Assessment on AnMBR for Removing Lipid and Carbohydrate from POME

Delft University of Technology

CIE5050-09 Additional Master Thesis

Qinying Zha (5237025)

Assessment Committee:

Prof. Dr. Ir. Jules van Lier

Prof. Dr. Ir. Ralph Lindeboom

PhD Candidate. Saqr Al-Muraisy

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Abstract

Palm oil is a popular ingredient in domestic products. The palm oil industry has been growing rapidly over the past decades, so that the amount of palm oil mill effluent (POME) generated from the palm oil production has been increasing as well. The anaerobic membrane bioreactor (AnMBR) is a treatment solution that can remove organic pollutants from POME while generating methane as an energy source. In comparison to conventional anaerobic digestors, the AnMBR technology has an additional membrane unit that can produce effluent with higher water quality. More specifically, if ultrafiltration is applied, the AnMBR will be able to effectively remove bacteria from the effluent, making it suitable for direct fertigation (Uman et al., 2021; Bray et al., 2021). However, in cases where infectious viruses are also present, further disinfection method might be required. In this experiment, a lab-scale AnMBR system was used for POME treatment. In order to evaluate how well the system can perform in terms of pollutant removal and methane production, under the controlled experimental conditions, several criteria were monitored: (1) chemical oxygen demand (COD) removal, (2) biomass growth, (3) biogas production, (4) digestion efficiency, and (5) volatile fatty acids (VFA) accumulation. A Long chain fatty acids (LCFA) analysis method was developed using the liquid chromatography/mass spectrometry (LC/MS), to elaborate on underlying conversion mechanisms. A COD balance analysis was also conducted. Factors that would potentially contribute to the COD gaps in the COD balance analysis were quantified and discussed in this paper as well to validate the experimental results. The solid retention time (SRT) was controlled at 140 days, and the organic loading rate (OLR) at 3 $gCOD \cdot L^{-1} \cdot d^{-1}$ during the first phase of the experiment, when synthetic POME and VFAs were added to the bioreactor. During the second phase, the SRT and the OLR of POME remained the same, whereas the VFAs were replaced by starch and the OLR of starch was increased, in order to simulate the real POME composition, because in addition to lipid, carbohydrate and protein are also found in POME. During Phase I, the AnMBR system could remove 98%-99% of the incoming COD, and produce about 5 L of methane each day. During Phase II, the microbes did not have enough time to adapt to the new experimental condition, but the stability of the AnMBR system could be achieved overtime, when the mixing is improved and the buffer solution is adjusted properly according to the pH variation. Although, based on the positive biomass net growth and the increased methane production, it could be predicted that adding carbohydrates to the feed for a more representative POME composition would promote biomass growth and methane production, suggesting that the AnMBR system would have higher potential when the real POME is used for energy recovery.

Key words: palm oil mill effluent (POME), anaerobic membrane bioreactor (AnMBR), anaerobic digestion (AD), ultrafiltration (UF), solid retention time (SRT), organic loading rate (OLR), chemical oxygen demand (COD), biomass, biogas, methane, volatile fatty acids (VFA), long chain fatty acids (LCFA), COD balance

Table of Contents

1. Introduction

1.1 Project Background

As the global palm oil industry expands, palm oil mill effluent (POME) treatment has become essential for wastewater reduction and energy recovery. Palm oil is a common cooking oil in developing countries (WWF, 2022). It is also widely used in food products, detergents, and cosmetics. Additionally, palm oil can be used as a resource for biodiesel production, which could reduce the consumption of fossil fuels (Shigetomi et al., 2020). 75.45 million metric tons of palm oil was produced globally in 2020, which is about 3% annual increase in comparison to the production of the previous year (Shahbandeh, 2021a). Indonesia and Malaysia are the world's largest producers of palm oil, contributing to 57.65% and 26.37% of the global palm oil production respectively (Shahbandeh, 2021b). Palm oil is produced by extracting crude palm oil (CPO) from fresh fruit bunches (FFBs) (Hasanudin, 2015). For each ton of CPO produced, approximately 2.5 to 3 m^3 of POME will be generated from the sterilization, clarification, purification, and hydro-cyclone processes.. Although, POME could be an energy source if treated properly, POME treatment is regularly considered a burden and discharged with insufficient treatment directly into the environment.

In this research project, a lab scale anaerobic membrane bioreactor (AnMBR) was used for POME treatment. The biological performance of the AnMBR was assessed in terms of COD removal, biomass growth, biogas production, digestion efficiency, and volatile fatty acids (VFA) accumulation. In addition, chemical oxygen demand (COD) balance and degradation pathway were discussed based on the experimental results. The filtration performance of the AnMBR, however, was not included within the scope of this research project.

1.2 Anaerobic Digestion

During anaerobic digestion, organic matters are converted into biogas through a series of reactions, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. This process has been illustrated in Figure 1, which was drawn based on two literature articles with modifications (Miyamoto et al., 2015; Ahmad, 2011).

First, complex organic polymers such as lipids, carbohydrates, and proteins are broken down into monomers and oligomers through hydrolysis, a biological process driven by extracellular enzymes (Ahmad, 2011). The enzymes are excretions released by fermentative bacteria. Lipases, cellulases, and proteases are enzymes that can disintegrate lipids, carbohydrates, and proteins, and produce long chain fatty acids (LCFAs) and glycerol, monosaccharides, and amino acids respectively.

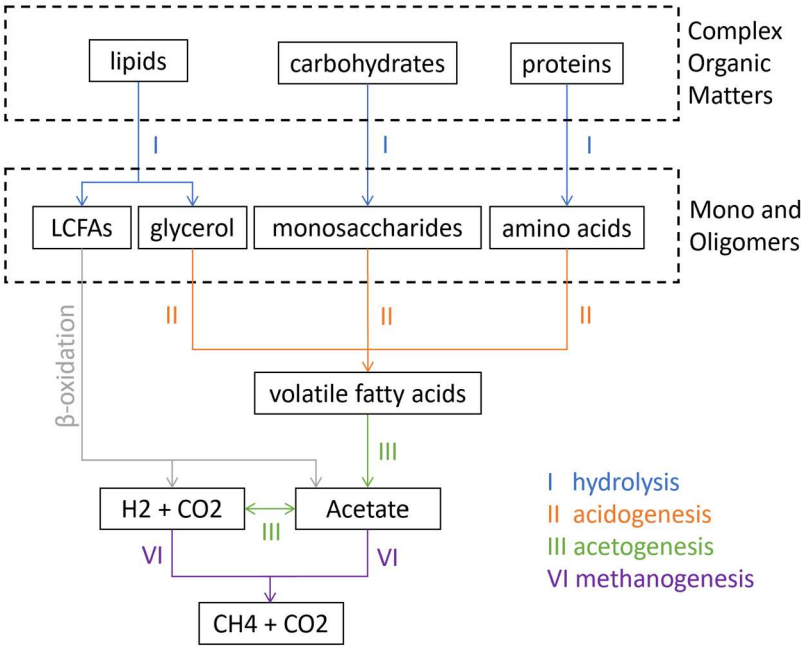

Figure 1. Anaerobic Degradation Pathway of Organic Matter

Subsequently, the hydrolysis products are converted into β-oxidation products (H_2, CO_2, and acetate) and acidogenesis products (volatile fatty acids). β-oxidation is the main degradation pathway of LCFAs (Ahmad, 2011). Acidogenesis, on the other hand, is a catabolic reaction processed by acidogenic bacteria that can convert soluble organic compounds into organic acids (Miyamoto et al., 2015; Ahmad, 2011). This process is also known as fermentation.

Volatile fatty acids (acetic acid, propanoic acid, butyric acid etc.) are the major intermediates produced during acidogenesis. They can be further oxidized into acetate by acetogenic bacteria via acetogenesis (Miyamoto et al., 2015). Moreover, acetate can be converted into H_2 and CO_2 through acetogenic oxidation, and H_2 and CO_2 can be converted into acetate through acetogenic reduction (Ahmad, 2011).

Methanogenesis is the final step where biogas is generated. The two pathways of methanogenesis are listed as follows (Miyamoto et al., 2015):

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \ (\Delta G^{0'} = -136\ kJ/reaction)$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \ (\Delta G^{0'} = -31\ kJ/reaction)$$

The operating conditions have significant influence on anerobic digestion, as the microorganisms are sensitive to pH and temperature. In case of acidification, when pH drops due to accumulation of VFAs in the sludge, methanogenic activities would be inhibited (Miyamoto et al., 2015). Maintaining the temperature of the bioreactor is as important as balancing the pH of the sludge. In order to achieve a higher reaction rate, thermophilic digestion is preferred when compared with mesophilic digestion (Miyamoto et al., 2015). The optimal temperature for thermophilic biodegradation is around 55 °C.

1.3 AnMBR

An AnMBR is an anaerobic digestor coupled with membrane filtration (Miyamoto, et al., 2015). In comparison to aerobic treatment, anaerobic digestion shows significant benefits in terms of low energy consumption and low sludge production. In addition, the biogas produced during anaerobic digestion can be further utilized as an energy source. However, anaerobic microorganisms grow much slower than aerobic microorganisms. Due to the slow growth of biomass, a long solid retention time (SRT) is required, and the conversion rate of the substrate is relatively low. Membrane filtration, on the other hand, is able to retain the biomass, increasing the biomass concentration in the sludge, as well as the conversion rate of the substrate. In the meantime, the permeate separated from the sludge after membrane filtration, can be reused or discharged depending on the quality of the effluent.

In this project, an external cross-flow AnMBR was used. There are three basic designs of AnMBR: external cross-flow, internal submerged, and external submerged (Miyamoto, et al., 2015). AnMBR is classified as an external cross-flow system, when the membrane unit is installed separately from the bioreactor, and the sludge retained by the membrane unit is pumped back to the bioreactor. The external cross-flow configuration enables direct control of fouling and high fluxes. However, the external cross-flow configuration has higher energy consumption, when compared with the internal submerged configuration. An internal submerged AnMBR is a system where the membrane unit is installed inside of the bioreactor, and the filtration is vacuum-driven. The internal submerged configuration consumes less energy than the external cross-flow configuration. Although, it is more difficult to clean or replace the membrane unit submerged inside of the bioreactor. An external submerged AnMBR overcomes this disadvantage by having the membrane unit installed separately from the bioreactor, similar to the external cross-flow configuration, but the filtration is still vacuum-driven. As a consequence, the structure of the external submerged AnMBR is more complicated than the other two configurations, which would make installation more difficult. The external cross-flow design was chosen for the AnMBR in this project, because direct control of membrane fouling and easy installation of the system were prioritized, in comparison to other factors.

1.4 POME

The composition and characteristics of POME have been studied in prior to the experiment. According to the data presented in a research paper (Gozan et al., 2018), calculations have been made in order to determine the concentration of each component in POME, as well as the corresponding mass composition. The calculations results have been summarized in Table 1. Meanwhile, another research paper has compared the physicochemical properties of POME based on different publications (Abdulsalam et al., 2018). Table 2 shows the typical range for the parameters that were compared in that research paper, and measured in this project as well.

Table 1. Composition of POME

Component	Formula	g/L	Mass Composition [%]
Carbohydrate	$C_6H_{10}O_5$	6.78	48%
Protein	$C_{16}H_{24}O_5N_4$	4.36	31%
Lipid	$C_{50}H_{90}O_6$	2.99	21%

Table 2. Characteristics of POME

Parameter	Range	Unit
COD	49,100 - 75,000	mg/L
pH	4.10 - 4.75	-
T	55.5 - 88	°C
TS	16,495 - 100,000	mg/L
TSS	18,000 - 59,350	mg/L
TVS	2600 - 80,000	mg/L

1.5 Research Question and Hypotheses

The research question of this project is: how would the AnMBR perform when it is co-fed with lipid and carbohydrate? The performance was assessed based on COD removal, biomass growth, biogas production, digestion efficiency, and VFA accumulation. The experiment consists of two phases. Phase I is the reference experiment, where the lipid-based POME was fed to the AnMBR along with the VFA feed (supplemental substrate). Phase II is the main experiment, where both lipid and carbohydrate were considered for more representative POME composition.

The hypotheses for Phase I were made upon the findings of the preliminary results of the same experiment conducted by other researchers, as different researchers have worked on the same research project under different conditions (with different SRTs and OLRs), and the findings of a similar research project, which also used an external cross-flow AnMBR, along with an UF membrane module, for POME treatment (Abdurahman et al., 2011). The hypotheses were established with the predicted values for different assessment criteria listed as follows:

- **COD Removal.** COD removal efficiency can be described as the amount of COD removed by the AnMBR system in comparison to the total influent COD. In this project, the COD removal efficiency was expected to reach 99%.
- **Biomass growth.** Biomass growth was measured as the net growth of volatile suspended solids (VSS) per day (in gVSS/d). A positive value for biomass net growth was predicted.
- **Biogas production.** Biogas production is a measurement of how much biogas can be produced per day (in L/d). Daily biogas production was expected to be around 6 L, without any normalization in correspondence with temperature.
- **Digestion efficiency.** Digestion efficiency was calculated as the ratio (in %) between the COD of the methane produced (in gCOD/d) and the total COD of

the feed (in gCOD/d). Approximately, the digestion efficiency would be ranged between 65% and 85%. Specific methanogenic activity (SMA) test was also conducted in order to verify the digestion efficiency of the sludge.

- **VFA accumulation.** VFA accumulation should be avoided in order to prevent acidification. Therefore, the VFA concentration in the sludge was monitored (in mg/L) throughout the project. VFA accumulation is more likely to occur at the beginning of phase II after the OLR is increased.

During Phase II, the SRT and the OLR of the lipid-based POME would not change, but the carbohydrate feed would be added to the AnMBR according to the real POME composition, so that the overall OLR would be higher for Phase II. The AnMBR might have similar performance in terms of COD removal efficiency and digestion efficiency; meanwhile, more biomass growth and biogas production would be observed under a higher OLR. VFA accumulation might occur if OLR is increased rapidly. Therefore, pH adjustment measures might be required, in order to avoid acidification caused by VFA accumulation.

LCFA analysis and COD balance were also discussed based on the experimental results. As mentioned in section 1.2, lipids from POME would be broken down into LCFAs and glycerol during hydrolysis. Then, LCFAs would be converted into acetate, H_2 and CO_2 via β-oxidation, while glycerol would be converted into VFAs via acetogenesis. β-oxidation is a rate limiting step for anaerobic digestion. Therefore, LCFA analysis was conducted in this experiment to (1) determine the LCFA concentrations and compositions in sludge and POME, and (2) evaluate the impact of LCFA precipitation on COD balance.

2. Materials and Methods

2.1 Materials

The main components of the AnMBR unit used in this project have been illustrated in Figure 2. POME feed was prepared with pure palm oil purchased from the market. VFA feed was added to the bioreactor (anaerobic digester) in order to maintain the growth of microorganisms. It was replaced with starch feed during the later stage of the project. The anaerobic digester in the middle was operated at 55 °C via an external water bath. The ultrafiltration (UF) unit contained a PVDF membrane with pore size of 30 nm. The final effluent (permeate) was collected in a plastic tank. Meanwhile, the biogas produced would go through a gas buffer tank for moisture removal before entering the gas meter. Unlike the permeate, the biogas was not collected. It was discharged directly to the ambient environment after being measured.

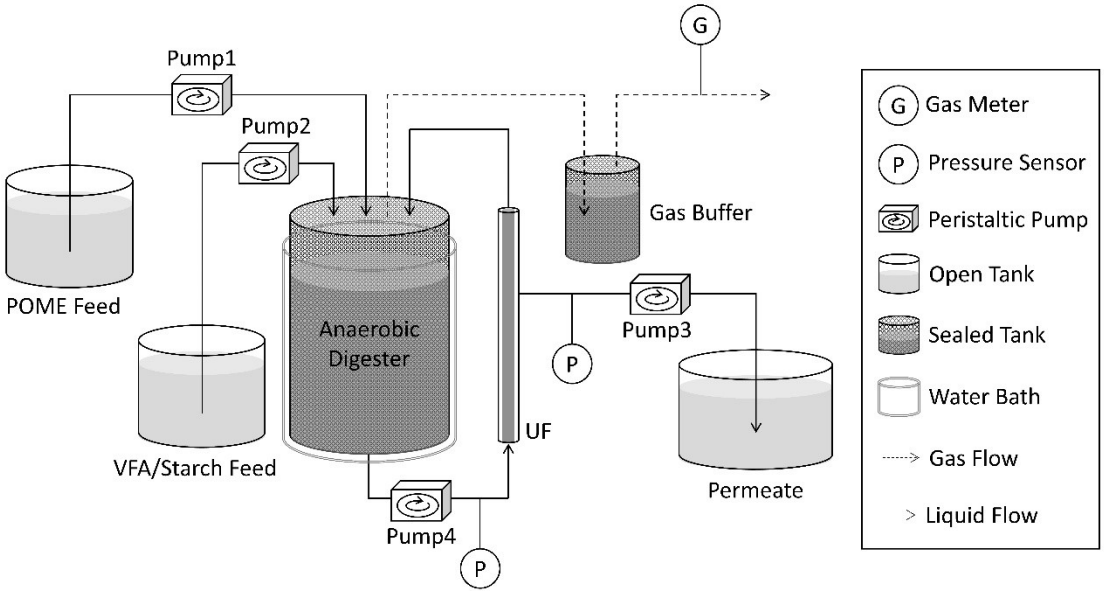

Figure 2. Schematic Design of the Experiment

Other laboratory instruments were used as well for measurement and sample preparation. The equipment used for measurement were mainly pH meter (WTW IDS 9430), Hach COD test kit (COD reagents, DRB200 reactor, and DR3900 spectrophotometer), gas chromatography (GC VFA and GC biogas, Agilent 7890A), and Liquid Chromatography/Mass Spectrometry (LC/MS, Waters Xevo TQ-S). In addition, the main tools that were used for sample preparation were centrifuge, magnetic mixer, sonicator, shaker, oven, and furnace.

2.2 Methods

2.2.1 Overview of the Experiment

The experiment consists of two phases. During the first phase, POME and VFA were fed to the bioreactor under the original experimental condition: SRT at 140 days, and OLR at 3 $gCOD \cdot L^{-1} \cdot d^{-1}$. Then, VFA was changed to starch during the second phase of the experiment, while the SRT was kept the same and the total OLR was increased to 4.3 $gCOD \cdot L^{-1} \cdot d^{-1}$. The overall flowrate of the feed was kept constant at 2.16 L/d for both phases, but the flowrate of each type of feed was distributed differently. The specific adjustments made for flowrate and OLR have been summarized in Table 3 and Table 4 respectively.

Table 3. Adjustment for Flowrate

Phase	Feed type	[L/d]
Phase I	VFA feed	0.86
	POME feed	1.30
Phase II	Starch feed	1.08
	POME feed	1.08

Table 4. Adjustment for OLR

Phase	Feed type	[gCOD·L^{-1}·d^{-1}]
Phase I	VFA feed	1.2
	POME feed	1.8
Phase II	Starch feed	2.5
	POME feed	1.8

2.2.2 Feed Preparation

In order to minimize the conglomeration of lipids in the POME feed, it was prepared every day during the workdays. The target COD concentration of the POME feed was 9 gCOD/L during the first phase of the experiment, and 10.83 gCOD/L during the second phase. In order to achieve the target COD concentrations, the pure palm oil (6.67 g for daily dose of Phase I and 8.02 g for daily dose of Phase II) was diluted with demineralized water (1.1 L for daily dose of Phase I and Phase II). Then, the mix of palm oil and water was sonicated for 35 minutes at 40% amplitude. After sonication, the sample was shaken for 24 hours at 55 °C and 150 rpm. The sample was taken out of the shaker on the next day, and filtrated with a 0.103 mm sieve. Finally, the filtrate was diluted according to its COD value.

The ingredients for VFA feed and starch feed have been shown in Appendix A (see Table A.1-A.5). The purpose of changing the VFA feed to the starch feed during the second phase of the experiment was to (1) simulate the carbohydrate content in the real POME (POME found in a practical situation), and (2) assess the effect of carbohydrate addition on the biomass growth. The phosphate buffer solution, macronutrient solution and micronutrient solution were added to the VFA feed and the starch feed as nutrient supplements. The COD:N:P ratio in the VFA feed was 140:5:1. When the VFA feed was replaced by the starch feed, the amount of nutrient supplements added per liter of feed remained the same, while starch and sodium bicarbonate (as a pH buffer) were used instead of VFAs (sodium acetate trihydrate, sodium propionate, and sodium butyrate). During the first phase of the experiment, 7 L of VFA was prepared weekly. The VAF feed was transitioned to the starch feed during the second phase of the experiment. In the beginning of the second phase (start-up phase), OLR was increased gradually, while the starch feed was prepared almost every day during the workdays (see Table A.5).

2.2.3 Operating Procedure

The key parameters related to the hypotheses were measured periodically. The detailed measurement procedures have been explained in the following sections (2.2.4-2.2.10). Additionally, daily monitoring and calibration were required (except for holidays and weekends). The POME feed and the VFA/starch feed were pumped to the anaerobic digester. The sludge was pumped from the bottom of the digester, into the UF unit, in order to create a cross-flow (recirculation flow). Finally, permeate was pumped out of the UF unit to the collection tank. The flowrates of these pumps were adjusted daily (except for the recirculation pump), in order to ensure that (1) the effective volume of

the digester was kept constant at 6.5 L, and (2) the flowrates of the influent and the effluent streams were controlled at the desired level (see Table 3). In order to maintain SRT of 140 days, 65 mL of sludge was also extracted from the digester every day during the workdays. The pH of the sludge inside of the digester, and the pH of the sludge sample extracted from the digester were both measured daily as well.

2.2.4 Measurement Procedure for COD

COD was measured in triplicates on Monday, Wednesday and Friday. Samples collected for the COD measurement were: POME feed, VFA/starch feed, unfiltered sludge (for total COD), supernatant of sludge, and filtered sludge (for soluble COD). The supernatant of sludge was obtained via centrifuge (18500 g for 10 minutes). Then, the supernatant was filtered with 0.2 μm filters, and the filtrate was collected as the filtered sludge for soluble COD measurement. The measuring range for each sample was determined by the estimated value. Dilution was also required when the estimated value exceeded the maximum measuring range available in the lab. After the samples were added to the test vials, a vortex mixer was used to mix the test vials, before they were transferred to the DRB200 reactor which had been preheated to 148 °C. After 2 hours of heating, the test vials were taken out of the reactor, inverted for three times, and let cool to room temperature. The DR3900 spectrophotometer was used in the end for the COD reading of each test vial.

The COD concentrations measured in this experiment were further used to calculate the COD removal efficiency based on the equation shown in Appendix B (see section B.1), in which case, the influent COD was the total daily COD of the POME feed and the VFA/starch feed, whereas the effluent COD was the daily COD of the permeate. The flowrates were controlled throughout the experiment (see Table 3).

2.2.5 Measurement Procedure for Solids

Solids in the sludge as well as the permeate were measured once a week. Total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS) were measured for the sludge sample in order to monitor the biomass growth in the sludge, while only TSS and VSS were measured for the permeate sample to ensure that the permeate (effluent) had low turbidity. Measurements of solids were also conducted in triplicates. The volume of each sample (sludge and permeate) used for the measurement has been shown in Table 5. The formulas to determine TS, VS, TSS, and VSS have been listed in Appendix B (see section B.2). The measurement results of VSS were used for biomass net growth calculations, which has been expressed in section B.3.

Table 5. Volume of Sample for Solids Measurement

Sample	Volume of Sample	Measured Parameters
Sludge	5 mL	TS, VS, TSS, VSS
Permeate	50 mL	TSS, VSS

2.2.6 Measurement Procedure for Biogas Production

The biogas produced by the digester was measured via a biogas meter. The measurement results were recorded once every day at noon. The biogas meter was reset to zero immediately after the reading was recorded on Friday. It was assumed that the production rate of biogas would be linear over the weekends, so that on Monday, the daily production of biogas was the accumulated biogas production divided by 3 days. The results presented in this paper for biogas production were not normalized with regard to temperature.

2.2.7 Measurement Procedure for SMA

The specific methanogenic activity (SMA) test was designed based on the parameters shown in Table 6. At least 600 mL of sludge was required for the test. Therefore, a few weeks before the VFA feed was changed to the starch feed, part of the sludge extracted from the digester would be saved on daily basis and stored in the designated container inside of a refrigerator. Once the total volume of the sludge exceeded 600 mL, it was mixed with a magnetic mixer before the VSS value was measured. SMA describes how capable the sludge is, in terms of producing methane using a specific substrate (Hussain & Dubey, 2017). Given the VSS value of the sludge determined in prior to the test (1.55 gVSS/L), SMA could be expressed in $gCH4\text{-}COD \cdot gVSS^{-1} \cdot day^{-1}$, which was calculated based on the maximum representative slope in the SMA graph. The detailed procedure to calculate the SMA has been explained in Appendix B (see section B.4).

Table 6. Design Parameters of the SMA Test

Parameter	Value	Unit
Temperature	55 ± 1	°C
Total volume	100	mL
SMA VSS	1.55	gVSS/L
SMA COD	2	gCOD/L
$C_2H_9NaO_5$	4.26	g/L
$C_2H_9NaO_5$	0.43	g per bottle
Nutrient Mix	2.12	g per bottle
Test Period	5 - 7	days

2.2.8 Measurement Procedure for GC Biogas

In addition to biogas production, biogas composition was measured as well on Monday, Wednesday, and Friday, via gas chromatography (GC) for biogas. During each measurement, 4 syringes (each with empty volume of 100 mL) was filled with biogas collected from the digester. Then, the biogas from each syringe was injected to the GC device (1 syringe for flushing and 3 syringes for triplicates). The measurement results were recorded by the GC device automatically.

2.2.9 Measurement Procedure of GC VFA

Gas chromatography (GC) was used not only for biogas composition, but also for VFA composition. The filtered sludge (the same sample used for soluble COD measurement) and the permeate were analyzed in terms of VFA composition on Monday, Wednesday, and Friday. The concentrations of VFA were monitored mainly to avoid acidification. During each measurement, 8 glass vials were used for sampling: 2 as blank samples, 3 as sludge samples, and 3 as permeate samples. First, 750 µL of ultra-pure water (UPW) was added to each blank sample, 750 µL of filtered sludge was added to each sludge sample, and 750 µL of permeate was added to each permeate sample. Then, 750 µL of pentanol (320 mg/L) and 10 µL of formic acid (99% v/v) were added to each of the 8 samples. Samples prepared on Monday, Wednesday, and Friday (24 samples) were placed in the GC device, which had been programed to measure and record the VFA concentrations automatically.

2.2.10 Measurement Procedure for LCFA

The method for LCFA measurement using the Waters Xevo TQ-S device for liquid chromatography/mass spectrometry (LC/MS) was not pre-defined. The method used in this project was still under the development phase. The steps can be divided into two stages. During the first stage, the organic phase of each sample was prepared according to the steps shown in Appendix B (see section B.5). During the second stage, different amounts of filtered organic phase were added to the new glass vials (each with empty volume of 1.5 mL), along with the internal standard mix (ISTD) and methanol. The specific dose of each ingredient can be found in Appendix A (see Table A.7-A.9) for the second preparation stage of the LCFA samples.

2.2.11 Changes to the VFA Feed

The AnMBR has been operated by other researchers from Day 1 to Day 341 under different experimental conditions. Satisfactory results were achieved at SRT of 90 days and 140 days, with OLR up to 2.8 $gCOD \cdot L^{-1} \cdot d^{-1}$. In this experiment, Day 342 to Day 377 is defined as Phase I, while Day 380 to Day 407 is defined as Phase II. The VFA feed was changed to the cellulose feed on Day 377 (as indicated by the dash line on the left in Figure 3-12 for the end of Phase I). On Day 379, the cellulose feed was changed back to the VFA feed due to precipitation of cellulose even after mixing was applied. On Day 380 (as indicated by the middle dash line in Figure 3-12 for the beginning of Phase II), the VFA feed was replaced by the starch feed as a substitute for cellulose, because the starch used in this experiment was soluble in water, unlike the cellulose.

2.2.12 Changes to the Flowrate and OLR

Changes to the flowrate (see Table 3) and OLR (see Table 4) were made on Day 390 after the VFA feed had been replaced by the starch feed already (as indicated by the dash line on the right in Figure 3-12 for changes to the flowrate and OLR). Although, the results for Day 390 were measured before the flowrates and the OLRs were changed.

3. Results

3.1 COD Removal

The COD removal efficiency during Phase I and Phase II of the experiment has been illustrated in Figure 3. During Phase I, the POME feed and the VFA feed were added to the bioreactor. The corresponding COD removal efficiency fluctuated between 98% and 99%. During Phase II, the VFA feed was replaced by the starch feed, and the COD removal efficiency became unstable as expected, but it was still above 95% in general.

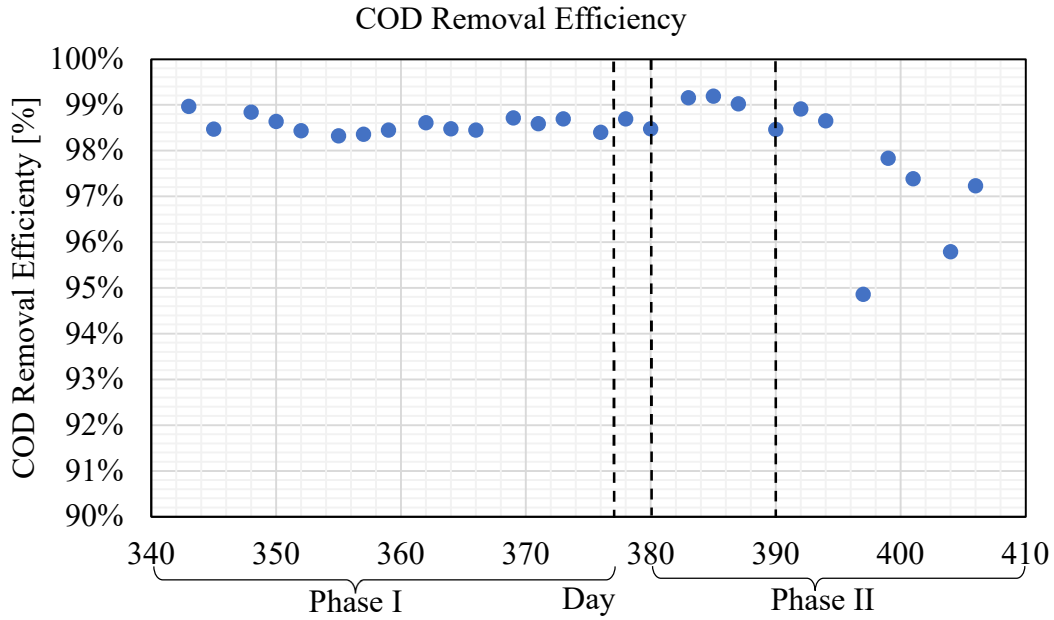

Figure 3. COD Removal Efficiency during Phase I and Phase II

3.2 Biomass Growth

Biomass net growth was monitored during both phases of the experiment. Each data point in Figure 4 was calculated based on the maximum range of the dataset that was available under the same feeding condition in terms of feed type and flowrate.

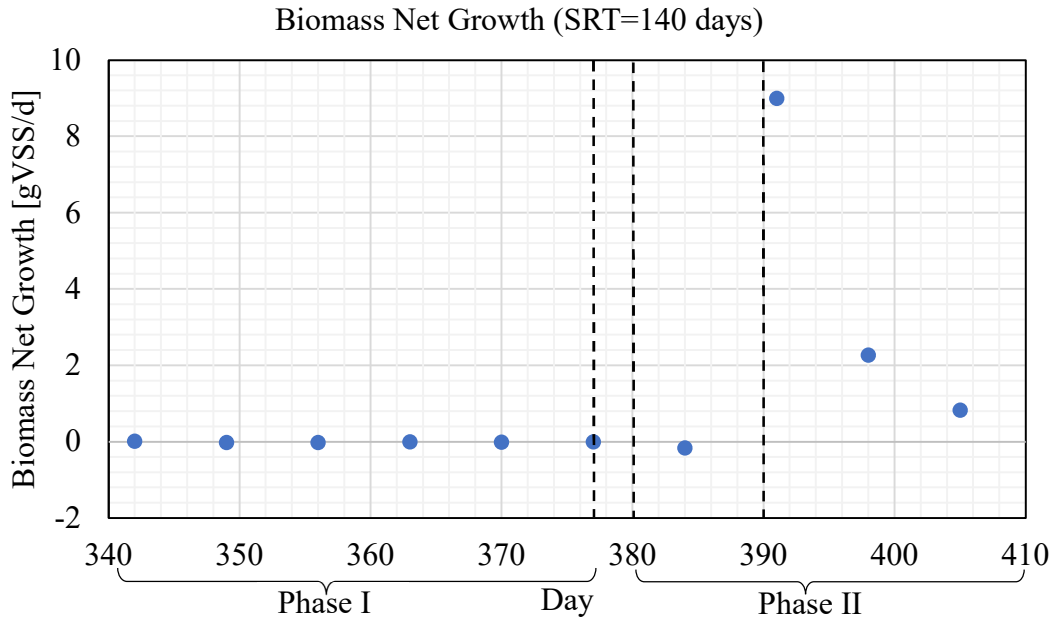

Figure 4. Biomass Net Growth during Phase I and Phase II

Before the VFA feed was replaced by the starch feed, the biomass net growth was around zero gVSS/d. However, as mentioned in section 2.2.3, 65 mL of sludge was extracted from the bioreactor every day during the workdays, which could be considered as the sludge yield of the bioreactor. During the second phase, after starch was added to the bioreactor instead of VFAs and the flowrate was increased to 1.08 L/day on Day 390, a significant increase in the net growth of biomass was observed on the next day. However, this increase could also be attributed to the accumulation of starch on the biomass.

3.3 Biogas Production

According to Figure 5, the biogas production ranged between 6.0 and 7.2 L per day during Phase I, and dropped to 4 L per day at the beginning of Phase II. Then, starting from Day 385, the biogas production increased steadily from 4.0 to 12.0 L per day. Although, on Day 404, the biogas production decreased. This phenomenon was further investigated via pH analysis during both phases of the experiment. In Figure 6, the measured pH refers to the pH value measured by an external pH meter (WTW IDS 9430), whereas the monitored pH is the pH value that was constantly monitored by the internal pH meter connected to the bioreactor. On Day 385 and Day 404, when the biogas production was low, pH of the sludge inside of the bioreactor was high.

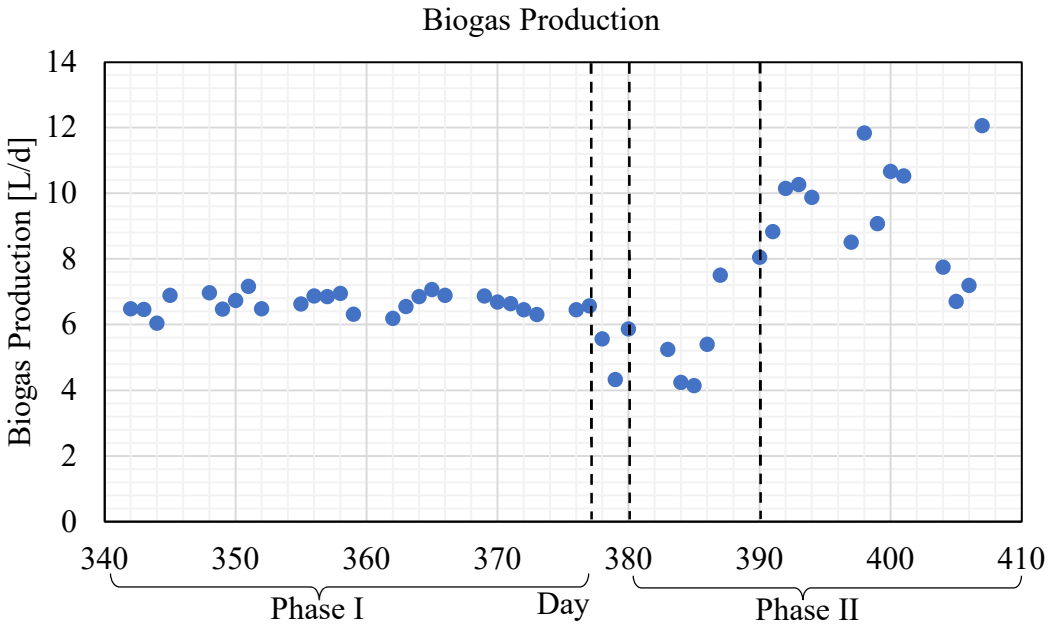

Figure 5. Biogas Production during Phase I and Phase II

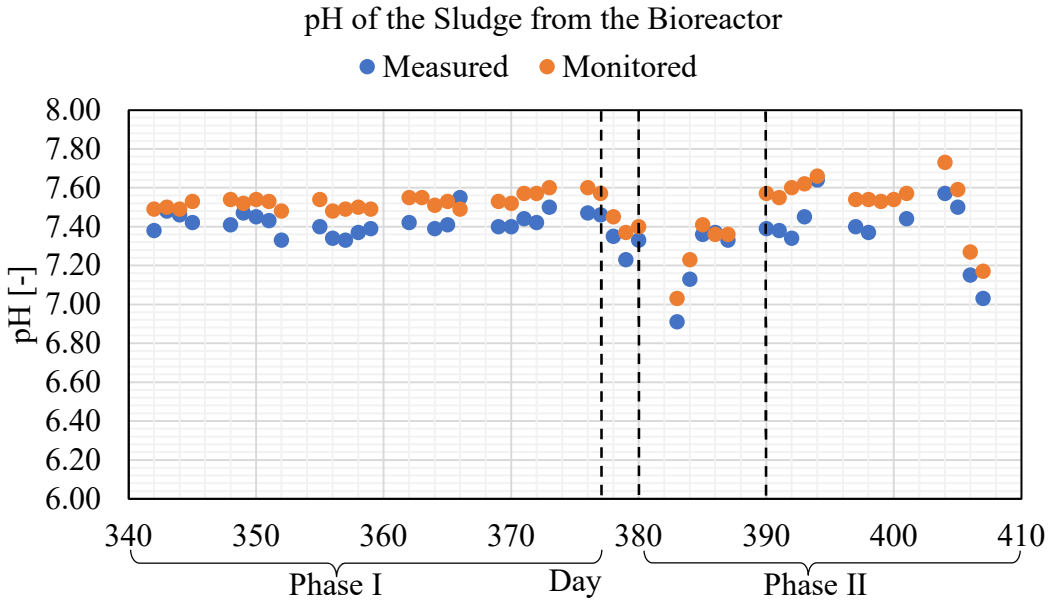

Figure 6. Change in pH of the Sludge during Phase I and Phase II

Besides the amount of biogas that the bioreactor was able to produce, the composition of the biogas was analyzed as well. Figure 7 shows the change in biogas composition depending on the CH_4 and CO_2 concentrations in the biogas that was collected from the bioreactor. During Phase I, about 75% of the biogas produced was CH_4, and the remaining 25% was CO_2. During Phase II, CH_4 and CO_2 tended to have equal compositions in the biogas.

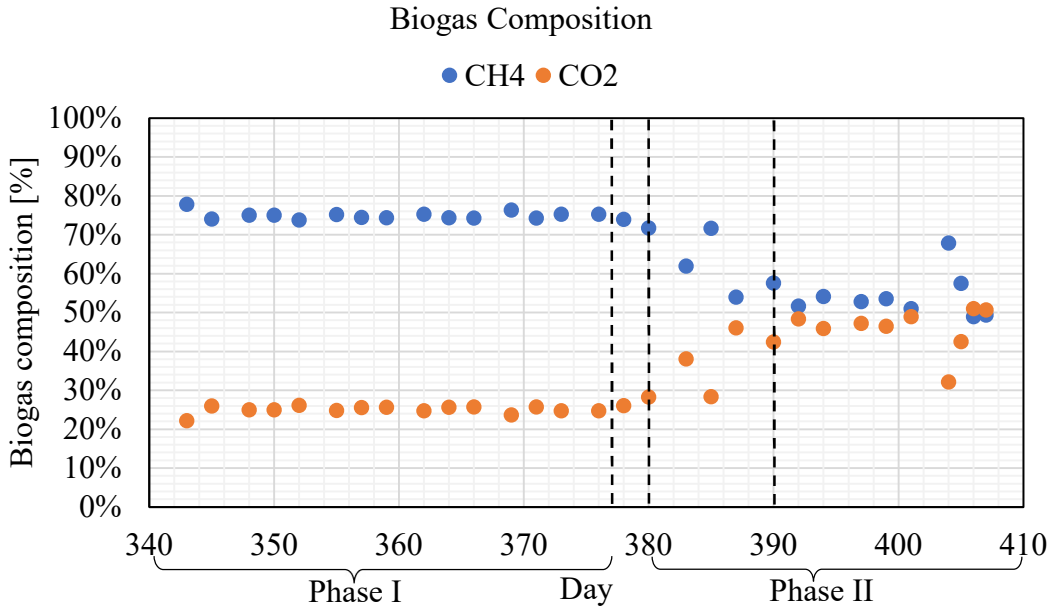

Figure 7. Biogas Composition during Phase I and Phase II

Based on the biogas production data and the biogas composition data, the change in methane production overtime was able to be determined (see Figure 8). During Phase I, the methane production was consistent around 5 L per day. On Day 385, when the biogas production was low, the methane production had a low peak as well. Therefore, the acidification that occurred at the beginning of Phase II might have inhibited the methane production. The molar ratio between $NaHCO_3$ and starch was increased from 2.1 to 3 on Day 386 to prevent further decrease in pH (see Table A.5 in Appendix A). Consequently, the methane production increased from 3 L per day on Day 385, to 4 L per day on Day 387. On Day 404, even though the biogas production dropped, the methane production did not, because the CH_4 concentration in the biogas increased while the CO_2 concentration decreased at a higher pH (see Figure 7). A lower $NaHCO_3$ to starch ratio was applied on Day 404 in order to reduce the pH, which resulted in the low methane production for the next two days. However, after the $NaHCO_3$ to starch ratio was increased again on Day 406, the methane production increased to 6 L per day on Day 407.

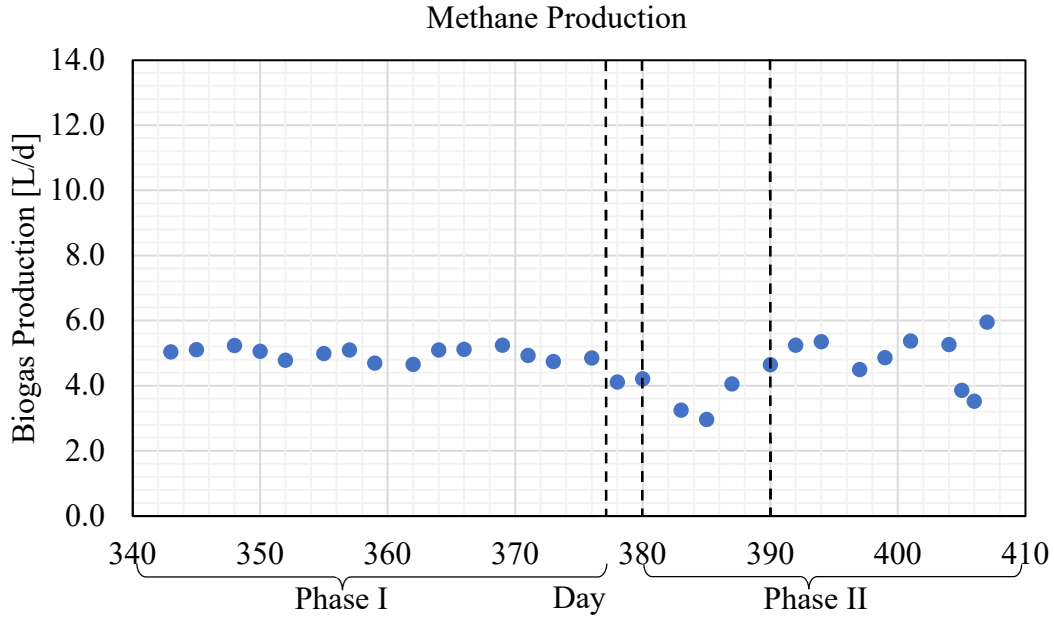

Figure 8. Methane Production during Phase I and Phase II

3.4 Digestion Efficiency

The digestion efficiency in terms of methane production, as shown in Figure 9, was between 70% and 85% during Phase I, and fluctuated at a lower percentage (around 50%) during Phase II. Although, positive biomass net growth was observed during Phase II, suggesting that more of the feed COD was converted for biomass growth during this period. According to Figure 10, during Phase I, the mass ratio between lipid and VSS was between 0.2 and 0.3; during Phase II, the range of ratio became larger (between 0.09 and 0.52). The data points in Figure 9 and Figure 10 suggest that when the digestion efficiency was high, the lipid to VSS ratio was low, and vice versa.

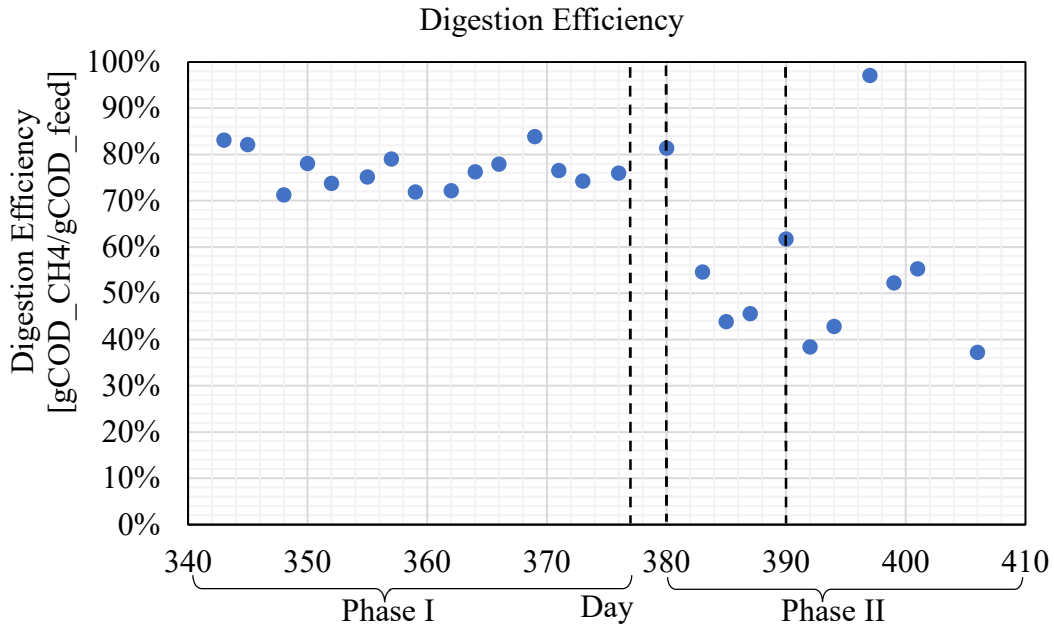

Figure 9. Digestion Efficiency during Phase I and Phase II

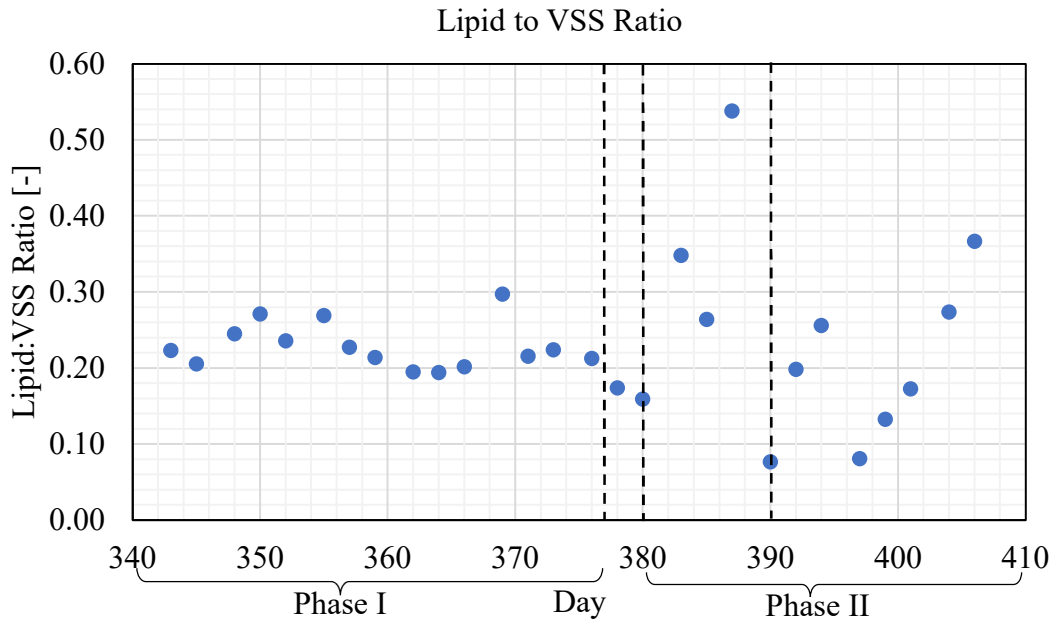

Figure 10. Lipid to VSS Ratio during Phase I and Phase II

The SMA results were also assessed in order to verify the performance of the bioreactor in terms of digestion efficiency. The black dash line in Figure 11 represents the SMA slope, which was approximately 0.57 gCH4-COD·gVSS^{-1}·d^{-1} at 55 °C using sodium acetate trihydrate ($C_2H_9NaO_5$) as the substrate for simulation of the methanogenic biodegradation process.

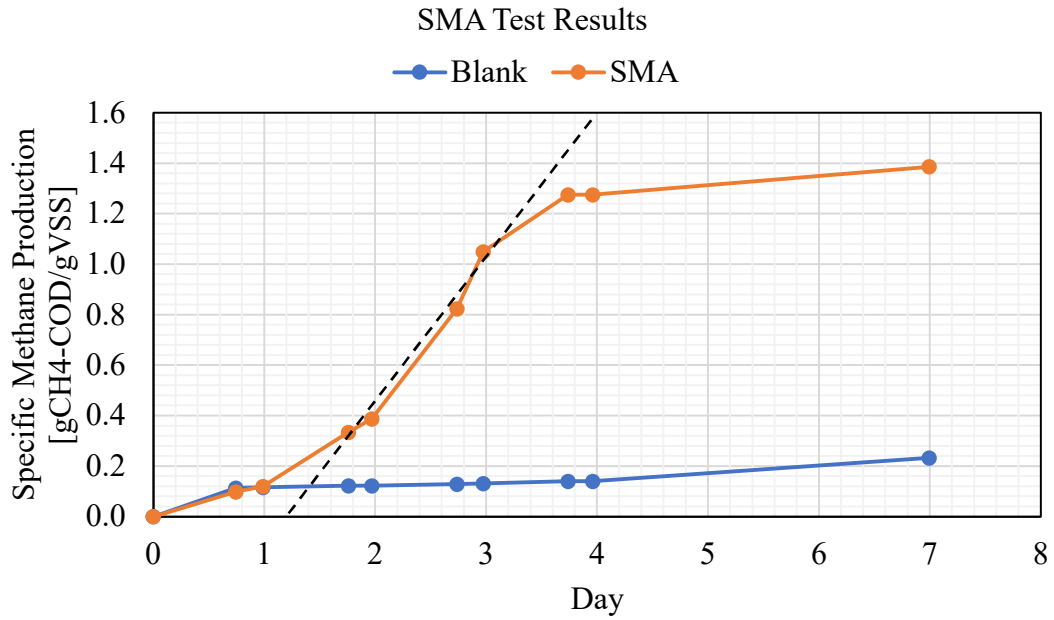

Figure 11. Specific Methanogenic Activity (SMA) Test Results

3.5 Volatile Fatty Acids (VFA) Accumulation

According to the GC-VFA test results, acetic acid was the dominant VFA in both sludge and permeate samples, followed by propionic acid. Therefore, the concentrations of acetic acid were plotted in Figure 12 for both phases of the experiment to check if there was any occurrence of VFA accumulation. During Phase I, both sludge and permeate samples had around 75 mg/L of acetic acid. During Phase II, VFA accumulation was observed. The accumulation rate based on the acetic acid concentration was about 8 to 9 mg/L per day for both sludge and permeate.

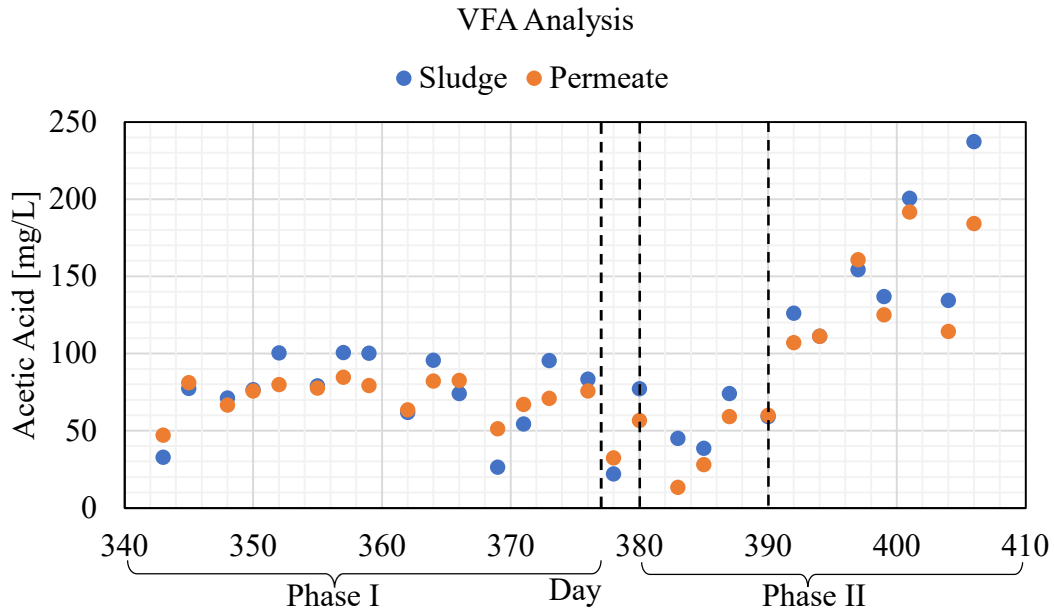

Figure 12. VFA Analysis during Phase I and Phase II

3.6 Long Chain Fatty Acids (LCFA) Analysis

Three LCFA tests were conducted during Phase II in order to obtain the reliable experimental results for LCFA concentrations and compositions in the original samples (collected from the AnMBR system). The results of these tests have been summarized in Table 7 to Table 9. In comparison to the 1st test, the 2nd test and the 3rd test had much smaller standard deviation (STD) and relative standard deviations (RSTD), meaning the 2nd test and the 3rd test had more reliable results than the 1st test. However, in the 3rd test, linolenic acid (C18:3) was the dominant LCFA in both sludge samples and POME samples, instead of palmitic acid, which was contradictory to the results reported by other researchers (Ahmad, 2011), where palmitic acid was the dominant LCFA in raw POME, followed by myristic acid and stearic acid. Therefore, only the results of the 2nd test were acknowledged for LCFA related calculations in this experiment.

Table 7. Results of the 1st LCFA Test w.r.t. Concentration and Composition

Compound	Capric Acid	Tridecanoic Acid (IS)	Myristic Acid	Palmitic Acid	Linolenic Acid (C18:3)	Linoleic Acid (C18:2)	Oleic Acid	Stearic Acid
Formula	$C_{10}H_{20}O_2$	$C_{13}H_{26}O_2$	$C_{14}H_{28}O_2$	$C_{16}H_{32}O_2$	$C_{18}H_{30}O_2$	$C_{18}H_{32}O_2$	$C_{18}H_{34}O_2$	$C_{18}H_{36}O_2$
Unit	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
S1	0.00	4.44E-03	0.00	40.79	0.00	2.74	8.82	0.00
S2	0.00	1.70E-03	3.70	35.40	0.00	0.67	3.60	0.00
S3	0.00	5.61E-04	0.37	20.58	0.00	0.00	1.74	13.32
Average	0.00	2.23E-03	1.36	32.26	0.00	1.14	4.72	4.44
STD	0.00	1.99E-03	2.03	10.47	0.00	1.43	3.67	7.69
RSTD	-	89.33%	149.95%	32.45%	-	125.77%	77.70%	173.21%
P1	0.00	1.36E-02	0.00	115.21	10.55	2.07	16.50	0.00
P2	0.00	4.51E-03	0.00	67.94	44.91	0.00	14.65	0.00
P3	0.00	4.61E-04	0.48	22.21	0.00	0.24	1.82	10.29
Average	0.00	6.19E-03	0.16	68.45	18.49	0.77	10.99	3.43
STD	0.00	6.72E-03	0.28	46.50	23.48	1.13	8.00	5.94
RSTD	-	108.64%	173.21%	67.94%	127.02%	146.89%	72.79%	173.21%
LCFA composition in sludge	0.00%	0.01%	3.09%	73.46%	0.00%	2.59%	10.75%	10.11%
LCFA composition in POME	0.00%	0.01%	0.16%	66.92%	18.07%	0.75%	10.74%	3.35%

Table 8. Results of the 2nd LCFA Test w.r.t. Concentration and Composition

Compound	Capric Acid	Tridecanoic Acid (IS)	Myristic Acid	Palmitic Acid	Linolenic Acid (C18:3)	Linoleic Acid (C18:2)	Oleic Acid	Stearic Acid
Formula	$C_{10}H_{20}O_2$	$C_{13}H_{26}O_2$	$C_{14}H_{28}O_2$	$C_{16}H_{32}O_2$	$C_{18}H_{30}O_2$	$C_{18}H_{32}O_2$	$C_{18}H_{34}O_2$	$C_{18}H_{36}O_2$
Unit	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
S1	0.00	2.09E-03	0.39	0.75	0.00	0.05	0.16	0.43
S2	0.00	1.89E-03	0.39	0.78	0.00	0.07	0.15	0.44
S3	0.00	1.94E-03	0.44	0.76	0.00	0.06	0.13	0.43
Average	0.00	1.97E-03	0.40	0.76	0.00	0.06	0.15	0.43
STD	0.00	0.00	0.03	0.02	0.00	0.01	0.02	0.00
RSTD	-	5.34%	6.86%	2.09%	-	12.73%	12.63%	0.87%
P1	0.00	3.19E-03	0.66	1.04	0.00	0.09	0.22	0.60
P2	0.00	1.55E-03	0.75	1.50	0.00	0.11	0.16	0.78
P3	0.00	1.54E-03	0.78	1.57	0.00	0.10	0.20	0.77
Average	0.00	2.09E-03	0.73	1.37	0.00	0.10	0.19	0.72
STD	0.00	9.49E-04	0.06	0.29	0.00	0.01	0.03	0.10
RSTD	-	45.34%	8.14%	21.20%	-	10.55%	13.79%	14.64%
LCFA composition in sludge	0.00%	0.11%	22.32%	42.16%	0.00%	3.29%	8.18%	23.95%
LCFA composition in POME	0.00%	0.07%	23.43%	44.03%	0.00%	3.19%	6.26%	23.02%

Table 9. Results of the 3rd LCFA Test w.r.t. Concentration and Composition

Compound	Capric Acid	Tridecanoic Acid (IS)	Myristic Acid	Palmitic Acid	Linolenic Acid (C18:3)	Linoleic Acid (C18:2)	Oleic Acid	Stearic Acid
Formula	$C_{10}H_{20}O_2$	$C_{13}H_{26}O_2$	$C_{14}H_{28}O_2$	$C_{16}H_{32}O_2$	$C_{18}H_{30}O_2$	$C_{18}H_{32}O_2$	$C_{18}H_{34}O_2$	$C_{18}H_{36}O_2$
Unit	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
S1	0.00	2.09E-03	0.29	0.64	3.59	0.06	0.23	0.33
S2	0.00	1.98E-03	0.30	0.64	8.16	0.06	0.21	0.34
S3	0.00	2.19E-03	0.29	0.50	10.62	0.06	0.13	0.18
Average	0.00	2.09E-03	0.29	0.59	7.46	0.06	0.19	0.28
STD	0.00	0.00	0.01	0.08	3.57	0.00	0.05	0.09
RSTD	-	5.15%	2.46%	14.01%	47.86%	5.12%	26.83%	31.04%
P1	0.00	2.84E-03	0.31	0.87	13.90	0.11	0.18	0.37
P2	0.00	2.67E-03	0.44	1.01	13.96	0.11	0.24	0.40
P3	0.00	2.96E-03	0.35	0.85	17.37	0.12	0.28	0.38
Average	0.00	2.82E-03	0.37	0.91	15.07	0.11	0.23	0.38
STD	0.00	1.43E-04	0.07	0.09	1.99	0.01	0.05	0.01
RSTD	-	5.08%	18.94%	9.75%	13.19%	5.37%	20.63%	3.51%
LCFA composition in sludge	0.00%	0.02%	3.29%	6.67%	83.99%	0.67%	2.18%	3.19%
LCFA composition in POME	0.00%	0.02%	2.15%	5.32%	88.27%	0.64%	1.37%	2.24%

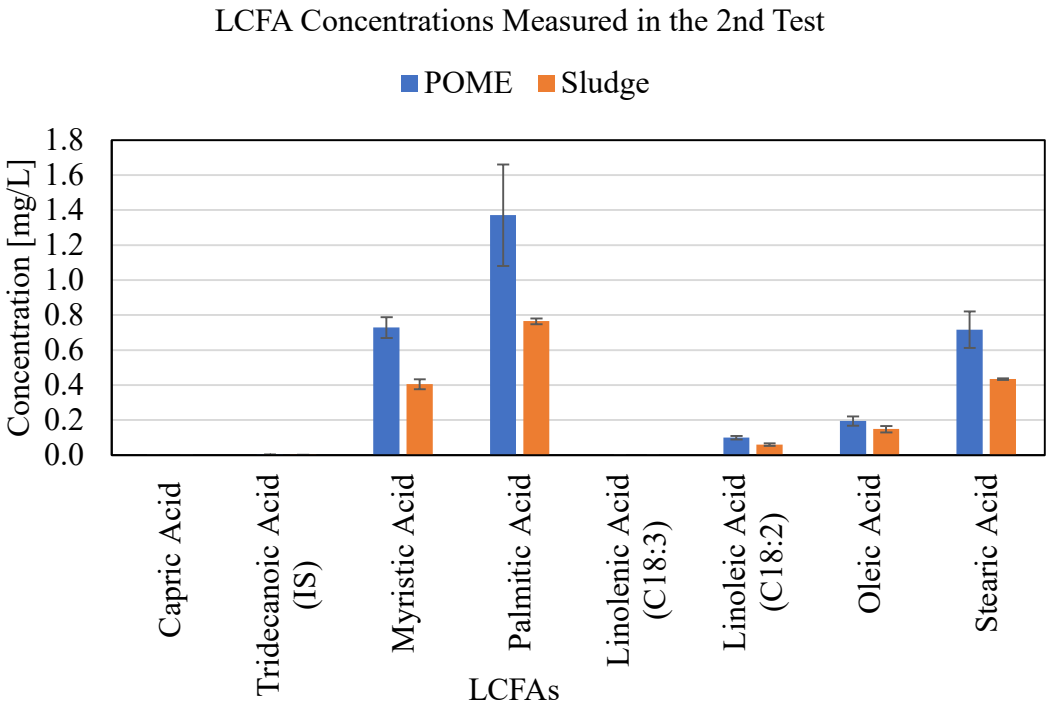

Figure 13. Analysis on LCFA Concentrations Measured in the 2nd Test

The measurement results of the 2nd test have also been plotted in Figure 13. For each of the five LCFAs detected in the POME and sludge samples, the degradation efficiency, i.e., the amount of LCFA that had been degraded in comparison to the total LCFA in POME was calculated as well (see Table 10).

Table 10. Degradation Efficiency

LCFAs	Degradation Efficiency
Myristic Acid	45%
Palmitic Acid	44%
Linoleic Acid (C18:2)	40%
Oleic Acid	24%
Stearic Acid	39%

LCFAs might also precipitate in the sludge due to the presence of divalent cations. In this experiment, only Mg^{2+} was able to cause LCFA precipitation. According to Table A.2, 7.23 mL of the macronutrient solution per 1 L of VFA/starch feed was added to the bioreactor. The flowrate of the VFA feed was 0.86 L per day until Day 390. After Day 390, the flowrate of the starch feed was 1.08 L per day. Therefore, the macronutrient solution that entered the bioreactor was 6.22 mL/d before Day 390 (Day 390 included) and 7.81 mL/d after Day 390. In the macronutrient solution, the concentration of $MgSO_4 \cdot 7H_2O$ was 9 g/L (see Table A.3), or 0.0365 mmol/mL when the molar weight of $MgSO_4 \cdot 7H_2O$ was applied (246.37 g/mol). Based on the 1:1 molar ratio of $MgSO_4 \cdot 7H_2O$ and Mg^{2+}, the amount of Mg^{2+} added to the bioreactor was 0.227 mmol/d before Day 390 and 0.285 mmol/d after Day 390.

In theory, the stoichiometric ratio between LCFA and the sum of divalent cations should be 2:1, but in practice, only 1.8:1 was achieved (Dereli et al., 2014). The amount of LCFA that precipitated in the sludge under the influence of Mg^{2+} could be estimated using the practical molar ratio. As a result, LCFA precipitation was 0.409 mmol/d before Day 390 and 0.513 mmol/d during after Day 390. In order to convert these values to gCOD/d, the molar weight (see Table 11) and the theoretical oxygen demand (see Table 12) of LCFA in sludge were calculated based on the LCFA compositions provided by the 2nd test. The amounts of LCFA precipitated in sludge before Day 390 and after Day 390 were calculated as follows:

$$Before\ Day\ 390 = \frac{0.409\ \text{mmol}}{d} \times \frac{259.8\ g}{mol} \times \frac{mol}{1000\ mmol} \times \frac{2.87\ gCOD}{g} = 0.305 \frac{gCOD}{d}$$

$$After\ Day\ 390 = \frac{0.513\ \text{mmol}}{d} \times \frac{259.8\ g}{mol} \times \frac{mol}{1000\ mmol} \times \frac{2.87\ gCOD}{g} = 0.383 \frac{gCOD}{d}$$

Table 11. Molar Weight (MW) of LCFA in Sludge (2nd Test)

LCFA	Formula	MW [g/mol]	Composition	Weighted MW [g/mol]
Capric Acid	$C_{10}H_{20}O_2$	172.26	0.0%	0.0
Tridecanoic Acid (IS)	$C_{13}H_{26}O_2$	214.34	0.1%	0.2
Myristic Acid	$C_{14}H_{28}O_2$	228.37	22.3%	51.0
Palmitic Acid	$C_{16}H_{32}O_2$	256.42	42.2%	108.1
Linolenic Acid (C18:3)	$C_{18}H_{30}O_2$	278.40	0.0%	0.0
Linoleic Acid (C18:2)	$C_{18}H_{32}O_2$	280.45	3.3%	9.2
Oleic Acid	$C_{18}H_{34}O_2$	282.50	8.2%	23.1
Stearic Acid	$C_{18}H_{36}O_2$	284.50	23.9%	68.1
			Total:	259.8

Table 12. Theoretical Oxygen Demand (ThOD) of LCFA in Sludge (2nd Test)

LCFA	Formula	ThOD [g/mol]	Composition	Weighted ThOD [g/g]
Capric Acid	$C_{10}H_{20}O_2$	2.60	0.0%	0.00
Tridecanoic Acid (IS)	$C_{13}H_{26}O_2$	2.76	0.1%	0.00
Myristic Acid	$C_{14}H_{28}O_2$	2.80	22.3%	0.63
Palmitic Acid	$C_{16}H_{32}O_2$	2.87	42.2%	1.21
Linolenic Acid (C18:3)	$C_{18}H_{30}O_2$	2.82	0.0%	0.00
Linoleic Acid (C18:2)	$C_{18}H_{32}O_2$	2.85	3.3%	0.09
Oleic Acid	$C_{18}H_{34}O_2$	2.89	8.2%	0.24
Stearic Acid	$C_{18}H_{36}O_2$	2.92	23.9%	0.70
			Total:	2.87

3.7 COD Balance

The COD balance of the overall treatment system has been demonstrated in Figure 14 and Figure 15, for Phase I and Phase II respectively. Factors considered for the COD balance analysis were:

- **Methane**: COD of methane was the major effluent COD in the system. Methane produced by the bioreactor was converted from L/day to gCOD/day based on 0.35 gCOD/L at standard temperature and pressure (STP) conditions.
- **Sludge sample**: 65 mL of sludge was extracted from the bioreactor every day during the workdays. It was converted to gCOD per workday according to the daily measurement result of the total COD in the sludge sample. Then, the COD output as a result of sludge extraction was normalized to gCOD/day throughout the whole week.
- **Permeate**: In order to determine the amount of COD remaining in the permeate stream, the daily COD measurement of the permeate stream (gCOD/L) was multiplied by the daily flowrate measurement of the permeate stream after the effluent pump had been calibrated (L/day), which would give the COD output that remains in the permeate stream (gCOD/day).
- **LCFA precipitation**: LCFA precipitation has been determined in section 3.6, which was 0.305 gCOD/day before Day 390 (Day 390 included), and 0.383 gCOD/day after Day 390.
- **POME conglomeration**: POME conglomeration was considered for the COD decline due to formation of clumps in the POME feed. An independent test was conducted in order to determine the rate of COD decline in the POME feed. The test result suggested that, 5.57% of the POME COD was reduced within a day. The OLR of the POME feed was controlled around 1.8 $gCOD \cdot L^{-1} \cdot d^{-1}$ while the volume of sludge was controlled at 6.5 L throughout the entire experiment. Therefore, after the POME COD was measured, approximately 0.652 g of COD could be further reduced each day because of conglomeration.
- **Adsorption of POME and starch on tubes**: Lipids and starch might also accumulate on the inner walls of the tubes depending on the corresponding absorptivity. Another independent test was conducted where some parts of the feeding tubes were replaced with new segments. The original weight of each segment and the final weight after 7 days were recorded. Based on the test results, 0.572 $g \cdot m^{-2} \cdot day^{-1}$ of POME and 2.174 $g \cdot m^{-2} \cdot day^{-1}$ of starch would be adsorbed on the feeding tubes. Along with other parameters as shown in Table 13, the daily loss of COD due to adsorption of POME and starch on tubes were estimated for each phase of the experiment: 0.053 gCOD/day for Phase I (only POME was considered), and 0.225 gCOD/d for Phase II (both POME and starch were considered).
- **Dissolved methane**: Part of the methane produced by the bioreactor might dissolve in the sludge. The COD contained in the dissolved methane was also considered for the COD balance.
- **Gap**: The COD gap was calculated as the difference between the total influent COD (the POME feed COD plus the VFA/starch feed COD) and the total effluent COD (when all the factors stated above were considered).

Table 13. Daily Loss of COD due to Adsorption of POME and starch on Tubes

Parameters	POME feed with small tubes	Starch feed with small tubes	Starch feed with large tubes
Measured Loss [g·m^{-2}·day^{-1}]	0.572	2.174	2.174
Inner Diameter [mm]	5.30	5.30	6.81
Total Length [m]	2.060	2.135	1.810
Total SA [m^2]	0.034	0.036	0.039
Daily Loss [g/d]	0.020	0.077	0.084
COD [gCOD/g]	2.700	1.067	1.067
Daily Loss [gCOD/d]	0.053	0.082	0.090

Furthermore, about the dissolved methane which could also contribute to the COD that was missing in the system, the calculation method has been explained in details, where Henry's Law (C = K·P) was used in this case to determine the concentration of methane dissolved in the sludge, and Henry's constant (K) was adjusted according to the sludge temperature inside of the bioreactor using the van 't Hoff equation:

$$\frac{d\ lnK}{d\ (\frac{1}{T})} = \frac{-\Delta_{sol}H}{R}$$

$$d\ lnK = \frac{-\Delta_{sol}H}{R}\, d\ (\frac{1}{T})$$

$$lnK_{T_2} - lnK_{T_1} = \frac{-\Delta_{sol}H}{R}(\frac{1}{T_2} - \frac{1}{T_1})$$

$$\ln\ (\frac{K_{T_2}}{K_{T_1}}) = \frac{-\Delta_{sol}H}{R}(\frac{1}{T_2} - \frac{1}{T_1})$$

$$\frac{K_{T_2}}{K_{T_1}} = \exp\left[\frac{-\Delta_{sol}H}{R}(\frac{1}{T_2} - \frac{1}{T_1})\right]$$

$$K_{T_2} = K_{T_1} \cdot \exp\left[\frac{-\Delta_{sol}H}{R}(\frac{1}{T_2} - \frac{1}{T_1})\right]$$

$where$:

$K = Henry's\ constant\ \left[\frac{mol}{m^3 \cdot Pa}\right]$

$\Delta_{sol}H = enthalpy\ of\ dissolution\ \left[\frac{J}{mol}\right]$

$R = ideal\ gas\ constant\ \left[\frac{J}{mol \cdot K}\right]$

$T_1 = reference\ temperature\ [298.15\ K]$

$T_2 = adjusted\ temperature\ [K]$

1600 K was used for $(-\Delta\_sol\,H)/R$, while 1.4×10^{-5} $[mol/(m\text{^}3 \cdot Pa)]$ was used for K_{T_1} i.e., Henry's constant at the reference temperature (Sander, 2015). Therefore, K_{T_2} at 55 °C could be calculated as:

$$K_{T_2} = 1.4 \times 10^{-5} \cdot \exp\left[1600 \cdot \left(\frac{1}{55 + 273.15} - \frac{1}{298.15}\right)\right]$$

$$= 8.572 \times 10^{-6} \frac{mol}{m^3 \cdot Pa}$$

Then, given the molar weight of methane (16.043 g/mol), K_{T_2} can be converted to:

$$K_{T_2} = 8.572 \times 10^{-6} \frac{mol}{m^3 \cdot Pa} \times 16.043 \frac{\text{g}}{\text{mol}} \times \frac{m^3}{10^3\ L} \times \frac{10^5\ Pa}{bar}$$

$$= 0.0138 \frac{g}{L \cdot bar}$$

In order to determine the concentration of dissolved methane (C) using Henry's Law (C = K·P), the partial pressure of methane in the bioreactor (P) was also required. In this experiment, methane composition could reach up to 75%, and the total pressure inside of the bioreactor ranged between 1019.55 mbar 1021.15 mbar (see Table 14). Therefore, taking 75% as the methane composition, the partial pressure of methane inside of the bioreactor was 764.66 mbar to 765.86 mbar, which was equivalent to 0.76 bar to 0.77 bar.

Table 14. Components of the Total Pressure inside of the Bioreactor

Range	Atmospheric Pressure [mbar]	Correction Factor [mbar]	Biogas Pressure [mbar]	Total Pressure [mbar]
Min	1013.25	5	1.3	1019.55
Max	1013.25	5	2.9	1021.15

Using 0.0138 g/(L·bar) for K and 0.76 bar for P, the dissolved methane concentration C could be calculated as 0.0105 g/L. When the COD of methane (4 gCOD/g) was applied, the COD contained in the dissolved methane was able to be determined: 0.042 gCOD/L. Finally, this value was multiplied by the sludge volume, which was controlled around 6.5 L throughout the experiment, so that the dissolved methane could account for 0.27 gCOD at maximum. The measurement results of methane composition were used to calculate the specific COD values of the dissolved methane for the days that were considered for COD balance.

Figure 14 and 15 were constructed in order to demonstrate COD balance for both phases of the experiment. During Phase I, the average COD gap was about 15%. The minimum COD gap was achieved on Day 369, which was 6% with regard to the total COD of the substrate fed into the bioreactor. The maximum COD gap was 25% on Day 317 and Day 334. Phase II had much larger COD gap than Phase I. The average COD gap during Phase II was 43%, which was significant for the closure of COD balance. However, if positive biomass net growth is considered for the COD balance of Phase II, the gap might be substantially smaller. According to the weekly measurement results of VSS, the COD uptake for biomass growth during Phase II was between -1% and 59% (around 17% on average). This amount has not been included in Figure 15 yet.

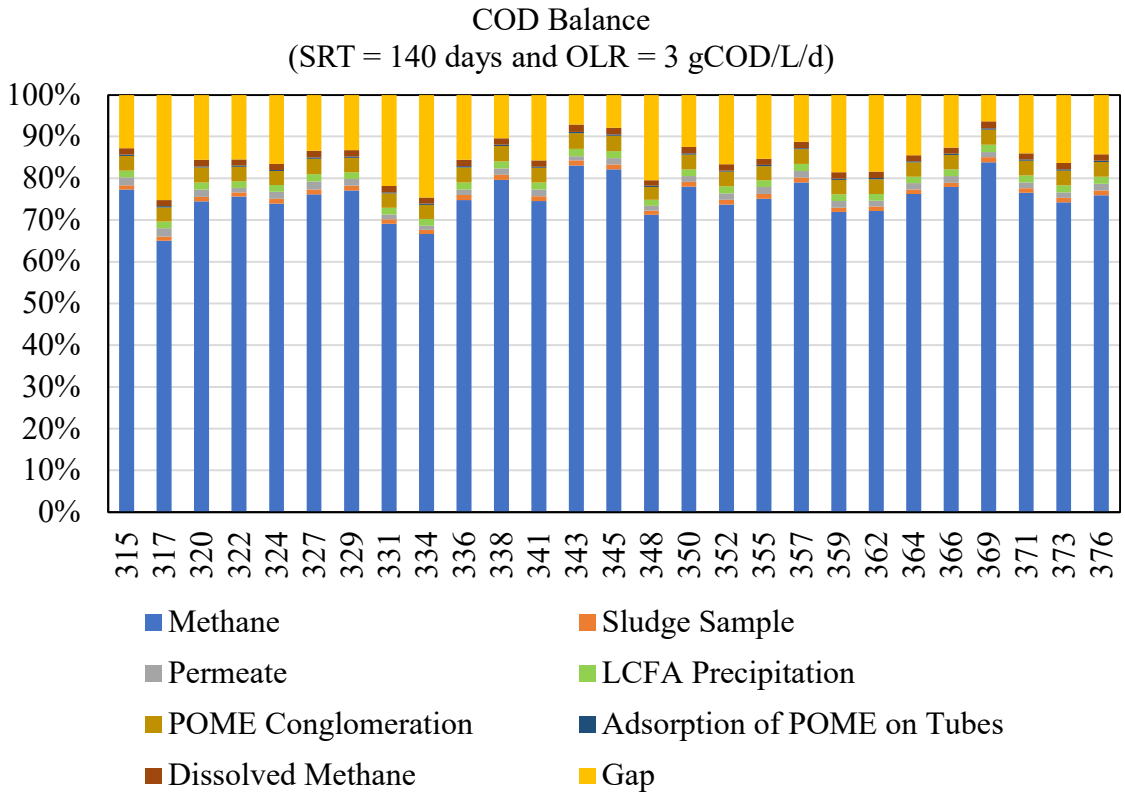

Figure 14. COD Balance during Phase I

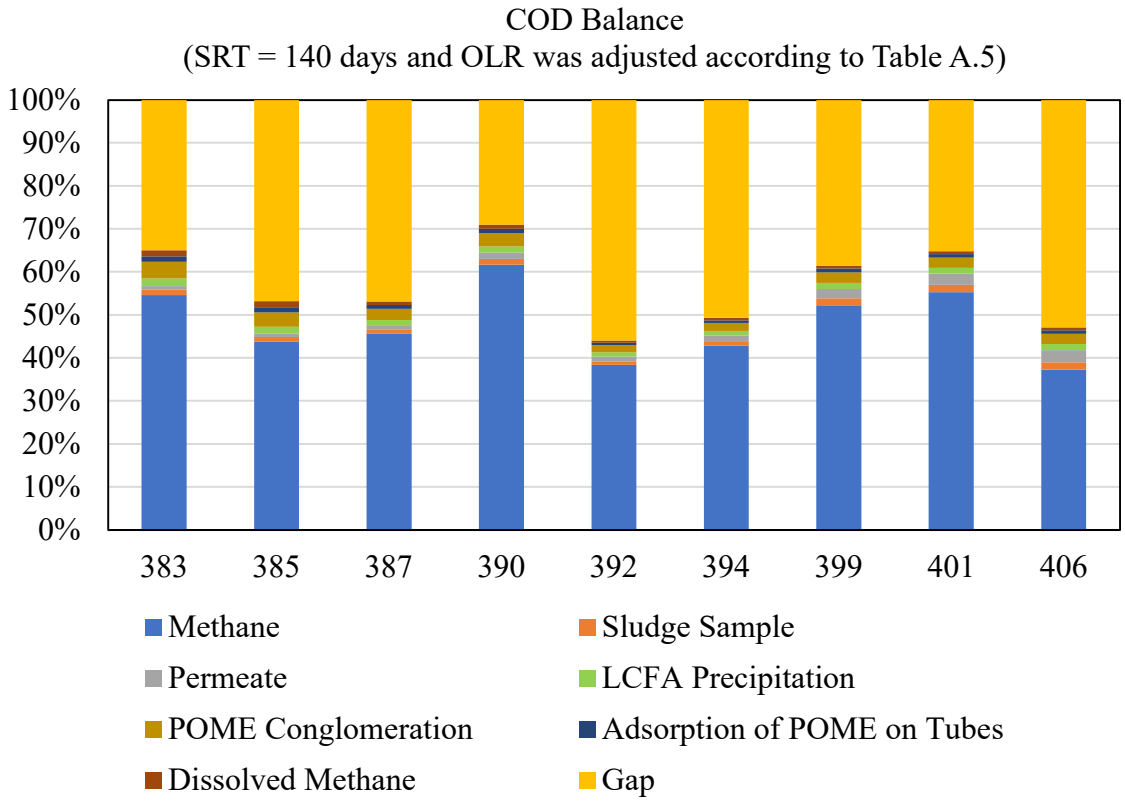

Figure 15. COD Balance during Phase II

4. Discussion

4.1 Biological Performance during Phase I & Phase II

The biological performance of the AnMBR system during Phase I and Phase II has summarized as follows:

Table 15. Summary of the Monitoring Results during Phase I and Phase II

Performance Indicators	Phase I	Phase II
COD removal	98% – 99%	95% – 99%
Biomass growth (Net VSS)	Zero (0 gVSS/d)	Positive (0 – 9 gVSS/d)
Biogas production	6 – 7.2 L/d (methane production was around 5 L/d).	4 – 12 L/d (methane production increased up to 6 L/d).
Digestion efficiency (COD_{CH4} / COD_{feed})	70% – 85%	Around 50% (37% – 97%)
VFA accumulation (Acetic acid in sludge)	Acetic acid is relatively constant (around 76 mg/L).	Acetic acid increased overtime (from 45 to 237 mg/L).

- **COD removal.** During both phases, the COD removal efficiency was able to reach 99%, which was consistent with the hypothesis. This result is higher than the values found in some of the other similar studies that also used AnMBR for POME treatment (Poh, P. E., & Chong, M. F., 2009).
- **Biomass growth.** The biomass net growth did not have the tendency to be positive during Phase I, as how the hypothesis predicted. Therefore, under the feeding conditions (1.2 $gCOD \cdot L^{-1} \cdot d^{-1}$ from VFA and 1.8 $gCOD \cdot L^{-1} \cdot d^{-1}$ from POME) and operational conditions (at 55 °C and SRT of 140 days) during Phase I, the microorganisms inside of the bioreactor was able to maintain the mass at a constant level. In addition, there was 65 mL of sludge withdrawn from the bioreactor on daily basis during the workdays, which could be considered as the waste stream or the biomass yield. During phase II, due to the increased COD of the influent, positive biomass net growth was observed. However, the VSS measurements during Phase II might also include the undegraded starch that was attached to the biomass, and the amount of the undegraded starch might accumulate overtime before it can be digested by the microbes completely.
- **Biogas production.** Biogas production was above the predicted value (6 L/d). According to section 3.3, the biogas production could reach up to 7.2 L/day under the experimental conditions during Phase I. Both biogas production and methane production increased during Phase II, when the OLR was increased.
- **Digestion efficiency.** Digestion efficiency improved when compared to the experimental results obtained previously by other researchers under the same operational conditions. As mentioned in hypothesis, 65%-85% of the COD added to the bioreactor would be converted to methane. In this experiment, 70%-85% was achieved during Phase I. The digestion efficiency in terms of methane production decreased during Phase II. Although, the overall COD

conversion efficiency could be compensated by the positive biomass net growth. SMA test was also conducted to determine how much methane the sludge was able to produce potentially. In this experiment, SMA of 0.57 gCH4-COD·gVSS^{-1}·d^{-1} was observed for phase II. In a previous study, where the SMA test was conducted using the sludge from the same AnMBR system that was been operated at SRT of 90 days and OLR of 3 gCOD·L^{-1}·d^{-1}, the most representative SMA slope was 0.26 gCH4-COD·gVSS^{-1}·d^{-1}. Therefore, the anaerobic sludge seemed to have higher capability of producing methane when SRT is longer. This phenomenon was also observed in another research project that was similar to this experiment (Szabo-Corbacho, 2021).

- **VFA accumulation.** VFAs are important intermediates during anaerobic digestion, and they can serve as precursors for methane production (Nghiem, 2017). VFAs in sludge and permeate were monitored in this experiment because VFA accumulation should be avoided for stable methane production. During Phase I, no VFA accumulation was observed for both sludge and permeate. During Phase II, the VFA level as indicated by the acetic acid concentration in the sludge was increasing overtime, suggesting the potential risk of acidification (methanogenesis could be inhibited in this case).

4.2 Impacts of Changes during Phase II

During Phase II, when the starch feed was added to the AnMBR system instead of the VFA feed, the biological performance of became unstable. This phenomenon might be caused by the adaptability of the microbes to the new feed as well as the feeding conditions:

- **Feed type.** In order to simulate the carbohydrates in real POME, cellulose was used in the beginning. However, cellulose is insoluble in water (Alves et al., 2016), and cellulose precipitation was observed even when continuous mixing was applied. Consequently, the measured COD of the cellulose feed was much lower than expected, probably because the inlet was at the layer with low cellulose concentration. Therefore, the cellulose feed was replaced by the starch feed starting on Day 380. Note that COD measurement results on Day 380 were obtained before the feed was changed. The starch used for feed preparation is soluble in water according to the manufacturer, but in practice, heating and overnight mixing were required to dissolve the starch powder. The starch feed bottles were left in a shaker for 24 hours at 70°C and 140 rpm before installed in the system.
- **Feeding conditions.** Besides the type of feed, the feeding conditions might also affect the stability of the biological performance, because the COD contained in the feed affects OLR directly. The theoretical OLR values were calculated based on the experimental design, whereas the measured OLR values were determined by (1) flowrates of the POME feed and the VFA/starch feed which were calibrated on daily basis, (2) COD measurement results of the POME feed and the VFA/starch feed, and (3) the sludge volume which was controlled around 6.5L. In case of inadequate mixing, the measured COD of the starch would not be consistent with the theoretical COD value, so that the measured

OLR and the theoretical OLR would have a large gap. Figure 16 and Figure 17 were developed based on the experimental results to show the OLRs during Phase I and Phase II respectively. During Phase I when VFA was added to the bioreactor, no mixing was required since VFAs dissolved in water completely, whereas during Phase II when starch was added to the bioreactor, an overhead stirrer was used for mixing. The stirrer had issues such as tilting. A magnetic mixer was proposed at the end of Phase II.

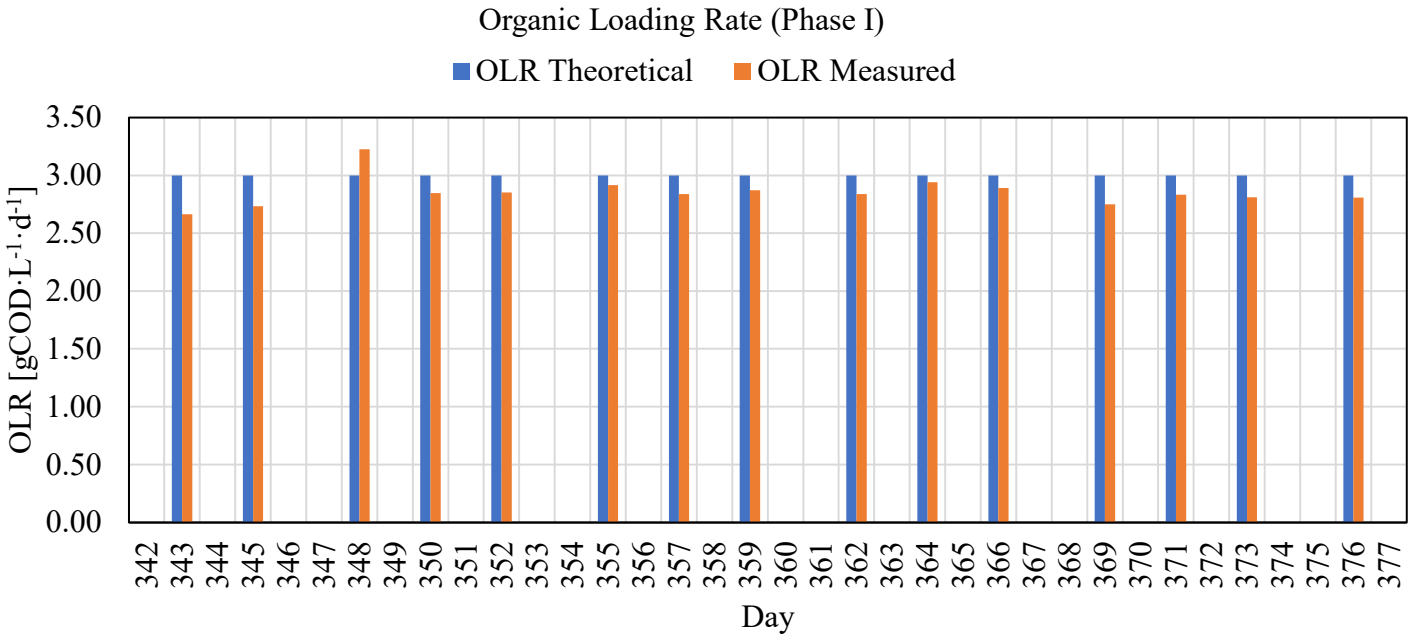

Figure 16. Organic Loading Rates (Theoretical vs. Measured) During Phase I

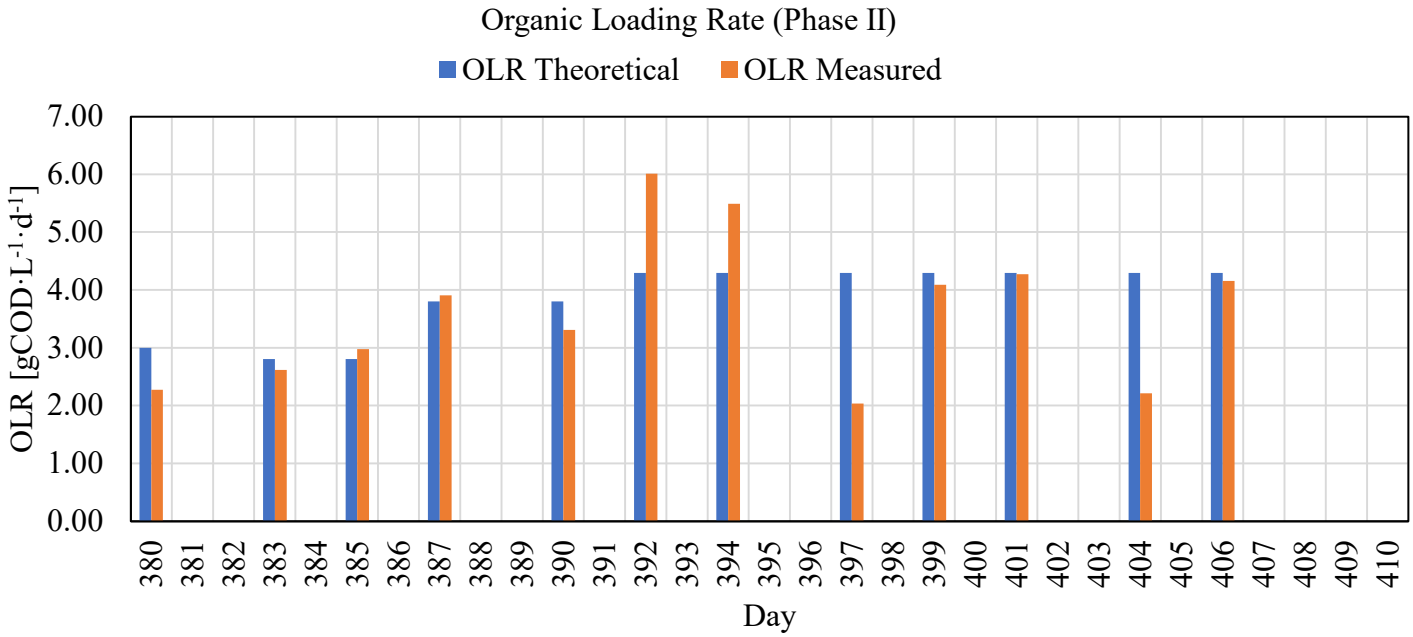

Figure 17. Organic Loading Rates (Theoretical vs. Measured) During Phase II

4.3 LCFA Analysis

In this experiment, both Ca^{2+} and Mg^{2+} were added to the bioreactor as part of the macronutrients (from $CaCl_2 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$ respectively). However, the affinity between Ca^{2+} and 2 Cl^- is greater than the affinity between Ca^{2+} and 2 $LCFA^-$. Therefore, $CaCl_2$ would bond together when dissolved in liquid instead of causing LCFA precipitation. On the other hand, affinity between Mg^{2+} and 2 $LCFA^-$ is greater than the affinity between Mg^{2+} and SO^{2-}, so that Mg^{2+} would bond with 2 $LCFA^-$ when dissolved in liquid and precipitate in the sludge. When LCFAs precipitate with divalent cations, they might adsorb on biomass and inhibit the methanogenic process of anaerobic digestion (Dereli, 2014).

The method developed in this experiment for LCFA measurement was able to provide satisfactory results. The 2nd test and the 3rd test had much reliable data when compared to the 1st test, whose total dilution factors for the sludge and POME samples were much larger than the ones used in the other two tests. The 2nd test and the 3rd test were conducted using the same method, except the samples used for the second stage of the 3rd test were the ones prepared during the 2nd test and stored in a freezer afterwards. Therefore, part of the palmitic acids might have been degraded during the storage period. Therefore, the 2nd test describes the LCFA conditions in the sludge better than the 3rd test.

4.3 COD Balance

Phase I had smaller and more stable COD gaps when compared with Phase II. Although, the COD gaps during Phase I could be further reduced by taking other factors into consideration, such as the adsorption of POME on glass materials (mainly the inner wall of the bioreactor). During Phase II, the large COD gaps might be caused by inadequate mixing and positive biomass net growth.

On Day 397 and Day 404, negative values were obtained for the COD gap. This phenomenon could be explained by Figure 17, where the measured OLR values for Day 397 and Day 404 were much less than the theoretical values. As mentioned in section 4.2, mixing had direct impact on the homogeneity of the starch feed. If the starch feed was not well mixed during sampling (sampling point was right before the starch feed entered the bioreactor), and then the mixing improved overtime, there would be more substrate entering the bioreactor than the amount projected by the measured COD of the starch feed. In this case, the total influent COD would be less than the total effluent COD, so that the COD gap would be negative. Day 397 and Day 404 were considered as outliers for the COD balance analysis, and they were not included in Figure 15.

On the other hand, the measured OLR values were much greater than the theoretical OLR values on Day 392 and Day 394, which might contribute to the large COD gaps observed on both days. The homogeneity of the starch feed was unstable during Phase II due to issues associated with mixing. The starch feed from the thick layer would have higher COD than the one from the thin layer. Therefore, if the starch feed from the thick layer was sampled for COD measurement before entering the bioreactor, and the

consistency of the starch feed changed overtime, the actual influent COD would be lower than anticipated, so would the actual effluent COD.

Furthermore, positive biomass net growth might also have a large impact on the COD gaps during Phase II. Based on Figure 4, positive biomass net growth was observed during Phase II. However, biomass net growth based on VSS was measured on weekly basis, and the measurement results might not represent the growth condition of each day throughout the week. Therefore, they were not included in the COD balance analysis for Phase II (see Figure 15). Although, as mentioned in section 3.7, -1% to 59% (with an average of 17%) of the total feed COD could be used for biomass net growth (including the amount of undegraded starch measured along with the biomass).

5. Conclusions and Recommendations

5.1 Conclusions

For wastewater treatment plants (WWTPs), especially the ones in Indonesia and Malaysia, where most of the palm oil is produced in the world, the AnMBR technology might be a solution to release the environmental burdens caused by lipid-rich POME. In this experiment, an external cross-flow AnMBR (an anaerobic bioreactor coupled with ultrafiltration membrane) was used to assess the biological performance of the system under SRT at 140 days and OLR at 3 gCOD·L-1·d-1 during Phase I. Then, during Phase II, starch was added to the POME feed (VFAs were replaced with starch) for a more representative POME composition where carbohydrates are also included. The effects of adding carbohydrates to the synthetic POME feed (mainly lipids) on the biological performance were evaluated.

During Phase I of the experiment, POME and VFAs were added to the bioreactor. 98% to 99% of the COD added to the system was able to be removed. The biomass concentration remained in the reactor was maintained at a constant level (no biomass net growth was observed). Biogas production ranged between 6.0 and 7.2 L per day, of which 75% was methane. Between 70% and 85% of the incoming COD was converted to methane. Furthermore, it was indicated by the VFA measurements that the acetic acid concentration was almost constant, so that the risk of acidification was low.

During Phase II, VFAs were replaced with starch mainly to assess how the biological performance of the AnMBR system changes due to the influence of adding carbohydrates, which might also be part of the POME found in practical situations. The COD removal efficiency was above 95% during Phase II, which was close to the values obtained during Phase I. Positive biomass net growth was observed, indicating that increasing OLR could accelerate the growth of the microbial community. Although, the VSS measurement results for calculating the biomass net growth might also include the undegraded starch that had not been digested yet. The biogas production and the methane production also increased during the second phase due to the increased overall OLR. The digestion efficiency (i.e., the amount of methane produced in relation to the total feed COD) decreased, while both the methane production and the total feed COD increased. Based on the positive biomass net growth, the decrease in the digestion efficiency might be caused by an increase in the COD uptake for biomass growth. In

comparison to a parallel study where OLR was the same but SRT was controlled at 90 days, the SMA of the sludge collected in this experiment was about 2 times higher, when the system was operated at SRT of 140 days. Increasing OLR during Phase II resulted in VFA accumulation in the sludge (and the permeate). Therefore, the dose of sodium bicarbonate was adjusted accordingly in order to prevent acidification. LCFA concentrations and compositions were measured as well in this experiment using LC/MS. Based on the LCFA analysis, the amount of LCFAs in the sludge and the POME feed was able to be monitored. LCFA compositions were also used to determine the amount of LCFAs precipitated due to the presence of divalent cations. Furthermore, according to the LCFA measurement results, oleic acid has less degradation efficiency when compared with other types of LCFAs. Finally, the COD balance was analyzed in the end, to evaluate the overall stability and efficiency of the system. The system has the potential to achieve stable outcomes, according to the preliminary results. Although, it would be crucial to improve mixing, and adjust the buffer solution depending on the pH of the sludge, at the later stage of Phase II.

5.2 Recommendations

This experiment could be further improved when the following suggestions are taken into consideration for any future attempt:

- The biogas produced from the AnMBR system could be collected, cleaned and used in other applications that generate energy with methane.
- For the SMA test, it would be better to release the biogas from the headspace at the end of each pressure measurement, because the gauge pressure might exceed the measuring range of the pressure meter. Moreover, the SMA calculation would be more accurate if the dissolved gasses were considered as well.
- Better mixing is required for the starch feed. A magnetic mixer was proposed at the end of the experiment.
- Adjustments to the buffer solution might be needed for the starch feed depending on the pH. Based on the previous experimental results, pH was between 7.25 and 7.50 when the stability of the treatment system was achieved, in spite of the operational conditions.
- LCFA could be measured more frequently using the method developed in this experiment. The biological performance of the system could also be monitored according to LCFA accumulation, in which case the methanogenic process might be inhibited.
- COD decline due to the adsorption of POME on glass materials could also be investigated for the COD balance analysis.
- For Phase II, VSS could be measured on the same days for COD measurement, so that the COD balance analysis would be more accurate when the biomass growth is included as well.
- Once the biological performance of the system becomes stable, the COD decline in the starch feed could be considered for closing the COD gap of Phase II, because some of the starch might be retained on the inner wall of the starch feed.

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7. Appendix

7.1 Appendix A: Supplementary Tables

Table A.1. Ingredients per Liter of VFA Feed

Chemical	Dose	Unit
Sodium Acetate Trihydrate	11.49	g
Sodium Propionate	1.26	g
Sodium Butyrate	1.54	g

Table A.2. Nutrient Supplements per Liter of VFA Feed or Starch Feed

Chemical	Dose	Unit
Macronutrient Solution	7.23	mL
Micronutrient Solution	3.61	mL
Phosphate Buffer Solution A (0.2M of $K_2HPO_4 \cdot 3H_2O$)	5.19	mL
Phosphate Buffer Solution B (0.2M of $NaH_2PO_4 \cdot 2H_2O$)	5.19	mL

Table A.3. Ingredients per Liter of Macronutrient Solution

Chemical	Dose	Unit
NH_4Cl	170.0	g
$CaCl_2 \cdot 2H_20$	8.0	g
$MgSO_4 \cdot 7H_20$	9.0	g

Table A.4. Ingredients per Liter of Micronutrient Solution

Chemical	Dose	Unit
$FeCl_3 \cdot 4H_2O$	2.0	g
$CoCl_2 \cdot 6H_2O$	2.0	g
$MnCl_2 \cdot 4H_2O$	0.5	g
$CuCl_2 \cdot 2H_2O$	30.0	mg
$ZnCl_2$	50.0	mg
HBO_3	50.0	mg
$(NH_4)_6Mo_7O_2 \cdot 4H_20$	90.0	mg
$Na_2SeO_3 \cdot 5H_2O$	100.0	mg
$NiCl_2 \cdot 6H_2O$	50.0	mg
EDTA	1.0	g
HCl 36%	1.0	mL
Resazurin	0.5	g
Yeast Extract	0.2	g

Table A.5. Ingredients for the Starch Feed during the Start-up Phase

Feeding Day*	Chemical	OLR [gCOD·L^{-1}·d^{-1}]	Target COD [gCOD/L]	Volume [L]	Mass [g]	Molar Ratio
380	NaHCO3	1	7.6	7.0	23.3	1.0
	Starch				44.9	
383	NaHCO3	1	7.6	2.0	23.6	3.0
	Starch				15.2	
384	NaHCO3	1	7.6	2.0	23.6	3.0
	Starch				15.2	
385	NaHCO3	1.5	11.3	2.0	23.0	2.1
	Starch				21.2	
386	NaHCO3	2	15.1	2.0	44.0	3.0
	Starch				28.3	
387	NaHCO3	2	15.1	5.0	110.0	3.0
	Starch				70.8	
390	NaHCO3	2.5	15.0	2.0	43.7	3.0
	Starch				28.1	
391	NaHCO3	2.5	15.0	4.5	98.3	3.0
	Starch				63.3	
394	NaHCO3	2.5	15.0	4.5	68.8	2.1
	Starch				63.3	
397	NaHCO3	2.5	15.0	2.0	30.6	2.1
	Starch				28.1	
398	NaHCO3	2.5	15.0	2.0	30.6	2.1
	Starch				28.1	
399	NaHCO3	2.5	15.0	2.0	30.6	2.1
	Starch				28.1	
400	NaHCO3	2.5	15.0	2.0	30.6	2.1
	Starch				28.1	
401	NaHCO3	2.5	15.0	8.0	122.4	2.1
	Starch				112.5	
404	NaHCO3	2.5	15.0	2.0	14.6	1.0
	Starch				28.1	
405	NaHCO3	2.5	15.0	2.0	0.0	0.0
	Starch				28.1	
406	NaHCO3	2.5	15.0	2.0	7.3	0.5
	Starch				28.1	
407	NaHCO3	2.5	15.0	4.0	17.5	0.6
	Starch				56.2	
411	NaHCO3	2.5	15.0	2.0	21.9	1.5
	Starch				28.1	

* The new starch feed of the feeding day was added to the bioreactor at around 3pm, after the COD measurements had been completed already.

Table A.6. Sample Preparation for SMA Test

Label	Sample	Sludge [g]	Nutrient Mix* [g]	$C_2H_9NaO_5$ ** [g]	Total Liquid [g]
1	Blank Sample	97.88	2.12	0.00	100
2	Blank Sample	97.88	2.12	0.00	100
3	Blank Sample	97.88	2.12	0.00	100
4	SMA Sample	97.45	2.12	0.43	100
5	SMA Sample	97.45	2.12	0.43	100
6	SMA Sample	97.45	2.12	0.43	100

* Nutrient mix means a mix of ingredients listed in Table A.2 with the same volume ratio. Since the total volume of the sample in each SMA bottle was 100 mL, 2.12 mL of the nutrient mix was required per bottle: [(7.23+3.6+5.19+5.19) mL/L_{feed}] × [(100/1000) L_{feed}] = 2.12 mL.

** For blank samples, sodium acetate trihydrate ($C_2H_9NaO_5$) was not included; for SMA samples, $C_2H_9NaO_5$ dose was calculated based on one of the design parameters (2 gCOD/L), the theoretical COD of the compound (0.47 gCOD/g$C_2H_9NaO_5$), and the sample volume in each bottle (0.1 L): [(2 gCOD/L) / (0.47 gCOD/g$C_2H_9NaO_5$)] × (0.1 L) = 0.43 g$C_2H_9NaO_5$..

Table A.7. Sample Preparation for LCFA Analysis (Stage II of the 1st Test)

Sample	Organic Phase [µL]	ITSD [µL]	Methanol [µL]	Total Volume [µL]	DF_1	DF_2	Total DF
Sludge 1 (S1)	10	10	480	500	4.75	50.00	237.50
Sludge 2 (S2)	20	10	470	500	4.75	25.00	118.75
Sludge 3 (S3)	50	10	440	500	4.75	10.00	47.50
POME 1 (P1)	5	10	485	500	4.75	100.00	475.00
POME 2 (P2)	10	10	480	500	4.75	50.00	237.50
POME 3 (P3)	50	10	440	500	4.75	10.00	47.50

Table A.8. Sample Preparation for LCFA Analysis (Stage II of the 2nd Test)

Sample	Organic Phase [µL]	ITSD [µL]	Methanol [µL]	Total Volume [µL]	DF_1	DF_2	Total DF
Sludge 1 (S1)	188	20	792	1000	4.75	5.32	25.27
Sludge 2 (S2)	188	20	792	1000	4.75	5.32	25.27
Sludge 3 (S3)	188	20	792	1000	4.75	5.32	25.27
POME 1 (P1)	130	20	850	1000	4.75	7.69	36.54
POME 2 (P2)	130	20	850	1000	4.75	7.69	36.54
POME 3 (P3)	130	20	850	1000	4.75	7.69	36.54

Table A.9. Sample Preparation for LCFA Analysis (Stage II of the 3rd Test)

Sample	Organic Phase [µL]	ITSD [µL]	Methanol [µL]	Total Volume [µL]	DF_1	DF_2	Total DF
Sludge 1 (S1)	188	20	792	1000	4.75	5.32	25.27
Sludge 2 (S2)	188	20	792	1000	4.75	5.32	25.27
Sludge 3 (S3)	188	20	792	1000	4.75	5.32	25.27
POME 1 (P1)	130	20	850	1000	4.75	7.69	36.54
POME 2 (P2)	130	20	850	1000	4.75	7.69	36.54
POME 3 (P3)	130	20	850	1000	4.75	7.69	36.54

7.2 Appendix B: Supplementary Equations and Measurement Procedures

B.1. Equation for COD Removal Efficiency

$$\varepsilon = \frac{COD_{in} - COD_{out}}{COD_{in}} = \frac{Q_1C_1 + Q_2C_2 - Q_3C_3}{Q_1C_1 + Q_2C_2}$$

$where$:
$\varepsilon = COD\ removal\ efficiency\ [\%]$
$COD_{in} = influent\ COD\ [g/day]$
$COD_{out} = effluent\ COD\ [g/day]$
$Q_1 = flowrate\ of\ the\ POME\ feed\ [L/day]$
$C_1 = COD\ concentration\ of\ the\ POME\ feed\ [g/L]$
$Q_2 = flowrate\ of\ the\ VFA/starch\ feed\ [L/day]$
$C_2 = COD\ concentration\ of\ the\ VFA/starch\ feed\ [g/L]$
$Q_3 = flowrate\ of\ the\ permeate\ [L/day]$
$C_3 = COD\ concentration\ of\ the\ permeate\ [g/L]$

B.2. Equations for TS, VS, TSS, and VSS

$$TS = \frac{W_3 - W_1}{V}\ in\ [g/L]$$
$$VS = \frac{W_3 - W_4}{V}\ in\ [g/L]$$

$where$:
$V = Volume\ of\ sample\ [L]$
$W_1 = Weight\ of\ aluminum\ plate\ [g]$
$W_2 = Weight\ of\ aluminum\ plate + sample\ [g]$
$W_3 = Weight\ of\ aluminum\ plate + sample\ after\ 24\ hours\ of\ heating\ at\ 105\ °C\ [g]$
$W_4 = Weight\ of\ aluminum\ plate + sample\ after\ 24\ hours\ of\ heatingat\ 105\ °C\ and\ 1\ hour\ of\ heating\ at\ 550\ °C\ (excl.the\ preheating\ time)\ [g]$

$$TSS = \frac{W_3^* - W_1^*}{V}\ in\ [g/L]$$
$$VSS = \frac{W_3^* - W_4^*}{V}\ in\ [g/L]$$

$where$:
$V = Volume\ of\ sample\ [L]$
$W_1^* = Weight\ of\ aluminum\ plate + filter[g]$
$W_2^* = Weight\ of\ aluminum\ plate + filter + sample\ [g]$
$W_3^* = Weight\ of\ aluminum\ plate + filter + sample\ after\ 24\ hours\ of\ heating\ at\ 105\ °C\ [g]$
$W_4^* = Weight\ of\ aluminum\ plate + filter + sample\ after\ 24\ hours\ of\ heatingat\ 105\ °C\ and\ 1\ hour\ of\ heating\ at\ 550\ °C\ (excl.the\ preheating\ time)\ [g]$

B.3. Equation for Biomass Net Growth

$$Biomass\ Net\ Growth = \frac{VSS_2V_2 - VSS_1V_1}{t_2 - t_1}\ in\ [g/day]$$

$where$:
$t_{1,2} = time\ of\ measurement\ 1\ and\ 2\ [day]$
$VSS_{1,2} = volatile\ suspended\ solids\ measured\ at\ t_1\ and\ t_2\ [g/L]$
$V_{1,2} = volume\ of\ sludge\ in\ the\ digester\ measured\ at\ t_1\ and\ t_2\ [L]$

B.4. Equation and Measurement Procedure for SMA

In order to establish a SMA graph, the gauge pressure of the methane produced in each bottle was measured over the course of the design period (5 – 7 days). Then, the ideal gas law was applied in order to convert the gauge pressure to the total number of gas molecules in mols (Childs, 2014), which was distributed according to the gas composition in order to obtain the amount of methane in the biogas produced. Given the VSS value and the amount of methane produced, SMA of the sludge in each bottle could be calculated using the following equation:

$$SMA = \frac{n \cdot MW \cdot ThOD}{VSS \cdot V \cdot t}\ in\ [gCH4\text{-}COD \cdot gVSS^{-1} \cdot day^{-1})]$$

$where$:
$n = moles\ of\ methane\ [mol]$
$MW = molar\ weight\ of\ methane\ [16.043\ gCH_4/mol]$
$ThOD = theoretical\ COD\ of\ methane\ [4\ gCOD/gCH_4]$
$VSS = volatile\ suspended\ solids\ in\ the\ sludge\ [gVSS/L]$
$V = volume\ of\ sample\ added\ to\ each\ bottle\ [L]$
$t = methane\ production\ time\ [day]$

The SMA test was conducted based on the following steps, for sample preparation and pressure measurement:

1. Prepare 6 glass serum bottles (each with empty volume of 120 mL), 3 for blank samples (without acetate) and 3 for SMA samples (with acetate);
2. Wash the bottles with demineralized water, and dry them completely in an oven;
3. Label each bottle with a number;
4. Measure the initial weight each bottle (with the rubber cap on);
5. Fill the bottles completely up with demineralized water, and then seal each bottle with the same rubber cap;
6. Dry the outside wall of each bottle and measure the weight again;
7. Empty the bottles, and dry them completely again in the oven;
8. Add ingredients to the bottles according to Table A.6 in Appendix A;
9. Flush the bottles with nitrogen gas and seal the bottles with aluminum vial caps immediately;

10. Place the bottles in a shaker (at 55°C and 100 rpm), and measure the atmospheric pressure and the gauge pressure of the head space twice a day for at least a week.

B.5. Measurement Procedure for LCFA

The LCFA measurement was conduced based on the following steps:

1. Prepare 6 glass vials (at least 10 mL each), 3 for POME samples and 3 for sludge samples, and add **2 mL** of sample in each vial;
2. Leave the vials in an oven for 12 hours at 85 °C;
3. Let the vials cool down to room temperature, and then add **1.5 mL** of HCl:1-propanol (25% v/v), **2 mL** of dichloromethane, and **2 mL** of UPW to each vial;
4. Mix the vials with a vortex mixer for about 1 minute;
5. Heat the vials at 100 °C for 3.5 hours;
6. Add **2 mL** of UPW to each vial;
7. Mix the vials again with the vortex mixer for about 1 minute;
8. Keep the vials in a stationary position for about 30 minutes;
9. Once the solution separates into 2 layers, take 1-2 mL of the solution from the upper layer (organic phase) of each vial with a 5 mL syringe;
10. Filter the sample in the syringe with a 0.2 μm filter.

More specifically, in step 2, the vials were heated to (1) break down the lipids contained in the solution, and (2) eliminate the remaining microbes; in step 3 and 6, UPW was added to the solution in order to promote separation in layers driven by the density difference. At the end of the first stage, the dilution factor was 4.75 for each sample (DF1 = 4.75).