Treatment of Synthetic Palm Oil Mill Effluent (POME) Using AnMBR: Biological and Filtration Performance

By

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Abstract

Palm oil mill effluent (POME) is a high organic pollution produced during the palm oil mill process, with a brownish color and stingy odor at high temperatures. Given the popularity in palm oil output over the years, the massive amount of POME causes growing concern. The enforcement of wastewater discharge standards and laws, as well as energy recycling of sustainability goals have facilitated the development of POME treatment processes. Several lab-scale studies have looked into the treatment of industrial wastewater using anaerobic membrane bioreactor (AnMBR) which has received considerable research interest due to its demonstrated potential for POME treatment.

In this study, the synthetic POME was treated by a lab-scale crossflow anaerobic membrane bioreactor system. This study tested the feasibility of thermophilic PVDF-AnMBR systems for synthetic POME treatment, and meanwhile evaluated the biological and filtration performance of AnMBR treating lipid-rich wastewater at different sludge retention times (SRTs = 60 days, 90 days, and 140 days).

AnMBR showed an adequate biological performance during the stabilizing state. The synthetic POME could be treated with over 98% of COD removal efficiency in all operational conditions. Plus, better digestion efficiency could be achieved at higher SRT (140 days). However, this study stresses that even though the membrane ensures biomass retention, the AnMBR process is still dodged by long-chain fatty acid (LCFA) accumulation and inhibition problems, especially at short SRT (60 days). The continuous reduction of biomass concentration during the stabilizing process of SRT at 60 days eventually resulted in the decreased methane production and system instability.

Under all operational conditions, sufficient filtration performance and net permeate fluxes between 8 and 11 LMH were achieved. The trans-membrane pressure (TMP) was under 200 mbar throughout operating process. No membrane cleaning was needed. The results showed that better sludge filterability could be achieved at SRT of 90 days. The sludge filterability was compared as per the standard methods, including specific resistance to filtration and capillary suction time, which did not show a linear relationship with SRTs. Meanwhile, the physical-chemical characteristics of the sludge during the operational phases, including TSS concentrations and SMP, have a close correlation with sludge filterability parameters, such as capillary suction time and supernatant filterability.

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1 Introduction

Globally, Malaysia is a leading producer as well as exporter of palm oil and related products. The palm oil industry satisfies a growing demand for oils and sustainable fats (MPOC, 2019). Admittedly, the palm oil industry spurs the economic growth, but it has severe impacts on the environment, including deforestation, greenhouse emissions, damage to precious ecosystems, resource waste, water pollution, etc. Among these, water pollution is environmentally hazardous situation. The palm oil industry releases significant quantities of brownish palm oil mill effluent (POME) that contaminates surrounding ecosystems, especially freshwater and groundwater. In detail, the POME comprises different compounds that pose significant environmental problems, including organic compounds, which can be proved by the biological oxygen demand (BOD) and chemical oxygen demand (COD), oil and grease, and total solids and suspended solids of POME ranging from 25,000 to 35,000 mg/L, 8,370 mg/L, 43,635 mg/L, and 19,020 mg/L, respectively (Abdullah & Sulaim, 2013). The organic content is far too high for it to be discharged safely without any treatment. Additionally, in 2019, the demand for palm oil in gobal was around 74.6 million tons, and it is continually growing (Grand View Research, 2020). It is therefore necessary to treat wastewater in order to counter the adverse environmental impacts of the palm oil industry.

When effluents contain high concentrations of organic matter, treating the effluents with anaerobic digestion is the best practice. In Malaysia, nearly 85% of the mills that treat POME have adopted facultative and anaerobic ponding systems (Rana et al., 2017). The ponding system offers vital advantages, such as low startup costs, ability to handle high organic loading rate and minimal maintenance. Yet, this system also has its disadvantages, say, a large surface area needed for treatment, long hydraulic retention time (HRT), and generation of methane gas which is a greenhouse emitter (Rana et al., 2017). Worse still, the sludge is most likely to absorb long-chain hydrocarbons, which decreases the bacteria's activity; consequently, the biological system lacks long-term efficiency, which inhibits the bacteria's activity (Ma et al., 2015). Numerous POME treatment methods are extensively applied to address these issues and boost the efficiency of treating effluents in the palm oil industry (Ohimain & Izah, 2017). To enhance the treatment capabilities, it is crucial to marry biological and physiochemical methods with membrane filtration because biological treatment alone cannot treat wastewater effectively.

In terms of recovering bioresources like biogas, membrane technology has proved to be extremely useful for treatment. With membrane technology, groups of microorganisms treat the POME actively via membrane filtration methods, such as microfiltration, ultrafiltration, and effective removal of suspended solids. Membrane technology combines active sludge processes based on the membrane separation process, which is a substitute clarifier to reduce the COD and BOD values to a range that is within the accepted standard while improving the productivity of biogas (Rana et al., 2017). Compared to conventional methods of treatment, membrane bioreactor technology offers significant improvements, such as short HRT, high-quality effluent, and higher organic loading rate (OLR). Nonetheless, it has a few disadvantages, such as membrane fouling, increased rate of energy consumption, increased membrane costs, and shorter lifespan of the membrane, which often inconvenience membrane bioreactor (MBR)users.

The biological and filtration performance of MBR systems is steadily improved for better treatment of concentrated organic effluents, oil, and grease (e.g. POME). Polyvinylidene fluoride was selected to fabricate membranes used in the filtration treatment process, and this polymer is preferred over other polymers thanks to its suitable mechanical characteristics and higher chemical resistance (Kim et al., 2015). However, there is one major drawback to system performance, which is fouling (Kasi et al., 2017). Exploring the characteristics of the PVDF membranes in the palm oil industry is crucial to optimizing the performance of filtration. Besides, the previous research shows that MBR treatment system is highly effective in treating POME. However, SRT is considered a key operational parameter in POME treatment. Its impact on response performance is so meaningful that it is worth further exploration.

This research project proposed for POME treatment process. POME was synthesized in a laboratory before being anaerobically digested into the bioreactor, combined with the biogas collection system and outside membrane for the retention of biomass. This study aimed to assess the performance of AnMBR treating synthetic POME at different OLRs and SRTs. The biological performance at different SRTs, as well as LCFA adsorption, degradation and inhibition processes were studied. Furthermore, the membrane filtration performance, including the long-term filtration performance, fouling rate and sludge filterability with respect to wastewater composition and operating condition were evaluated.

2 Literature review

2.1 POME wastewater

2.1.1 Introduction of Palm Oil Processing

The fresh fruit bunches are used to extract palm oil by four processes including sterilization, stripping, threshing, digestion and extraction in palm oil mills (Rupani & Singh, 2010). Sterilization is the first step while extracting the crude palm oil, and the whole process should be conducted by stream in 140 °C for 75-90 mins inside of the autoclave (Mohammad, Baidurah, Kobayashi, Ismail, & Leh, 2021). The main purpose of this step is inactivating the hydrolase, breaking down the oil into free fatty acids and loosening the fruit. However, the stream condensate coming out of the sterilizer could be the main sources of wastewater in this step. After sterilization, the stripping or threshing will help the fruits separate the empty fruits bunches part and become available for further heating and mesocarp oily cells breaking in the next digestion step. Afterwards, the homogeneous oil mash after digestion needs to remove fine solids, water, and other substances. Therefore, vibrating screens, hydrocyclones and decanters will be used for purification. However, it is inevitable that the main waste decanting wastewater and decanting cake will be generated at this stage. Then, the oil will be purified by centrifugation and drying before store in the oil tank.

The oil palm mill generates a large amount of wastes, for every 1 ton crude palm oil, 2.5-3.5 tone of POME is produced (Madaki & Seng, 2013). The extraction of oil results in the production of a liquid waste known as palm oil mill effluent (POME). Palm oil mill effluent is mainly produced by the process of mill's oil extraction, washing, and cleaning, and it comprises cellulosic material, fat, oil and grease (FOG), among other things. The leaf, stem, decanter cake, empty fruit bunch, seed shells, and the mesocarp fibre are solid waste created during the extraction process (Rupani & Singh, 2010).

2.1.2 Characteristics of POME

During palm oil milling, a high level of organic pollution called POME is released, which has a brownish color and a strong odor at high temperatures (80-90°C). And it is also a main source of river pollution which is highly acidic in nature and has a high biological oxygen demand, chemical oxygen demand, along with unsafe levels of oil and grease, suspended solids, and total nitrogen content (Ahmed et al., 2015). Besides, POME composition varies in different factories and seasons, as the characteristic of POME is highly dependent on process design, operations, and quality control in the palm oil mill (Bello et al., 2013).

Given the rising trend in palm oil output year after year, the massive amount of POME gets even more concern. Around six tones of water are needed to generate one tone of crude palm oil, with an average of three tons of fresh water ending up as POME (A. L. Ahmad, Ismail, & Bhatia, 2003; Nnaji, 2016). In this project, the POME in Malaysia is focused on, the Table A.1 in appendix A lists the typical characteristics of raw POME, which can be referred to make a better choice of

POME treatment technology. The Malaysia experience in effluent control of the palm oil industry demonstrate that a set of effective in controlling industrial pollution in a developing country (Igwe & Onyegbado, 2007). The Table A.2 in appendix A shows the comparison between discharge limit from Malaysian Department of Environment (DOE) and guidelines for wastewater reuse from WHO, which are good references for the evaluation of water treatment methods.

Other physical and chemical features of POME, in addition to the composition and amount indicated above, are critical for the selection of treatment method and resource utilization in the bioprocessing and fermentation sectors (Alrawi et al., 2013). The overall oil droplet size of oily wastewater in agriculture was in the range of 0.8 - 1.3 μm, which fit into the category of emulsified oil (size 20 μm) in oily wastewater. Besides, it was also reported that because of the lipid hydrolysis, the oily water particle size distribution and composition change over time (Zhong, Xing, & Zhang, 2013).

2.1.3 Current treatment and Superiority of biological anaerobic treatment

The enforcement sewage discharge standards and laws have facilitated the development of POME treatment processes. The three main modern treatment are physical, chemical and biological process, which including the anaerobic process, aerobic process, membrane separation, water evaporation, and solid removal by coagulation-flocculation in detail (Hamzah et al., 2020). Among them, the physical and chemical treatment process cannot achieve satisfactory performance, and the investment and maintenance costs are too high, it has not been used on a full scale.

Anaerobic and aerobic procedures are used in biological treatment. They are a more effective and long-term solution for POME treatment. A number of studies have found the anaerobic digestion in thermophilic condition could achieve the satisfactory performance of suspended solids, BOD and COD removal while treating POME (Chan, Chong, & Law, 2012). Moreover, anaerobic treatment is considered to be an efficient and stable environment-friendly process, because the methane produced by the reaction can be effectively used as a recycled energy source. Simultaneously, POME is a rich source of nutrition for microbes due to its high organic content, hence methane production via anaerobic digestion has a great deal of potential (Hamzah et al., 2020).

There are many great advantages of anaerobic digestion while comparing with aerobic technology. Firstly, the anaerobic digestion has less sludge production, accompanying low cost of management and disposal. Second, higher loading rate could be applied, and smaller reactor size is required. Besides, the biogas produced by the digestion could be the energy supply, no aeration leads to less energy required. In addition, no additional chemical dosage or low needed, the unconsumed nitrogen and phosphate could be recovered (van Lier et al., 2012). The numerous benefits of anaerobic digestion make it widely used for high strength wastewater treatment (Poh et al., 2016).

2.2 Anaerobic digestion

2.2.1 Long chain fatty acid (LCFA) characterization and degradation

Hydrolysis, acidogenesis, acetogenesis and methanogenesis are the four sequential steps in anaerobic digestions which can convert the organic matter such as proteins, carbohydrates, and lipids to methane. Lipids are a primary organic pollutant found in POME. Lipids are glycerol, they bond to LCFAs, alcohols, and other groups via an ether or ester linkage. During the process of anaerobic treatment, hydrolytic extracellular lipases hydrolyze lipids into glycerol plus LCFAs (Cirne et al., 2007). Glycerol is broken down through acidogenesis, meanwhile, LCFAs are broken down via β-oxidation process (syntrophic acetogenesis) to acetate, H_2, and CO_2. Finally, methanogenesis converts them to CH_4 or CO_2 (Figure 1). Throughout the process, lipid hydrolysis did not serve as the rate-limiting step. Instead, the breaking down of LCFAs via β-oxidation or through the physical processes of dissolution and mass transfer of these acids limited the overall conversion rate (A. Ahmad et al., 2011).

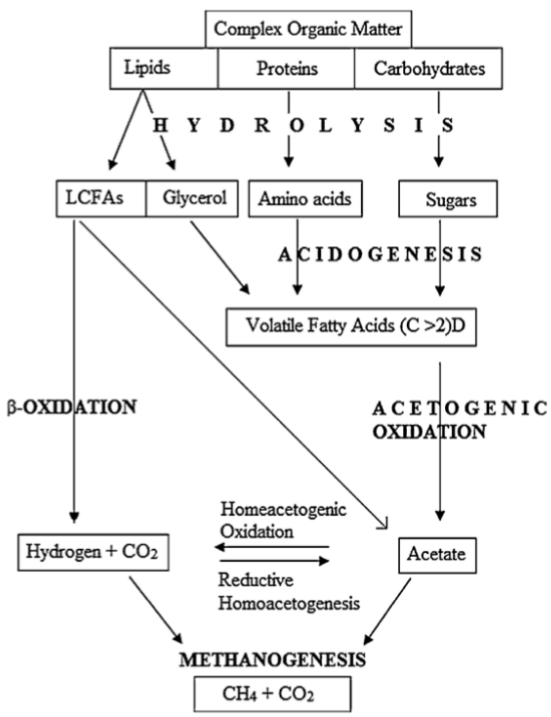

Figure 1. Food web of methanogenic anaerobic digestion (A. Ahmad et al., 2011)

There are various fatty acids in POME, including saturated, monounsaturated, and polyunsaturated long-chain fatty acids (LCFA) from C8 to C20, among these the top quantitative types comprise C16 and C18 (Habib et al., 1997) The types of LCFA in POME are listed in table A.3 in appendix A. Syntrophic groups of acetogenic bacteria are needed in the process of anaerobically degrading LCFAs. These communities of bacteria perform fatty acid β-oxidation and methanogenic archaea, which deplete hydrogen and acetate to lower the concentrations (Schink, 1997).

Currently, around 14 species that have the capability to degrade fatty acids in syntropy with methanogens have been identified. Out of these, only seven can utilize LCFAs comprising more than twelve carbon atoms (Schink, 1997). Besides, several biochemical and molecular methods have been utilized to classify the groups of microbials involved in anaerobic degradation, particularly to study those in anaerobic reactors. And the anaerobic communities responsible for breaking down LCFAs are being explored.

2.2.2 Application of anaerobic digestion on POME treatment

Among POME wastewater treatment methods, the ponding system is the most extensively utilized. Because of its many advantages such as a low cost of development and upkeep, high system reliability, and simple design (Mohammad et al., 2021). The de-oiling tank, acidification pond, anaerobic pond and facultative or aerobic pond are included in the pond system. However, this system requires a long hydraulic retention time, generates a lot of greenhouse gas and lack energy recovery (Mohammad et al., 2021). Due to the above problems, research interest in alternative production strategies to achieve outdated open ponding systems partial replacement has increased.

POME treatment is realized using an Anaerobic filter. Due to the packing, the biomass attaches on the surface once raw POME feed passes from the bioreactor's bottom. Meanwhile, treated effluent and produced biogas exit from the bioreactor's top. Regarding POME treatment, the maximum COD removal efficiency was 94%, 63% of methane could be produced (OLR of 4.5 kg COD/m^3/day). Meanwhile, the general COD exclusion efficacy reached up to 90 percent, containing the methane composition is around 60% (R Borja & Banks, 1994).

POME treatment has proven to be a success in an Up-flow anaerobic sludge blanket (UASB) reactor, and it has provided a COD exclusion efficacy of 98.4% with a maximum operational OLR of 10.63 kg COD/m3day. The high quantity of daily POME discharge from the milling process necessitates the treatment system to operate at a greater OLR. POME treatment utilizing a two-stage UASB system aiming to avoid the inhibition of granule creation at greater OLRs and does not require the removal of solids from POME before the treatment process is effective, the analysis recommended that OLR of 30 kg COD/m3day will guarantee 90% COD lessening and effective methane conversion (Khemkhao et al., 2012).

UASB and anaerobic filter has been incorporated to create a hybrid bioreactor – Up-flow anaerobic sludge fixed-film (UASFF) reactor. The hybrid reactor merges the benefits of the individual reactors whilst overcoming respective limitations. In general, the hybrid reactor can tolerate OLRs greater than UASB and anaerobic filter. There are no reports on clogging in studies that examined how a hybrid reactor performs. Besides, UASFF can also attain a minimum COD removal efficiency of 70%, and also produces a satisfactory quantity of methane while treating the POME (Najafpour et al., 2006).

Continuous stirred tank reactor (CSTR) is identical to a closed-tank digester accompanied by a mixer. The mechanical agitator expands the area of contact with the biomass, thereby elevating gas production. Trisakti et al., (2012) used CSTR to demonstrate a COD exclusion efficacy of around 77%. In addition, POME treatment has also utilized the advanced anaerobic expanded granular sludge bed (EGSB). Reportedly, the use of industrial-scale pilot EGSB reactors in POME treatment exhibited a constant COD exclusion efficacy of 94.89%. Moreover, the reactor generated 27.65 m^3 of biogas per one m^3 of POME, the gas was used to generate electricity (Trisakti, Wongistani, & Tomiuchi, 2012).

The table 1 compares the biological performance of different anaerobic digestion applications while treating the POME. At the same time, more and more good treatment applications are being explored with the development of the technology.

Table 1. POME treatment performance of anaerobic digestion applications.

Parameter	**(Trisakti et al., 2012)**	**(Khemkhao et al., 2012)**	**(Trisakti et al., 2012)**	**(R Borja & Banks, 1994)**	**(Najafpour et al., 2006)**
Application	CSTR	UASB	EGSB	Anaerobic filtration	UASFF
OLR (kg COD/m3/d)	2	10.63	-	4.5	11.58
HRT (d)	8	4	10	15	3
Methane composition (%)	64	54.2	65-70	63	71.9
COD removal (%)	77	98.4	94.89	94	97

2.3 AnMBR

2.3.1 AnMBR application for the treatment of industrial wastewater

Recently, AnMBR has received considerable research interest due to its demonstrated potential for POME treatment (Abdulsalam et al., 2018). The interest is driven by the benefits provided by coupling anaerobic digestion and membrane filtration. Besides the benefits of anaerobic digestion, AnMBR achieves complete biomass retention. A smaller reactor with the ability to treat a variety of different forms of industrial wastewater in harsh conditions, and effluent free of solids allow AnMBR to outperform other anaerobic technologies, which would otherwise lead to failure of other anaerobic technologies (Ariunbaatar et al., 2021). With improved anti-fouling membrane qualities becoming more commonly available, this technology will be investigated and implemented at a wider scale (Le-Clech et al., 2006).

The simple definition of AnMBR regards it as a biological treatment process that operates in the absence of oxygen, it uses a membrane to provide the separation between solid and liquid (Lin et al., 2013). There are a variety of AnMBR setups, depending on the membrane positioning and the permeate driving force used. Liquid and biogas recirculation can provide the shear force required to flush the membrane surface in AnMBRs. Sludge is transported to the membranes at a high velocity in external cross-flow AnMBRs to prevent cake layer development and fouling (Abdelrahman et al., 2020). In addition, to force liquid through the membrane pores, the cross-flow pump produces driving pressure. Membrane can be immersed in the reactor or in an external chamber in the submerged configuration. The permeate is suctioned through the membrane pores using a vacuum pump, and the membrane surface is cleaned with biogas in the sparging process. Both setups have their own set of benefits and drawbacks. In cross-flow condition, external cross-flow AnMBRs can be replaced and cleaned easily. However, the energy consumed for liquid recirculating may be significantly higher. For submerged configuration, the biogas recirculation pipeline must be strengthened to prevent leakage and fire protection, which will increase the construction cost (Skouteris et al , 2012)

Several lab-scale research have looked into the treatment of industrial wastewaters using AnMBRs. The following table summarizes the treatment and membrane performance of AnMBRs used to treat a variety of industrial wastewaters.

Table 2. The treatment and membrane performance of AnMBRs used for the treatment of various industrial wastewaters.

Parameter	(Ramos et al., 2014)	(Dereli et al., 2014)	(Dereli et al., 2019)	(Szabo-Corbacho et al., 2019)	(Basset et al., 2016)
Wastewater Type	Snack factory wastewater	Lipid rich corn-to-ethanol thin stillage	Cheese whey	Synthetic dairy wastewater with high lipid content	Winery wastewater
Reactor Volume (m3)	0.76	12	0.01	0.01	0.0035
Temperature	30-36	37	37	35	35
OLR (kg COD/m3/d)	5.1	4.5-7	5	4.7	3.4
HRT (d)	2.8	16	-	2.2	2.3±0.3
TSS (g/L)	7.9-10.4	24	40	12.4±0.4	4.78±1.9
SRT (d)	-	200	50	40	560
COD Removal (%)	97	98	95	99	96.7±2.7
Specific CH4 Production (m3/kg CODremoved)	-	0.31	0.3	0.32±0.02	0.147±0.05
Membrane Type and Properties	Hollow fibre (0.4 µm, 2 m2)	Flat sheet (0.08 µm, 18 m2)	Tubular PVDF (0.03 µm, 0.0114 m2)	Tubular PVDF (0.03 µm, 0.049 m2)	Flat sheet (0.2 um, 0.01 m2)
Membrane Configuration	Submerged	Submerged	Side Stream Cross Flow	Side Stream Cross Flow	Side Stream Cross Flow
TMP (bar)	-	0.1-0.2	0.4	0.4	0.64
Crossflow Velocity (m/s)	-	0.003-0.013	0.5	0.5	0.64
Flux (LMH)	6.5-8	4.3±1.1	8—11	10	20.2±8.5

2.3.2 Factors influencing biological and filtration performance of AnMBR

Biological performance and filtration performance of AnMBR should be invested while treating the industrial wastewater. Inseparable from filtration performance, membrane fouling is a critical obstacle that limits the widespread practical application of AnMBR. These factors could diminish the efficiency of the system and necessitate more frequent cleaning. This would increase the cost of replacing the membrane as well as the energy required for gas or liquid cleaning. When the membrane material and the sludge suspension interact, membrane fouling occurs (Lin et al., 2013). Both performance are influenced by a variety of factors such as temperature, SRT, OLR and others. Besides, factors like the composition of the substrate and the operating conditions of the bioreactor have an indirect impact on the fouling by affecting the characteristics of the sludge, the factors related to the membrane material and operation are directly related to the fouling of the membrane.

2.3.2.1 Temperature: Thermophilic anaerobic digestion

Mesophilic (30-37°C) and thermophilic (50-60°C) temperatures are suitable to treat the POME since the wastewater from a palm oil mill processing system is discharged at a relatively high temperature (80-90°C) (Choorit & Wisarnwan, 2007). In Malaysia, mesophilic condition is used by the majority part of the anaerobic POME treatment. However, thermophilic treatment is popular due to its efficacy in energy recovery due to the higher substrate degradation rate and biogas production rate. On the one hand, thermophilic treatment is conducive to avoid pre-cooling and post-heating, which occurs in wastewater treatment processes and subsequent reusing processes, it reduces redundant energy input for heat exchange (Lin et al., 2013). On the other hand, the faster reaction led to the high biogas recovery efficiency and electricity energy generation, which could deliver the quickest return on investment. The studies about exploring the feasibility of high strength wastewater in thermophilic temperature showed that the system worked well in the thermophilic temperature range, with POME treatment rates more than four times faster than in the mesophilic range (Poh & Chong, 2009).

The effect of the high temperature on the performance of anaerobic digestion steps are explored. Algapani et al., (2016) found that higher temperatures are favorable for the first step of hydrolysis but unsuitable for the second acidogenesis and production of hydrogen. In other words, higher temperatures do not simultaneously improve hydrolysis and acidogenesis. Besides, higher temperatures can induce the synthesis of brown-colored matter, and it cannot be readily converted into methane (Sun et al., 2014). Therefore, it can be hypothesized that an optimized temperature is beneficial for bio-hydrolysis, acidogenesis, and hydrogen production. However, an optimum temperature range for synthetic POME AD has not been elucidated so far. Even more, the thermophilic condition need the more precise and stable temperature management is needed to avoid the problems of biomass washout and reaction inhibition caused by the failure of temperature control (Poh & Chong, 2009). The potential risks determine that the thermophilic anaerobic digestion must be applied to the technology with high operation stability.

Temperature has the potential to affect membrane filtration by influencing the permeate viscosity (Mulder & Kragl, 1997). The research on the effect of temperature on MBR fouling was carried out at two sets of temperatures, which have different hydraulic resistances. Experiments have shown that at low temperatures, strong deflocculation tends to occur, thereby reducing the size of biomass flocs and releasing EPS into the solution. Additionally, at low temperatures, the particle velocity calculated using the Brownian diffusion coefficient (which is linearly related to temperature) is less. Furthermore, as the temperature drops, COD biodegradation slows, resulting in the larger solute and particulate COD concentrations (Jiang et al., 2005). At last, it was also discovered that when AnMBR was operated at 20°C rather than 30°C, the greater SMP levels were measured. All these elements are linked to membrane fouling, it's likely that more material will deposit on the membrane surface at lower temperatures (Le-Clech et al., 2006).

2.3.2.2 Substrate composition

Multiplex processes involved in anaerobic digestion which need different microorganisms participate together. However, the microbial activity directly depends on the nutrient content of the substrate, the type of substrate used is more concerned. In detail, the biogas generation, methane percentage, biodegradability, and degradation kinetics of the biomass involved are all affected by the makeup of these substrates (Nwokolo et al., 2020). Carbohydrates, protein, and fat are the most essential nutrients of the substrate, among them, lipids are a primary organic pollutant found in POME.

Lipid-rich substrates have a higher potential for methane generation, however, lipid-rich wastes like POME still have obvious shortcomings when used as the only carbon resource for anaerobic digestion. Operational issues such as blockage and biomass flotation, as well as inhibitory issues induced by the presence of long-chain fatty acids in lipids, are the two main drawbacks. Anaerobic co-digestion, on the other hand, is said to have greater benefits than single substrate digestion, such as increased organic waste degradation and dilution of inhibition compounds (Hu et al., 2018). As a result, an increasing number of scientists are looking into the co-digestion of high lipid waste and other waste materials.

LCFA plays a role in the ultrafiltration membrane fouling through adsorption of undissociated fatty acids on the surface of the membrane or pore walls, resulting in considerably declining the flux, especially, in acidic solutions having a low level of acid dissociation (Amin et al., 2010). An elevated level of fouling condition in the membrane is seen in fatty acids with a larger carbon number, moreover, the shape of the chemical structure is critical to the adsorption of fatty acid.

Traditionally, Lipids are considered to be rather troublesome due to their hydrophobic character and propensity for accumulating and fouling in the polymeric membranes in MBRs via hydrophobic exchanges. Lipids can influence the fouling in MBRs directly through hydrophobic exchanges with the membrane, or indirectly influence the biological and sludge features. Al-Halbouni et al. demonstrated how LCFAs that originate from incoming wastewater bacteria could adhere to ultrafiltration membranes, thereby impacting the formation of fouling layers in MBRs. Ramos et al., (2014) noticed greater concentrations of lipids in the fouling layer than the bulk sludge of an AnMBR that treats effluent abundant in lipids.

2.3.2.3 Organic Loading rate

Organic loading rate is a significant parameter in the operational process of anaerobic digestion. Higher organic loading rates could improve the processing efficiency of anaerobic inhibition digestion; however, it may follow shortcomings such as direct inhibition, VFAs overload, and physical fouling of equipment. In addition, the sudden changes of organic loading rate might cause the instability of anaerobic process (Ferguson, Coulon, & Villa, 2016).

In theory, the high OLR and a short HRT could be applied in AnMBR system. According to the research about exploring the biological and filtration performance of high strength lipid wastewater treatment by AnMBR, the system had the ability to treat wastewater containing between 4.6 and 36 g oil and grease per liter, and the system kept stable for about 2.8 d without any inhibition when the OLR at roughly 17 kg COD/(m3 d) (Ramos et al., 2014). Besides, the research about palm oil mill wastewater treatment by AnMBR indicated that the great COD removal efficiency (96%) was achieved when the OLR is 1-11kg COD m^3/d and the HRTs are around 7 – 600 days (Abdurahman et al., 2011). However, a study about slaughterhouse wastewater treatment ability of AnMBR system indicated the worse biological performance as the VFA accumulation when the OLR was increased to 16.3 kg COD/m^3/d (Saddoud & Sayadi, 2007).

2.3.2.4 SRT

In high-rate bioreactors, SRTs have an equivalent doubling time associated with the rate limiting biomass, or it could be greater than three times. Moreover, in view of doubling times in the range of four-ten days for acetotrophic methanogenic biomass, anaerobic high-rate reactors usually adopts an SRT that exceeds twenty days. Meanwhile, industrial sludge bed reactors are typically categorized by SRTs in the range of hundred-two hundred days, or higher. An SRT comprehensively free from HRT is much more controllable in AnMBRs than alternative kinds of anaerobic reactors, regardless of the standard of the sludge. Generally, the SRTs adopted in AnMBRs tend to be between thirty-three hundred days, which is identical to industrial-scale high-rate sludge bed reactors.

In POME treatment, SRT is considered a key operational parameter since it determines the LCFA degradation, accumulation, and methanogenic activities of the sludge. Every coin has two sides. The increased SRT creates additional opportunities for degradation and accumulation reduction. As these slow-growing bacteria, which are engaged in the biodegradation of LCFA, have a prolonged residency time in the system, they could benefit from it. However, the higher SRTs also might lead to LCFAs accumulation due to the reduced wastage of these compounds with the sludge waste (Szabo-Corbacho et al., 2019). The SRT will play a significant role in establishing appropriate circumstances for LCFA accumulation or non-accumulation inside the system.

The influence of SRT on biological performance of AnMBR while treating high lipid wastewater is investigated nowadays. In the case that treat high lipid synthetic dairy wastewater, SRT of 20 and 40 are used. The result showed the 99% COD removal rate with the OLR of 4.7 g COD/L/d for both SRT operations. However, SRT of 40 days performed the better biological conversion and specific methanogenic activity (Szabo-Corbacho et al., 2019). In addition, the biological performance in three different sludge retention times (20, 30 and 50 days) were compared in the study which explored the potential of AnMBR for the lipid-rich corn-to-ethanol thin stillage treatment. The COD removal efficiency of up to 99% was achieved by AnMBR, and very good effluent quality was obtained in all SRT operating conditions. At the same time, when SRT was increased, greater biodegradation efficiency can be achieved. However, extreme inhibition of

LCFA was observed at 50 days of SRT, which may be due to its large dissolution in the reactor inhibited methanogenic biomass (Dereli et al., 2014).

The impaction of solid retention time on the filtration performance and fouling of the AnMBR system is studied previously. Modifying the solid retention time (SRT) in an AnMBR used to process lipid-rich corn-to-ethanol thin stillage treatment also explored the impaction of the sludge filterability. According to this study, SRT is definitely one of the most critical elements determining the filterability of sludge in AnMBR. SRT can impact the accumulation of fine particles and solutes, which has an impact on the flux of membrane reactor and fouling. Better filterability was reported at 20 days of SRT compared to increased SRT as 50 days (Dereli et al., 2014). In addition, LCFA inhibition at high SRTs promoted floc breakdown and SMP release in high lipid wastewater treatment. Sludge floc's hydrophobicity and fouling propensity were altered as a result of the deposited LCFA, with less fouling as a result of a higher hydrophobicity (Dereli et al., 2015).

3 Knowledge Gaps, Research Objectives and Research Questions

3.1 Knowledge Gaps

1. The filtration performance, fouling condition of PVDF AnMBR systems in synthetic POME filtration process.
2. The influence of SRT on POME LCFA degradation and accumulation, removal mechanisms for thermophilic digestion of synthetic POME in AnMBRs.

3.2 Research Objectives

The objective of this study is to investigate the feasibility of thermophilic PVDF-AnMBR systems for synthetic POME treatment. To achieve this objective, the biological and filtration performance of AnMBRs treating lipid-rich wastewater at different SRTs are evaluated. Priority is given to the system performance and stability as well as LCFA degradation, accumulation, inhibition and adaptation processes.

3.3 Main Research Questions & Sub-Questions

Main research questions: **How can the advances in understanding anaerobic digestion at thermophilic condition (55 °C) using AnMBR be used to improve the treatment of synthetic POME at various SRTs?** The main research is further explored by the following two research questions, which contains the different evaluation elements including biological performance (RQ1) and filtration performance (RQ2). Then, the final evaluation is based on the combination of the performance of these two aspects and the limiting factors that may occur during the operation.

RQ1: What's the biological performance: degradation efficiency, biogas production and inhibition process of thermophilic (55 °C) AnMBR system when treating synthetic POME, at different OLRs and SRTs?

- What is the optimal OLR value in thermophilic condition considering the effluent quality, biogas quality/quantity, and inhibition process at different SRTs?
- What is the biological degradation rate of adsorbed LCFAs in different operating conditions?
- What is the impact of presence and accumulation of LCFAs in different operating conditions?

RQ2: What's the filtration performance: removal efficiency, fouling property, sludge filterability of PVDF membrane for synthetic POME filtration?

- What are the characteristics of permeate from membrane filtration of the system?
- What is the impact of changing SRTs on sludge filterability in AnMBR?

- How to describe the change trend of TMPs, permeate yield and quality? At which parameter state the membrane should be chemically cleaned?

4 Method

4.1 Reactor set-up

The study used a lab-scale AnMBR (Figure 2). The reactor's effective volume was kept at 6.5L. PVDF was used to build the tubular ultrafiltration membrane module, with an average pore diameter of 0.03 μm and the total surface area of about 0.00987m^2, and was set up in a side-stream configuration. Table B.1 in Appendix B presents the detailed information of the membrane. The feed flows were provided by the influent pumps (120U, WATSON MARLOW, United Kingdom), and the crossflow pump (620U, WATSON MARLOW, United Kingdom) was used to recirculate the mixed liquor at 1000L/d between the bioreactor and membrane module. The permeate pump helped to permeate water from membrane to storage and backwashing. Filtration, backwash, and idle time were conducted for 500 seconds, 20 seconds, and 5 seconds, respectively. The maximum crossflow velocity was 0.7 m/s. The sensors (ATM-800, AE sensors, the Netherlands) were used to measure the transmembrane pressure, a pH sensor (Memosens, Germany) and a temperature sensor (ATM-800, AE sensors, the Netherlands) were inserted into the reactor to monitor the pH and temperature in real time. A biogas meter was used to measure the biogas production and the data was recorded online. The water bath (Tamson instruments, the Netherlands) was utilized to keep the temperature of the AnMBR at 55 °C by providing a constantly recirculated flow on the periphery.

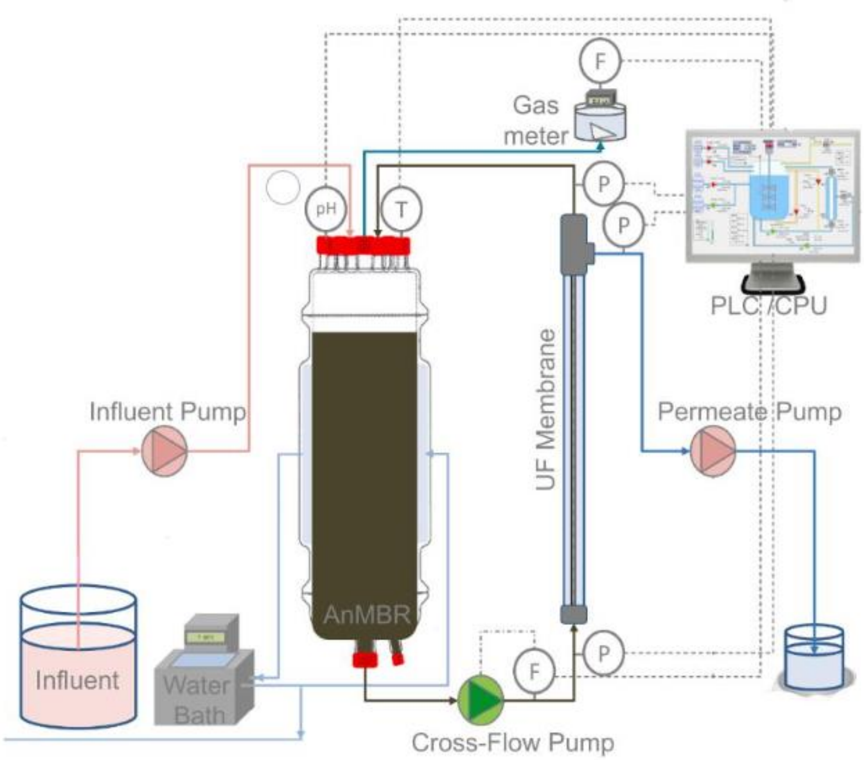

Figure 2. Schematic drawing of AnMBR reactor setup (Muñoz Sierra et al., 2019).

4.2 Synthetic wastewater and operational conditions of AnMBR

The feed contains volatile fatty acids (VFA) and synthetic POME. The VFA (the COD concentration ratio of acetate:propionate:butyrate is 3:1:1) was periodically prepared and stored in the fridge at 4 °C. For the synthetic POME feed, 6 g unrefined palm oil (Brand: KTC Ltd, U.K.) was added into 1 L glass bottle and then filled the demi-water to 1L tick mark. After that, the mixture was shaken for 24 hours at 180 rpm at 55°C in the shaker (New BrunswickTM Innova 40, the Netherlands), and sonicated for 30 minutes at 40% amplitude in a Sonifier. To remove solid oil aggregate, the mixture was filtered using a 0.103 mm sieve (INTERL AB-BV) once it cooled to room temperature. (The characteristics of the synthetic POME are detailed in Table 3). Finally, the COD concentration measurement was applied to determine the dilution times for controlling the synthetic POME feed concentration, and the feed was stored and used in room temperature (22°C). Buffer solution K_2HPO_4 and NaH_2PO_4 as well as micronutrients and macronutrients were supplied in accordance with a COD/N/P ratio of 350:5:1.

Table 3. Characteristics of Synthetic POME Feed after Shake, Sonication and Sieve.

	UNIT	**AVERAGE VALUES**	**STDEV [%]**
PH	-	5.373	0.13
CONDUCTIVITY	uS/cm	18.3	0.32
TURBIDITY	NTU	5807	1.55
TCOD	mg/L COD	14977	0.50
SCOD	mg/L COD	2080	1.54
TN	mg/L N	5.91	0.88
NH4	mg/L NH4-N	0.20	0.78
TS	g/L	3.75	0.69
TDS	g/L	1.85	0.16
TSS	g/L	1.90	4.57

The infinite SRT (140 days) was applied for the startup period, which means no other sludge was taken out except for testing. For the startup phase, the reactor was operated initially at OLR of 1 g COD/L/d VFA, and then the OLR of synthetic POME was increased stepwise until reaching the targeted OLR of 1.5 g COD/L/d (the total OLR is 2.5 g COD/L/d). After the first 70 days of the startup phase, the three operational phases are all operated at the total OLR of 2.5 g COD/L/d, with different SRTs (SRTs for operational phase A, B and C are 140 days, 60 days and 90 days, respectively. According to the limitation of biomass growth in operational phase C, the OLR was increased to 1.2 times (total 3 g COD/L/d; VFA: 1.2 g COD/L/d, POME: 1.8 g COD/L/d) by increasing the concentration of both feeds in operational phase D. The HRT for operational phase was 3 days, and each operational phase lasted around 12 HRTs. More detailed information on the operational conditions of AnMBR is shown in Table 4.

Table 4. Operate information about duration, objective OLR and SRT in different conditions of AnMBR.

		Day	Objective OLR [g COD/L/d]	SRT [d]
Start-up phase		1-70	1-2.5	140
Operational phases	A	70-105	2.5	140
	B	119-155	2.5	60
	C	155-190	2.5	90
	D	190-225	3	90

4.3 Membrane de-conditioning and cleaning

The membrane de-conditioning was applied with NaClO (500 ppm) for 2 hours, and then washed with demineralized water prior to the start of the experiments. When the TMP exceeded 350 mbar, the fouled membranes were physically and chemically removed from bioreactors. The contaminated membrane was physically cleaned by flushing the cake layer with running demineralized water. After physical cleaning, the membrane was chemically cleaned by NaClO (500 ppm) for 6 hours and citric acid (1 w/v %) solutions for 12 hours to remove the irreversible fouling.

4.4 Analytical methods

In order to monitor the feed, permeate, and sludge properties, various analyses were performed. The permeate and feed were collected every other day, and the sludge was applied to the daily sludge taken based on the current SRT. pH measurement was conducted manually after sample collection. COD and VFA were measured three times a week, where COD analysis and VFA analysis were conducted by Hach Lange kits and gas chromatography (GC VFA, Agilenttech 7890A, the Netherland). Supernatant samples of sludge were prepared by centrifuging 18,500 g (ST16R, Thermo Scientific, the Netherland) of sludge for 10 minutes and then decanting the sample into a separate container. After the sample was filtered with 0.45 um syringe filters (Chromafil Xtra), soluble parameters were measured. By subtracting soluble COD from supernatant COD, colloidal COD was calculated. Routing parameters, such as total phosphorus (TP), phosphate phosphorus (PO_4^{3-}), total nitrogen (TN), and ammonium nitrogen were all measured with Hach Lange kits.

SM Titrino 701 auto titrator determined the sludge and permeate alkalinity to pH endpoints of 4.3 in triplicates The Beckman Coulter LS230 laser particle size analyzer was used to run the tests on the sludge particles. Every measurement was carried out in triplicates. The viscosity was measured by Anton Paar viscometers at 55 °C. Biogas production was measured with a Ritter Milli Gas counter, which was reported after correction to 0 °C and 1 atm. The composition of outlet biogas was measured at regular intervals by taking 8-10 mL biogas which was produced by the reactor and injecting it into the GC (Gas Chromatograph) (Agilenttech 7890 A, the Netherland).

Weekly analyses of total suspended solids (TSS) and volatile suspended solids (VSS) were performed. Sludge activity tests was performed to monitor the anaerobic methanogenic activity and compare different operational phases. The experiment used acetate as substrate, and was conducted in serum bottles (120 ml) by monitoring the pressure increase and measuring the headspace biogas composition. The tests were carried out in the 1.5 g COD-acetate /L, and the total liquid volume in the serum bottles was 50 ml. In each serum bottles, the VSS concentration applied was based on the real reactor VSS concentration and corresponding methanogenic activity, ensuring that the experiment could be completed in 2-3 days. The blank test, which consisted of biomass and tap water only, had to be run in order to determine endogenous biomass activity. After flushing the headspace with a mixture of N_2: CO_2 (70% : 30% v/v), the serum bottles were sealed by a rubber stopper and aluminum crimp cap. All bottles were inculcated at 160 rpm and 55 °C (New Brunswick Innova 43, the Netherland). Several parameters were determined in order to characterize the initial and final conditions, including COD, TS, VS, TSS, VSS, VFA and pH.

The daily methane generation (as g COD) divided by the daily total load is used to calculate digestion efficiency (as g COD). Furthermore, assessing the LCFAs accumulated in anaerobic reactors directly can provide further information about LCFA inhibition. However, at times, the LCFA measurement was not accessible. For the current study, the LCFA concentration equivalence was roughly estimated based on the total COD, soluble COD, and VSS concentration. The LCFA in COD was roughly determined by considering the difference between the total COD and the sum of soluble COD and VSS-COD (1 g of VSS/L = 1.42 g COD/L). All calculated LCFA concentrations (in COD) were transferred to palmitic acid equivalents (2.88g COD / g Palmitic acid), as the palmitic acid is the primary LCFA during the anaerobic POME digestion in both concentration and importance aspects. Although the primary fatty acids in POME are palmitic acid (C16:0) and oleic acid (C18:1), which are the by-products of oil and fat after hydrolysis. Even when oleic acid was fed to an EGSB reactor, palmitic acid was the central LCFA that accumulated onto the anaerobic sludge (Pereira et al., 2005).

4.5 Fouling potential measurement

The total membrane resistance was calculated by the TMP, filtration flux and dynamic viscosity based on the equation below from Xing et al. (2019). TMP is the transmembrane pressure (Pa) during filtration and J is the filtration flux (m/s).

$$R_t = \frac{TMP}{\mu J}$$

A series of standard analyses were performed to determine the filtration properties of sludge at room temperature (20-22°C). The sludge capillary suction time was measured in triplicates by Triton Capillary Suction Timer (304M) and standard filter paper (Whatman No.17).

Specific resistance to filtration (SRF) was determined using a dead-end filtration cell (Millipore 8050). For starters, the sludge was diluted with permeate to a concentration of 10 g/L TSS (when the TSS concentration was less than 10 g/L, no-dilution sludge was employed). The diluted sample was then filtrated under 0.5 bar pressure without stirring to create a cake layer on 0.7 µm standard filter paper (Whatman GF/F). For 30 minutes, the volume of the filtrate was measured against time. The specific resistance to filtration was then calculated by graphing the ratio of filtration time to filtrate volume (t/V) against the filtrate volume (V). The SRF was calculated according to the following formula (Police et al., 2008):

$$SRF = \frac{2 \cdot \Delta P \cdot A^2 \cdot b}{\mu \cdot C}$$

ΔP : Pressure (Pa), A : Effective filtration area (m^2), b : Slope ($s \cdot L^{-2}$), μ: Viscosity of filtrate (Pa·s), C: TSS concentration ($kg \cdot m^{-3}$)

A centrifuge (ST16R, Thermo Scientific) at 18,500 g for 10 minutes was used to separate the sludge from the supernatant. The filterability of the supernatant was tested in a stirred dead-end filtration cell (Millipore 8050). The supernatant was stirred during the test to prevent the buildup of soluble macromolecules and immediate membrane pore blockage. In this test, MF-Millipore 0.22 m filter paper was utilized. For 10 minutes, the permeate was collected on a balance for analysis. In order to calculate filterability, a 5-minute flow rate average was used.

4.6 Mixed liquor property analysis

Extracellular polymeric substance (EPS) was processed as heat extraction at 100 °C for one hour (Chang & Lee, 1998). Then, the samples were centrifuged (ST16R, Thermo Scientific) at 18,500 g for 10 minutes and filtered by 0.45 µm filter (Chromafil Xtra). Sludge samples of soluble microbial products (SMP) were centrifuged (ST16R, Thermo Scientific) at 18,500 g for 10 minutes and then filtered by 0.45 µm filter (Chromafil Xtra) but without heat treatment. Proteins (BCA Protein Assay Kit, Sigma Aldrich) and polysaccharides were used to determine the total EPS and SMP (DuBois et al., 1956). For standardization, bovine serum albumin and d-glucose were utilized in protein and polysaccharide assays. Each sample was measured in triplicates during the sample measurement.

5 Results

5.1 Biological performance

5.1.1 Biological performance in different operational conditions

5.1.1.1 Start-up period

The OLR increased from around 1 kg COD/m^3/d to 2.5 kg/m^3/d during the start-up period (Day 1 – 70) at SRT of 140 days. During this period, the permeate COD concentration decreased from 3,810 mg/L to 580 mg/L, while the COD removal rate of the system increased from around 47% to 91%. The TSS and VSS concentrations dropped from 63.79 g TSS/L and 34.77 g VSS/L to 30.89 g TSS/L and 17.54 g VSS/L, respectively, during the start-up period.

The VFA concentration in the reactor is an excellent predictor of the anaerobic treatment performance. Besides, it is also suitable for monitoring acetogenic and methanogenic bacterial activity (Issah & Kabera, 2020). Figure 5 illustrates the VFA concentration in the reactor as a function of the reactor's operating time. During the start-up period, the VFA concentration decreased with a stable OLR at 1 kg COD/m^3/d. On Day 23, the POME was dosed to the reactor to increase OLR, the VFA concentration increased and accumulated briefly. Following a short VFA accumulation period, the concentration started to decrease when the OLR increased from 1.7 to 2.5 kg COD/m^3/d (Day 58-70). In most cases, an accumulation of VFAs reflects an imbalance between acid producer and consumer and is usually connected to a decrease in pH and a breakdown of the sludge buffering capacity (Akuzawa et al., 2011). The reduction in pH can cause the inhibition of the growth of methanogens. However, there was no evidence for any significant differences in the pH in this test, which was 7.66±0.07.

The biogas production was converted to standard temperature and pressure (0 °C and 1 atm), which did not indicate any increase during the first stage of the OLR increase process (Day 23-40) combined with VFA accumulation. Afterwards, the biogas production increased with the increasing OLR in the later part of the start-up period. Concurrently, the biogas methane content decreased from 83.6±2.3% to 76.4±0.5% as the proportion of POME increased in the OLR of the substrate. The rapid accumulation of VFA and low digestion efficiency (Day 23-40) can be attributed to the limited growth rate of methanogens combined with the inhibitory effect of LCFA on microbial activity when we started to dose POME to the reactor to increase OLR.

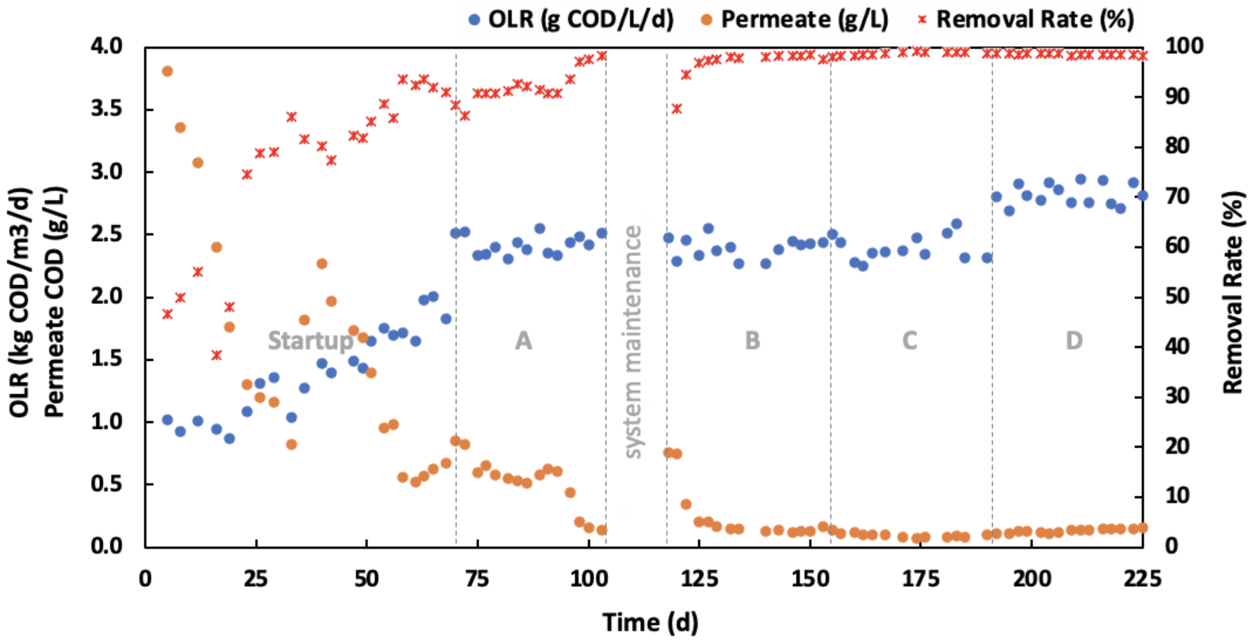

Figure 3. The OLR (kg COD/m3/d), permeate COD (g/L) and COD removal efficiency (%) of reactor at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90).

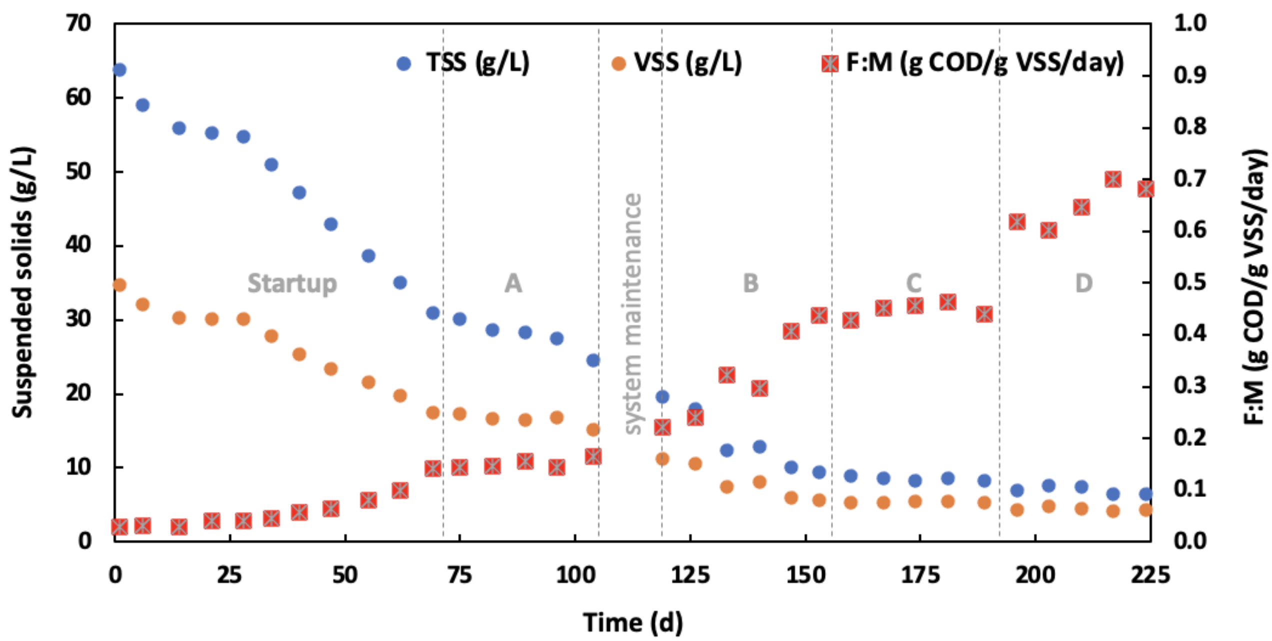

Figure 4. Total suspended solids (g/L), volatile suspended solids (g/L), and food to biomass ratio (g COD/g VSS/day) at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90).

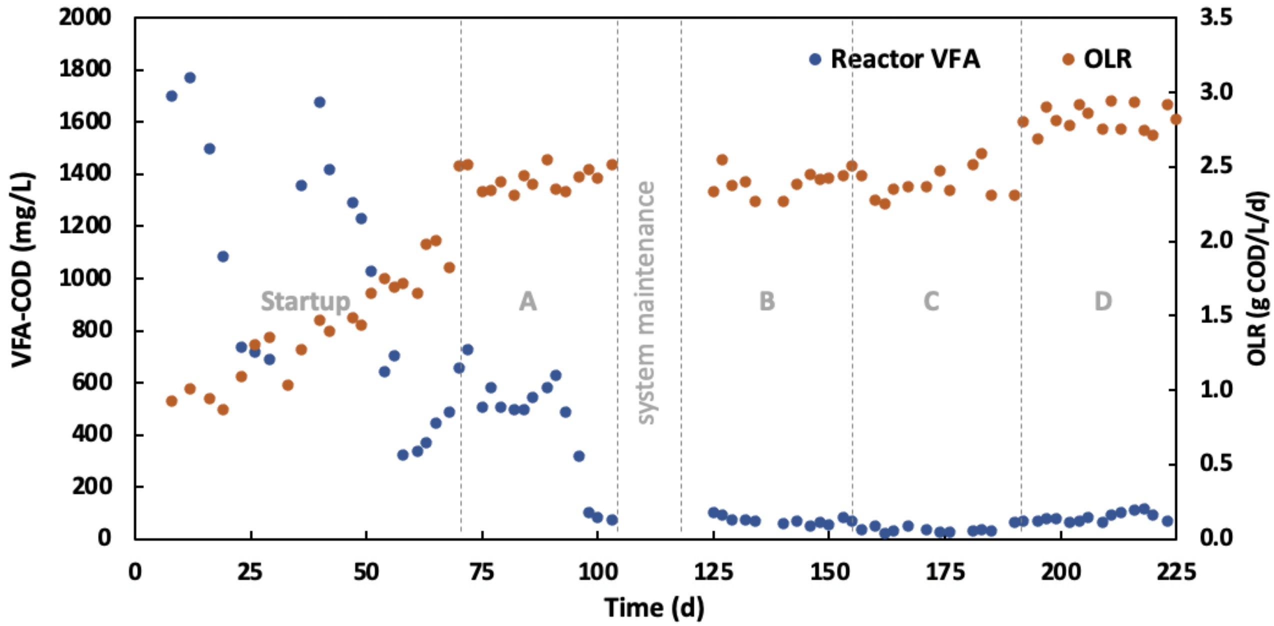

Figure 5. Volatile Fatty acids concentration (in mg COD/L) and correspond OLR (g COD/L/d) of sludge at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90).

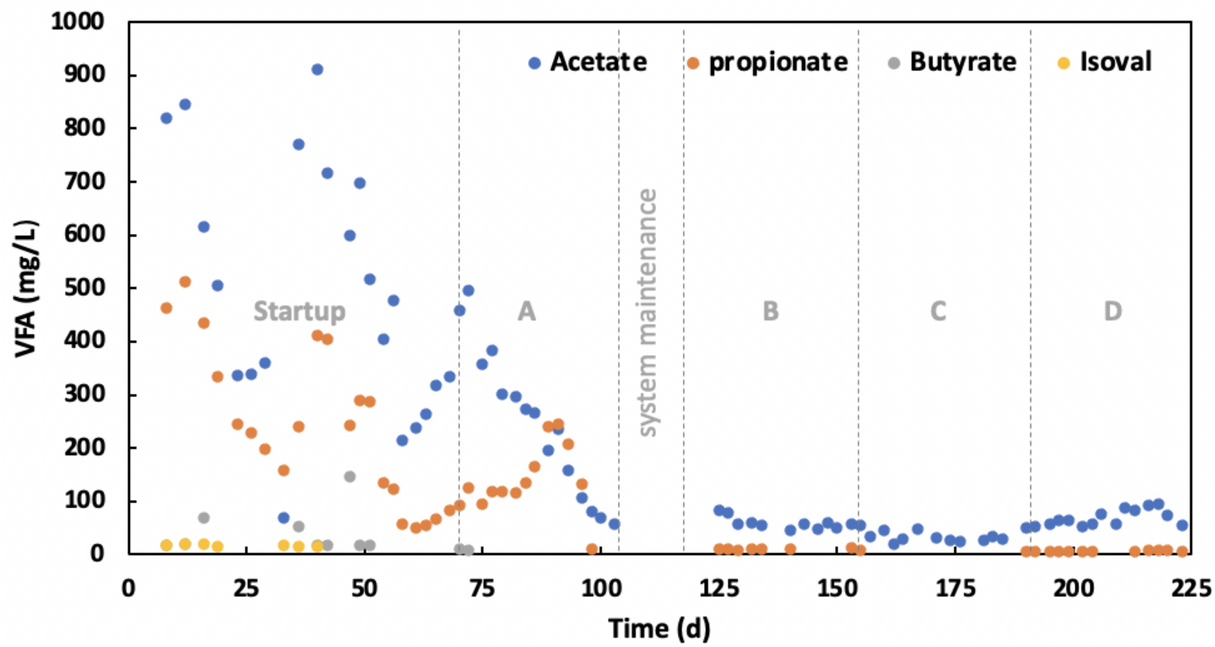

Figure 6. VFAs (Acetate, Propionate, Butyrate and Isoval) and concentrations (mg/L) of the sludge at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90), (OLR: Start-up = 1 – 2.4 g COD/L; Phase A, B and C= 2.4 g COD/L, D = 2.8 g COD/L).

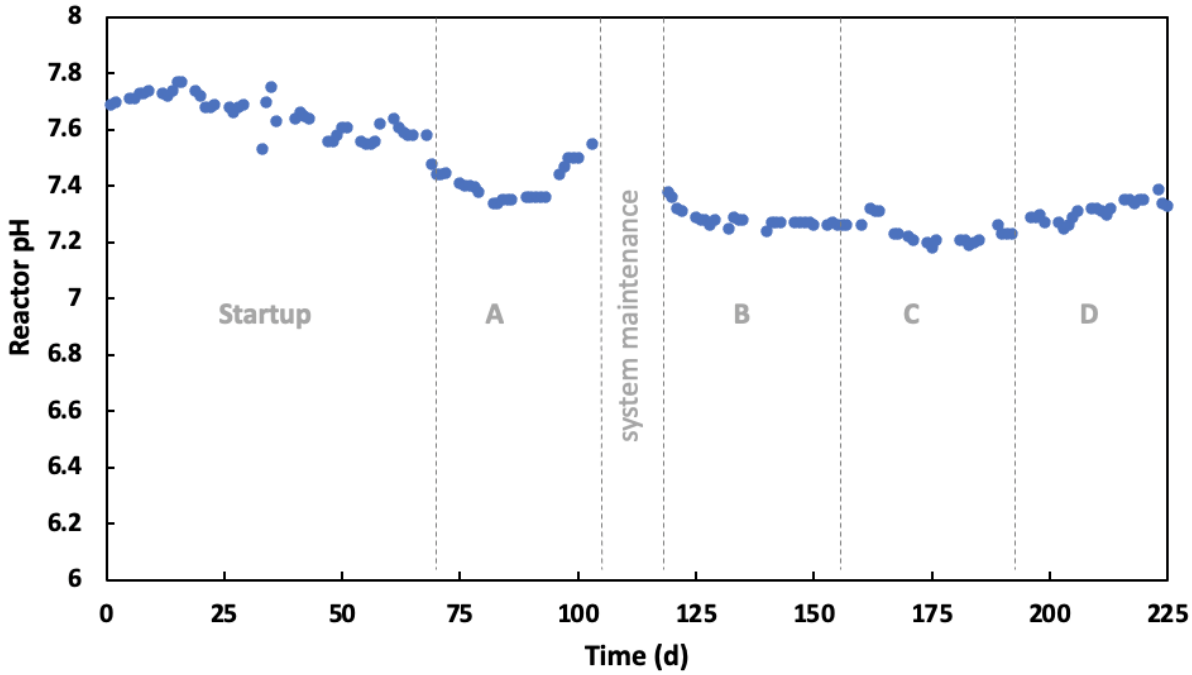

Figure 7. pH of the sludge at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90).

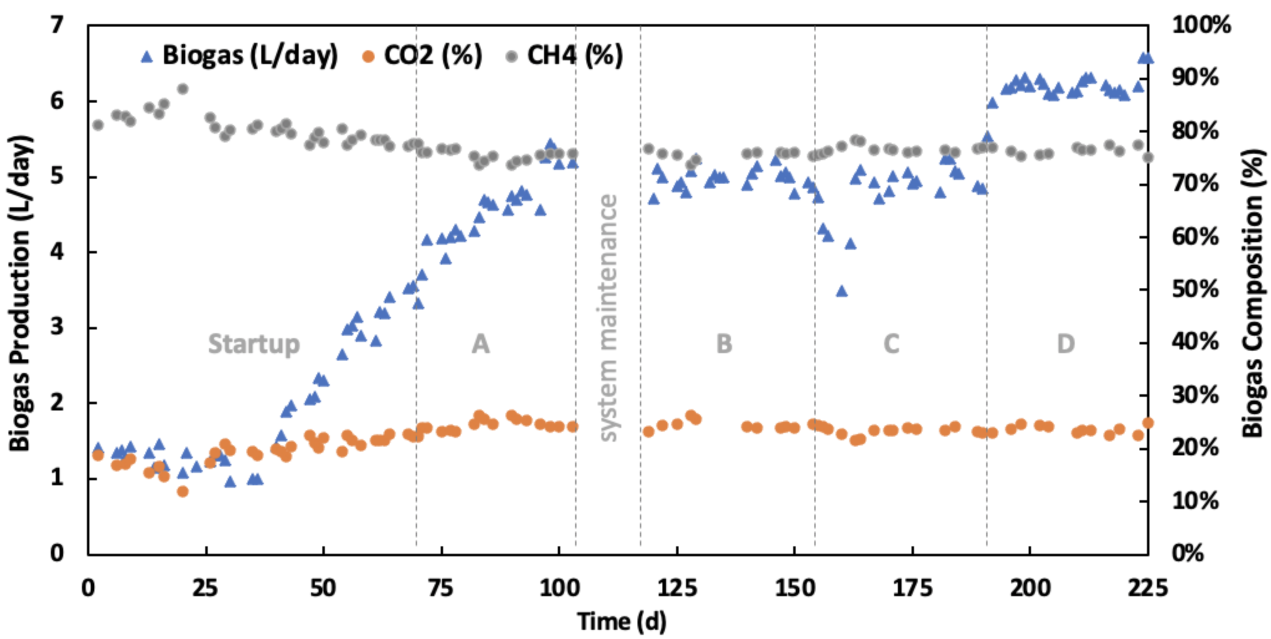

Figure 8. Biogas production (L/day at temperature and pressure 0 °C, 1atm), and composition (%) at start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90), (Operational phase A, B and C: OLR = 2.4 g COD/L, D: OLR = 2.8 g COD/L).

5.1.1.2 Operational phase A

In operational phase A (Day 70 – 104), 2.4±0.1 kg COD/m^3/d OLR was applied at an SRT of 140 days. A stabilizing condition was associated with operation phase A. Specifically, after the first three HRTs in the phase, the permeate COD concentration decreased from 577 mg/L to 135 mg/L, and the COD removal rate increased from 91% to 98%. The TSS and VSS concentration stabilized at 28.7±1.1 g TSS/L and 16.8±0.4 g VSS/L. Throughout the entire evaluation, acetate and propionate were the primary VFA constituents. During this phase, the VFA concentration increased slightly from 508 mg COD/L to 629 mg COD/L, and the increasing propionate concentration contributed to the total increase of VFA. Finally, after the propionate concentration started decreasing, the VFA concentration in the reactor decreased to 71 mg COD/L. However, the pH is 7.4±0.1, and the alkalinity ratio is 0.1, which falls in the safe range of anaerobic digestion (Issah & Kabera, 2020). Concurrently, the biogas production grew from around 4.2 L to 5.2 L in the stable OLR. During the stable phase, the biogas methane composition was approximately 76%. It remained stable at 76.0±1.0% throughout the entire operation phase.

The accumulated LCFA and VFA combined with low biogas yield during the start-up period and increasing OLR prior to this phase led to the longer stabilizing time requirement. During phase A, the constant improvement in the system performance arose from the anaerobic biomass adaptation of LCFA. Besides, the biomass was capable of top biodegradation, and the accumulated LCFA, which reduced VFA concentrations, increased the biogas yield. However, the phase terminated owing to system breakdown, which was caused by the accumulation of clumps at the bottom of the reactor that stopped the system's crossflow recirculation. During the last part of the phase, the COD removal rate still exhibited an increasing trend. It is expected that a higher COD removal rate could be achieved than the actual performance at the end of this phase under this operational condition in case of longer operational period.

5.1.1.3 Operational phase B

In operational phase B (Day 119 – 155), 2.4±0.1 kg COD/m^3/d OLR was applied, and the SRT was kept at 60 days. In the stabilizing condition, the permeate COD concentration was 141 mg/L, and the COD removal efficiency based on permeate quality was 98.02±0.27%. The average VFA concentration was 66±9 mg COD/L with a stable pH of 7.3, and the biogas production was 5.0±0.1 L. Nevertheless, the TSS and VSS concentrations decreased gradually and reached 8,860 mg/L TSS and 5,570 mg/L VSS, which resulted from the slow biomass growth rate and the higher biomass wastage for maintaining the SRT.

5.1.1.4 Operational phase C

In operation phase C (Day 155 – 190), 2.4±0.1 kg COD/m^3/d OLR was applied, and the SRT was maintained at 90 days. The permeate COD concentration in stabilizing operational condition C was 84 mg/L. The COD removal efficiency based on permeate quality was 98.88±0.16%. Both TSS and VSS concentrations became stable at 8,540±240 mg/L and 5,330±90 mg/L, respectively. The average VFA concentration was 34±8 mg COD/L and the pH was 7.2 during the stabilizing process of the operation phase C. However, the biogas production dropped sharply at the first stage of this phase, then recovered and stabilized at 5.0±0.2 L.

The initial decline of biogas production could be caused by various reasons. The limited degradation process of hydrolysis, acidogenesis, acetogenesis, methanogenesis, or even the unsuitable environment during reaction, are all probable causes (Atelge et al., 2020). However, the pH in this phase was stable at around 7.2, which is still a good range for the thermophilic anaerobic digestion (Suryawanshi, Chaudhari, & Kothari, 2010). Moreover, the stable and low VFA concentration also showed no inhibition conditions for methanogenesis and acetogenesis, as the restricted reaction process always comes with the accumulation of VFA.

5.1.1.5 Operational phase D

In operation phase D, 2.8±0.1 kg COD/m^3/d OLR was applied, and the SRT was kept at 90 days. The permeate COD concentrations in stabilizing operational condition D was 135 mg/L. The COD removal efficiencies based on permeate quality were higher than 98.52±0.15%. As the OLR increased, the biomass concentration decreased slightly before stabilizing at 6,990±480 mg/L TSS and 4440±270 mg/L VSS. The sudden rise in OLR could lead to the accumulation and adsorption of LCFAs, which inhibit biomass production and cause a decrease in TSS and VSS concentrations.

In operation phase D, the VFA concentration showed a slow growth before starting a downward trend. The average VFA concentration of the entire stabilizing process was 86.44±19 mg COD/L, the pH 7.3 and the alkalinity 0.1. Besides, the reactor produced 6.2±0.1L biogas in operation phase D. No obvious inhibition process took place after the increase in OLR, probably because the previous LCFA pulse exposure increased the tolerance of biomass (Palatsi et al., 2009).

5.1.3 COD Mass Balance

Reportedly, COD removal effectiveness is not always consistent with the biodegradation of COD in the treatment of lipid-rich wastewater (Hwu et al., 1998). If LCFAs aren't converted to methane, then the reactor could accumulate LCFAs when absorbed onto the sludge. According to Hwu et al. (1998), an EGSB reactor that treats synthetic wastewater abundant in LCFAs exhibited a much lower methane conversion efficiency in mesophilic conditions compared to an EGSB reactor that treats COD-abundant synthetic effluent. It implies that physicochemical mechanisms such as precipitation and biosorption play a substantial role in LCFA removal. Therefore, when the removal efficiency of COD is high, it is not always analogous to effective biodegradation. Permeate quality needs to be assessed alongside the efficacy to convert COD to methane to assess performance more realistically.

Table 5 summarizes the results of the COD mass balance calculations obtained for each operational phase. Overall, around 20% of COD in the mass balance happened in all operational phases. Operational phase A, B and C are compared, which had similar OLRs but different SRTs. The conversion efficiency improved with increasing SRTs. However, there is no obvious relationship between the missing fraction of COD in the mass balance and the SRTs. In the meantime, the amount of biomass is constantly changing during the operational phases A and B and the unstable state makes it difficult to calculate the data on wasted sludge accurately. In addition, the missing fraction of COD in the mass balance decreased at higher OLRs when the operational phases C and D were compared.

Table 5. COD mass balance

Phase Stream	A g COD/d	 %	B g COD/d	 %	C g COD/d	 %	D g COD/d	 %
Influent	15.9±0.2		15.7±0.6		15.6±0.6		18.4±0.5	
Effluent	0.27±0.07	1.7	0.3±0.1	2.0	0.2±0.0	1.3	0.3±0.0	1.6
Wasted sludge	1.4±0.1[a]	9.0	1.7±0.3[a]	10.6	0.8±0.1	5.1	0.6±0.1	3.3
Methane	11.4±0.4	71.8	10.8±0.3	69.2	11.1±0.3	70.9	13.6±0.3	74.1
Total		84.0		81.8		77.3		79.0

[a] Unsteady condition with the variable biomass concentration.

Compared to another study which also used AnMBR to treat high lipid content wastewater, the higher COD percentage (80 %) was digested into biogas, and less missing COD percentage (4% - 10%) in the COD mass balance (Dereli, van der Zee, et al., 2014). The better digestion efficiency in their study might be attributed to the lower lipid/mass ratio (max 0.1 kg lipid/kg VSS/d). And as biomass concentration decreased in our study, the lipid/mass ratio was achieved 0.15 kg lipid/kg VSS/d during the last phase. Palatsi et al. (2009) also indicated that one of the best approaches for dealing with an LCFA-inhibited which happened in thermophilic manure reactor was to raise the biomass/LCFA ratio. For the COD missing part, scum formation in the reactor is likely to be one of the reasons. The scum build-up occurred and even caused the blockage of gas lines and pipelines in another study which treated POME by theomorphic anaerobic digestion (Ibrahim, Yeoh, & Cheah, 1985). Corresponding to the system breakdown period of the reactor operation, the clumps, which accumulated at the bottom of the reactor obstructed the recirculation of the system. These clumps were removed and used as a substrate to perform BMP experiments, and it was found that each gram of clump contained 0.037 g CH_4-COD.

5.1.4 LCFA concentration, accumulation, and inhibition

The association between palmitic acid concentration and methane production is shown in Figure 9. In the initial start-up stage, the production of methane exhibited a slowly decreasing trend when the OLR was increased in the form of POME. Meanwhile, the palmitic acid concentration also increased. When the equivalent concentration of palmitic acid dropped to around 5 g/L, the production of methane began to increase continuously. However, the methane production reduced sharply following operational phase B at SRT of 60 days, even though the palmitic acid concentration was relatively low.

LCFA:biomass ratio is also a very important entry point to explore inhibition process. This study also probed into the ratio relationship between palmitic acid equivalence/VSS and methane production of the reactor. During the first stage, the LCFA was absorbed by the sludge, and then it was consumed, leading to increased biogas production during the start-up period. After operational phase B at SRT of 60 days, the palmitic acid equivalence/VSS increased continually as the biomass reduced, contributing to a sharp decrease in biogas production. Next, the palmitic acid equivalence/VSS started to decrease after the SRT increased to 90 days during operational phase C, and biogas production was recovered. During operational phase D, as OLR increased, the palmitic acid equivalence/VSS ratio increased and was close to the previous inhibition point. However, LCFAs only accumulated briefly and were accompanied by a steady output of methane.

Better biological performance was observed when the microbial community structure could easily cope with the rise of OLR and the accumulation of LCFA.

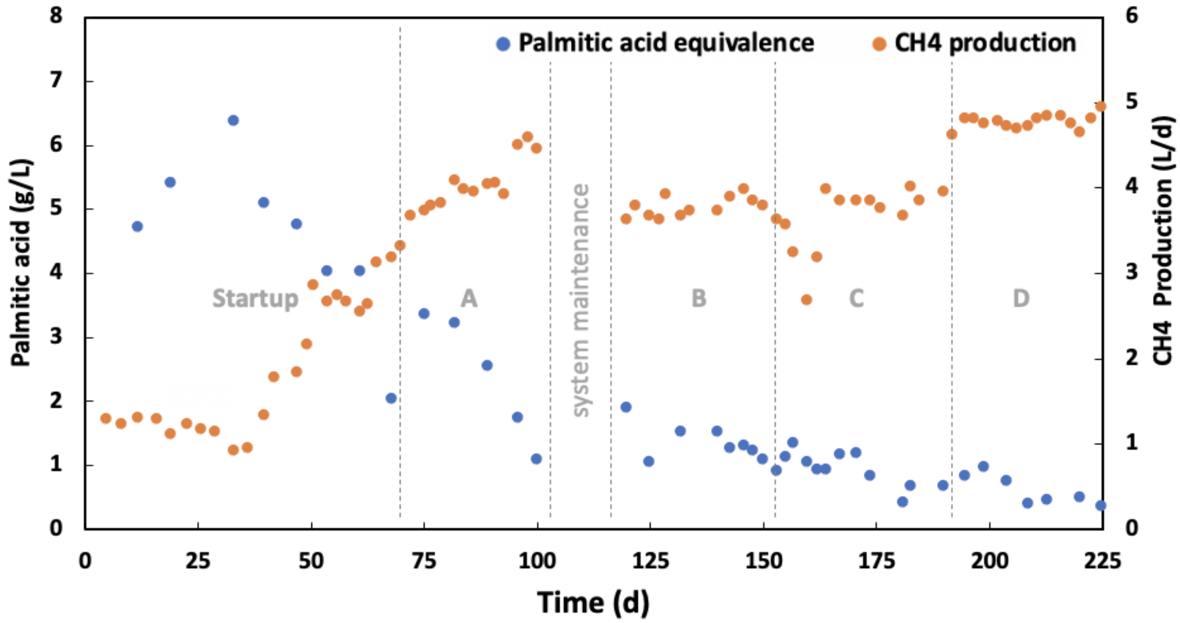

Figure 9. Equivalent concentration of palmitic acid (g/L) and methane production (L/d) at the start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90) (Operational phases A, B and C: OLR = 2.4 g COD/L, and operational phase D: OLR = 2.8 g COD/L).

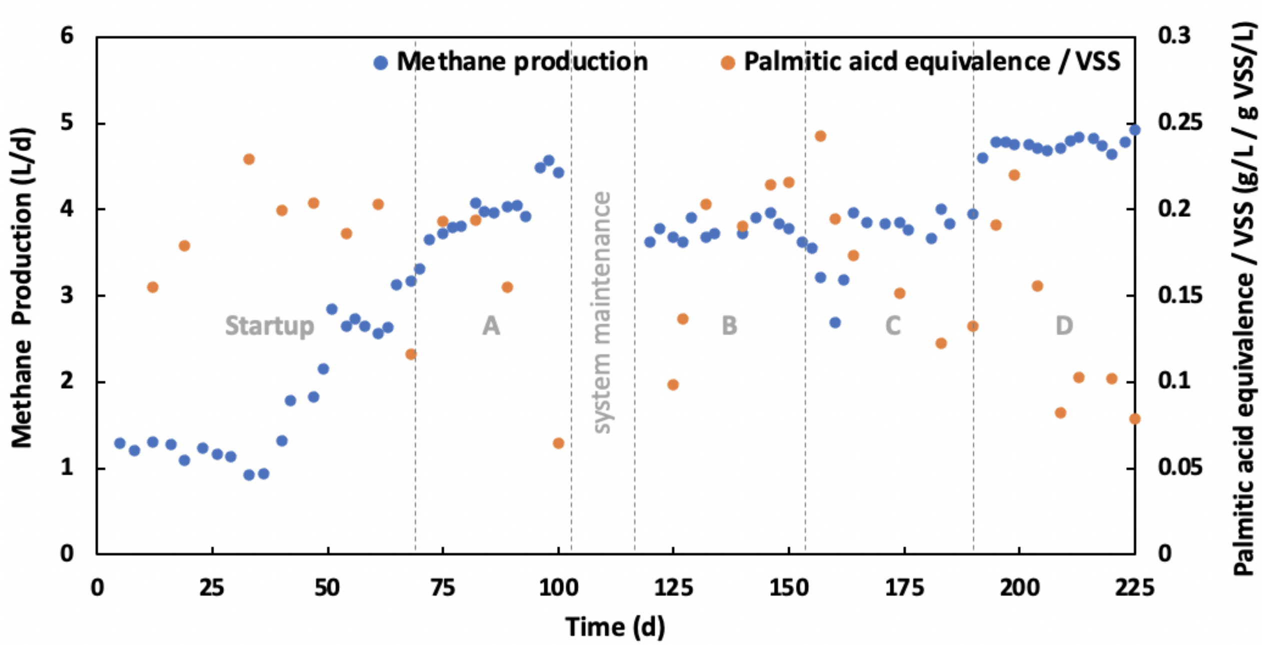

Figure 10. Equivalent concentration of palmitic acid and biomass ratio (g/L/ g VSS/L) and methane production (L/d) at the start-up period and operational phases A (R-140), B (R-60), C (R-90) and D (R-90) (Operational phases A, B and C: OLR = 2.4 g COD/L, and operational phase D: OLR = 2.8 g COD/L).

5.2 Filtration Performance

5.2.1 Long-term filtration performance

The operational flux, TMP, and membrane permeability of the lab-scale crossflow AnMBR are illustrated in Figure 11. Generally, all operational phases showed a stable filtration performance. Throughout the entire operational period, the operating flux values in the operational phases ranged from 8 to 11 $Lm^{-2}H^{-1}$, and there was a gradual increase in permeability.

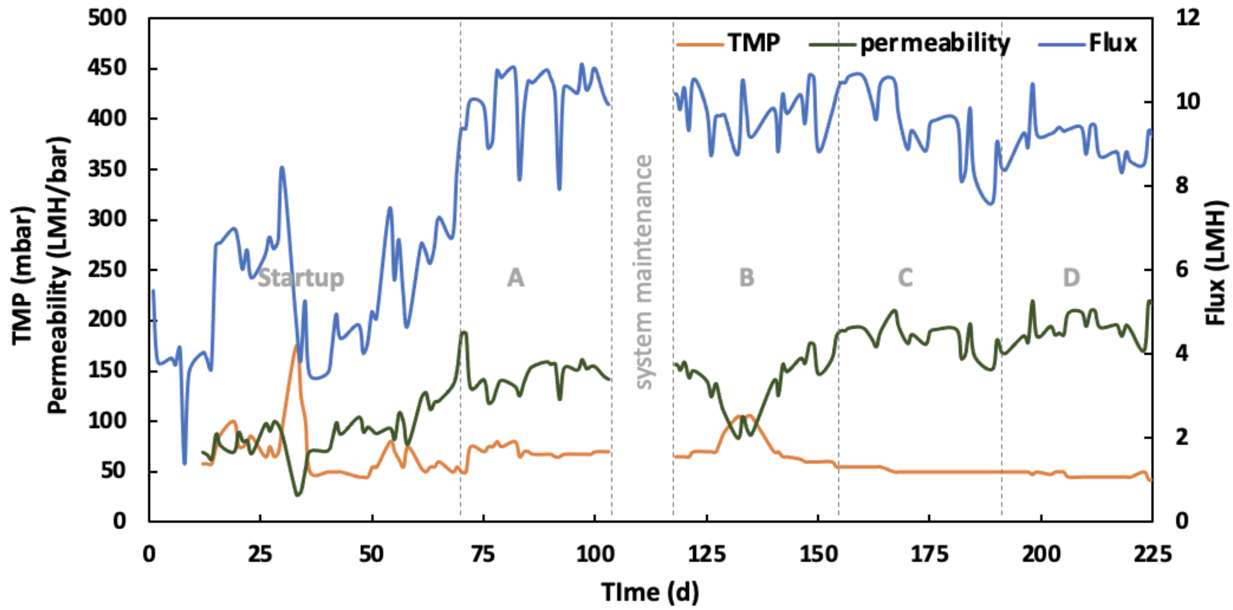

Figure 11. Long-term filtration performance: TMP (mbar), permeability (LMH/bar) and flux (LMH) of the reactor at the start-up and operational phases A (R-140), B (R-60), C (R-90), and D (R-90).

5.2.2 Sludge characteristics

5.2.2.1 EPS and SMP

SMP and EPS are detected as carbohydrates and proteins related to observed membrane fouling (Meng et al., 2009). Therefore, the concentrations of SMP and EPS were measured throughout the operational process of the reactor. Firstly, both SMP and EPS concentrations decreased when the reactor changed the SRTs from 140 days to 60 days. The reduced accumulation of the compounds within the reactor explains the decrease in the concentration, and the reduced accumulation is caused by the higher discharge of sludge at reduced SRTs. Moreover, the ever-decreasing biomass in the reactor could be another critical reason for the reduction in SMP and EPS concentrations. Afterward, when the SRT increased to 90 days, both concentrations exhibited a slight increase, and then further decreased in operational phases C and D. Finally, there was another slight increment in both concentrations during the final part of operational phase D. The accumulation of LCFA which impacts the biomass could have resulted in the two latter increments. Reportedly, LCFAs could have a bactericidal effect on anaerobic biomass (Ma et al., 2015). Thus, the LCFA accumulation in the operational phase could lead to cell lysis, which releases bacterial decay products to the bulk liquid. Studies have also stressed that bacteria might release more SMPs in unstable operational settings (Le-Clech et al., 2006).

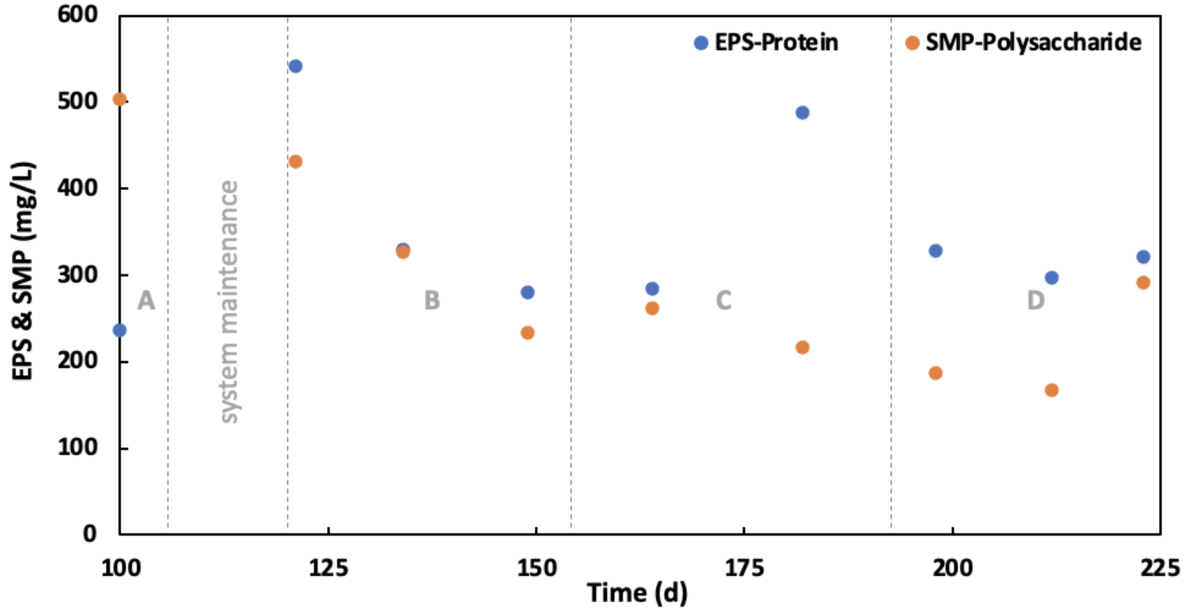

Figure 12. Total EPS (mg protein/L) and SMP (mg polysaccharide/L) of the sludge in the reactor in operational phases A (R-140), B (R-60), C (R-90) and D (R-90) (Operational phases A, B and C: OLR = 2.4 g COD/L, and operational phase D: OLR = 2.8 g COD/L).

5.2.2.2 Particle size distribution (PSD)

The particle size showed a small decrease in operational phases A and B. The reduction in the median particle size could be caused by the crossflow pump disrupting the sludge flocs. Numerous studies have emphasized that the application of shear stress to scour the particles from the surface of the membrane reduces the particle size in AnMBRs (Jeison, Telkamp, & van Lier, 2009). However, the PSD in an unstable condition occurred during operational phases C and D. For operational phase C, the median particle size of the sludge was 5.87 µm after the operation of the first three HRTs. Then after the operation of another four HRTs in this condition, the PSD had two peaks at 1.73 µm and 16.37 µm. The bimodal PSD might be caused by the growth of acidogens after the SRT increased from 60 days to 90 days. Dereli et al. (2015) also held that the PSD of the sludge was bimodal because the acidogens proliferate and grew dispersedly at a low COD: TKN ratio. Besides, considering the reduction in biogas, the unstable reactor at the first stage of operational phase C, and the increase in OLR in operational phase D, LCFA may cause toxicity on biomass and have an impact on PSD (Hwu, Donlon, & Lettinga, 1996). Reportedly, numerous instabilities such as temperature, sudden organic load, and pH shocks could lead to a floc breakage, which would reduce the particle size in AnMBRs (Akram & Stuckey, 2008).

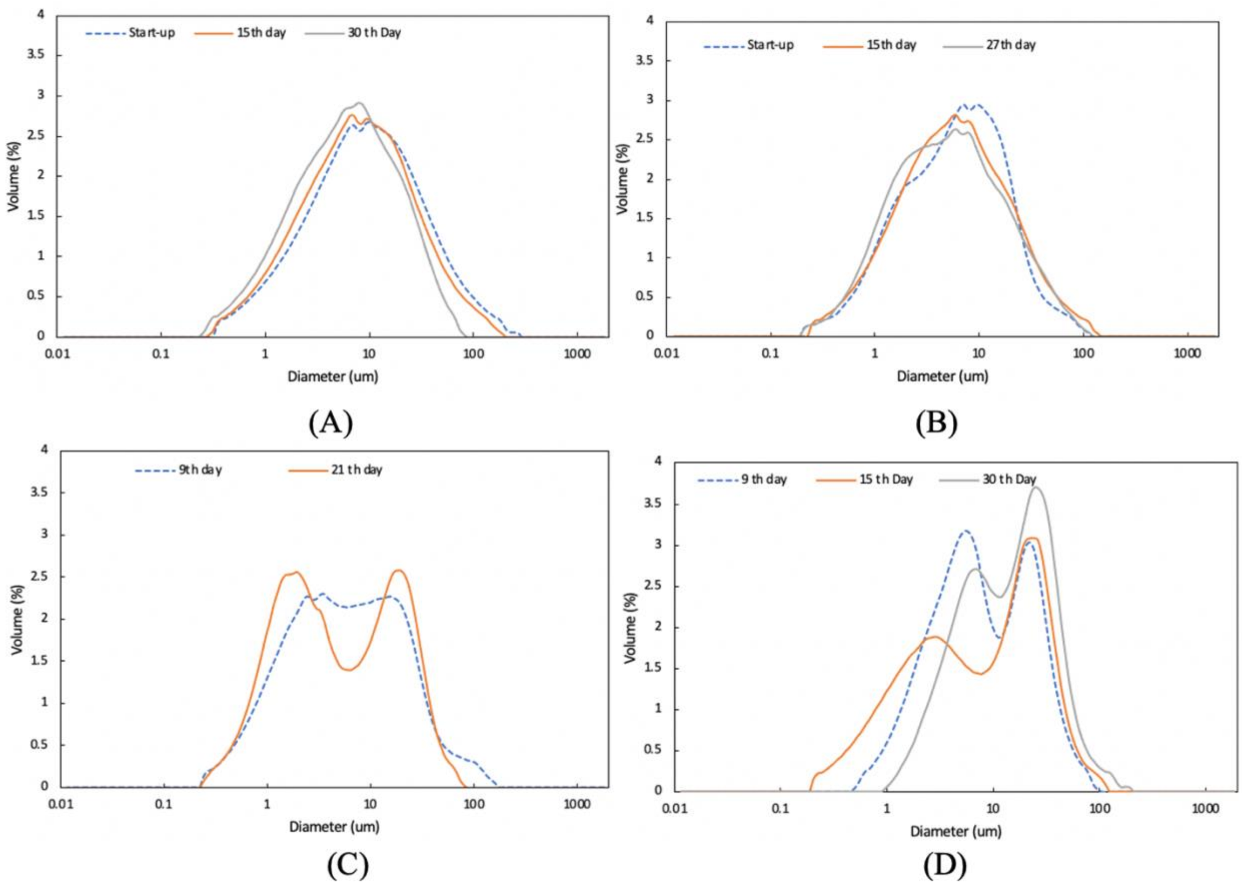

Figure 13. PSD of sludge in operational phases A (R-140), B (R-60), C (R-90) and D (R-90) (Operational phases A, B and C: OLR = 2.4 g COD/L, and operational phase D: OLR = 2.8 g COD/L).

5.2.3 Sludge filtration characteristics

5.2.3.1 Specific resistance to filtration (SRF)

In general, cake layer formation is considered the most crucial fouling mechanism in AnMBRs. Thus, the parameter SRF, which indicates the filterability of the sludge, could also specify the quality of cake accumulating on the surface of the membrane. Accordingly, a high SRF corresponds to the formation of a compact and a less porous cake layer comprising small-sized particles. Meanwhile, a low SRF is indicative of a cake layer with a higher level of porosity. Figure 14 presents the SRF from the end of operational phase A to operational phase D. The overall variable trend is decreasing from 1180 E^{12} m/kg to 333E^{12} m/kg throughout the entire operational process without correlating with SRT. In a separate study, Yurtsever et al. (2017) presented the average SRT values in AnMBR as 1080±410 E^{12} m/kg for all infinite, 60, and 30 day-SRTs, all of them are consistent with the SRF values in the present study without correlation with SRT. Besides, the SRF showed a remarkable increasing trend in operational phase B, which could be caused by instabilities in biological performance due to the LCFA inhibition. Moreover, Dereli et al. (2015) found that the LCFA inhibition during the AnMBR reactor operation led to a significant increase in SRF of the sludge. Besides, the SRF did not show any obvious correlation with SRT, and kept on decreasing throughout the operational process, except for the remarkable increment mentioned above.

The SRF was reported to have a relationship with PSD in many cases. According to Chang and Kim (2005), the SRF depended on the particle size and cake porosity associated with the sludge. The particle size decreased slightly throughout the entire operational process, meaning that the small size particles had a slightly higher fraction, and that the SRF should be increased. Moreover, Dereli et al. (2014) showed that a reactor operating at a SRT of 20 days had a significantly higher PSD. Additionally, the SRF measured in the said reactor was less than that of an reactor at a SRT of 50 days with lower PSD. However, in the present study, the SRF and particle size were not significantly correlated, probably because the difference in particle size is not obvious, making the impact on SRF insignificant. Several other parameters, such as EPS, degree of dispersion, and shear sensitivity, could influence SRF. Therefore, these factors need to be considered (Liu et al., 2012; Mikkelsen & Keiding, 2002).

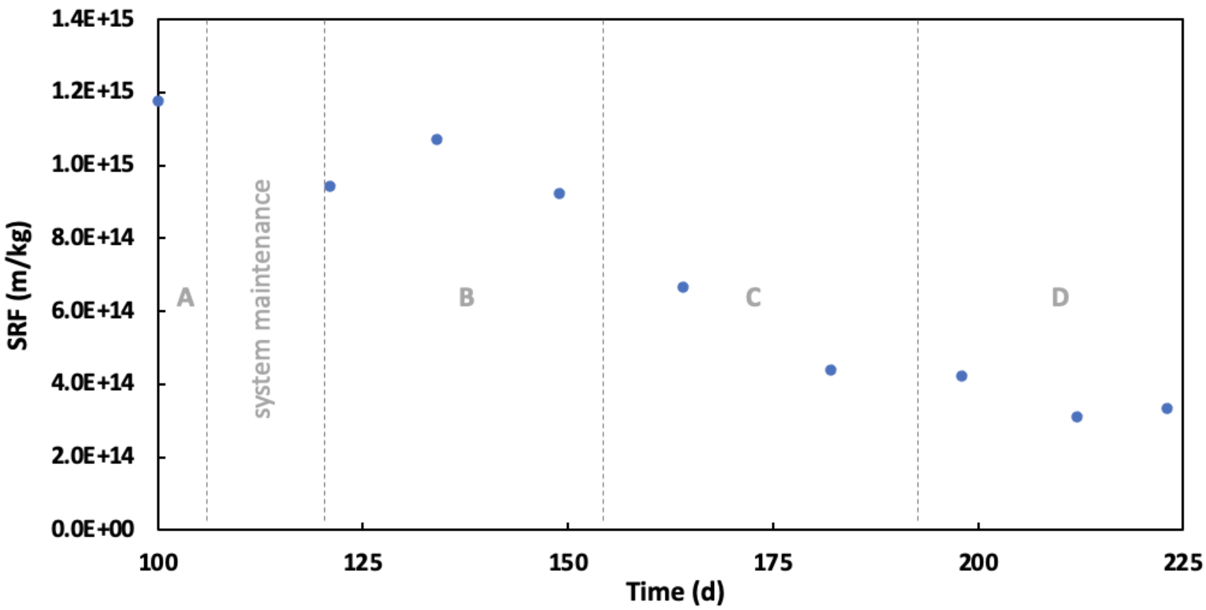

Figure 14. Evolution of SRF (m/kg) of the sludge in operational phases A (R-140), B (R-60), C (R-90) and D (R-90) (Operational phases A, B and C: OLR = 2.4 g COD/L, and operational phase D: OLR = 2.8 g COD/L).

5.2.3.2 Capillary suction time (CST)

CST is a common parameter to assess sludge dewaterability. It followed an identical trend and exhibited a significant correlation with TSS concentration. And it decreased from 1, 816 s to 82 s (from the end of operational phase A to the end of operational phase D). The CST values normalized to TSS were 66.0 s L/g and 12.6 s L/g, respectively. There was no sign of any correlation with SRTs. According to Dereli et al. (2014), in AnMBR, at SRTs of 20, 30, and 50 days, the CST was 951, 1,743, and 2,414 s, respectively. The normalized CST values were 61, 90, and 86 s L/g, respectively, which are 1–6 times the values obtained in the present research. Reportedly, many characteristics influence the sludge dewaterability, including protein, particle size, polysaccharide, and protein/polysaccharide ratio in EPS of sludge flocs (T. Wang, Chen, Shen, & An, 2016). Variations in these factors could elevate the dewaterability of the sludge. Additionally, CST could be a potential marker to assess the filterability of the sludge and its fouling propensities. Owing to the ease of measurement, the correlation of CST with other fouling indicators was highlighted. For instance, Wang et al. (2006) published results outlining a significant correlation with critical flux (correlation coefficient, $R^2 = 0.94$). Hence, CST has the potential to serve as a quick marker to assess the filterability of the sludge and could be useful for determining the operational flux.

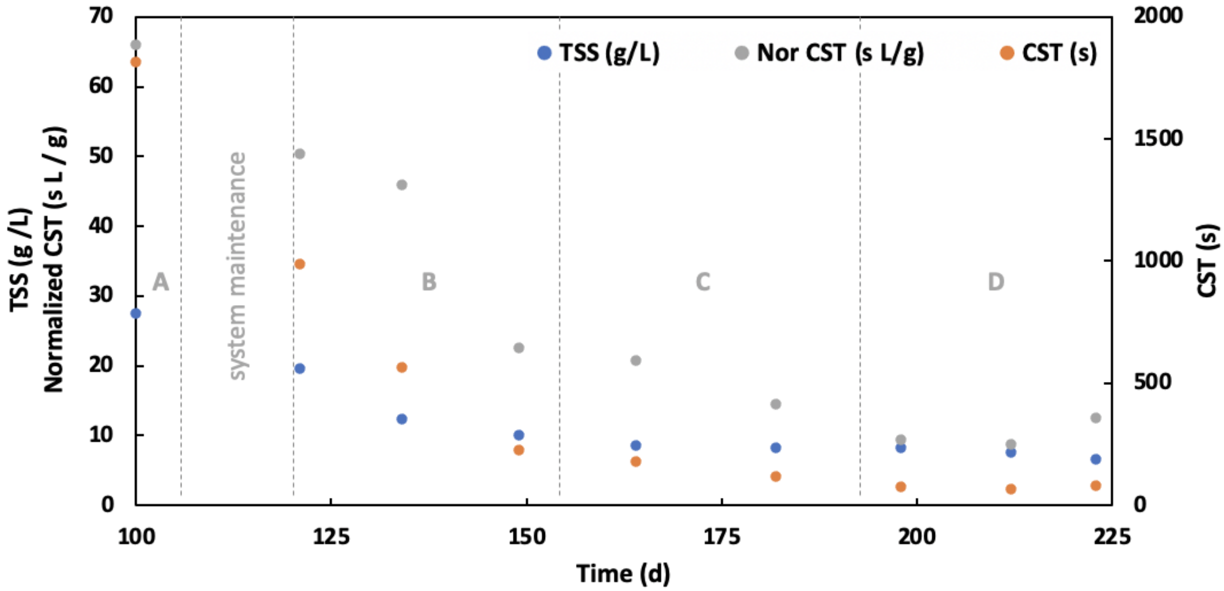

Figure 15. TSS (g/L), CST (s) and normalized CST (s L/g) of the sludge in operational phases A (R-140), B (R-60), C (R-90) and D (R-90) (Operational phases A, B and C: OLR = 2.4 g COD/L, and operational phase D: OLR = 2.8 g COD/L).

5.2.3.3 Supernatant filterability

Supernatant filterability gives insights into the fouling tendency of fine particles and solutes, such as colloids and SMP, yielding pore blockage in the membranes (Le-Clech et al., 2006; Meng et al., 2009). The supernatant filterability reflected the overall increasing trend throughout the entire operational period without any significant correlation with SRT. However, Dereli et al. (2015) suggested that the supernatant filterability improves at shorter SRTs because solutes and fine particles are washed out with sludge wasting. In the present case, the reactor was not maintained in a stable condition and the SRT was changing. Variations in other factors caused by changes in SRT could become a crucial reason for determining changes in supernatant filterability. Concurrently, the SMP and colloids also exhibited a decreasing trend throughout all operational phases.

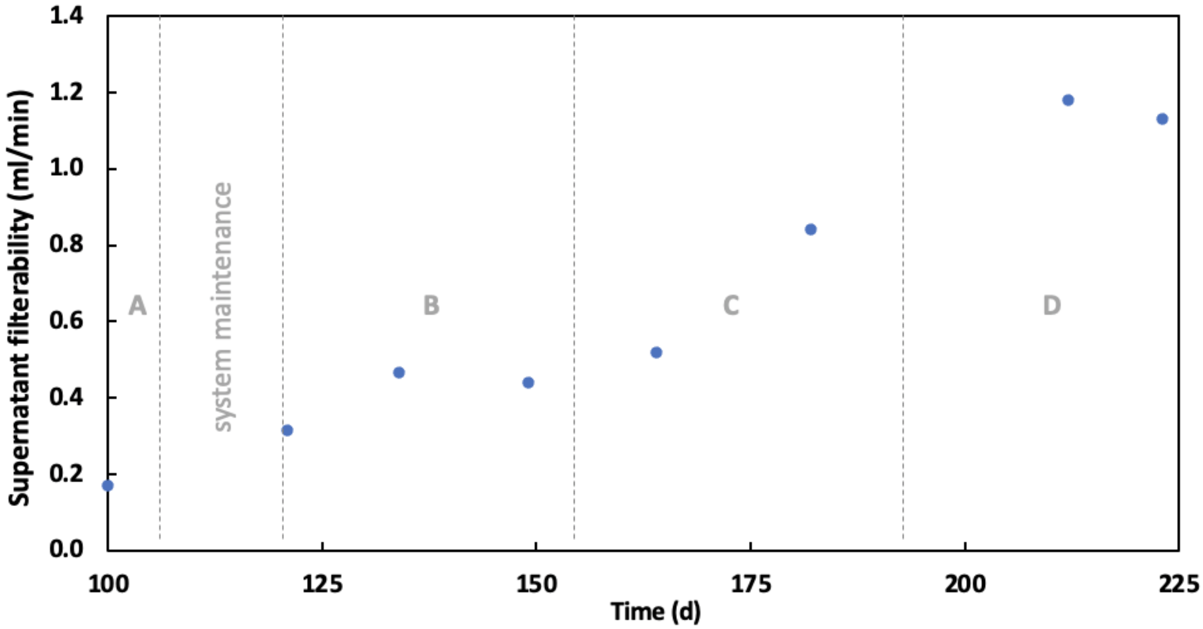

Figure 16. Supernatant filterability (ml/min) of the sludge in operational phases A (R-140), B (R-60), C (R-90) and D (R-90), (Operational phases A, B and C: OLR = 2.4 g COD/L, and operational phase D: OLR = 2.8 g COD/L).

5.2.4 Relationship between sludge characteristics and real membrane performance

Table 6 presents the correlation between operational membrane filtration resistance and sludge filtration characteristics. According to the results, a positive correlation exists between membrane filtration resistance and EPS-protein, TSS, SMP-polysaccharide, Non-VFA soluble COD concentrations, SRF, and CST. It is established that CST, soluble carbohydrate, SCOD, and proteins have inter-correlations. Moreover, it has also been found that the parameters mentioned above have an effect on the filtration resistance of the membrane (Wu et al., 2007). The filtration resistance and the filterability of the supernatant had a negative correlation, indicating that when the supernatant filterability increases, it could lessen the resistance of the operational membrane. It is a reasonable outcome, particularly given that the supernatant fraction of the sludge primarily comprises fine particles and solutes, which are instrumental in membrane fouling (Gao et al., 2013). Based on numerous studies, Dereli et al. (2015) summarized that the supernatant's contribution to the overall filtration resistance was in the range of 17-81 percent. The notable differences in the reported outcomes regarding the supernatant's effect on fouling can be ascribed to various processes adopted for operational conditions of the membrane, bioreactor, and sludge fractionating (Dereli et al., 2015; Le-Clech et al., 2006).

Table 6. Relationship between sludge characteristics and real membrane performance.

	TSS Concentration	*EPS-Protein*	*SMP-Polysaccharide*	*Non-VFA soluble COD*	*CST*	*Supernatant filterability*	*SRF*
EPS-Protein	-0.001						
SMP-Polysaccharide	0.918	0.070					
Non-VFA soluble COD	0.977	0.078	0.916				
CST	0.993	-0.059	0.934	0.965			
Supernatant filterability	-0.773	0.009	-0.733	-0.867	-0.749		
SRF	0.786	-0.102	0.766	0.877	0.786	-0.945	
Filtration resistance	0.290	0.155	0.439	0.408	0.337	-0.386	0.615

The sludge filterability indicators did not show a strong correlation with the filtration resistance of the membrane. Dereli et al., (2015) indicated that indicators of the sludge filterability, including TSS concentration, CST, and SRF did not show any significant correlation with the long-term membrane filtration resistance for the AnMBR operating at SRTs of 20 days and 30 days, for which there are various explanations. Firstly, the operating flux of the membranes was lower than the critical flux with regular backwash. Such operational variables could restrict cake compaction and decrease the accumulation of filtration resistance. It resulted in a weak association between membrane filtration resistance and characteristics of sludge filterability. Besides, the hydrodynamic conditions related to certain filterability tests (i.e., SRF) could be completely different from the actual membrane filtration. When compared with dead-end filtration, the formation of the cake layer under crossflow filtration has a superior resistance, primarily because of the selective deposition of fine particles on the membrane's surface (Dereli et al., 2015; Le-Clech et al., 2006). CST is a straightforward and complete variable to characterize the comprehensive effect of various variables for indicating the sludge's filterability and fouling of the membrane. However, contradictory results have been reported. Supposedly, following a couple of years of comprehensive tracking and evaluation of data, Lyko et al. (2008) failed to establish a positive association between the filterability of the sludge and CST in a full-scale MBR. However, it could be difficult to establish direct correlations in a full-scale plant, primarily because the membranes are most likely to operate at a secure flux level with fixed operational variables. Besides, there is a high level of difficulty associated with assessing the membrane history's influence (Dereli et al., 2015).

There is no specific parameter to represent the filterability of the sludge. Therefore, it is more reliable to use a combination of parameters to predict the filterability and fouling tendency (Van den Broeck et al., 2011). Hence, it is necessary to evaluate a set of parameters to gain deeper insights into membrane filtration. Noticeably, some parameters are not completely independent. On top of that, a lack of observable correlations does not imply that an association does not exist between these parameters. Thus, the association could be non-linear and more intricate.

6 Discussion

6.1 Biological Performance

6.1.1 Comparison of performance in different operational conditions

Table 7 summarizes the biological performances in the stabilizing state of operation phases at different SRTs or OLRs. The comparison among phases A, B, and C aims to investigate the influence of SRT on LCFA degradation and AnMBR biological performance. The comparison between phases C and D is designed to explore the impaction by OLR. Phase A was dynamic due to the changing biological performance, and the data in the last two HRTs will be used for comparing with other operational phases.

Table 7. Comparison of the stabilizing state performance of reactor at different operational phase. The performance is represented by the data after first three HRTs in each phase as (mean±standard deviation), or (beginning → end point) for the continuously changing parameter.

Parameter	Unit	Phase A	Phase B	Phase C	Phase D
SRT	d	140	60	90	90
OLR	kg COD/m3/d	2.42±0.08	2.40±0.08	2.40±0.1	2.82±0.08
TSS	g/L	28.66±1.10	18.04→9.41	8.54±0.24	6.99±0.48
VSS	g/L	16.82±0.4	11.17→5.57	5.33±0.09	4.44±0.27
F/M ratio	kg COD/kg VSS/d	0.15	0.22→0.44	0.45±0.01	0.66±0.04
Permeate COD	mg/L	577→135	141±18	84±9	135±14
COD removal efficiency based on permeate quality	%	90.7→98.2	98.02±0.27	98.88±0.16	98.52±0.15
Methane production[a]	L/d	3.24→4.00	3.77±0.10	3.82±0.12	4.75±0.12
Digestion efficiency[b]	%	60.2→71.8	69.2±2.6	70.9±2.9	74.1±2.2

[a]At standard temperature and pressure (0 °C, 1atm)

[b]Daily methane production (as g COD) divided by daily total load (as g COD)

When comparing the operational phases A, B, and C with the same OLR and different SRTs, all of the average COD removal efficiencies were above 98%, a higher COD removal efficiency based on permeate quality was achieved in phase C (R-90). Additionally, according to the result, the average methane conversion efficiencies in phase A (R-140), phase C (R-90), and phase B (R-60) were 72%, 71%, and 69%, respectively. Apparently, slightly higher digestion efficiencies were obtained at high SRTs during stabilizing processes. It could be ascribed to the higher biomass concentration in longer SRTs which also make retaining a slower growth rate for biomass in the bioreactor possible (Nilusha, Yu, Zhang, & Wei, 2020). According to the study which probed into the effect of sludge retention time on the biological performance of AnMBR while treating high lipid content wastewater, the stable operation conditions were achieved after the long-term continuous operation at SRT at 20, 30 and 50 days (Dereli et al., 2014). They suggested that better biological degradation efficiencies could be achieved at higher SRTs, which is identical to our study.

When comparing the operational phases C and D, which operated at the same SRT of 90 days but different OLRs, a higher COD removal efficiency based on permeate quality could be achieved at a lower OLR. However, higher digestion efficiency was attained with the application of higher OLR. The increased COD loading rate might cause an increased level of organic substrates available for conversion to biogas resulting in CH_4 production, which could be a reason for higher digestion efficiency. Moreover, Jiang et al. (2020) also indicated that the higher digestion efficiency could be attained with the application of higher OLRs (0.5 – 9 g VS/L/d) in thermophilic anaerobic digestion, and a slight decrease of digestion efficiency was observed at OLR as 11 g VS/L/d as the overloading. Besides, the sufficient food supply might also break the limited biomass growth scenario in phase C might be another reason for increasing the digestion efficiency. However, the concentration of biomass did not show an increasing trend in phase D in 12 HRTs. Since the sudden increase in OLR could also cause the accumulation of LCFAs and produce an inhibitory effect on microbial growth during the early stage of this condition (Pereira et al., 2005).

The performance was represented by the data of nine HRTs after the first three HRTs in each phase. The small standard division values indicate that the system tended to stabilize gradually, which means the data represents the stabilization and adaptation process. However, less operational time is the limiting factor for showing the effect of SRTs and OLRs on biological performance more extremely. On the one hand, due to LCFA and VFA accumulation from the start-up period and the shorter operation time in operational phase A, the system performance was not reaching the optimal performance limit until the end of this phase. The constant improvement in the system performance arose from the anaerobic biomass adaptation of LCFA suggested the better biological performance could be achieved at SRT at 140 days. Specifically, the COD removal efficiency based on the permeate quality was expected to achieve higher than reality. On the other hand, the risk of operating at SRT as 60 days did not lead to the most intuitive inhibitory performance during the limited operational time, so the satisfactory biological performance could be achieved while biomass concentration was decreasing and LCFA was accumulating. However, it is not difficult to infer that if the long-term operation is possible, the system adaptation process cannot proceed and then tend to be the stable state under this operating condition.

The appropriate SRT selection and optimal OLR dosage are significant in anaerobic digestion while treating high-strength wastewater. In light of the reported biological results, operating the AnMBR at an SRT of 140 days and 90 days with satisfactory treatment performance and system adaptability would be advisable. Besides, higher digestion efficiency was attained with the application of OLR as 2.8 g COD/L/d with adequate COD removal efficiency based on permeate quality. The maximum OLR that the thermophilic anaerobic membrane bioreactor system can handle without causing overloading is worth exploring in the future.

6.1.2 LCFA accumulation and impaction

The palmitic acid concentration equivalence of the sludge was connected to the biomass concentration and methane production, which could provide better insights into the impaction of presence and accumulation of LCFA in different operating conditions.

According to the result, the palmitic acid concentration equivalence increased to 6.5 g/L with the increasing OLR during the start-up period. The higher POME dosage resulted in excessive LCFAs, which inhibited the methanogenic activity, caused VFA accumulation, and depressed methane production. The reason could be that the accumulation of LCFA on the sludge could form a physical obstacle and impede the transfer of products and substrates, slowing down the initial methane generation (Pereira et al., 2005). Besides, LCFAs have marked restricting influences on the microorganisms in anaerobic digestion, specifically for methanogens (Rinzema et al., 1994). Moreover, the previous study highlighted how the restricting influences of LCFA become prominent at low concentrations, specifically at 50 mg/L. The restrictive effects of LCFAs on the generation of biogas and the defensive effect on membrane bioreactor showed that the concentrations of palmitic at 3.0 g/L led to greater than 50 percent inhibition of the generation of biogas (Dasa et al., 2016). However, in our study, the result indicated that the palmitic acid concentration equivalence of 6.5 g/L led to restricting the generation of biogas.

The methane production reduced sharply following operational phase B at SRT of 60 days. The palmitic acid concentration equivalence to VSS ratio was 0.25 g/L / gVSS/L, even though the palmitic acid concentration was relatively low. The decline in methane production could result from limited acidogenic oxidation and/or ß-oxidation processes as the VFA concentration was not accumulated. The most likely reasons for the slow biomass growth rate and the high biomass wastage at SRT as 60 days could be the most likely reasons. Besides, the difference between the rates of these two factors could result in a reactant–product imbalance and subsequent LCFA accumulation over time, thus inhibiting microbial activity (Ma et al., 2015). Expressly, the physical absorption of LCFAs into the surface of microbial cell membranes could limit mass transfer and further impact product diffusion and nutrient uptake (Ma et al., 2015). Based on the observation from Cirne et al. (2007), the inhibition of methanogenesis at 0.15-0.33 g COD / TS concentrations of palmitate showed a similar threshold value in our case (the palmitic acid concentration equivalence to TS ratio – 0.3 g COD/ g TS). For the palmitate concentration and LCFA: biomass ratio for methanogenesis inhibition, the difference threshold value between other studies and here may be due to the error in the equivalent concentration obtained through calculation, or it may be caused by the difference in the LCFA concentration that can be tolerated under different operational conditions.

Based on the comparison, the LCFA: biomass ratio is more closely related to inhibition than the LCFA concentration in the current scenario, particularly in lower biomass concentrations. Besides, it is reliable to use it as an indicator as the previous two inhibition processes were observed in very similar LCFAs: biomass ratio. However, it is still necessary and meaningful to explore the inhibition relationships more in-depth by using the specific LCFA concentration through experimental tests.

To sum up, while operating the reactor at shorter SRT or increasing the OLR of the system, the accumulation of LCFA could cause an inhibitory effect on microbial activity, the most obvious manifestation was the decrease in methane production. Admittedly, the equivalent LCFAs: biomass ratio can serve as a valuable indicator for reactor control to help predict and reduce risk, even under a rough calculation. So it would be advisable to operate the reactor at longer SRTs (90 days and 140 days) and use the LCFAs: biomass ratio as an indicator to avoid the inhibition caused by the accumulation of LCFA when increasing OLR.

6.2 Filtration performance

6.2.1 Long-term filtration performance

The long-term membrane filtration result showed the overall stable filtration performance in all operational phases. According to the result, the net permeate flux between 8 and 11 LMH, TMP was under 200 mbar and no membrane cleaning was needed during the operation. Besides, there was a gradual increase in permeability. Based on these, it could be preliminarily confirmed that using the PVDF membrane to process POME by AnMBR at the long-term laboratory operation level is feasible. The outcome could be ascribed to two factors: the frequent backwash operation and sub-critical flux operation. Consequently, the permanent fouling and cake compaction are limited (Dereli et al., 2014). Dereli et al. (2014) also indicated that the great filtration performance and net permeate flux between 9 and 13 LMH could be achieved by PVDF membrane in AnMBR system while treating high-lipid wastewater.

There are many factors that lead to the satisfactory filtration performance in terms of limit TMP and improved permeability. On the one hand, the constantly reducing biomass concentration could be one reason for the permeability gain, and the permeability increased in operational phase B together with the reduced biomass concentration. Bin et al. (2004) suggested that based on data from measurements, the permeate flux decreased with increased MLSS concentration. They believed that this occurs because the rapid development of a fouling cake layer at high MLSS concentrations decreases permeate flux. However, the impact of MLSS on permeability is controversial, according to the previous research observations, and it appears that a fouling index which is based solely on the MLSS concentration is inadvisable and should always be evaluated together with other system characteristics (Gkotsis & Zouboulis, 2019). On the other hand, the reduced SMP concentration could be another factor that impacted the performance of TMP and permeability at various operational conditions. The foulant that primarily contributes to membrane fouling is known as SMP and impacts major fouling indices, such as TMP, membrane resistance, and permeability. This is because SMP is mostly responsible for the presence of such indices due to its hydrophilic and gelling characteristics, and it is shown by polysaccharides and causes them to adhere strongly to the membrane surface (Gkotsis & Zouboulis, 2019). Chen et al. (2017) also indicated that the SMP and EPS could impact the TMP and membrane resistance at different OLRs by generating the pore blockage and cake layer. Therefore, in our study, the reduced SMP during the whole operational period helped relieve the fouling tendency.

However, the operation period of the reactor is no longer enough to reach a completely stable state was a limitation for judging the overall filtration performance as there are still some variables such as the concentration of the biomass, mixed liquor and so on. It is hard to make an utterly definite summary of the long-term filtration performance in the steady state. In addition, the solids concentration of sludge in the reactor was relatively low for most of the operational period. The conclusions obtained in this study may not be fully applicable to sludge with high solids concentration.

6.2.2 Effect of SRT on sludge filterability

The better sludge filtration characteristics were observed in the operational phase D when the SRT was 90 days. According to the research from Meng et al. (2009), an optimum SRT exists for all individual cases in MBRs, corresponding to the changes in the composition of the substrate, the set-up of the reactor, and membrane operation. Besides, according to the results, the variation of sludge filtration characteristics did not correlat with SRT. In the study which evaluated SRT's impact (infinite, 60, and 30 days) on the treatment and filtration characteristics of sequential anaerobic sulfate-reducing MBRs for treating textile wastewater, the reactor was operated in six conditions: SRT at infinity (20 ml sludge taken in every two weeks which could be neglected as the reactor working volume was 4L), 60 days, 60 days, 30 days, infinity, and infinity. The bioreactor's CST, SRF, and supernatant filterability also did not show any correlation with SRT (Yurtsever et al., 2017). Moreover, the biomass concentration decreased during the operational period, which is similar to our case. However, Dereli et al. (2015) indicated that better sludge filtration characteristics were observed at a shorter SRT (20 days) compared to the other two reactors which were operated at SRTs of 30 and 50 days. The article concluded that the improvement could be attributed to the washout of SMP and fine particles with the wasted sludge, which have been identified as the central membrane foulants. Improved supernatant filterability, larger sludge particle size, and lower supernatant COD concentrations are consistent with higher operating fluxes and enhanced sludge filterability.

The optimal SRT for the optimum filterability of the sludge is 90 days in our study. However, the optimal SRT can be different from that for system feasibility and biological parameters. Hence, it is necessary to find a compromise between biological performances and the membrane. Notably, there are other methods to improve the filterability of the sludge, such as adding adsorbents and coagulants that are conducive to reducing membrane fouling by improving attainable flux (Akram & Stuckey, 2008).

6.3 Synthetic POME treatment by AnMBR

Synthetic POME was successfully treated in thermophilic AnMBR at SRTs of 140 and 90 days with satisfactory biological and filtration performance. Over 98% COD removal efficiencies could be achieved by AnMBR system, which is higher than many other anaerobic applications while treating the POME such as CSTR, EGSB, UASFF (77%, 95%, 97%, respectively) (Trisakti et al., 2012; Najafpour et al., 2006). Coupling anaerobic digestion and membrane filtration did provide the benefits to the POME treatment process. The most central point is that the complete biomass retention helped retain the sufficient quantity of active biomass so that the system can maintain relatively strong adaptability and an adequate treatment performance even under unfavorable conditions with the slow growth rate of microorganisms. According to the study about treatment of POME by membrane anaerobic system from Noor et al. (1999), 91.7% to 94.2% of COD was removed at the same HRT (3 days) and similar SRT range (162 to 77 days) with us, in high OLRs (14.2 – 21.7 g COD/L/d) by the ultrafiltration membrane bioreactor. The slightly higher COD removal rate could be achieved here might because of the low OLR (maximum 2.8 g COD/L/g) applied during the operation. Although the low OLR applied did not meet the requirement of the POME industry, the exploration of the system's performance at different SRTs in our study could help select the optimal operating condition in POME treatment by AnMBR.

While combining anaerobic digestion and membrane filtration brings benefits, they also jointly determine the limitations of the system. Based on reported biological results, satisfactory treatment performance and system adaptability could be achieved at SRT as 140 days and 90 days in synthetic POME treatment by AnMBR. Meanwhile, the overall stable filtration performance can be obtained in the operational phases under different conditions (SRT at 60, 90 and 140 days). Therefore, the appropriate operational condition selection was mainly based on deterring factor from the anaerobic digestion while treating POME by AnMBR system in our case.

However, in actual industrial applications, it is still necessary to maximize the membrane utilization efficiency and reduce the cost as much as possible on the premise of ensuring the effluent quality could meets the water standards. Membrane fouling has the potential to significantly reduce the lifespan of membrane modules and impact the investment and operating cost (Stoller & Ochando-Pulido, 2014). Noor et al. (1999) also indicated that the membrane fouling led to the flux rate deterioration in the POME treatment process, which is the important limitation of ultrafiltration module. The system operating strategies could help to prevent the membrane fouling. Firstly, the critical flux is a crucial metric for determining the flux range which could used in the filtration process. The membrane system should be operated at low or near the critical flux, as is important for fouling control as well as establishing a high selectivity rate in order to avoid deposits on the membrane (Bacchin et al., 2006). Besides, the critical flux value can be used to confirm the membrane area that should be used for filtration to improve effectiveness and reduced the capital investment. Secondly, the fouling could be minimized by operate the system in the appropriate MLSS range. The study which explored the fouling behavior of the AnMBR for POME treatment suggested that the biofilm growth on the membrane surfaces was triggered by the increasing cake layer, which resulted from the high MLSS concentration (Treatment et al., 2021). Thirdly, the deposition of particles and formation of cake layer on the membrane surface could be limited by a higher crossflow velocity and regular backwash. (Dereli et al., 2015; Fakhru’l-Razi & Noor, 1999).

7 Conclusion and Outlook

7.1 Conclusion

The conclusions for the research questions are presented as follows: How can the advances in understanding the anaerobic digestion at thermophilic condition (55 °C) using AnMBR be used to improve the treatment of synthetic POME at various SRTs?

Overall conclusion: Synthetic POME was successfully treated in thermophilic AnMBR at SRTs of 140 and 90 days with satisfactory biological performance and filtration performance.

RQ1: What's the biological performance: degradation efficiency, biogas production of thermophilic (55 °C) AnMBR system when treating synthetic POME, in different OLRs and SRTs?

Synthetic POME could be treated with COD removal efficiencies over 98% and digestion efficiencies from 69% to 72% at an OLR of 2.4 kg COD/m^3/d in AnMBR systems at different SRTs. The methane fraction in biogas was 76% and the specific methane production was from 0.242 Nm^3/kg COD to 0.254 Nm^3/kg COD. Improved digestion efficiency (around 72%) could be achieved at a longer SRT (140 days). However, this study underlines that even though membrane can ensure the biomass retention, the AnMBR process is still dogged by the LCFA accumulation and inhibition problems, especially at a short SRT (60 days). Besides, the continuous reduction in biomass concentration even cannot reach a constant value in the stabilizing process of 60 days SRT, and eventually led to a decrease in methane production.

OLR of up to 2.8 kg COD/m^3/d at 90 days SRT of also can be treated and achieved the with satisfactory biological performance, that is, over 98% COD removal efficiency and 74% digestion efficiency, which are significantly higher than 71% when OLR is 2.4 kg COD/m^3/d and the SRT is the same. The specific methane production was also improved to 0.26 Nm^3/kg COD. A higher OLR might break the limit of biomass growth that contributes to higher digestion efficiency.

RQ2: What is the filtration performance (removal efficiency, long-term membrane filtration, and sludge filterability of PVDF membrane for synthetic POME filtration under different operational conditions?

Under all operational conditions, this work achieved satisfactory filtration performance and net permeate fluxes between 8 and 11 LMH. TMP was under 200 mbar during the whole operational process, and no membrane cleaning was needed during the operation. For the filtration characteristics, PSD showed a slight decrease in operational phases A and B, and became unstable in operational phases C and D. The EPS and SMP concentrations of the sludge changed in different operational conditions. Overall, CST decreased from 1,816 s to 82 s, and the normalized CST values were 66.0 s L/g and 12.6 s L/g, respectively. SRF decreased from 1,180 E^{12} m/kg to 333 E^{12} m/kg, and the supernatant filterability increased from 0.2 ml/min to 1.2 ml/min throughout the entire operational phase.

The results revealed that better sludge filterability could be achieved at 90 days SRT. The sludge filterability was compared based on the standard methods including CST and SRF, which did not show a linear relationship with SRTs. The physical-chemical characteristics of the sludge during the operational phases, including TSS concentrations and SMP have a close correlation with sludge filterability parameters, such as CST and supernatant filterability. The sludge filterability was strongly affected by short SRTs, accompanied by the rapid decrease in TSS concentrations and the gradual accumulation of LCFAs.

7.2 Experiment Suggestions

Based on the experimental results and weaknesses, the suggestions for further research are offered below:

1. For a better understanding the accumulation and inhibition of LCFAs in different operational conditions, and to better control the reactor, the concentrations and specific types of LCFAs should be accurately measured by experiments. Besides, the precise oil and individual LCFA removal efficiency ranges of membrane under different operational conditions can be explored in this study.

2. It is recommended to conduct microbial community analysis under different operational conditions to fully explore the changes of microorganisms involved in the anaerobic reaction under different conditions, the inhibition of LCFAs, and the limitations of the overall reaction.

3. It is recommended to conduct the sequential cleaning procedure of the membrane after each operational phase, so that different fouling resistances could be calculated, and cleaning efficiency could be evaluated. Additionally, membrane autopsy and SEM tests of the fouling on PVDF membranes are recommended to analyze different fouling morphologies and determine the fouling mechanism.

4. It is recommended to measure and calculate the energy supply needed for AnMBR by considering the production of biogas gained in the empirical analysis of AnMBR at a laboratory level as well as energy consumption to further assess the industrial application of the AnMBR system.

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Appendix

A POME characteristics and Treatment Guideline

Table A.1. The physicochemical characteristics of POME.

Parameter	Unit	Value	Reference
pH	-	3.9-5	(Habib et al., 1997)
DO (Dissolved oxygen)	mg/L	2.57-4.69	(Elijah, Sylvester, & Nimi, 2013)
EC (Electrical Conductivity)	µs/cm	137	(Elijah et al., 2013)
Total COD	mg/L	42900-88250	(Elijah et al., 2013; Tabassum, Zhang, & Zhang, 2015; Wood, Lai, & Rajara, n.d.)
Soluble COD	mg COD/L	45000-88000	(Fang, O-Thong, Boe, & Angelidaki, 2011; Yoochatchaval et al., 2011)
BOD	mg/L	17000-65714	(Chin, Poh, Tey, Chan, & Chin, 2013; Tabassum et al., 2015; Wood et al., n.d.)
TS (Total solids)	mg/L	30000-100000	(Rafael Borja et al., 1996; Chin et al., 2013; Tabassum et al., 2015)
VS (Volatile solids)	mg/L	24300-80000	(Borja et al., 1996)
VSS (Volatile suspended solids)	mg/L	8100-28500	(Borja et al., 1996)
SS (suspended solids)	mg/L	14100-50000	(Wood et al., 1979)
Alkalinity	mg/L	148-536	(Fang et al., 2011; Tabassum et al., 2015)
T-protein	mg COD/L	8830-21150	(Yoochatchaval et al., 2011)
T-sugar	mg COD/L	15320-26330	(Yoochatchaval et al., 2011)
Oil and grease	mg/L	4000-15900	(Chin et al., 2013; Yoochatchaval et al., 2011)
Lipid	mg/L	8400	(Fang et al., 2011)
Ethanol	mmol/L	12.45	(Fang et al., 2011)
Butyric acid	mmol/L	0.18	(Fang et al., 2011)
Acetic acid	mmol/L	52.62	(Fang et al., 2011)
Propionic acid	mmol/L	0.78	(Fang et al., 2011)
Total VFA	mg/L	3300	(Fang et al., 2011)
TN (Total nitrogen)	mg/L	500-900	(Rafael Borja et al., 1996; Chin et al., 2013; Wood et al., n.d.)
TKN	mg/L	3200	(Fang et al., 2011)
SO_4	mg/L	60-66	(Awotoye, Dada, & Arawomo, 2011; Rafael Borja et al., 1996)
NO3	mg/L	262.26	(Awotoye et al., 2011)
K	mg/L	1281-1928	(Wood et al., 1979)
Mg	mg/L	254-344	(Wood et al., 1979)
Na	mg/L	225-332	(Awotoye et al., 2011)
Ca	mg/L	252-605	(Ohimain & Izah, 2017; Wood et al., n.d.)
Al	mg/L	120	(Rafael Borja et al., 1996; Ohimain & Izah, 2017)
B	mg/L	0.9	(Rafael Borja et al., 1996; Ohimain & Izah, 2017)
N	mg/L	365-800	(Wood et al., 1979)
P	mg/L	17-165	(Pechsuth, Prasertsan, & Ukita, 2001; Wood et al., n.d.)
Cd	mg/L	0.01-0.03	(Elijah et al., 2013; Wood et al., n.d.)
Cu	mg/L	0.6-2.44	(Elijah et al., 2013; Ohimain, Seiyaboh, Izah, Oghenegueke, & Perewarebo, 2012; Wood et al., n.d.)
Fe	mg/L	6-205	(Rafael Borja et al., 1996; Elijah et al., 2013; Wood et al., n.d.)
Cr	mg/L	0.05-0.43	(Wood et al., 1979)
Zn	mg/L	1.2-6	(Rafael Borja et al., 1996; Wood et al., n.d.)
Mo	mg/L	0.1	(Borja et al., 1996)
Mn	mg/L	2-34	(Wood et al., 1979; Awotoye et al., 2011)
Ni	mg/L	1.2	(Rafael Borja et al., 1996; Ohimain & Izah, 2017)
Si	mg/L	55	(Rafael Borja et al., 1996; Ohimain & Izah, 2017)
Ba	mg/L	0.3	(Rafael Borja et al., 1996; Ohimain & Izah, 2017)
Co	mg/L	0.01-0.06	(Rafael Borja et al., 1996; Wood et al., n.d.)

Table A.2. Guidelines for wastewater reuse provided by WHO (Saleem, Bukhari, & Akram, 2011; Azmi et al., 2013) and discharge in Malaysia (A. L. Ahmad et al., 2003).

Parameter	Unit	Discharge limit	Reuse limit
pH	-	5-9	6-9
Turbidity	NTU	-	-
Oil and grease	mg/L	50	8.0
BOD	mg/L	100	200
COD	mg/L	-	500
TDS	mg/L	-	1500
TSS	mg/L	400	150
Total nitrogen	mg/L	150	70

Table A 3. Types of LCFA in POME.

LCFA	Unit	Value	Reference
Linoleic Acid (C18:2)	mg COD/L	400	(Yoochatchaval et al., 2011)
Oleic Acid (C18:1)		2500	
Stearic Acid (C18:0)		400	
Palmitic Acid (C16:0)		2880	
Myristic Acid (C14:0)		20	

B Membrane Characteristics

Table B 1. Membrane characteristics.

Membrane Characteristics		
Parameter	Value	Unit
Pore Size	30	nm
Brand	Pentair	
Type	Tubular	Inside-Out
Diameter	5.2	mm
Length	640	mm
Cross-Sectional Area	2.10E-05	m^2
Membrane Area	0.01	m^2
Cross-flow Velocity	0.6	m/s