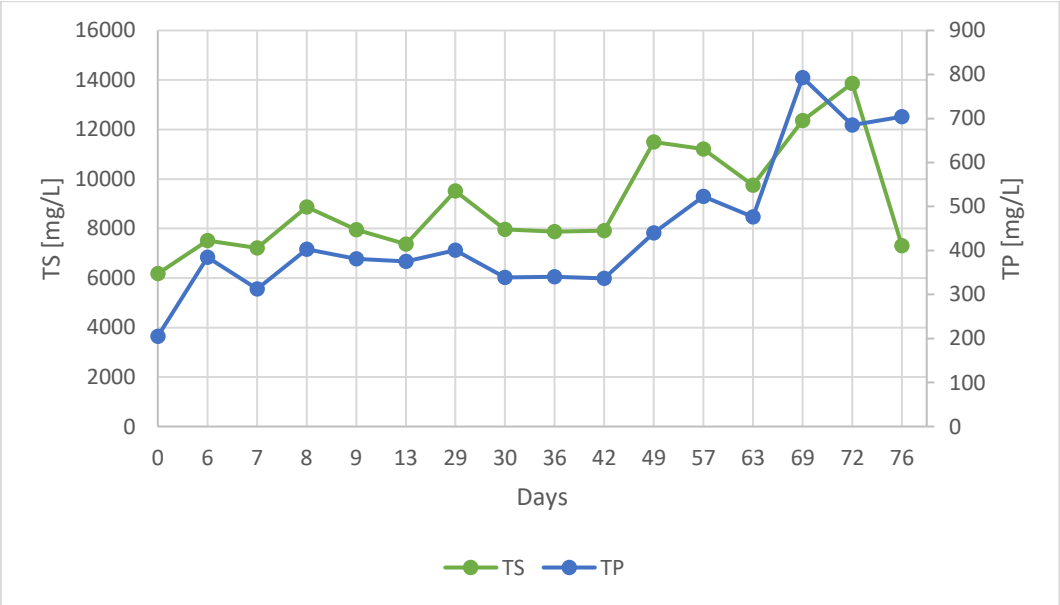

Figure 24: Changes in TS and TP concentrations over time in the sludge from SBR

Regarding the sludge quality, it is rather stabile from around day 5 until day 55. At the very beginning, the ratio is at the lowest. The clogging issues could be the reason behind this. At around 60 days the sludge quality is increasing, which is most likely caused by a 30% increase in both acetate and phosphate in the feeding solution. The aim of this increase was to increase the sludge quality, to have more luxury-P in the biomass, and it seems to have responded well.

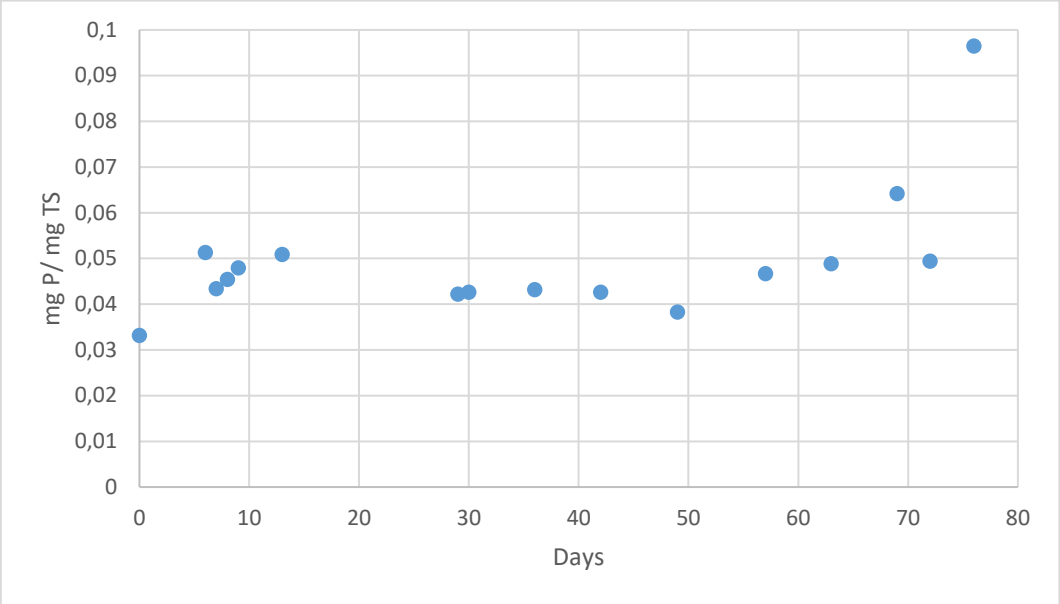

Figure 25: Changes in Sludge Quality over time in SBR sludge

4.2.2. Sludge from CMBBR Pilot

Figure 26 shows how the TS and TP concentrations in the Pilot sludge changes over the time period the sludge was used in experiments. Figure 27 shows how the sludge quality changes over the same time period.

The concentration of TS and TP decreases between day 2 and 14. This is the period where the sludge collection method had to be changed. This may have influenced the TS and TP concentration in the collected sludge.

Changes in TS and TP could also be affected by the weather conditions. If the wastewater is diluted due to rain, the TP could be lower since less phosphate is available for uptake by the PAOs. Lack of carbon and nutrients could also prohibit growth of biomass, which may affect the TS. Diluted wastewater does not have the same effect on the SBR sludge, since there is a feeding solution being pumped in, containing both acetate and phosphate.

The sludge quality ratio is rather stabile through the whole timeline. It is decreasing somewhat towards the end of the time line. This could be the result of decreasing TP between day 19 and day 20.

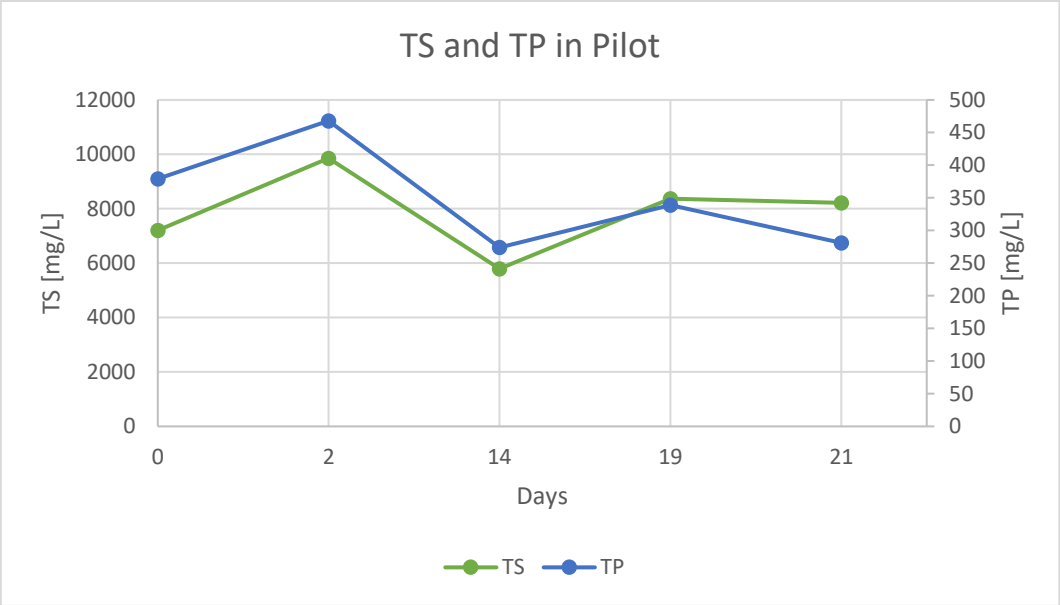

Figure 26: Changes in TS and TP concentrations over time in the sludge from CMBBR Pilot

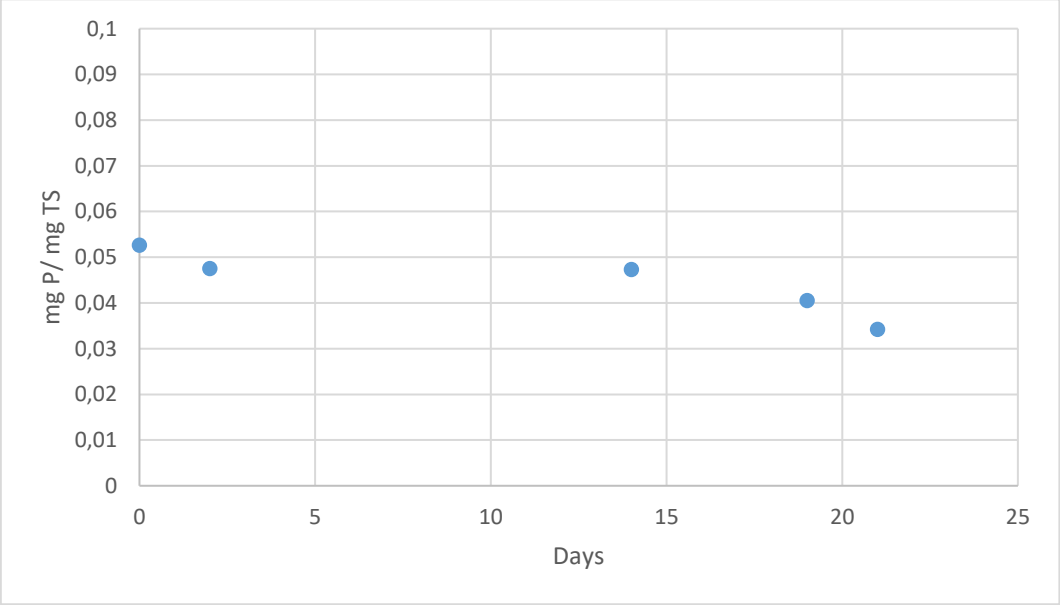

Figure 27: Changes in Sludge Quality over time in CMBBR Pilot sludge

4.3. P-stripping experiments

The aim of the P-stripping experiments was to have high phosphorus release. Release was enhanced by adding carbon sources of different kinds and doses. All but two experiments were conducted in colder conditions, with a temperature between 11 and 13°C. The reason for doing the experiments in colder temperatures was to mimic winter conditions in northern countries. The other two experiments were conducted in room temperature.

4.3.1. SBR Sludge

Table 12 gives an overview of the P-release in all the P-stripping experiments with sludge from SBR. The P-release is calculated as both mass and as a percentage of luxury-P. Luxury P was found by assuming Ordinary Heterotrophic Organisms contain 0,015 mg P/ mg TS (Wentzel, et al., 2008).

Table 12: P-release in all P-stripping experiments with sludge from SBR

Date	Name	P-release [mg]	P-release [% of luxury-P]	Carbon source	Day FPSS extracted	Dosing	Temperature
25.jan	S1	112,68	43,11	FPSS	2	100 mg/L*h	Room
29.jan	S2	107,18	38,66	FPSS	2	100 mg/L*h	Cold
15.feb	S3	88,13	34,64	FPSS	Not known	100 mg/L*h	Cold
21.feb	S4	74,51	21,71	Glucose	-	100 mg/L*h	Room
27.feb	S5	38,62	19,22	FPSS	Not known	500 mg/L	Cold
06.mar	S6	76,69	32,97	FPSS	8	200 mg/L*2nd hour	Cold
14.mar	S7	64,40	19,64	FPSS	4	200 mg/h*h	Cold
26.mar	S8	214,43	22,34	Acetate	-	100 mg/L*h	Cold
29.mar	S9	170,72	22,74	Acetate	-	200 mg/L*h	Cold
02.apr	S10	106,23	19,34	FPSS	8	100 mg/L*h	Cold

Figure 28 shows the P-release in all the experiments in terms of mass. This representation of release does not take into account the sludge quality, and thus does not take into account the potential P-release. Comparing these results, experiments S8 and S9, where acetate was used as carbon source, gives the highest mass release of phosphorus. The increase in added COD from 100 mg/L*h to 200 mg/L*h does not increase the P-release. It does not seem like the PAOs take up more sCOD when more is added.

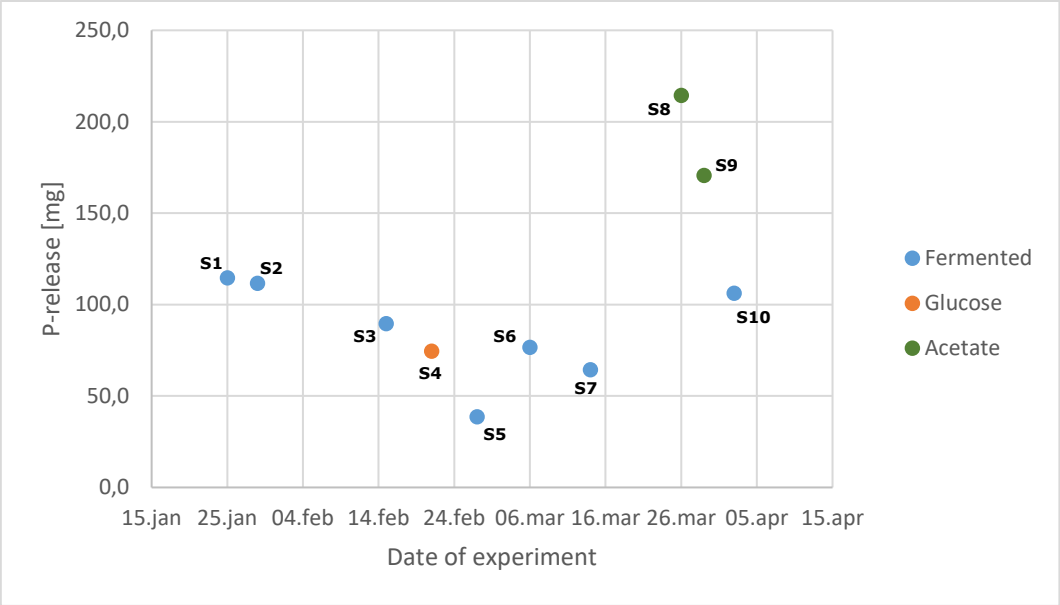

Figure 28: P-release in terms of mass in all P-stripping experiments with SBR sludge

Figure 29 also shows P-release in all the P-stripping experiments with SBR sludge. Here P-release is represented as a percentage of the luxury-P. This takes into account the sludge quality ratio, making the results more comparable. The potential P-release is not reached in any of the experiments, as they are between 19 and 43%. However, this is quite good considering the experiments were run in cold temperatures.

The experiments with best results are the ones with FPSS as carbon source and with doses of 100 mg/L*h and 200 mg/L*2nd h. Experiment S1 has the highest release in terms of luxury-P release, at 43%. This experiment was conducted in room temperature. In S1 and S2 the same FPSS was used, and the dosing was the same. The only difference was the temperature. Even though S2 was conducted in cold temperatures, the P-release was at 38% and thus not far behind the results in room temperature.

Even though acetate gave the highest P-release in mass, the percentage of luxury-P release was not the highest. This could indicate that there are VFAs in the FPSS that the PAOs prefer over acetate. This will be discussed closer in a later chapter. Another reason why acetate doesn't work as well as FPSS could be due to more competition from GAOs, when acetate is the only carbon source. According to Lopez-Vazquez, et al. (2009), the precence of only acetate does not favor PAOs or GAOs.

Which day of the fermentation FPSS is taken out may influence the P-release. The VFA distribution changes over the course of fermentation. In S1 and S2, the FPSS was taken after 2 days of fermentation. These have the highest P-release, which may indicate that the best time to take the FPSS out is after fewer days. The FPSS for S6 was taken out after 8 days of fermentation, and the P-release is one of the highest. For some of the FPSS, the day of extraction is unknown. It is therefore difficult to draw any clear conclusion on what time FPSS should be extracted from fermenter.

The experiment where all the carbon was added at the beginning gave the poorest result, with only 19% release of luxury-P. The P-release is higher when the carbon source is added evenly through the experiment. There are exceptions to this, such as S7 and S10.

Glucose gave results in one of the lowest P-releases, even though the experiment was conducted in room temperature. This corresponds well with previous studies where glucose as only carbon source in EBPR has shown poor results (Oehmen, et al., 2007).

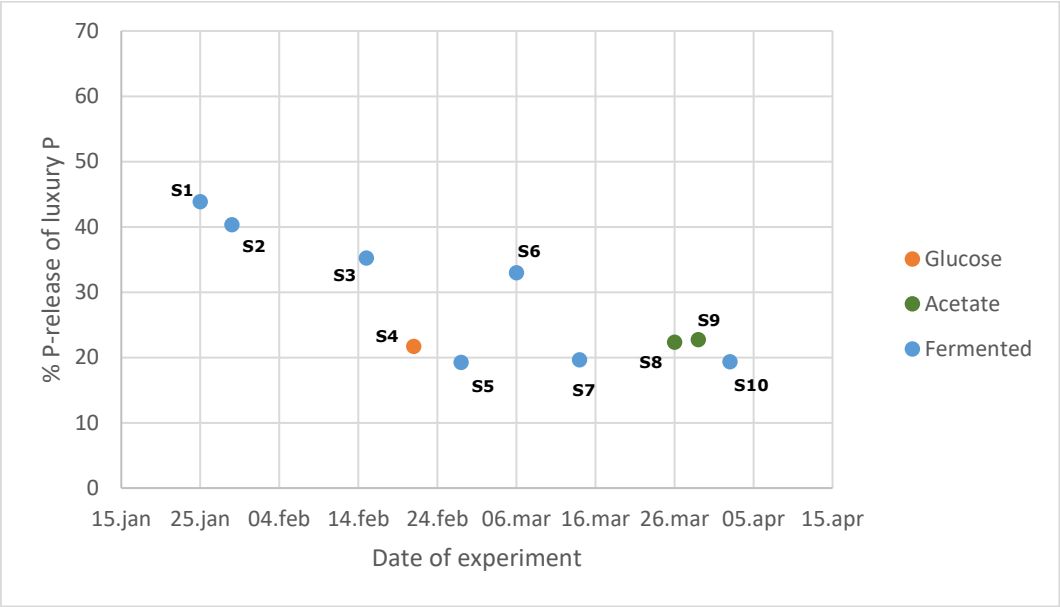

Figure 29: P-release as a percentage of luxury-P with SBR sludge

A plot with results from P-stripping experiments from Fiksdal (2018) can be seen in Figure 30. Sludge from the same SBR was used in all these experiments and they were run in room temperature and acetate was used as carbon source. A few experiments were also run with FPSS, but the needed numbers to calculate the percentage of P-release of luxury-P was not found.

The P-release is overall better in the P-stripping experiments conducted by Fiksdal (2018), than the experiments from this study. However, it is important to remember that Fiksdal (2018) used room temperature in the experiments, which means a somewhat higher P-release can be expected (Mulkerrins, et al., 2004).

The best P-release results by Fiksdal (2018) was found for experiment D1, D2, D3 and D7, with more than 40% release of luxury-P for all. For all of these, except D2, the carbon was added as a pulse, with doses between 100 mg/L*h and 200 mg/L*h. For experiment D2 all the carbon (500mg/L) was added at the beginning of the experiment. In D8 the same dose was added, but the P-release was much less. It is therefore hard to conclude which one of these is the exception.

In the experiment were the pulse doses were less than 100 mg/L*h, the P-release was less. Using higher doses, such as 1788 mg/L in D9, did not result in a higher P-release. Using high doses like this will therefore be a waste of carbon source.

The results obtained from this study shows similar results. Adding the carbon over time enhanced the P-release compared to adding all in one dose at the beginning of experiment and the ideal dose is between 100 mg/L*h and 200 mg/L*h.

Table 13: P-release in all P-stripping experiments with SBR sludge by Fiksdal (2018)

Date	Name	P-release [mg]	P-release [% of luxury-P]	Carbon source	Dosing	Temperature
11.04.2019	D1	376,8	65,84833911	Acetate	100 mg/L*h	Room
13.04.2019	D2	302,72	47,09084131	Acetate	500 mg/L	Room
17.04.2018	D3	513,92	55,97720473	Acetate	200 mg/L*h	Room
19.04.2018	D4	199,52	18,74342406	Acetate	50 mg/L*h	Room
26.04.2018	D5	22,208	1,798708986	No addition	-	Room
30.04.2018	D6	265,888	19,30810124	Acetate	75 mg/L*h	Room
04.05.2018	D7	648,752	41,99911983	Acetate	183,3 mg/L*h	Room
11.05.2018	D8	364,576	25,5686346	Acetate	500 mg/L	Room
12.05.2018	D9	458,56	30,85037661	Acetate	1788 mg/L	Room

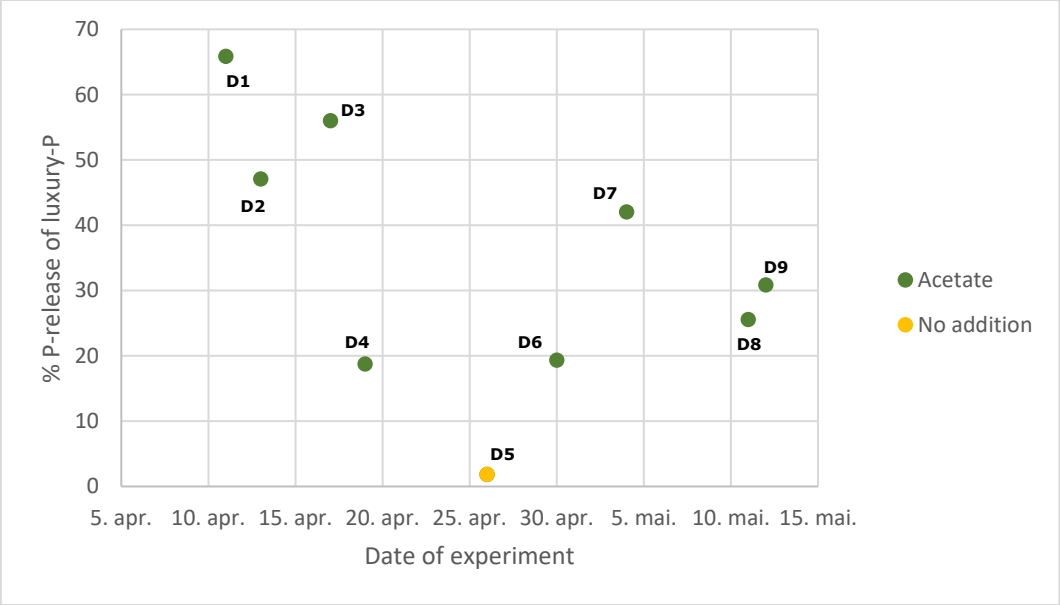

Figure 30: Percentage P-release of luxury-P results from P-stripping experiments with SBR sludge conducted by Fiksdal (2018)

4.3.2. CMBBR Pilot sludge

Table 14 gives an overview of the P-release in all the P-stripping experiments with sludge from CMBBR pilot. The P-release is calculated as both mass and as a percentage of luxury-P.

Table 14: P-release in all P-stripping experiments with sludge from CMBBR Pilot

Date	Name	P-release [mg]	P-release [% of luxury-P]	Carbon source	Day FPSS extracted	Dosing	Temperature
20.mar	P1	41,0	13,90	FPSS	8	200 mg/L*h	Cold
22.mar	P2	97,4	29,45	FPSS	Not known	100 mg/L*h	Cold
03.apr	P3	57,7	30,45	FPSS	8	100 mg/L*h	Cold
08.apr	P4	41,2	12,23	Glucose	-	100 mg/L*h	Cold
10.apr	P5	85,3	34,39	Acetate	-	100 mg/L*h	Cold

Figure 31 shows the P-release in experiments as a mass, while Figure 32 shows the P-release as a percentage of luxury-P. P-release as a mass is highest in experiment P2, where FPSS was used as carbon source with doses of 100 mg/L every hour. Experiment P5, with acetate as carbon source, also has a high P-release.

Dosing 200 mg/L every hour in P1 did not enhance that much P-release. It therefore seems like the best dosing for the pilot sludge should be 100 mg/L*h. However, more experiments with different carbon doses should be tested to make any clear conclusions.

Using glucose gave the poorest P-release, both as mass and percentage of luxury-P. The experiments with SBR sludge gave a similar result with glucose.

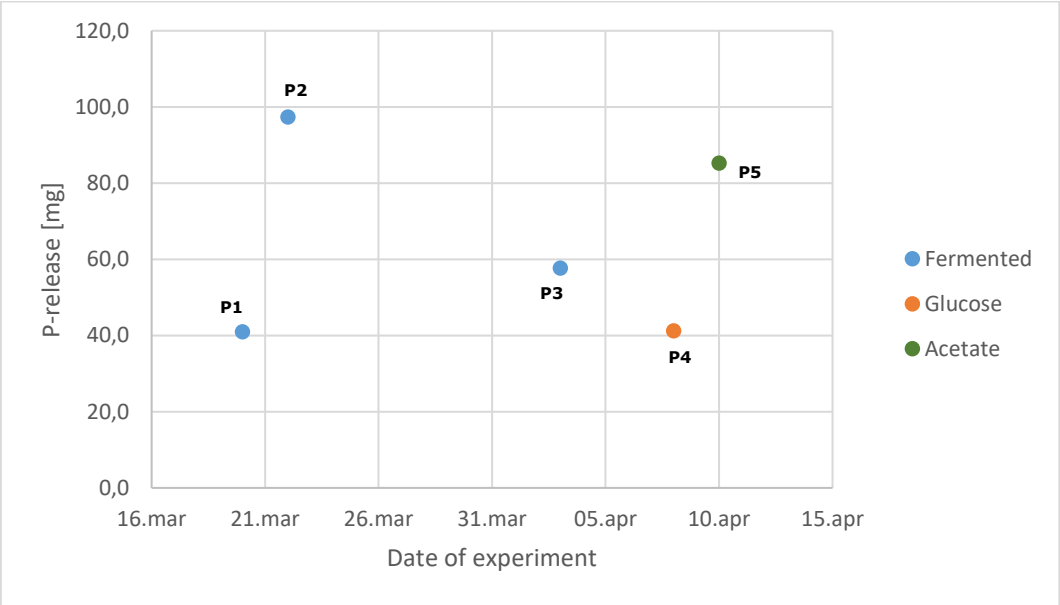

Figure 31: P-release in terms of mass in all P-stripping experiments with CMBBR Pilot sludge

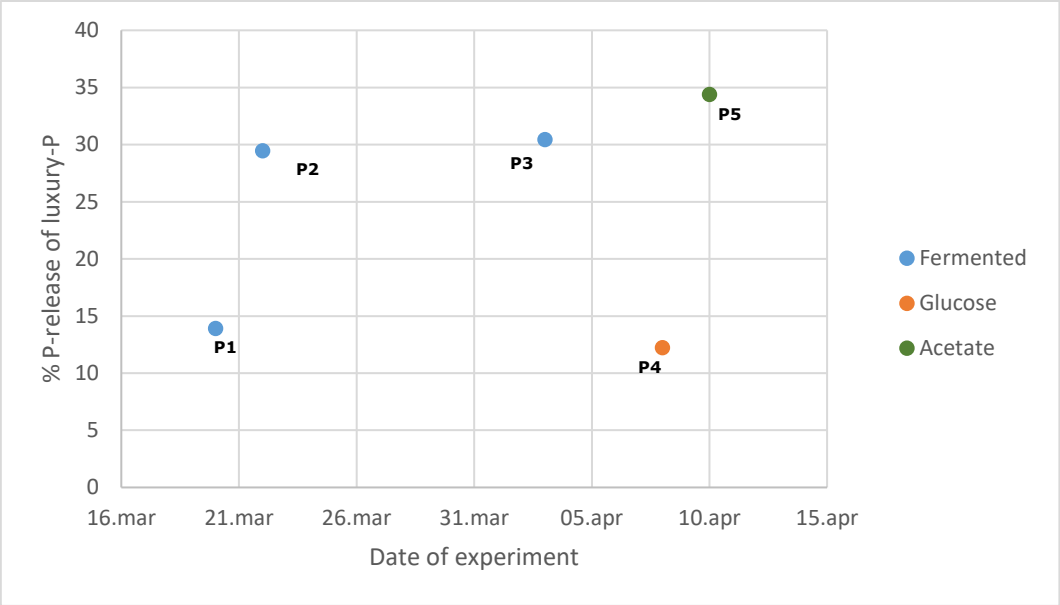

Figure 32: P-release as a percentage of luxury-P in P-stripping experiments with CMBBR Pilot sludge

Looking at the release of luxury-P, the majority of the experiments had a release of 29-35%. This is not that different from the P-release with SBR sludge, which had between 30 and 43% P-release as the best results.

There is not that much difference in P-release between FPSS and acetate as carbon source. The biomass present in the pilot may not have that much of a preference when it comes to type carbon source.

The biomass from the CMBBR Pilot seems to release phosphorus in the presence of both acetate and FPSS. However, it will be interesting to test an FPSS extracted after 24 hours of fermentation, since there is other types of sCOD in addition to VFA, which may enhance phosphorus release.

4.3.1. VFA consumption

Figure 33 and Figure 34 presents the VFA consumption in two P-stripping experiments with sludge from SBR and sludge from CMBBR Pilot. The same FPSS was used for both experiments, and the used FPSS with 2% TS was made by Svendby (2019). The VFA distribution is the same as in Figure 19.

To see which type of VFA the PAOs prefer, the consumption was calculated as a percentage of the available VFA. That way the numbers are comparable. An example of the calculations behind these figures can be found in Appendix E.

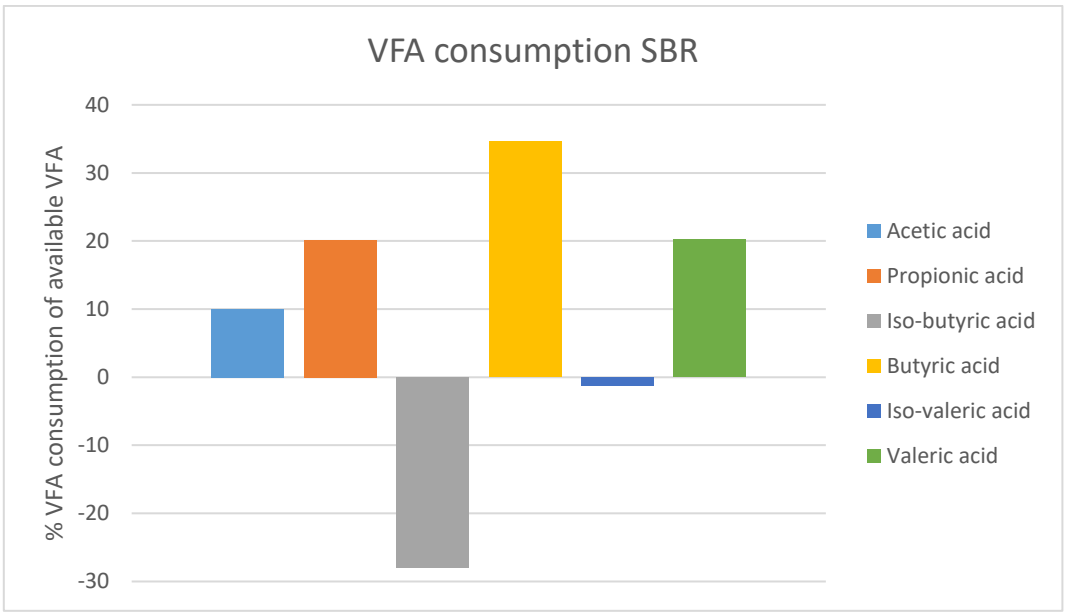

Figure 33: VFA consumption presented as percentage of available VFA in P-stripping experiment with sludge from SBR

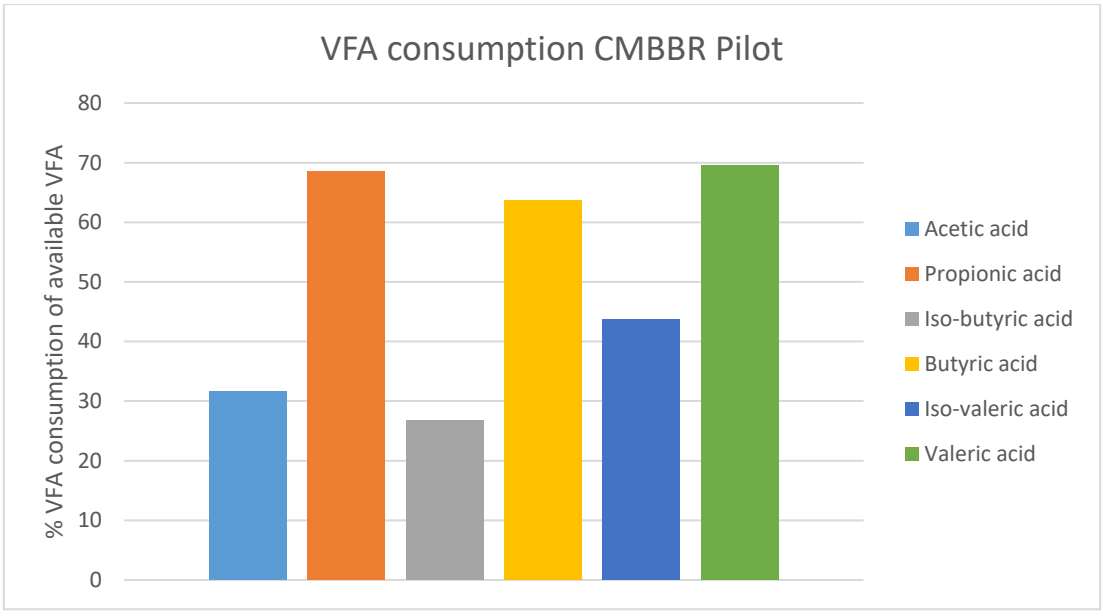

Figure 34: VFA consumption presented as percentage of available VFA in P-stripping experiment with sludge from CMBBR Pilot

Butyric acid is the preferred VFA for the SBR sludge and valeric acid is similar to propionic acid. The biomass in the SBR does not seem to prefer acetic acid. This corresponds well with the observed P-release in experiments with acetate, which was less than the observed P-release with FPSS.

Iso-butyric acid and Iso-valeric acid has negative consumption, which means the concentrations of these acids, are higher at the end of the experiment, than when it was started. There must be some bacteria producing these acids during the experiment, for example fermenting bacteria. This is an expected observation, since fermenting bacteria has not been discovered in the pilot previously.

There is no production of VFA in the experiment with Pilot sludge. The bacteria responsible for fermentation in the SBR sludge may not be present in the Pilot sludge. The consumption is greatest for valeric acid, propionic acid and butyric acid.

The biomass in the CMBBRpilot sludge, seems to have less preferences when it comes to VFA carbon sources. There is a rather high consumption of propionic acid, butyric acid and valeric acid, with over 60% consumption of the available acids. There is less consumption of acetic acid, iso-butyric acid and iso-vaelric acid, but still higher than the consumption in SBR sludge.

It is difficult to make a clear conclusion about the VFA consumption in the P-stripping experiments, since only samples from one experiment of each sludge were analyzed. With more VFA analyzes to compare, a pattern or trend in VFA consumption could have been established.

4.4. Correlation with P-release

The conductivity and concentrations of cations were measured in the P-stripping experiments, since cations such as magnesium and potassium acts like counter-ions in EBPR. Cations are released simultaneously as phosphate. The conductivity is affected by the changes in ion concentrations (Aguado, et al., 2006).

Figure 35 through Figure 40 shows the correlation between the release of PO_4-P and changes in magnesium, potassium and conductivity in P-striping experiments with both SBR sludge and pilot sludge. The R^2 indicates how well the correlations are.

The correlation between PO_4-P release and release of magnesium are the best for both SBR and CMBBR pilot sludge. The fit is rather good, as the R^2 values are above 0,7. The correlation between PO_4-P and K release is not as good, but the R^2 is above 0,5 for the CMBBR pilot sludge.

The poor correlation between P-release and changes in conductivity is surprising. The R^2 values are quite low for both sludge types. The correlation between conductivity and P-release for Fiksdal (2018) gave a R^2 of 0,94. Other Studies has also found strong correlations between conductivity and phosphorus concentrations. Aguado, et al. (2006) obtained R^2 values of 0,99.

The reason behind the bad correlation found in this study is not known. There might have been errors with the measuring equipment or instability in the way conductivity was measured, that has caused this.

Conductivity is very sensitive to temperature according to Aguado, et al. (2006). The cold temperature in these experiments could therefore have affected the measured conductivity.

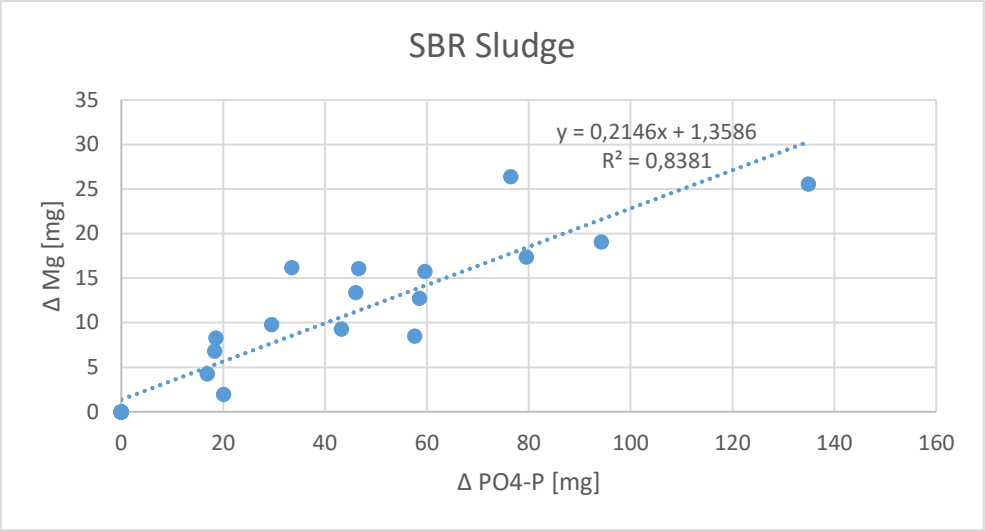

Figure 35: Correlation between PO4-P and Mg release in P-stripping experiments with SBR sludge

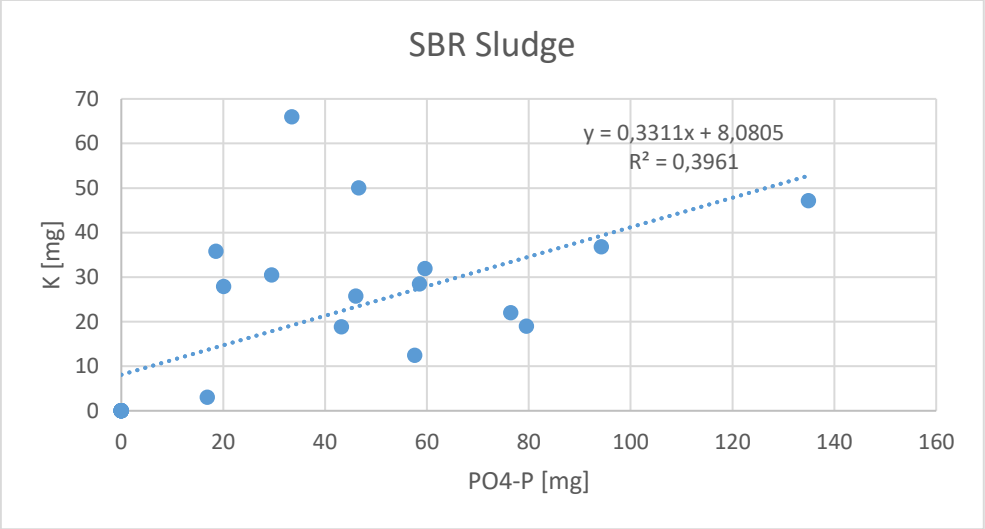

Figure 36: Correlation between PO4-P and K release in P-stripping experiments with SBR sludge

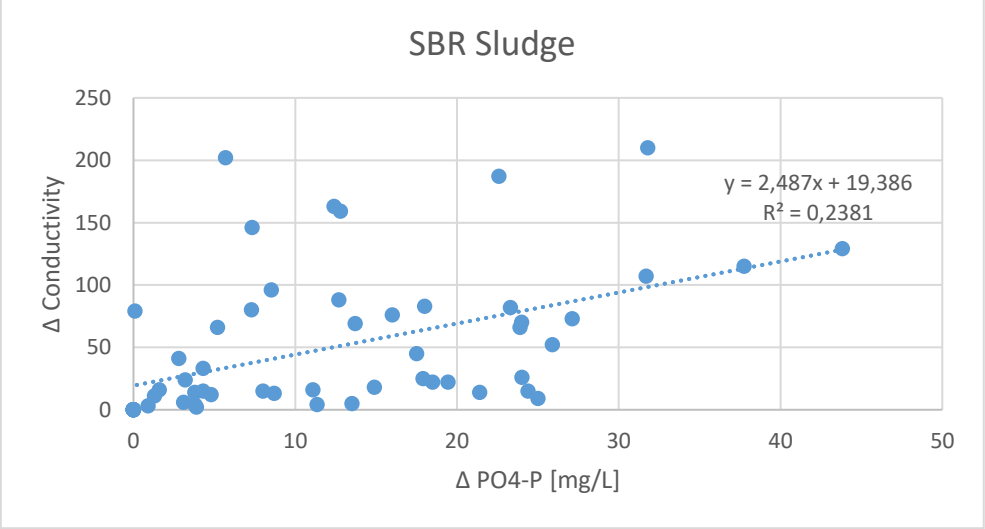

Figure 37: Correlation between PO4-P release and conductivity in P-stripping experiments with SBR sludge

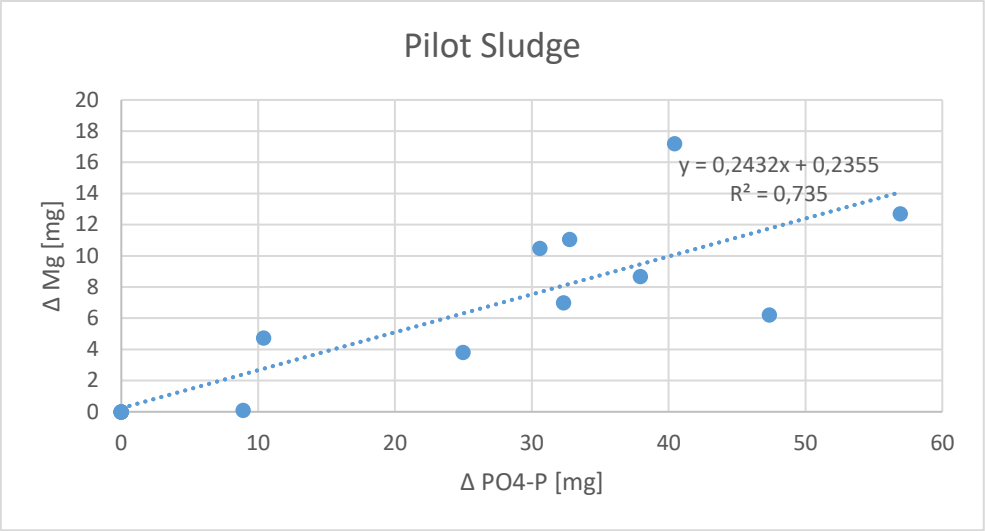

Figure 38: Correlation between PO4-P and Mg release in P-stripping experiments with Pilot sludge

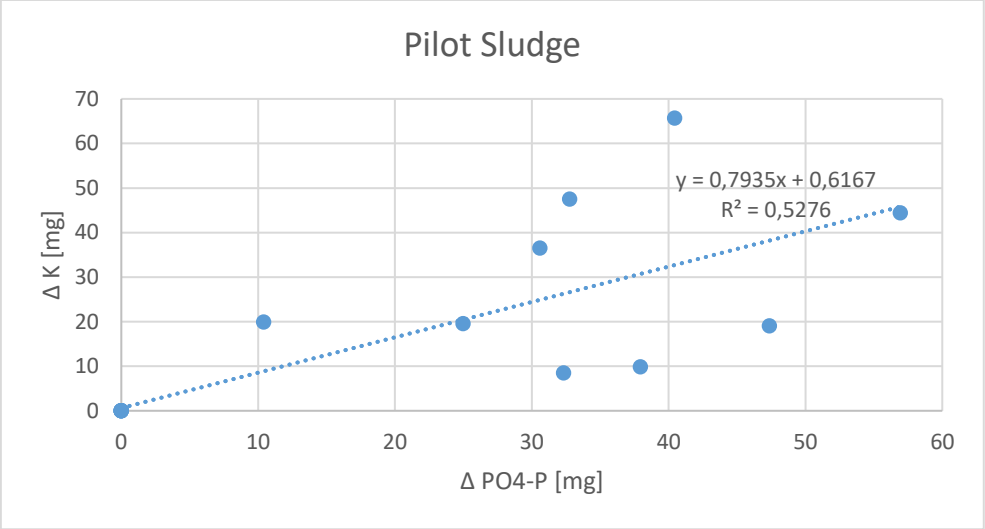

Figure 39: Correlation between PO4-P and K release in P-stripping experiments with Pilot sludge

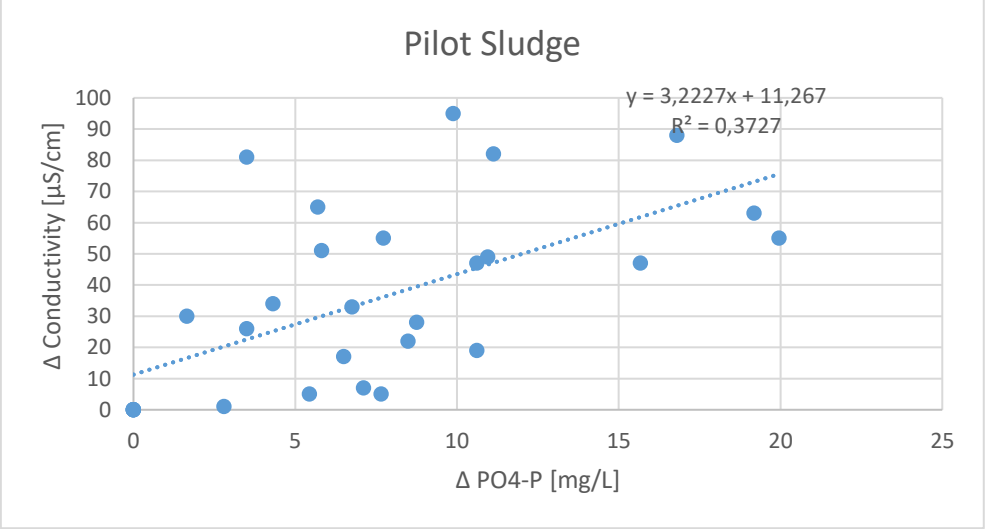

Figure 40: Correlation between PO4-P release and conductivity in P-stripping experiments with pilot sludge

5. Conclusion

The aim of this master thesis was to see if P-stripping with Bio-P sludge would work in colder temperatures. Sludge from two different EBPR processes were tested. Sludge from a Sequence Batch Reactor and sludge from a CMBBR pilot, based on the Hias Process, were extracted for the experiments. Carbon sources were added to the reactor to enhance the P-stripping. The goal with P-stripping was to decrease the P-content in the sludge to prevent struvite precipitation in sludge treatment, and to have a high phosphate concentration in the supernatant, making it ideal for P-recovery.

Results from P-stripping experiments shows that P-release in cold temperatures (11-13°C) is working. There is still more luxury-P left in the biomass, and the release is not as high as observed in room temperature. However, the observed P-release is still quite good. The best results obtained a P-release of between 30 and 40% of luxury-P. The best strategy for carbon dosing is adding doses every hour with sCOD concentrations between 100 mg/L and 200 mg/L.

FPSS, acetate and glucose were all tested as carbon sources in the P-stripping. Using glucose was not unexpectedly, the least efficient carbon source for P-stripping.

FPSS has proven to be a carbon source just as efficient as acetate, and in some cases even better. This is encouraging, since FPSS is a cheaper and more sustainable carbon source compared to acetate. During fermentation, a trend of decreasing NH_4-N concentrations was observed. This is beneficial, when the FPSS is used as carbon source, since too high ammonium concentration may increase the potential for struvite formation. It is therefore important to keep an eye on the NH_4-N concentration in the FPSS.

VFA analyzes from two P-stripping experiments gave information about what type of VFA the biomass liked. The biomass in the CMBBR pilot sludge has fewer preferences when it comes to carbon sources, compared to the biomass in the SBR sludge. Production of iso-butyric and iso-valeric acid was also observed during the P-stripping with SBR sludge. There must be bacteria in the sludge producing the acids. These bacteria does not exist in the CMBBR pilot sludge, as no acid production was observed with this sludge.

VFA analyzes for samples taken during fermentation has given interesting information. The fermentations conducted in this study had HRT of 4 and 8 days. However, the highest VFA production occurred in the first 24 hours of the fermentation. The HRT could therefore be reduced. Shortening the HRT, means that more primary sludge can be fermented.

The release of phosphorus that has been observed in the experiments will decrease the concentration of phosphorus in the bio-P sludge. Hence, the potential of uncontrolled struvite precipitation is reduced. Release of magnesium has also been observed simultaneously with P-release. This also reduces the potential for struvite precipitation. The supernatant from P-stripping experiments could be used for phosphorus recovery if the concentrations of phosphorus are high enough. The aim of the P-stripping is therefore fulfilled, even in colder temperatures and with the use of FPSS.

6. Future Work

There is still much to investigate around the subject discussed in this master thesis. More P-stripping experiments in colder conditions should be conducted. The results presented in this master shows that P-stripping works in cold temperature, with up to 30% release of luxury-P. Further work could be increasing this release even more. This could be done by testing more carbon sources, especially FPSS. Testing FPSS taken from fermenter earlier, could increase the P-release.

Sludge from both the SBR and the CMBBR Pilot can be used in future studies. Making a more permanent sludge collection from the pilot would help getting a more similar sludge for all experiments.

Doing more VFA analyzes would give useful information, both for VFA production in fermentation and VFA consumption in P-stripping experiments. Looking at VFA consumption, a lot of information can be obtained, about what type of carbon the organisms like. Knowing this information, the P-release can be optimized by using more of the carbon they like. Finding the optimal process design for fermentation in order to have the highest production of the carbon source the organisms like the most, can also increase the P-release.

Taking it further in the direction of phosphorus recovery would be very interesting. The supernatant that is left after the P-stripping experiments has a high content of phosphate. There is an opportunity to investigate the possibilities for phosphorus recovery by struvite precipitation.

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Appendix A – Carbon source calculation for P-stripping experiments

25.01.2019- Sludge from SBR

Carbon source: FPSS (1860 mg sCOD/L)

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 doses

Volume at the end of experiment: 1395 mL

$$V_{Dose} = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1395\ mL}{1860\ {}^{mg\ sCOD}/_{L}} = 75\ mL$$

$$Total\ V_{fermented} = 5 \cdot 75\ mL = 375\ mL$$

$$V_{start} = 1395\ mL - 375\ mL = 1020\ mL$$

A 75 mL dose was by mistake also added after 30 minutes. This means, 6 doses were added and that the actual volume at the end of experiment was 1470 mL.

29.01.2019 – Sludge from SBR

Carbon source: FPSS (1860 mg sCOD/L)

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 doses

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{1860\ {}^{mg\ sCOD}/_{L}} = 86\ mL$$

$$Total\ V_{fermented} = 5 \cdot 86\ mL = 430\ mL$$

$$V_{start} = 1600\ mL - 430\ mL = 1170\ mL$$

15.02.2019 – Sludge from SBR

Carbon source: FPSS (2010 mg sCOD/L)

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 doses

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{2010\ {}^{mg\ sCOD}/_{L}} = 80\ mL$$

$$Total\ V_{fermented} = 5 \cdot 80\ mL = 400\ mL$$

$$V_{start} = 1600\ mL - 400\ mL = 1200\ mL$$

21.02.2019 – Sludge from SBR

Carbon source: Glucose

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 doses

Volume: 1600 mL

To have a shot of 16 mL:

$$sCOD\ Concentration = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{16\ mL} = 10\,000\ {}^{mg\ sCOD}/_{L} = 10\ {}^{g\ sCOD}/_{L}$$

$$Glucose\ concentration = \frac{10\ {}^{g\ sCOD}/_{L}}{1{,}07\ {}^{g\ sCOD}/_{g\ glucose}} = 9{,}3\ {}^{g}/_{L}$$

Making a solution of 100 mL:

$$Needed\ glucose = \frac{9{,}3\ g}{1000\ mL} \cdot 100\ mL = 0{,}93\ g$$

27.02.2019 – Sludge from SBR

Carbon source: FPSS (1251 mg sCOD/L)

Target sCOD concentration in reactor: 500 mg/L

Dosing only at beginning: 1 dose

Volume: 1600 mL

$$V_{Dose} = \frac{500\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{1251\ {}^{mg\ sCOD}/_{L}} = 640\ mL$$

$$V_{start} = 1600\ mL - 640\ mL = 960\ mL$$

06.03.2019 – Sludge from SBR

Carbon source: FPSS (1370 mg sCOD/L)

Target sCOD concentration in reactor: 200 mg/L

Dosing every second hour: 3 dose

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{200\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{1370\ {}^{mg\ sCOD}/_{L}} = 233\ mL$$

$$Total\ V_{fermented} = 3 \cdot 233\ mL = 699\ mL$$

$$V_{start} = 1600\ mL - 699\ mL = 901\ mL$$

14.03.2019 – Sludge from SBR

Carbon source: FPSS (2605 mg sCOD/L)

Target sCOD concentration in reactor: 200 mg/L

Dosing every hour: 5 dose

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{200\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{2605\ {}^{mg\ sCOD}/_{L}} = 123\ mL$$

$$Total\ V_{fermented} = 5 \cdot 123\ mL = 615\ mL$$

$$V_{start} = 1600\ mL - 615\ mL = 985\ mL$$

20.03.2019 Sludge from Pilot

Carbon source: FPSS (3385 mg sCOD/L)

Target sCOD concentration in reactor: 200 mg/L

Dosing every hour: 5 dose

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{200\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{3385\ {}^{mg\ sCOD}/_{L}} = 95\ mL$$

$$Total\ V_{fermented} = 5 \cdot 95\ mL = 475\ mL$$

$$V_{start} = 1600\ mL - 475\ mL = 1125\ mL$$

22.03.2019 – Sludge from Pilot

Carbon source: FPSS (1488 mg sCOD/L)

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 dose

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{1488\ {}^{mg\ sCOD}/_{L}} = 108\ mL$$

$$Total\ V_{fermented} = 5 \cdot 108\ mL = 540\ mL$$

$$V_{start} = 1600\ mL - 540\ mL = 1060\ mL$$

26.03.2019 – Sludge from SBR

Carbon source: Acetate

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 dose

Volume: 1600 mL

Molar mass acetate: 59 g/mol

Molar mass sodium acetate trihydrate: 136,08 g/mol

To have a shot of 16 mL:

$$sCOD\ Concentration = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{16\ mL} = 10\,000\ {}^{mg\ sCOD}/_{L} = 10\ {}^{g\ sCOD}/_{L}$$

$$Acetate\ concentration = \frac{10\ {}^{g\ sCOD}/_{L}}{1{,}0845\ {}^{g\ sCOD}/_{g\ acetate}} = 9{,}22\ {}^{g}/_{L}$$

Making a solution of 100 mL:

$$Needed\ acetate = \frac{9{,}22\ g}{1000\ mL} \cdot 100\ mL = 0{,}922\ g$$

Correction with molar masses because acetate is added as sodium acetate trihydrate:

$$Needed\ sodium\ acetate\ trihydrate\ in\ 100\ mL = \frac{136{,}08\ {}^{g}/_{mol}}{59\ {}^{g}/_{mol}} \cdot 0{,}922\ g = 2{,}12\ g$$

29.03.2019 – Sludge from SBR

Carbon source: Acetate

Target sCOD concentration in reactor: 200 mg/L

Dosing every hour: 5 dose

Volume: 1600 mL

Molar mass acetate: 59 g/mol

Molar mass sodium acetate trihydrate: 136,08 g/mol

To have a shot of 16 mL:

$$sCOD\ Concentration = \frac{200\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{16\ mL} = 20\,000\ {}^{mg\ sCOD}/_{L} = 20\ {}^{g\ sCOD}/_{L}$$

$$Acetate\ concentration = \frac{20\ {}^{g\ sCOD}/_{L}}{1{,}0845\ {}^{g\ sCOD}/_{g\ acetate}} = 18{,}44\ {}^{g}/_{L}$$

Making a solution of 100 mL:

$$Needed\ acetate = \frac{18{,}44\ g}{1000\ mL} \cdot 100\ mL = 1{,}844\ g$$

Correction with molar masses because acetate is added as sodium acetate trihydrate:

$$Needed\ sodium\ acetate\ trihydrate\ in\ 100\ mL = \frac{136{,}08\ {}^{g}/_{mol}}{59\ {}^{g}/_{mol}} \cdot 1{,}844\ g = 4{,}25\ g$$

02.04.2019 – Sludge from SBR

Carbon source: FPSS (1375 mg sCOD/L)

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 dose

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{1375\ {}^{mg\ sCOD}/_{L}} = 116\ mL$$

$$Total\ V_{fermented} = 5 \cdot 116\ mL = 580\ mL$$

$$V_{start} = 1600\ mL - 580\ mL = 1020\ mL$$

03.04.2019 – Sludge from Pilot

Carbon source: FPSS (1375 mg sCOD/L)

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 dose

Volume at the end of experiment: 1600 mL

$$V_{Dose} = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{1375\ {}^{mg\ sCOD}/_{L}} = 116\ mL$$

$$Total\ V_{fermented} = 5 \cdot 116\ mL = 580\ mL$$

$$V_{start} = 1600\ mL - 580\ mL = 1020\ mL$$

08.04.2019 – Sludge from Pilot

Carbon source: Glucose

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 dose

Volume: 1600 mL

To have a shot of 16 mL:

$$sCOD\ Concentration = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{16\ mL} = 10\,000\ {}^{mg\ sCOD}/_{L} = 10\ {}^{g\ sCOD}/_{L}$$

$$Glucose\ concentration = \frac{10\ {}^{g\ sCOD}/_{L}}{1{,}07\ {}^{g\ sCOD}/_{g\ glucose}} = 9{,}3\ {}^{g}/_{L}$$

Making a solution of 100 mL:

$$Needed\ glucose = \frac{9{,}3\ g}{1000\ mL} \cdot 100\ mL = 0{,}93\ g$$

10.04.2019 – Sludge from Pilot

Carbon source: Acetate

Target sCOD concentration in reactor: 100 mg/L

Dosing every hour: 5 dose

Volume: 1600 mL

Molar mass acetate: 59 g/mol

Molar mass sodium acetate trihydrate: 136,08 g/mol

To have a shot of 16 mL:

$$sCOD\ Concentration = \frac{100\ {}^{mg\ sCOD}/_{L} \cdot 1600\ mL}{16\ mL} = 10\,000\ {}^{mg\ sCOD}/_{L} = 10\ {}^{g\ sCOD}/_{L}$$

$$Acetate\ concentration = \frac{10\ {}^{g\ sCOD}/_{L}}{1{,}0845\ {}^{g\ sCOD}/_{g\ acetate}} = 9{,}22\ {}^{g}/_{L}$$

Making a solution of 100 mL:

$$Needed\ acetate = \frac{9{,}22\ g}{1000\ mL} \cdot 100\ mL = 0{,}922\ g$$

Correction with molar masses because acetate is added as sodium acetate trihydrate:

$$Needed\ sodium\ acetate\ trihydrate\ in\ 100\ mL = \frac{136{,}08\ {}^{g}/_{mol}}{59\ {}^{g}/_{mol}} \cdot 0{,}922\ g = 2{,}12\ g$$

Appendix B – VFA calculations

The results from NMBU were given in mmol/L.

The conversion from mmol/L to COD is done in this appendix.

Acetic acid

$$C_2H_4O_2 + 2O_2 \rightarrow 2CO_2 + 2H_2O$$

2 oxygen needs to balance the equations.

$$2 \cdot (16g + 16g) = 64g\ oxygen$$

$$^{64g}/_{60g} = 1{,}07 \frac{g\ oxygen}{g\ acetic\ acid}$$

Propionic acid

$$C_3H_6O_2 + 3{,}5O_2 \rightarrow 3CO_2 + 3H_2O$$

3,5 oxygen is needed:

$$3{,}5 \cdot (16 + 16) = 112g\ oxygen$$

$$^{112g}/_{74g} = 1{,}51 \frac{g\ oxygen}{g\ propionic\ acid}$$

Iso-butyric acid

$$C_4H_8O_2 + 5O_2 \rightarrow 4CO_2 + 4H_2O$$

5 oxygen is needed:

$$5 \cdot (16 + 16) = 160g\ oxygen$$

$$^{160g}/_{88g} = 1{,}82 \frac{g\ oxygen}{g\ iso - butyric\ acid}$$

Butyric acid

$$C_4H_8O_2 + 5O_2 \rightarrow 4CO_2 + 4H_2O$$

5 oxygen is needed:

$$5 \cdot (16 + 16) = 160g\ oxygen$$

$$^{160g}/_{88g} = 1{,}82 \frac{g\ oxygen}{g\ butyric\ acid}$$

Iso-valeric acid

$$C_5H_{10}O_2 + 6{,}5O_2 \rightarrow 5CO_2 + 5H_2O$$

6,5 oxygen is needed:

$$6{,}5 \cdot (16 + 16) = 208g\ oxygen$$

$$^{208g}/_{102g} = 2{,}04 \frac{g\ oxygen}{g\ iso - valeric\ acid}$$

Valeric acid

$$C_5H_{10}O_2 + 6{,}5O_2 \rightarrow 5CO_2 + 5H_2O$$

6,5 oxygen is needed:

$$6{,}5 \cdot (16 + 16) = 208g\ oxygen$$

$$^{208g}/_{102g} = 2{,}04 \frac{g\ oxygen}{g\ valeric\ acid}$$

Appendix C – Data collections from fermentations

Table C-1: Measurements for TS and VS in fermented mix at start

	W_dish [g]		W_sample [g]		V-Sample [ml]		W_105 [g]		TS [g/l]		TS [%]		W_550 [g]		VS [%]	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
PS BATCH 1	96,3246	93,9636	38,8083	40,128	40	40	98,6822	96,5604	58,94	64,92	6,075	6,471	96,4514	94,0978	94,62	94,83
BATCH 1	96,098	96,1044	39,0453	38,8946	40	40	96,4436	96,4376	8,64	8,33	0,885	0,857	96,127	96,1328	91,61	91,48
BATCH 2	90,1834	96,1034	39,8145	39,4443	40	40	90,528	96,3816	8,61	6,96	0,866	0,705	90,2236	96,1346	88,33	88,79
BATCH 3	96,1076	81,0074	38,3727	39,3292	40	40	96,4332	81,4056	8,14	9,96	0,849	1,012	96,1412	81,041	89,68	91,56
BATCH 4	96,1038	96,3256	39,1894	39,726	40	40	96,4392	96,744	8,38	10,46	0,856	1,053	96,127	96,3528	93,08	93,50

Table C-2: Data from fermentation

Fermentation start 13.02.19

Roomtemperature

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [∘C]	Information
13.02.2019	0	10:00	166	4,93	36,71	9300	-	-	7,503	17,8	
	5	15:00	270	5,75	39,9				7,208	19,7	
14.02.2019	22,5	08:30	360	7,72	35,85				6,987	19,5	
	29	15:00	350	7,15	37,75				6,895	20,8	
15.02.2019	48	10:00	405	6,1	32,5				6,792	19,8	

BATCH 2

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]	Information
15.02.2019	0	11:00	240	3,83	25,47	6400	22,15	119,5	7,160	17,6	
	4	15:00	234	3,84					6,987	21,1	
16.02.2019	27,5	13:30	302	2,91	17,5				6,867	20,6	
17.02.2019	48	10:00	244	0,5	5,47				6,825	19,5	

BATCH 3

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]	Information
17.02.2019	0	10:00	188	2,08	14,52	3500	24,1	172	7,096	17,1	
	5	15:00	253	2,51						19,2	
18.02.2019	22,5	08:30	384	2,98	10,67				6,530	20,1	
	29	15:00	401	2,75					6,420	21,2	
19.02.2019	48	10:00	499	3,216	4,35				6,191	20,0	
19.02.2019		10:00	463	3,171	4,35						Supernatant

BATCH 4

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]	Information
28.02.2019	0	10:00	310	3,34	17,82	5000	30,7	148,6	7,081	17,4	
	5	15:00	358	3,72					6,851	19,8	
01.03.2019	22,5	08:30	458	3,62	15,19				6,616	19,5	
	29	15:00	496	3,8					6,533	20,0	
02.02.2019	48	10:00	427	2,83	8,92				6,402	19,4	

Table C-3: Measurements for TS and VS in fermented mix at start

	W_dish [g]		W_sample [g]		V-Sample [ml]		W_105 [g]		TS [g/l]		TS [%]		W_550 [g]		VS [%]	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
BATCH 1	94,9742	81,0068	38,5162	38,7114	40	40	95,4238	81,4552	11,240	11,210	1,167	1,158	95,0090	81,0384	92,26	92,95
BATCH 2	96,3230	90,1950	39,7723	40,2923	40	40	96,8194	90,7486	12,410	13,840	1,248	1,374	96,3562	90,2300	93,31	93,68
BATCH 3	95,2716	91,3614	39,9520	39,1532	40	40	95,7472	91,846	11,890	12,115	1,190	1,238	95,3096	91,5980	92,01	51,18
BATCH 4	96,3254	96,1036	39,8692	39,4439	40	40	96,7746	96,5618	11,230	11,455	1,127	1,162	96,3580	96,1348	92,74	93,19

Table C-4: Data from fermentation

Fermentation start 22.02.2019

High temperature

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [∘C]	Information
22.02.2019	0	09.00	174	5,20	35,08	5800	31,5	183	7,302	16,0	
	6	15:00	342	6,97	38,04				6,855	25,9	
23.02.2019	29	14:00	506	7,50	36,42				6,521	28,0	
24.02.2019	48	10:00	462	4,20	16,88				6,431	27,9	Supernatant

BATCH 2

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]	Information
24.02.2019	0	10:00	323	5,67	26,80	-	37,1	241	6,733	21,9	
	5	15:00	509	6,93					6,516	28,1	
25.02.2019	22,5	08:30	610	8,40	29,72				5,914	27,4	
	29	15:00	800	9,38							
26.02.2019	48	10:00	736	9,16	23,55						
26.02.2019		09:00	851	9,99	26,23						Supernatant

BATCH 3

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]	Information
26.02.2019	0	09:00	459	6,6	23,7	1710	21	152	6,412	21,5	
	5	13:30	540	7,68					6,154	27,9	
27.02.2019	22,5	08:30	727	9,04	22,12				5,543	28,0	
	29	15:00	785	9,88					5,440	27,5	
28.02.2019	48	10:00	1024	10,28	19,88				5,259	27,5	Supernatant

BATCH 4

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]	Information
28.02.2019	0	10:00	475	6,42	19,38	6960	32,1	171	6,103	21,4	
	5	15:00	566	8,43	21,19				5,993	26,5	
01.03.2019	22,5	08:30	705	9,39	17,89				5,478	26,9	
	29	15:00	621	8,21					5,394	28,6	
02.02.2019	48	10:00	685	7,82	10,69				5,256	27,5	Supernatant

Table C-5: Measurements for TS and VS in fermented mix at start

	W_dish [g]		W_sample [g]		V-Sample [ml]		W_105 [g]		TS [g/l]		TS [%]		W_550 [g]		VS [%]	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
BATCH 1	91,6764	91,3650	39,7178	39,6506	40	40	92,2522	91,956	14,395	14,775	1,450	1,491	91,709	91,398	94,34	94,42
BATCH 2	96,1092	85,8332	39,3488	39,8036	40	40	96,6014	86,377	12,305	13,595	1,251	1,366	96,1392	85,8654	93,90	94,08

Table C-6: Data from fermentation

Fermentation start 07.03.2019

High temperature

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [∘C]	Information
07.03.2019	0	09.00	237	5,50	33,21	9890	37,5	179	6,979	16,9	
	5	14:00	509						6,637	27,7	
08.03.2019	23,5	08:30	792	13,28	53,10				6,390	26,9	
	30	15:00	661						6,515	26,6	
09.03.2019	51	12:00	542,5	8,27	28,23				6,373	27,2	
10.03.2019	78	15:00	733,5						5,806	27,1	
11.03.2019	95,5	08:30	1090	7,59	0,402				5,518	26,1	
	96,5	09:30	927	7,36	0,556						Supernatant

BATCH 2

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [∘C]	Information
11.03.2019	0	09:30	662	8,38	27,36	13630	53,4	270	6,335	21,8	
	3,5	13:00	615,5						6,116	27,6	
12.03.2019	23	08:30	945	12,54	30,41				5,551	26,6	
	28,25	13:45	1314						5,470	27,3	
13.03.2019	47	08:30	1258	13,86	35,58				5,325	26,8	
	52,5	14:00	1466						5,317	26,5	
14.03.2019	72,5	10:00	1758	16,14	40,88				5,264	26,4	
	77,5	15:00	1475						5,234	27,4	
15.03.2019	96	09:30	1557	15,06	38,16				5,175	27,0	
		09:30	1440	14,28	36,34						Supernatant

Table C-7: Measurements for TS and VS in fermented mix at start

	W_dish [g]		W_sample [g]		V-Sample [ml]		W_105 [g]		TS [g/l]		TS [%]		W_550 [g]		VS [%]	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
BATCH 1	91,6760	96,3234	39,9921	38,4310	40	40	92,0348	96,627	8,97	7,59	0,897	0,790	91,7042	96,3552	92,14	89,53

Table C-8: Data from fermentation

Fermentation start 25.03.2019

High temperatures

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [∘C]	Information
25.03.2019	0	08:30	161	3,30	15,69	9300	27,1	133,5	7,083	13,7	
26.03.2019	24	08:30	452	6,39	21,55				6,178	26,2	
27.03.2019	48	08:30	526	6,82	18,94				5,805	28,7	
28.03.2019	72	08:30	648	6,48	14,40				5,654	28,3	
29.03.2019	96	08:30	584	5,98	7,36				5,500	28,7	
			588,5	5,92	7,56						Supernatant

Appendix D – P-stripping Experiments

Experiment 25.01.2019 – SBR sludge

Initial conditions FPSS	
PO4-P	26,4 mg/L
sCOD	1860 mg/L
NH4-N	78,9 mg/L

Initial conditions sludge	
TS	7955 mg/L
VS	69,17 %
TP	381 mg/L
PO4-P	5,45 mg/L
NH4-N	-
sCOD	22 mg/L
Conductivity	465 µS/cm
mg Pns/ mg TS	0,047209

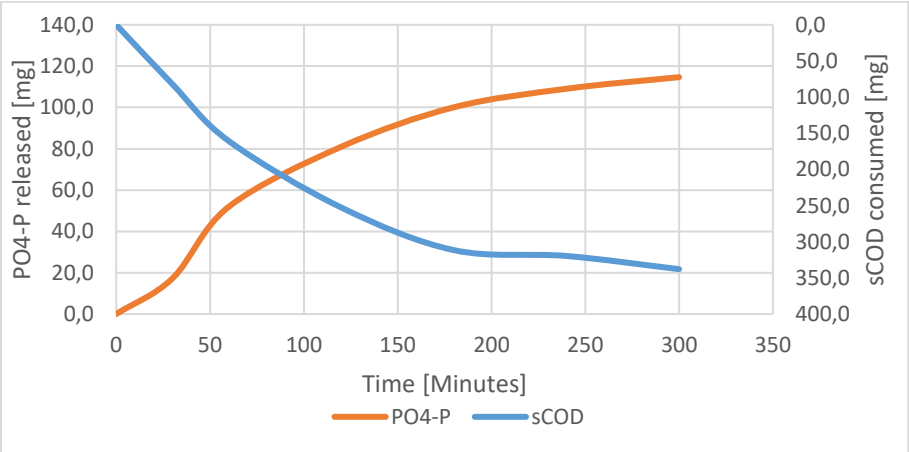
sCOD dose: 100 mg/L*h

NB: Added FPSS after 30 minutes also

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Conductivity [µS/cm]
T0	0	88	7,58	5,96	465
T1	30	140	23,5	-	-
T2	60	183	51,4	-	594
T3	120	210	71,9	-	-
T4	180	259	83,1	-	701
T5	240	339	86,3	-	725
T6	300	326	90,1	27,03	729

Corrected for dilution and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	82,3	17,3
T2	60	160,8	52,0
T3	120	252,8	81,0
T4	180	311,6	100,1
T5	240	319,7	109,1
T6	300	337,9	114,7



Experiment 29.01.2019 – SBR sludge

Initial conditions FPSS	
PO4-P	26,4 mg/L
sCOD	1860 mg/L
NH4-N	78,9 mg/L

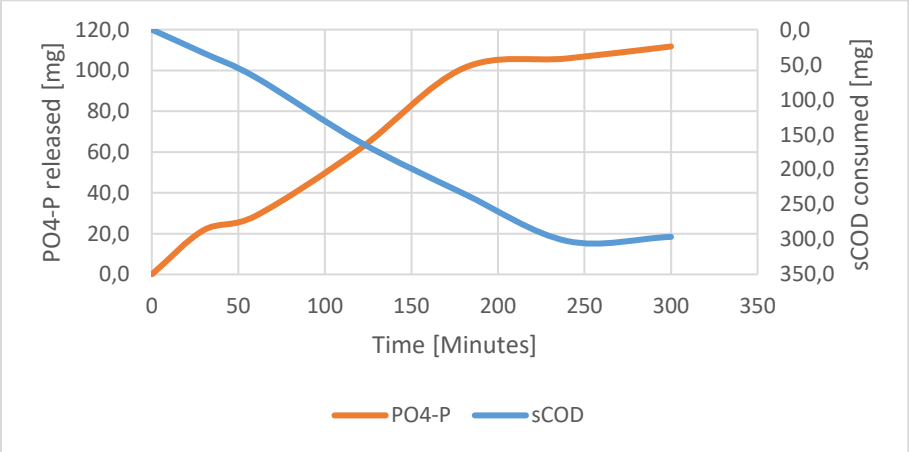
Initial conditions sludge	
TS	7370 mg/L
VS	70,64 %
TP	375
PO4-P	5,37
NH4-N	-
sCOD	28,5
Conductivity	464 µS/cm
mg Pns/ mg TS	0,050153

sCOD dose: 100 mg/L*h

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	70	13,71	7,37	464	13,2
T1	30	41	32,5	-	-	12,7
T2	60	139	37,7	-	534	12,3
T3	120	181	61,6	-	600	13,3
T4	180	231	87,5	-	652	12,2
T5	240	279	87,2	-	671	12,7
T6	300	283	91,1	26,02	673	12,5

Corrected for dilution and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	33,5	21,7
T2	60	68,2	28,7
T3	120	160,5	61,4
T4	180	234,2	101,1
T5	240	302,3	105,9
T6	300	296,3	111,7



Experiment 15.02.2019 – SBR sludge

Initial conditions FPSS	
PO4-P	9,15 mg/L
sCOD	2010 mg/L
NH4-N	23,77 mg/L

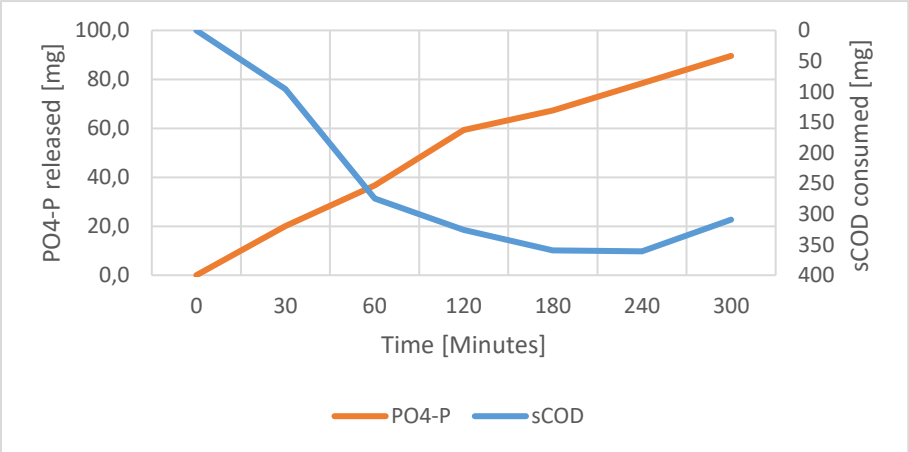
Initial conditions sludge	
TS	7957,5 mg/L
VS	71,82 %
TP	339 mg/L
PO4-P	7,62 mg/L
NH4-N	-
sCOD	19 mg/L
Conductivity	856 µS/cm
mg Pns/ mg TS	0,0416437

sCOD dose: 100 mg/L*h

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	131	9,47	-	7,55	12,84	856	12,7
T1	30	56	25,2	-	-	-	846	11,6
T2	60	42	38,7	-	-	-	851	11,9
T3	120	120	53,6	-	16,47	33,02	869	11,6
T4	180	202	56,7	-	-	-	875	11,4
T5	240	296	61,5	-	-	-	887	11,6
T6	300	414	65,4	7,42	20,12	47,14	726	11,4

Corrected for dilution and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	96,0	20,1
T2	60	274,7	36,7
T3	120	326,1	59,3
T4	180	359,2	67,3
T5	240	361,0	78,4
T6	300	309,3	89,6



Experiment 21.02.2019 – SBR sludge

Glucose

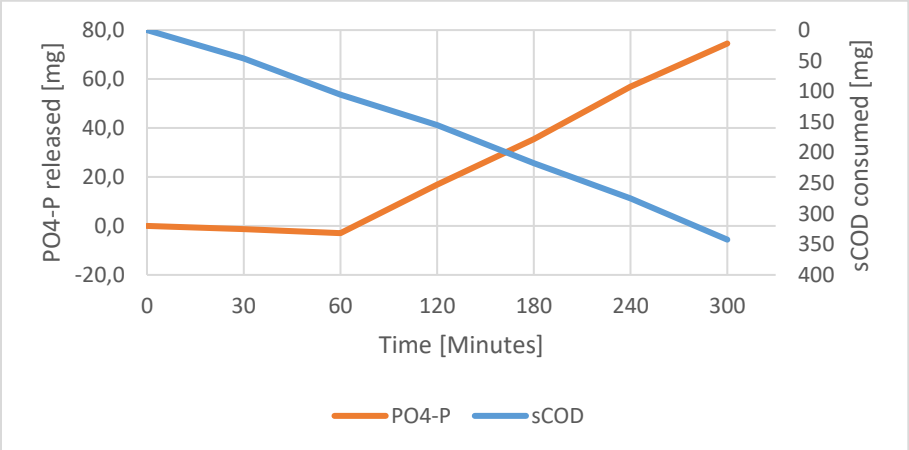
sCOD dose: 100 mg/L*h

Initial conditions sludge	
TS	7872,5 mg/L
VS	73,00 %
TP	340 mg/L
PO4-P	7,38 mg/L
NH4-N	-
sCOD	22 mg/L
Conductivity	588 µS/cm
mg Pns/ mg TS	0,0422508

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Conductivity [µS/cm]
T0	0	109	10,63	under range	10	21,59	588
T1	30	80	9,85				-
T2	60	43	8,79				578
T3	120	112	21,2		12,66	23,5	582
T4	180	173	32,8				608
T5	240	237	46,2				617
T6	300	295	57,2	under range	17,97	31,3	632

Corrected for volume:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	46,4	-1,2
T2	60	105,6	-2,9
T3	120	155,2	16,9
T4	180	217,6	35,5
T5	240	275,2	56,9
T6	300	342,4	74,5



Experiment 27.02.2019 – SBR sludge

Initial conditions FPSS	
PO4-P	17,62 mg/L
sCOD	1251 mg/L
NH4-N	50,4 mg/L

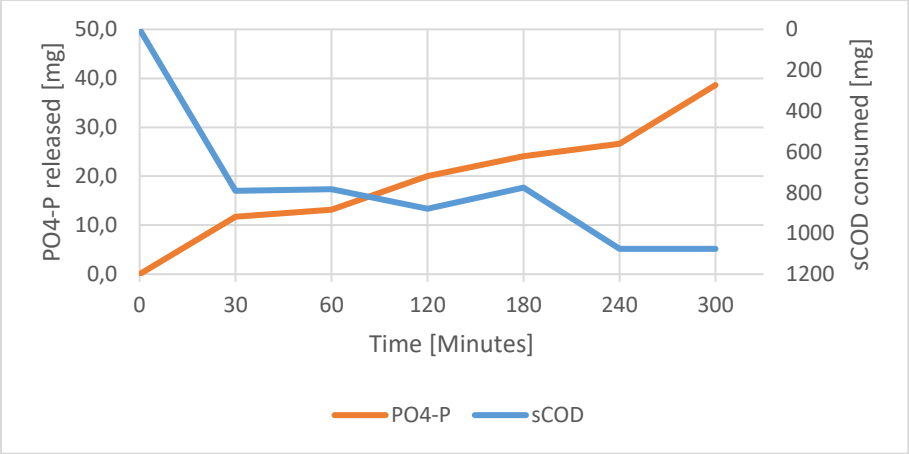
Initial conditions sludge	
TS	7912,5 mg/L
VS	74,40 %
TP	337 mg/L
PO4-P	9,02 mg/L
NH4-N	-
sCOD	16,6 mg/l
Conductivity	537 µS/cm
mg Pns/ mg TS	0,04145087

sCOD dose: 500 mg/L

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	532	16,06	23,8	11,6	24,68	537	13,0
T1	30	537,5	23,4				683	12,0
T2	60	543	24,3				686	11,5
T3	120	482,5	28,6		12,83	42,13	719	12,3
T4	180	548	31,1				705	11,4
T5	240	360	32,7				721	12,0
T6	300	360	40,2	18,02	18,02	64,52	715	11,7

Corrected for volume and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	791,8	11,7
T2	60	783,0	13,2
T3	120	879,8	20,1
T4	180	775,0	24,1
T5	240	1075,8	26,6
T6	300	1075,8	38,6



Experiment 06.03.2019 – SBR sludge

Initial conditions FPSS	
PO4-P	22,26 mg/L
sCOD	1370 mg/L
NH4-N	44,10 mg/L

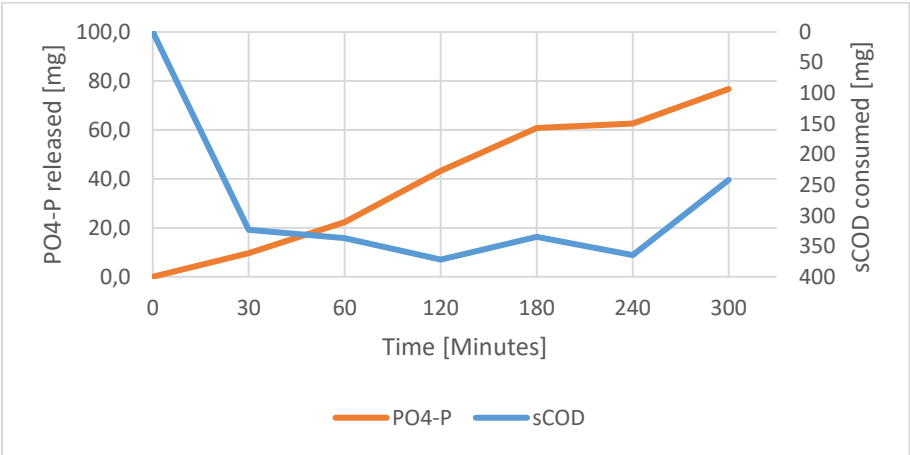
sCOD dose: 200 mg/L*2nd h

Initial conditions sludge	
TS	11495 mg/L
VS	71,30 %
TP	440 mg/L
PO4-P	9,42 mg/L
NH4-N	-
sCOD	19,7 mg/L
Conductivity	528 µS/cm
mg Pns/ mg TS	0,037458025

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	285	16,77	10,01	10,94	36,12	86	528	12,3
T1	30	212,5	25,3					624	12,4
T2	60	200,5	36,4					640	12,0
T3	120	169,5	54,9		19,15	52,74	76,5	662	12,0
T4	180	401,5	62,2					742	12,0
T5	240	379,5	63,5					753	12,0
T6	300	600,5	66,3	23,7	23,7	78,6	104,5	794	12,4

Corrected for volume and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	323,2	9,7
T2	60	336,8	22,3
T3	120	372,0	43,2
T4	180	334,5	60,8
T5	240	364,6	62,6
T6	300	241,8	76,7



Experiment 14.03.2019 – SBR sludge

Initial conditions FPSS	
PO4-P	35,94 mg/L
sCOD	2605 mg/L
NH4-N	96 mg/L

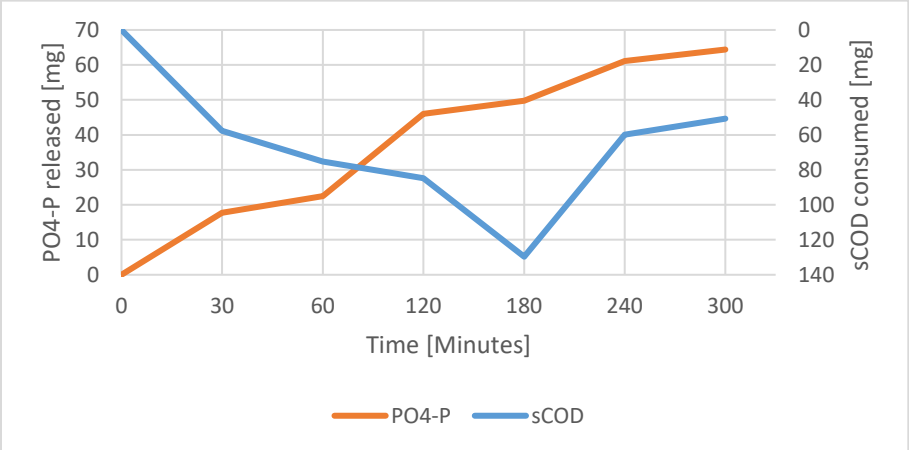
Initial conditions sludge	
TS	11265 mg/L
VS	72,61 %
TP	523 mg/L
PO4-P	22 mg/L
NH4-N	2,7 mg/L
sCOD	37 mg/L
Conductivity	475 µS/cm
mg Pns/ mg TS	0,044702208

sCOD dose: 200 mg/L*h

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	245	27,0	11,2	10,8	44,06	51	475	13,2
T1	30	193	43,0					551	12,6
T2	60	177	47,3					566	11,7
T3	120	412	65,3		20,6	60,56	98,5	649	11,6
T4	180	578	65,4					728	11,9
T5	240	794	70,6					794	11,7
T6	300	939	70,0	40,74	20,1	36,02	106,4	838	12,1

Corrected for volume and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	57,6	17,7
T2	60	75,3	22,5
T3	120	84,7	46,0
T4	180	129,7	49,8
T5	240	60,0	61,1
T6	300	50,7	64,4



Experiment 20.03.2019 – Pilot sludge

Initial conditions FPSS	
PO4-P	36,6 mg/L
sCOD	3385 mg/L
NH4-N	98,31 mg/L

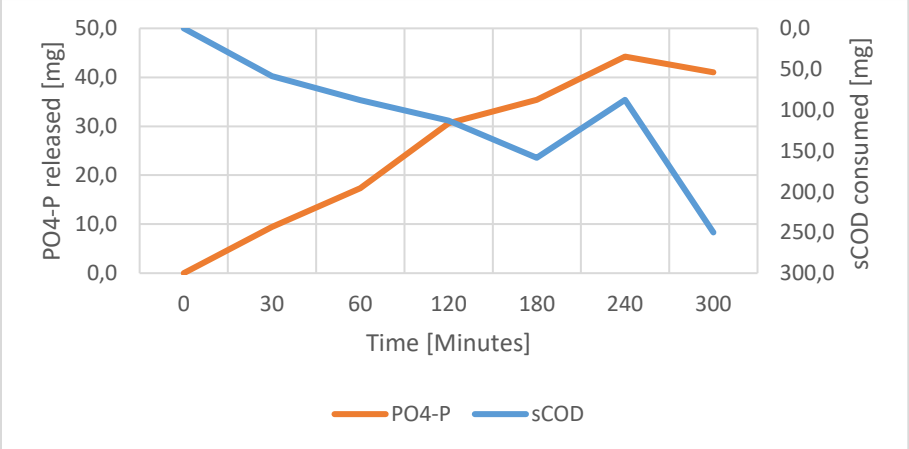
sCOD dose: 200 mg/L*h

Initial conditions sludge	
TS	7202,5 mg/L
VS	79,61 %
TP	379 mg/L
PO4-P	8,99 mg/L
NH4-N	24,3 mg/L
sCOD	37 mg/L
Conductivity	514 µS/cm
mg Pns/ mg TS	0,05137244

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	310	10,28	29,72	7,6	58,72	54,4	514	13,6
T1	30	262	18,00					569	11,5
T2	60	238	24,50					586	11,8
T3	120	446	35,45		15,02	82,28	68,5	635	11,9
T4	180	612	38,95					716	11,5
T5	240	834	44,65					781	11,7
T6	300	884	42,15	42,47	15,3	80,06	85,2	843	11,9

Corrected for volume and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	58,6	9,4
T2	60	87,8	17,3
T3	120	113,3	30,6
T4	180	158,4	35,4
T5	240	87,8	44,2
T6	300	250,1	41,0



Experiment 22.03.2019 – Pilot sludge

Initial conditions FPSS	
PO4-P	17,07 mg/L
sCOD	1488 mg/L
NH4-N	39,81 mg/L

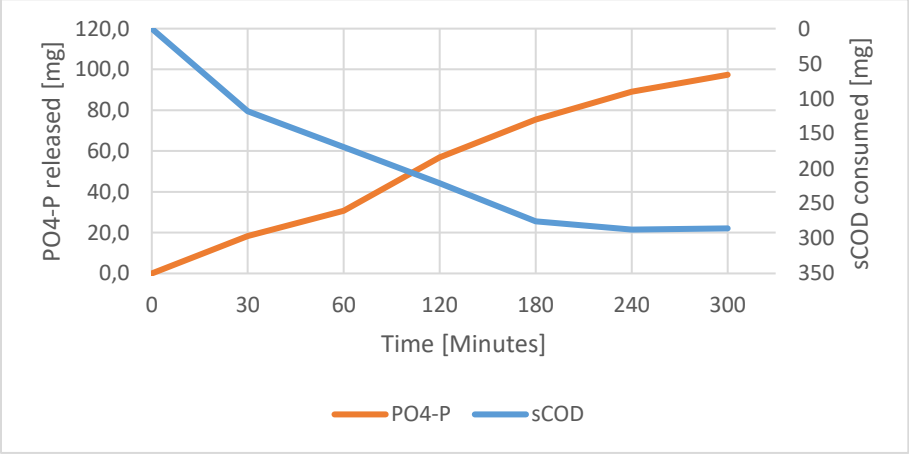
Initial conditions sludge	
TS	9857,5 mg/L
VS	77,78 %
TP	468 mg/L
PO4-P	8,28 mg/L
NH4-N	8,13 mg/L
sCOD	28 mg/L
Conductivity	376 µS/cm
mg Pns/ mg TS	0,046636571

sCOD dose: 100 mg/L*h

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	219	7,17	12,04	5,88	19,58	50,6	376	13,4
T1	30	118	22,84					423	12,0
T2	60	74	33,45					442	11,9
T3	120	153	52,63		15,33	52,74	59,8	505	12,1
T4	180	218	63,24					552	11,6
T5	240	302	69,05					603	12,2
T6	300	383	70,70	23,15	22,98	83,14	72,3	633	12,0

Corrected for volume and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	118,0	18,3
T2	60	169,4	30,7
T3	120	221,3	56,9
T4	180	275,5	75,5
T5	240	287,3	89,1
T6	300	285,8	97,4



Experiment 26.03.2019 – SBR sludge

Acetate

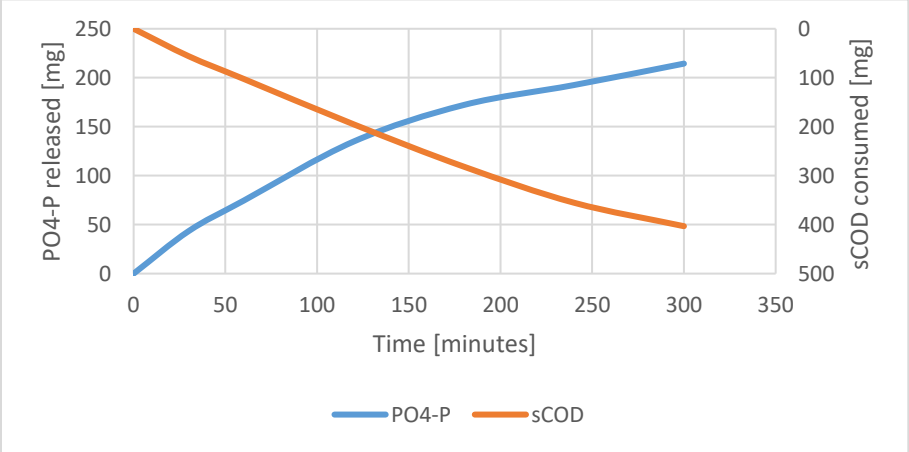
sCOD dose: 100 mg/L*h

Initial conditions sludge	
TS	12355 mg/L
VS	72,13 %
TP	793 mg/L
PO4-P	7,85 mg/L
NH4-N	0,348 mg/L
sCOD	34 mg/L
Conductivity	483 µS/cm
mg Pns/ mg TS	0,06354917

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	105	24,48	0,348	8,16	24,51	52,8	483	13,9
T1	30	70	51,60					556	13,0
T2	60	41	71,05					578	12,7
T3	120	83	108,80		24,13	54	66,3	693	12,5
T4	180	129	132,10					775	13,0
T5	240	183	144,80					863	13,0
T6	300	253	158,50	3,39	35	65,88	68,2	932	12,9

Corrected for volume:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	56,0	43,4
T2	60	102,4	74,5
T3	120	195,2	134,9
T4	180	281,6	172,2
T5	240	355,2	192,5
T6	300	403,2	214,4



Experiment 29.03.2019 – SBR sludge

Acetate

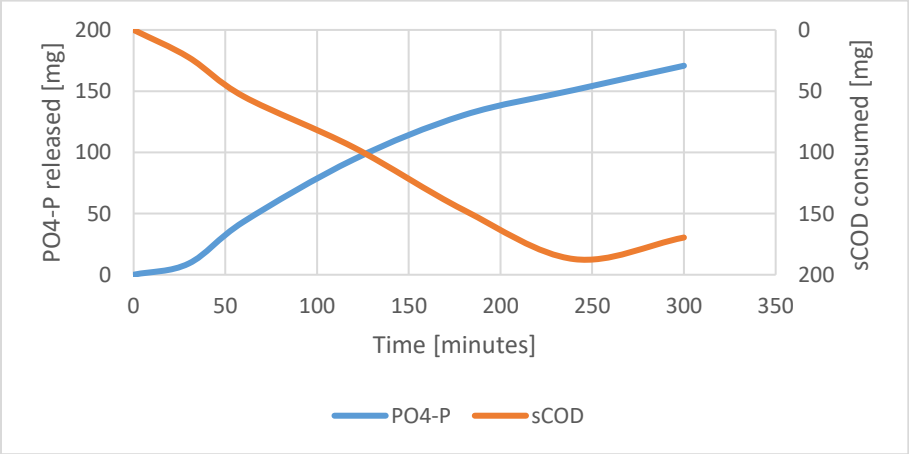
sCOD dose: 200 mg/L*h

Initial conditions sludge	
TS	13865 mg/L
VS	71,73 %
TP	685 mg/L
PO4-P	7,86 mg/L
NH4-N	1,106 mg/L
sCOD	25 mg/L
Conductivity	477 µS/cm
mg Pns/ mg TS	0,048838082

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	200	53,90	1,106	13,82	33,89	64,4	477	13,9
T1	30	186	59,60					679	12,7
T2	60	166	81,00					693	12,5
T3	120	340	112,80		25,73	56,92	73,7	903	12,6
T4	180	508	135,40					1090	12,8
T5	240	683	148,20					1249	12,7
T6	300	894	160,60	4,15	42,23	70,66	81,4	1412	12,8

Corrected for volume:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	22,4	9,1
T2	60	54,4	43,4
T3	120	96,0	94,2
T4	180	147,2	130,4
T5	240	187,2	150,9
T6	300	169,6	170,7



Experiment 02.04.2019 – SBR sludge

Initial conditions FPSS	
PO4-P	17,4 mg/L
sCOD	1375 mg/L
NH4-N	40,86 mg/L

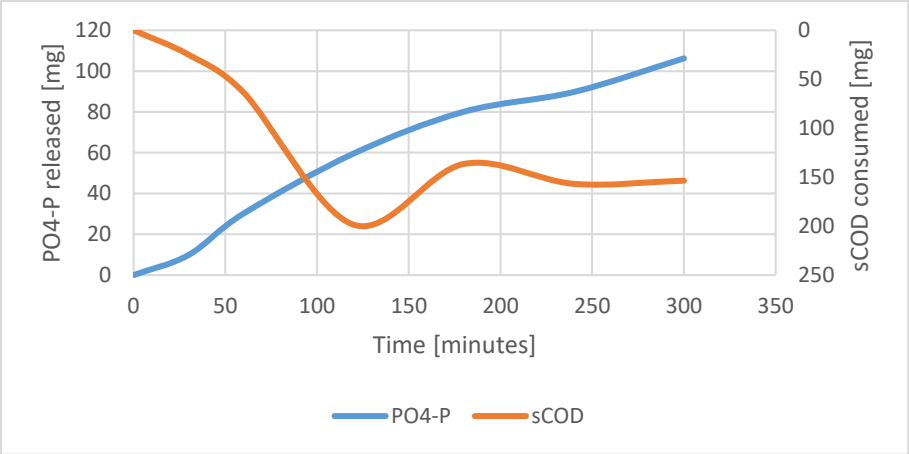
Initial conditions sludge	
TS	7300 mg/L
VS	72,56 %
TP	704 mg/L
PO4-P	56 mg/L
NH4-N	6,19 mg/L
sCOD	46 mg/L
Conductivity	558 µS/cm
mg Pns/ mg TS	0,088767123

sCOD dose: 100 mg/L*h

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	144	55,30	9,24	14,7	46,34	67,5	558	13,4
T1	30	122	64,00					571	12,7
T2	60	88	81,90					596	12,8
T3	120	168	99,40		25,94	67,56	76,2	641	12,6
T4	180	253	107,40					656	12,4
T5	240	327	106,90					686	13,3
T6	300	405	110,70	20,87	30,36	84,16	85,6	700	12,9

Corrected for volume and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	25,0	9,9
T2	60	63,6	30,2
T3	120	198,6	59,6
T4	180	136,5	80,1
T5	240	156,8	89,8
T6	300	153,6	106,2



Experiment 03.04.2019 – Pilot sludge

Initial conditions FPSS	
PO4-P	17,4 mg/L
sCOD	1375 mg/L
NH4-N	40,86 mg/L

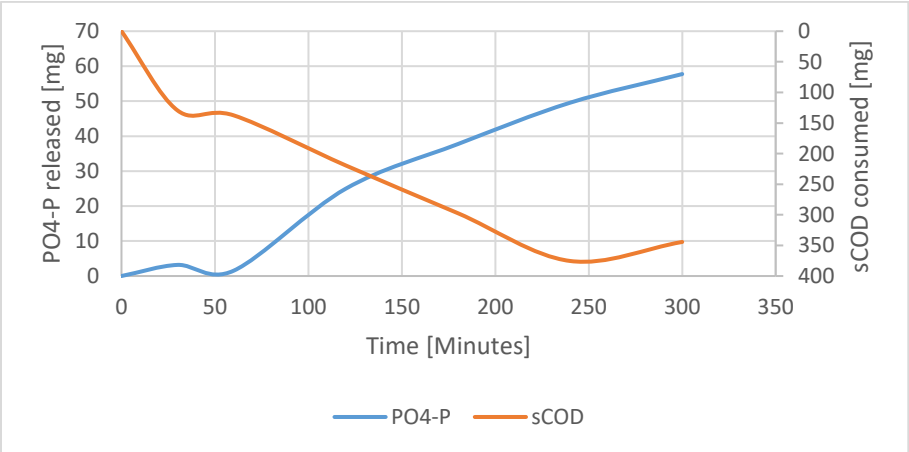
Initial conditions sludge	
TS	5795 mg/L
VS	76,16 %
TP	274 mg/L
PO4-P	1,214 mg/L
NH4-N	0,036 mg/L
sCOD	32 mg/L
Conductivity	415 µ/cm
mg Pns/ mg TS	0,047072649

sCOD dose: 100 mg/L*h

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	164	2,91	3,72	9,47	18,92	67,9	415	13,5
T1	30	50	5,71					416	12,5
T2	60	43	4,25					396	12,7
T3	120	101	24,20		11,64	32,8	67,9	451	12,9
T4	180	152	32,95					479	13,3
T5	240	195	39,70					512	13,2
T6	300	300	43,20	16,02	16,02	55,38	76,4	538	13,2

Corrected for volume and added FPSS:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	129,5	3,2
T2	60	137,5	1,5
T3	120	219,4	25,0
T4	180	297,4	37,7
T5	240	375,4	49,6
T6	300	344,3	57,7



Experiment 08.04.2019 – Pilot sludge

Glucose

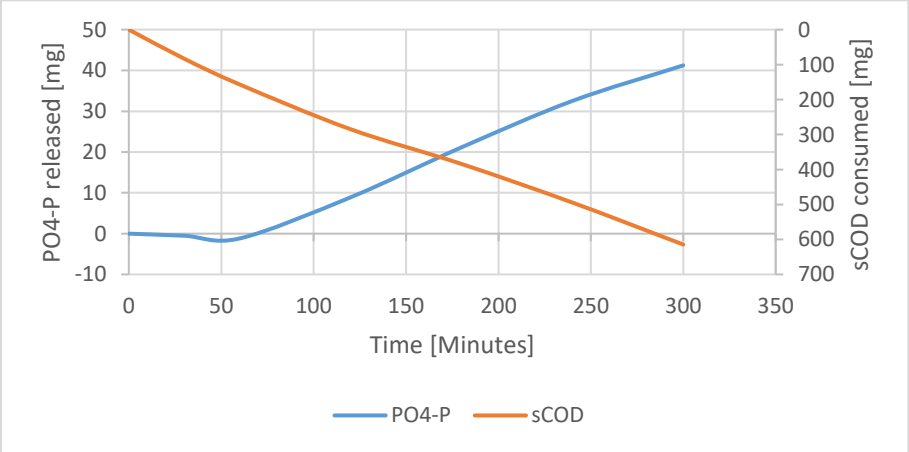
sCOD dose: 100 mg/L*h

Initial conditions sludge	
TS	8372,5 mg/L
VS	77,05 %
TP	339 mg/L
PO4-P	2,74 mg/L
NH4-N	2,34 mg/L
sCOD	49 mg/L
Conductivity	458 µS/cm
mg Pns/ mg TS	0,040162437

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	145	2,57	2,34	9,94	21,33	56,1	458	13,4
T1	30	93	2,25					442	12,6
T2	60	47	1,84					435	12,7
T3	120	67	8,14		10	17,66	61,1	428	12,9
T4	180	105	15,79					433	12,6
T5	240	136	22,90					440	12,9
T6	300	161	28,34	0,235	14,37	22,97	59,5	445	12,9

Corrected for volume:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	83,2	-0,5
T2	60	156,8	-1,2
T3	120	284,8	8,9
T4	180	384,0	21,2
T5	240	494,4	32,5
T6	300	614,4	41,2



Experiment 10.04.2019 – Pilot sludge

Acetate

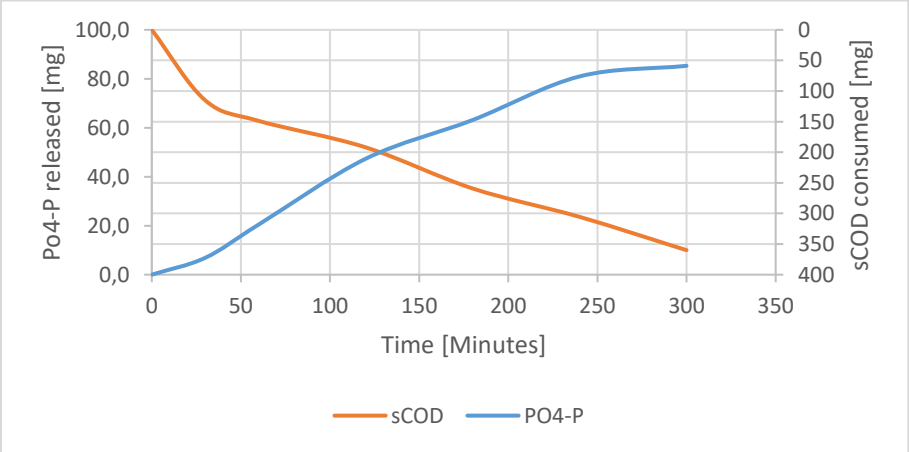
sCOD dose: 100 mg/L*h

Initial conditions sludge	
TS	8215 mg/L
VS	79,23 %
TP	281 mg/L
PO4-P	2,78 mg/L
NH4-N	6,09 mg/L
sCOD	44 mg/L
Conductivity	463 µS/cm
mg Pns/ mg TS	0,033867316

Sample	Time [min]	sCOD [mg/L]	PO4-P [mg/L]	NH4-N [mg/L]	Mg [mg/L]	K [mg/L]	Ca [mg/L]	Conductivity [µS/cm]	Temp [°C]
T0	0	135	2,04	6,09	10	27,97	58,4	463	13,2
T1	30	63	6,35					497	12,6
T2	60	42	14,84					519	12,7
T3	120	115	31,64		13,89	39,89	52	607	12,6
T4	180	173	41,52					702	12,9
T5	240	244	52,65					784	12,6
T6	300	310	55,35	6,19	19,32	46,04	51,8	676	12,9

Corrected for volume:

Sample	Time [min]	sCOD consumed [mg]	PO4-P released [mg]
T0	0	0,0	0,0
T1	30	115,2	6,9
T2	60	148,8	20,5
T3	120	192,0	47,4
T4	180	259,2	63,2
T5	240	305,6	81,0
T6	300	360,0	85,3



Appendix E – VFA Consumption Calculations

One example of the calculations behind Figure 33 and Figure 34 about VFA and sCOD consumption in P-stripping experiments.

The example will show how the consumption of acetic acid in experiment with SBR sludge was calculated.

Information about FPSS	
Acetic acid in FPSS	325,86 mg/L
Volume of one FPSS dose	0,116 L
Number of doses	5

Available acetic acid through the whole experiment is then:

$$Available\ acetic\ acid = 325{,}86\frac{mg}{L} \cdot 5 \cdot 0{,}116L = 188{,}99\ mg$$

Time [min]	**Measured acetic acid**	**Volume in reactor**	**Acetic acid [mg]**
0	24,60 mg sCOD/L	1,136 L	24,60 mg/L*1,136L = 27,9 mg
120	29,18 mg sCOD/L	1,252 L	29,18 mg/L*1,252L = 36,5 mg
300	100,12 mg sCOD/L	1,6 L	100,12 mg/L*1,6L = 160,2 mg

$$Acetic\ acid\ added\ every\ hour = 0{,}116L \cdot 325{,}86\frac{mg}{L} = 37{,}8\ mg$$

Time [min]	**Acid consumption [mg]**
0	0 mg
120	27,9 mg – 36,5 mg + 37,8 mg = 29,2 mg
300	29,2 mg + (36,5 mg – 160,2 mg)+(3*37,8 mg) = 18,9 mg

$$\%\ acetic\ acid\ consumption\ of\ available\ acetic\ acid = \frac{18{,}9\ mg}{188{,}99\ mg} \cdot 100 = 10\%$$