# Investigation of Readily Degradable Carbon Source Production by Hydrolyzing Sludge and Reject water at Ängen planned WWTP

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Water and Environmental Engineering
Department of Chemical Engineering
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by

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Picture on front page: Experimental setup on hydrolysis batch. Photo by Kartika Novitasari Pranoto

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# Preface and Acknowledgement

This master thesis project was done as a completion of my study in the Master's program Water Resources Engineering in LTH. During 2 years of my study, I have learned and gain a lot of knowledge. This thesis is not only the end of my study in LTH but also the beginning of my new journey. Working on wastewater treatment topic on my thesis has opened my knowledge on sustainable, cost effective and efficient treatment plant. As an LPDP awardee, I am expected to be able to bring the solution to Indonesian's environmental problem. I am very grateful to be able to work with this topic and in the chemical engineering department. I was enjoying my time working there, thanks for the friendly atmosphere. Therefore, I want to wish my gratitude to those people that supporting me to finish this master thesis in the middle this pandemic.

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# Summary

As form of effort to mitigate the increase of pollution level that caused by human activity, Ängen wastewater treatment plant (WWTP) will be built in Lidköping as part of the European LIFE project (LIWE LIFE). Ängen WWTP will be operated based on enhanced biological phosphorus removal (EBPR) technology. This treatment plant is projected to be cost effective and resource efficient, therefore this treatment plant will rely on the biological process to recover the energy and the resources from the treatment plant's stream. In EBPR technology, a readily degradable carbon source availability is needed to have an efficient removal process. To maintain the removal process the availability of readily degradable carbon source is need to be fulfilled. Volatile fatty acids (VFAs) are readily degradable carbon that results from the hydrolysis process on the sludge mixture. By introducing the VFAs back to the EBPR system, the necessity of the readily degradable carbon source can be fulfilled.

This study is investigating the impact of the sludge mixture on the hydrolysis yield, rate, and VFAs production. The sludge mixture used was activated sludge (AS) / primary sludge (PS) and activated sludge (AS) / reject water (RW) from the primary sludge thickener. The sludge mixture was hydrolyzed for 10 days under temperature 13°C. The experiment with AS/PS mixture resulting in higher hydrolysis rate, hydrolysis yield, and VFAs production compared to the experiment with the AS/RW mixture. The highest hydrolysis rate and hydrolysis yield were generated by 50% AS : 50% PS composition. The hydrolysis yield rate is 84 mg/g TS and 0.44 mg/g TS×$h^{-1}$ respectively. The highest hydrolysis rate and hydrolysis yield that was obtained by AS/RW are 38-40 mg/g TS and 0.21-0.23 mg/g TS×$h^{-1}$ respectively by adding 5-10% of reject water in the experiment.

The sludge mixture with more primary sludge composition is producing more VFAs and less phosphate ($PO_4^{3-}$) and ammonia ($NH_4^+$). While the sludge with more activated sludge composition generating more phosphate ($PO_4^{3-}$) and ammonia ($NH_4^+$) and fewer VFAs. The small amount of reject water addition in the experiment batch is improving the hydrolysis and generating more VFAs compared to the VFAs generated by only activated sludge. This result shows the potential and the benefits of the sludge mixture hydrolysis to achieve the cost effective and resources efficient wastewater treatment plant.

***Keyword: EBPR, activated sludge, primary sludge, reject water, hydrolysis rate, hydrolysis yield, VFAs, pH***

# Abbreviations

| | |
|---|---|
| **AS** | Activated Sludge |
| **BOD** | Biological Oxygen Demand |
| **COD** | Chemical Oxygen Demand |
| **EBPR** | Enhanced Biological Phosphorus Removal |
| **GAO** | Glycogen Accumulating Organism |
| **GC** | Gas Chromatography |
| **PAO** | Polyphosphate Accumulating Organism |
| **PHB** | Poly-hydroxy-butyrate |
| **PS** | Primary Sludge |
| **RW** | Reject Water |
| **S.COD** | Soluble Chemical Oxygen Demand |
| **SCFA** | Short Chained Fatty Acids |
| **SRT** | Solid Retention Time |
| **SS** | Suspended Solid |
| **T.COD** | Total Chemical Oxygen Demand |
| **TS** | Total Solid |
| **VFA** | Volatile Fatty Acid |
| **VS** | Volatile Solid |
| **WWTP** | Wastewater Treatment Plant |

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# 1 Introduction

Lidköping municipality is receive water from river Lidan and lake Vänern, those water body are the natural habitat for 38 species of fish. Due to the increasing number of human activities and pollutants, some numbers of fish species are on the verge of extinction (EU LIWE LIFE, 2020). The existing wastewater treatment in this municipality, currently is lack of the efficiency in the treatment process. The present capacity in Lidköping WWTP is designed for 45000 PE and 850 $m^3$/h (Amundsson, 2020). Therefore, as a part of the European LIFE project (LIWE LIFE), a new wastewater treatment equipped with advanced treatment technologies will be built. The new treatment plant will be named Ängen wastewater treatment plant.

Ängen WWTP will be built based on the EBPR technology and phosphorus recovery via struvite. This technology will enable the optimization of the process in order to achieve a cost effective and resource efficient wastewater treatment. To achieve the goals, this treatment plant should have a minimum to none additional resources. E.g. utilizing the biological process to recover nutrients such as phosphorus, ammonium and VFAs would be beneficial.

Ängen wastewater treatment projected to be able to generate over 700 MWh of energy by anaerobic digestion. This treatment plant also projected to have more efficiency by recovering resources (e.g. nutrients and carbon source) though the process. This study was performed to investigate one essential process in the resources recovering process. The main resource recovery investigation is the possible yield of readily degradable carbon source in the form of volatile fatty acids from different sludge mixture combination will be applied in Ängen WWTP. To perform this study, sludge was obtained from Lundåkra wastewater treatment in Landskrona that also uses EBPR technology.

The readily degradable carbon source is required in the phosphorus removal process, and it is common to use additional carbon sources such as VFAs to maintain the process efficiency. Generally, the production of VFAs and nutrients in the modern treatment plant utilizes the hydrolysis process to produce the VFAs and nutrients. The studies about hydrolysis process of the sludge mixture resulting in volatile fatty acid production, nutrients release in the form of phosphate and ammonium, pH changes, and hydrolysis rate were also included in this report.

## 1.1 Aim

This study aims to provide the data about the possibility to perform the hydrolysis process to recover the carbon source in the form of VFAs (e.g. acetic, propionic, butyric, and valeric acids). It was also purposed to find the hydrolysis yield and hydrolysis rate potential that can be applied at Ängen WWTP. These findings were done to investigate:

1. Which sludge composition can produce the most VFAs?
2. Is the addition of reject water from primary sludge thickener beneficial in the hydrolysis process?
3. What is the retention time (SRT) to produce the maximum amount of VFAs?

# 2 Wastewater treatment

## 2.1 Wastewater Treatment in General

Wastewater treatment in Sweden has been developed since the late 1800s to manage the pollution problems that potentially degrading the Swedish water body. Wastewater treatment is started by mechanical separation then continued by secondary treatment and finalized by tertiary treatment. Mechanical separation is done using screens, grit chambers, and sedimentation. The mechanical separation utilizes the size and weight of the pollutant particle. Large pollutant debris such as leaves and branches will be trapped on screen, while small and heavy particle such as sand and gravel will be collected in the grit chamber. Rest of the pollutant that pass-through screen and grit chamber will be separated in the primary sedimentation chamber (Naturvårdsverket, 2016).

Secondary treatment or biological treatment is a treatment process to remove chemical pollutants like nitrogen, phosphorus, and organic matter. Biological treatment is consisting of several chambers with different environmental conditions: anaerobic, anoxic, and aerobic. This condition will be affecting the microorganism and stimulate pollutant degradation. The conventional biological treatment example is activated sludge and trickling filter; however, the development of a biological treatment is very vast nowadays by modifying the carrier for the microorganism or by changing the environment condition.

The tertiary treatment in wastewater treatment usually finalizes by chemical treatment. The chemical treatment process is a precipitation process using metal salts such as aluminum and iron to precipitate phosphorus. The addition of the metal salts will coagulate and settle the polluter down, the settled sludge will be treated as chemical sludge in sludge treatment. Nowadays tertiary treatment not only able to remove phosphorus but also pollutant that cannot be treated in biological treatment such as, micro plastic and pharmacy residue.

Ängen wastewater treatment will be using EBPR for its secondary treatment. Therefore, this report mostly discussing about EBPR and the parameter affecting the phosphorus removal process.

## 2.2 Enhanced Biological Phosphorus Removal (EBPR)

Phosphorus removal in the wastewater treatment process becomes gradually important to reduce the potential of eutrophication at the receiving water body. EBPR removal relies on Polyphosphate Accumulating Organisms (PAOs) and this process works under anaerobic and aerobic environment consecutively (Sathasivan, 2009). The microorganism metabolism will run differently under the aerobic condition and anaerobic conditions as described in Figure 2.1. Under anaerobic conditions, the organic matter in the form of BOD-COD or VFAs are consumed and stored as PHB (Poly-hydroxy-butyrate). In absence of oxygen the microorganism utilizes the poly-P and glycogen as an energy source while phosphorus is released ($PO_4$-P). Meanwhile, in the aerobic environment, the oxygen will be the energy source to take up the phosphate that released and the PHB that was stored before then forming Poly-P, glycogen, and regenerate new cell (US EPA, 2013).

The EBPR process relies on enhancing microorganism's ability to consume more phosphorus into their cells. In the EBPR process, the availability of the carbon source is very important to maintain PAOs metabolism and new cells material. During the carbon shortage situation, PAOs metabolism will be deteriorated. In order to prevent the metabolism imbalance additional carbon source such as VFAs is often needed (US EPA, 2013).

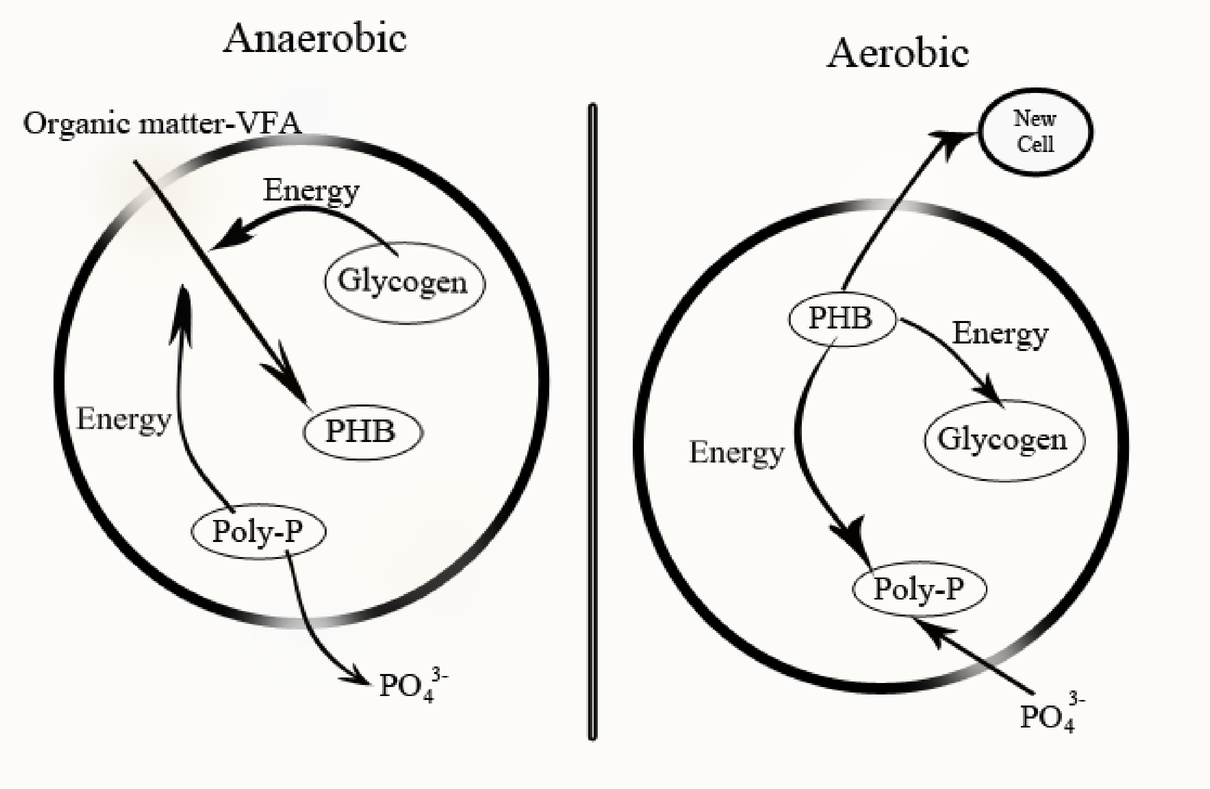

*Figure 2.1. EBPR microorganism metabolic process. Left side: the anaerobic phase where the organic matter is consumed, and phosphate released. Right side: Aerobic phase where phosphate is consumed and stored as poly-P, the regeneration of new cells also occurs in the aerobic phase. ('Mino model')*

EBPR is very effective in phosphorus removal. A study stated that EBPR efficiency can reach 97% of phosphorus removal (Ge, Zhao, Zhang and Chen, 2013). Although the EBPR technology has very high efficiency, this process is very sensitive to composition changes in the wastewater and the environment temperature (Mulkerrins, Dobson and Colleran, 2004). Heavy rainfall can dilute the organic matter concentration in the wastewater and can deteriorate the metabolism of PAOs due to insufficient carbon sources. High sugar concentration in the influent will stimulate the Glycogen Accumulating Organisms (GAOs) (Satoh, Mino and Matsuo, 1994). GAOs presence will suppress PAOs and resulting in lower phosphorus removal rates.

## 2.3 Volatile Fatty Acids

The Influent of the wastewater highly fluctuates while the EBPR process is very sensitive towards changes. One of the parameters that influencing the EBPR is the organic matter or carbon source. Due to the fluctuation in organic matter, sometimes external carbon source needs to be added to maintain the removal process stability. The ratio of carbons source and phosphorus should be around 10-20 mg COD/mg P to achieve a satisfactory phosphorus removal (Oehmen et al., 2007). In the process of external carbon sources selection, several aspects should be considered such as the rate fluctuations, origins of the external carbon source, and supply availability (Hatziconstantinou, Yannakopoulos, and Andreadakis, 1996).

Volatile Fatty Acids (VFAs) are readily degradable carbon sources that can be obtained in the resource recovery process. This process is a way to regain water, carbon source, and energy to be used in the wastewater treatment process. Hydrolysis and fermentation methods are frequently used in the source recovery process. Anaerobic fermentation is a metabolic process to convert organic matter into methane. The fermentation begins with hydrolysis where the organic matter is degraded into VFAs. Then the process continues under high temperature (37°C) to produce methane that can be used as energy source (Li et al., 2018). VFAs production can be used to substitute the needs of external carbon source for the phosphorus removal (Gidstedt, 2017). VFAs is defined as Short Chain Fatty Acids (SCFAs) for carbon chain amount between C2 – C6 or it can be defined by the existence of acetic acid (C2), propionate acid (C3), Butyrate acid (C4), valeric acid or pentanoic acid (C5), and caproic acid or hexanoic acid (C6). VFAs production and composition depend on hydrolysis rate and acidification, while this rate is a function factor of pH, temperature, substrate loading, hydraulic retention time (HRT) and solids residence time (SRT) (Veeken and Hamelers, 1999).

Many studies were performed to measure the impact of alkalinity on VFAs production. Based on the experiment that was done by Wu, et.al (2010), Alkaline condition was beneficial for VFAs production during the primary sludge hydrolysis process. In that experiment, pH value was set at 10-11 and the retention time was set at 5 days Another study that was done by Yuan et.al (2006) also showed a similar trend. This report concludes that high VFAs production was generated by higher soluble COD production, this situation was caused by inhibited methanogens under the alkaline environment.

## 2.4 Ion Composition

Ion composition in the wastewater is very essential for maintaining the effluent quality. Low ammonium ($NH_4^+$), phosphate ($PO_4^{3-}$), nitrate, and nitrite concentration are significant to fulfil the standard of effluent quality and minimizing the environmental impact like eutrophication. In Sweden, the effluent demands vary in every wastewater treatment plant. Lundåkra wastewater treatment is having the effluent standard 10 mg/l yearly concentration for total nitrogen and 0.4-0.5 mg/l yearly concentration for total phosphorus (Lundåkraverket, 2018). EU effluent standard is having the yearly total nitrogen concentration less (than 25 mg/l while yearly phosphorus concentration should be less than 3 mg/l. (EU, 2016). The new wastewater treatment in Lidköping is expected to produce better effluent quality, 6 mg/l yearly concentration for nitrogen and 0.2 mg/l yearly concentration for phosphorus (Dahlberg and Kamp, 2018).

Ion concentration in the wastewater depends on the ion concentration in the influent. During the treatment process, the ion concentration is changing due to the biochemical reaction, temperature, and pH influence. Nitrogen in the wastewater treatment can be found in the form of ammonia ($NH_3$), ammonium ($NH_4^+$), and organic bond nitrogen. The ammonia and ammonium balance are depending on the pH. When the pH is acidic to neutral, the ammonium-ammonia balance is shifted to ammonium ($NH_4^+$). When pH is on range 9.4 or higher, the balance is shifted to ammonia ($NH_3$) (Ji et al., 2014).

The biochemical reaction highly depends on on oxygen existence. Under the anaerobic environment, phosphate is released. On the contrary in aerobic environment phosphate is taken and the nitrifiers bacteria are also convert the ammonium and release nitrate and nitrite. Under the anoxic environment, the denitrifying process occurs to convert the nitrate and nitrite into nitrogen gas.

Cations concentration is useful to sustain the removal process and stability in the EBPR process. The concentration and composition of $Ca^{2+}$, $Mg^{2+}$, and $K^{+}$ are very influential at phosphorus uptake and for the binding mechanism by activated sludge (Pattarkine and Randall, 1999). The co-transport of $Mg^{2+}$ and $K^{+}$ are prominent for the poly-P intracellular stability. Other studies by Shönborn et al. in 2001 reported that artificial enhancement of magnesium can increase the Phosphorus removal efficiency. In the EBPR process, the ion calcium does not have any impact on the microbial population however, when $Ca^{2+}$ concentration is elevated, the extracellular precipitation of calcium phosphate also increased. These changes also affect the ratio of the phosphorus released, acetate uptake, glycogen degradation, and poly-β-hydroxyalkanoates synthesized during the anaerobic phase and lead to shifting the metabolism from phosphate accumulating metabolism to glycogen accumulating metabolism (Zhang et al., 2015).

## 2.5 Temperature

Temperature is one of the most important parameters during the operating process. The temperature is affected by seasonal changes and resulting in the changes in microorganism's metabolism. Temperature is also affecting the reaction rate changes. The hydrolysis rate is constantly increasing in consequence to the temperature increase (Veeken and Hamelers, 1999). The temperature is also affecting the competition of PAOs and Glycogen Accumulating Organisms (GAOs) in low temperature 10°C until moderate temperature 20°C PAOs is dominating. However, VFA production in 20°C is not favorable unless high pH (7.5) is applied to the experiment. At higher temperatures, 30°C PAOs were inhibited causing the EBPR system to deteriorate (Lopez-Vazquez et.al., 2009). VFA production in the lower temperature also is very slow and this condition can be compensated by extending the retention time of hydrolysis.

## 2.6 Hydrolysis yield

The anaerobic hydrolysis rate is derived from the biochemical degradation process that resulting in ammonium, phosphate, soluble COD and VFAs release. In this report, the hydrolysis yield calculation is based on the empirical method of total soluble COD. The first method is based on ammonium release during protein degradation. two moles of ammonium are produced by the degradation of one mol protein, and the generated formula is given in equation 1:

$$\Delta COD_N = 11.4 \times \Delta NH_4\text{-}N \qquad (1)$$

Where;

$\Delta COD_N$ : Soluble COD generated by ammonium release (mg/g TS)

$\Delta NH_4$-N : Ammonium concentration generated throughout the hydrolysis process (mg/g TS)

The second method is based on the phosphate release from the PAOs metabolism under anaerobic environment. The ratio presented in equation (2) is derived from the fermentation of biomass converted into acetate. In the second method, it is assumed that all soluble COD consist of VFAs (Jensen et al., 2017). The derivation formula is given in equation 2:

$$\Delta COD_P = 2.5 \times \Delta PO_4\text{-}P + \Delta sCOD \qquad (2)$$

Where:

$\Delta COD_P$ : Soluble COD generated by phosphate release (mg/g TS)

$\Delta PO_4$-P : Phosphate concentration generated throughout the hydrolysis process (mg/g TS)

ΔsCOD : Soluble COD generated by measurement throughout the hydrolysis (mg/g TS)

Table 2.1 and Table 2.2 shows some previous studies about hydrolysis yield and hydrolysis rate. The listed study shows clear hydrolysis experiment in various sludge mixture, different environmental condition, and different unit measurement. The different unit measurement presented in Table 2.1 and Table 2.2 can be adjusted by using the same assumption that used to develop the second method, where all soluble COD contains VFA. The hydrolysis yield value listed below is the maximum yield value, while the hydrolysis rate value is the initial hydrolysis rate. Initial hydrolysis rate is the highest rate that happening in the early time step of the hydrolysis and slows down after 48 hours (Jensen et al., 2017).

*Table 2.1. Previous studies on hydrolysis yield in different sludge composition and environment condition.*

| **Author** | **Sludge composition** | **Environmental conditions** | **Hydrolysis yield** |
|---|---|---|---|
| (Gidstedt, 2017) | 100% PS<br>75%PS:25% RW | T: 14.5°C<br>Anaerobic | 78 mg $COD_{VFA}$/g VS<br>110 mg $COD_{VFA}$/g VS |
| (Jönsson and Jansen, 2006) | AS (EBPR) | T: 20°C<br>Anaerobic | 18 mg soluble COD/g VS |

*Table 2.2. Previous studies on the hydrolysis rate in different sludge composition and environment condition.*

| **Author** | **Sludge composition** | **Environmental conditions** | **Hydrolysis rate** |
|---|---|---|---|
| (Henze and Mladenovski, 1991) | Raw wastewater: AS | T: 20°C<br>Anaerobic<br>Anoxic<br>Anaerobic | 0.115 g COD/(g TKN.d)<br>0.034 g COD/(g TKN.d)<br>0.060 g COD/(g TKN.d) |
| (Jensen et al., 2017) | AS | T: 15°C<br>Anaerobic:<br>$NH_4^+$ method<br>$PO_4^{3-}$ method | 0.13 ΔCOD, mg-$O_2$/(gVSS×h)<br>0.23 ΔCOD, mg-$O_2$/(gVSS×h) |
| (Gidstedt, 2017) | 100% PS<br>75%PS:25% RW | T: 14.5°C<br>Anaerobic | 1.4 mg $COD_{VFA}$/(g VS×h)<br>2.1 mg $COD_{VFA}$/(g VS×h) |
| (Jönsson and Jansen, 2006) | AS (EBPR) | T: 20°C | 0.34 (mg sCOD/(g VS×h)<br>0.04 (mg NH4-N/(g VS×h) |

# 3 Experiment Setup

## 3.1 Lundåkra WWTP and Ängen WWTP Comparison

Ängen wastewater treatment will be located in Lidköping. This new wastewater treatment is planned to be based on EBPR removal and phosphorus recovery via struvite. The carbon source for EBPR will be obtained from the hydrolysis sludge process. Since there is no sludge production in Ängen wastewater treatment yet, the sludge that was used in this laboratory experiment was taken from the Lundåkra WWTP located in Landskrona. This consideration was based on the Lundåkra WWTP is also using EBPR removal. However, there are some differences between Lundåkra and Ängen WWTP system that requires to be noticed for further discussion.

In Lundåkra WWTP, the primary sludge is hydrolyzed in the primary clarifier tank. Then the sludge that returns to the EBPR tank is coming from the secondary clarifier. Lundåkra wastewater treatment plant is also using only chemical precipitation for its tertiary treatment. As for the Ängen wastewater treatment plant, there is no hydrolysis process for primary sludge. The primary sludge will be treated in the anaerobic digester to produce energy. Ängen WWTP has been projected to be able to generate over 700 MWh by the anaerobic digester process (EU LIWE LIFE, 2020). Thus, the hydrolysis process is planned to use activated sludge as the major component, while a small amount of primary sludge and reject water from the primary sludge thickener are used to improve the VFA production.

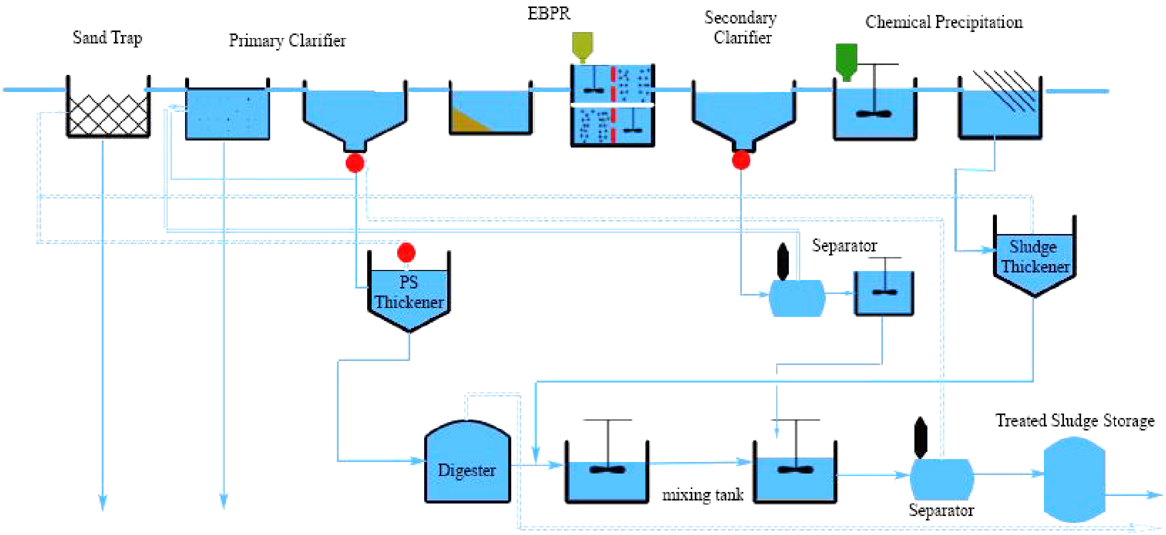

*Figure 3.1. Lundåkra WWTP scheme; primary sludge hydrolysis takes in the primary clarifier tank. The sampling points (red dots) are the primary sludge thickener, primary clarifier, and EBPR tank.*

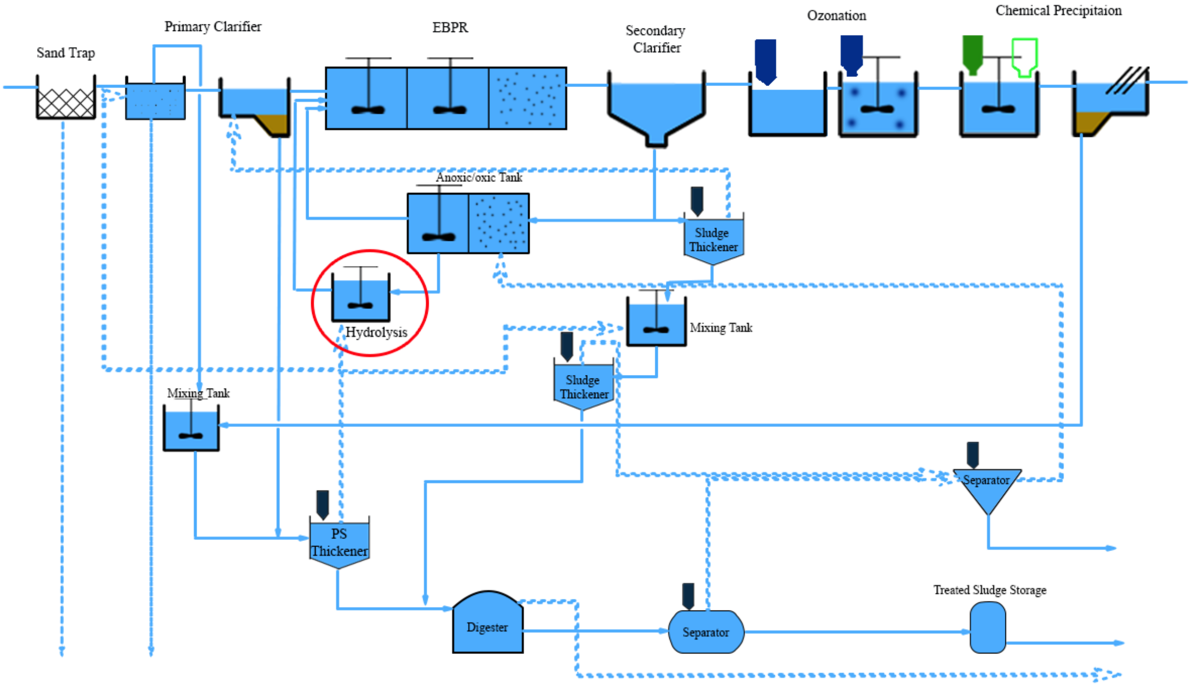

*Figure 3.2. Lidköping wastewater treatment scheme.*

## 3.2 Methodology

The hydrolysis process was applied to the sludge mixture consisting of activated sludge, primary sludge, and reject water from the primary sludge thickener with different compositions. The reject water has the least amount of organic matter compared to activated and primary sludge. However, the impact of reject water addition in the sludge composition was observed and expected to get an insight about the best composition mixture.

## 3.3 Experimental Setup

Two sets of experiments with various sludge compositions were done to produce the reliable result of nutrients and VFAs production. Figure 3.3 shows the experimental setup. The hydrolysis process was done for 10 days and run on Kemira flocculator that consists of 6 jar tests with 1 liter volume capacity for each jar. The flocculator run under constant stirring 50 rpm. To maintain the temperature stability, the jars were placed in the water bath that set on 13-15$^{o}$C. Nitrogen gas was used along with the hydrolysis. The nitrogen gas connected using hoses that secured on the flocculator lid. On the end of each hose, a diffuser head was attached to prevent vigorous bubbling. The diffuser head was dipped to the surface layer of the sludge mixture. The purpose of this set up is to minimize the oxygen presence in the jars and prevent spilling due to gas bubbling. The sludge mixture composition listed in Table 3.1, the composition is based on the % volume of the sludge.

*Figure 3.3. Experimental Setup.*

*Table 3.1. Sludge composition for each experimental set based on volume; AS: Activated Sludge, PS: Primary Sludge, RW: Reject water.*

| **Jar** | **Experiment 1** | | **Experiment 2** | |
|---|---|---|---|---|
| | **Sludge** | **Composition** | **Sludge** | **Composition** |
| **1** | AS | 100 | AS | 100 |
| **2** | AS : PS | 90 : 10 | AS : RW | 95 : 5 |
| **3** | AS : PS | 75 : 25 | AS : RW | 90 : 10 |
| **4** | AS : PS | 50 : 50 | AS : RW | 80 : 20 |
| **5** | AS : PS | 25 : 75 | AS : RW | 70 : 30 |
| **6** | PS | 100 | AS : RW | 50 : 50 |

## 3.4 Analyses

The hydrolysis run for 10 days and the result of the hydrolysis process are the nutrients that consist of phosphate and ammonium, and VFAs which is composed of acetic acid, propionic acid, butyric acid, valeric acid. The samples were categorized into three categories: centrifuged and filtered sample, centrifuged sample, and unseparated unfiltered sample. The centrifuged and filtered sample was obtained from 15 ml sample from the jar then the sample placed inside 50 ml falcon tube to be centrifuged in centrifuge Sigma 3-16K with velocity set at 4500 rpm, temperature 15$^o$C for 7 minutes. The sample then filtered using syringe filter Sartorius Rc 0.45 $\mu m$ or vacuum filter using Wattman filter paper 0.45 $\mu m$

The centrifuged sample was obtained from 1.3 ml sample, then placed in the Eppendorf tube and centrifuged in Hermle Z 216 that set at 13 000 rpm for 10 minutes. While the unseparated unfiltered sample was obtained directly from the jar without any separation method. The sampling processes were done only on the working days starting on Monday and the sample marked as time 0” to time 8”. In the first day, there were 2 samplings that marked as time 0” and time 1”. Time 0” was the condition before hydrolysis process started, time 1” was the condition several hours after the hydrolysis started, Then the rest sampling was done once a day until day 10 or Time 8”.

*Table 3.2. Analysis schedule list*

| **Time / Day** | **Mon** | | **Tue** | **Wed** | **Thurs** | **Fri** | **Mon** | **Tue** | **Wed** |
|---|---|---|---|---|---|---|---|---|---|
| **Test** | **0”** | **1”** | **2”** | **3”** | **4”** | **5”** | **6”** | **7”** | **8”** |
| **TS** | X | | | | | | | | X |
| **VS** | X | | | | | | | | X |
| **Total COD** | X | | | | | | | | X |
| **Soluble COD** | X | X | X | X | X | X | X | X | X |
| **Temperature** | X | X | X | X | X | X | X | X | X |
| **Dissolved $O_2$** | X | X | X | X | X | X | X | X | X |
| **pH** | X | X | X | X | X | X | X | X | X |
| **Ion composition** | X | X | X | X | X | X | X | X | X |
| **VFAs Analysis** | X | X | X | X | X | X | X | X | X |

### 3.4.1 Analysis list

Table 3.2 shows the schedule of the analysis during the 10 days of hydrolysis. The analysis explanation that used to monitor the hydrolysis process and VFAs production are given in the section below:

- TS & VS

The total solids and volatile solids were done by taking the unseparated unfiltered sample and place the sample into a VWR disposable aluminium weigh dish 75 ml, 65 mm inner diameter, and the weighed using an analytical scale Sauter RE 1614. To calculate the total solid, the sludge needs to be dried overnight inside the oven with temperature 105°C. In order to calculate the volatile solids, the sample the dried sample was ignited inside the furnace with temperature 550°C for an hour. The total solids and volatile solids then calculated using the equation (3) to (5).

$$\%TS = \frac{dry\ sludge\ weight - container\ weight}{sludge\ weight - container\ weight} \tag{3}$$

$$\%VS = \frac{dry\ sludge\ weight - ignited\ sludge\ weight}{sludge\ weight - container\ weight} \tag{4}$$

$$\%VS\ (as\ \%TS) = \frac{dry\ sludge\ weight - ignited\ sludge\ weight}{dry\ sludge\ weight - container\ weight} \tag{5}$$

- Total COD

0,5 ml unseparated unfiltered samples were analyzed using Hach Lange COD cuvette LCK-014. Then the cuvettes were heated using heating block, Hach lange heater LT-200 for two hours at 148°C. After being heated the cuvette rested until it cools down to the room temperature and finally scanned using Hach lange Reader DR 2800.

- Soluble COD

In the same way as the total COD method, the 2 ml of centrifuged and filtered samples used the Hach Lange cuvettes. The used cuvette for the soluble COD level is varied according to the projected COD level value as listed in Table 3.3. The heating and reading process were done using the same methods as total COD analysis.

*Table 3.3. Hach Lange cuvettes and its concentration sensitivity range*

| Hach Lange COD Cuvette | Concentration sensitivity range (mg/l $O_2$) |
|---|---|
| LCK-014 | 1000-10000 |
| LCK-314 | 15-150 |
| LCK-614 | 50-300 |
| LCK-114 | 150-1000 |

- Temperature and pH

The pH and temperature measurement were measured directly in the jar using the pH meter WTW pH 3116 set 2. This pH meter probe is equipped with thermometer probe that able to measure both pH and temperature at the same time.

- Oxygen level

This measurement was done using Hach HQD Field Case. Due to the limitation on the setup, this measurement could not be performed in the jar directly, so the measurement was immediately done after the sample being taken from the jar at the 50 ml falcon tube to minimize the oxygen contact.

- Ion composition ($PO_4^{3-}$, $NH_4^+$, $NO_3^-$, and $NO_2^-$)

Ion composition was analyzed using ion chromatography system by Metro Ohm ECO IC for cation and anion. The centrifuged and filtered samples were diluted based on the criterion of the lower and upper region limit listed in Table 3.4. the sample required for this analysis was 4 ml.

*Table 3.4. Range value that can be accurately analyzed on Ion Chromatography*

| Substance | Lower limit | Upper region | Unit |
|---|---|---|---|
| **$NO^{2-}$** | 0,02 | 3 | mg N/L |
| **$NO^{3-}$** | 0,05 | 15 | mg N/L |
| **$PO_4^{3-}$** | 0,1 | 15 | mg P/L |
| **$NH_4^+$** | 0,1 | 15 | mg N/L |

- VFAs composition (acetic acid, propionic acid, butyric acid, valeric acid)

VFA composition analysis was done using GC Agilent system. 900 $\mu l$ centrifuged sample mixed with 100 $\mu l$ phosphoric acids in the GC vials then analyzed using GC Agilent system. The phosphoric acid acts as preservatives for the sample. To determine the concentration value of the VFA, standardized curves for those VFAs were specified and the samples were calculated based on standardized curves. The standardized curve was made based on standardized solutions from various concentrations starting at 25, 50, 100, 200, 500, and 1000 mg/l COD with control concentrations 20 and 750 mg/l COD. The resulting area then plotted based on the linear regression method, the trendline intercept was set at 0, to fit the line equation in the form of $y = kx$.

### 3.4.2 Experimental problem

The nitrogen gas was supplied by a nitrogen gas tube that attached to the experimental setup. In Experiment 1 the diffuser head attachments were not installed. Due to gas tube regulator instability and tube leakage, the gas supply inside the jar becoming unstable and caused a vigorous bubbling resulting in sludge spill. Moreover, this problem can run out the nitrogen quickly and could not sustain the whole experiment. In Experiment 2, the spillage problem was managed by diffuser attachment. However, the gas regulator instability and tube leakage problem were still occurred.

# 4 Result and Discussion

## 4.1 Experiment 1

Experiment 1 was run on 10-19th of February and this experiment focused on the combination of primary and activated sludge. Unlike in the Ängen planned treatment plant, Lundåkra applies hydrolysis process to the primary sludge. The primary sludge character normally has thicker consistency than activated sludge. The experiment originally started with nitrogen gas without the diffuser head attached to the system, and gas was run out after 3 hours after the experiment started. Due to the absence of diffuser and instability of gas tube regulator and leakage, the spillage problem was happened due to vigorous bubbling. Figure 4.1 shows that the spillage problem happens the most at jar 5. To minimize the oxygen contact, the hole on the lid was sealed using tape, the average $O_2$ concentration measured was 0.174 mg/L, and the average temperature was 13°C.

Based on the visual appearance that showed in Figure 4.1, the color of the sludge after 10 days hydrolysis was darker than before hydrolysis especially in the jars with more primary sludge (jar 5 and jar 6). Table 4.1 shows that the total solids (TS) and volatile solids (VS) after hydrolysis was lower than the TS and VS before hydrolysis. This is because some solids being degraded into smaller particle and forming SCFAs. Total organic matter can be measured by COD and suspended solids. During the hydrolysis, the total organic matter should remain constant. In the first experiment, the total COD were decreasing after hydrolyzed in 10 days. The Cuvettes that used in total COD measurement (LCK – 014) have a wide range of concentration sensitivity rate (1000-10000 mg/l). This wide sensitivity range can cause the measurement error as shown by total COD decrease in Table 4.1. The value of the volatile solid portions (VS as %TS) that correlates to the COD remains constant. This value reassures that the total organic matters are unchanged despite the spillage problem.

*Table 4.1. Total Solids, Volatile solids and total COD before and after hydrolysis; Experiment 1 with the combination of primary sludge and activated sludge.*

| | **Before** | | | | **After** | | | |
|---|---|---|---|---|---|---|---|---|
| **Sludge Composition (AS:PS)** | **TS (%)** | **VS (%)** | **VS as %TS (%)** | **T.COD (mg/l)** | **TS (%)** | **VS (%)** | **VS as %TS (%)** | **T.COD (mg/l)** |
| 100% AS | 0.68 | 0.50 | 74 | 8400 | 0.62 | 0.48 | 78 | 7900 |
| 90% : 10% | 0.66 | 0.51 | 77 | 9600 | 0.60 | 0.45 | 76 | 7700 |
| 75% : 25% | 0.71 | 0.56 | 78 | 10400 | 0.63 | 0.47 | 75 | 8000 |
| 50% : 50% | 0.81 | 0.63 | 77 | 16200 | 0.68 | 0.52 | 77 | 9000 |
| 25% : 75% | 0.83 | 0.64 | 77 | 17900 | 0.77 | 0.57 | 74 | 8000 |
| 100% PS | 1.01 | 0.76 | 75 | 16700 | 0.77 | 0.59 | 77 | 13500 |

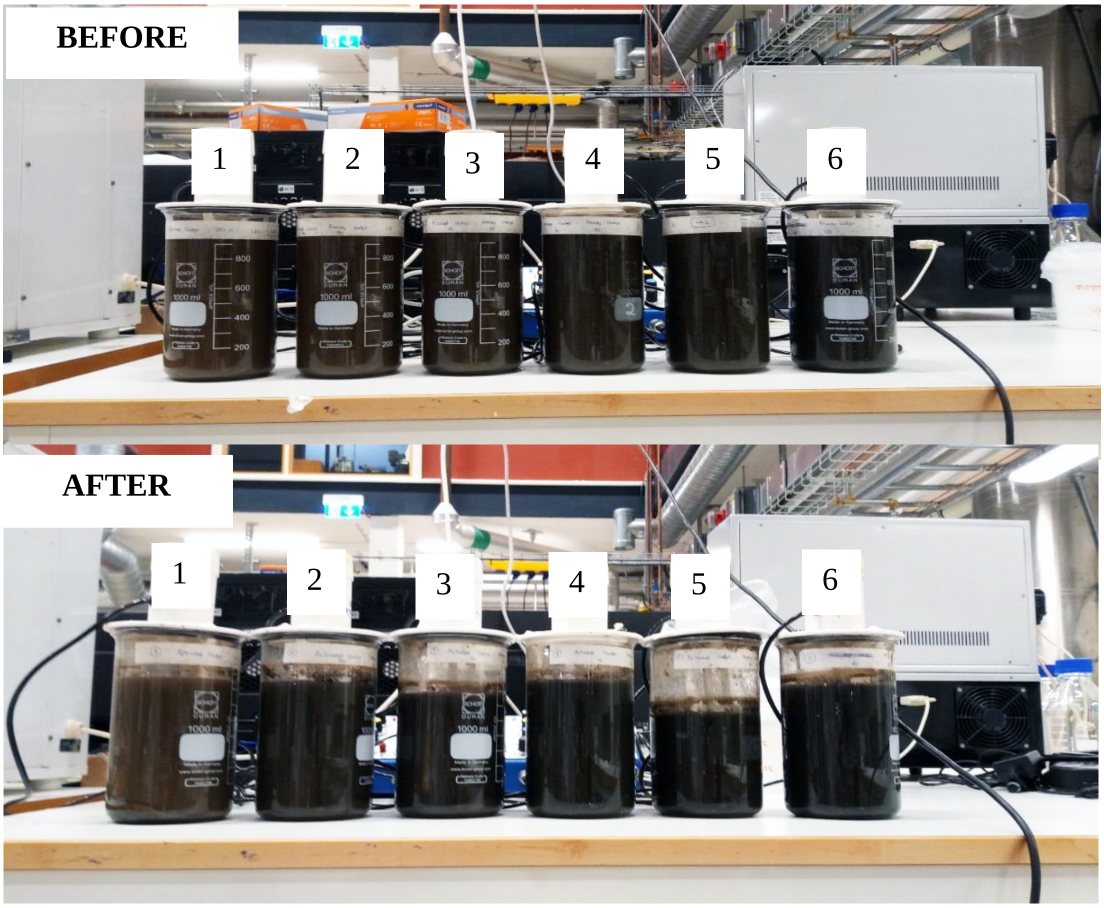

*Figure 4.1. The before and after hydrolysis and the spilling problem that happened in the jars.*

The hydrolysis process happens at the fast rate in the first 48 hours, then the rate slows down along with longer hydrolysis time (Jensen et al., 2017). Hydrolysis rate was tracked by the changes of soluble COD (sCOD) concentration, Ion concentration that exists in the jar, and VFA that formed after hydrolysis. During the hydrolysis process, the sCOD is increasing in consequence with the time. Figure 4.2 shows that the sCOD increased along the time, however different compositions of sludge affects the sCOD release. The jars with high primary sludge content have more sCOD. Primary sludge is rich in organic matter that being degrade during the hydrolysis process. Degradation of organic matter resulting in sCOD and eventually increase the sCOD concentration.

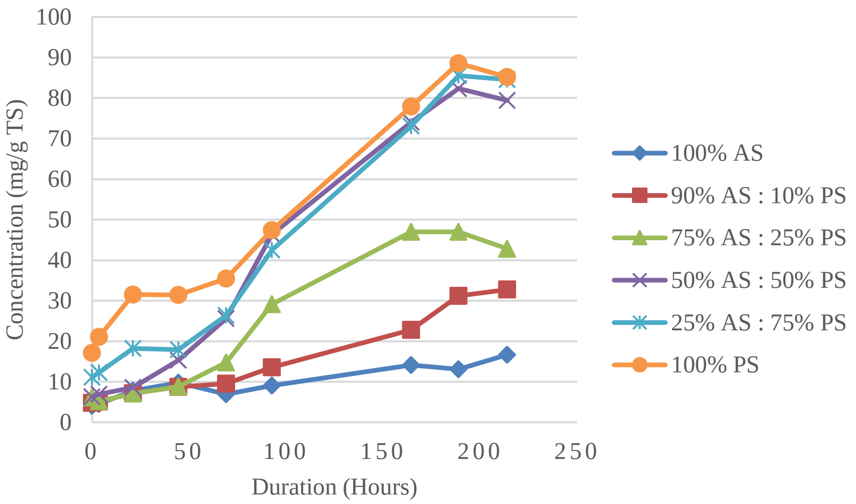

*Figure 4.2. Soluble COD concentration during hydrolysis in Experiment 1.*

Under anaerobic condition, ammonium and phosphate are released resulting in concentration increase during hydrolysis. Figure 4.3 shows that the ammonium concentration has a modest increase in the jars with higher content of activated sludge during the experiment. After 92 hours, the jars with higher primary sludge (PS) content have decreased trend of ammonium concentration. The hydrolysis process was operated under low oxygen concentration, the average dissolved oxygen measured is 0.174 mg/l. Low oxygen concentration and low temperature conditions are limiting the metabolism of nitrifiers bacteria that resulted in low nitrate and nitrite concentration measured during the hydrolysis process. The nitrate and nitrite measured in this experiment were very low and the data are presented in the appendix Table. A.6 and Table A.7.

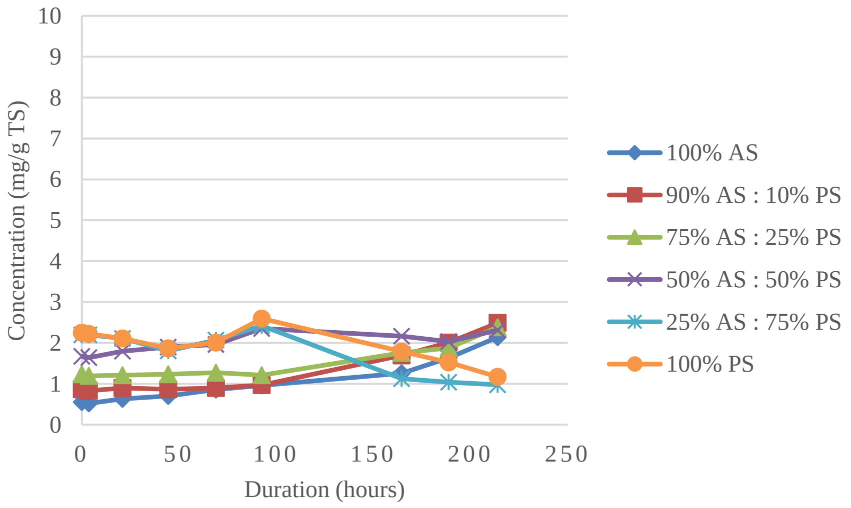

*Figure 4.3. Ammonium concentration during hydrolysis in Experiment 1.*

The measured phosphate concentration shows the increasing trend in Experiment 1. The low oxygen presence enables the anaerobic metabolic process that releases the phosphate. Figure 4.4 shows that phosphate concentration increases rapidly during the first 5 days. Jars with more activated sludge, releasing the higher phosphate. In the other hand sludge with more primary sludge content had a modest increase. In the EBPR process, activated sludge is rich in PAOs. During the anaerobic condition, PAOs releases phosphate. Due to hydrolysis was done for 10 days, the microorganism degradation also happens in this process. This degradation resulting in phosphate and ammonium release. The detailed comparison of phosphorus and ammonium concentration released showed in Figure 4.5

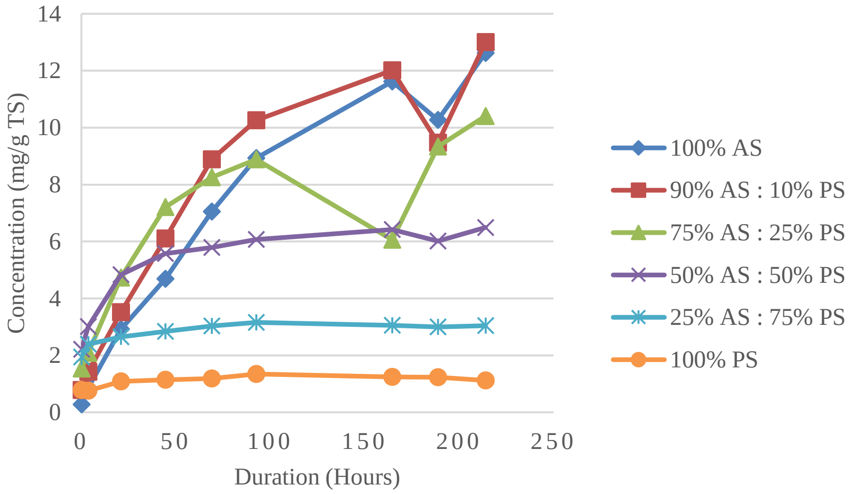

*Figure 4.4. Phosphate concentration during hydrolysis in Experiment 1.*

Figure 4.5 shows a clear comparison between the phosphate concentration and ammonium concentration in different jars with various sludge concentration. This figure also shows that more primary sludge content releasing less ammonia and phosphate. The minimum amount of phosphate and ammonium release can be seen at the jar test with compositions 25%AS:75%PS and 100% PS. Both Figure 4.5 and Figure 4.3 shows the ammonium concentration in the jar with 25%AS:75%PS and 100% PS was decreasing. During the hydrolysis, organic matter in the primary sludge being degraded into sCOD. P and N in primary sludge, usually in the form of organic bond nitrogen or phosphorus. Therefore, the hydrolysis primary sludge producing a high concentration of sCOD and low concentration nutrients. The decreasing value is caused by low ammonium concentration and measurement error. In the ion composition measurement, the sample needs to be adjusted by dilution process to fits the sensitivity range that stated in Table 3.4. Every dilution step will add the degree of uncertainty to the measurement.

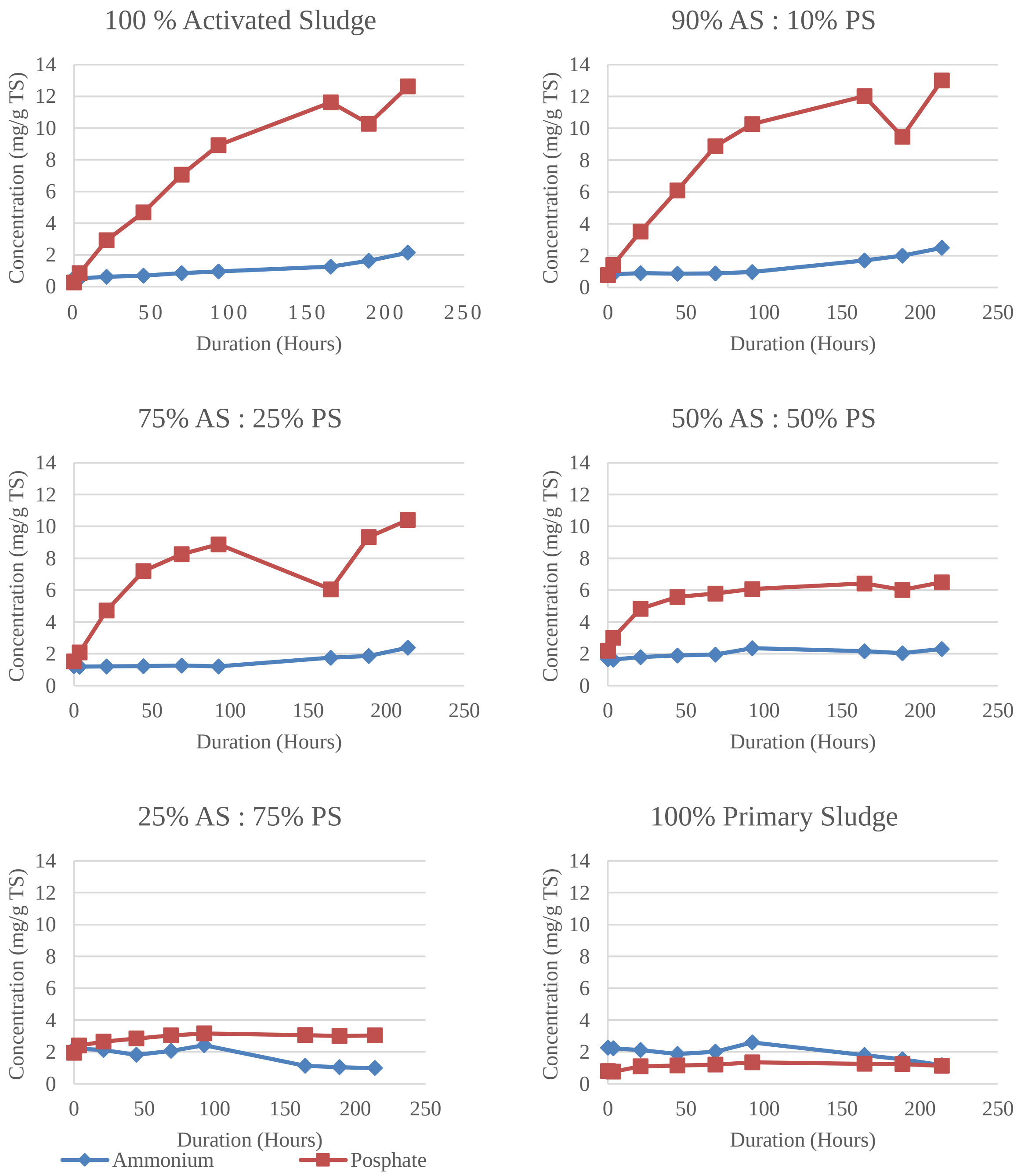

*Figure 4.5. Comparison of phosphate and ammonium concentration in different sludge composition*

In Experiment 1, hydrolysis yield calculation was done using the method based on phosphate release due to the anomaly in ammonium concentration data. The calculated data then compared in Figure 4.6 side by side with produced sCOD during the hydrolysis. The highest rate hydrolysis yield obtained in composition 50% activated sludge and 50% primary sludge. Figure 4.6 also shows that the COD yield that based on phosphate has a higher value compared to the actual measured COD yield.

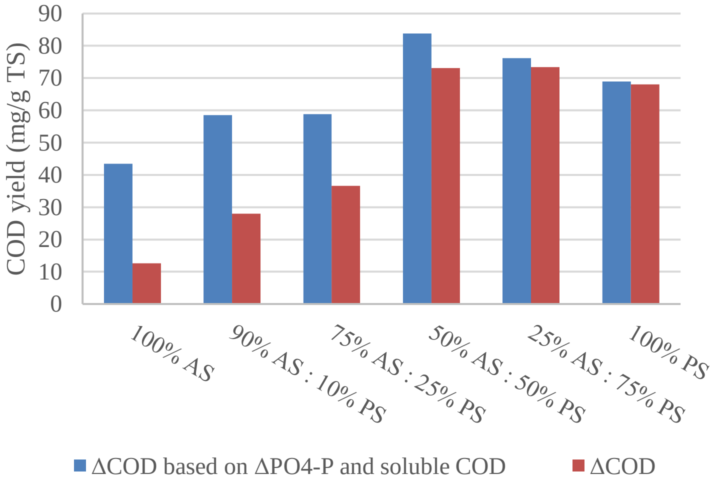

*Figure 4.6. Hydrolysis yield on various sludge composition based on phosphate release and soluble COD measured.*

The hydrolysis yield value then used to calculate the hydrolysis rate by plotting it on time vs COD yield and analyze it using the linear regression method. The hydrolysis rate data and comparison are presented in Table 4.2. As shown in Table 4.2 the hydrolysis rate shows that the hydrolysis rate by phosphate and by sCOD were having different rates for the more activated sludge. While the hydrolysis rate high primary sludge concentration is having similar value both from phosphate method and sCOD generates. This similar value is caused by low phosphate and ammonium release in the experiment with high primary sludge concentration.

*Table 4.2. Hydrolysis rate to various compositions based on phosphate-COD yield and soluble COD.*

| **Sludge Composition (%Volume) AS:PS** | **Hydrolysis rate by phosphate release (mg sCOD/g TS × h$^{-1}$)** | **Hydrolysis rate by soluble COD (mg sCOD/g TS×h$^{-1}$)** |
|---|---|---|
| 100% AS | 0.22 | 0.08 |
| 90% : 10% | 0.28 | 0.15 |
| 75% : 25% | 0.32 | 0.24 |
| 50% : 50% | 0.44 | 0.42 |
| 25% : 75% | 0.37 | 0.43 |
| 100% PS | 0.35 | 0.46 |

Compared to the previous study about hydrolysis yield value listed in Table 2.1, the yield obtained by the measurement of sCOD in 100% AS is similar compared to the value obtained on the previous study. The value obtained in this experiment is 13 mg sCOD/g TS or 17 mg sCOD/g VS. The previous study stated 18 mg sCOD/g VS under temperature 20$^o$C (Jönsson and Jansen, 2006). This similar value shows that the temperature difference under 20$^o$C is not affecting the hydrolysis yield due to the domination of PAOs. PAOs existence is dominant in low to moderate temperature between 10-20$^o$C (Lopez-Vazquez et.al., 2009).

The hydrolysis rate by phosphate release that obtained by 100% AS sludge composition is 0.22 mg sCOD/g TS × $h^{-1}$ or 0.29 mg sCOD/g VS × $h^{-1}$. This value is slightly lower compared to the previous study 0.34 mg sCOD/g VS × $h^{-1}$ under temperature 20$^o$C (Jönsson and Jansen, 2006). This different rate might be caused by temperature difference that was used in the previous study and Experiment 1. Hydrolysis rate is constantly increasing consequently to the temperature increase (Veeken and Hamelers, 1999).

The yield obtained by 100% primary sludge is 69 mg sCOD/g TS or 91 mg sCOD/gVS, while the rate obtained is 0.35 mg sCOD/(g TS×$h^{-1}$) or 0.47 mg sCOD/(g VS×$h^{-1}$). The PS hydrolysis yield and rate that obtained in the previous study are 78 mg $COD_{VFA}$/g VS and 1.4 mg $COD_{VFA}$/(g VS×$h^{-1}$) (Gidstedt, 2017). By assuming all the sCOD is containing VFAs, these values can be compared. Both experiments were run under similar temperature. However, the resulted yield obtained by 100% primary sludge in Experiment 1 is higher compared to the previous study while the rate is lower. This condition might be caused by different organic matter amount in the sludge that used. The sludge in this experiment was taken from Lundåkra WWTP where the primary sludge was hydrolyzed in the primary clarifier.

The VFAs production shows in Figure 4.7 is consistent with the degrading organic matter that showed by sCOD in Figure 4.4. Figure 4.4 shows that the most sCOD was obtained is 89 mg/g TS or 894 mg COD /l. this value was obtained by composition 100% PS in 9 days hydrolysis. The VFAs generated were 233 mg COD/l of acetate, 166 mg COD/l of propionate and 47 mg COD/l butyrate. For the sludge composition with high PS content, half of the sCOD were in the form of VFAs. However, only a third of sCOD is in the form of VFAs for sludge composition with more AS content. The VFAs produced by 100% activated sludge is below the detection limit 25 mg/l and the addition of primary sludge is improving the VFA production.

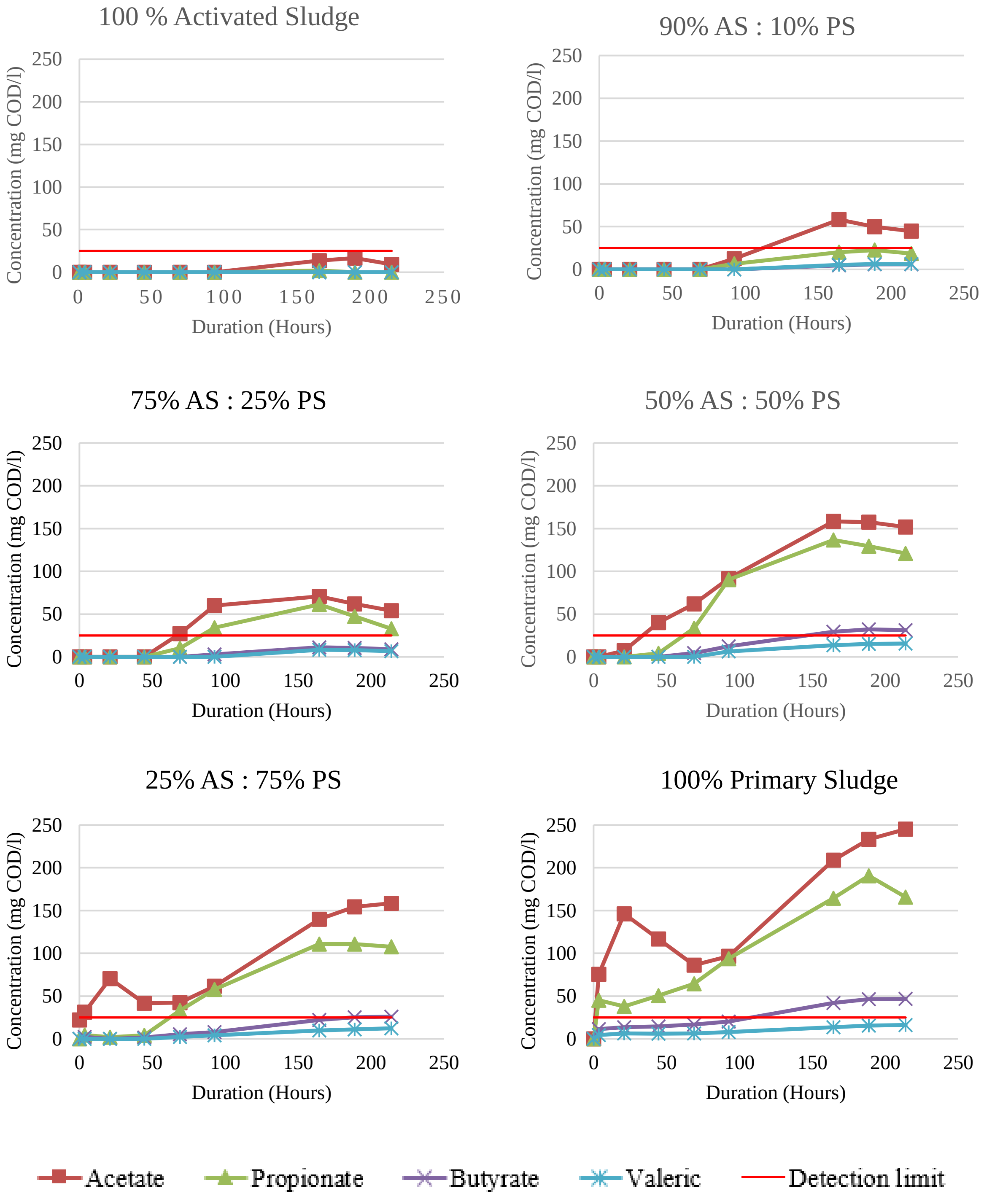

*Figure 4.7. Comparison of VFAs production in different activated sludge and primary sludge composition*

The VFAs production also increasing the acid concentration inside the jars and lowering the pH. As shown in Figure 4.7 most VFAs are produced by jars with high primary sludge content. The higher concentrations of VFAs are reducing the pH in those jars as shown in Figure 4.8. The pH value before and after hydrolysis for the jar with 100% activated sludge remains unchanged. While the pH value before and after hydrolysis with 100% Primary sludge content is decreased significantly.

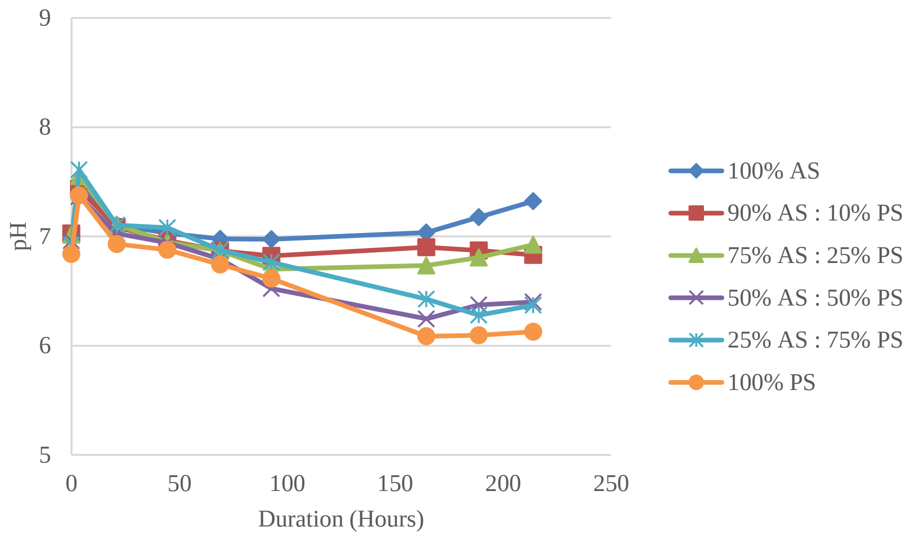

*Figure 4.8. pH reduction as an impact of VFAs production in Experiment 1. The pH axis started from 5 to show the anomaly at the beginning of the experiment.*

## 4.2 Experiment 2

Continuing Experiment 1, Experiment 2 was done with the focus on the influence of addition reject water from primary sludge thickener towards the hydrolysis. This experiment was run on 9-19th of March, and the sludge composition only consists of activated sludge as a major component and reject water from the primary clarifier. This experiment also started with nitrogen gas with diffusers attachment to prevent vigorous bubbling. However, the nitrogen gas supply also very unstable in this experiment. In Experiment 1, the nitrogen gas was run out in 1st day. A new tube came right after, but the new tube has only lasted for 2 days. This situation was not sustainable for the experiment. Therefore, the experiment was continued without nitrogen gas. The average oxygen level measured is 0.147 mg/L, and the average temperature is 13.5 $^{o}$C.

*Table 4.3. Total solids, Volatile solids and total COD before and after hydrolysis; Experiment 2 with the combination of activated sludge and reject water from primary sludge thickener.*

| | **Before** | | | | **After** | | | |
|---|---|---|---|---|---|---|---|---|
| **Sludge Composition (AS:RW)** | **TS (%)** | **VS (%)** | **VS as %TS (%)** | **T.COD (mg/l)** | **TS (%)** | **VS (%)** | **VS as %TS (%)** | **T.COD (mg/l** |
| 100% AS | 0.62 | 0.46 | 75 | 8900 | 0.56 | 0.41 | 74 | 7800 |
| 95% : 5% | 0.62 | 0.46 | 75 | 8600 | 0.56 | 0.42 | 74 | 6800 |
| 90% : 10% | 0.60 | 0.45 | 75 | 9000 | 0.54 | 0.40 | 74 | 7600 |
| 80% : 20% | 0.55 | 0.41 | 74 | 6200 | 0.49 | 0.35 | 72 | 6200 |
| 70% : 30% | 0.50 | 0.37 | 74 | 6400 | 0.47 | 0.34 | 72 | 6000 |
| 50% : 50% | 0.37 | 0.26 | 71 | 3400 | 0.34 | 0.23 | 69 | 4600 |

Table 4.3 in Experiment 2 shows some similarity to Table 4.1 in Experiment 1 result. Total COD after hydrolysis in Experiment 2 are showing unstable result due to the measurement.

Due to the unstable total COD, the total organic matter in Experiment 2 was also determined by the value of the volatile solid portions (VS as %TS).

*Figure 4.9. Before and after hydrolysis Experiment 2, the diffuser attachment successfully eliminates the spilling problem.*

In Experiment 2, the diffuser attachments successfully eliminate the spillage problem that happens in Experiment 1. As shown by Figure 4.9 the volume after 10 days is uniform in all jars after the hydrolysis. The sludge color after hydrolysis is darker compared to before hydrolysis except for jar 5 and 6. The color difference is caused by a high concentration of reject water. Jar 5 and 6 is filled with 70%AS:30%RW and 50%AS:50%RW respectively.

Figure 4.10 to Figure 4.12 are showing the hydrolysis profile generated by sCOD, ammonium concentration, and phosphate concentration, respectively. There are two charts in each figure, the chart on the left shows the measured value of the sCOD, ammonium concentration, and phosphate concentration, while the right chart shows the calculated value of the sCOD, ammonium concentration, and phosphate concentration based on the measured 100% activated sludge content and the activated sludge fraction. The calculated value was obtained by assuming the reject water from primary sludge thickener is containing a very minimum amount to none of the organic matter. The calculated wavlue were obtained by multiplication of the activated sludge fraction with the measured value of sCOD, ammonium concentration, and phosphate concentration resulted by 100% activated sludge. Both charts were placed side by side to show the comparison between the measurement result and the assumption.

Figure 4.10 shows that measured sCOD concentration is mostly increasing during the 10 days hydrolysis process. The comparison between the measured value and the calculated value shows that the addition of a certain amount of the reject water is beneficial to generate more sCOD. From Experiment 2, the sludge which has 90% activated sludge and 10% reject water is producing the most sCOD measured. This condition might be caused by the reject water content. Reject water is containing 0.09% TS, 0.03% VS, a third of the total solids is in the form of volatile solid.

Figure 4.11 shows that the measured ammonium concentration changes are higher in the jar with higher reject water content. The measured phosphate concentration also shows a similar trend to ammonium release shown by Figure 4.12. Similar to the sCOD, the comparison between the measured value and the calculated value shows that reject water addition in the experiment is increased the ammonium and phosphate concentration. This condition is caused by the initial ammonium concentration measured in the jars with high reject water content is higher compared to other jars with less reject water to none. Jar with composition 50% of activated sludge and 50% of reject water is having the highest sCOD, phosphate, and ammonium concentration.

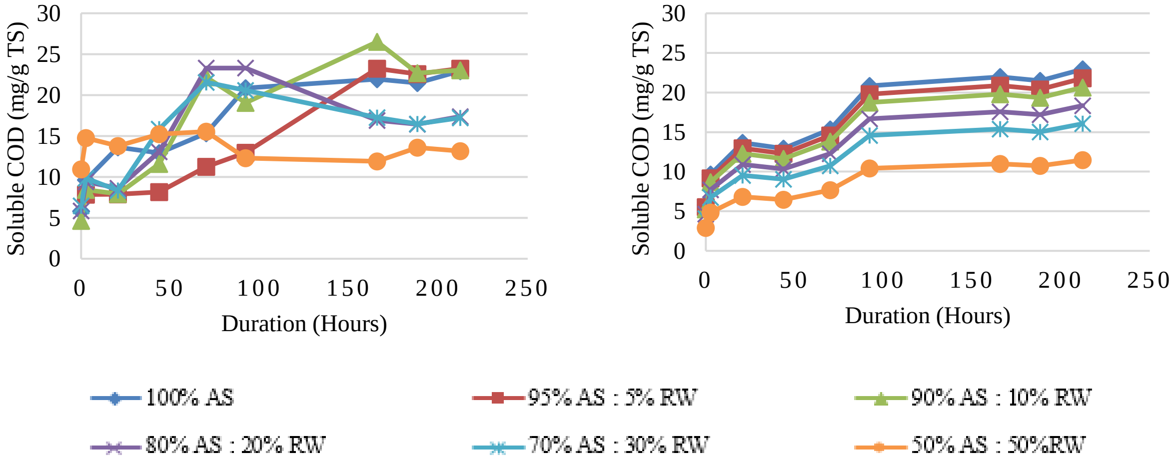

*Figure 4.10. Left: the measured sCOD concentration (mg/g TS), Right: Calculated soluble COD concentration that obtained by multiplication of the activated sludge fraction and measured sCOD in 100% activated sludge.*

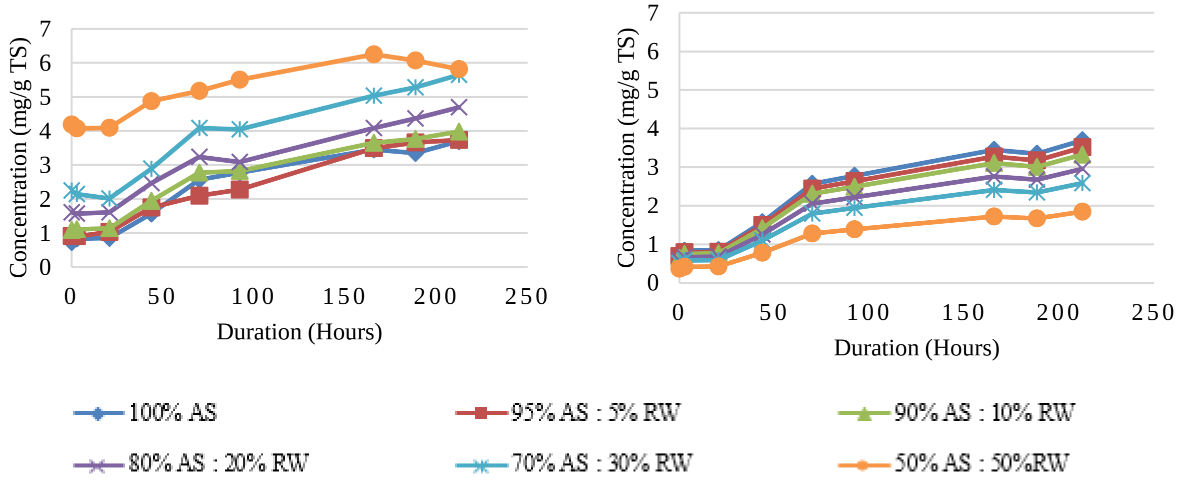

*Figure 4.11. Left: the measured ammonium concentration (mg/g TS), Right: Calculated ammonium concentration that obtained by multiplication of the activated sludge fraction and measured ammonium in 100% activated sludge.*

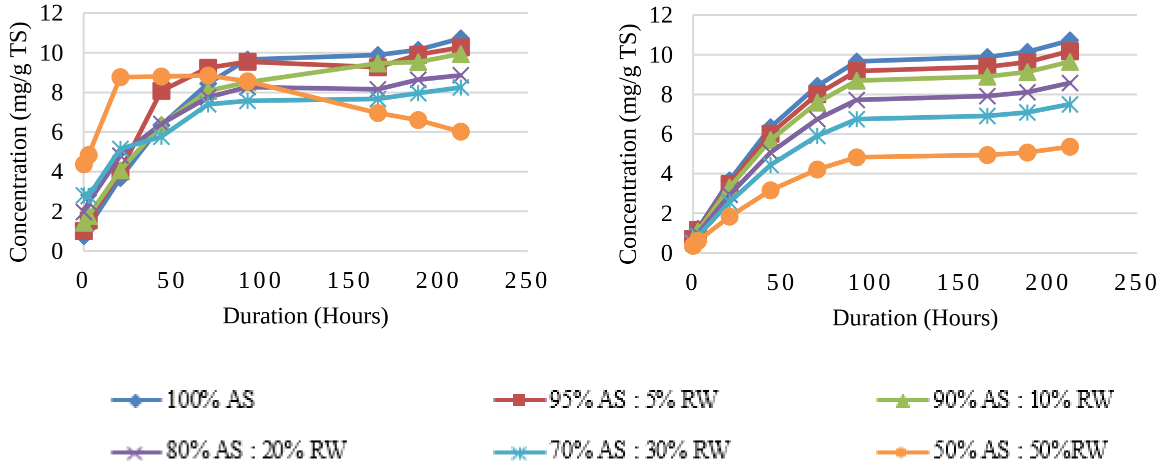

*Figure 4.12. Left: the measured phosphate concentration (mg/g TS), Right: Calculated phosphate concentration that obtained by multiplication of the activated sludge fraction and measured phosphate in 100% activated sludge.*

In Experiment 2 the hydrolysis yield and rate were calculated based on ammonium and phosphate method. The value used in this calculation was the measured value of ammonium, phosphate and sCOD. The hydrolysis yield results then compared with sCOD yield that obtained from the measurement in Figure 4.13. This figure shows that jar with 100% activated sludge is having the highest COD yield. The yield generated by phosphate method is having the highest value compared to the yield generated by ammonium and sCOD value. This condition caused by the assumption on ammonium and phosphate method. The ammonium method only based on protein degradation and exclude the degradation of lipids and carbohydrates. The phosphate method is based on the degradation of the organic matter, phosphate release from PAOs activity and sCOD measured.

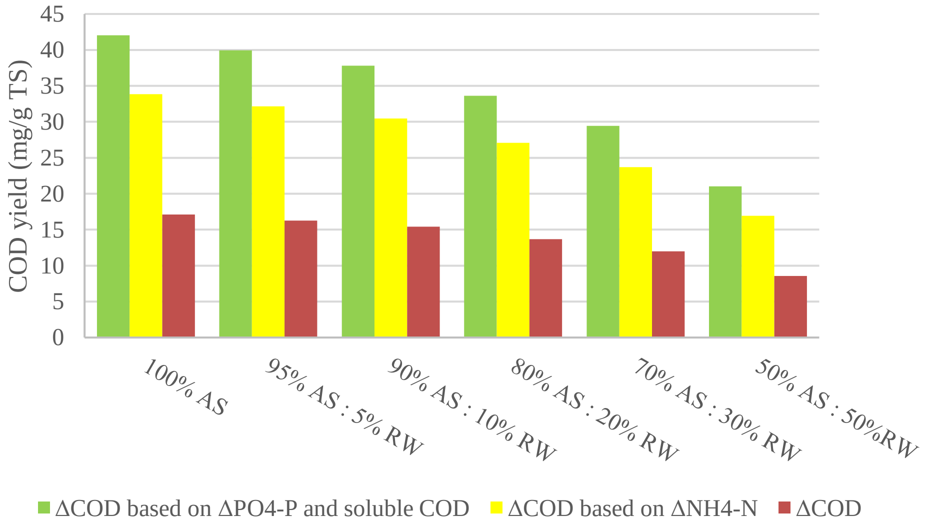

*Figure 4.13. Hydrolysis yield based on phosphate ammonium and soluble COD release in Experiment 2*

The hydrolysis rate in Experiment 2 was calculated and presented in Table 4.4. The hydrolysis rate was calculated from the hydrolysis yield. Therefore, the hydrolysis rate by phosphate release is higher compared to the ammonium and sCOD release. The hydrolysis yield and rate that were obtained by 100% activated sludge is having similar value to the hydrolysis yield that obtained in Experiment 1, presented in Figure 4.6 and Table 4.2.

*Table 4.4. Hydrolysis rate on various compositions based on phosphate, ammonium and soluble COD.*

| **Sludge Composition (%Volume) AS:RW** | **Hydrolysis rate by phosphate release (mg/g TS × $h^{-1}$)** | **Hydrolysis rate by ammonium release (mg/g TS × $h^{-1}$)** | **Hydrolysis rate by soluble COD (mg/g TS × $h^{-1}$)** |
|---|---|---|---|
| 100% AS | 0.25 | 0.18 | 0.13 |
| 95% : 5% | 0.23 | 0.17 | 0.13 |
| 90% : 10% | 0.21 | 0.16 | 0.12 |
| 80% : 20% | 0.19 | 0.14 | 0.11 |
| 70% : 30% | 0.17 | 0.12 | 0.09 |
| 50% : 50% | 0.12 | 0.09 | 0.06 |

The addition of reject water in this experiment did not increase the hydrolysis yield and hydrolysis rate. The hydrolysis yield and rate is decreasing consequently to the addition of reject water. In the previous study showed that additional reject water is increasing the yield and rate of the hydrolysis (Gidstedt, 2007). This condition might be caused by different sludge composition. The previous study was using primary sludge and reject water while this.

Figure 4.14 shows that the VFAs produced in 100% AS was very low despite having the highest hydrolysis yield and rate. A possible reason that causing this condition is the PAOs consuming the VFAs right after its produced (Jönsson and Jansen, 2006). The highest VFAs production resulted from an addition 5% of reject water that is 50 mg COD/l in 6 days. The sCOD obtained in this sludge composition and duration was 23 mg/g TS or 143 mg COD/l. This value is corresponded to the Experiment 1 result where the a third of sCOD is in the form of VFAs for high activated sludge composition. Reject water with composition 50%AS:50%RW was not generated enough VFAs to pass the detection limit.

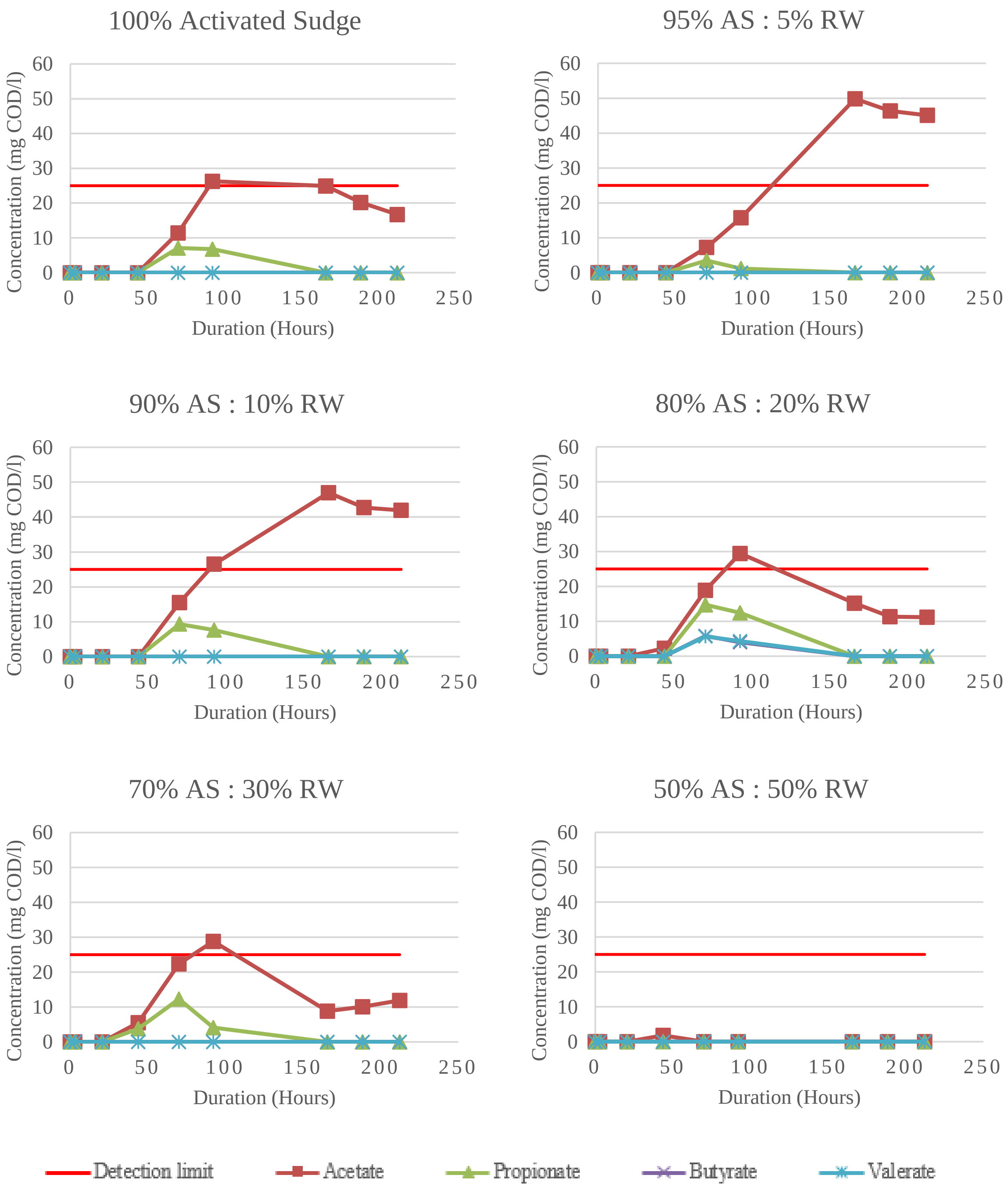

*Figure 4.14. Comparison of VFAs production in different activated sludge and reject water composition*

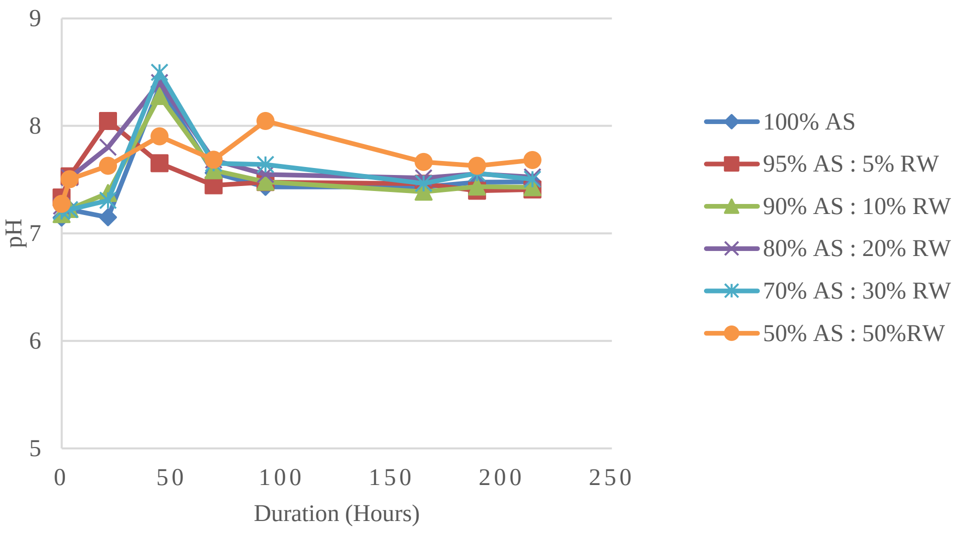

*Figure 4.15. pH level during the hydrolysis in Experiment 2, there are no significant changes in pH level. The pH axis started from 5 to show the anomaly at the beginning of the experiment*

Figure 4.15. also shows that there is some pH fluctuation at the beginning of the experiment. This fluctuation timeline is consistent with the nitrogen gas availability. The nitrogen gas supply was very unstable, the gas was running out on the first day that makes the experiment left without nitrogen gas for a night. The new nitrogen gas tube was connected the next day and due to the leaking problem and instability, the gas supply only lasts for 2 days. The pH was more stable after the experiment runs without the nitrogen gas for the rest of the hydrolysis process. This condition also happens in Experiment 1, in Figure 4.8. shows that there was a fluctuation at the beginning of the experiment. The timeline of the fluctuation in Experiment 1 also consistent with the spillage problem that happens around that time. The pH fluctuation might not be happening if the nitrogen supply is reliable to support the whole of the hydrolysis experiment for 10 days. The experiment can also be done without the nitrogen gas supply and a closed environment that prevent oxygen contact.

# 5 Conclusion

Experiment 1 and Experiment 2 was studying the VFAs production under two different sludge mixture. The sludge mixture that was used in the 1st experiment was activated sludge and primary sludge, while the 2nd experiment was using activated sludge and reject water from primary sludge thickener. The hydrolysis experiment was done under 10 days of experiment and temperature 13°C.

The first experiment shows that the combination of activated sludge and primary sludge is very beneficial for recovering the carbon source. The highest hydrolysis yield and rate were obtained by sludge mixture 50%AS:50%PS. The hydrolysis yield and rate value are 84 mg/g TS and value 0.44 mg/g TS× $h^{-1}$ respectively. The VFAs concentration was consisting of 160 mg COD/l acetic acid and 140 mg COD/l propionic acid with retention time 6 days. The most VFAs was obtained by 100% primary sludge with VFA concentration 245 mg COD/l of acetic acid and 166 mg COD/l propionic acid.

From Experiment 2 results, it can be concluded that the additional reject water in certain compositions is beneficial for recovering the carbon source. The reject water from the primary sludge thickener is containing 0.09% TS, 0.03% VS, a third of the total solid is in the form of volatile solid. Highest VFAs production was obtained by adding 5% to 10% reject water and the VFAs concentration was 50 mg COD/l of acetic acid in 6 days of retention time. The hydrolysis rate and hydrolysis yield obtained were 38-40 mg/g TS and 0.21-0.23 mg/g TS.×$h^{-1}$ respectively.

# 6 Future Studies

This thesis project was done in a peculiar situation. The corona pandemic emerged during this thesis project resulting in a cancellation of the last planned experiment. The last experiment was planned to study the hydrolysis process on activated sludge, primary sludge, and reject water sludge mixture.

Ängen WWTP is planning to utilize more activated sludge in the side stream hydrolysis, while the majority of the primary sludge will be processed in an anaerobic digester to produce energy. Based on the 2nd experiment, most VFAs were produced by 95% activated sludge and 5% reject water. This result shows that the reject water addition in the experiment is improving the hydrolysis yield. A recommended future study about this project can use sludge comparison such as:

*Table 6.1. Sludge composition for future study based on volume; AS: Activated Sludge, PS: Primary Sludge, RW: Reject water.*

| Jar | Sludge | Composition |
|---|---|---|
| **1** | AS | 100 |
| **2** | AS : PS : RW | 90 : 5 : 5 |
| **3** | AS : PS : RW | 80 : 10 :10 |
| **4** | AS : PS : RW | 80 : 5 : 15 |
| **5** | AS : PS : RW | 70 : 15 :15 |
| **6** | AS : PS : RW | 70 : 10 : 20 |

Sludge compositions listed in Table 6.1 are based on the Experiment 2 data where most VFAs are produced by 90% activated sludge. With the small addition of primary sludge higher hydrolysis yield and rate is expected.

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# Appendix

### VFAs Calibration table and calibration curve

*Table A.1. Raw data of VFAs calibration measurement in Experiment 1*

| Concentration (mg/l) | Acetate Area | | Average Acetate Area | Propionate Area | | Average Propionate Area |
|---|---|---|---|---|---|---|
| 25 | 88 | 93 | 91 | 106 | 109 | 108 |
| 50 | 193 | 188 | 191 | 230 | 226 | 228 |
| 100 | 379 | 370 | 374 | 451 | 452 | 452 |
| 200 | 758 | 756 | 757 | 920 | 924 | 922 |
| 500 | 1919 | 1971 | 1945 | 2325 | 2359 | 2342 |
| 1000 | 3981 | 3960 | 3970 | 4789 | 4779 | 4784 |
| Control 20 | 80 | 82 | 81 | 110 | 109 | 109 |
| Control 750 | 2952 | 2935 | 2944 | 3546 | 3544 | 3545 |
| **Concentration (mg/l)** | **Butyrate Area** | | **Average Butyrate Area** | **Valerate Area** | | **Average Valerate Area** |
| 25 | 104 | 111 | 107 | 95 | 102 | 98 |
| 50 | 235 | 230 | 232 | 233 | 225 | 229 |
| 100 | 462 | 472 | 467 | 465 | 480 | 472 |
| 200 | 953 | 960 | 957 | 969 | 978 | 974 |
| 500 | 2388 | 2407 | 2397 | 2418 | 2439 | 2428 |
| 1000 | 4891 | 4862 | 4876 | 4972 | 4946 | 4959 |
| Control 20 | 105 | 102 | 104 | 108 | 100 | 104 |
| Control 750 | 3618 | 3610 | 3614 | 3662 | 3662 | 3662 |

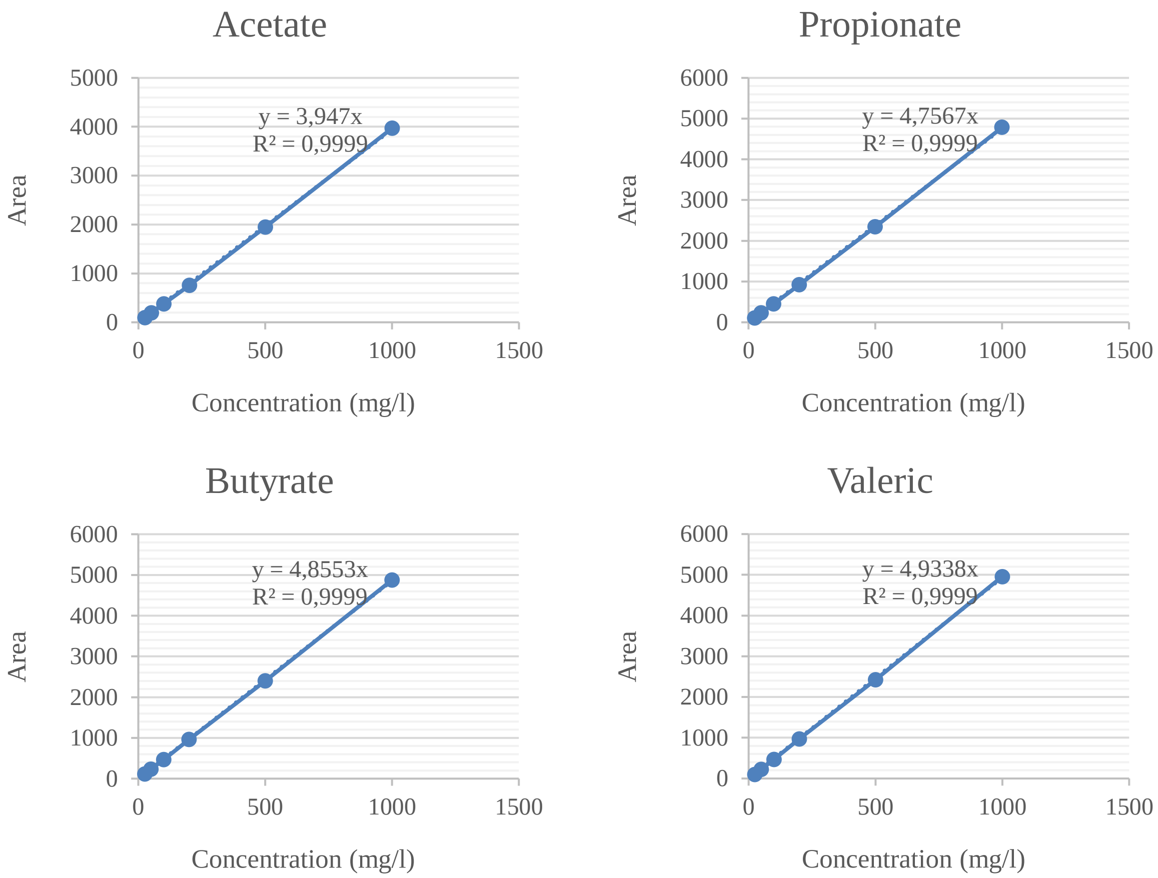

*Figure A.1. VFAs calibration curve measurement in Experiment 1*

*Table A.2. Raw data of VFAs calibration measurement in Experiment 2*

| **Concentration (mg/l)** | **Acetate Area** | | **Average Acetate Area** | **Propionat Area** | | **Average Propionate Area** |
|---|---|---|---|---|---|---|
| 25 | 103 | 108 | 106 | 134 | 123 | 128 |
| 50 | 205 | 205 | 205 | 242 | 244 | 243 |
| 100 | 373 | 374 | 374 | 452 | 456 | 454 |
| 200 | 756 | 781 | 769 | 914 | 933 | 924 |
| 500 | 1832 | 1884 | 1858 | 2227 | 2271 | 2249 |
| 1000 | 3810 | 3720 | 3765 | 4613 | 4523 | 4568 |
| Control 20 | 75 | 71 | 73 | 88 | 87 | 88 |
| Control 750 | 2562 | 2558 | 2560 | 3159 | 3089 | 3124 |

| **Concentration (mg/l)** | **Butyrate Area** | | **Average Butyrate Area** | **Valerate Area** | | **Average Valerate Area** |
|---|---|---|---|---|---|---|
| 25 | 135 | 128 | 132 | 120 | 114 | 117 |
| 50 | 249 | 256 | 253 | 234 | 237 | 236 |
| 100 | 473 | 476 | 475 | 475 | 479 | 477 |
| 200 | 947 | 958 | 953 | 953 | 960 | 957 |
| 500 | 2308 | 2344 | 2326 | 2328 | 2358 | 2343 |
| 1000 | 4772 | 4704 | 4738 | 4812 | 4763 | 4788 |
| Control 20 | 95 | 93 | 94 | 87 | 88 | 88 |
| Control 750 | 3332 | 3170 | 3251 | 3341 | 3135 | 3238 |

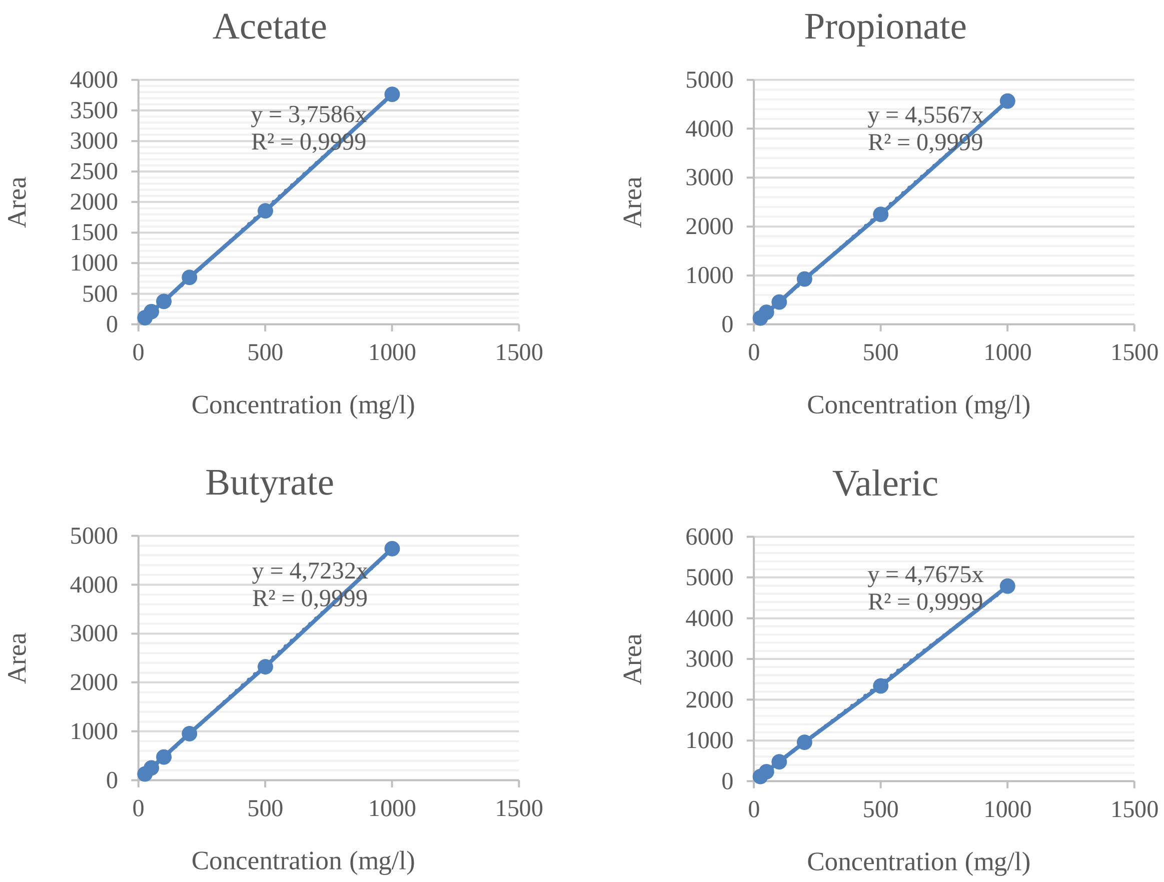

*Figure A.2. VFAs calibration curve measurement in Experiment 2*

## Raw data of TS and VS measurement in Experiment 1

*Table A.3. Raw data TS and VS measurement before hydrolysis in the Experiment 1*

| | **100% AS** | **AS:PS (90%:10%)** | **AS:PS (75%:25%)** | **AS:PS (50%:50%)** | **AS:PS (25%:75%)** | **100% PS** |
|---|---|---|---|---|---|---|
| Container (g) | 1.746 | 1.741 | 1.742 | 1.729 | 1.741 | 1.720 |
| Filled (g) | 35.555 | 38.052 | 40.748 | 39.356 | 39.168 | 39.836 |
| Dried (g) | 1.975 | 1.982 | 2.020 | 2.033 | 2.051 | 2.105 |
| Ignited (g) | 1.805 | 1.796 | 1.803 | 1.798 | 1.812 | 1.815 |

*Table A.4. Raw data TS and VS measurement after hydrolysis in the Experiment 1*

| | **100% AS** | **AS:PS (90%:10%)** | **AS:PS (75%:25%)** | **AS:PS (50%:50%)** | **AS:PS (25%:75%)** | **100% PS** |
|---|---|---|---|---|---|---|
| Container (g) | 1.843 | 1.823 | 1.851 | 1.735 | 1.847 | 1.819 |
| Filled (g) | 39.783 | 41.749 | 38.347 | 42.000 | 40.344 | 39.764 |
| Dried (g) | 2.077 | 2.061 | 2.079 | 2.008 | 2.143 | 2.113 |
| Ignited (g) | 1.895 | 1.880 | 1.908 | 1.799 | 1.924 | 1.888 |

*Table A.5. TS conversion from % to g/l in Experiment 1*

| | **100% AS** | **AS:PS (90%:10%)** | **AS:PS (75%:25%)** | **AS:PS (50%:50%)** | **AS:PS (25%:75%)** | **100% PS** |
|---|---|---|---|---|---|---|
| **TS (%)** | 0.007 | 0.007 | 0.007 | 0.008 | 0.008 | 0.010 |
| **TS (g/l)** | 6.780 | 6.630 | 7.130 | 8.090 | 8.280 | 10.090 |

## Raw data of $NO_3^-$ and $NO_2^-$ measurement in Experiment 1

*Table A.6. Raw data soluble $NO_3^-$ measurement (mg/l)*

| **Duration (hours)** | **100% AS** | **AS:PS (90%:10%)** | **AS:PS (75%:25%)** | **AS:PS (50%:50%)** | **AS:PS (25%:75%)** | **100%PS** |
|---|---|---|---|---|---|---|
| 0 | 0.09 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4 | 0.07 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 |
| 21 | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 45 | 0.11 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 |
| 69 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| 93 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| 165 | 0.05 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| 189 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 214 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

*Table A.7. Raw data soluble $NO_3^-$ measurement (mg/l)*

| Duration (hours) | 100% AS | AS:PS (90%:10%) | AS:PS (75%:25%) | AS:PS (50%:50%) | AS:PS (25%:75%) | 100%PS |
|---|---|---|---|---|---|---|
| 0 | 0.07 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4 | 0.08 | 0.05 | 0.03 | 0.02 | 0.00 | 0.00 |
| 21 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| 45 | 0.06 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| 69 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 93 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 165 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 189 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 214 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

## Raw data of COD measurement in Experiment 1

*Table A.8. Raw data soluble COD measurement (mg/l)*

| Duration (hours) | 100% AS | AS:PS (90%:10%) | AS:PS (75%:25%) | AS:PS (50%:50%) | AS:PS (25%:75%) | 100%PS |
|---|---|---|---|---|---|---|
| 0 | 28 | 32 | 44 | 51 | 92 | 173 |
| 4 | 31 | 34 | 37 | 56 | 102 | 213 |
| 21 | 53 | 47 | 51 | 70 | 151 | 318 |
| 45 | 67 | 58 | 63 | 124 | 148 | 317 |
| 69 | 47 | 63 | 105 | 207 | 218 | 358 |
| 93 | 61 | 90 | 208 | 375 | 352 | 478 |
| 165 | 96 | 151 | 335 | 599 | 605 | 786 |
| 189 | 89 | 207 | 335 | 666 | 708 | 894 |
| 214 | 113 | 217 | 305 | 642 | 700 | 860 |

*Table A.9. Soluble COD concentration (mg/g TS) based on the TS*

| Duration (hours) | 100% AS | AS:PS (90%:10%) | AS:PS (75%:25%) | AS:PS (50%:50%) | AS:PS (25%:75%) | 100%PS |
|---|---|---|---|---|---|---|
| 0 | 28 | 32 | 44 | 51 | 92 | 173 |
| 4 | 31 | 34 | 37 | 56 | 102 | 213 |
| 21 | 53 | 47 | 51 | 70 | 151 | 318 |
| 45 | 67 | 58 | 63 | 124 | 148 | 317 |
| 69 | 47 | 63 | 105 | 207 | 218 | 358 |
| 93 | 61 | 90 | 208 | 375 | 352 | 478 |
| 165 | 96 | 151 | 335 | 599 | 605 | 786 |
| 189 | 89 | 207 | 335 | 666 | 708 | 894 |
| 214 | 113 | 217 | 305 | 642 | 700 | 860 |

## Raw data of $NH_4^+$ measurement in Experiment 1

*Table A.10. Raw data soluble $NH_4^+$ measurement (mg/l)*

| Duration (hours) | 100%AS | AS:PS (90%:10%) | AS:PS (75%:25%) | AS:PS (50%:50%) | AS:PS (25%:75%) | 100%PS |
|---|---|---|---|---|---|---|
| 0 | 4 | 6 | 9 | 14 | 18 | 23 |
| 4 | 4 | 5 | 9 | 13 | 18 | 22 |
| 21 | 4 | 6 | 9 | 15 | 17 | 21 |
| 45 | 5 | 6 | 9 | 15 | 15 | 19 |
| 69 | 6 | 6 | 9 | 16 | 17 | 20 |
| 93 | 7 | 6 | 9 | 19 | 20 | 26 |
| 165 | 9 | 11 | 13 | 18 | 9 | 18 |
| 189 | 11 | 13 | 13 | 16 | 9 | 15 |
| 214 | 15 | 16 | 17 | 19 | 8 | 12 |

*Table A.11. Soluble $NH_4^+$ concentration (mg/g TS) based on the TS*

| Duration (hours) | 100%AS | AS:PS (90%:10%) | AS:PS (75%:25%) | AS:PS (50%:50%) | AS:PS (25%:75%) | 100%PS |
|---|---|---|---|---|---|---|
| 0 | 1 | 1 | 1 | 2 | 2 | 2 |
| 4 | 1 | 1 | 1 | 2 | 2 | 2 |
| 21 | 1 | 1 | 1 | 2 | 2 | 2 |
| 45 | 1 | 1 | 1 | 2 | 2 | 2 |
| 69 | 1 | 1 | 1 | 2 | 2 | 2 |
| 93 | 1 | 1 | 1 | 2 | 2 | 3 |
| 165 | 1 | 2 | 2 | 2 | 1 | 2 |
| 189 | 2 | 2 | 2 | 2 | 1 | 2 |
| 214 | 2 | 2 | 2 | 2 | 1 | 1 |

## Raw data of $PO_4^{3-}$ measurement in Experiment 1

*Table A.12. Raw data soluble $PO_4^{3-}$ measurement (mg/l)*

| Duration (hours) | 100%AS | AS:PS (90%:10%) | AS:PS (75%:25%) | AS:PS (50%:50%) | AS:PS (25%:75%) | 100%PS |
|---|---|---|---|---|---|---|
| 0 | 2 | 5 | 11 | 18 | 16 | 8 |
| 4 | 6 | 9 | 15 | 24 | 20 | 8 |
| 21 | 20 | 23 | 34 | 39 | 22 | 11 |
| 45 | 32 | 40 | 51 | 45 | 24 | 12 |
| 69 | 48 | 59 | 59 | 47 | 25 | 12 |
| 93 | 61 | 68 | 63 | 49 | 26 | 14 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 165 | 79 | 80 | 43 | 52 | 25 | 13 |
| 189 | 70 | 63 | 67 | 49 | 25 | 12 |
| 214 | 86 | 86 | 74 | 53 | 25 | 11 |

*Table A.13. Soluble $PO_4^{3-}$ concentration (mg/g TS) based on the TS*

| **Duration (hours)** | **100%AS** | **AS:PS (90%:10%)** | **AS:PS (75%:25%)** | **AS:PS (50%:50%)** | **AS:PS (25%:75%)** | **100%PS** |
|---|---|---|---|---|---|---|
| 0 | 0 | 1 | 2 | 2 | 2 | 1 |
| 4 | 1 | 1 | 2 | 3 | 2 | 1 |
| 21 | 3 | 4 | 5 | 5 | 3 | 1 |
| 45 | 5 | 6 | 7 | 6 | 3 | 1 |
| 69 | 7 | 9 | 8 | 6 | 3 | 1 |
| 93 | 9 | 10 | 9 | 6 | 3 | 1 |
| 165 | 12 | 12 | 6 | 6 | 3 | 1 |
| 189 | 10 | 9 | 9 | 6 | 3 | 1 |
| 214 | 13 | 13 | 10 | 6 | 3 | 1 |

## Hydrolysis rate calculation in Experiment 1

*Table A.14. Hydrolysis rate calculation based on $PO_4^{3-}$ method*

| Duration (hours) | 100% AS | | | | | 90% AS : 10% PS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **$PO4^{3-}$** | **ΔP** | **sCOD** | **Δ COD** | **COD yield** | **$PO4^{3-}$** | **ΔP** | **sCOD** | **ΔCOD** | **COD yield** |
| 0 | 0 | 0 | 4 | 0 | 0 | 1 | 0 | 5 | 0 | 0 |
| 4 | 1 | 1 | 5 | 0 | 2 | 1 | 1 | 5 | 0 | 2 |
| 21 | 3 | 3 | 8 | 4 | 10 | 4 | 3 | 7 | 2 | 9 |
| 45 | 5 | 4 | 10 | 6 | 17 | 6 | 5 | 9 | 4 | 17 |
| 69 | 7 | 7 | 7 | 3 | 20 | 9 | 8 | 10 | 5 | 25 |
| 93 | 9 | 9 | 9 | 5 | 27 | 10 | 9 | 14 | 9 | 32 |
| 165 | 12 | 11 | 14 | 10 | 38 | 12 | 11 | 23 | 18 | 46 |
| 189 | 10 | 10 | 13 | 9 | 34 | 9 | 9 | 31 | 26 | 48 |
| 214 | 13 | 12 | 17 | 13 | 43 | 13 | 12 | 33 | 28 | 58 |

| Duration (hours) | 75% AS : 25% PS | | | | | 50% AS : 50% PS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **$PO4^{3-}$** | **ΔP** | **sCOD** | **Δ COD** | **COD yield** | **$PO4^{3-}$** | **ΔP** | **sCOD** | **ΔCOD** | **COD yield** |
| 0 | 2 | 0 | 6 | 0 | 0 | 2 | 0 | 6 | 0 | 0 |
| 4 | 2 | 1 | 5 | -1 | 0 | 3 | 1 | 7 | 1 | 3 |
| 21 | 5 | 3 | 7 | 1 | 9 | 5 | 3 | 9 | 2 | 9 |
| 45 | 7 | 6 | 9 | 3 | 17 | 6 | 3 | 15 | 9 | 17 |
| 69 | 8 | 7 | 15 | 9 | 25 | 6 | 4 | 26 | 19 | 28 |
| 93 | 9 | 7 | 29 | 23 | 41 | 6 | 4 | 46 | 40 | 50 |
| 165 | 6 | 5 | 47 | 41 | 52 | 6 | 4 | 74 | 68 | 78 |
| 189 | 9 | 8 | 47 | 41 | 60 | 6 | 4 | 82 | 76 | 86 |
| 214 | 10 | 9 | 43 | 37 | 59 | 6 | 4 | 79 | 73 | 84 |

| Duration (hours) | 25% AS : 75% PS | | | | | 100% PS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **$PO4^{3-}$** | **ΔP** | **sCOD** | **Δ COD** | **COD yield** | **$PO4^{3-}$** | **ΔP** | **sCOD** | **ΔCOD** | **COD yield** |
| 0 | 2 | 0 | 11 | 0 | 0 | 1 | 0 | 17 | 0 | 0 |
| 4 | 2 | 0 | 12 | 1 | 2 | 1 | 0 | 21 | 4 | 4 |
| 21 | 3 | 1 | 18 | 7 | 9 | 1 | 0 | 32 | 14 | 15 |
| 45 | 3 | 1 | 18 | 7 | 9 | 1 | 0 | 31 | 14 | 15 |
| 69 | 3 | 1 | 26 | 15 | 18 | 1 | 0 | 35 | 18 | 19 |
| 93 | 3 | 1 | 43 | 31 | 34 | 1 | 1 | 47 | 30 | 32 |
| 165 | 3 | 1 | 73 | 62 | 65 | 1 | 0 | 78 | 61 | 62 |
| 189 | 3 | 1 | 86 | 74 | 77 | 1 | 0 | 89 | 71 | 73 |
| 214 | 3 | 1 | 85 | 73 | 76 | 1 | 0 | 85 | 68 | 69 |

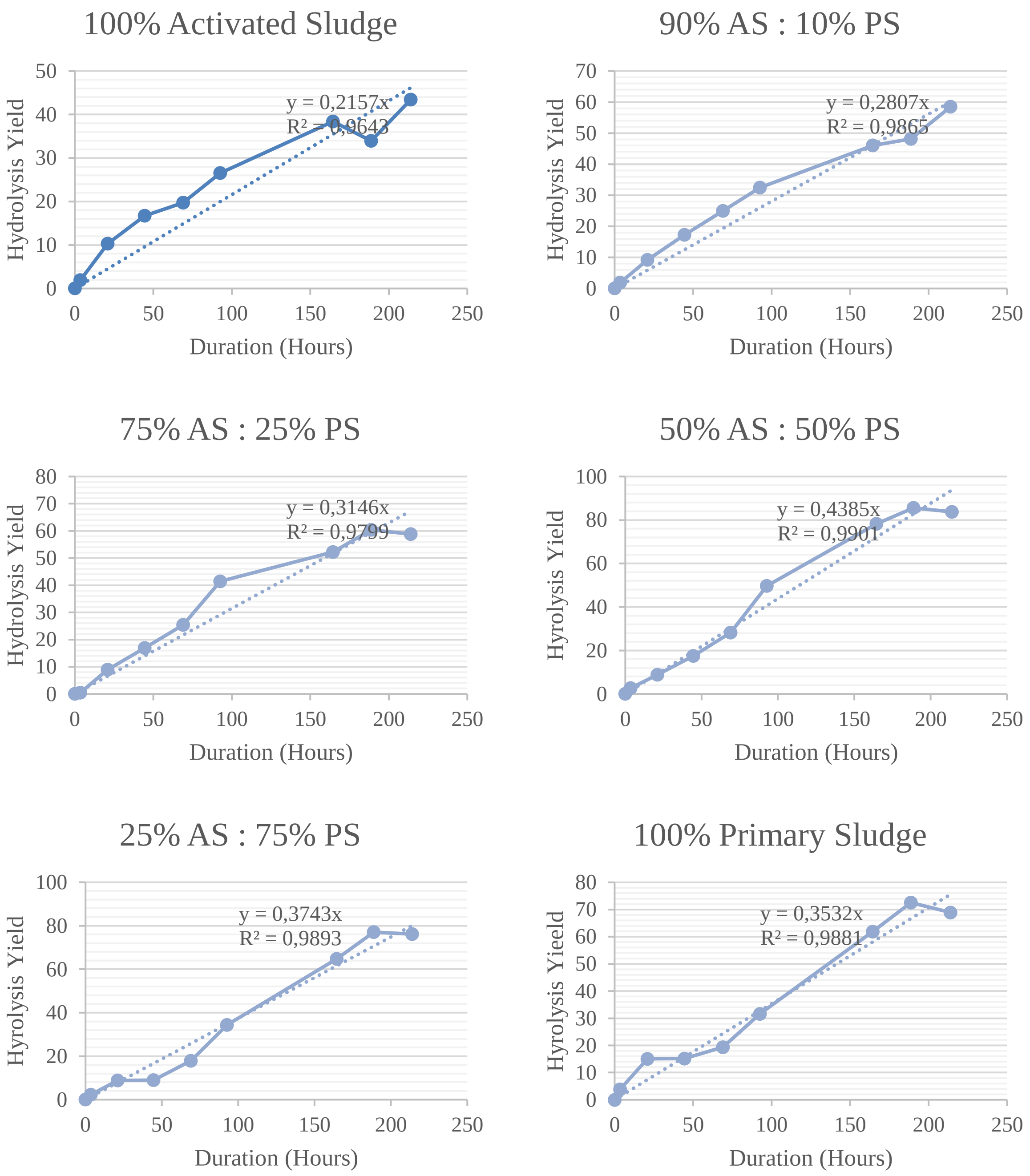

*Figure A.3. Hydrolysis rate plot based on COD yield from phosphorus method from Experiment 1. The hydrolysis rate is the constant (k) on the libe equation (y=kx).*

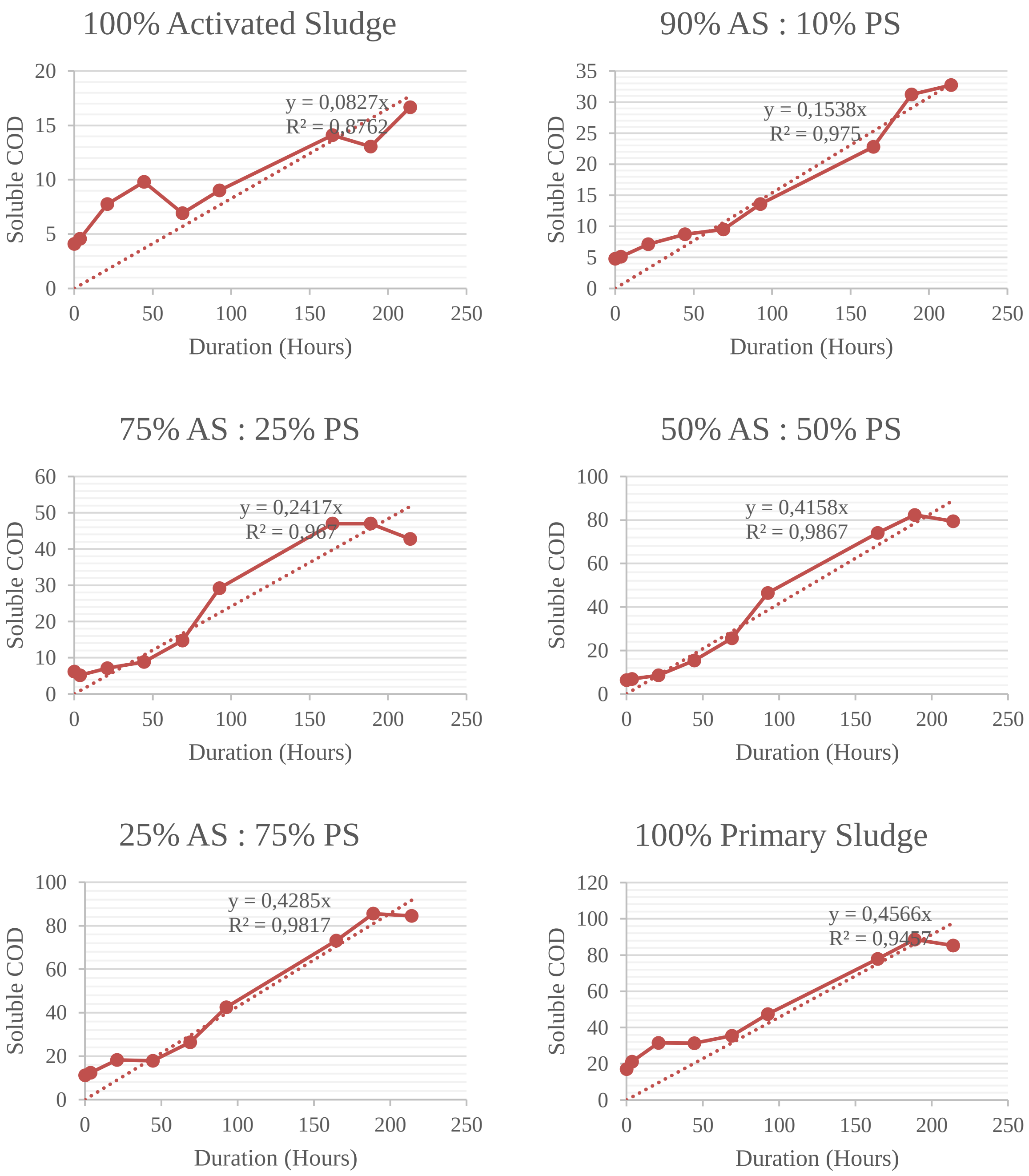

*Figure A.4. Hydrolysis rate plot based on soluble COD from Experiment 1. The hydrolysis rate is the constant (k) on the libe equation (y=kx).*

## Raw data of TS and VS measurement in Experiment 2

*Table A.15. Raw data TS and VS measurement before hydrolysis in the Experiment 2*

| | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) | RW |
|---|---|---|---|---|---|---|---|
| Container (g) | 1.725 | 1.745 | 1.717 | 1.747 | 1.720 | 1.720 | 1.720 |
| Filled (g) | 40.910 | 41.549 | 39.094 | 39.425 | 39.881 | 40.764 | 39.957 |
| Dried (g) | 1.968 | 1.990 | 1.943 | 1.954 | 1.910 | 1.866 | 1.753 |
| Ignited (g) | 1.787 | 1.806 | 1.773 | 1.800 | 1.770 | 1.763 | 1.742 |

*Table A.16. Raw data TS and VS measurement after hydrolysis in the Experiment 2*

| | 100% AS | AS:PS (90%:10%) | AS:PS (75%:25%) | AS:PS (50%:50%) | AS:PS (25%:75%) | 100% PS |
|---|---|---|---|---|---|---|
| Container (g) | 1.743 | 1.747 | 1.733 | 1.748 | 1.727 | 1.740 |
| Filled (g) | 38.113 | 39.375 | 38.449 | 38.104 | 40.229 | 37.535 |
| Dried (g) | 1.945 | 1.959 | 1.933 | 1.926 | 1.910 | 1.861 |
| Ignited (g) | 1.796 | 1.802 | 1.784 | 1.797 | 1.778 | 1.777 |

*Table A.17. TS conversion from % to g/l in Experiment 2*

| | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---|---|---|---|---|---|---|
| **TS (%)** | 0.006 | 0.006 | 0.006 | 0.005 | 0.005 | 0.004 |
| **TS (g/l)** | 6.19 | 6.15 | 6.04 | 5.49 | 4.96 | 3.72 |

## Raw data of $NO_3^-$ and $NO_2^-$ measurement in Experiment 1

*Table A.18. Raw data soluble $NO_3^-$ measurement (mg/l)*

| Duration (hours) | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---|---|---|---|---|---|---|
| 0 | 0.08 | 0.03 | 0.07 | 0.05 | 0.04 | 0.02 |
| 3 | 0.22 | 0.11 | 0.12 | 0.08 | 0.06 | 0.06 |
| 21 | 0.16 | 0.07 | 0.04 | 0.04 | 0.04 | 0.02 |
| 44 | 0.06 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 |
| 70 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 92 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 166 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 188 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 212 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

*Table A.19. Raw data soluble $NO_2^-$ measurement (mg/l)*

| Duration (hours) | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---:|---:|---:|---:|---:|---:|---:|
| 0 | 0.07 | 0.04 | 0.05 | 0.05 | 0.04 | 0.02 |
| 3 | 0.07 | 0.05 | 0.05 | 0.04 | 0.02 | 0.02 |
| 21 | 0.07 | 0.06 | 0.02 | 0.03 | 0.03 | 0.03 |
| 44 | 0.07 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 |
| 70 | 0.09 | 0.05 | 0.00 | 0.00 | 0.00 | 0.05 |
| 92 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08 |
| 166 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 |
| 188 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 |
| 212 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 |

## Raw data of COD measurement in Experiment 2

*Table A.20. Raw data soluble COD measurement (mg/l)*

| Duration (hours) | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---:|---:|---:|---:|---:|---:|---:|
| 0 | 36 |  | 28 | 32 | 32 | 41 |
| 3 | 60 | 48 | 51 | 52 | 49 | 55 |
| 21 | 84 | 49 | 48 | 47 | 41 | 51 |
| 44 | 80 | 50 | 70 | 72 | 79 | 57 |
| 70 | 95 | 69 | 134 | 128 | 107 | 58 |
| 92 | 129 | 80 | 115 | 128 | 102 | 46 |
| 166 | 136 | 143 | 160 | 93 | 86 | 44 |
| 188 | 133 | 139 | 137 | 90 | 82 | 51 |
| 212 | 142 | 143 | 139 | 96 | 85 | 49 |

*Table A.21. Soluble COD concentration (mg/g TS) based on the TS*

| Duration (hours) | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---:|---:|---:|---:|---:|---:|---:|
| 0 | 6 |  | 5 | 6 | 6 | 11 |
| 3 | 10 | 8 | 8 | 9 | 10 | 15 |
| 21 | 14 | 8 | 8 | 9 | 8 | 14 |
| 44 | 13 | 8 | 12 | 13 | 16 | 15 |
| 70 | 15 | 11 | 22 | 23 | 22 | 16 |
| 92 | 21 | 13 | 19 | 23 | 21 | 12 |
| 166 | 22 | 23 | 27 | 17 | 17 | 12 |
| 188 | 21 | 23 | 23 | 16 | 16 | 14 |
| 212 | 23 | 23 | 23 | 17 | 17 | 13 |

## Raw data of $NH_4^+$ measurement in Experiment 2

*Table A.22. Raw data soluble $NH_4^+$ measurement (mg/l)*

| Duration (hours) | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---|---|---|---|---|---|---|
| 0 | 4 | 6 | 7 | 9 | 11 | 16 |
| 3 | 5 | 5 | 7 | 9 | 11 | 15 |
| 21 | 5 | 6 | 7 | 9 | 10 | 15 |
| 44 | 10 | 11 | 12 | 14 | 14 | 18 |
| 70 | 16 | 13 | 17 | 18 | 20 | 19 |
| 92 | 17 | 14 | 17 | 17 | 20 | 20 |
| 166 | 21 | 21 | 22 | 22 | 25 | 23 |
| 188 | 21 | 23 | 23 | 24 | 26 | 23 |
| 212 | 23 | 23 | 24 | 26 | 28 | 22 |

*Table A.23. Soluble $NH_4^+$ concentration (mg/g TS) based on the TS*

| Duration (hours) | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---|---|---|---|---|---|---|
| 0 | 1 | 1 | 1 | 2 | 2 | 4 |
| 3 | 1 | 1 | 1 | 2 | 2 | 4 |
| 21 | 1 | 1 | 1 | 2 | 2 | 4 |
| 44 | 2 | 2 | 2 | 2 | 3 | 5 |
| 70 | 3 | 2 | 3 | 3 | 4 | 5 |
| 92 | 3 | 2 | 3 | 3 | 4 | 6 |
| 166 | 3 | 3 | 4 | 4 | 5 | 6 |
| 188 | 3 | 4 | 4 | 4 | 5 | 6 |
| 212 | 4 | 4 | 4 | 5 | 6 | 6 |

## Raw data of $PO_4^{3-}$ measurement in Experiment 2

*Table A.24. Raw data soluble $PO_4^{3-}$ measurement (mg/l)*

| Duration (hours) | 100% AS | AS:RW (95%:5%) | AS:RW (90%:10%) | AS:RW (80%:20%) | AS:RW (70%:30%) | AS:RW (50%:50%) |
|---|---|---|---|---|---|---|
| 0 | 5 | 6 | 9 | 11 | 14 | 16 |
| 3 | 8 | 10 | 11 | 14 | 14 | 18 |
| 21 | 23 | 25 | 25 | 26 | 26 | 33 |
| 44 | 39 | 50 | 38 | 35 | 29 | 33 |
| 70 | 52 | 57 | 49 | 43 | 37 | 33 |
| 92 | 60 | 59 | 51 | 45 | 38 | 32 |
| 166 | 61 | 57 | 57 | 45 | 38 | 26 |
| 188 | 63 | 61 | 58 | 47 | 39 | 25 |
| 212 | 66 | 63 | 60 | 49 | 41 | 22 |

*Table A.25. Soluble $PO_4^{3-}$ concentration (mg/g TS) based on the TS*

| **Duration (hours)** | **100% AS** | **AS:RW (95%:5%)** | **AS:RW (90%:10%)** | **AS:RW (80%:20%)** | **AS:RW (70%:30%)** | **AS:RW (50%:50%)** |
|---:|---:|---:|---:|---:|---:|---:|
| 0 | 1 | 1 | 1 | 2 | 3 | 4 |
| 3 | 1 | 2 | 2 | 2 | 3 | 5 |
| 21 | 4 | 4 | 4 | 5 | 5 | 9 |
| 44 | 6 | 8 | 6 | 6 | 6 | 9 |
| 70 | 8 | 9 | 8 | 8 | 7 | 9 |
| 92 | 10 | 10 | 9 | 8 | 8 | 9 |
| 166 | 10 | 9 | 9 | 8 | 8 | 7 |
| 188 | 10 | 10 | 10 | 9 | 8 | 7 |
| 212 | 11 | 10 | 10 | 9 | 8 | 6 |

## Hydrolysis rate calculation in Experiment 2

*Table A.26. Hydrolysis rate calculation based on* $PO_4^{3-}$ *method*

| Duration (hours) | 100% AS | | | | | 95% AS : 5% RW | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $PO_4^{3-}$ | ΔP | sCOD | Δ COD | COD yield | $PO_4^{3-}$ | ΔP | sCOD | ΔCOD | COD yield |
| 0 | 0 | 0 | 6 | 0 | 0 | 1 | 0 | 6 | 0 | 0 |
| 3 | 1 | 1 | 10 | 4 | 7 | 1 | 0 | 9 | 4 | 5 |
| 21 | 4 | 4 | 14 | 8 | 17 | 3 | 3 | 13 | 7 | 14 |
| 44 | 6 | 6 | 13 | 7 | 23 | 6 | 5 | 12 | 7 | 20 |
| 70 | 8 | 8 | 15 | 10 | 31 | 8 | 7 | 15 | 9 | 27 |
| 92 | 10 | 10 | 21 | 15 | 39 | 9 | 8 | 20 | 14 | 35 |
| 166 | 10 | 10 | 22 | 16 | 41 | 9 | 9 | 21 | 15 | 37 |
| 188 | 10 | 10 | 21 | 16 | 41 | 10 | 9 | 20 | 15 | 37 |
| 212 | 11 | 11 | 23 | 17 | 44 | 10 | 9 | 22 | 16 | 40 |
| Duration (hours) | 90% AS : 10% RW | | | | | 80% AS : 20% RW | | | | |
| | $PO_4^{3-}$ | ΔP | sCOD | Δ COD | COD yield | $PO_4^{3-}$ | ΔP | sCOD | ΔCOD | COD yield |
| 0 | 1 | 0 | 5 | 0 | 0 | 1 | 0 | 5 | 0 | 0 |
| 3 | 1 | 0 | 9 | 3 | 5 | 1 | 0 | 8 | 3 | 4 |
| 21 | 3 | 3 | 12 | 7 | 14 | 3 | 2 | 11 | 6 | 12 |
| 44 | 6 | 5 | 12 | 6 | 19 | 5 | 4 | 10 | 6 | 17 |
| 70 | 8 | 7 | 14 | 9 | 26 | 7 | 6 | 12 | 8 | 23 |
| 92 | 9 | 8 | 19 | 14 | 34 | 8 | 7 | 17 | 12 | 30 |
| 166 | 9 | 8 | 20 | 15 | 35 | 8 | 7 | 18 | 13 | 31 |
| 188 | 9 | 8 | 19 | 14 | 35 | 8 | 8 | 17 | 13 | 31 |
| 212 | 10 | 9 | 21 | 15 | 38 | 9 | 8 | 18 | 14 | 34 |
| Duration (hours) | 70% AS : 30% RW | | | | | 50% AS : 50% RW | | | | |
| | $PO_4^{3-}$ | ΔP | sCOD | Δ COD | COD yield | $PO_4^{3-}$ | ΔP | sCOD | ΔCOD | COD yield |
| 0 | 1 | 0 | 4 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| 3 | 1 | 0 | 7 | 3 | 4 | 1 | 0 | 5 | 2 | 3 |
| 21 | 3 | 2 | 10 | 5 | 11 | 2 | 1 | 7 | 4 | 8 |
| 44 | 4 | 4 | 9 | 5 | 15 | 3 | 3 | 6 | 4 | 11 |
| 70 | 6 | 5 | 11 | 7 | 20 | 4 | 4 | 8 | 5 | 14 |
| 92 | 7 | 6 | 15 | 11 | 26 | 5 | 4 | 10 | 8 | 19 |
| 166 | 7 | 6 | 15 | 11 | 27 | 5 | 5 | 11 | 8 | 19 |
| 188 | 7 | 7 | 15 | 11 | 27 | 5 | 5 | 11 | 8 | 20 |
| 212 | 8 | 7 | 16 | 12 | 29 | 5 | 5 | 11 | 9 | 21 |

*Table A.27. Hydrolysis rate calculation based on $NH_4^+$ method*

| Duration (hours) | 100% AS | | | 95% AS : 5% RW | | |
|---|---|---|---|---|---|---|
| | **$NH_4^+$** | **ΔN** | **COD yield** | **$NH_4^+$** | **ΔN** | **COD yield** |
| 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 3 | 1 | 0 | 1 | 1 | 0 | 1 |
| 21 | 1 | 0 | 1 | 1 | 0 | 1 |
| 44 | 2 | 1 | 10 | 1 | 1 | 9 |
| 70 | 3 | 2 | 21 | 2 | 2 | 20 |
| 92 | 3 | 2 | 23 | 3 | 2 | 22 |
| 166 | 3 | 3 | 31 | 3 | 3 | 29 |
| 188 | 3 | 3 | 30 | 3 | 2 | 28 |
| 212 | 4 | 3 | 34 | 4 | 3 | 32 |
| **Duration (hours)** | **90% AS : 10% RW** | | | **80% AS : 20% RW** | | |
| | **$NH_4^+$** | **ΔN** | **COD yield** | **$NH_4^+$** | **ΔN** | **COD yield** |
| 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 3 | 1 | 0 | 1 | 1 | 0 | 1 |
| 21 | 1 | 0 | 1 | 1 | 0 | 1 |
| 44 | 1 | 1 | 9 | 1 | 1 | 8 |
| 70 | 2 | 2 | 19 | 2 | 1 | 17 |
| 92 | 2 | 2 | 21 | 2 | 2 | 19 |
| 166 | 3 | 2 | 28 | 3 | 2 | 25 |
| 188 | 3 | 2 | 27 | 3 | 2 | 24 |
| 212 | 3 | 3 | 30 | 3 | 2 | 27 |
| **Duration (hours)** | **70% AS : 30% RW** | | | **50% AS : 50% RW** | | |
| | **$NH_4^+$** | **ΔN** | **COD yield** | **$NH_4^+$** | **ΔN** | **COD yield** |
| 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | 1 | 0 | 1 | 0 | 0 | 1 |
| 21 | 1 | 0 | 1 | 0 | 0 | 1 |
| 44 | 1 | 1 | 7 | 1 | 0 | 5 |
| 70 | 2 | 1 | 15 | 1 | 1 | 11 |
| 92 | 2 | 1 | 16 | 1 | 1 | 12 |
| 166 | 2 | 2 | 22 | 2 | 1 | 16 |
| 188 | 2 | 2 | 21 | 2 | 1 | 15 |
| 212 | 3 | 2 | 24 | 2 | 1 | 17 |

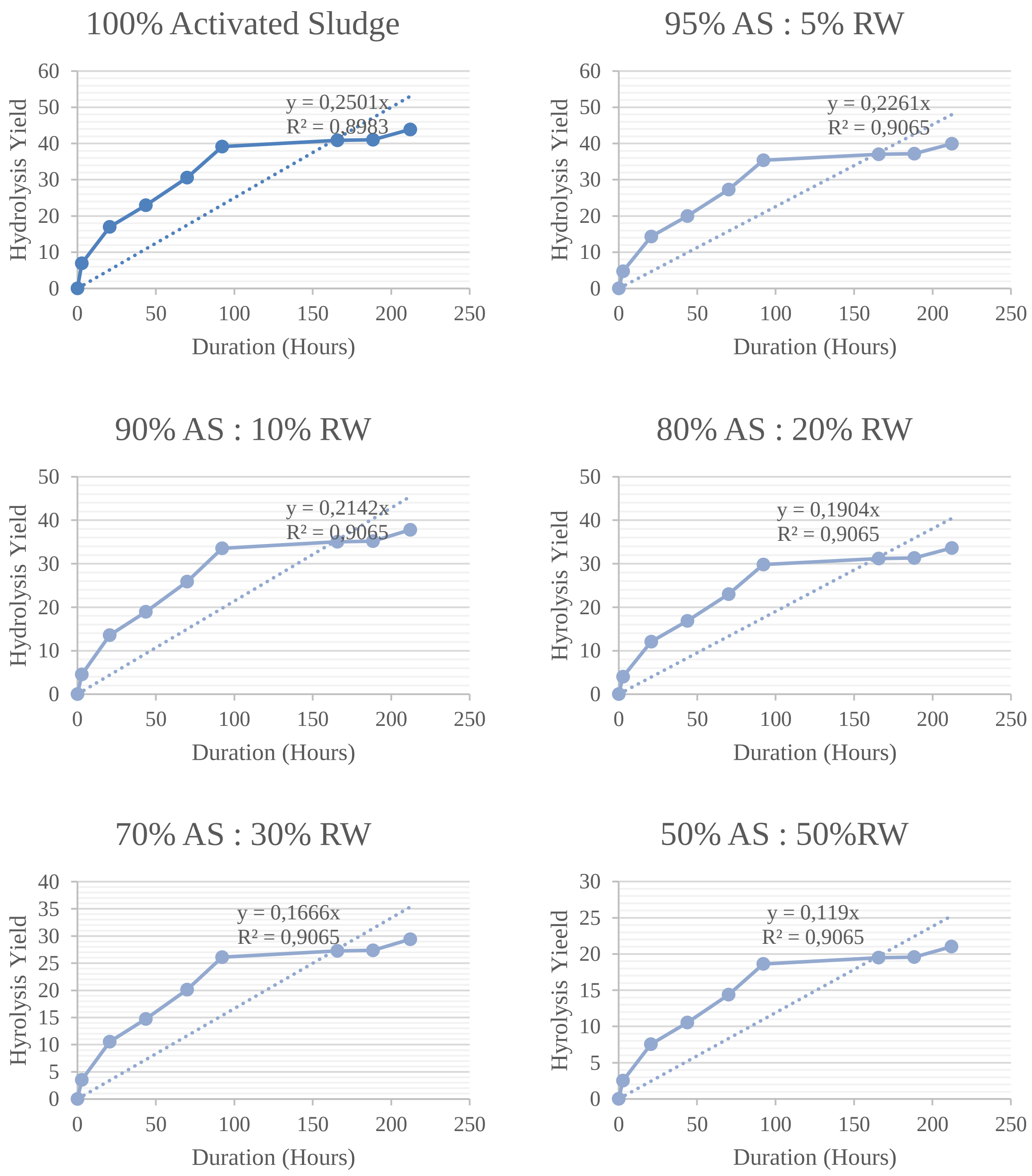

*Figure A.5. Hydrolysis rate plot based on hydrolysis yield from phosphate method from Experiment 2. The hydrolysis rate is the constant (k) on the libe equation (y=kx).*

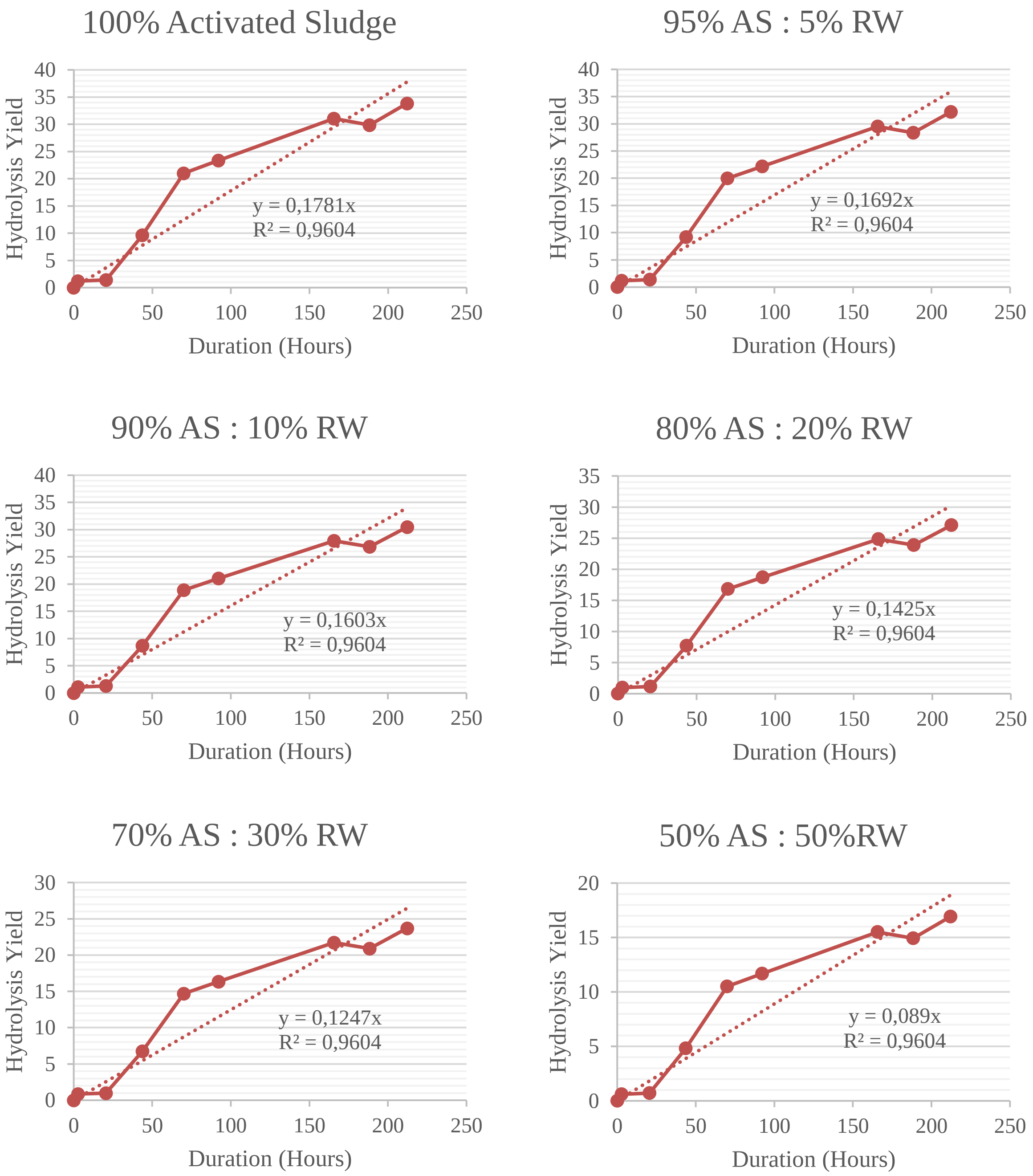

*Figure A.6. Hydrolysis rate plot based on hydrolysis yield from ammonium method from Experiment 2. The hydrolysis rate is the constant (k) on the libe equation (y=kx).*

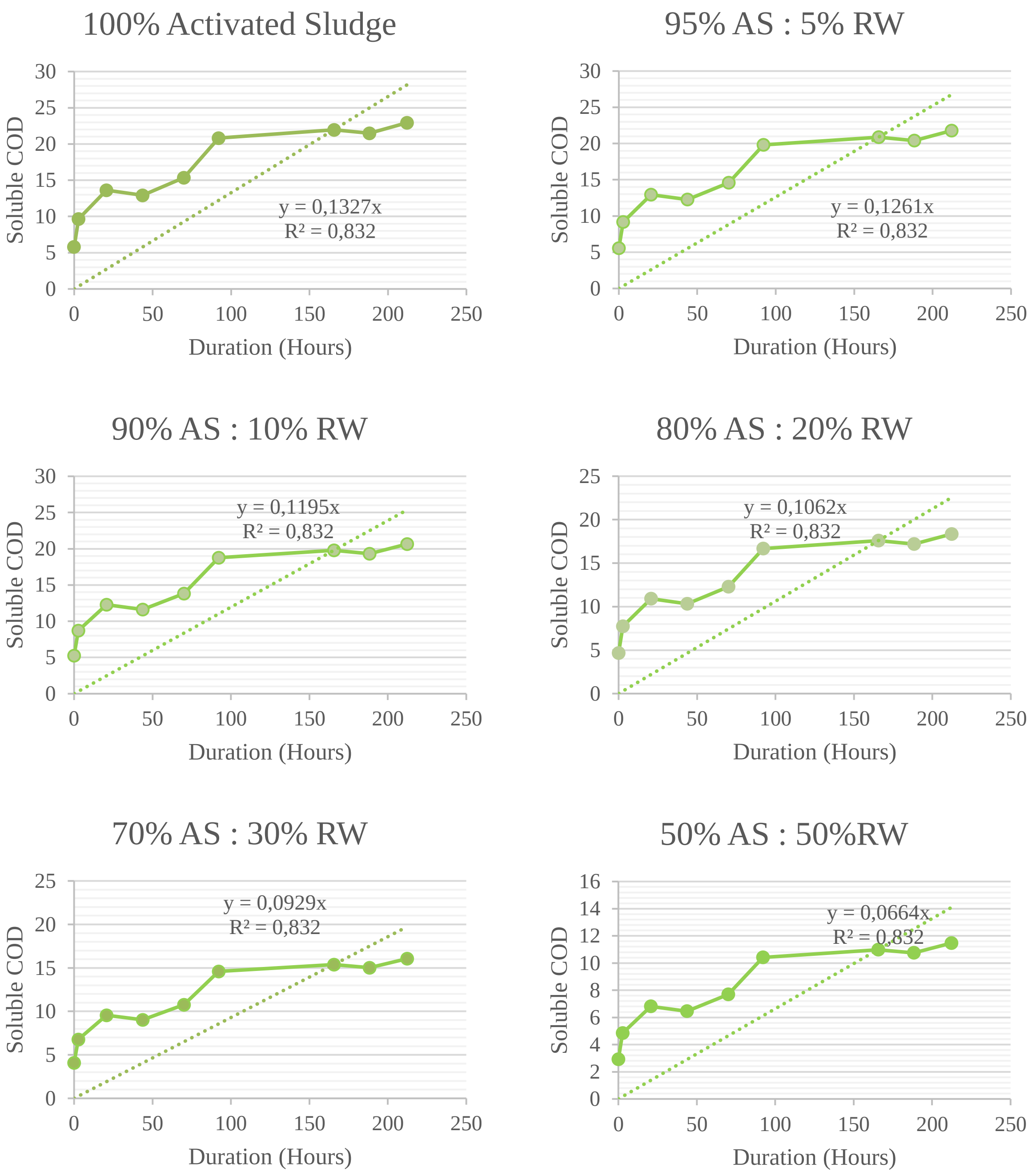

*Figure A.7. Hydrolysis rate plot based on soluble COD from Experiment 2. The hydrolysis rate is the constant (k) on the libe equation (y=kx).*

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# Carbon Source Recovery on Wastewater Treatment Plant

Wastewater treatment in Sweden has been developed since the late 1800s to mitigate the pollution problem caused by human activity. One of the biggest pollution problems that have been the eutrophication caused by nutrients such as phosphorus and ammonium in the water body. The wastewater treatment process is an expensive process; therefore, it is very important to develop an energy effective and resources efficient treatment plant.

Enhanced Biological Phosphorus Removal (EBPR) is one of the best technologies on phosphorus removal. EBPR efficiency on phosphorus removal process can reach 97%. This process relies on enhancing the Polyphosphate Accumulating Organisms (PAOs) ability to remove the phosphorus in the wastewater. The removal efficiency requires the availability of readily degradable carbon source such as Volatile Fatty Acids (VFAs). To create an efficient treatment plant, it is necessary to recover the resources such as VFAs through a resource recovery process.

Hydrolysis is a degradation process that breaks down the organic matter into smaller substances (VFAs). The VFAs production is depended in the sludge composition, temperature, and pH. Primary sludge is rich in organic matter, and very beneficial for energy recovery by producing methane. Activated sludge in the EBPR process is rich with PAOs. The combination of these two sludges is beneficial to generate VFAs and nutrients. In the other hand, reject water from primary sludge thickener is having the lowest organic matter content compared to activated sludge and primary sludge.

What we have done in this study? In this study, we investigated which sludge composition that generating the most VFAs. Two experiments with different sludge mixture were hydrolyzed for 10 days to provide the data about the carbon recovery possibility. The first experiment was using the sludge mixture that contains activated sludge and primary sludge with various concentration. The second experiment was using the mixture that contains activated sludge and reject water with various concentration. This study concludes that activated sludge and primary sludge mixture generated the most VFAs. Addition of a certain amount reject water is beneficial for the hydrolysis process. Addition of 10% reject water into activated sludge mixture generates more VFAs than 100% activated sludge.