



Biological Performance of Palm Oil Mill Effluent (POME) Treatment using AnMBR

Additional Thesis Project

By

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Abstract

Palm Oil Mill Effluent (POME) is an attractive medium for biogas production in an anaerobic membrane bioreactor (AnMBR) because of its high lipid content. Long-chain fatty acid (LCFA) accumulation is toxic and considered harmful for the biological performance within the reactor as they can be absorbed by biomass particles causing sludge flotation and biomass washout from the reactor. Membrane fouling can be caused by LCFA inhibition through adsorption on membrane walls. The biodegradation efficiency and filterability are affected by several factors such as solids retention time (SRT), and an organic loading rate (OLR). The objective of this research was to determine biological performance and LCFA inhibition while operating the AnMBR system at SRT of 90 days and an OLR of 3 g COD/ L/d under thermophilic condition (55°C).

It was observed that successful operation was achieved with high COD removal efficiencies over 98% and average biogas production of 5 NL/d. Acidification occur in the sludge causing signification drop in pH, biomass concentration and methane production. The reactor slowly recovered back normal after adding sodium bicarbonate in the VFA feed. In addition, acetic and propionic acid were the major VFA constituent presented in the sludge.

Keywords: Anaerobic membrane bioreactor, long-chain fatty acids (LCFA), POME, membrane fouling, solid retention time, organic loading rate

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1. Introduction

Palm oil cultivation is mainly dominated by Indonesia and Malaysia. Currently, 39% of the world palm oil come from Malaysia and counted as 44% of the world export (Mohammed & Chong, 2014). In 2015, more than half of all palm oil production was consumed by India, China, Malaysia, and Pakistan (Intelligence, 2016). In the current market, palm oil is considered one of the efficient and reasonably-priced resources and is used in many products such as cooking oil. However, compared to vegetable oil made from rapeseed or soybeans, palm oil has higher saturated fatty acids which are harmful to human health (Matthäus, 2007).

The extraction of one ton of crude palm oil (CPO) from fresh fruit bunches (FFB) requires 5-7 tons of water, and more than half of the water used ends up as POME (Ahmad et al., 2003). POME is viscous, brownish in color, and rich in both biochemical oxygen demand (BOD) and chemical oxygen demand (COD). It is 95-96% water, 0.6-0.7% oils, and 2-4% suspended solids (Mohammed & Chong, 2014).

Oily wastewater such as POME is toxic to aquatic life because of its high BOD which causes oxygen depletion, contaminates drinking and groundwater, and causes overall destruction to the natural landscape and ecosystem (Madaki & Seng, 2013). The treatment of POME has become an urgent and challenging problem (Yu et al., 2017). POME treatment and disposal have become problematic in the production of palm oil due to its great abundance which requires effective treatment techniques.

Anaerobic membrane reactors (AnMBRs) is being one of the alternative techniques and innovative methods to treat POME, recover renewable energy, for instance, biogas, and achieving a zero-liquid discharge concept within the palm oil industry (Madaki & Seng, 2013). This report will discuss different methods for the efficient removal of COD, suspended solids (TSS and VSS), and the production of biogas from biologically treated POME using PVDF membrane at 90 days SRT and OLR 3 g COD/ L/d under thermophilic condition (55°C). Membrane treatment is good at retaining biomass and oil degradation resulting from slow growing microorganisms (β -oxidizers).

2. Literature Review

2.1. Characteristics of POME

Raw POME is characteristically an acidic, viscous, colloidal suspension and has an unpleasant smell (Bello et al., 2013; Lam & Lee, 2011). Several factors influence the composition of POME such as operational methods, weather, raw materials, etc. Fresh POME contains a brownish colloidal mixture of water, oil, and suspended solids.

Table 1. Characteristics of raw POME (Loh et al., 2013)

Parameters	Concentration (mg/L)
Potassium (K)	1154.8 ± 3.14
Magnesium (Mg)	287.8 ± 8.41
Calcium (Ca)	286 ± 4.39
Zinc (Zn)	1.98 ± 0.74
Iron (Fe)	65.7 ± 1.09
Copper (Cu)	0.85 ± 0.05
Ammoniacal nitrogen (NH ₃ -N)	36 ± 1
Total Kjeldahl nitrogen (TKN)	60 ± 6

According to Mohammed & Chong (2014), the brownish color of the palm oil come from pigments and soluble fiber content such as 8 mg/L of carotene, 3400 mg/L of pectin, tannin, 5800 mg/L of phenolic compounds, and 4700 mg/L of lignin. The temperature of POME is between 80-90 degrees Celsius, a total solid of 40.5 g/L. The Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) are 50000 mg/L and 25000 mg/L. These two parameters are an indicator for the degree of water pollution and are usually measured in mg/L. According to Ngan (1999), the oil and grease content in POME is 4 g/L. POME is acidic due to the presence of complex organic acid, which later can be used as the source of carbon that comes from the fermentation process which has a pH of about 4.5. Besides oil residue, POME also contains short palm fiber, cell walls, carbohydrates (sugar, nitrous compounds, protein, amino acids) and minerals (Ugoji, 1997).

2.2. POME Treatment Process

2.2.1. Ponding system

The ponding system, or land application, is considered as one of the most cost-effective techniques in treating POME due to its potential in reduction of organic properties (Yaser et al., 2009). The design scheme of the ponding system consists of an oil-trapping pond, acidification pond, cooling pond, aerobic ponds, and facultative pond. An open ponding system showed significantly high removal of COD, BOD, and ammoniacal nitrogen. However, this system emitted a high amount of methane gas into the atmosphere. According to Wang et al. (2011), methane traps 84 times more GWP (Global Warming Potential) than carbon dioxide and accounts for 20% of total radiative forcing. To minimize the emission of methane when producing renewable energy such as biogas, the company used technology utilizing a gas retrieval device to capture the resulting methane. However, the implementation of a methane capturing system is relatively expensive.

This treatment system is simple, reliable, affordable, energy-efficient, and low maintenance (Loh et al., 2013). According to Zahrim et al.(2014) and Chin et al.(2013), the effluent treatment from the ponding system can meet the standard discharge requirement. Even though the implementation of the ponding system seems to provide a lot of benefits, it also has various disadvantages such as a large land requirement of about 30-40 acres and a long hydraulic retention time (HRT) of between 100-160 days. Moreover, in practice it does not always meet discharge limits.

2.2.2. Biological Treatment

The biological treatment technique of POME involved the presence of microbial communities such as fungi, algae, bacteria, and other different kinds of microorganisms. Compared to the ponding system, anaerobic treatment techniques show a higher removal of COD, BOD, TSS, color, oil, and grease. It also performs more economically on the industrial scale (Chan et al., 2012). The current anaerobic treatment techniques are anaerobic fluidized bed reactor, membrane separation, up-flow anaerobic sludge blanket (UASB), aerobic suspended growth processes, etc. These techniques often result in instability of effluent organic matter content and additional post-treatment steps are necessary. Chan et al.(2012) had come up with the idea of integrating anaerobic and aerobic treatment known as “ The Integrated Anaerobic-Aerobic Bioreactors (IAAB)” operated under steady thermophilic conditions with OLR of 30 g COD/L using a mesophilic bacterial seed. Table 2 illustrates the different results of each treatment system.

Several factors make anaerobic treatment differ from aerobic treatment. These differences include that no aeration is required which leads to lower operational cost, lower sludge yield, lower carbon dioxide/methane production from organic matter, no chemical energy loss, and higher energy production while requiring less surface area (Lin et al., 2013). However, additional post-treatment is required for anaerobic treatment to meet discharge standard requirements (Cakir & Stenstrom, 2005).

Phytoremediation and microbial fuel cell (MFC) are considered alternative biological treatments of POME. Phytoremediation of POME use different types of plants such as *Typha latifolia* (Purwanti et al., 2014), *Vetiveria zizanioides* (Darajeh et al., 2014), *Ipomoea aquatica* Forsk (Zulfahmi et al., 2021), and *Spirogyra sp.* (J. Biot., 2017) to reduce the COD and BOD concentration in the POME. This technique shows good performance in the removal of heavy metals such as chromium III (Cr^{3+}) (Chen et al., 2010), lead (Pb) (Bedabati Chanu & Gupta, 2016), nitrate (NO_3^-), phosphate (PO_4^{3-}) and textile dyes. This technique is economical, eco-friendly, practical and feasible (Effendi et al., 2018).

Microbial Fuel Cell (MFC) was operated at room temperature (20-25 °C) at a low substrate concentration to produce electricity from POME. The main factors which affect the MFC removal capacity were the type of substrates, reactor designs, operational cost, and technical parts of electron transfer (Islam et al., 2016). However, this treatment method removed only about 45% of COD (Baranitharan et al., 2013).

Table 2. Biological treatment of POME using the different anaerobic treatment system

Treatment system	IAAB	CSTR	EGSB	UASB-HCPB	Anaerobic co-digestion
Microbes	Mesophilic-thermophilic bacterial seed	Thermophilic mixed culture	Inoculum from open anaerobic ponds of POME	Thermophilic mixed culture	<i>Moringa oleifera</i> extract
HRT (d)	115	6	9.8	36	9.8
Reactor Volume (L)	75	2.5	-	4.5	0.45
COD removal(%)	99.6	90	94.89	86.30	85
TSS removal(%)	99	-	-	98.30	89
CH ₄ production (%)	45-60	64	65-70	52	69
Reference	(Chan et al., 2012)	(Yacob et al., 2005)	(Hwang T.K et al., 1978)	(Poh & Chong, 2014)	(Yap et al., 2021)

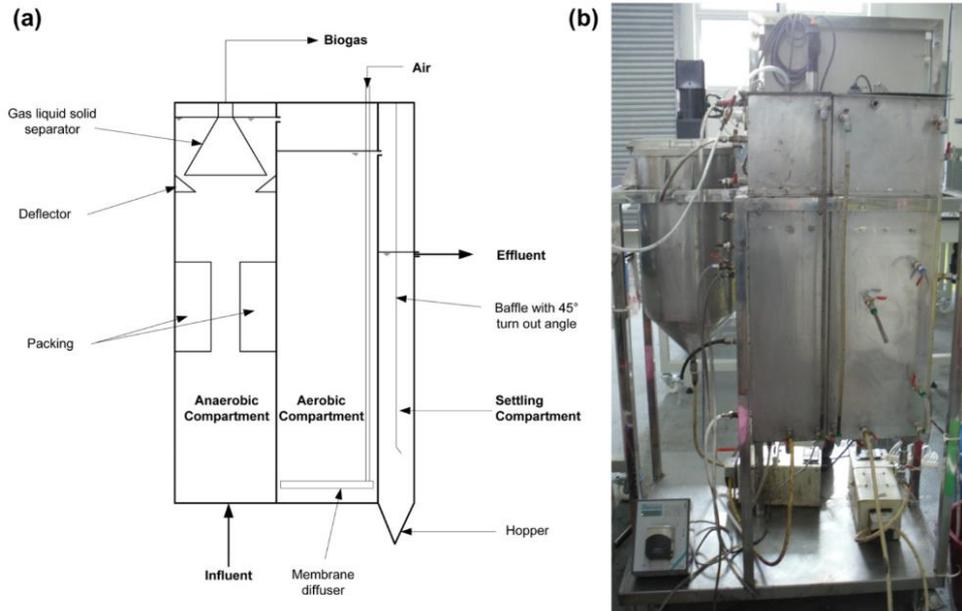


Figure 1. Reactor set up of IAAB, (a) schematic drawing, (b) actual view (Chan et al., 2012)

2.2.3. AnMBR application

Anaerobic membrane bioreactor (AnMBR) treatment systems combine anaerobic and membrane technology which provides unique advantages over conventional anaerobic and aerobic membrane bioreactors in various wastewater applications. In anaerobic treatment, there is no oxygen needed and low sludge production makes it more economical than aerobic treatment. Membrane technology (MBR) is able to handle wide changes in the quality of influent, reduce footprint and enhance the effluent quality (Lin et al., 2013). Regarding the rapid development of AnMBR technology, the problem of membrane fouling remains as one of the main challenges in the AnMBR system. Abdulsalam et al. (2018) reported that the AnMBR is suitable for POME treatment with high removal efficiency ranging from 98-99%.

2.2.4. Membrane materials

The AnMBR is made from different materials such as polymer, metal, and ceramic. Ersu & Ong (2007) and R.W. Baker (2000) reported that membranes can be backwashed using ceramic or inorganic materials and they are anti-corrosive, abrasion and fouling resistant, and have high flux (200-250 L/m² h). Metallic membranes tolerate oxidation, have high impact force, prevent fouling, and have good hydraulic performance (Kim & Jung, 2007; Zhang et al., 2005). The polymeric membrane is cheaper compared to the metallic and ceramic membrane. About 75% of total polymeric membrane products are made from polyvinylidene difluoride (PVDF), polyethersulfone (PES), and 25% are made from polyethylene (PE), polypropylene (PP), and polysulfone (PSF). The UF or MF membrane's configuration can be hollow, flat, or tubular.

Researchers and the commercial communities have gained more interested in the flat membrane for its stability, ease of cleaning, and ability to replace a damaged membrane (Lin et al., 2013). Submerged membrane bioreactors (SMBRs) used mostly hollow membranes because of their high packing density and cost-effectiveness. Tubular membranes have low-fouling and many of the same advantages as flat membranes, while they have the disadvantages of high capital and operational costs for pumping, high dead end volume, and low packing density. In AnMBR applications, it is preferable to choose low-cost membrane filters since the membrane is only capable of separating liquid and solid. Also, it does not contribute to the improvement of the effluent water quality. In this study, PVDF ultrafiltration membrane was chosen to treat POME.

2.3. Anaerobic Digestion

The anaerobic ponging system treats POME through the anaerobic decomposition and involves four main processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, complex organic molecules, namely fats, protein, and carbohydrates, break down into soluble organic molecules such as fatty acids, amino acids, and sugar respectively. Then in the following step, acidogenesis, fermented bacteria converts carbon monomer into volatile fatty acids (VFA), carbonic acid, alcohols, and a trace amount of by-product. Long-chain fatty acids (LCFA) are produced by lipids within this process. Acetogenesis functions using two groups of acetogenins; obligate hydrogen-producing acetogenins (OHPA) and homoacetogen. These microorganisms reduce the volatile fatty acids into acetic acid, hydrogen, and carbon dioxide. Methanogenesis is the final metabolic phase in anaerobic digestion. The hydrogenotrophic methanogens consume hydrogen and carbon dioxide and produce methane and water. The acetoclastic methanogens are carried out by *Methanosarcina* and *Methanosaeta* splits acetate into methane and carbon dioxide, and produces about two-thirds of total methane production (Magonigal et al., 2003).

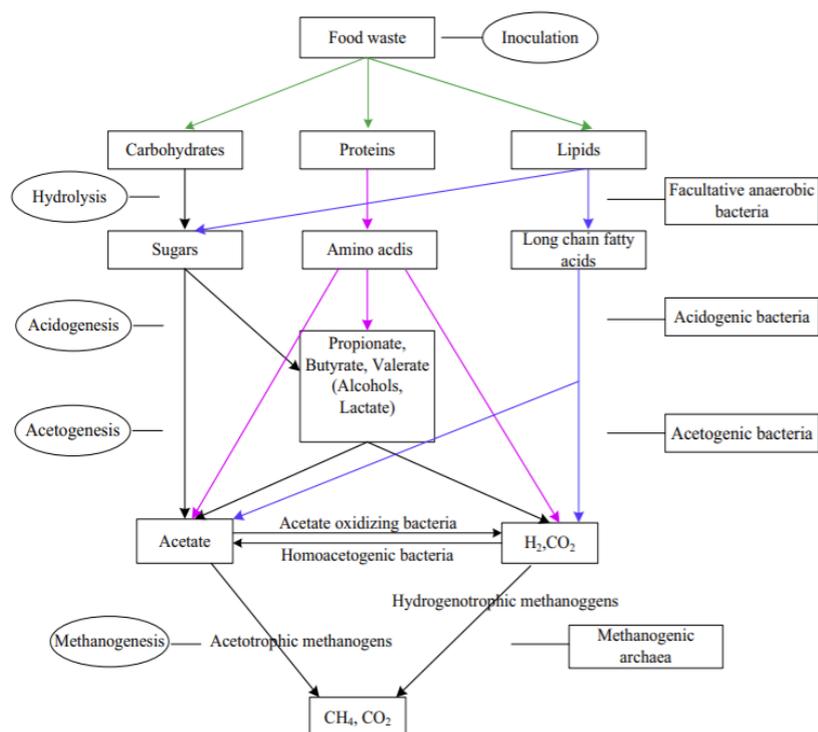


Figure 2. Pathways of Anaerobic Digestion present in food waste (Kumar Jha et al., 2011)

2.4. Operational Condition for AnMBR

The factors that influence the performance of the reactor include HRT, SRT, OLR, pH, temperature, and hydrodynamic conditions such as cross-flow velocity (CFV). High CFV harms the microbial community and causes disruption of the syntrophic between acidogenesis and methanogenic bacteria which leads to the dissipation of sludge activity (Evren et al., 2011; Seghezzi et al., 1998).

The treatment performance and membrane fouling are controlled by the SRT. Trzcinski & Stuckey (2010) operated the reactor with SRT of 300 days and 30 days, and the results showed that high soluble COD was removed by long SRT. However, according to Baek et al. (2010) changing SRT didn't affect the removal efficiency and reactor performance. HRT could potentially remove the pollutant to a limited extent. Mostly in AnMBR operation, long HRT and SRT show significantly high methane recovery, good treatment performance, and less sludge production (Ho & Sung, 2009). The optimum interval pH for AnMBR is from 7.0-8.0. However, to achieve these values, extra chemical dosage such as sodium bicarbonate ($NaHCO_3^-$) need to be done for neutralization because in anaerobic digestion, hydrolysis and acidogenesis reduce the sludge pH (Weiland, 2010).

Mostly the applied OLR for Continuous Stir Flow Treatment (CSRT) AnMBR is below 10 kg/COD/m³/d with low biomass concentration. However, for UASB, hybrid UASB, and EGSB, the preferred organic loading rate is above 10 kg/COD/m³/d (Lin et al., 2013).

Temperature is classified into three different classes: psychrophilic (0-20 °C), mesophilic (20-42 °C), and thermophilic (42-75 °C) (Rajeshwari et al., 2000). Meanwhile, thermophilic temperatures have gained interest from researchers due to their efficiency in increasing methane potential, specifically for wastewater coming from the paper and pulp industry which contains a high amount of organic matter and other toxic compounds. Psychrophilic and mesophilic conditions have proved to accumulate more LCFA which leads to membrane fouling (Martinez-Sosa et al., 2011).

3. Knowledge Gaps, Research Goal and Research Questions

3.1. Knowledge Gaps

1. The biological performance, fouling condition, and energy consumption of PVDF membrane.
2. The effect of SRT and OLR on the LCFA accumulation and digestion efficiency.

2.2. Research Goal

Overarching research goal: analyze the biological performance of the anaerobic membrane bioreactor (AnMBR) treating POME.

2.3. Main Research Questions, Hypothesis, Research Expectations, and Sub-Questions

Research Question: What's the biological performance: digestion efficiency, and biogas production of thermophilic (55 °C) AnMBR system when treating synthetic POME at SRT of 90 days and applying OLR of 3 g COD/L/d?

Hypothesis: AnMBR treatment of POME at SRT of 90 days and applying OLR of 3 g COD/L/d at 55°C is a suitable operating condition for efficient COD removal (> 98%).

Research Expectation: It is expected that the effluent from the AnMBR is capable enough to meet the wastewater discharge requirements and the biogas composition of methane is between 60%-80%. By operating the reactor under thermophilic conditions (55 °C), the biological treatment and biogas production are stable and have high COD removal efficiency (97%-99%).

Sub questions:

- How stable is a continuous operation in terms of pH, VFA, TSS, VSS?
- What is the digestion efficiency of the AnMBR and SMA?
- What is the COD removal rate (based on feed COD vs permeate COD)? How long did it last?

4. Methodology

4.1. Reactor Setup

The experimental study began with a lab-scale reactor operated at an SRT of 90 days and 3 g COD/L/d OLR at thermophilic conditions (55°C). To maintain the reactor at a constant temperature, a water bath (Tamson instruments, the Netherlands) was applied to the setup. The schematic representation of the lab-scale reactor is shown in Figure 3. The reactor is made up of a double-walled glass reactor with 6.5 L effective volume and PVDF tubular cross-flow ultrafiltration membrane with a mean pore size of 30 nm and total surface area of 0.00987 m². The system is equipped with two influent pumps, one for POME and VFA, a cross-flow recirculation pump, and an effluent pump. The transmembrane pressure is monitored by a sensor, a pH sensor, and a temperature sensor which is plugged into the reactor to measure the real-time sludge pH and temperature. Table 3 indicates the different sensor and pump models for each application. The biogas meter is installed at the top of the reactor to measure biogas production and the data is transferred into the monitoring computer. The reactor is controlled by a computer running LabView software. The backwashing process was done via the permeate pump which has 500 seconds filtration time, 20 seconds for backwashing, and 5 seconds for idle time. The PVDF membrane had a maximum crossflow velocity (CFV) of 0.7 m/s.

Table 3. Sensor and Pump model

Application		Equipment Model	Country
Pump	POME	120U, Watson Marlow	United Kingdom
	VFA	110U, Watson Marlow	
	Permeate	140U, Watson Marlow	
Recirculation	Sludge	520 Du, Watson Marlow	The Netherlands
Pressure sensor	Influent & Effluent	AE sensor, ATM-800..600 mBar, Serial number: 1186435	
Temperature	Sludge	ATM-800, AE sensors	
pH	Sludge	Memosens	Germany
SMA	Sludge	Digital Pressure Meter, GMH 3151	
Shaker	POME	New Brunswick Scientific, Innova 43	
Gas Production	Biogas	Ritter-MilliGascounter (MGC)	
SMA	Sludge	Digital Pressure Meter (GMH 3151)	
Sonicator	POME	Digital Sonifier, Model S-450D, Branson	The United States of America
Centrifuge	Sludge	ST16R, Thermo Scientific	
Gas Chromatography	Biogas Composition	Agilenttech 7890 A	
	VFA		
Alkalinity	Sludge	702 SM Titrino, Metrohm	Switzerland
	Permeate		

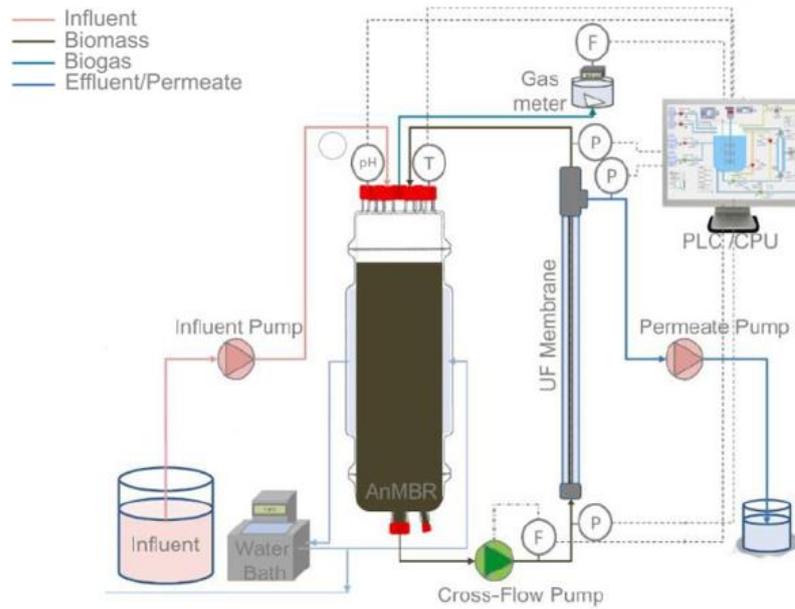


Figure 3. Schematic drawing of the AnMBR (Muñoz Sierra et al., 2019)

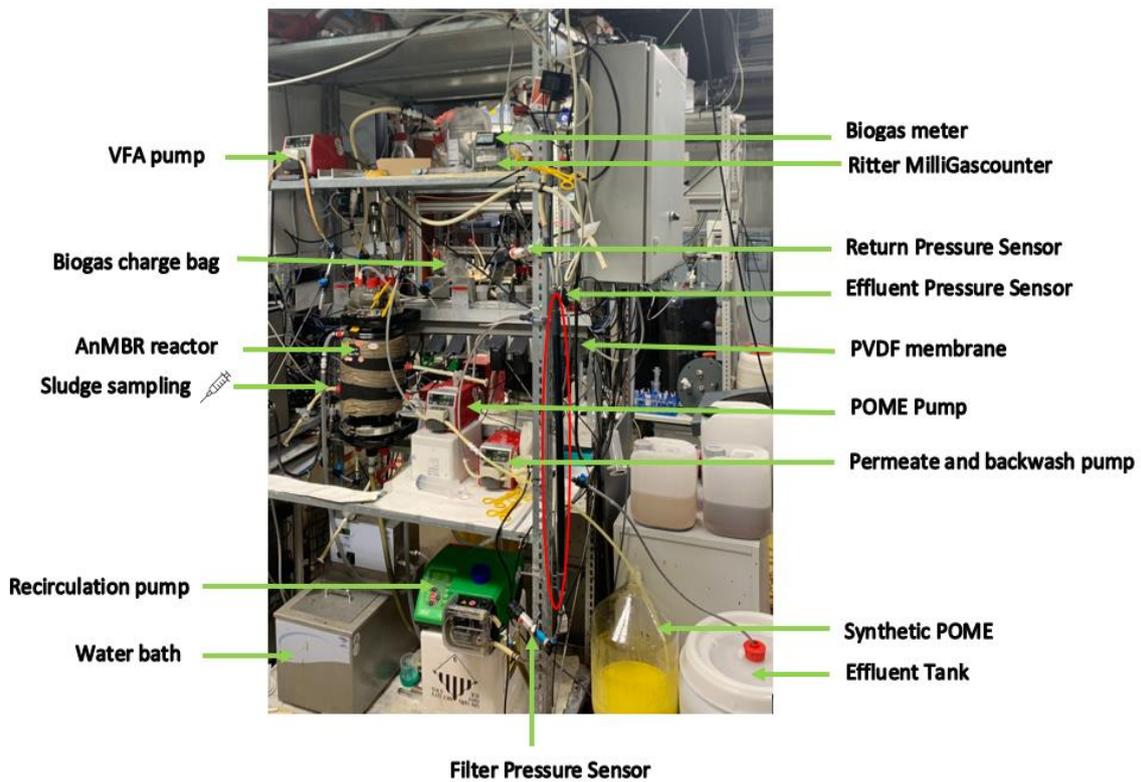


Figure 4. Actual View of the AnMBR system

4.2. Experimental Methods

4.2.1. Analytical Frequency

Frequency analyses of feed, sludge, and permeate were made to evaluate the reactor performance as shown in Table 4.

Table 4. Parameters analysis frequency

Parameters	Sample	Frequency	Objective
Flow rate	feed permeate	5/week 2/day	Controlling Reactor volume
TS, VS, TSS, VSS	sludge	1/week	Microorganism/biomass concentration; VS < 40g VS/L
pH	sludge permeate feed	3/week	Reflect digestion process, accumulation of VFA
Alkalinity	sludge	1 (in every 2 weeks)	Maintain sufficient alkalinity to buffer the effect of acids; >3 g CaCO ₃ /L
Total COD	sludge permeate feed	3/week	COD removal efficiency; Influent = Permeate + Wasted sludge + Biogas + Missing
Soluble COD	sludge		
Total P, N	sludge permeate feed	1 (in every 2 weeks)	Biological Performance, removal efficiency; NH ₄ ⁺ <2.5 g N/NH ₄ ⁺ /L
VFA	sludge permeate	3/week	Indicator of LCFA inhibition; an indicator of anaerobic treatment performance; <1 g Acetate /L
Biogas production/composition	biogas	2/week	Methane yield and quality; Digestion efficiency = (Daily methane production (gCOD/d))/(Daily total load (g COD/d))
SMA	sludge	1 per 2 months	Determine the sludge methane production capability

4.2.2. Feeds preparation

4.2.2.1. Synthetic POME

The synthetic POME was made by adding 6.6 g of crude palm oil (Brand: KTC unrefined palm oil 500 ml) mixed with demineralized water in a 1.1 L glass bottle. Then, the bottle was put to incubate for one day in a 55 °C shaker set 150 rpm (revolutions per minute). After 24 hours, the bottle was sonicated for 30 minutes with an amplitude of 40% to emulsify the oil. The sample was let to cool down at room temperature. After cooling down, the 0.103 mm siever was used to separate the aggregate solid oil. Lastly, the POME COD was measured using the Hach-Lange Test kit ranging from 1000-10000 mg COD/L with 2 times dilution factor.

4.2.2.2. VFA feed

The Volatile Fatty Acid (VFA) was prepared once every week with the chemical shown in Table 5 added with 7 L demineralized water. The sample was prepared according to the COD/N/P ratio of 350:5:1.

Table 5. VFA feed compound of 7 L solution

Chemical	Mass or Volume for 9 g COD/L
Sodium Acetate trihydrate	80.43 g
Sodium Propionate	8.85 g
Sodium Butyrate	10.81 g
Macronutrients	50.6 ml
Micronutrients	25.3 ml
Solution A	36.3 ml
Solution B	36.3 ml

4.3. Analytical Methods

The experiments were done in triplicates to validate the results and all data are expressed as the mean \pm standard deviation. The pH of the feed, sludge, and permeate was measured using the pH meter to ensure the optimal pH of the sludge remained between 7.0-8.0 and if pH went out of this range, extra acid or base dosage is required to neutralize the sludge. The VFA and POME flow rate were analyzed once per day and twice per day for permeate. During the collection of permeate flow, 20 seconds backwash and 5 seconds idle time were not counted.

The Chemical Oxygen Demand (COD) for each sample was conducted three times per week. For the total phosphorus (TP), phosphate (PO_4^{3-}), total nitrogen (TN), and ammonium (NH_4^+), the measurement was done every two weeks as summarized in Table 6. To measure the soluble COD, and supernatant COD, 10 mg of the sludge is placed in a 15 ml tube and centrifuge for 10 minutes with a setting of 18500 g. After that, a portion of the supernatant was used to measure supernatant COD, and the remaining was used to measure soluble COD by filtering through 0.45 μm using a 10 ml syringe.

Table 6. COD, TN, TP, PO_4^{3-} , NH_4^+ data analysis

Sample		Parameter	Dilution Factor	Test Kit range (mg/L)
POME		COD	-	1000-10000
VFA				
		TP	8	2-20
		TN	8	20-100
Permeate		COD	-	100-2000
		TP	5	2-20
		TN	5	20-100
		PO_4^{3-}	5	2-20
		NH_4^+	5	2-47
Sludge	Total	COD	-	1000-10000
	Soluble		-	100-2000
	Supernatant		-	100-2000

Solids and suspended solids were measured weekly for sludge and permeate. The parameters are total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS). The aluminum plate was used for TS and VS. Meanwhile, for TSS and VSS, another paper filter with $0.7 \mu m$ pore size was placed on the plate. The experiment started by putting all the plates in a $550^\circ C$ oven to remove particulate matter on the plate and filter paper. After heating, the sample was put in the desiccator for cooling about 10 minutes before weighing. All the empty plates and plates with filters were measured. For the TS and VS, 5 g of sludge will be added. A vacuum device is needed to measure the TSS and VSS. The plates were placed in the vacuum cells using tweezers and a small spatula aid. The filters were wet with demineralized water and 5 ml of sludge or permeate were added. Then, all the plates were put in the $105^\circ C$ oven and evaporation was allowed for 24 hours. Next, the plates were let to cool down in the desiccator and their weight was measured. Lastly, the plates were put in the $550^\circ C$ oven and, after the plates cooled down, the final weight was measured.

The total alkalinity analysis of sludge and permeate involved titration. In this test, titration is the addition of small and precise quantities of hydrochloric acid (HCl) which is used as a reagent until the sample reaches an endpoint pH of 4.3.

Daily biogas production was measured using the Ritter MilliGascounter (MGC) which is filled with a clear, colorless and odorless synthetic oil known as Silox. The lab environment keeps the reactor at a constant temperature of $55^\circ C$. Therefore, the biogas data was corrected to $0^\circ C$ at 1 atm. To measure the biogas composition, the gas was collected from the exhaust gas nozzle in the MGC and injected into the GC-biogas with the method “ INJ-MANUAL TEST2 NO HEADSPACE.M”

The volatile fatty acids (VFA) of sludge and permeate were analyzed by gas chromatography three times per week with a dilution time of 14. The sample was prepared by adding 0.75 ml of sludge or permeate, filled with 0.75 ml of 320 mg/L pentanol solution. Then an additional of 10 μ L of 98% formic acid was added before being mixed with a vortex mixer.

The COD removal efficiency was calculated based on permeate quality:

$$COD\ removal\ efficiency(\%) = \left(1 - \frac{COD_{effluent}(g\ COD/L)}{COD_{influent}(g\ COD/L)} \right) \times 100 \quad (1)$$

The specific methanogenic activity (SMA) was done by using acetate as the only substrate in a sealed serum bottle (120 ml). The sample was prepared by adding 100g of sludge and add a specific amount of acetate (0.43g/bottle) with an organic load of 2 g COD/L, then the headspace was flushed 3 minutes with nitrogen gas before sealing with a T-shaped butyl rubber stopper and aluminum crimp seal immediately to avoid oxygen contact. Subsequently, the bottles were placed in a temperature-controlled incubator. The pressure was measured using a digital pressure meter twice per day for 3 days. The methane production curve was made by using the slope of the linear regression line.

5. Results

The results below were obtained from operating the AnMBR reactor from the day 226 till 287. The reactor's pH was stable in the range of 7.2 to 7.4 and dropped significantly from day 276 to day 282 due to acidification in the reactor, and slowly recovered back to normal after a few days after injecting a small amount of sodium bicarbonate into the VFA in the sludge. The average pH during the operation was 7.3 ± 0.1 .

The total suspended solids (TSS) and volatile suspended solids (VSS) of the sludge has an average value of 4.4 ± 0.9 g TSS/L and 3.2 ± 0.6 g VSS/L. The food to mass ratio (F:M) is known as the amount of food coming into the reactor and was calculated by dividing OLR by VSS, which represents the biomass concentration in the reactor. The average F:M was 0.9 ± 0.2 g COD/g VSS/d. There was a high F:M ratio on day 280, which led to inactive biomass and a decrease in VSS content of about 38.6% relative to the day 272.

The activity of methanogenic and acidogenic bacteria can be monitored through the VFA concentration (Rajeshwari et al., 2000; van Lier et al., 2015). The sludge VFA was analyzed using GC analysis. The results showed the average VFA in the reactor was 209.1 mg COD/L and the major VFA constituents throughout the entire operation were acetic acid and propionic acid with average values of 158.9 mg COD/L and 46.7 mg COD/L, respectively. It's worth noting that the feed VFA (propionate and butyrate) was added to the reactor for two reasons. Firstly, the VFA production via β -oxidation is slow, and it is the rate limiting factor. That's why an additional VFA with an OLR of 1 g/L/d is needed to supply to the methanogens. Secondly, methanogens are sensitive to the amount of feedstock and feeding frequencies (Yee et al., 2019), and they are slow growing, therefore additional VFA is necessary to prevent the loss of methanogens in the reactor. The average VFA/alkalinity ratio was 0.04 ± 0.01 .

The COD removal rate remained effective till day 276 and dropped significantly from 98.8% to 70.5% at the end of the operational period. COD content in the permeate also increased between the day 281 and 286. The average daily biogas production was 5.4 ± 1.8 L/d and composed of 75.1% of methane gas. Low methane production from day 276 has caused a decrease in digestion efficiency from 80% to 4%.

The SMA test was monitored using the reactor's sludge from day 258 to day 275 . The initial pH before starting the test was reported as 8.38. After 9 days of incubation, the blank pH decreased from 8.38 to 8.27 ± 0.04 , and the SMA sample increased to 8.5 ± 0.4 . The SMA total COD decreased 6% and soluble COD increased by 40%. The maximum slope of SMA was

55.63 g CH₄-COD/g/VSS/d and an average of 7.73 CH₄-COD/g/VSS/d. A similar figure shows the maximum blank sample which represents sludge digestibility of 6.34 CH₄-COD/g/VSS/d and an average of 4.77 CH₄-COD/g/VSS/d.

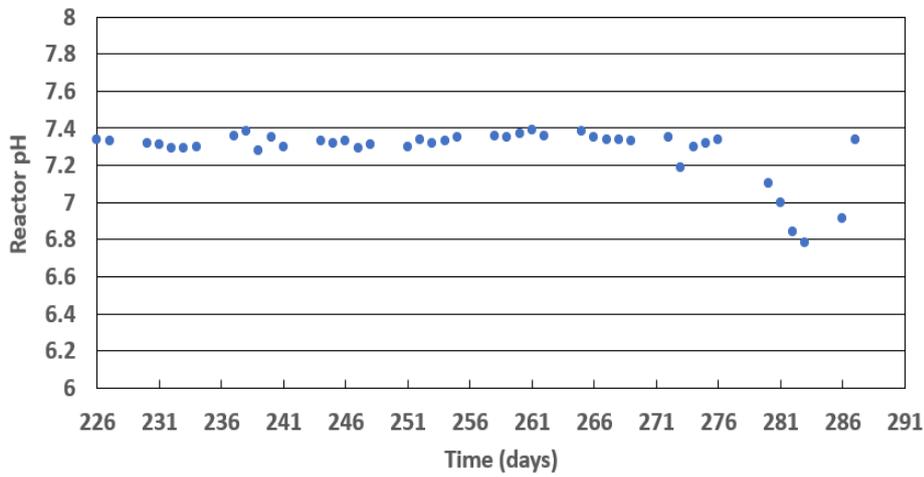


Figure 5. Reactor's pH

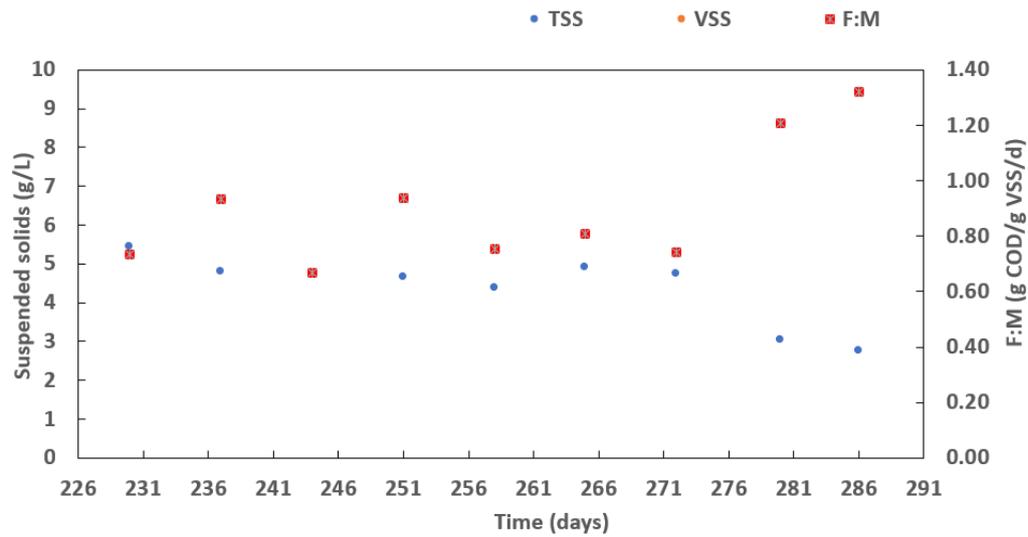


Figure 6. Total Suspended Solids (TSS) (g/L), Volatile Suspended Solids (VSS)(g/L), and Food to Mass Ratio (F:M) (g COD/g VSS/d)

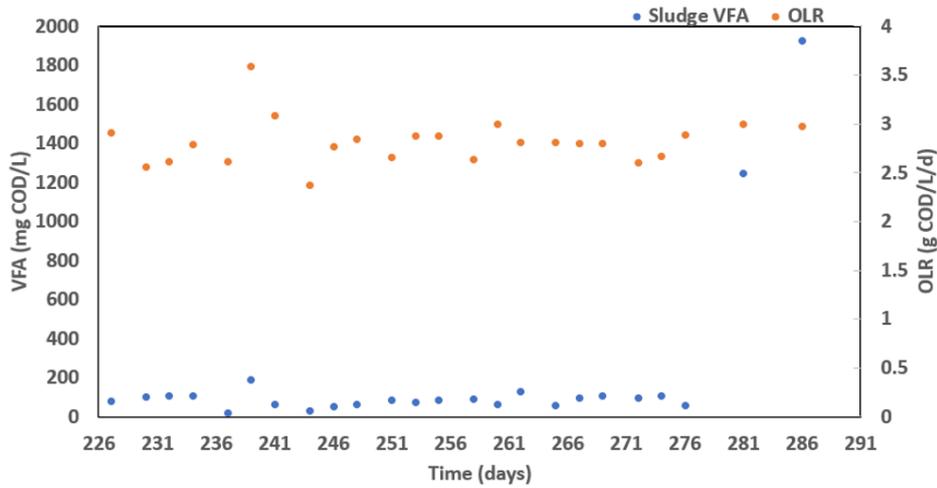


Figure 7. Sludge VFA (mg COD/L) and organic loading rate (g COD/L/d)

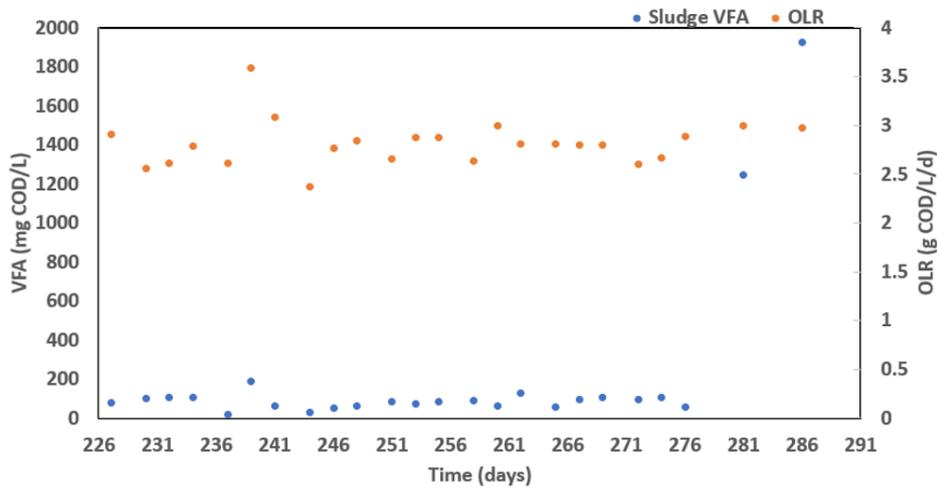


Figure 8. OLR (g COD/L/d), Permeate COD (g COD/L), and COD removal rate (%)

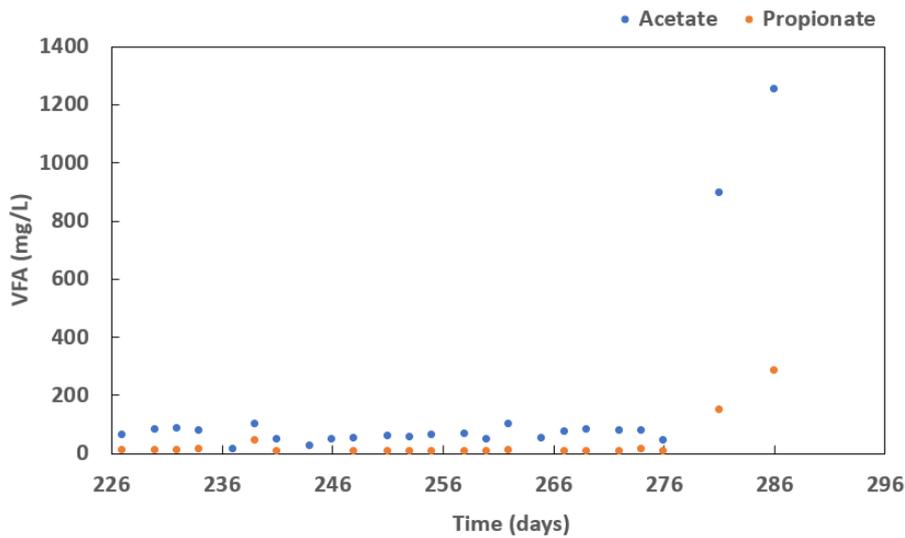


Figure 9. Sludge acetate (mg/L) and propionate (mg/L)

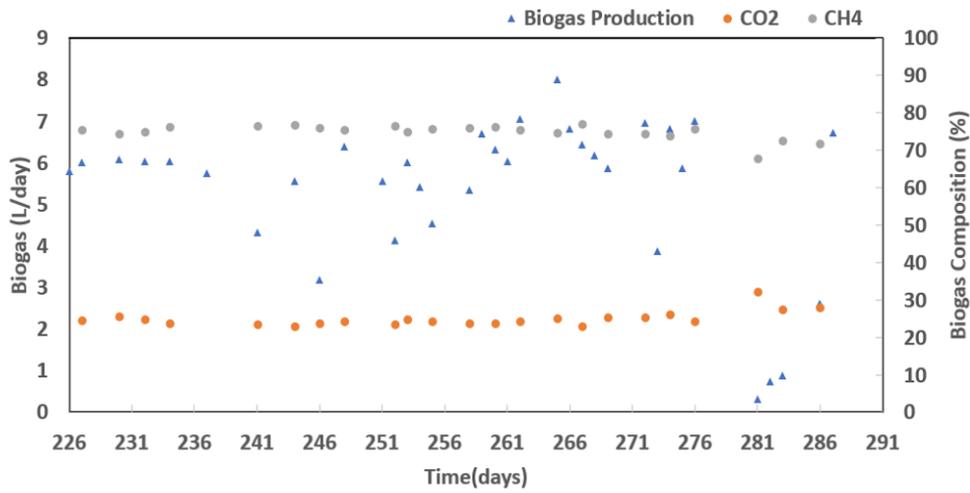


Figure 10. Daily biogas production (L/day), CO₂(%), and CH₄(%)

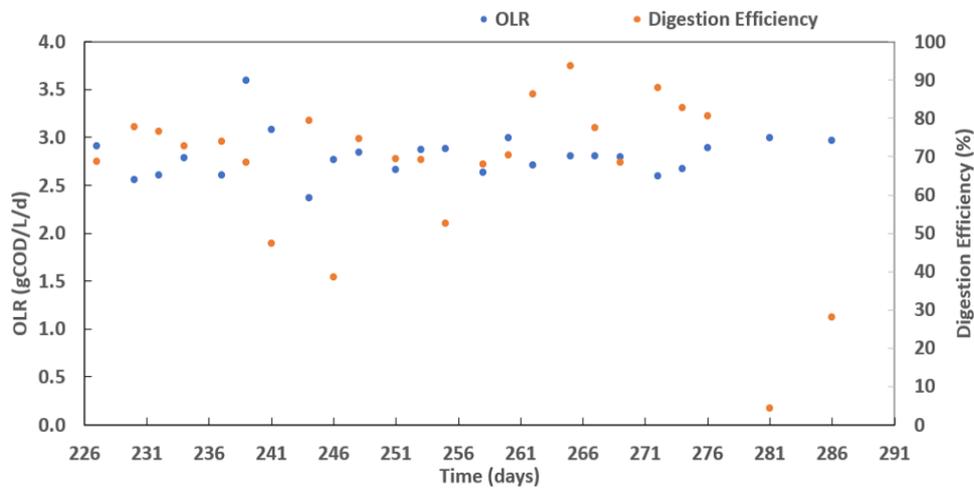


Figure 11. Organic loading rate (g COD/L/day) and digestion efficiency (%)

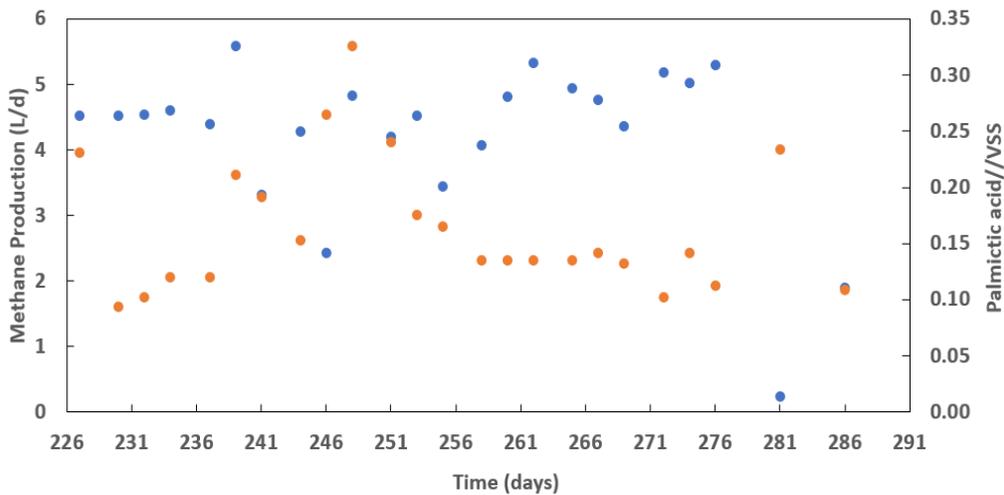


Figure 12. Methane production (NL/day) and palmitic acid equivalent/VSS ratio

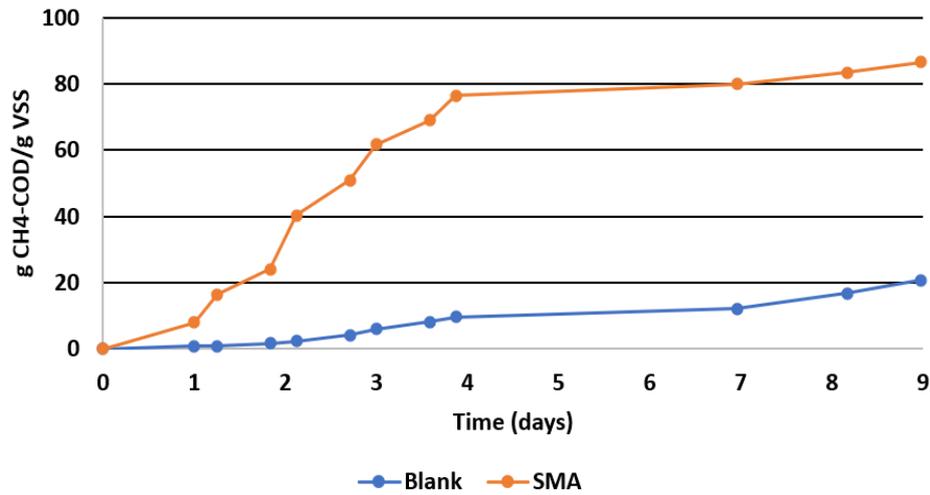


Figure 13. Sludge digestibility and specific methanogenic activity

Table 7. COD, Total phosphorus, Total Nitrogen, Ammonium, Phosphate of feed (POME, VFA), Permeate, and Sludge

Sample		Parameter	Result (mg/L)
POME		COD	8.4 ± 0.5
			8.3 ± 0.3
VFA		TP	0.03 ± < 0.01
		TN	0.12 ± < 0.01
Permeate		COD	0.3 ± 0.6
		TP	0.03 ± < 0.001
		TN	0.1 ± 0.01
		PO_4^{3-}	0.03 ± < 0.001
		NH_4^+	0.1 ± 0.01
Sludge	Total	COD	7 ± 0.5
	Soluble		0.7 ± 0.7
	Supernatant		0.7 ± 0.7

6. Discussion

The results obtained from the biological performance showed significant changes in the reactor between day 276 and 286. These changes were caused by the failure of the transmembrane pressure sensor which required system maintenance, and the reactor took some time to recover back to its normal level. In the meantime, it was noticed that there was an accumulation of solids and clumps of lipids occurring at the bottom of the reactor forming a cake layer that may influence the flow. In addition, the reactor level affects the performance of the microbes causing changes in pH and biogas production. As the level rises, this could be attributed to too much food going into the reactor at one time. The microbes were unable to digest the excessive food causing a build up which may temporarily inhibit the microbial growth performance. When there is overloading, some microbes may not survive in new conditions, which shows by the rate of decay.

Another reason could be the accumulation of LCFA in the reactor which affects the sludge methanogenic activity and reduces the pH level. The VSS content in the reactor decreased which can be another result of excess food inside of the reactor (expressed as the F: M ratio) causing loss of biomass or the biomass to become inactive. On the day 265, higher digestion efficiency was achieved along with high methane production. Palmitic acid (C16) is the intermediate of the oleic acid degradation, and oleic acid inhibits the methanogens more than saturated LCFAs (Lalman & Bagley, 2000). Therefore, to overcome the limitation of methane production, it was suggested that the conversion of palmitic acid to acetate and hydrogen is needed (Deaver et al., 2020). The VFA/total alkalinity ratio was in a safe range (≤ 0.4) with less risk of acidification in anaerobic digestion process (Issah & Kabera, 2021). Rosato (2015) stated that if the VFA/total alkalinity ratio >0.3 , there is instability and poor digestion.

The results obtained from the sludge methanogenic activity using acetate as sole substrate showed a decrease in TCOD and an increase in SCOD. Souto et al.(2010) reported that the stirring speed and incubation temperature influence the sludge activity. The amount of acetate added to the sludge as the sole substrate would also influence the methane production result. Overdosing on acetate concentration could cause sludge inhibition.

The test which was performed at 35 °C showed that methane production happened immediately, but after decreasing temperature to 15 °C, there were six days of lag phase. An orbital continuous stirring was required to minimize deflocculation formed by biomass. In this research case, the SMA was performed at 55 °C, and there was immediate methane production.

Considering the obtained results from the entire operation, high effluent quality was obtained when operating with AnMBR which can be further treated and used for various purposes such as irrigation in the agricultural sector or for reuse in industry (Perle M et al., 1995).

Earlier data from day one till the day 225 of the reactor made by Jianyi Tang during her thesis can be found in the appendix (Tang, 2021). Jianyi operated the reactor in 4 phases with different SRT: phase A (R-140), B (R60), C (R-90), and D (R-90). The start up OLR was 1-2.4 g COD/L/d, then 2.5 g COD/L/d for phase A,B,C and increases to 3 g COD/L/d in phase D. Jianyi found that at 90 days and 140 days SRT, there was high biological degradation efficiency, high COD removal rate, and high biogas production.

Table 8 Comparison of phase D (Tang,2021) and current study

Parameter	Unit	Phase D	Current study
SRT	d	90	90
OLR	kg COD/m ³ /d	2.82 ± 0.08	2.81 ± 0.24
TSS	g/L	6.99 ± 0.48	4.39 ± 0.89
VSS	g/L	4.44 ± 0.27	3.18 ± 0.62
F/M ratio	kg COD/kg VSSS/d	0.66 ± 0.04	0.90 ± 0.23
Permeate COD	mg/L	135 ± 14	297 ± 0.6
COD removal efficiency based on permeate quality	%	98.52 ± 0.15	96.74 ± 6.38
Methane production	L/d	4.75 ± 0.12	5.35 ± 1.84
Digestion efficiency	%	74.1 ± 2.2	67.38 ± 20.17

As shown in Table 8, there was decreased in biomass content in the reactor comparing the current study to phase D. TSS and VSS decreased 37% and 28%, respectively. F:M ratio, permeate COD and methane production in the current study is higher than phase D.

7. Conclusion

Overall, POME was successfully treated with AnMBR using a PVDF ultrafiltration membrane at an SRT of 90 days and an OLR of 3 g COD/ L/d under thermophilic conditions (55 °C).

Research Question: What's the biological performance: digestion efficiency, and biogas production of a thermophilic (55 °C) AnMBR system when treating synthetic POME at SRT of 90 days and an OLR of 3 g COD/ L/d ?

The digestion efficiency was 72% with a COD removal rate of 98%. The daily biogas production was 5.86 L/day. The sludge pH was 7.3. TSS and VSS concentrations were 4.4 g TSS/L and 3.2 g VSS/L, which resulted from some biomass loss or inactivity resulting in a high decay ratio. The maximum SMA was 55.63 g CH₄-COD/g/VSS/d and an average of 7.73 CH₄-COD/g/VSS/d. The reactor's performance remained stable for 50 days (day 226 - 276). The treatment of POME with PVDF membrane was economical and high effluent quality was achieved.

8. Recommendation

The operational changes can be observed using sludge filterability methods such as capillary suction time (CST), specific resistance to filtration (SRF), extracellular polymeric substances (EPS), sludge relative hydrophobicity (SRH), and particle size distribution (PSD). LCFA measurement can be done to measure the LCFA inhibition and fouling caused by sludge in the anaerobic reactor. In addition to SMA testing, the biochemical methane potential (BMP) analysis can be made to determine the production yield of methane. A mechanical stirring device can be mounted to the top of the reactor to mix the sludge and to prevent accumulation at the bottom of the reactor. It worth to test the experiment with different membrane type such as $\alpha\text{-Al}_2\text{O}_3$ to check the treatment efficiency. In addition, life cycle analysis (LCA) can be made to analyze the environmental impact of POME associated with the current anaerobic membrane technology.

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Appendix

A. SMA results

Table A-1 Summary of SMA result before and after measurement

		pH	Acetic acid		Propionic acid		TCOD	SCOD
		-	Average mg/L	COD mg/L	average mg/L	COD mg/L	mg/L	mg/L
Blank SMA	initial	8.38	71.9	77.6	7.9	12.1	6668.3	396.1
	final	8.265	9.503	1.01	-	-	6071	381
		8.472	21.246	2.50	74.609	5.61	6221.3	556.5

B. Previous data of the reactor at different SRT

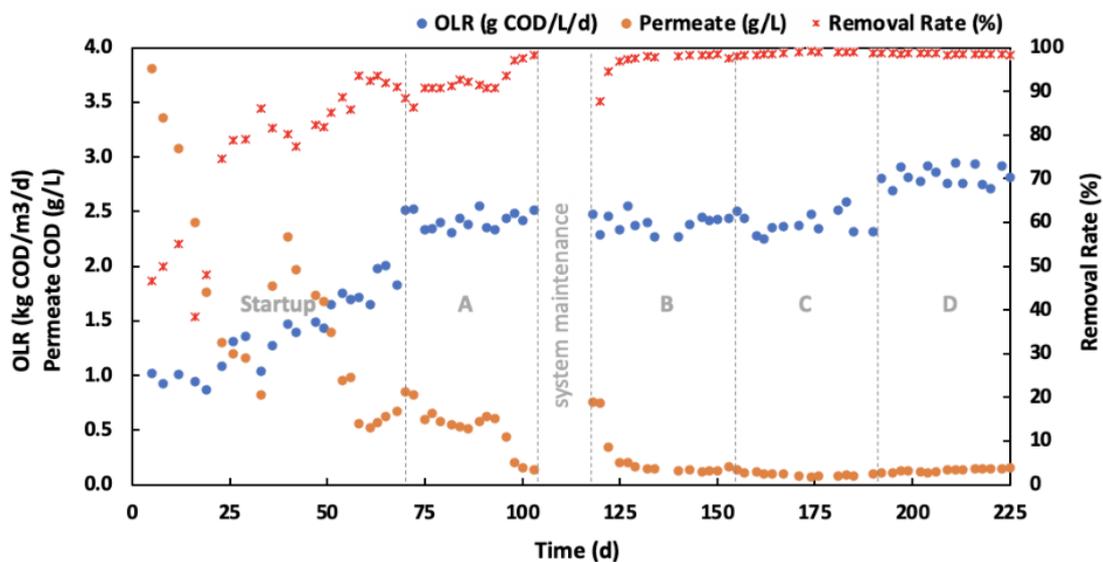


Figure B-1. The OLR (kg COD/m³/d), permeate COD (g/L) and COD removal efficiency (%) of reactor at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90)

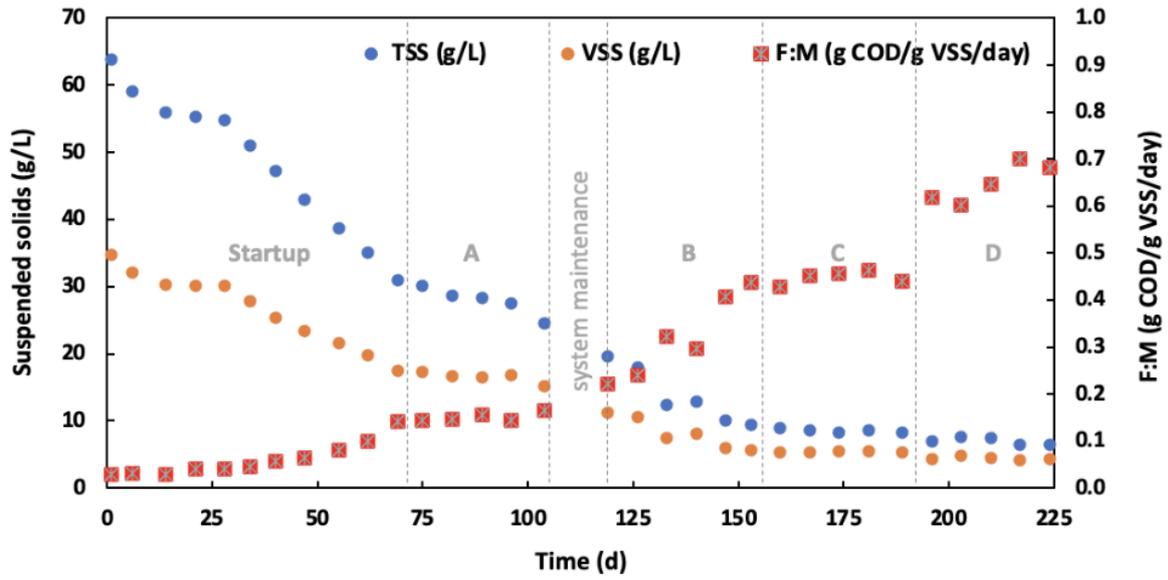


Figure B-2. Total suspended solids (g/L), volatile suspended solids (g/L), and food to biomass ratio (gCOD/gVSS/day) at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90).

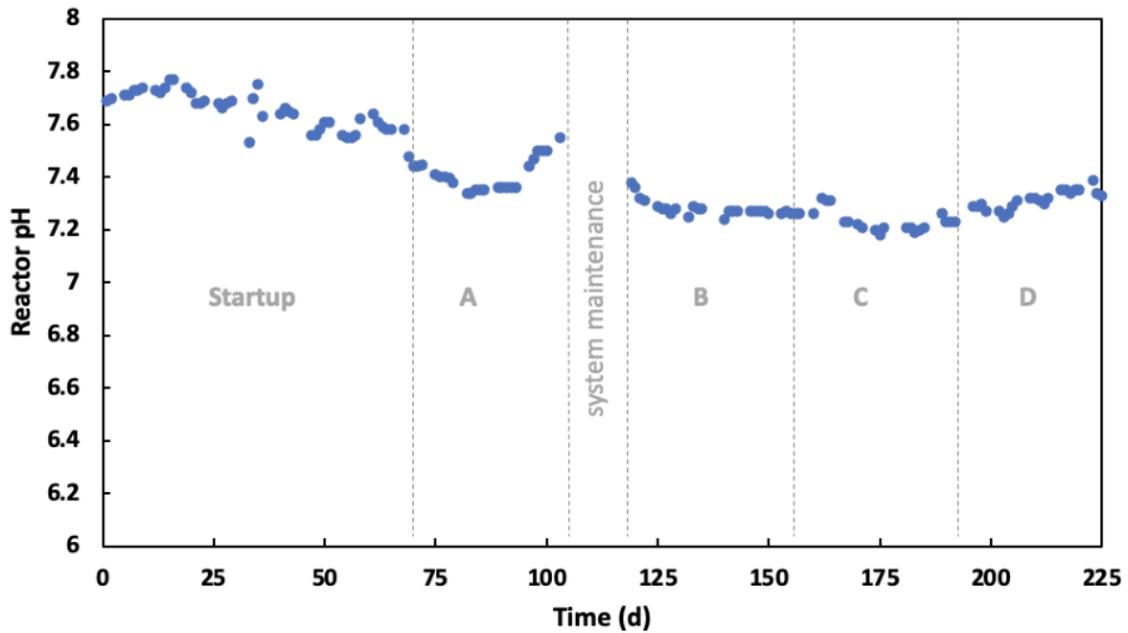


Figure B-3. pH of the sludge at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90).