# SUBMERGED ANAEROBIC MEMBRANE BIOREACTOR FOR PALM OIL MILL EFFLUENT: IDENTIFICATION OF MAIN DETERMINING MECHANISMS FOR CONTROL AND PERFORMANCE

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**A THESIS SUBMITTED AS A PART OF THE REQUIREMENTS**
**FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**
**IN ENVIRONMENTAL TECHNOLOGY**

**THE JOINT GRADUATE SCHOOL OF ENERGY AND ENVIRONMENT**
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## ABSTRACT

The aim of this study was to investigate the effect of sludge retention time (SRTs) on treatment performances of two-stage submerged anaerobic membrane bioreactors (SAnMBR) treating palm oil mill effluent (POME). The characteristics of sludge and microbial on filterability and biofouling were evaluated at different SRTs. SAnMBR with SRTs of 15, 30 and 60 d were setup for treating POME at hydraulic retention times (HRT) 2 day. The average permeate flux was fixed at 2.4 $L/m^2 \cdot h$. During operation, the membrane was regenerated by using two steps: membrane rinsing during each experiment as soon as trans-membrane pressure (TMP) reached 125-130 mbars, and backwashing and chemical cleaning at the end of each experiment when analyzing the membrane surface and foulant material. The results indicated that total COD removal efficiencies higher than 97% was achieved at all operating conditions. Maximum biogas production rate was 0.35 L $CH_4$/g $COD_{remove}$ at SRT 30 d. An increase in SRT enhanced growth of biomass and accumulation of soluble microbial products (SMP), which accelerated membrane fouling. The fouling occurred was the cake deposit, especially for SRT 60 d. Scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), atomic force microscopy (AFM), Fourier transform infrared (FTIR) analysis indicated that fouled membrane surfaces were covered with a cake layer containing organic and inorganic elements whose concentrations were higher when working at a higher SRT. In these experiments the soluble microbial products (SMP) and extracellular polymeric substances (EPS) played a secondary role because of the dominant effect of the cake layer.

**Keyword:** Submerged anaerobic membrane bioreactors, Membrane fouling, Solids retention time, Palm oil mill effluent, Trans-membrane pressure.

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## NOMENCLATURES

| | | |
|---|---|---|
| POME | = | Palm Oil ill effluent |
| $^{\circ}C$ | = | Degree Celsius |
| STP | = | Standard Temperature Pressure |
| BOD | = | Biological Oxygen Demand |
| TCOD | = | Total Chemical Oxygen Demand |
| SS | = | Suspended Solids |
| SAnMBR | = | Submerged Anaerobic Membrane Bioreactor |
| AnMBR | = | Anaerobic Membrane Bioreactors |
| TSS | = | Total Suspended Solids |
| TCOD | = | Total Chemical Oxygen Demand |
| US.EPA | = | United States Environmental Protection Agency |
| HRT | = | Hydraulic Retention Time |
| SRT | = | Sludge Retention Time |
| UASB | = | Up-Flow Anaerobic Sludge Blanket |
| UASFF | = | Up-Flow Anaerobic Sludge Fixed-Film |
| EGSB | = | Expanded Granular Sludge Bed |
| OLR | = | Organic loading Rate |
| MLSS | = | Mixed liquor Suspended Solid |
| MLVSS | = | Mixed liquor Suspended Volatile Suspended Solid |
| BMP | = | Biochemical Methane Potential |
| BD | = | Ultimate Biodegradability |
| $\lambda$ | = | Lag Phase |
| SMA | = | Specific Methanogenic Activity |
| TS | = | Total Solid |

## NOMENCLATURES (Cont')

| | | |
|---|---|---|
| VS | = | Volatile Solid |
| SCOD | = | Soluble Chemical Oxygen Demand |
| OLR | = | Organic Loading Rate |
| F/M | = | Food/Microorganism ratio |
| $CO_2$ | = | Carbon Dioxide |
| $CH_4$ | = | Methane |
| VFA | = | Volatile Fatty Acids |
| ADM | = | Anaerobic Digestion Model |
| CPO | = | Crude Palm Oil |
| PKO | = | Palm Kernel Oil |
| FFB | = | Fresh Fruit Bunch |
| LMH | = | Liter per square meter per hour |
| SMP | = | Soluble microbial product |
| EPS | = | Extracellular Polymeric Substances |
| FFB | = | Fresh Fruit Bunch |
| TMP | = | Transmembrane Pressure |
| SEM | = | Scanning Electron Microscopy |
| AFM | = | Atomic Force Microscopy |
| RMS | = | Root-Mean-Square |
| PN | = | Proteins |
| PS | = | Polysaccharides |
| MF | = | Microfiltration |

## NOMENCLATURES (Cont')

| | | |
|---|---|---|
| UF | = | Ultrafiltration |
| MWCO | = | Molecular weight Cut Off |
| PVDF | = | Polyvinylidene Fluoride |
| PE | = | Polyethylene |
| PAN | = | Polyacrylonitrile |
| PES | = | Polyethersulfone |
| SDS | = | Sodium Dodecyl Sulfate |
| CIL | = | Cleaning in Line where Chemical Solutions are Generally Backflow (under gravity) Inside The Membrane |
| CIP | = | Cleaning in Place where Membrane Tank is Isolated and Drained; The Module is Rinsed Before Being Soaked in The Cleaning Solution and Rinsed to Remove Excess of Chlorine |
| VSS | = | Volatile Suspended Solids |
| NaOCl | = | SodiumHypochlorite |
| PSD | = | Particle Size Distribution Analysis |
| EDX | = | Energy Dispersive X-ray |
| HP-SEC | = | High Performance Size Exclusion Chromatography |
| FTIR | = | Fourier transforminfrared |
| TKN | = | Total Kjeldahl Nitrogen |
| $NH_4^+$-N | = | Ammonium-Nitrogen |
| TSS | = | Total Suspended Solid |
| SS | = | Suspended Solid |
| TS | = | Total Solid |
| LCFA | = | Long Chain Fatty Acid |
| DE | = | Differential Equations |

## NOMENCLATURES (Cont')

| | | |
|---|---|---|
| IN | = | Inorganic Nitrogen |
| FID | = | Flame Ionization Detector |
| TCD | = | Thermal Conduct Detector |
| IWA | = | International Water Association |
| OMW | = | Olive Mill Wastewater |
| OMSW | = | Olive Mill Solid Waste |
| TCM | = | Traditional Chinese Medicine |
| UASFB | = | Upflow Anaerobic Sludge Filter Bed |

# CHAPTER 1
# INTRODUCTION

## 1.1 Rationale/Problem Statement

Nowadays, palm oil has received much attention in terms of alternative and sustainable energy resources as well as the local economy. In Thailand, the total production trend of crude palm oil in 2008-2014 has increased 8.20 to 22.50 billion [1]. Consequently, the number of palm oil mills has rapidly increased from 49 mills in 1995 to 72 operated mills in 2010. Typically, palm oil mills with wet milling process are accounted for major production of palm oil due to its suitability for use in large-scale productions and for producing better quality palm oil [2]. Concerning the extraction of wet process, its operation requires significantly large quantities of water for steam sterilizing the palm fruit bunches and clarifying the extracted oil. This results in high discharge of wastewater or palm oil mill effluent (POME).

Palm oil mill effluents (POME) are constantly associated with environmental burdens due to the voluminous discharge of the wastewater during the milling process [3]. It is estimated that about 1.5 $m^3$ of water are needed to process one ton of the fresh fruit bunches (FFB), half of this amount ends up as palm oil mill effluent (POME). POME appears as a complex wastewater. It is acidic, colored and discharged at high temperature, 80- 90 $^{O}C$ [3]. It contains oil and grease 8.2-9.6 g/L [3], presents high (10–44 g/L) biological oxygen demand, BOD, more than 50 times the concentration in domestic wastewater, high (16–100 g/L) total chemical oxygen demand TCOD, high (5–54 g/L) suspended solids (SS) concentrations[4]. By comparing POME production and domestic wastewater volume, in term of BOD rate, POME appears as equivalent to the annual BOD rate generated by 3 million people [2]. Thus if such effluents are discharged without treatment, they cause significant and dramatic environmental impacts.

The most common POME treatment systems are pond systems and open tank digesters. More than 85% of palm oil mills use pond systems due to their low costs[5]. Nevertheless, these technologies for POME treatment present several disadvantages such as long hydraulic retention time HRT, 45–60 days[6], bad odor, difficulty in collecting and

utilizing the methane generated by local anaerobic fermentation and detrimental greenhouse effect on the environment [7].

In order to solve these problems, improved high-rate anaerobic bioreactors were investigated [8, 9], such as anaerobic fluidized bed reactors [10], anaerobic baffled reactors [11, 12] and upflow anaerobic sludge blanket reactors UASB [13, 14]. High-rate anaerobic bioreactors allow the enhancement of bioreactor and their consequences, i.e. the treatment time is largely shortened, use land is required, increase of COD removal efficiency treatment (90-95%) and methane production [15]. However, despite the above advantages, the complexity of POME content (high pollutant concentrations with numerous molecules, presence of oil and grease, refractory colored molecules, low pH, large range of composition) and influent variable induces frequent destabilization of the biological system with significant risks of biomass washout.

The development of two-stage systems has improved the functioning of high-rate anaerobic bioreactors with better pH control [16]. Nevertheless, the washout of biomass remains the main problem of the process when a bad equilibrium appears between acidogen and methanogen activities. More recently, in analogy with aerobic membrane bioreactors MBRs, porous membrane barriers were coupled to anaerobic systems to (i) avoid washout of microorganisms whatever the functioning conditions and (ii) increase sludge retention time in the bioreactor to favor organic matter degradation [17-23].

Submerged anaerobic membrane bioreactors (SAnMBR) have attracted much interest, because of the positive experiences gained from the successful application of industrial wastewater treatment at extreme conditions, such as high salinity, high temperature, high concentrations of suspended solids (SS) and the presence of toxics [24-28]. SAnMBR achieves a good degradation yield of organic matter (over 90%) and produces biogas, which can be converted to energy (0.2–0.4 $m^3$ $CH_4/kg_{converted\ COD}$) [17-28]. Reports on SAnMBR treatment of industrial dairy effluents show advantages performance compared to anaerobic biological processes such as better-treated water quality compared to non-membrane purification treatment and increased biomass concentration [17-23], thus enabling a higher organic mass loading rate.

Although SAnMBRs offer many advantages in terms of biodegradation and energy production, membrane fouling is the main drawback to their application. Even the first studies point out the role of solids in suspension SS and soluble microbial products (EPS

and SMP) on fouling rates and intensity, the identification of the foulants and their interactions with membrane surface remain still limited.

This thesis consists of 10 chapters:

Chapter 1 briefly introduces the current POME wastewater treatment practices and potential benefits of SAnMBR processes, the thesis introduction and literature review. A general review of SAnMBR is given. This is followed by a summary of studies conducted on SAnMBR for the treatment of wastewater.

Chapter 2 presents the background theory on palm oil mill extraction, anaerobic treatment fundamental (principle, type and important factor of anaerobic process), the state of the art on anaerobic membrane bioreactor SAnMBR processes for wastewater applications (principle, type of SAnMBR, classified membrane fouling, mitigation of membrane fouling and membrane cleaning methods)

Chapter 3 presents the methodology employed and the results of a study of SAnMBR performance, including carbon and nitrogen removal, biogas production, methods and membrane fouling.

Chapter 4 focuses on the SMA and BMP methods, which were applied to selected the active seed sludge and suitable POME concentration for use in SAnMBRs.

Chapter 5 discusses the optimum of intermittent filtration time to fouling control was studied in order preliminary data to reference in SAnMBR.

Chapter 6 discusses the treatment performance of start-up and SAnMBR is identified.

Chapter 7 discusses the behavior membrane fouling in SAnMBR is evaluated.

Chapter 8 discusses the information of treatment performance of SAnMBR and membrane fouling from experiment from Chapter 6 was simulated by using the commercial software GPS-X.

Lastly, Chapter 9 presents the conclusion and recommendation for future work derived from the investigations of the objectives of the research. The emphasis here is to relate findings from Chapters 4-9 that are necessary to facilitate the transfer of data found in laboratory to full-scale process, recommendations for further study and suggestions for improvements are presented.

## 1.2 Literature Review

### 1.2.1 Palm Oil Trend

Palm oil can be widely used as the raw material for various products, i.e. cooking, product ingredients. In addition, palm oil has been supported as a source of renewable energy known as biodiesel, worldwide. Therefore, palm oil production has rapidly increased. Palm oil is a biomass resource presenting a high potential to become renewable energy as bio-diesel. The total productions trend of crude palm oil in Thailand from the Ministry of Agriculture and Cooperative prediction in 2004-2014 has increased from 8.20 to 22.50 billion tons in Figure 1.1 [1]. So the palm oil industry is one of the most important agro-industries in Thailand. The demand of palm oil production rapidly increases in agreement with growing of the palm oil mills. The number of palm oil mills in Thailand has rapidly increased from about 50 mills in 1995 to more than 70 operating mills in 2010, in order to fulfill consumption demands [2].

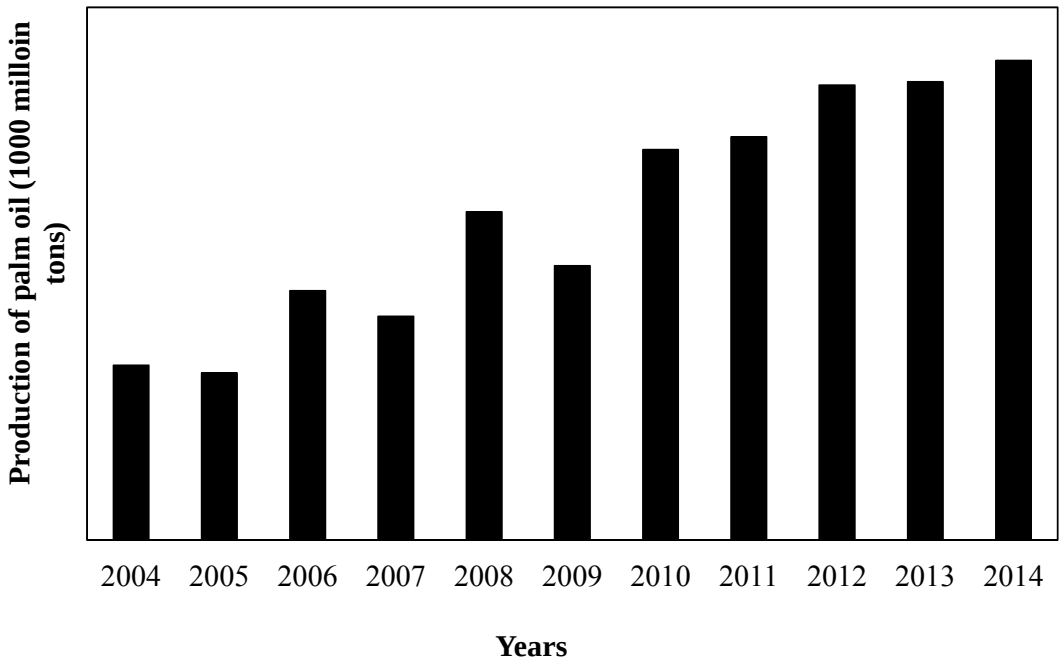


**Figure 1.1** Forecasted production of palm oil for the year 2004–2014 in Thailand [1].

### 1.2.2 Characteristics of Palm Oil Mill Effluents

The wet extraction process produces high quality and high quantities of crude palm oil, but the by-products of the process generate large amounts of effluent (POME). In 2010, it was estimated that the total quantity of wastewater generated from the extraction process of palm oil mills in Thailand had reached an average amount of 0.12 $m^3$/ton[29]. In general, the characteristic of raw POME depend on the processing techniques, indeed the activities of the palm oil mill [5] affect the quality and quantity of POME. Characteristic of POME in Thailand is a thick brownish liquid discharged at temperatures between 30-75$^oC$. It is acidic (average pH: 3.9–4.8), high organic concentrations in term, a biological oxygen demand (BOD) which is 100 times more polluting than domestic sewage and chemicals and the total chemical oxygen demand (TCOD), suspended solids (SS) and contains oil and grease[30]. A detailed description of the composition of the wastewater and sludge is summarized in Table 1.1.

**Table 1.1** Characteristics of POME[3].

| Parameters | Concentration range (g/L) |
|---|---|
| pH | 4.15-4.45 |
| Temperature | 36-77 |
| $BOD_5$ | 21.50-28.50 |
| SCOD | 20.50-24.50 |
| TCOD | 45.50-65 |
| Suspended solids (SS) | 18.40-31 |
| Total solid (TS) | 33.79-37.23 |
| Total nitrogen [20] | 0.50-0.80 |
| Ammonia nitrogen ($NH_3$-N) | 0.02-0.08 |
| Oil&grease | 1.07-8.50 |

**Remark:** All parameters are in units of mg/L except pH and temperature ($^oC$).

### 1.2.3 Treatment of Palm Oil Mill Effluent

In the past decades, several technologies applied for the treatment of POME include physicochemical treatments (simple skimming devices, chemical coagulation and flotation), membrane filtration (ultrafiltration and reverse osmosis), land disposal, aerobic and anaerobic biological processes and other specialized treatments (Bioelectricity)[13, 31]. The most common POME treatment systems are ponding systems. More than 85% of palm oil mills use solely ponding systems for POME treatment [5]. The pond system that has been applied for the treatmentof POME was classified as waste stabilizationpond. The configuration of this system consists of essentially anumber of ponds of different functions. Thus, anaerobic ponds are one of the mosteffective treatments that are being applied inpond system. This is because it has considerableadvantages such as (a) it demands less energy, (b) sludgeformation is minimal, (c) unpleasant odors are minimised and (d) anaerobic bacteria efficiently break down the organic substances to methane. Nonetheless, these methods for treatment of POME have several disadvantages such as long hydraulic retention time (HRT), large areas of lands or digester are required and difficulty in collecting and utilizing the methane generated, which causes a detrimental greenhouse effect to the environment [7].

In order to obviate these problems, suggested high-rate anaerobic treatment processes for POME include anaerobic suspended growth processes, attached growth anaerobic processes (immobilized cell bioreactors, anaerobic fluidized bed reactors and anaerobic filters), and anaerobic sludge blanket processes (up-flow anaerobic sludge blanket reactors and anaerobic baffled reactors) [7, 11, 32, 33]. High-rate anaerobic bioreactors are one of the most applied in laboratory-scaled POME treatment such as in up-flow anaerobic sludge blanket (UASB) reactors[10], up-flow anaerobic sludge fixed-film (UASFF) reactors [34], anaerobic contact digesters [35] and continuous stirred tank reactors (CSTR) [36].

In addition to applications of anaerobic treatment processes, design configurations can be operated as single-phase or two-phase systems. Single-phase systems involve only one reactor for the microorganisms to digest the organic matter[37], whereas two-phase systems separate the hydrolysis and acidogenic carried out in a former reactor, and methanogenic reactions in a second reactor. Since the nutrient and growth requirements of the acidogenic and methanogenic organisms may be different, the two-phase system can be operated to provide optimal conditions for the microorganisms in each phase for greater

efficiency in digestion. In the first phase, acidogenic organisms digest organic solids and complex soluble organics, converting them to volatile fatty acids (VFAs). In the second phase, methane-producing microorganisms (methanogens) utilize the VFAs to produce methane and carbon dioxide [38].

The acidogenic bacteria perform the hydrolysis and acidogenesis step of anaerobic digestion, whereas the optimum pH is 5.2-6.5 at the acidogenesis step. At the second step, the products of the first step which cannot be metabolized by methanogenic bacteria such as propionate and butyrate are degraded to acetate and $H_2$ at an optimum pH of 6.6-7.6 [11, 39]. Finally the optimum pH environment for methanogens is within the range 7.5-8.5 [40]. Treating wastewater in two phases allows the development of specifc biomass in each reactor and then optimizes environmental conditions for each phase because in comparison with a single phase process where both classes of organisms are forced to operate in a common environment [41]. Some examples of reported performances obtained with single or two-phase anaerobic wastewater treatment are given in Table 1.2.

Yeoh [42] indicates that three times more methane yield was obtained in a two-stage CSTR in comparison with a single stage reactor for sugar cane molasses stillage treatment. However, the COD removal efficiency of the two-stage system is only 65% while the five day biochemical oxygen demand (BOD) removal is about 85%. This difference in COD and $BOD_5$ removal could be due to the composition of molasses containing hardly biodegradable melanoidine pigments which largely contribute to COD value. It should be noted that COD and BODremoval efficiencies of hydrolytic reactor are less than 8%, but the methanogenic reactor exhibited high removal efficiency. [42]Also illustrated that two-stage anaerobic system could tolerate higher loading rates without affecting the removal efficiency over that of single stage system.

Demirer and Chen [43] summarized the advantages of a two-phase system over a one-stage as: (1) better selection and enrichment of different bacteria in each phase, then in the first phase, complex pollutants were degraded by acidogenic bacteria into VFA, subsequently converted to $CH_4$ and $CO_2$ by acetogenic and methanogenic bacteria in the second phase, (2) increase process stability by controlling the acidification phase in order to prevent overloading and built up of toxic materials and (3) easiness to buffer the prior acid phase and prevent any pH shocks to the methanogenic population.

Borja et al [13] applied the concept of a two-stage treatment by using a pair of up-flow anaerobic reactors, namely acidogenic and methanogenic UASB reactors, to treat

POMEs and evaluate the effect on sludge granulation. They found that loadings as high as 60 $kg_{COD}/m^3/d$ resulted in a significant decrease in COD removal efficiency as well as in conversion of long-chain fatty acids to $CH_4$.

Among the reported studies, Zinatizadeh et al [44] and Najafpour et al [34] have obtained COD removal efficiencies of more than 85% and methane yield of 0.30 to 0.35 $m^3$ $CH_4$/kg $COD_{removed}$ by using hybrid anaerobic high rate reactors.

In general, by using conventional anerobic systems in the range of conventional COD loading rates (6-10 $kg_{COD}/m^3/d$), the COD removal is in the range of 80-90% for low organic loading rates (< 2 $kg_{COD}/m^3/d$) and the removal efficiency can be higher than 95%.

**Table 1.2** Examples of performances of single and two-phase conventional anaerobic systems.

| Types of wastewater | Reactor types | T (°C) | HRT (d) | OLR ($kg/m^3.d$) | MLSS or MLVSS | $COD_{In}$ (g/L) | COD Removal Efficiency (%) | Methane yield $m^3CH_4/kgCOD_{removed}$ | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Cane molasses alcohol stillage | Single and Two-stage CSTR | 55 | 36-9<br>5.6-33 | 3.45-14.5<br>4.65-20.02 | - | 130 | -<br>65 as COD | 0.06<br>0.19 | [42] |
| Unscreened dairy manure | Two-phase anaerobic digestion | 35 | 2-10 | 15.06 | - | 40-160 | 71 | 0.35 | [45] |
| POME | Two-stage UASB | 35 | 1.02 | 30 | 19 (VS)<br>16 (VS) | 30.6 | 90 | 0.30-0.33 | [13] |
| POME | Upflow anaerobic sludge fixed film | 38 | 1.5-3 | 2.63-23.15 | 42 (VS) | 42-56 | 85 | 0.35 | [34] |
| POME | Upflow anaerobic sludge fixed film | 38 | 1-6 | 0.88-34.73 | - | 5-35 | 81-99 | 0.35 | [44] |
| Potato processing | Two-Stage UASB | 35<br>55 | - | 11<br>36 | - | - | - | 0.41<br>0.49 | [46] |

**Table 1.2** Examples of performances of single and two-phase conventional anaerobic systems (cont').

| Types of wastewater | Reactor types | T (°C) | HRT (d) | OLR ($kg/m^3.d$) | MLSS or MLVSS | $COD_{In}$ (g/L) | COD Removal Efficiency (%) | Methane yield $m^3CH_4/kgCOD_{removed}$ | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Synthetic fruit canning | Two upflow UASB | - | 4-5h | 15 | - | - | 80-90 | - | [47] |
| Olive mill wastewater | Completely stirred tank | 37 | 15-108 | 1.5–11 | 26 (VSS) 4.2-80(VS) | 5.25-89.93 | 77–97 | 0.24 | [48] |
| Olive mill wastewater | Sequencing semi-continuous digesters | 37 | 14-24 | 5.54-14 | - | 133 and 196 | 86 | - | [49] |
| Municipal landfill leachate | Two-UASB-CSTR | 37 | 4.5 | 16 | - | 20 | 79 | | [50] |
| Dairy WW | UASB Continuous | 35 | 1 | 12.48 | - | 12.48 | 90 | - | [51] |
| Cheese whey | Two UASB | - | 4.95 | 11.1 | 107, 99 | 55.1 | 95 | 0.42 | [12] |
| Dairy manure | UASB Two | 35 | 2 | 8.9 | - | 17.8 | - | - | [52] |
| Malt whisky WW | UASB Two | 35-1 | 1.22 | 17.2 | - | 20.92 | 92 | - | [53] |

### 1.2.4 Biochemical Methane Potential (BMP) & Specific Methanogenic Activity (SMA) Assay

Anaerobic biological processes are most applied for treating POME, because they can operate at high volumetric organic loadings. They can well perform with high efficiency for treating POME by converting organic matter to biogas. The application of anaerobic processes for wastewater treatment is usually recommended to operate and monitor several parameters such as pH, alkalinity, temperature, etc [54]. Substrate concentration is one of the important parameters on efficiencies methane production in the anaerobic digester, which can be investigated by specific methanogenic activity (SMA) and biochemical methane potential (BMP) testing. The SMA and BMP assays are widely accepted for selection of the inoculum used in the startup of the whole process, evaluation of the inhibitory potential or the degree of degradability of various compounds and/or maximum applicable loading rate of certain sludge [55, 56]. Table 1.3 present the main results for biochemical methane potential.

**Table 1.3** Examples of the main results for biochemical methane potential.

| Substrates | Temp (°C) | Characteristics of substrates | | | | $CH_4$ (%) | Maximum $CH_4$ yield ($LCH_4$/g VS $_{added}$) | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | T COD (g $O_2$/kg) | SCOD (g $O_2$/kg) | TS (g/kg) | VS (g/kg) | | | |
| Household solid waste | 37 | - | - | 35 | 26 | - | 0.50 | [57] |
| Sunflower oil cake | 35 | - | 12.1 g/L | - | 30 | - | 0.22 | [55] |
| Winter wheat | 35 | - | - | 363-835 | 347-811 | | 0.36 | [58] |
| Sewage sludge | 35 | 45 g/L | 4.3 g/L | 460 | 310 | 77 | 0.70 | [59] |
| Whole corn stillage | 35 | 253 g/L | 51 g/L | 1240 | 190 | 65 | 0.75 | [60] |
| Palm oil mill effluent | 55 | 45-97 | 34-88 | 12.9-57.3 | 19.7-67.3 | - | 0.61 | [61] |
| Co-digested dairy manure with an array of food residues | 35 | 27.8-2880 | - | 49.2-991 | 35.4-988.8 | - | 0.65 | [62] |
| Wheat straw | 35 | 1078 | - | 922 | - | - | 0.43 | [63] |
| Food waste with dairy manure | 35 | 148-648 g/L | - | 3.97-224 g/L | 1.73-275 g/L | 49.6-73.5 | 0.29 | [64] |
| Olive mill solid waste | 35 | 331 | 143 | 265 | 228 | - | 0.39 | [65] |
| Meat-processing wastes | 37 | 1774-1846 | - | - | - | - | 0.70 | [66] |
| Bamboo waste | 37 | 902 g/L | 35-129 g/L | 93.3-94.5 | 77.3-90 | - | 0.23 | [67] |

### 1.2.5 Limitations of Anaerobic Treatment for POME

Even though anaerobic systems have been widely applied to treat POMEs, they still have many limite:

The anaerobic systems with suspended cultures and no final liquid-solid separation step were not able to decouple HRT and solid retention time (SRT). When working with high loading rates and high influent COD levels, insufficient HRT led to some accumulation of volatile fatty acids (VFAs) in the reactor due to the inbalance between acid formation and methane generation [44]. Higher HRT and reactor volume are then required, especially when treating complex wastewaters. Moreover, oil and grease present in POME can also be the origin of scum formation and limitation of soluble compounds transfer to biomass. Such phenomena lead to a significant reduction of substrate conversion.

The anaerobic systems, such as UASB, allowed the separation of SRT and HRT, because of sludge granulation and retention in the bioreactor. Such a system had the same behavior as a fixed culture reactor. Nevertheless, when working in a one-step configuration, UASB can become unstable under stressful conditions such as overloading (more than 15 kg COD/$m^3$) with a possible (i) acidification of the bulk and a dissolution of granules or (ii) sludge flotation caused by a combination of high SS concentrations in the digester and a rapid gas production [5]. Moreover, specific compounds present in POME in the form of fat, protein and cellulose have adverse impact on UASB reactor performances and can cause deterioration of microbial activities and wash out of active biomass [68].So, the applications of conventional anaerobic systems for of complex wastewaters containing organic compounds like particulates, proteins, fats, and fibers are then limited if working under high organic loading rates. These complex wastewaters are hard to degrade by anaerobic way since the hydrolysis of such compounds appears difficult. Moreover,the degradation kinetics of compounds like fats and solids are very slow as growth of granular pellets and settling velocities of such biomass remain poor under those circumstances according also to local shear stresses [69]. Under such conditions, acidogenic population may be weakly fixed in aggregates and easily washed out from the reactor [70], the organic hydrolysis rate then decreases gradually [71], as the total performances of the bioreactor.

In general, effluent water discharged from conventional anaerobic systems treating POMEs still contain much oil and grease 0.13 g/L, COD4.82 g/L, BOD0.61 g/L, total solids 10.36 g/L, suspended solid concentrations 4.68g/L, and final effluent quality was

unable to meet the discharge water standard set by the Thai Department of Environment, so further treatment is still needed [6].

According to such problems of effluent qualities and difficulties in controling the reactors performance, a perfect control of the biomass retention inside the bioreactor then becomes a key factor to ensure high and constant performances of anaerobic intensive systems when treating complex wastewater. To overcome the problems, it is important to fine new reactor able to maintain high biomass retention and high SRT whatever the functioning conditions are. The assisted membrane separation offers a possible alternative. The membrane coupled with an anaerobic digestion process would be expected to alleviate most of these problems.

### 1.2.6 Anaerobic Membrane Bioreactor for Wastewater Treatment

The most recent development in high rate anaerobic treatment is using membranes to separate biomass from the effluents. Such anaerobic membrane bioreactors (AnMBRs) offers high effluent quality free of solids and pathogens due to the membrane barrier cut-off insuring a complete retention of biomass, regardless its settling and/or granulation properties. The membrane characteristic of AnMBRs used have been mainly flat sheet, hollow fiber, or tubular. The materials used were mainly polymers, as polyethersulphone, poly-ethylene, poly-vinylidene fluoride, poly-tetrafluoroethylene and ceramics [72].Regarding membrane cut-off (or average pore size), both microfiltration (MF) and ultrafiltration (UF) membranes have been used. Therefore, the removals of dissolved organic and inorganic contaminants in wastewater by UF and MF are not significant. However, UF and MF are capable of removing colloids matter, suspended particles and macromolecules [71-75]. Furthermore, the application of AnMBR can be efficient for wastewater treatment is expected to have benefit [29] such as:

1. Allowing particulate substrates to remain longer in digesters, thereby allowing more time for the slowly biodegradable material to breakdown and enhance bio-availability
2. Retaining biomass to increase the population of slowly growing methanogenic bacteria for a given digester volume.
3. Retaining extracellular enzymes to create an active environment for biochemical reactions.
4. Allowing digesters to operate at higher feed rates to reduce digester volume and associated digester heating and operational costs.

5. Enabling concurrent thickening of sludge during the digestion process to decrease handling in downstream processing.
6. Increasing net energy production per given sludge flow and digester volume.

Therefore, such a technology may present an attractive option to treat municipal wastewaters and industrial wastewaters, and/or slurries at extreme conditions, such as high salinity, high temperature, high concentrations of suspended solids (SS) and possible presence of toxicity that hamper granulation in UASB or reduce biological activities. Recent studies about the application of this technology at lab-, pilot- and full-scale reactors are summarized in Tables 1.4–1.7 and commented on as follows:

- The most important target to achieve by an AnMBR operation is to reduce the organic carbon content in the influent before its discharge in to the environment. Influent COD concentrations ranged from low values, about 1g/L, to high values, 64 g/L for sauerkraut brine or even 18 g/L for high-strength petrochemical effluent, mainly loaded with short-chain (C2 to C6) fatty acids.
- Hydraulic retention time (HRT) values ranged from a few hours, i.e. ~2 h to a few days, i.e. 20 d, whereas solids retention time (SRT) values ranged from a few days, i.e. 18 d or 30 d to about a year, i.e. 300 d or even more, indicating that no sludge purging practically took place during the MBR operation. Most researchers worked at SRT values higher than 150 d.
- Most AnMBRs were operated at around 35 $^{o}$C in the mesophilic range or at around 55 $^{o}$C in the thermophilic range, even though psychrophilic temperatures of around 20 $^{o}$C were also tested.
- Saddoud and Sayadi [73] investigated the treatability of slaughterhouse wastewater by an AnMBR at relatively high organic loading rates between 4.4 and 13.3 kg COD/m$^3$ day. They experienced a process failure at an OLR of 16.3 kg$_{COD}$/m$^3$/d due to VFA accumulation and not due to the separation step.
- Concerning palm oil mill wastewater, an AnMBR achieved very high COD removal performance (>96%) at OLRs of 1–11 kg$_{COD}$/m$^3$/d and HRTs of 7–600h[27].
- COD removal efficiencies have varied from 76% [40] up to 99% [23,54] (Tables 1.4–1.7). BOD$_5$ removal efficiencies. Of course, TSS removal efficiencies appeared to be very high, more than 99% and regarding pathogens, namely *Escherichia coli* and *Enteroccoci*, total removal can be achieved, so most of the time, the effluents

appear to be suitable for re-use in unrestricted crop irrigation, which is officially defined as the use of treated wastewater to grow crops that are normally eaten raw.

- The methane production (0.13 to 0.35 $m^3CH_4/kgCOD_{removed}$, the theoretical value is 0.37) appeared to depend the experimental conditions and the type of wastewater.

**Table 1.4** Examples of treatment performance of lab-scale AnMBR used for the treatment of various high strength wastewaters.

| Types of wastewater | Scale | Reactor volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T ($^oC$) | Specific $CH_4$ production ($m^3$/kg COD $_{removed}$) | Influent COD (g/L) | Effluent COD (g/L) | COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palm oil mill | L | 50 | 50.8-56.6 | 14.2-21.7 | 2.82-3.15 | 77-161 | 35 | 0.24-0.28 | - | - | 91.7-94.2 | [74] |
| Domestic | L | 17.7 | 16-22.5 | 0.5-12.5 | 4-6 | 150 | - | 0.13-0.42 | 0.097-2.60 | - | 97 | [18] |
| Alcohol fermentation | L | 5 | 2 | 3-3.5 | 1 | - | 55 | - | 38.40 | - | 93-97 | [75] |
| Sauerkraut brine | L | 7 | 25-60 | 2-8.6 | - | - | 30 | 0.2-0.34[g] | 40.70-64.60 | 0.050-0.10 | >90 | [76] |
| Food processing | L | 400 | 6-8 | 0.88-4.52 | 60 | 50 | 37 | 0.136[f] | 0.244-13.40 | 0.41-0.55 | 81-94 | [77] |
| Cheese whey-based | L | 20 | 6.4-10 (VSS) | 3-19.78 | 1-4 d | - | 35-39 | 0.3 | 68.60 | - | 98.5 | [78] |
| Slaughter house | L | 50 | 10.1 | 1.59-16.32 | 30-80 | - | 37 | 0.13-0.3 | 15.88 | - | >99 | [73] |

[a] Reported as $m^3$/kg COD/day [f] Reported as biogas: $m^3$/kg COD [g] Reported as $m^3CH_4$/kg $COD_{fed}$

**Table 1.4** Examples of treatment performance of lab-scale AnMBR used for the treatment of various high strength wastewaters (cont').

| Types of wastewater | Scale | Reactor volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T ($^oC$) | Specific $CH_4$ production ($m^3/kg$ $COD_{removed}$) | Influent COD (g/L) | Effluent COD (g/L) | COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Landfill leachate | L[a] | 29 (total) | -[b] | 0.7-4.9 | 24-168 | - | 35 | - | 0.500 | 0.42 | 95 | [79] |
| Petrohemical | L | 23 | >30 | 14.6 | 31.5 | 175 | 37 | - | 19.10 | 0.61 | 98 | [80] |
| Wastewaters containing suspended solids | L | 3.8 | 40 (TSS) | 10 | - | - | 30 | - | 10.00 | 0.15-0.20 | >98 | [25] |
| Landfill leachate | L | 50 | <3 (VSS) | 1-6.27 | 7 d | - | 37 | - | 15.00-41.00 | 0.96-4.10 | >92 | [81] |
| Kraft evaporator condensate | L | 10 (total) | 8.3 | 2.3-13.3 | 5.8d | 230 | 37-56 | 0.35±0.05 | 9.50-10.50 | 0.074-0.27 | 99 | [22] |
| Volatile fatty acid | L | 2 | <21(VSS) | 10-55 | - | 120 | 55 | - | 10.00 | - | - | [82] |

**Table 1.4** Examples of treatment performance of lab-scale AnMBR used for the treatment of various high strength wastewaters (cont').

| Types of wastewater | Scale | Reactor volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T ($^oC$) | Specific $CH_4$ production ($m^3/kg$ $COD_{removed}$) | Influent COD (g/L) | Effluent COD (g/L) | COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Landfill leachate | L | 3 | 7.2-10.8 | 8-11.8 | 1.1-19 d | 30-300 | 10-35 | - | - | - | >95 (soluble COD) | [83] |
| Landfill leachate | L | 50 | <3 | 6.27 | 7 | - | 37 | - | 0.04 | 0.01 | 90.7 | [28] |
| Kraft evaporator condensate | L | 3.5 | 2.1-24 | 1-24 | - | - | 36-38 | 0.35±0.05 | 5.60-10.00 | 0.05-0.20 | 99 | [84] |
| Thermo-chemical whitewater | L | 10 | 4.9-10.7 | 2.0-2.8 | - | 280 | 36-38 | 0.35-0.41 | 2.78-3.35 | <0.30 | 90 | [85] |
| Whey+sucrose | L | 11 | 5.5-20.4 (VSS) | 1.5-13 | - | 30-40 | 35 | - | - | - | - | [23] |

**Table 1.4** Examples of treatment performance of lab-scale AnMBR used for the treatment of various high strength wastewaters (cont').

| Types of wastewater | Scale | Reactor volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T ($^oC$) | Specific $CH_4$ production ($m^3$/kg COD $_{removed}$) | Influent COD (g/L) | Effluent COD (g/L) | COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maltose+glucose + volatile fatty acid | L | 0.6 | 19.5 (VSS) | 2.5 | 14 d | - | 35 | - | 25.00 | - | 95.1 | [86] |
| Thermo-chemical whitewater | L | 10 | 6.7-11.3 | 2.6-4.8 | - | 280 | 36-38 | 0.25-0.30 | 2.78-3.46 | 0.28-0.42 | 90 | [87] |
| Brewery wastewater | L | 4.5 | 12-25 | 12 | - | - | 30 | - | 2.30 | 0.19 | 99 | [88] |
| Palm oil mill | L | 50 | 11.8-20.8 | 1-11 | 6.8-600 | 12.1-1000 | - | 0.25-0.57[a] | 60.00-87.00 | 0.99-1.40 | 96-99 | [27] |

**Table 1.5** Examples of AnMBR performance for municipal wastewater.

| Types of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Real municipal | L[a] | 12.9 | -[b] | 2.36 | 2.6-12 | - | 15-20 | 0.16-0.60 | - | - | [81] |
| Primary effluent from WWT plant | L | 10 | 7.3-(Max) | 0.02-2.11 | 48 | 18-233 | 32 | 0.023-0.11 | 0.02-0.03 | 76 | [89] |
| Raw and UASB effluent | P[a] | 849 | - | - | 6 | - | - | 0.28-0.56 | 0.02-0.04 | 90 | [90] |
| Organic waste mixture | L | 0.5-0.6 | - | - | 2-20 d | - | 35 | - | - | - | [86] |
| Real municipal | L | 5-15 | 1.05-2.4 | - | - | - | 33-37 | 0.48 | 30-50 | 98 | [91] |
| Final effluent containing nitrates | L | 5.6 | 1.32-1.97 | - | 3 | 20 | 25-28 | 0.04-0.07 | 0.001 | 72 | [92] |
| Real municipal | L | 50 | - | 0.8-1.2 | - | - | 37 | 0.41-0.90 | - | 76 | [93] |
| Municipal waste | L | 3 | 8.3-21 | - | 4.4 | 300 | 34-36 | - | - | - | [94] |
| Secondary effluent | L | 2.4 | - | 1.1-3.7 | 3-8 d | - | 33-37 | - | - | - | [95] |

[a]L: Laboratory; P: Plot; [b]Value not reported. Modified from Skouteris et al., 2012 [96].

**Table 1.6** Examples of AnMBR performance for synthetic wastewaters.

| Types of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sucrose-based | L[a] | 3 | 11.45-16.12 ($VSS^A$) | 6-16 | 6-40 | 250 | 34-36 | 4.00 | 0.03-0.48 | 98 | [97] |
| Sucrose-based | | 3 | 1.68-9.69 (VSS) | 4-4.8 | 15-80 | 150 | 34-36 | 4.00 | 0.16-0.24 | 96 | [98] |
| Meat extract/peptone-based | L | 3 | 2.5-3.9 (VSS) | -[b] | 6 | 150 | 34-36 | 0.43-0.47 | 0.001-0.02 | 96 | [99] |
| Synthetic sewage | L | 10 | - | 5 | 24 | 50 | 30 | 0.50 | 0.020 | >96 | [85] |
| Synthetic simulating municipal | L | 4 | 6-14 | 1 | 12 | - | 14-26 | 0.50 | 0.04-0.20 | 95 | [100] |
| Synthetic simulating municipal | | 5 | 5-11.24 | 1.1-1.65 | 8-12 | 30-infinite | 25-30 | 0.55 | - | 97 | [101] |

[a]L: Laboratory; P: Plot; [b]Value not reported. Modified from Skouteris et al., 2012 [96].

**Table 1.6** Examples of AnMBR performance for synthetic wastewaters (cont').

| Types of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Synthetic simulating municipal | | 3 | 4.3-5.02 | - | 3-24 | - | 35 | 0.46 | 0.027-0.047 | 95 | [102] |
| Low-strength | L | 5 (total) | 4.3-5.72 | 1.1 | 12 | 30-60 | 25-30 | 0.55 | 0.05 | 99 | [103] |
| Volatile fatty acid mixtures | L | 3.7 | 37-43 | - | - | - | 30-55 | - | - | - | [104] |
| Volatile fatty acid mixtures | L | 3.7 | 35-40 | 10-70 | - | - | 30 | 5.00-10.00 | - | - | [105] |
| Volatile fatty acid mixtures | L | 3.7 | 35-40 | 10-40 | - | - | 55 | 5.00-10.00 | - | - | [106] |
| Volatile fatty acid mixtures | L | 3.8 | 13-35 | <15 | - | - | 30-55 | 10.00 | - | - | [25] |
| Volatile fatty acid mixtures | L | 2 | 41 (Final) | 10-15 | - | - | 55 | 10.00-17.00 | - | - | [82] |

[a]L: Laboratory; P: Plot; [b]Value not reported. Modified from Skouteris et al., 2012 [96].

**Table 1.6** Examples of AnMBR performance for synthetic wastewaters (cont').

| Types of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Synthetic simulating alcohol distillery wastewater | L | 4.5 | 1.3-1.9 | 4 | 6.5 d | - | 54-56 | 4.20-5.80 | - | >84 | [107] |
| Sodium acetate/Sodium propionate-based | L | 2 | - | 4.1-6.2 | 1.8-3 | - | 35 | 0.51 | 0.03-0.11 | 99 | [108] |
| Synthetic containing formic acid | L | 10.9 | 1.03-1.81 | - | 8 | - | 31-35 | - | - | - | [109] |
| Synthetic simulating municipal | | 50 | 0.5-4 | 1 | - | - | 37 | 0.80-1.20 | - | - | [110] |
| Whey/Sucrose-based | L | 11 | 5.5-20.4 | 1.5-13 | - | 30-40 | 34-36 | - | - | - | [23] |
| Synthetic of COD of 800 mg/L | L | 25 (total) | 4-10 | 0.46-5.76 | 10.4 | Infinite | - | 0.80-2.50 | - | 85 | [111] |

[a]L: Laboratory; P: Plot; [b]Value not reported. Modified from Skouteris et al., 2012 [96].

**Table 1.6** Examples of AnMBR performance for synthetic wastewaters (cont').

| Types of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Synthetic sewage | L | 3 | - | 2 | 20 | 250 | 34-36 | 0.44-0.48 | - | 98.8 | [24] |
| Synthetic with nitrate | L | 4.8 | 2.23 | - | 2 d | 35 | - | 0.08-0.19 | - | - | [112] |
| Glucose-based | | 3 | 3.5-5.5 | - | 3-48 | - | 35 | 0.15-0.92 | 21.76-50.38 | 95 | [113] |
| Molasse-based | L | 9 | 1.6-10 (VSS) | 5-12.2 | - | - | 27-33 | 0.70-24.20 | 0.081 | - | [114] |

[a]L: Laboratory; P: Plot; [b]Value not reported. Modified from Skouteris et al., 2012 [96].

**Table 1.7** Examples of lab-scale AnMBR performance for the treatment of various industrial wastewaters.

| Type of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Specific $CH_4$ production ($m^3/kg\ COD_{removed}$) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Landfill leachate | L[a] | 29 (total) | -[b] | 0.7-4.9 | 24-168 | - | 35 | - | 5.00 | 0.42 | 95 | [79] |
| Landfill leachate | L | 3 | 7.2-10.8 (VSS) | 8-11.8 | 1.1-19 d | 30-300 | 10-35 | - | - | - | >95 (soluble COD) | [83] |
| Landfill leachate | L | 50 | <3(VSS) | 1-6.27 | 7 d | - | 37 | - | 15.00-41.00 | 0.96-4.10 | >92 | [28] |
| Thermo-chemical whitewater | L | 10 (total) | 4.9-10.7 | 2.0-2.8 | - | 280 | 36-38 | 0.35-0.41 | 0.27-3.35 | 0.30 | 90 | [26] |
| Thermo-chemical whitewater | L | 10 | 6.7-11.3 | 2.6-4.8 | - | 280 | 36-38 | 0.25-0.30 | 2.78-3.46 | 0.28-0.42 | 90 | [87] |

[a] Reported as $m^3/kg\ COD/day$ [f] Reported as biogas: $m^3/kg\ COD$ [g] Reported as $m^3CH_4/kg\ COD_{fed}$

**Table 1.7** Examples of lab-scale AnMBR performance for the treatment of various industrial wastewaters (cont').

| Type of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Specific $CH_4$ production ($m^3/kg\ COD_{removed}$) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kraft evaporator condensate | L | 10 (total) | - | 2.3-13.3 | - | - | 37-56 | 0.35±0.05 | 9.50-10.50 | 0.07-0.27 | 99 | [22] |
| Kraft evaporator condensate | L | 10 | 3.7-5.7 | - | - | - | 36-38 | - | 5.50-10.00 | 0.06-0.19 | - | [115] |
| Kraft evaporator condensate | L | 3.5 | 2.1-24 | 1-24 | - | - | 36-38 | 0.35±0.05 | 5.60-10.00 | 0.05-0.20 | 99 | [84] |
| Cheese whey | L | 20 | - | 3-19.78 | 1-4 d | - | 35-39 | 0.3 | - | - | 98.5 | [78] |
| Slaughter house | L | 50 | 10.1 | 1.59-16.32 | 30-80 | - | 37 | 0.13-0.3 | 15.88 | - | >99 | [73] |

[a] Reported as $m^3/kg$ COD/day [f] Reported as biogas: $m^3/kg$ COD [g] Reported as $m^3CH_4/kg\ COD_{fed}$

**Table 1.7** Examples of lab-scale AnMBR performance for the treatment of various industrial wastewaters (cont').

| Type of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Specific $CH_4$ production ($m^3/kg\ COD_{removed}$) | Influent COD (mg/L) | Effluent COD (mg/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Brewery wastewater | L | 4.5 | 12-25 (VSS) | 12 | - | - | 30 | - | 0.230 | 0.19 | 99 | [88] |
| Fischer Tropsch acid water | L | 23 | 30 | 25 (Max) | 31.5 | 175 | 37 | - | 19.10 | 0.61 | - | [116] |
| Dairy manure-based | P[a] | 200 | - | 2.4 (kg $VSS/m^3/d$) | 9 d | 28 | - | - | - | - | 92 | [117] |
| Swine manure | L | 5 | - | 1-2 (kg $VSS/m^3/d$) | 6 | 118-211 | - | - | - | - | >95 | [118] |
| Food processing | L | 500 (total) | 6-8 | 0.88-4.52 | 2.5 | 50 | 33-39 | 0.136[f] | 2.44-13.40 | 0.41-0.55 | 81-94 | [77] |

[a] Reported as $m^3/kg\ COD/day$ [f] Reported as biogas: $m^3/kg\ COD$ [g] Reported as $m^3CH_4/kg\ COD_{fed}$

**Table 1.7** Examples of lab-scale AnMBR performance for the treatment of various industrial wastewaters (cont').

| Type of wastewater | Scale | Working volume (L) | MLSS (g/L) | OLR ($kg/m^3/d$) | HRT (h) | SRT (d) | T (°C) | Specific $CH_4$ production ($m^3/kg\ COD_{removed}$) | Influent COD (g/L) | Effluent COD (g/L) | Maximum COD removal (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Palm oil mill | L | 50 | 50.8-56.6 | 14.2-21.7 | 2.82-3.15 | 77-161 | 35 | 0.24-0.28 | | | 91.7-94.2 | [74] |
| Palm oil mill | L | 50 | 11.8-20.8 | 1-11 | 6.8-600 | 12.1-1000 | - | 0.25-0.57[a] | 60.00-87.00 | 0.99-1.40 | 96-99 | [27] |
| Alcohol fermentation | L | 5 | 2 | 3-3.5 | 1 | infinite | 55 | - | 38.40 | - | 93-97 | [75] |

[a] Reported as $m^3/kg\ COD/day$ [f] Reported as biogas: $m^3/kg\ COD$ [g] Reported as $m^3CH_4/kg\ COD_{fed}$

### 1.2.7 Filtration performances of AnMBR

Some membrane permeability of pilot- and full-scale during AnMBRs operations are presented in Table 1.8.

In general, fluxes were reported to be higher with cross-flow membrane configurations than with submerged membrane configurations. Trans-membrane pressure (TMP) values were found to be higher when working with hollow fiber membranes than with flat sheet membranes when operating under similar biological conditions, making hollow fiber membranes more susceptible to fouling. Even though, under normal conditions, there should not be any difference in TMP values in membranes having the same surface area, material and pore size [119].

Cross-flow velocity and gas sparging area key technical performance are used to limit the fouling rate [105]. The cross flow velocity was in the range of 0.5-5 m/s. The gas sparging rate was applied about 0.2-1.5 L/min. Fakhru'l-Razi and Noor [74]reported that cross-flow velocities over 1.5 m/s would be desirable to limit solids deposition on the membrane surface. Xie et al [84] reported that the membrane critical flux of a submerged AnMBR increased and the fouling rate decreased when the biogas sparging rate was increased from 0.3 to 0.75 L/min. Similar results for submerged AnMBRs, were presented by Choo et al.[120], they have also pointed out that the resistance due to cake layer formation can be decreased by increasing the cross-flow velocity. Since, membrane fouling is the key drawback for AnMBR development, it is important to understand this problem.

**Table 1.8** Membrane performance of lab-scale AnMBRs used for the treatment of various industrial wastewaters.

| Wastewater type | Membrane type and properties | Membrane configuration | TMP (bar) | Cross-flow velocity (m/s) | Gas sparging rate (L/min) | Flux(L/m$^2$/h) | Ref. |
|---|---|---|---|---|---|---|---|
| Palm oil mill | UF (MWCO[a]: 200 kDa, 0.1 µm, tubular, 0.024 m$^2$) | Cross-flow | 1.5-2 | - | - | - | [27] |
| Thermomechanical pulping pressate | UF (MWCO: 70 kDa, flat sheet, 0.03 m$^2$) | Submerged | - | - | 1.5 | 5.7-6.9 | [121] |
| Thermomechanical pulping whitewater | MF (MWCO: 70 kDa, flat sheet, 0.03 m$^2$) | Submerged | <0.4 | - | 0.75 | 4.8-9.1 | [87] |
| Brewery with surplus yeast | MF (0.2 µm, tubular) UF (0.03 µm, tubular) | Gas-lift | - | - | 0.2-0.35[b] | 6-20 | [88] |
| Thermomechanical pulping whitewater | MF (MWCO: 70 kDa, flat sheet, 0.03m$^2$) | Submerged | <0.3 | - | 0.75 | 4.3-5.2 | [85] |
| Kraft pulp mill evaporator condensate | MF (MWCO: 70 kDa, flat sheet, 0.03 m$^2$) | Submerged | <0.3 | - | 0.4 | 5.3±1 | [115] |
| Kraft evaporator condensate | MF (MWCO: 70 kDa, flat sheet, 0.03 m$^2$) | Submerged | <0.3 | - | 0.3-0.75 | 5.6-12.5 | [84] |
| Simulated petrochemical | MF (0.45 µm, flat sheet, 0.351 m$^2$) | Submerged | 0.005 | - | - | 1.5-4.5 | [116] |

**Table 1.8** Membrane performance of lab-scale AnMBRs used for the treatment of various industrial wastewaters (cont').

| Wastewater type | Membrane type and properties | Membrane configuration | TMP (bar) | Cross-flow velocity (m/s) | Gas sparging rate (L/min) | Flux ($L/m^2/h$) | Ref. |
|---|---|---|---|---|---|---|---|
| Acidified cheese whey | MF (0.2 µm, 0.4$m^2$) | Cross-flow | 1.25-2.25 | 5 | - | 137-140 | [78] |
| Slaughterhouse | UF (MWCO: 100kDa, 1$m^2$) | Cross-flow | 1 | 3 | - | 2-8 | [73] |
| Food processing | UF (MWCO: 20-70kDa, flat sheet, 0.32$m^2$) | Cross-flow | 2 | 1.02-1.09 | - | 13.1-18.9 | [77] |
| Slaughterhouse | UF (0.06-3µm) | Cross-flow | - | - | - | 40-100 | [122] |
| Sauerkraut brine | MF (0.2 µm, 0.126 $m^2$) | Cross-flow | - | 2-3 | - | 5-10 | [76] |
| Slaughterhouse | MF (0.2 µm, 0.126 $m^2$) | Cross-flow | - | 2-3 | - | 5-10 | [76] |
| Alcohol fermentation | MF (0.14 µm,tubular, 0.0113 $m^2$) | Cross-flow | 0.6 | 3 | - | 70-85 | [75] |
| Palm oil mill | UF (MWCO: 200 kDa) | Cross-flow | 1.5 | 2.3 | | 26.4-30.3 | [74] |
| Alcohol-distillery | UF (MWCO: 20 kDa, flat sheet,0.0168 $m^2$) | Cross-flow | 0.5-3 | 0.5-1.5 | - | 10-40 | [123] |
| Landfill leachate | UF (0.1µm, hollow fiber, 0.46 $m^2$) | Cross-flow | - | 2 | - | ~9 | [79] |

**Table 1.8** Membrane performance of lab-scale AnMBRs used for the treatment of various industrial wastewaters (cont').

| Wastewater type | Membrane type and properties | Membrane configuration | TMP (bar) | Cross-flow velocity (m/s) | Gas sparging rate (L/min) | Flux ($L/m^2/h$) | Ref. |
|---|---|---|---|---|---|---|---|
| Synthetic | UF (0.4μm, hollow fiber, 0.05 $m^2$) | - | - | - | - | 24 (Initial) | [111] |
| domestic wastewaters | UF (0.1μm, hollow fiber, 0.091 $m^2$) | - | 0.1–0.35 | - | - | 7–10 | [108] |

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### 1.2.8 Membrane Fouling

#### 1.2.8.1 Mechanisms and Fouling Types

The main drawback of the successful application of AnMBRs for wastewater treatment is membrane fouling and associated cost-efficient operation. Membrane fouling is characterized as a reduction of permeate flux (and/or TMP increase) as a result of membrane permeability decline due to the retention on/in the membrane material of a lot of compounds according to the membrane cut-off. Such phenomenon obliges to practice membrane regeneration methods. The nature and intensity of membrane cleaning depends on the origin and intensity of membrane fouling, such cleaning plays an important role on membrane lifetime and maintenance costs [124].

So far, extensive research to understand, quantify and model membrane fouling were undertaken and have unveiled the main fouling factors as follows:

- Cake Formation

Cake formation on the membrane surface (external fouling) generated by complementary mechanisms as the adsorption of soluble organic and biopolymers, deposit of particles, attachment of microbial cells and possible biofilm development, colloids and deposition of inorganic precipitates [81].

Liao et al [125]stated that in comparison to conventional (MBR) operations, the high concentrations of MLSS in AnMBRs increase cake deposition dynamics. According to Rosenberger et al [126], the relationship between MLSS and flux is complex. An increase in MLSS at low MLSS levels (< 6g/L) resulted in reduced fouling while an increase beyond a critical MLSS level (15 g/L) exacerbated fouling.

Lee et al [127] and Wang et al [128] mentioned that membrane fouling was mainly caused by the sludge cake layer formed on membrane surfaces which includes sludge particles and biopolymers such as proteins, polysaccharides and humic substances.

Jeison et al [105] reported that biomass concentration showed to be an important factor determining cake formation in mesophilic MBRs. Under mesophilic conditions, biomass concentration affects linearly critical flux. An increase from 25 to 50 g TSS/L reduces critical flux from 21 $L/m^2 \cdot h$ to 9 $L/m^2 \cdot h$.

Lin et al [22] performed a study on a lab-scale anaerobic submerged membrane bioreactor (AnMBR) for 3.5 months with kraft evaporator condensate. They observed that the cake layer formed in the thermophilic SAnMBR contained higher levels of both organic and inorganic foulants, smaller particle sizes, and especially, a denser and more

compact sludge cake structure. These results indicate that floc size, SMP, BPC, bound EPS as well as cake layer structures are the major factors governing membrane fouling in SAnMBR systems.

Gao et al [26] reported that the particle size of the cake layers decreased with an increase in operating temperature and increased with an increase of cake age, while the quantity of bound EPS in the cake layer decreased with an increase in operating temperature and cake age.

Zhang et al [129] have explained cake formation mechanism in an anaerobic membrane bioreactor at a high flux of 65 L/($m^2$ h). The dynamic membrane was formed by the sludge particles and the solutes and colloids content such as SMP and EPS. The compounds accumulation could be divided into three stages of formation: at the first stage, SS, SMP, EPS had a rapid accumulation on the membrane surface. At the second stage, the cake layer on the membrane surface showed a stable growing rate, with little change of SS/cake volume. At the third stage, the growth of SS surpassed that of thickness, indicating the compaction of cake layer.

- Decrease of Internal Membrane Porosity

Decrease of internal membrane porosity as a result of membrane pore clogging by large soluble compounds and adsorption of small molecules onto the internal surface of pore channels, corresponds mainly to the adsorption of extracellular polymeric substances EPSs and soluble microbial products SMPs which are mostly by-products of microbial activity, particularly SMP often cited as the primary internal foulants [24, 87].

Meng et al [130] and Liang et al [131] have reported that soluble biopolymers had had a considerable influence on membrane fouling. The studies showed a significant effect of soluble carbohydrates on flux however they have shown no significant relationship between soluble protein and flux.

Huang et al [132] observed an increase in the fouling propensity of sludge with increase of the ratio “soluble carbohydrate/protein”. Conversely, Lin et al [22] observed an increase in fouling propensity of sludge with a decrease of the bound carbohydrate to protein ratios respectively.

Gao et al [26] reported that the bound extracellular polymeric substances (EPS) in bulk sludge, soluble microbial products (SMP), and colloidal particles content increased with an increase in operating temperature of SAnMBR. Temperature shocks had modest impact on bound EPS and SMP in bulk sludge.

Kang et al [75] and An et al [81] showed that inorganic materials in solution could be responsible for irreversible membrane fouling by precipitating within the membrane pores as well as accumulating on the membranes surface. In addition, floc-associated and solution cations have been shown to play a role in consolidation of biomass cakes and further enhancement of the compactness of the fouling layer. This may be caused by charge neutralization of functional ionizable anionic groups such as carboxylic and phosphate groups, deposits of metal salts and/or bridging between deposited biopolymers on the membrane surface.

Zhang et al [118] calculated the saturation index to determine the potential for the precipitation of inorganic salts during the AnMBR treatment of swine manure. The results identified struvite, hydroxyapatite ($Ca_{10}(PO_4)_6)(OH)_2$), dolomite ($CaMg(CO_3)_2$) and Calcite ($CaCO_3$) as the major contributors of inorganic precipitates within a digester and on the membrane surface.

Therefore, the control of membrane fouling during operations remains the main challenge in AnMBRs. Due to filtration, a lot of compounds present in suspension are retained by the membrane barrier and interact with the membrane material. The membrane permeability is drastically modified, obliging the use of energy to maintain it at a sustainable level (gas bubbling, backwashing, chemical consumption), and the intensity and the frequency of membrane cleaning can significantly shorten the membrane lifetime [88].

Several physical cleaning methods such as air bubbling, backwashing, and sponge scrubbing for membrane fouling control in the aerobic submerged membrane bioreactor (SMBR) [24, 81, 85] have been developed for the control of membrane fouling. However for AnMBR, this strategy cannot directly be applied and it still has some limited due to the several differences between anaerobic and aerobic biomass such as the high mixed liquor suspended solid (MLSS) concentration in the reactor, the size reduction of the biomass and the size distribution of bio-solid particles [115]. These biomass characteristics affect from the mechanical sheer stress, the operating conditions and the inorganic precipitate generated during anaerobic digestion, which was making different mechanism of membrane fouling [115]. There is a need more investigate and apply technique influencing on membrane fouling in AnMBR.

#### 1.2.8.2 Membrane Fouling Mitigation in AnMBR

The immediate effect of fouling is to cause a reduction in the permeate flux or an increase of TMP. The long-term effect may lead to irreversible fouling from biological organic and inorganic foulants and the reduction of the membrane lifetime, because of the necessity to practice severe chemical cleaning. To maintain the economic viability of a membrane process, membrane fouling has to be kept to a minimum. Different strategies and methods have been evaluated to minimize fouling in AnMBR, they include:

- Modifying the Membrane Properties

The membrane properties were modified to increase back transport of foulants away from the membrane surface into the bulk solution [120, 133]. Stuckey [134] reported that modification of the surface characteristics of hydrophobic membranes by coating or grafting is one of the means to obtain hydrophilicity. Then, Bailey et al [133] smoothed the membrane surface with a precoat layer of diatomaceous earth powder that reduced anaerobic bacteria accumulation. Choo et al [120] reported that modifying the hydrophobic membrane surface to become hydrophilic by graft with 2-hydroxyethyl methacrylate (HEMA) led to a 35% flux increase. Sainbayar et al [135] reported that 13.5% of flux enhancement with a modified surface of polypropylene have 70% degree of grafting with HEMA.

- Pretreatment of the Feed Solution

The feed solution was pretreated by the absorbents, such as the addition of powdered or granular activated carbon. The PAC can absorb and coagulate dissolved organics and fine colloids. In addition PAC has a higher scouring effect and lower specific cake resistance[120]. Choo et al.[120]confirmed that addition of powdered activated carbon (PAC) to the reactor contributed to the reduction of a polymeric membrane fouling caused by organic adsorption and fine colloid deposition by sorbing and/ or coagulating dissolved and colloidal matter present in the bioreactor. It was the same results for Akram and Stuckey (2008) who showed that the addition of PAC increased the flux of flat sheet submerged anaerobic membrane bioreactor treating synthetic wastewater (4 g COD/L) from 4 to 9 LMH.

- Increasing of cross flow velocity

Cross flow velocity may be reduced to external particle deposition on the membrane surface. Padmasiri et al [136] studied long-term methanogenic population dynamics and performance of an AnMBR treating swine manure at high cross-flow

velocities (0.9–2 m/s). They concluded that sudden changes in shear rate can have a negative effect on biomass activity but they noticed an improvement of membrane performance due to a decreased of cake layer resistance. This was confirmed by Choo et al.[120]and Akram and Stuckey [137]who reported that cross-flow velocities over 1.5 m/s would be desirable to limit solid deposition on the membrane surface. However, this situation contrasts the negative effect of shear rate on anaerobic biomass activity and particle size as reported by many researchers.

- Working in Subcritical Flux Conditions

Operations below the critical flux are expected to have little or even no effect for causing external fouling [20]. By operating two SAnMBRs, they presented a new operation strategy based on a continuous critical flux determination; hence, avoiding excessive cake-layer accumulation on the membrane surface. But observations have proven that fouling takes place even below the critical flux [138].

- Gas Sparging Injections

Gas sparging injections are the most common ways to provide shear stresses over the membrane surface in order to disrupt the formation of the cake layer or to restrict their interaction with the membrane [24, 84]. These authors reported that membrane fouling could be promoted when gas sparging was turned from a continuous mode into an intermittent one. They concluded that reduction of continuous biogas sparging to intervals of 10 min on and 5 min off resulted in a slight increase in the TMP values by 0.025 bar during their experiments. Xie et al [84] reported that the membrane critical flux of a submerged AnMBR increased and the fouling rate decreased when the biogas sparging rate was increased from 0.3 to 0.75 L/min. Moreover, the efficiency of this technique should depend on the biogas sparging rate applied. Similar results, also for submerged AnMBRs, were presented by Jeison and van Lier [20].

- Membrane Relaxation

Periods of relaxation (interruption of the permeation cycle to allow deposits to relax) have been used as a way of controlling fouling. Vallero et al [19] reported that the working without any relaxation resulted in an immediate increase of fouling rate (137 mbar/d), in opposite the introduction of relaxation resulted of fouling rate limited to about 18.5 mbar/d. The relaxation of the membranes was shown to slow the fouling in the membranes. Hulse et al.[139]defined an optimal cycle of filtration composed by 9 minutes of permeation followed by 1 minute of relaxation (flat sheet AnMBR treating potato

wastewater). Similar results were obtained by Lin et al.[87], for submerged AnMBRs, with a cycle of 4 min filtration/1 min relaxation, a gas sparging 0.75 l/min was practiced to intensify the solid detachment from the membrane surface.

- Backwashing

Backwashing is pumped of permeate in the reverse direction through the membrane to remove the upper layer or irreversible foulants, and to maintain a given flux productivity and higher instantaneous fluxes. In the previous studies for optimization of backwashing parameters, optimal intervals and durations of backwashing for fouling mitigation have been investigated. Wu et al.[30]investigated to identify the effect of backwashing parameters including backwashing strength for the same net permeate productivity. It found that the low backwashing flux of 30 L/($m^2$· h) featured the lowest resistance after 24 h.

### 1.2.9 Biological Modeling of SAnMBR

The two-stage SAnMBR has two main biochemical stages: the acidogenesis and methanogenesis stages. Since acidogenic and methanogenic organisms require different kinetic parameters and optimum pH for growth, two reactors were used to create suitable environment for each group of organisms [140]. In order to describe the effects of operating variables on such system performances, it is important to choose a dynamic model tool to calibrate the kinetics parameters and optimize the system design. There are only a few studies available on the modelling of two-stage SAnMBRs.

Several static and dynamic models describing anaerobic digestion processes were developed during the last three decades [141]. Early models were very simple and considered organic matter as a simple substrate and did not take into account the complex composition of the feedstock [142, 143]. After that a development of models for anaerobic digestion processes considered complex feed compositions (carbohydrate, protein, volatile fatty acids (VFA) and other organics) yielding more accurate results [144, 145]. Nowadays, the increasing knowledge on anaerobic digestion and the interactions of the multiple functional species involved require more complex models to simulate the impact of the changing environmental conditions on complex biological treatment systems. The latest developed model is the International Water Association (IWA) Anaerobic Digestion Model No. 1 (ADM1), published in 2002[140].

Anaerobic Digestion Model No. 1 (ADM1) was developed by the IWA Task Group for Mathematical Modeling on Anaerobic Digestion. It consisted of a number of processes

to simulate all possible reactions occurring in anaerobic sludge including not only biological reactions but also physicochemical reactions [146]. The extended applications of ADM1 model as a basic model concept for further development for dynamic simulation of different anaerobic reactor may be applied with different forms of Equations. An overview of adaptations of ADM1 and their field of application are presented in Table 1.9 and Table 1.10 that give comparisons of kinetic parameters for different wastewater and different anaerobic processes as following;

1. Single-stage anaerobic process: co-substrate anaerobic digestion process of olive mill wastewater (OMW) with olive mill solid waste (OMSW) in semi-continuous tubular digester [49]; dog food and flour in anaerobic sequencing batch reactor (ASBR) [147]; opium alkaloid effluent in lab-scale upflow anaerobic sludge bed reactor (UASBR) [119]; municipal solid wastes (OFMSW) in continuous stirred-tank reactor (CSTR) and upflow sludge blanket (UASB)[148].
2. Two-stage anaerobic process: traditional Chinese medicine (TCM) wastewater [149]; olive pulp [150, 151] and grass silage [152]; acidified sorghum extract generated from a hydrogen producing bioreactor in a two-stage CSTR [153].
3. Hybrid anaerobic reactor: wastewater coming from wine residue after distillation in hybrid upflow anaerobic sludge filter bed (UASFB) [154].

**Table 1.9** Modifications and applications of ADM1.

| Substrate | Reactor | Parameter measurements | | | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | SCOD | TCOD | TVFA | Acetic Acid | Propionic acid | Butyric acid | Valeric acid | |
| municipal sewage sludge | two-stage mesophilic /thermophilic (35/55 °C) | - | - | - | - | 0.15-3 | 0.2-1 | - | - | [155] |
| sludge | upflow anaerobic sludge bed (UASB) | 7-7.2 | - | - | - | 0.1-0.2 | - | - | - | [156] |
| Olive mill wastewater and solid waste | Thermopilic anaerobic co-digestion | 7-7.5 | - | - | 0.5-1 | - | - | - | - | [157] |
| Olive mill wastewater and solid waste | Thermopilic anaerobic co-digestion | 7-7.5 | - | - | 0.5-1 | - | - | - | - | [158] |
| municipal solid wastes | anaerobic co-digestion | 7.2-7.7 | 2.5-3 | 18-20 | 0.012-0.030 | - | - | - | - | [159] |

Acetic, Propionic, Butrylic and Valeric Acid unit (kg COD/$m^3$/d); biogas production ($m^3$/d)

**Table 1.9** Modifications and applications of ADM1 (cont').

| Substrate | Reactor | Parameter measurements | | | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | SCOD | TCOD | TVFA | Acetic Acid | Propionic acid | Butyric acid | Valeric acid | |
| traditional Chinese medicine | CSTR and UASB | 4.8-5.2 6.5-7 | - | 1.4-1.7 | - | 0.2-0.8 | 0.06-0.17 | 0.07-0.34 | 0.15 | [149] |
| Activated sludge | Anaerobic batch reactors | 7.2-7.5 | - | - | - | 0.2-0.4 | 0.7-0.9 | 0.05-0.2 | 0.05-0.2 | [154] |
| Evaporator condensate (EC) | CSTR | 7.2-7.5 | 2-8 | - | - | - | 0.5-3 | - | - | [160] |
| co-substrate composed of dog food and flour | anaerobic sequencing batch reactor (ASBR) | - | - | - | - | 0.5-1 | - | - | - | [147] |
| grass silage | - | - | - | - | - | 0.05-3 | - | 0.1-2 | - | [161] |
| Opium alkaloid | UASB | 8.1-8.5 | - | 0.5-3.5 | - | - | - | - | - | [162] |

Acetic, Propionic, Butrylic and Valeric Acid unit (kg COD/$m^3$/d); biogas production ($m^3$/d)

**Table 1.9** Modifications and applications of ADM1 (cont').

| Substrate | Reactor | Parameter measurements | | | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | SCOD | TCOD | TVFA | Acetic Acid | Propionic acid | Butyric acid | Valeric acid | |
| grass silage | 2-stage CSTR | 7.5-7.8 | - | - | 0.25-0.35 | 0.1-0.15 | - | - | - | [152] |
| Chlorella vulgaris | Anaerobic digestion | 7-7.5 | 0.5-1 | 6-8 | 0.1-0.3 | 0.1-0.5 | 0.1-0.2 | <0.1 | <0.1 | [163] |
| municipal solid wastes | two-stage anaerobic digester | 7.5-7.8 | - | - | 2-6 | 0.2-1.4 | 1.5-3 | 0.2-1.8 | 0.05-1.4 | [148] |
| waste sludge | Continuous digesters | - | - | 28-33 | - | - | - | - | - | [164] |
| wine and pig manure | hybrid UASB–AF | 7-8 | 2-6 | 5-10 | - | 0.01-0.06 | - | - | - | [165] |
| grass silage | 2-stage CSTR | 7.5-7.8 | - | - | 0.25-0.35 | 0.1-0.15 | - | - | - | [152] |
| Chlorella vulgaris | Anaerobic digestion | 7-7.5 | 0.5-1 | 6-8 | 0.1-0.3 | 0.1-0.5 | 0.1-0.2 | <0.1 | <0.1 | [163] |

Acetic, Propionic, Butrylic and Valeric Acid unit (kg COD/$m^3$/d); biogas production ($m^3$/d)

**Table 1.10.** Comparison of kinetic parameters at mesophilic conditions in the anaerobic system.

| Kinetic parameters | Name | Unit | Calibrated values | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Bastone et al. [140] | Siegrist et al. [122] | Blumensaat and Keller [150] | Lee et al. [147] | Dereli et al. [162] | Lubken et al. [166] | Yu et al. [148] | Souza et al. [164] |
| $K_{dis}$ | Disintegration constant | $d^{-1}$ | - | - | 0.5/1 | 0.9/0.4 | - | 0.05/0.02 | 0.5 | 0.24 |
| $K_{hyd\_CH}$ | Maximum specific hydrolysis rate of carbohydrates | $d^{-1}$ | - | - | - | - | - | - | 10 | 2.38 |
| $K_{hyd\_PR}$ | Maximum specific hydrolysis rate of protein | $d^{-1}$ | - | - | - | - | - | - | 10 | 4.42 |
| $K_{hyd\_LI}$ | Maximum specific hydrolysis rate of lipid | $d^{-1}$ | - | | | | | - | 10 | 1.49 |
| $k_{m,su}$ | Maximum uptake rate for sugar utilizers | COD/ COD/d | 30 | - | - | - | 30/20 | - | 30 | - |
| $k_{m,aa}$ | Maximum uptake rate for amino acid utilizers | COD/ COD/d | 50 | - | - | - | 50/40 | - | 50 | - |

**Table 1.10** Comparison of kinetic parameters at mesophilic conditions in the anaerobic system (cont').

| Kinetic parameters | Name | Unit | Calibrated values | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Bastone et al. [140] | Siegrist et al. [122] | Blumensaat and Keller [150] | Lee et al. [147] | Dereli et al. [162] | Lubken et al. [166] | Yu et al. [148] | Souza et al. [164] |
| Kdec_su | Decay rate for sugar | $d^{-1}$ | 0.02 | - | - | - | - | - | 0.02 | - |
| Kdec_aa | Decay rate for amino acid | $d^{-1}$ | 0.02 | - | - | - | - | - | 0.02 | - |
| $k_{dec}$ | Decay rate for biomass death | $d^{-1}$ | | - | - | 0.03 | 0.02/0.05 | - | | |
| $k_{m,fa}$ | Maximum uptake rate for fatty acid utilizers | COD/COD/d | 6 | - | - | - | 6/4 | - | 20 | - |
| $k_{m,c4}$ | Maximum uptake rate for valerate and butyrate utilizers | COD/COD/d | 20 | - | - | - | 20/13 | - | 13 | |
| $k_{m,pro}$ | Maximum uptake rate for propionate utilizers | COD/COD/d | | - | 16/9 | 45/12.5 | | - | 0.4 | - |

**Table 1.10** Comparison of kinetic parameters at mesophilic conditions in the anaerobic system (cont').

| Kinetic parameters | Name | Unit | Calibrated values | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Bastone et al. [140] | Siegrist et al. [122] | Blumensaat and Keller [150] | Lee et al. [147] | Dereli et al. [162] | Lubken et al. [166] | Yu et al. [148] | Souza et al. [164] |
| $k_{m,ac}$ | Maximum uptake rate for acetate utilizers | COD/ COD/ d | | - | 25/9 | 14/6.5 | 8/4 | - | 8 | - |
| $k_{m,h2}$ | Maximum uptake rate for hydrogen utilizers | COD/ COD/ d | 35 | - | - | - | 35/25 | - | 35 | - |
| $Y_{ac}$ | acetate acid degraders yield | $d^{-1}$ | 0.05 | - | - | - | - | - | 0.05 | - |
| $Y_{h2}$ | hydrogen degraders yield | $d^{-1}$ | 0.06 | - | - | - | - | - | 0.06 | - |
| $K_{dec\_ac}$ | Decay rate for acetate acid | $d^{-1}$ | 0.02 | - | - | - | - | - | 0.02 | - |
| $K_{dec\_h2}$ | Decay rate for hydrogen | $d^{-1}$ | 0.02 | - | - | - | - | - | 0.02 | - |

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## 1.3 Research Objectives

AnMBR presents a great interest to treat concentrated wastewater coming from agro-industry, because of its biological performances due to a total retention of biomass in the bioreactor. Its application for the treatment of complex POME may represent a great challenge to minimize environmental impact of such waste by recovering high quality of treated water and energy through biogas production.

Nevertheless, the main bottleneck of such system is the control of membrane fouling dynamics during operation mainly linked to the biological suspension characteristics (suspended solid concentration TSS and soluble exopolymeric substances EPS) and the filtration conditions (filtration cycle, permeate flux, trans-membrane pressure, temperature and local shear stresses notably) chosen for a defined membrane module configuration.

Literature has shown that the solid retention time (SRT) may have a great influence on TSS and EPS concentrations directly at the origin of fouling intensity in defined filtration conditions.

The main objectives of this study was to confirm the interest of SAnMBR to treat POMEs consisting of three main studies: (1) the role of SRT on biological performance, (2) identification of membrane fouling origin, and (3) interest of ADM to simulate AnMBR performance. To fullfill such an objective, the sub-objectives were the defined as follows:

1. Selection of the highest active inoculum and determination by SMA and BMP assays of concentration of organics in POME that can be anaerobically converted to $CH_4$
2. Investigation of the influence of SRTs on treatment performances of two-stage SAnMBR for the treatment of POMEs.
3. Simulation of the dynamic anaerobic biodegradability behaviour and the methane potential of a two-stage SAnMBR treatment process treating POMEs at different SRTs using the modified ADM1 model.
4. Quantification of the membrane fouling dynamics and identification of the main fouling mechanisms (reversible and irreversible mechanisms) using TMP

evolution measurement and calculation of the corresponding hydraulic resistance (Darcy's law).

5. Investigation of the influence of intermittent filtration frequency on filtration step control
6. Analyses of the properties of foulants on membrane surface and identification of the major factors governing membrane fouling at different SRTs using a variety of analysis techniques, including biomass concentration, SMP, EPS, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), atomic force microscopy (AFM), Fourier transform infrared (FTIR) spectroscopy and particle size analyzer.
7. Understanding and evaluation of the filtration characteristic and fouling behavior of a SAnMBR system at different SRTs using the in-series resistance model that was supported by direct (experimental) measurements of the intrinsic membrane resistance, cake resistance and fouling resistance.

## 1.4 Scopes of Research Work

To accomplish the objective of experiments were carried out in a stepwise. First, the investigation of biochemical methane potential (BMP) and specific methanogenic activity (SMA) in palm oil mill effluent (POME): an effect of organic fraction and concentration (Chapter 4) was to investigate and select the highest active inoculum on methane production from three industries by specific methanogenic activity (SMA). After that the best anaerobic seed sludge from SMA assay was applied to evaluate methane production potential from filtrated and raw POME used by BMP assay.

After determination of the best anaerobic seed sludge activity and optimum POME concentration, Chapter 5 was focused on (i) the influence of intermittent filtration frequency on filtration step control and (ii) to quantify the main origin of membrane fouling. (Chapter 6 and 7), the hypothesis tested in this study was that two-stage SAnMBRs operated under several SRTs would result in different removal efficiency and sludge properties and thus might lead to different membrane fouling behaviors (Chapter

8), the modified ADM 1 model predicted to explain the efficiency treatment and methane gas production of data obtained from laboratory-scale during the start-up and SAnMBR reactors treating POMEs.

# CHAPTER 2
# THEORIES

The theories presented in this study are divided into five sections. In the first section, a general review of palm oil characteristic and extraction processing is given. This is followed by a summary of fundamental anaerobic wastewater treatment, biochemical methane potential methods and anaerobic membrane bioreactors. The last section discusses the filtration and anaerobic digestion model (ADM1) to be applied in the experiment.

## 2.1 Palm Oil

Palm oil is extracted from the fresh fruit of the oil palm. The palm fruit is about the size of a large plum and grows in large bunches. Each bunch of fruit weighing 40-50 kilograms can have up to 2000 individual fruits. Figure 2.1 shows that each fruit consists of a hard kernel (seed) inside a shell (endocarp), which is surrounded by a fleshy mesocarp. Oil is extracted from both the pulp and palm of the fruit, which is used for edible purposes while the palm kernel is used for non-edible purposes such as making soaps, cosmetics and detergents. Crude palm oil (CPO) is the primary product derived from the red fruits of the oil palm, while palm kernel oil (PKO) derived from the fruit's nut is considered to be a secondary product [167].


**Figure 2.1** The fruit flesh of the oil palm [168].

### 2.1.1 Palm Oil Extraction Processing

In general, the palm oil milling process can be classified into a dry and a wet (standard) process. The wet process of palm oil milling is the major way of extracting palm oil in Thailand, which is suitable for large-scale productions and produces better quality palm oil [169]. The processing of in the oil palm mill involves four major unit operations: (1) sterilization, (2) threshing and stripping of fruits, (3) digestion, (4) oil extraction. Figure 2.2 presents a typical process flow diagram for the extraction of crude palm oil.

Stage 1: Fresh Fruit Bunches (FFB) are sterilized by saturated steam under a pressure of $3x10^5$ Pa for 40-60 min at 120-130 $^oC$. The sterilization process breaks down the rapid formation of free fatty acids during the pulping process. In addition, this process helps loosen the fruits from their bunches so that the oil can be extracted easily. Sterilizer condensate generates POME, which is about 0.9 tons for each produced ton of crude palm oil [169].

Stage 2: The sterilized fresh fruit bunches (FFB) are then fed continuously into a rotary drum machine in order to strip and separate the fruits from the bunch. The fruits then pass along channel bars running longitudinally along the drum, while the empty bunches are eventually discharged at the end of the drum for incineration [169].

Stage 3: After stripping, the fruits are fed continuously into a digester in a heated vessel at about 80-90 $^oC$, which converts the fruits into a homogeneous oil mash suitable for pressing.

Stage 4: A homogeneous oil mash from the digesters is pushed through a screw press, and the oil is thus separated from the spent mesocarp and the nuts. During the oil extraction phase, the digested mashes are pressed under pressure, either hydraulically or mechanically to extract CPO. The CPO extracted from pressing still has impurities, including water, vegetable matter and total solids. The contaminants of CPO are removed by settling and centrifuging. The sludge from settling and centrifuging is moved to a sludge separator or centrifuge which amounts to approximately 1.5 tons of sludge waste obtained per ton of crude palm oil produced. Finally, after separation of the fiber from the nuts, the palm kernel is still had the empty shells. This processing section separates kernels by hydrocyclone. For this processing approximately 0.1 tons of liquid effluent per ton of produced crude palm oil is generated [169].

According to Yacob et al [7], palm oil mills discharge about 2.5 $m^3$ POME/t CPO produced or 0.5 $m^3$ POME/t FFB (fresh fruit bunches) processed. Sumanthi et al [170] found that the average amount of POME produced in palm oil mills in Indonesia is 3.5$m^3$ POME/tCPO or 0.7$m^3$POME/t FFB, and Basri et al [171] stated that approximately 1.5 $m^3$ POME/t FFB is typically produced by palm oil processing.

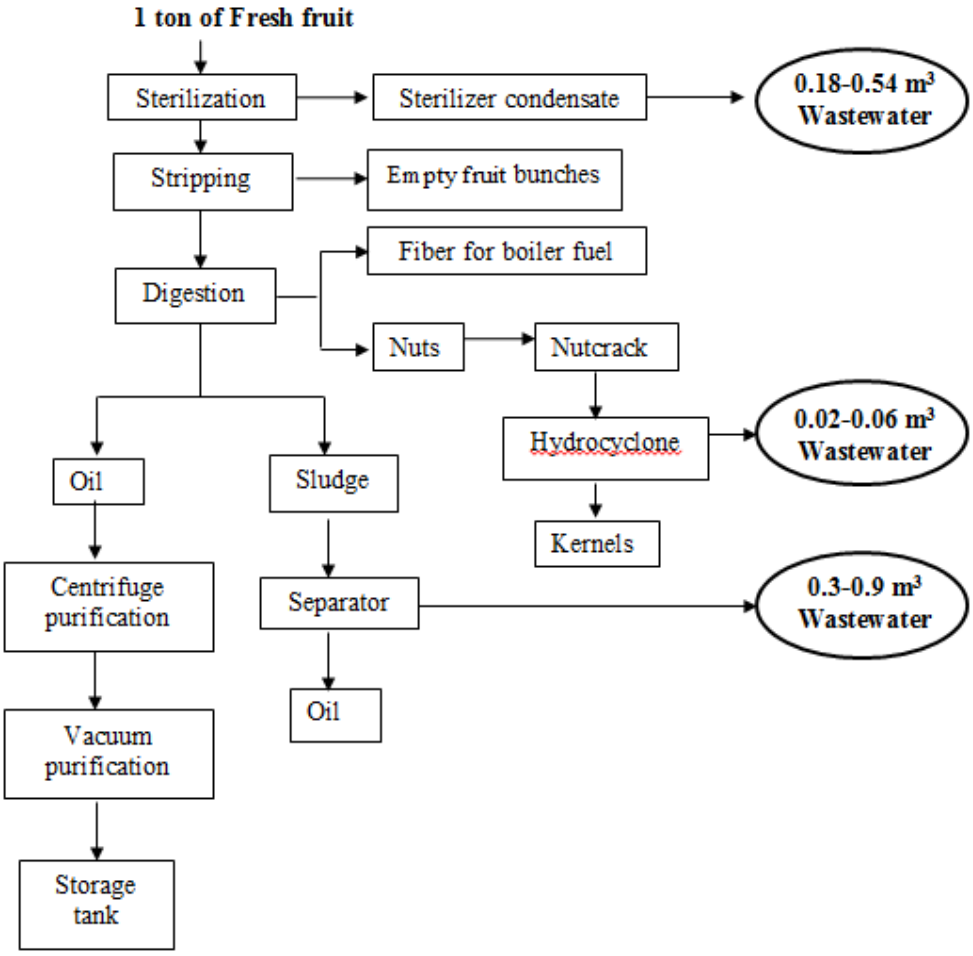



**Figure 2.2** The palm oil extraction process[5].

## 2.2Anaerobic Wastewater Treatment Process

### 2.2.1 Fundamentals of Anaerobic Wastewater Treatment

The anaerobic degradation process can be separated into two main stages in Figure 2.3. The first is acid formation, and the second, methane formation. The acid formation consists of three steps: hydrolysis, acidogenesis and acetogenesis. In the hydrolysis step

large organic molecules such as proteins, poly-saccharides and fats are degraded into small and soluble components (sugars, amino-acids, fatty acids) by excreted enzymes of fermentative bacteria. After that, soluble organic components (products of hydrolysis) are converted into volatile organic acids, alcohols, aldehydes, formate, acetate, carbon dioxide, etc, by the action of acid forming or acid fermentative bacteria (acidogens in the acidification step). The products of acidogenesis (fatty acids) are converted into aceticacid, formate, $H_2$ and $CO_2$ by the action of obligate hydrogen producing acetogenic bacteria, which are considered as acetogens in the acetogenesis step. All intermediate products produced from the previous stages are converted mainly into methane and carbon dioxide by the action of methanogens or methanogenic bacteria (obligate anaerobes).

In the final phase of anaerobic decomposition, the products of the first three phases: acetic acid, $H_2$ and $CO_2$, formic acid and methanol are converted into methane and $CO_2$. In this phase, the actual COD removal takes place. During all phases, a part of the organic matter is also transformed into new biomass.

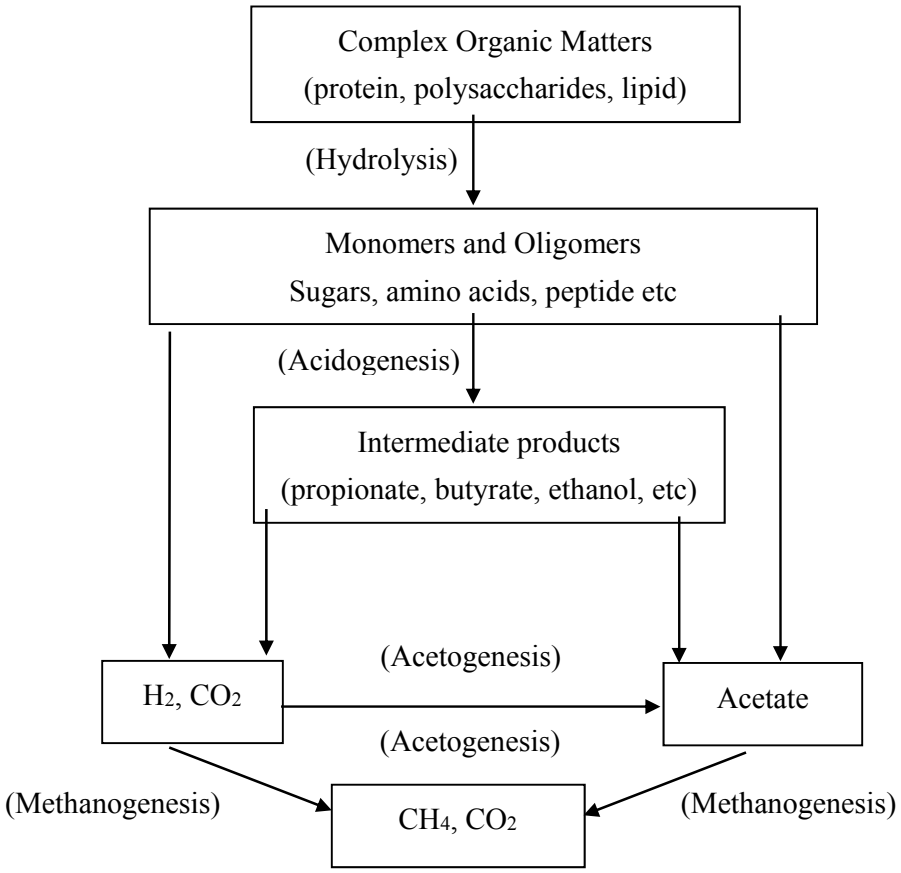



**Figure 2.3** Anaerobic degradation process [172].

### 2.2.2 Types of Anaerobic Wastewater Treatment

In anaerobic digestion, acidogenic and methanogenic microorganisms have different optimal growth conditions with different physiologies, growth kinetics, nutrient requirements and sensitivity levels to environmental changes [173]. Acidogenics grow relatively faster, and are less sensitive to pH variation than methanogenics. This usually results in the accumulation of organic acids and the lowering of pH, which leads to the inhibition of methanogenic activities when producing high VFA concentration in the bulk. This might lead to a reactor failure [174]. Therefore, the acidogens and methanogens have different physical, biochemical and environmental requirements [175]. Another alternative for anaerobic treatment of wastewater could be the two-stage system.

The bioreactors used in anaerobic wastewater treatment can then be configured according to different levels and complexity. When no separation step is present

downstream of the reactor (by settling notably) or in the reactor (fixed culture in fixed or fluidized bed), HRT is equal to SRT and the systems are relatively extensive. When a separation step exists to differentiate HRT from SRT, the reactor can be more intensive.

- Single-Stage Digestion System

A single-stage digestion system is one of the biological steps occurring within a single sealed reactor or holding tank. Utilizing a single stage reduces construction costs, but offers less control of the reactions occurring within the system. Acidogenic bacteria, through the production of acids, reduce the pH of the tank. Methanogenic bacteria, as outlined earlier, grow in a strictly defined pH range. Therefore the biological reactions of the different species in a single stage reactor can be in direct competition with each other and the reactor design must be made according to the slowest processes. Another one-stage reaction system is an anaerobic lagoon. These lagoons are pond-like earthen basins used for the treatment and long-term storage of manures. Here the anaerobic reactions are contained within the natural anaerobic sludge in the pool.

- Two Stage or Multi-Stage Digestion System

The different digestion vessels are optimized to bring maximum control over the bacterial communities living within the digesters. In the first stage, specific bacteria hydrolyze the particulate organic matter in sugars, fatty acids and amino acids by extracellular enzymes (generally the hydrolysis of particulate organic matter is the slowest step). These relatively simple compounds are then fermented to form short-chain fatty acids, alcohols, carbon dioxide and hydrogen, which are subsequently converted to $CH_4$ and $CO_2$ by acetogenic and methanogenic microorganisms in the second stage where the methanogenic reaction is generally the slowest step.

The acidogenic reactor has the advantage of protecting the sensitive methanogens from VFA resulting in a drastic fall in pH. The variations in influent characteristics, such as pH shock, rapid increasing of organic loading and shorter hydraulic retention time, are all factors that are favorable to establishing the acidogenic phase. The establishment of the methanogens process can be smaller and more cost efficient [39]. In addition, the acidogenic reactor prevents particulate suspended solids, which blocks granulation and inhibits the production of methane [176].

The different growth rates and optimal pH for the acidogenic bacteria is grown in the acidogenic reactor where the pH is naturally low (between 5.5 and 6.5). The second group, the methanogenic bacteria, is grown in the methanogenic reactor where the pH is natural (around pH 7), thus different requirements regarding reactor conditions have led to the development of two-stage anaerobic digestion processes [173].

Two-stage systems for the anaerobic degradation of organic waste have been shown to have several advantages over conventional processes:

1. They permit the selection and enrichment of different microorganisms in each digester.
2. They increase the stability of the process by controlling the acidification stage and preventing overloading and the formation of toxic materials.
3. The first stage may act as a metabolic buffer preventing pH shock to the methanogenic population [39].

### 2.2.3 Important factors in anaerobic treatment

#### 2.2.3.1 pH and Alkalinity

The pH is a significant factor for the enzymatic activities or digester's performance. Alkalinity acts as a buffer that prevents rapid change in pH. The optimal pH microbial community of anaerobic treatment requires different ranges for growth. Acceptable pH for acid-producing bacteria is above 5, while acceptable pH for methane-producing bacteria should not go below 6.2. The optimal pH is between 6.8-7.2 in which anaerobe bacteria consuming volatile acid are converted to methane and carbon dioxide. The pH values out of the 6.3-8 range are toxic to methane-producing bacteria. However, the digesting sludge has a tendency to become acidic and decrease alkalinity if methane-producing bacteria is inhibited. This results in (1) excessive accumulation of organic acids or volatile acids in excess of 1,800-2,000 mg/L (2) composition and concentration of influent.

The pH value can be neutralized by the addition of alkali. Sodium bicarbonate and potassium bicarbonate are perhaps the best choices of chemicals, because of their desirable solubility, handling, and minimal adverse impact within the digester [54, 177].

#### 2.2.3.2 Temperature

Temperature has generally been classified into three ranges, psychrophilic (5-25$^{o}$C), mesophilic (25-38$^{o}$C), and thermophilic (50-70$^{o}$C). The rate of anaerobic digestion and gas production, the most methane-forming bacteria, are active in the mesophilic range at 30 to 35$^{o}$C and the thermophilic range of 50 to 60$^{o}$C. The methane-forming bacteria are inhibited between 40$^{o}$C and 50$^{o}$C. Methane-forming bacteria grow slowly and are sensitive to small changes in temperature. The acclimation must proceed very slowly. The rates of anaerobic digestion and methane production are considerably faster in thermophilic conditions than in mesophilic ones. However, thermophilic anaerobes are very sensitive to rapid changes in temperature. Therefore, fluctuations in digester temperature should be controlled, because of the impact on enzymatic activity and reaction. Therefore, increasing of temperature results in more enzymatic activity, whereas decreasing temperature results in less enzymatic activity [54, 177].

#### 2.2.3.3 Mixing

Mixing within the digester provides good contact between the microbes and the substrates, reduces resistance to mass transfer, minimizes buildup of inhibitory intermediates and stabilizes environmental conditions. Mixing can be accomplished through mechanical mixing, biogas recirculation or through slurry recirculation. Mechanical mixers are more effective than gas recirculation, but they often become clogged or fouled with digester solids. Rapid mixing does not encourage methane-producing bacteria as it induces methane-producing bacteria to be washed out in the effluent if the final separation step is not sufficiently reliable. Mixing during start-up is not beneficial as the pH digester lowered resulting in performance instability as well as leading to a prolonged start-up period. Mixing in palm oil mills are less efficient compared to mechanical mixing because the digesters do not mix perfectly. The investigation of the biogas production mixing effects on POME should be undertaken to obtain a suitable mode of mixing for the best digester performance [54, 177].

#### 2.2.3.4 Nutrients

Anaerobic microorganisms require nutrients for growing and producing new cells. Nutrients can be classified into two groups. Macronutrients, for example nitrogen and phosphorus, are required in relatively large quantities. Micronutrients, for example

cobalt and nickel, are required in relatively small quantities. Nitrogen and phosphorus must be available in the digester. Their amounts can be determined from the quantity of substrate or COD in the feed. The optimal nutrient requirements are in the ratio of COD:N:P about 100:1.1:0.2. Additionally, the micronutrients are required by methane-producing bacteria to convert acetate to methane. [178].

#### 2.2.3.5 Toxic Substances

Toxicity in the anaerobic digester may be acute or chronic. Acute toxicity results from the rapid exposure of an unacclimated population of bacteria to a relatively high concentration of toxic waste. Chronic toxicity results from the gradual and relatively long exposure of an unacclimated population of bacteria to toxic waste. Some substances, if at too high concentration, are toxic to the bacteria, for example ammonia concentrations > 1.50 g/L at high pH result in digester failure. Reduction of sulfide toxicity inhibits the metabolic activity of anaerobic bacteria and also other toxic substances, such as heavy metals, cyanide, and chlorinated hydrocarbons.

#### 2.2.3.6 Retention Time

There are two important retention times in anaerobic digesters, affecting system performance as they relate to the growth rate of microorganisms and to effluent concentrations. The solid retention time (SRT) is the period that bacteria and solids are retained in the digester. The hydraulic retention time (HRT) is the time that the liquid phase remains in the digester. When no liquid solid phases separation exists (sttler downstream the bioreactor or presence of fixed culture inside the bioreactor), HRT is equal to SRT, in opposite, in presence of a separation step between biomass and liquid phase, SRT can be greatly more important than HRT, which is favorable to reactor intensification. When no separation step exists, the methanogenic bacteria require a minimum retention time of more than 15 days, due to their growth rates as compared with aerobic bacteria and facultative anaerobic bacteria. The designs of digesters normally aim at a minimum retention period of 25-30 days. If SRT< 10 days while increasing flow-through or the water content of raw sludge then the HRT decrease, and there was significant washout of the microbial biomass and an associated reduction in performance [54, 177].

### 2.2.4 Microorganisms in Anaerobic Treatment

Anaerobic treatment is the biological process in which microorganisms break down biodegradable material in the absence of oxygen. In order to achieve these anaerobic treatments, it is necessary to understand microorganism community structure in this process. Anaerobic treatment has the various microorganisms growing in a symbiotic relationship. Not only some fungi and protozoa were found in anaerobic treatment but also bacteria which play an important role in anaerobic wastewater treatment. Anaerobic digesters have the suspended and the fixed-film bacteria growth for degrading pollutants in wastewater. There are three important bacteria groups in anaerobic treatment. The first is hydrolytic bacteria, which can degrade complex molecules into simple molecules. The second group is divided into two groups of fermentative bacteria. Acidogenic microorganisms convert simple molecules to organic acids. Acetogenic bacteria produce acetate and hydrogen. Finally, methanogenic bacteria produce biogas from acetic, hydrogen and carbon dioxide [54, 177].

#### 2.2.4.1 Hydrolytic Bacteria

Hydrolytic bacteria consist of a consortium of gram-positive, rod-shaped, facultative anaerobic bacteria that can break down complex molecules (carbohydrates, lipids, and proteins) into simple molecules (sugars, fatty acids, and amino acids). Hydrolytic bacteria produce exoenzymes such as cellulose (hydrolyze starches or carbohydrates), lipases (hydrolyzed lipids), and proteases (hydrolyzed proteins). An example of hydrolytic bacteria, Clostridium, are mostly found in thermophilic environments [54, 177].

#### 2.2.4.2 Acidogenic Bacteria

The acidogenic bacteria, such as Enterobacteriaceae, Baccillsceae, Lactobaccillaceae, and Streptococcaceae, convert simple molecules from hydrolytic bacteria to (1) organic acids (acetate, butyrate, formate, and lactate), (2) alcohols (ethanol and methanol) (3) acetones, and (4) carbon dioxide, hydrogen and water. These groups are mostly found in anaerobic digesters, because they can utilize various substrates and have a high growth rate [54, 177].

#### 2.2.4.3 Acetogenic Bacteria

Acetogenic bacteria produce acetate and hydrogen that can be used directly by methanogenic bacteria. Acetogenic bacteria convert several of the fatty acids, propionates, and alcohols that are produced by the acidogenic bacteria to acetate, hydrogen, and carbon dioxide. Examples of acetogenic bacteria are Clostridium and Acetobacterium.

1. Hydrogen Producing Acetogenic Bacteria

These bacteria can degrade soluble organic substrates from hydrolysis, such as alcohol and fatty acids, converting them to acetate and hydrogen.

2. Homoacetogenic Bacteria

These bacteria are a mixotrophic methabolism and catabolize both $H_2/CO_2$ or multicarbon compounds (e.g. sugars) [54, 177].

#### 2.2.4.4 Methanogenic Bacteria

These bacteria can be found as individual rods, curved rods, spirals, and cocci, or grouped as irregular clusters of cells, chains of cells or filaments, and sarcina or cuboid arrangements. The range in diameter sizes of individual cells is 0.1-15 μm. All methanogenic bacteria obtain energy by reducing simplistic compounds or substrates, such as carbon dioxide and acetate, to methane. There are three groups of methanogenic bacteria, as shown in Equation 2.1-2.3 [54, 177].

$$\text{Acetate} \xrightarrow{\text{Acetoclastic methane-forming bacteria}} CH_4 + CO_2 \quad (2.1)$$

$$H_2 + CO_2 \xrightarrow{\text{Hydrogenotrophic methane-forming bacteria}} CH_4 + 2H_2O \quad (2.2)$$

$$\text{Methanol} \xrightarrow{\text{Methyltrophic methane-forming bacteria}} 2CH_4 + 2H_2O \quad (2.3)$$

## 2.3 Biochemical Methane Potential (BMP) Test

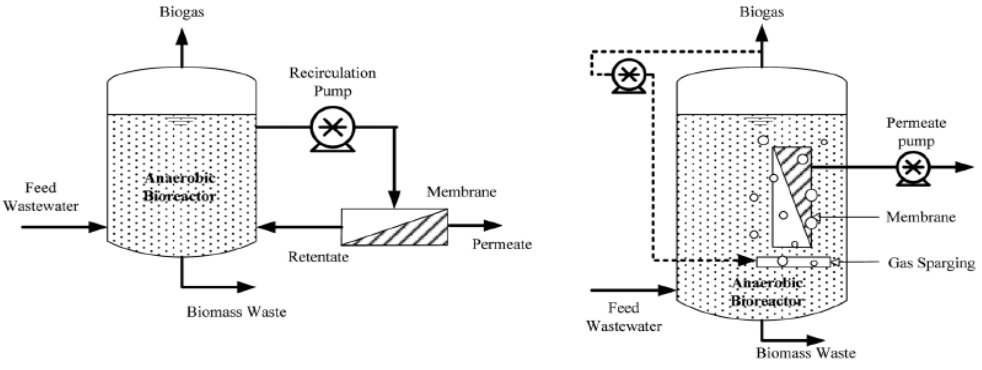
Owen et al [179] developed the BMP test by combining the theory and procedures of the anaerobic Warburg with serum-bottle techniques for the cultivation of anaerobes. The Warburg apparatus is an analytical instrument for measuring the pressure of gases and

vapors from biochemical reactions. The Warburg apparatus is based on the principle that, at constant temperature and gas volume, any changes in the amount of gas can be measured by changes in its pressure. It consists of a detachable flask for placing the sample equipped with one or more sidearms for additions of chemicals and a manometer containing a liquid of known density. The BMP test was defined as a measure of substrate biodegradability determined by monitoring cumulative methane production from a sample anaerobically incubated in a chemically defined medium. The set-up includes 250 ml reagent bottles and rubber serum caps, gassed with a mixture of 30% carbon dioxide ($CO_2$) and 70% nitrogen ($N_2$) for 15 minutes, then stoppered and equilibrated at incubation temperature. This was done before introducing samples, defined media and inocula. Respective gas productions were monitored volumetrically using the syringe method of Nottingham and Hungate [180]. The method comprises a needle attached to a 10 ml syringe being inserted into the test vessel. The volume of gas forced into the syringe as the needle penetrates the stopper is noted. The methane contribution resulting from sample decomposition was determined by subtracting background values, obtained from seed blanks, from the sample totals. BMP is referenced to either the sample volume ($m^3$ $CH_4/m^3$ sample), sample mass ($m^3$ $CH_4$/kg sample) or sample organic content ($m^3$ $CH_4$/kg COD).

## 2.4 Anaerobic Membrane Bioreactor (AnMBR)

**2.4.1 AnMBR Fundamentals** The AnMBR process is an anaerobic biological treatment coupled with a membrane to provide the complete retention of biomass and the solid-liquid separation process [21]. The AnMBR process operated under pressure or vacuum, which can be classified into four major configurations according to the location of membrane units, as seen in Figure 2.4. The membrane may be operated under pressure or it may be operated under a vacuum. In the first approach, the membrane is separated from the bioreactor and a pump is required to push the bioreactor effluent into the membrane unit which makes permeate to come through the membrane. This configuration is often called as an external cross-flow membrane bioreactor (Figure 2.4(a)). When the membrane is immersed into the bioreactor and operated under a vacuum (Figure 2.4(b)), instead of under direct pressure, the configuration is called

submerged membrane bioreactor due to the location of the membrane. Recently, some submerged AnMBR application studies were reported [87, 115]. To reduce cake formation on the membranes in submerged AnMBRs, the produced biogas is recirculated and used instead of air bubbling in aerobic submerged MBRs [87, 115]. The membrane may be immersed directly into the bioreactor or immersed in a separate chamber (Figure 2.4(c)). The latter configuration now looks like an external membrane, and will likely require a pump to return the retentate to the bioreactor. However, unlike the external cross-flow membrane, the membrane here is operated under a vacuum instead of under pressure. The external chamber configuration is used for full-scale aerobic wastewater treatment plants, because it provides for easier cleaning of the fouled membranes, as the chambers can be isolated instead of the membranes being physically removed. More studies are conducted in order to enhance the performance of AnMBR. The configuration in Figure 2.4(d) shows that the system is operating intermittently under a semi dead-end mode to reduce the continuous pumping cost and to minimize the harmful effects, such as biomass activity reduction, of sludge pumping [181].




(a) External cross-flow

(b) Submerged

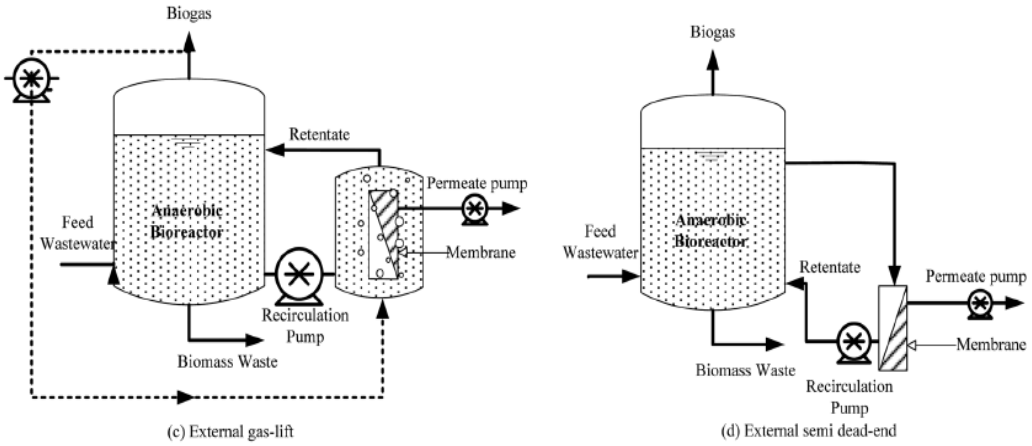



**Figure 2.4** AnMBR configurations [182].

Another AnMBR reactor configuration is the two-stage reactor configuration, as show in Figure 2.5 a-b. In a single-stage reactor, where both of the processes take place inside, the maintenance of optimum conditions for the acid formation and methane formation is impossible. The biological reactions of the different species in a single stage reactor can be in direct competition with each other in Figure 2.5a. In a two-stage treatment system two reactors are operating with the optimized conditions of the respective bacteria to bring maximum control of the bacterial communities living in the reactor. In two-stage reactor configuration the reactions of hydrolysis, acetogenesis and acidogenesis occur within the first reactor named as the hydrolytic (or acidogenic) reactor, followed by methanogenic reactor where the methanogenic process take place (Figure. 2.5b). The methanogenic reactor that facilitates for the methanogens operates in a strictly defined optimum pH range for the growth of the microorganisms. In the past, operation of two-stage anaerobic system was hindered by difficulties in solid-liquid separation and the maintenance of separate and distinct biomass populations in each reactor [183]. Yet the membrane coupled bioreactors provides the applicability of the two-stage anaerobic degradation both with excellent separation and high biomass retention.

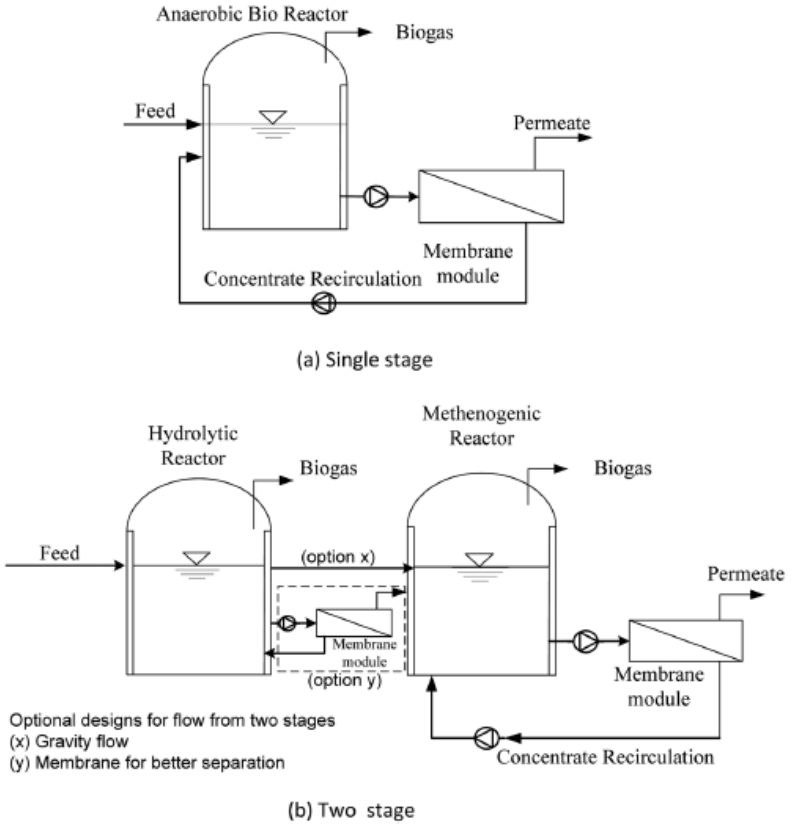



**Figure 2.5** Single and two stage AnMBR configurations [182].

### 2.4.2 Advantages of AnMBR

The AnMBR has many advantages over conventional wastewater treatment processes. These include the excellent effluent quality due to the retention of all suspended matter and most soluble compounds within the bioreactor. Included is reduced footprint and/or sludge production through maintaining a high biomass concentration in the bioreactor. The system is also capable of handling wide fluctuations in influent quality, and the effluent can be reused directly for nonpotable purposes, because filtration efficiency is such that a high disinfection level is generated.

### 2.4.3 Fundamental of Membrane Fouling

Membrane fouling is definitively the main drawback of the application of MBRs for wastewater treatment [184]. Membrane fouling results in a reduction of the permeate flux or an increase of transmembrane pressure (TMP), depending on the operation mode,

requiring more frequent membrane cleaning and replacement. This operating costs. Membrane fouling can be caused by several phenomena, the most evident is the accumulation of compounds onto the membrane surface due to the selective cut-off of the membrane, it is at the origin of polarization layer and deposit closed to or on the membrane surface. This phenomenon is completed by reversible and irreversible that can become dominant according to the operational conditions: adsorption of organic matter, precipitation of inorganic matter and colloids, adhesion of microbial cells within/on membrane and biofilm development [17].

#### 2.4.3.1 Classification of Membrane Fouling

##### 2.4.3.1.1 Removable and Irremovable Fouling

Membrane fouling can be classified into three types according to cleaning methods: (1) removable fouling, (2) irremovable fouling and (3) irreversible fouling in Figure 2.6. The removable fouling, known as reversible fouling, is caused by loosely attached foulants. Sludge flocs and colloids are much larger than the membrane pores and they tend to form a cake layer on the membrane surface. The removable fouling can be easily removed by physical cleaning (e.g. tangential shear stresses, relaxation and backwashing). On the other hand, the irremovable fouling is caused by the adsorption of dissolved matter smaller than the membrane pores. They can absorb and block the membrane pores and can strongly attach foulants during filtration. The irremovable fouling can only be removed by chemical cleaning. Irreversible fouling is permanent and cannot be removed by any cleaning approaches. This processe should not be noticed as it corresponds to a bad choice of membrane material.

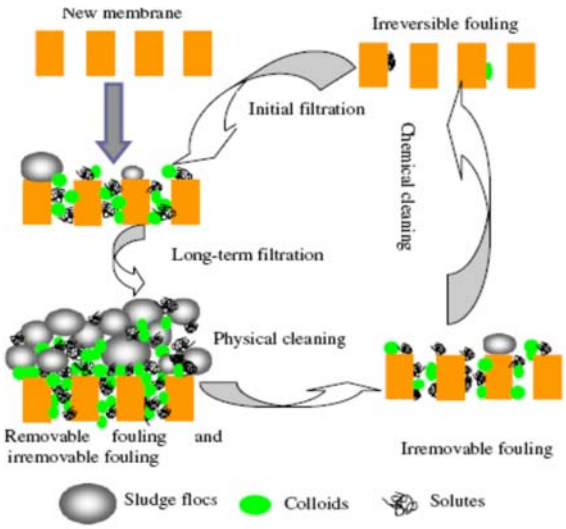



**Figure 2.6** Schematic illustration of the formation of removable and irremovable fouling in MBRs [185].

#### 2.4.3.1.2 Biofouling

Biofouling is specific to biological activities directly in contact with the membrane (interactions of soluble microbial products with the membrane materials, and biofilm development on the membrane surface). Biofilm development is due to the deposition, growth and metabolism of bacteria cells or flocs on the membranes, arousing significant concern in membrane filtration. Biofouling is a major limitation in microfiltration and ultrafiltration for treating wastewater, because most foulants (microbial flocs) in MBRs are much larger than the membrane pore size. Microbial products, SMP and EPS, secreted by bacteria are at the origin of biofilm formation and structuring, but also at the origin of internal fouling by progressive pore blocking [186, 187].

Biofouling can be identified by techniques such as scanning electron microscopy (SEM), atomic force microscopy (AFM), and direct observation through the membrane. These techniques are helpful for understanding the mechanism of floc/cell deposition and the microstructure or architecture of the cake layer.

- Organic Fouling

Organic fouling is the deposition of biopolymers (i.e. proteins and polysaccharides) on the membrane surface. Organic fouling can be identified by Fourier transforminfrared (FTIR) spectroscopy,and high performance size exclusion chromatography(HP-SEC). These techniques are powerful analytical tools. The major component of biopolymer from FTIR analysis was identified as proteins and polysaccharides [56]

- Inorganic Fouling

As seen in Figure 2.7, inorganic fouling has two origins namely chemical precipitation and biological precipitation ($COO^-$,$CO_3^{2-}$ , $SO_4^{2-}$, $PO_4^{3-}$, OH). A great number of cations and anions, such as $Ca^{2+}$, $Mg^{2+}$, $Al^{3+}$, $Fe^{3+}$, $CO_3^{2-}$ , $SO_4^{2-}$, $PO_4^{3-}$, $OH^-$ and others, are still present in MBRs. Inorganic fouling can be identified by Energy dispersive X-ray (EDX) spectroscopy. Inorganicfouling can result in severe irremovable fouling and chemicalcleaning is more effective than physical cleaning for removal of inorganic precipitation. Chemical cleaning agents such as EDTA might efficiently remove inorganics on the membrane surface [188].

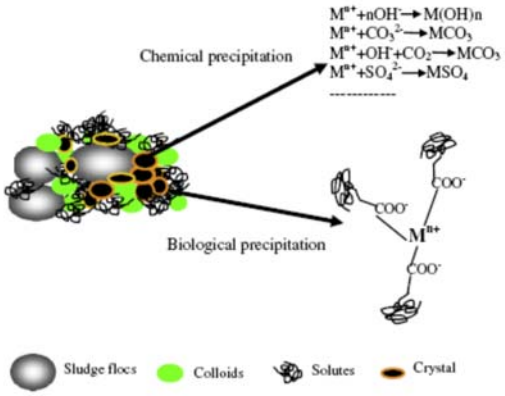



**Figure 2.7** Schematic illustration of the formation of inorganic fouling in MBRs [185].

### 2.4.3.2 Fouling Factors

The fouling membrane factors can be classified into four groups [189]: membrane materials, module configurations and associated local turbulences, feed water characteristics, and sludge characteristics depending on (i) biological parameters (OLR,

HRT, SRT, pH, Temp) that influence the type of bacterial population, TSS and EPS/SMP concentrations in the suspension, and (ii) level of turbulence that influence particle size and biopolymer clusters (BPC), all of which have been identified as affecting membrane fouling.

**1. Membrane Materials**

- Pore Size and Distribution

The effect of pore size on membrane fouling is strongly related to the biological conditions imposed in the bioreactor (TSS and EPS/SMP concentrations, nature of biological population and floc size distribution) and the wastewater characteristics. Based on a short-term study, working with small pores would reject a wider range of materials with the formation of a denser cake layer that could present a higher resistance compared with the use of large pore membranes and oblige to work under lower permeate flux or higher pressure. However, this type of fouling is external to the membrane material and easier to remove by external shear stresses and relaxation at the opposite to internal fouling that obliges to use backwashing, and above all, more frequent chemical cleaning. The fouling due to deposition of organic and inorganic materials onto and into the membrane is the main cause of the poor long-term performances of larger pore sized membranes. Therefore, large pore membranes like MF would present a higher fouling propensity compared to UF membranes [189].

- Porosity/Roughness

The surface of larger MWCO is rougher than that of the membrane with smaller MWCO. The rougher membranes are more prone to creating fouling layers, while fewer smaller “crevices” are observed on smoother membranes [189].

- Hydrophobicity

Membrane hydrophobicity is considered another important factor for membrane fouling. Membrane fouling occurs more rapidly on hydrophobic membranes than on hydrophilic ones, because of the hydrophobic interactions between solutes, microbial cells and membrane materials. Clech et al [189] reported that the contact angle measurement showed that the hydrophobicity of the PES membranes decreased with the increase in MWCO.

- Materials

The membrane materials always show different fouling properties due to their different pore size, morphology and hydrophobicity. Polyvinylidene fluoride (PVDF) membranes are better for the prevention of irremovable fouling than are polyethylene (PE) membrane in terms of MBRs used for the treatment of municipal wastewater [190]. Regarding MBR processes, the fouling behaviour of the membrane used is determined by the affinity between foulants (e.g., EPS/SMP) and membrane material. Zhang et al [191] studied the affinity between EPS and three polymeric ultrafiltration membranes. The results showed that the affinity and capability of the three membranes were in the following order: Polyacrylonitrile (PAN) < PVDF < Polyethersulfone (PES). It suggested that among these membranes, the PAN membrane is more fouling-resistant. Inorganic membranes, such as aluminum, zirconium, and titanium oxide, have been successfully used for several MBR applications. Especially as a potential alternative for the treatment of high temperature wastewater [192] as it could obtain a higher permeate flux, superior hydraulic and thermal, and chemical resistance. But these inorganic membranes are not the preferred option for large-scale MBR plants because of their high costs. In addition, inorganic membranes can induce severe inorganic fouling. So, the inorganic membranes might be used only in some special applications such as for high temperature wastewater treatment.

- Module Configuration

The current study in MBR design tends to favor submerged over side-stream configurations in the majority of the studies dealing with domestic wastewater treatment. In submerged MBR processes, the membrane can be configured as vertical flat plates, vertical or horizontal hollow fine fibers (filtration from out-to-in), or more rarely as tubes (filtration from in-to-out). The hollow fiber modules are generally cheaper to manufacture and they allow a high membrane density. They great advantage on flat sheet membranes is they can tolerate vigorous backwashing. Moreover, flat plate and tubular types may probably be easier to control. Due to the heterogeneity of fluid distribution through hollow fibers, these systems may be more prone to fouling and require more frequent washing and cleaning. An interesting discussion of the relative performances of hollow fibers and flat plate membranes were initiated by Gunder and Krauth [189].

**2. Feed–Biomass Characteristics**

- Nature of Feed and Concentration

Wastewater properties have a direct effect on membrane fouling. For example, the protein fraction measured in the extracted EPS (eEPSp) has been found to be significantly lower when biomass was fed with synthetic feed (chemical oxygen demand: COD of 460 mg/l) rather than with real sewage (COD of 140 mg/L).

**3. Biological suspension characteristics**

- MLSS Concentration

The MLSS concentrations have significant effects on membrane fouling. A rise in MLSS seems to decrease fouling when working at low MLSS concentrations (<6 g/L), more fouling is expected as the MLSS concentration increases to above 15 g/L. If the MLSS concentration is high (30–40 g/L), it has a major influence on membrane fouling. However, the level of MLSS does not appear to have a significant effect on membrane fouling when it is between 8 and 12 g/L if tangential shear stresses are adapted to the filtration conditions.

- Floc Characteristics

Due to the differences between floc size distribution in the mixed liquor (in the range of 1.2 to 600 μm [193]) and the pore size distribution of the membranes (notably when using UF membranes, average pore size lower than 50 nm), the size of flocs should not appear as a determining criterion. However, the shear stresses due to local suspension circulation (cross-flow filtration and gas injection in SMBRs) induce the breakdown of the biological flocs, generating fine colloids and individual cell formation, which then form a denser cake layer on the membrane, even increase the production of EPS that influence the internal fouling dynamics. According to Cicek et al [194] the average diameter of particles in a side-stream MBR system can decrease to 3.5 μm. with 97% of the particles being smaller than 10 μm, whereas the ASP mixed liquor contained flocs ranging from 20 to 120 μm. This result is consistent with a study by Wisniewski and Grasmick [195]. The suspension produced after floc breakup consisted mainly of particles having a size of around 2 μm, which was responsible for flux decline.

- Extracellular Polymeric Substances (EPS)

EPS are large molecular weight compounds secreted by microorganisms. They have been found during microorganism activity (outside the cell surface and intercellular space of microbial aggregates) [189]. EPS, bound or soluble, consists of proteins, polysaccharides, nucleic acids, lipids, humic acids, etc. The relationship between the specific cake resistances established a functional Equation in which the specific cake resistance was proportional to the EPS concentration. Therefore, bound EPS on the membrane surface influenced membrane fouling.

- Soluble Microbial Products (SMP)

SMP can be defined as soluble cellular components that are released from substrate metabolism, biomass decay, the simple substrates and cell lysis. SMP adsorb on the membrane surface, block membrane pores and/or form a gel structure on the membrane surface where they provide a possible nutrient source for biofilm formation and a hydraulic resistance to permeate flow [189].

- Solid Retention Time (SRT)

SRTs are the one of the most important operating parameters affecting MBR performance, including membrane fouling [196]. Masse et al [197] found that are too short might SRTs harm the membrane performance. Overly long SRTs were also found to result in excessive membrane fouling due to large amounts of foulants, and high corresponding TSS concentrations increased sludge viscosity. Consequently, the resistance force of the membrane surface increased rapidly and the water flux reduced significantly. The optimum SRT of MBRs should be controlled at 20–50 d depending on HRT and feed water.

- Sludge Loading Rate

The sludge loading rate is related to increasing organic loading rate (OLR), feed concentration, and hydraulic retention time (HRT). Inversely, HRT and OLR can govern both the F/M in the bioreactor and the MLSS concentration. In general, short HRT can induce large OLR. Thus, HRT is correlated not only to the expected treatment efficiency but with OLR they are also two main operating parameters affecting the production of bound EPS since they directly govern biomass growth and decay. Some reports showed that there were high bound EPS concentrations and high sludge viscosity as F/M ratios increased. The formation of bound EPS is growth-related and is produced in direct

proportion to substrate utilization. Thus, the increase of the organic loading rate or F/M ratio induces the generation of more bound EPS [198].

### 2.4.4 Fouling Control

The fouling control includes the improvement of membrane materials and modules, the adjustment of sludge characteristics and the optimization of operational parameters (flux, cross-flow velocity, aeration, backwashing, cleaning, etc.). Adding a certain carrier with specific characteristics to MBR was also one attempt to control membrane fouling. Powdered activated carbon was the most applied carrier due to its good adsorption capacity of dissolved organic substances, which were considered to be the most important contributors to membrane fouling.

The optimizing of hydrodynamic conditions, operating the membrane system below critical flux, pre-treating the feed water, or conducting air scour, membrane backwashing and cleaning are recommended in MBR. Innovative methods, such as membrane coating, addition of porous carriers for attached growth, flocculation of activated sludge by adding additives, and modification of the suspension by adsorption, are well performed. Recently, various chemicals including synthetic or natural polymers, metal salts, resins, granular or power activated carbon have been tested for filterability and fouling reduction in MBR mixed liquors through batch test and dead-end filtration processes [185].

#### 2.4.4.1 Cleaning of membrane fouling

One fouling results in a significant reduction in the separation efficiency by decreasing the permeate flux and increasing the pressure drop across the membrane. Therefore, membrane cleaning is an essential step to the recovery of membrane filtration. There are a number of different chemical and physical cleaning methods currently used for membrane cleaning.

- Physical Cleaning

Physical cleaning technologies depend upon mechanical forces to dislodge and remove foulants from the membrane surface. Physical methods used include forward flushing, reverse flushing, backwashing, vibrations, air sparking and $CO_2$ back permeation. According to contain reports, the production interval between cleaning periods and the duration of backwash and pressure during forward flush are significant factors affecting physical cleaning. For example, less frequent, but longer backwashing (600 s filtration/45 s

backwashing) was found to be more efficient than more frequent backwashing (200 s filtration/15 s backwashing) [199].

- Chemical Cleaning

Chemical cleaning methods depend upon chemical reactions to weaken the cohesion forces between the foulants and the adhesion forces between the foulants and the membrane surface. Chemical reactions involved in cleaning include hydrolysis, peptization, saponification, solubilisation, dispersion, and chelation. Each of the four main MBR suppliers (Kubota, Memcor, Mitsubishi and Zenon) proposes their own chemical cleaning recipes, which differ mainly in terms of concentration and methods (Table 2.1). Cleaning efficiency varies with respect to the conditions applied during cleaning, namely, type of cleaning agent, cleaning solution pH, cleaning agent dose, cleaning time, crossflow velocity during cleaning, and cleaning solution temperature. Furthermore, cleaning efficiency, even at fixed cleaning conditions, is also influenced by the conditions applied during fouling [189]. Examples of the types of cleaning agents applied are as follows:

- Sodium hypochlorite (for organic foulants) and citric acid (for inorganics). Sodium hypochloride hydrolyzes the organic molecules, and therefore, loosens the particles and biofilm attached to the membrane surface. The effects of chemical cleaning agents, such as NaOCl, on the microbial community have also been recently studied for modeled MBR processes.

- Alkaline solution, such as NaOH (pH 11.0), metal chelating agent as disodium ethylenediaminetetraacetate ($Na_2$-EDTA), and surfactants, such as sodium dodecyl sulfate (SDS) are used to clean the organic-fouled membranes. Alkaline solutions clean organic-fouled membranesby hydrolysis and solubilization. Alkaline solutions increasethe solution pH, and therefore increase the negative chargeand solubility of the organic foulant.

- Metal chelating agents, such as EDTA, remove divalent cations from the complexed organic molecules and improve the cleaning of the fouled membrane.

- Surfactants are compounds that have both hydrophilic and hydrophobic groups, and are semisoluble in both organic and aqueous solvents. Surfactants can solubilize macromolecules by forming micelles around them, and help to remove the foulants from the membrane surface.

**Table 2.1** Intensive chemical cleaning methods for four MBR suppliers [189].

| MBR suppliers | Type | Chemicals | Concentration (%) | Protocols |
|---|---|---|---|---|
| Mitsubishi | CIL | NaOCl<br>Citric acid | 0.3<br>0.2 | Backflow through membrane (2 h) + soaking (2 h) |
| Zenon | CIP | NaOCl<br>Citric acid | 0.2<br>0.2–0.3 | Backpulse and recirculate |
| Memcor | CIP | NaOCl<br>Citric acid | 0.01<br>0.2 | Recirculate through lumens, mixed liquors and in-tank air manifolds |
| Kubota | CIL | NaOCl<br>Oxalic acid | 0.5<br>1 | Backflow and soaking (2 h) |

CIL: cleaning in line where chemical solutions are generally backflowed (under gravity) inside the membrane. CIP: cleaning in place where the membrane tank is isolated and drained. The module is rinsed before being soaked in the cleaning solution and rinsed to remove excess chlorine. The exact protocol for chemical cleaning can vary from a plant to another.

## 2.5 Biological Model

### 2.5.1 Model Description

Anaerobic digestion model no.1 (ADM1) was presented by the IWA Task Group for the mathematical modelling of the anaerobic digestion process. The ADM1 is structured on the basic biochemical steps including disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis steps presented in Figure 2.8. The physico-chemical process is also included describing the ion association and dissociation, and gas liquid transfer [146]. Process inhibition is also considered including pH, hydrogen and free ammonia [140]. In this structured model, the first order kinetics is applied to explain the disintegration and hydrolysis process as well as biomass death. The ADM1 model employs a large number of constants and coefficients. It describes 7 groups of bacteria and archea, catalyzing 19 biochemical kinetic processes, coupled to 3 gas – liquid mass transfer Equations and 8 algebraic variables [200]. The complexity of the model was acknowledged

by Parker [201] when trying to calibrate the model against, previously published reports on anaerobic digestion processes.

Disintegration and hydrolysis are non-biological extracellular solubilization steps. The disintegration step converts composite particulate substrates in inert carbohydrates, proteins and lipids. The enzymatic hydrolysis which is the following step, will convert carbohydrates, proteins and lipids into monosaccharides, amino acids and long chain fatty acids (LCFA), respectively. Both disintegration and hydrolysis processes are described by the first order kinetics [2]. There are seven bacterial groups in the biochemical conversion step.

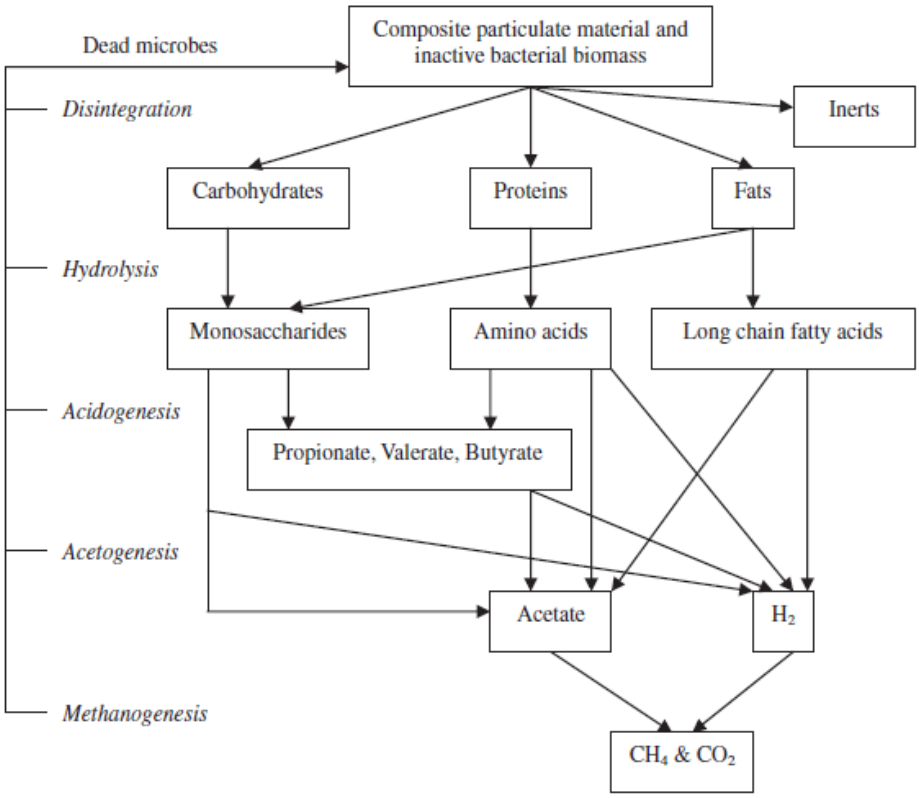



**Figure 2.8** The reaction paths described in ADM1[140].

Monosaccharides and amino acids will be converted by two groups of acidogenic to mixed organic acids, hydrogen and carbon dioxide. Subsequently, Long chain fatty acid (LCFA), butyrate and valerate, and propionate will be converted by three groups of

acetogenic to acetate, hydrogen and carbon dioxide. Generated Hydrogen and acetate will be then used by hydrogen-utilising methanogenic group and aceticlastic methanogenic group, respectively. Every step of intracellular biochemical reactions is described by substrate-based uptake Monod-type kinetics. Death of biomass is described by the first order kinetics. The dead biomass is maintained in the system as composite particulates. Inhibition functions include pH (all bacterial groups), hydrogen (acetogenic groups) and free ammonia (aceticlastic methanogenic group). Mechanisms describing physico-chemical processes are acid-base reactions for identifying hydrogen ion concentration, free ammonia and carbon dioxide, and non-equilibrium liquid-gas transfer.

### 2.5.2 Model Equation

The ADM1 is a structured mathematical model with 32 dynamic state concentration variables and 19 biochemical rate processes. The set of differential Equations (DE) of the ADM1 model are as follows: 10 (DE) to model the evolution of soluble matter concentrations in the liquid phase and two (DE) to model inorganic carbon (IC) and inorganic nitrogen (IN) levels in the liquid phase. Twelve (DE) to depict the behavior of particulate matter and biomass concentrations in liquid phase; two (DE) to model cation and anion levels in liquid phase and an additional six (DE) for acid–base reactions in order to determine the pH of effluent and calculate ionized forms of VFA, free ammonia nitrogen and carbon-dioxide concentrations (Batstone et al., 2002). More details about the elaboration of these differential Equations (DE) are shown below.

#### 2.5.2.1 Liquid Phase Equations

The mass balance equations used by the ADM1 model to describe the dynamic behavior of soluble substrates components and particulate substrates components in the liquid phase are given below by Equations 2.4-2.5.

$$\frac{dS_{liq,i}}{dt} = \frac{Q}{V_{liq}} \cdot \left(S_{in,i} - S_{liq,i}\right) + \sum_{j=1-19} \rho_j v_{i,j} \qquad i = 1, \dots, 12; i = 25 - 26 \qquad (2.4)$$

$$\frac{dS_{liq,i}}{dt} = \frac{Q}{V_{liq}} \cdot \left(X_{in,i} - X_{liq,i}\right) + \sum_{j=1-19} \rho_j v_{i,j} \qquad i = 13, \dots, 24 \qquad (2.5)$$

where $S_{liq,i}$ is the concentration of each soluble state variable, $X_{liq,i}$ is the concentration of each particulate and biomass state variable, $V_{liq}$ is the liquid reactor volume, Q is the flow into and out of the reactor, $S_{in,i}$ is the input concentration of soluble components, $X_{in,i}$ is the input concentration of particulate and biomass components and the term $\Sigma_{j=1\text{-}19}\rho_j\nu_{i,j}$ is the sum of the specific kinetic rates $\rho_j$ for process j multiplied by the stoichiometric coefficients $\nu_{i,j}$.

#### 2.5.2.2 Gas Phase Equations

Three major gaseous components are modelled by the ADM1 in the gas phase. These gases are hydrogen, methane and carbon-dioxide. The transfer rate of carbon dioxide, methane and hydrogen into the gas phase was determined from the general theory of two-film mass transfer [202]. All gases were assumed to obey the ideal gas law and exist at a temperature equivalent to the liquid phase temperature in a constant volume (completely mixed) and a constant pressure headspace [140]. Using these assumptions, the general dynamic gas phase concentration equation of each gas component ‘‘i” can be written as Equation 2.6.

$$\frac{dS_{gas,i}}{dt} = -\frac{q_{gas}}{V_{gas}}S_{gas,i} + \frac{V_{liq}}{V_{gas}}\rho_{T,i} \tag{2.6}$$

where $q_{gas}$ (l/day) is the gas flow; $V_{liq}$ (l) is the liquid reactor volume; $V_{gas}$ (l) is the gas reactor volume; $S_{gas,i}$ (mol/l) is the gas phase concentration of gas component ‘‘i” and $\rho_{T,i}$ is the specific mass transfer rate of gas ‘‘i” expressed as follows in Equation 2.7:

$$\rho_{T,i} = k_{La} \cdot \left(K_H \cdot P_{gas,i} - S_{liq,i}\right), \qquad i = CH_4, CO_2 \text{ and } H_2 \tag{2.7}$$

where $k_{La}$ ($d^{-1}$) is the volumetric gas–liquid mass transfer coefficient, $K_H$ (M $bar^{-1}$) is the Henry’s law coefficient and $S_{liq,i}$ (M) is the liquid phase concentration of gas component ‘‘i” and $P_{gas,i}$ (bar) is the gas phase pressure of each gas component ‘‘i” calculated from the ideal gas law as follows in Equations 2.8-2.10:

$$P_{gas,H_2} = S_{gas,H_2} \cdot \frac{R \cdot T_{op}}{16} \tag{2.8}$$

$$P_{gas,CH_4} = S_{gas,CH_4} \cdot \frac{R \cdot T_{op}}{64} \quad (2.9)$$

$$P_{gas,CO_2} = S_{gas,CO_2} \cdot R \cdot T_{op} \quad (2.10)$$

The gas production rate can be calculated by Equation 2.11:

$$q_{gas} = \frac{R \cdot T_{op}}{P_{atm} - P_{gas,H_2O}} V_{liq} \left(\frac{\rho_{T,H_2}}{16} + \frac{\rho_{T,CH_4}}{64} + \rho_{T,CO_2}\right. \quad (2.11)$$

**2.5.2.3 pH Equation**

The charge balance equation of the original ADM1was modified to take into account the contribution of soluble phenolic compounds into acid–base reactions as follows Equation 2.12:

$$S_{H^+} - S_{OH^-} = S_{HCO_3^-} + \frac{S_{ac^-}}{64} + \frac{S_{pro^-}}{112} + \frac{S_{bu^-}}{160} + \frac{S_{va^-}}{208} \quad (2.12)$$

where $S_{H^+}$ is the concentration of hydrogen ions in the liquid phase reactor, $S_{OH^-}$ is the concentration of hydroxide ion, $S_{ac^-}$ , $S_{pro^-}$ , $S_{bu^-}$ , $S_{va^-}$ , $S_{NH4^+}$and $S_{HCO^-3}$ are the concentrations of ionised forms of buffer components expressed as dynamic state variables and implemented in the ADM1 model as kinetic rate equations.

**2.5.3 The modification of the ADM model**

The ADM model was constructed to describe two-phase characteristics including liquid (POME wastewater) and gas (biogas) in anaerobic digesters. ADM1 was implemented in GPS-X version 6.1, which is a computer program for mathematical modeling and simulation of aquatic systems. ADM1 requires a detailed influent characterization, process configuration, operational condition and dynamics of population. A modified ADM model is proposed.

General useful ADM model

1. Anaerobic Digestion Model (ADM) explains the complex substrates through their principal components [140]. It includes several steps that describe the biochemical and physicochemical processes involved in the anaerobic biodegradation of organic compounds.
2. Mathematical modeling supports tool for design, operation and control of activated sludge systems [166].

3. It can be used to predict process behavior in different situations and to assist operational management in order to develop strategies that improve stability [160]. These predictions can not only improve operational decision making in agricultural biogas plants but also assist the planning of research experiments [203].

Specific useful ADM model in this study

4. The response of an anaerobic digester, such as COD effluent, TSS and VSS, during the start-up period under different organic loads and SAnMBR under different SRT treating POME was estimated through fitting of the model equations using GPS-X version 6.1 software for the implementation of the ADM1.
5. In this study the ADM1 was applied to predict the methane production during the start-up as compared with SAnMBR using POME as the substrate.

# CHAPTER 3

# METHODOLOGY

This chapter presents the overview which consists of five parts of materials and methods (analytical procedures, sampling technique and instruments), as given in Figure 3.3.

(3.1) Investigation of SMA and BMP in POME

(3.2) Influence of relaxation frequency on membrane fouling control in SAnMBR

(3.3) Influence of SRT on SAnMBR efficiency when treating POME

(3.4) Effect of SRT on membrane fouling intensity in SAnMBR treating POME

(3.5) Modelling the effect of OLR and SRT on the performance in start-up period and SAnMB treating POME using GPS-X.

## 3.1 Investigatigation of SMA and BMP in POME

The investigation of SMA and BMP assays in this research were carried out in batch conditions. The SMA assay was used to investigate the methanogenic activity of anaerobic sludge from three full-scale palm oil industries. This testing used acetic acid as substrate and try out with triplicate samples for each industry. During SMA test, the biogas production and biogas composition were collected every 1 hr. After that, the good anaerobic seed sludge from SMA test was select to evaluate methane potential from different diluted levels 25%, 50%, 75% and 100% of POME using by BMP assay. During BMP test, samples were collected everyday from serum bottle, and also analyzed biogas production and biogas composition. The good anaerobic seed sludge from SMA and the suitable POME concentration on methane production from BMP was used in SAnMBR. The results and discussion of SMA and BMP are given in Chapter 4.

### 3.2 Influence of Relaxation Frequency on Membrane Fouling Control in SAnMBR

The intermittent filtrations were investigated to obtain an appropiate membrane fouling control, which was possible for the SAnMBRs in this study. The intermittent filtrations composed of 5 L SAnMBR reactor with an immersed the hollow-fiber membrane module with area of 0.1 $m^2$, as shown in Figure 3.1. It was provided from Shanghai Jofur Advanced Materials Co., Ltd, China. Hollow fibers were made of hydrophilic polyvinylidene fluoride (PVDF) with a pore size of 0.1 μm, and internal/external diameter of 0.7/1.3mm. The membrane module was conducted from 30 hollow-fibers with a length of 30 cm. The top of module were fixed by epoxy for merged fibers together, whereas was free at the end of module. However, at the end of each fiber was also collapsed by epoxy for prevented the influence water. POME and anaerobic microorganism, including solids particulates and bacteria, remain on the outside, while permeate is drawn through the side surface of membrane to the inside of the fibers. The membrane maintained constant permeates flux and following TMP variable with time.

**Figure 3.1** Hollow-fiber membrane module.

The intermittent filtrations operated in the range of supra-critical conditions (about 20 L/$m^2$h) according to Jeison and van Lier et al. [20]. The filtration was shut down when the TMP reached a value close to 250 mbar (25kPa). The membrane module was then taken off from the SAnMBR, and three successive steps of cleaning (physical, backwash with water and chemical cleaning) were practiced. The rinsed water was

collected to identify the main origins of membrane fouling from EPS and SMP, which typically consisted of carbohydrates (PC), proteins (PN). Protein concentration was analyzed according to the colorimetric method defined by the modified Lowry procedure of Peterson [16], using bovine serum albumin (BSA) as a standard protein. Carbohydrate concentration was analyzed by the phenol-sulfuric acid methods with D-glucose used as a standard carbohydrate. Carbohydrate samples analyses were measured at 480 nm [17]. The sludge cake layer on the membrane surface was rinsed with DI water and centrifuged for 30 min at 6000rpm and then the extracted supernatant was filtrated through a membrane with a mean pore size of 0.45 μm. The filtrate of the centrifuged supernatant represented the concentration of SMP. The remaining pellet was washed and suspended again with saline water (0.9% NaCl solution). The sludge cake layer was then subjected to heat treatment (100$^{o}$C, 1 h) and centrifuged again under the same operating conditions. The centrifuged supernatant was clarified as an EPS solution, as shown in Figure 3.2. The result and discussion of influence of relaxation frequency on membrane fouling control in SAnMBR are explained in Chapter 5.

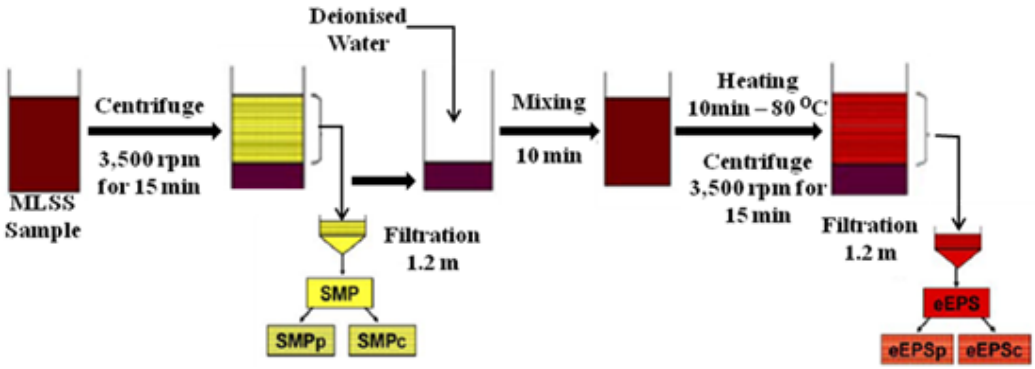



**Figure 3.2** Method for EPS and SMP extractions [189].

### 3.3 Influence of SRT on SAnMBR Efficiency When Treating POME

This topic is presented in two main parts: the treatment efficiency and methane production (1) during start-up, and (2) SAnMBR, which the seed sludge and raw POME obtained from section 3.1 was used for this investigation. During start-up consist of two reactors; (1) an acidogenic reactor and (2) a methanogenic reactor. At start-up period,

OLR was progressively increased by decreasing the daily HRT. After the start-up period finish, a third tank containing porous membranes was set up downstream of the methanogenic bioreactor to constitute a selective barrier to separate solid and soluble phases. This stage (SAnMBR) was operated at various 3SRTs. The influent and effluent taken from three reactors evaluated efficiency treatment by the parameter, displayed in Table 3.1. The result and discussion of the role of SRT on SAnMBR efficiency when treating POME explained in Chapter 6.

**Table 3.1** Parameters measured and analytical methods.

| Parameters | Acidogenic reactor | | Methanogenic reactor | Membrane reactor | Analytical method | Frequency |
|---|---|---|---|---|---|---|
| | Influent | Effluent | Effluent | | | |
| pH | ✓ | ✓ | ✓ | ✓ | pH meter | Daily |
| Temperature | ✓ | ✓ | ✓ | ✓ | Thermometer | Daily |
| TCOD and SCOD | ✓ | ✓ | ✓ | ✓ | Close reflux* | Three times a week |
| $NH_3$-N | ✓ | ✓ | ✓ | ✓ | Distillation, titration* | Once a week |
| TKN | ✓ | ✓ | ✓ | ✓ | Distillation, titration* | Once a week |
| Alkaline | ✓ | ✓ | ✓ | ✓ | Titration method* | Three times a week |
| VFA | ✓ | ✓ | ✓ | ✓ | Titration method* and Gas chromatograph | Three times a week |
| SS and VSS | ✓ | ✓ | ✓ | ✓ | Dried 103-105 $^{O}$C Dried 550 $^{O}$C | Once a week |
| Biogas compounds | ✓ | ✓ | ✓ | - | Gas chromatograph | Three times a week |

*All analytical methods are followed to Standard Methods for the Examination of Water and Wastewater [207].

### 3.4 Effect of SRT on membrane fouling intensity in SAnMBR treating POME

Submerged anaerobic membrane bioreactors (SAnMBRs) treating palm oil mill effluents (POME) were analysed in terms of membrane fouling when working at three different sludge retention times (SRTs of 15, 30 and 60 d). The average permeate flux

was controled at 2.4 L/$m^2$·h. The duration time for each experiment was 90, 130 and 125 days for the three SRT values. Membrane fouling dynamics were analysed in terms of hydraulic resistances, observations of membrane surface (SEM, EDX, AFM and FTIR) spectroscopy as explained in topic 3.4.1-3.4.4 and biological suspension characteristics (SMP and EPS analysis) as shown in Figure 3.2. The result and discussion of the role of SRT on membrane fouling intensity in SAnMBR treating POME explained in chapter 7.

### 3.4.1. Hydraulic Resistances

The membrane fouling dynamics were quantified by TMP evolution measurement and calculation of the corresponding hydraulic resistance (Darcy's Law). When the TMP reached a critical value close to 125-130 mbars, the filtration was stopped and the membrane module was taken off from the SAnMBRs bioreactor to be rinsing with water. The filtration was shut down when the TMP reached a value close to 250 mbar (25kPa), and the membrane module was then taken off from the SAnMBR and and then three successive steps of cleaning (physical, backwash with water and chemical cleaning) were practiced.

### 3.4.2 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Spectroscopy

The fouled membrane module was taken out from SAnMBR at the end of each operation when the TMP reached about 125-130 mbar. Samples of virgin and fouled membranes were cut into small sizes at the end of each operation. The samples were fixed with 3.0% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2 for 2 h. The fixed samples were washed with buffer solutions three times taking 10 min for each washing series. Then samples were dehydrated in a graded ethanol series (10–30–50–70–90–100%) for 20 min at each concentration, and then dried for 5 h at 30 $^oC$ (modified method from Lin et al., 2009). Aqueous carbon used to fix the specimens onto SEM (JEOL JSM-5800 LV) mounts before gold splutter coating (30 mA for 2.5 min, vacuum 0.2 Torr). Additionally, the EDX analyzer (Oxford) was employed to investigate the inorganic components of the cake layer at the end of each operation.

### 3.4.3 Atomic Force Microscopy (AFM)

AFM imaging allowed the comparison of the surface roughness of clean and fouled membranes. An AFM analysis was used to get images of the membrane's top

surface. The images were obtained by using the Nano Scope IV AFM system (Digital Instruments, USA) operating in contact mode. Before AFM analyses, each membrane was dried by freezing. The membrane surfaces were imaged at a scan size of 25 μm × 25 μm. The membrane surfaces were characterized in terms of the mean roughness (Ra), Equation 3.1, root-mean-square (RMS) roughness ($R_q$), Equation 3.2 [41]. AFM imaging allowed the comparison of the surface of clean and fouled membranes.

$$R_a = \frac{1}{n}\sum_{i=1}^{n} z_i \quad (3.1)$$

$$R_q = \frac{1}{n}\sum_{i=1}^{n} z_i^2 \quad (3.2)$$

where $Z_i$ is height at point i, n is number of points in the image, and $Z_{max}$ and $Z_{min}$ are the highest and the lowest $Z$ values, respectively [42].

### 3.4.4 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared is the technique passing of IR radiation through a sample. Some of the infrared radiation is absorbed by the sample and some of it is transmitted. An infrared spectrum represents a fingerprint of a sample with absorption or transmission peaks which correspond to the frequencies of vibrations between the bonds of the atoms foulant. The fouled membrane module was taken and placed in a dryer at 70°C for 48 h to obtain dry foulants. The dry matter was analyzed by FTIR spectroscopy (Bruker EQUINOX 55). In this study, FTIR used to detect the major functional groups of biofouling and organic fouling in the cake layer. FTIR spectra of the fouled membranes were collected over the wave number range of 650–4000 $cm^{-1}$ using the ATR method. The instrument resolution was adjusted to 4.0 $cm^{-1}$ and the scan speed was 0.2 cm/s. prior to ATR-FTIR analysis, the clean and fouled membranes were dried overnight in a desiccator at room temperature.

### 3.5 Modelling the effect of OLR and SRT on the performance in start-up period and SAnMB treating POME using GPS-X

The biological treatment efficiency and biogas production data during the start-up period and SAnMBR treating POME were applied to simulate the relationship between the OLR and 3 SRTs by the ADM1 model. Before beginning simulation should be prepared three input data consists of (1) influent characterization, (2) operational condition and (3) kinetic parameter of anaerobic microorganism. These data were inserted to GPS-X software for simulating the ADM model. However, the model parameters, as displayed in section 2.5, had more complexity. So, this study aimed to focus on the evolution of organic matter in liquid phase to gas phase during growth and decay stages of four anaerobic microorganism processes (i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis).

The start-up period was conducted at organic loading rates (OLRs) of 4.79, 5.70, 7.08, 9.73, 19.18 and 28.59 kgTCOD/$m^3$/d. Submerged anaerobic membrane bioreactors (SAnMBRs) worked three different sludge retention times (SRTs of 15, 30 and 60 d). The results and discussion of modelling the effect of OLR and SRT on the performance in start-up period and SAnMB treating POME using GPS-X are given in Chapter 8.

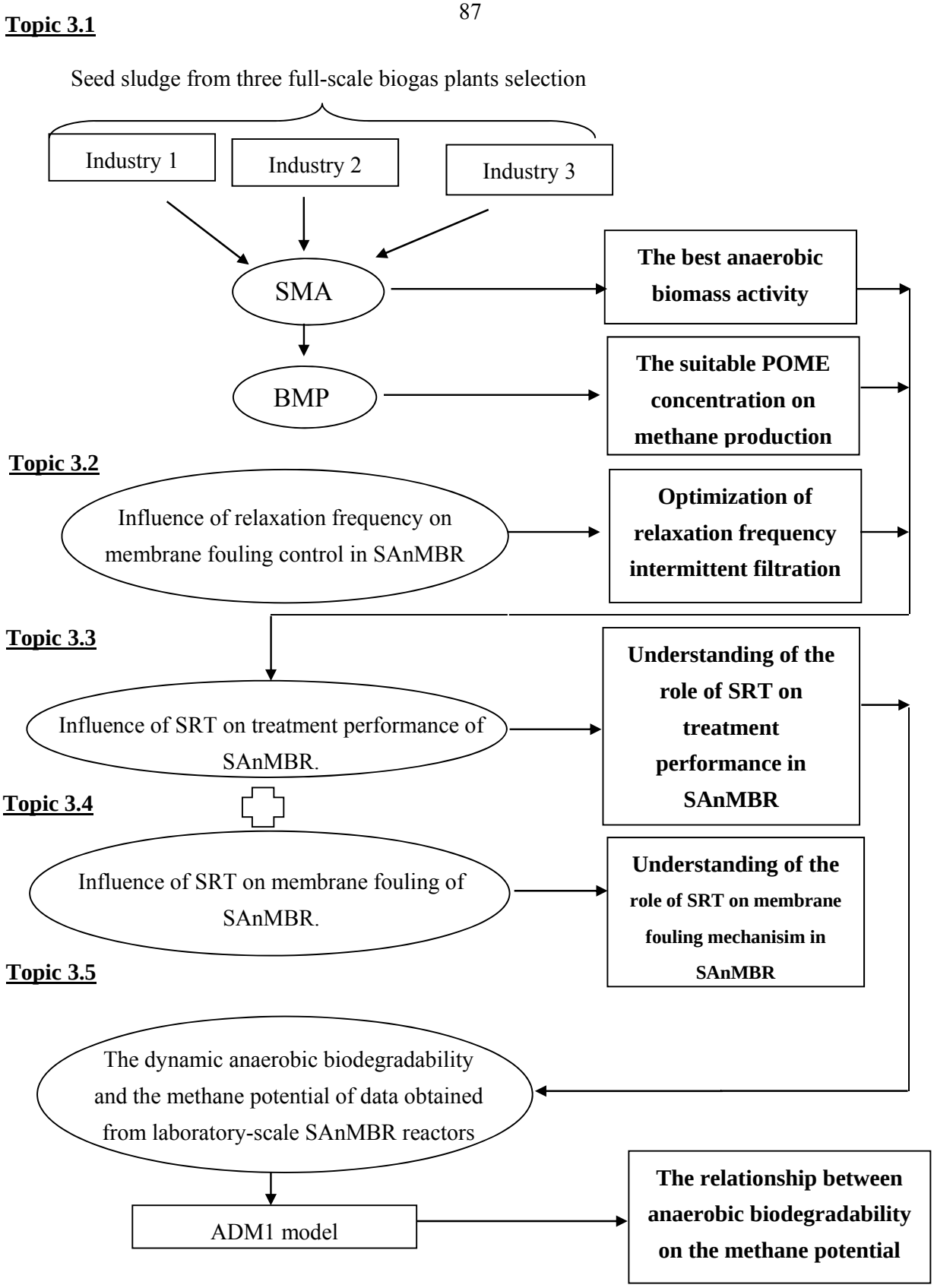



**Figure 3.3** Scope of all experiments.

# CHAPTER 4
# INVESTIGATION OF SPECIFIC METHANOGENIC ACTIVITY AND BIOCHEMICAL METHANE POTENTIAL IN PALM OIL MILL EFFLUENT

This chapter presents the effects of organic fraction and concentration on the methane production potential of palm oil mill effluent (POME) using the specific methanogenic activity (SMA) and biochemical methane potential (BMP) assay. It was investigated in batch tests maintained temperature at 35±1$^{o}$C and continuously mixed at 180 rpm. Cumulative methane production and methane yield data at four concentration levels of raw and filtrated raw POME were evaluated and fitted with gompertz equation.

## 4.1 Introduction

Palm oil mill effluents (POME) contain high values of organic matter, including suspended solids, oil and grease and nutrients [204]. They have a negative impact on microbial activities and wash out of active biomass when developing biological ways of treatment [44]. Palm oil industry wastewater is often discharged directly into rivers with a significant impact on the environment. Nevertheless, over the past decade, several anaerobic treatment technologies have been developed for the treatment of POME. There are important operating factors which affect the efficiency and performance of anaerobic digestion such as pH, temperature, hydraulic and solid retention times.

Generally, little attention has been paid the composition and activity of the microbial community compared to the conventional parameters during the operation of anaerobic reactors. However, an interdependent microbial community anaerobic rectors are highly sensitive to sudden changes in environmental any imposed stress may lead to a change in species types, their relative population levels and their activity, which are ultimately reflected in the rector performance [199]. Therefore, maintenance of active methanogenic populations in an anaerobic reactor is critical for stable performance [199]. Consequently, an understanding of both the microbial ecology and its activity are essential to operate the anaerobic reactors effectively. Therefore, it is necessary to determine the amount of active methanogenic populations in anaerobic reactors. In this respect SMA had been proposed

and offered more benefits, i.e. lower cost and skill required than that of the other methods. The activity assay is based on the measurement of methane production during digestion of known-amount of biomass and substrate in a mineral medium. This SMA assay is widely accepted for selection of the inoculums used in the startup of the process, determine the anaerobic sludge activity in various anaerobic processes and evaluation of the inhibitory potential or the degrees of degradability of various compound [199].

In addition, a more efficient alternative method to study the kinetics and efficiency of the anaerobic digestion process is needed. Owen et al. [179] proposed to quantify the biochemical methane potential (BMP) as a relevant method to evaluate the anaerobic treatability of organic wastewater. The BMP method is widely accepted as a standardized tool to determine the ultimate biodegradability (BD) of an organic substrate and its associated methane yield during anaerobic fermentation. Moreover, it can be applied to evaluate the maximum applicable loading rate or the inhibitory potential of specific substrates [55]. The BMP measurement can underline the attention of the dilution of food wastewater at 25% and 50% to induce positive effects on methane yield production [205]. Moreover, BMP measurements can allow the identification of influencing organic composition in wastewater on methane production, total acetate (over 375 mg/L) in food wastewater affecting negatively the ultimate practical methane yields [205]. Similarly, Labatut et al [62] investigated the biodegradability of complex organic substances from dairy manure using a BMP assay. Highly lipid and easily-degradable carbohydrates presented a higher methane yield than recalcitrant lignocelluloses [62]. Therefore, it requires a suitable concentration of substrate and inoculums to have an ideal balance in order to overcome biomass limitation and avoid organic matter overloading. Thus, a rapid and easily applicable method for estimating the optimal POME concentration for maximum $CH_4$ yield by BMP assay application is an interesting choice.

### 4.2 Research Objective

1. SMA assay was used to investigate the performance of methanogenic activity in 3 anaerobic sludges.

2. BMP assay was used to evaluate the optimum POME concentration by determining on methane production.

The best sludge and optimum POME concentration were selected for SAnMBR.

## 4.3 Materials and Methods

### 4.3.1 Palm Oil Mill Effluent (POME)

POME samples used in this study were taken from the clarification tank of a palm oil plant in Southern Thailand. They were stored at a temperature of 4$^{o}$C before testing with BMP assay. The characteristics of POME samples were analyzed by using parameters, such as pH, temp, TCOD, SCOD, BOD, total Kjeldahl Nitrogen (TKN), alkalnity, volatile Fatty Acids (VFA), total Solids (TS), suspended Solids (SS), and Oil and Grease, according to Standard Methods, as shown in Chapter 3.The characteristics of deoiled POME were given in Table 4.1. The deoiled POME is acidic with pH 4.07-4.56 and the temperatures approximately are 48–58 $^{o}$C.

POME used for BMP assays obtained from two sources of palm oil mill effluent: (i) Raw POME (ii) Filtrated raw POME (filtering the raw POME through GF/C pore size 0.45 μm filter paper, named soluble POME or filtrated POME). Raw POME and filtrated raw POME were diluted at different levels as 25%, 50%, 75% and 100% respectively.

**Table 4.1** Characteristics of deoiled POME from three industries.

| Parameters | Unit | Industry 1 | Industry 2 | Industry 3 |
|---|---|---|---|---|
| pH | - | 4.56±0.01 | 4.39±0.02 | 4.07±0.02 |
| Temp | $^oC$ | 58±2 | 48±3 | 56±3 |
| TCOD | g/L | 57.60±6.74 | 64.25±7.56 | 76.80±8.12 |
| SCOD | g/L | 20.40±2.35 | 18.80±1.85 | 28.80±2.37 |
| TCOD/SCOD | - | 2.82 | 3.40 | 2.67 |
| TKN | g/L | 0.72±0.12 | 0.71±0.11 | 0.82±0.14 |
| $NH_3$-N | g/L | 0.14±0.01 | 0.07±0.01 | 0.05±0.01 |
| Oil&Grease | g/L | 2.98±0.32 | 3.53±0.45 | 2.51±0.37 |
| TS | g/L | 71.39±5.12 | 69.47±5.54 | 77.42±5.15 |
| SS | g/L | 21.52±3.47 | 27.84±3.78 | 21.32±4.12 |
| Alkalinity | g/L as $CaCO_3$ | 1.18±0.23 | 0.95±0.56 | 0.97±0.42 |
| VFA | g/L as $CaCO_3$ | 5.32±3.12 | 4.66±3.76 | 4.435±3.75 |

Remark: All parameters were analyzed by following to the Standard Methods for the Examination of Water and Wastewater [206].

### 4.3.2 Specific Methanogenic Activity (SMA) Tests of Anaerobic Biomass

The biomass sludge from full-scale biogas palm oil plants was used as inoculums for the SMA and BMP tests. After collecting biomass sludge, they were incubated at 35$^o$C for approximately one week in order to reduce the biodegradable content in the sludge. The inoculum was pH in the range of 7.40-7.78, VSS (11,400 mg/L) and TSS (13,600 mg/L) are given in Table 4.2. MLVSS/MLSS ratio from industry 1 and 2 have in range of the generally of the MLVSS/MLSS ratio (0.7-0.8, as given in Table 4.2) except anaerobic seed sludge from industry3. If ratios below 0.55 mean that a large amount of inert non-biodegradable solids have accumulated in the system [207].

The SMA test was the practical method for anaerobic biomass activity evaluation. The SMA value derived was 0.37±0.002 g $CH_4$ COD/gVSS/d. This indicated that the seed sludge had good specific methanogenic activity [208]. Therefore, inoculums from palm oil plant were selected for the next BMP assay study.

**Table 4.2** Characteristics of inoculums of biomass sludge from three full-scale biogas plants.

| Parameters | Unit | Industry 1 | Industry 2 | Industry 3 |
|---|---|---|---|---|
| TS | g/L | 55.01±5.57 | 58.87±6.12 | 53.66±5.23 |
| TVS | g/L | 18.44±2.34 | 21.35±2.11 | 17.97±1.54 |
| SS | g/L | 16.67±1.67 | 17.66±1.55 | 17.23±1.68 |
| VSS | g/L | 13.12±1.14 | 12.67±1.12 | 11.95±1.09 |
| VSS/SS | - | 0.78 | 0.71 | 0.69 |

Remark: All parameters were analyzed according to Standard Methods for the Examination of Water and Wastewater [206].

### 4.3.3 Kinetic Analysis

A modified gompertz equation, as shown in Eq. (4.1), was applied for the kinetic parameters calculation for the different dilution levels of TCOD and SCOD concentrations on the cumulative methane production in the BMP assay.

$$H(t) = P \times exp\left\{-exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right\} \quad (4.1)$$

Where H(t) is cumulative methane production time (ml)

P is methane production potential (ml)

$R_m$ is maximum methane production rate (ml/day)

t is fermentation time (h)

e is exp (eq 4.1) = 2.71

λ is the lag phase or fermentation time (day)

The three parameters P, $R_m$ and λ were estimated by using a nonlinear regression fitted to the experimental data using the solver function in Excel (version 2007, Microsoft) with a Newtonian algorithm.

### 4.3.4 Experimental Set-up

In this study, the anaerobic biomass was collected from three full-scale biogas plants. These biomasses were investigated by SMA and BMP assays, which was the selection of biomass and POME concentrations for the next experiments in SAnMBR.

#### 4.3.4.1 Specific Methanogenic Activity (SMA) Assay

The SMA test is the practical method for anaerobic biomass activity evaluation, which was carried out following the previous report [8] in Figure 4.1. This testing used 300 ml serum bottles with acetic acid as substrate and try out with triplicate samples for each industry. Each serum bottle contains seed 4 g/l of volatile solids (VS), 15 ml of nutrient solution, and 2.5 ml of 1 M acetate acid. In control bottle, it was added only with biomass without substrate. After adjusting pH to 7 by 5 M of $NaHCO_3$, the final volume was adjusted to 200 ml by using deionized water. Oxygen in the liquid was purged by $N_2$ gas mixture for 5 min. Then serum bottles were sealed with butyl rubber stoppers and incubate in a shaker at 180 rpm and temperatures of 35 $^oC$. The composition of this nutrient and trace element solution are as follows:

- Nutrient solution **:** $NH_4Cl$ 1.4 g/L; $K_2HPO_4$ 1.25g/L; $MgSO_4 \cdot H_2O$ 0.5 g/L; $CaCl_2 \cdot 2H_2O$ 0.05 g/L; yeast extract 0.5 g/L; trace element solution 5 ml/L
- Trace element solution: $FeCl_2 \cdot 4H_2O$ 2 g/L; $H_3BO_3$ 0.05 g/L; $ZnCl_2$ 0.05 g/L; $CuCl_2 \cdot 2H_2O$ 0.04 g/L; $MnCl_2 \cdot 4H_2O$ 0.5 g/L; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.05 g/L; $AlCl_3 \cdot 6H_2O$ 0.09 g/L; $CoCl_2 \cdot 6H_2O$ 2 g/L

#### 4.3.4.2 Biochemical Methane Potential (BMP) Assay

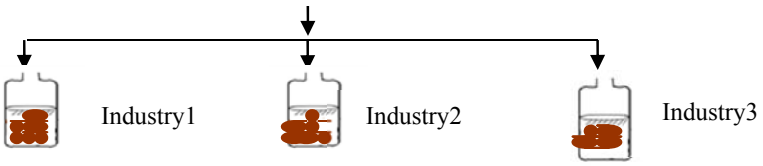
After the collection of POME samples, they were stored at 4 $^oC$ before usage. The BMP assay was set up to determine the methane potential of POME at different concentrations. The experiments were carried out in batch conditions, using 300 mL glass bottles and 200 mL working volume. The method of BMP test was carried out as previously reported [54] in Figure 4.2.

This experiment was also conducted in triplicate. In each bottle, 100 mL of seed contained 10 g/l volatile solids (VS) at diluted levels of 25%, 50%, 75% and 100% of raw wastewater and filtrated wastewater concentrations of POME. The control bottle was prepared by containing only seed volume. After adjusting pH to 7 by 5 M of $NaHCO_3$, the final volume was adjusted to 200 ml by using deionized water. The bottle headspace was

flushed with $N_2$ for approximately 5 min and then closed with rubber stopper. The reactors were manually mixed every day and maintained at constant temperature at mesophilic conditions (35 $^{o}$C) and stirring rate 180 rpm.

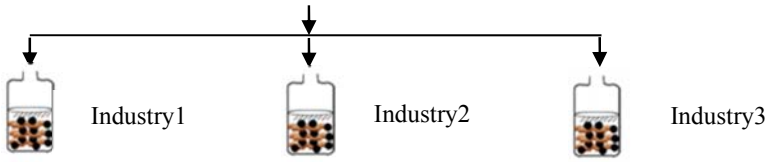
Step 1

Adding 100 ml of seed sludge (4 g/L VS) was incubated 1 week at 35 ºC



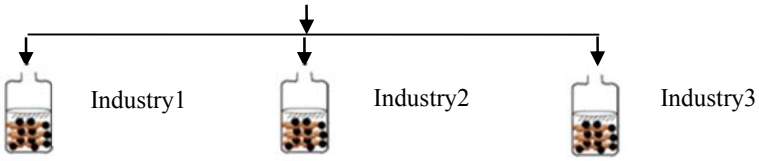

Step 2

Adding 15 ml of nutrient solution, and 2.5 ml of 1 M acetate acid



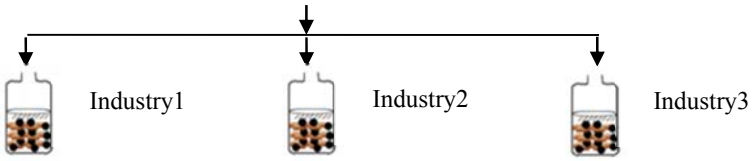

Step 3

Adjust the pH of samples to 7-8 using diluted (0.1 M) $NaHCO_3$



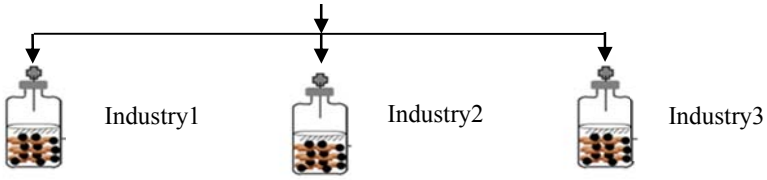

Step 4

Flush with nitrogen gas for approximately 5 min




Step 5

Close the septum and three way value




Step 6

Incubated at 35 ºC, stirring rate at 180 rpm and measured the volume of biogas every 1-2 hours, analyzed biogas composition by gas chromatography

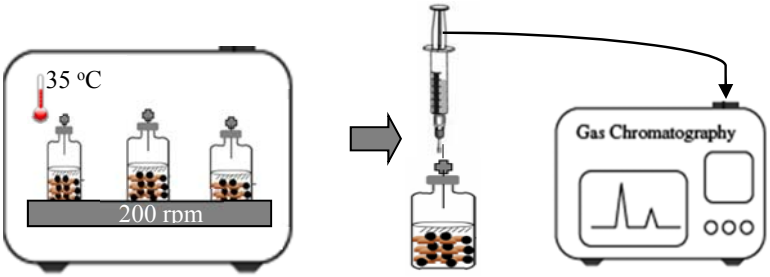



**Figure 4.1** Method of SMA assay.

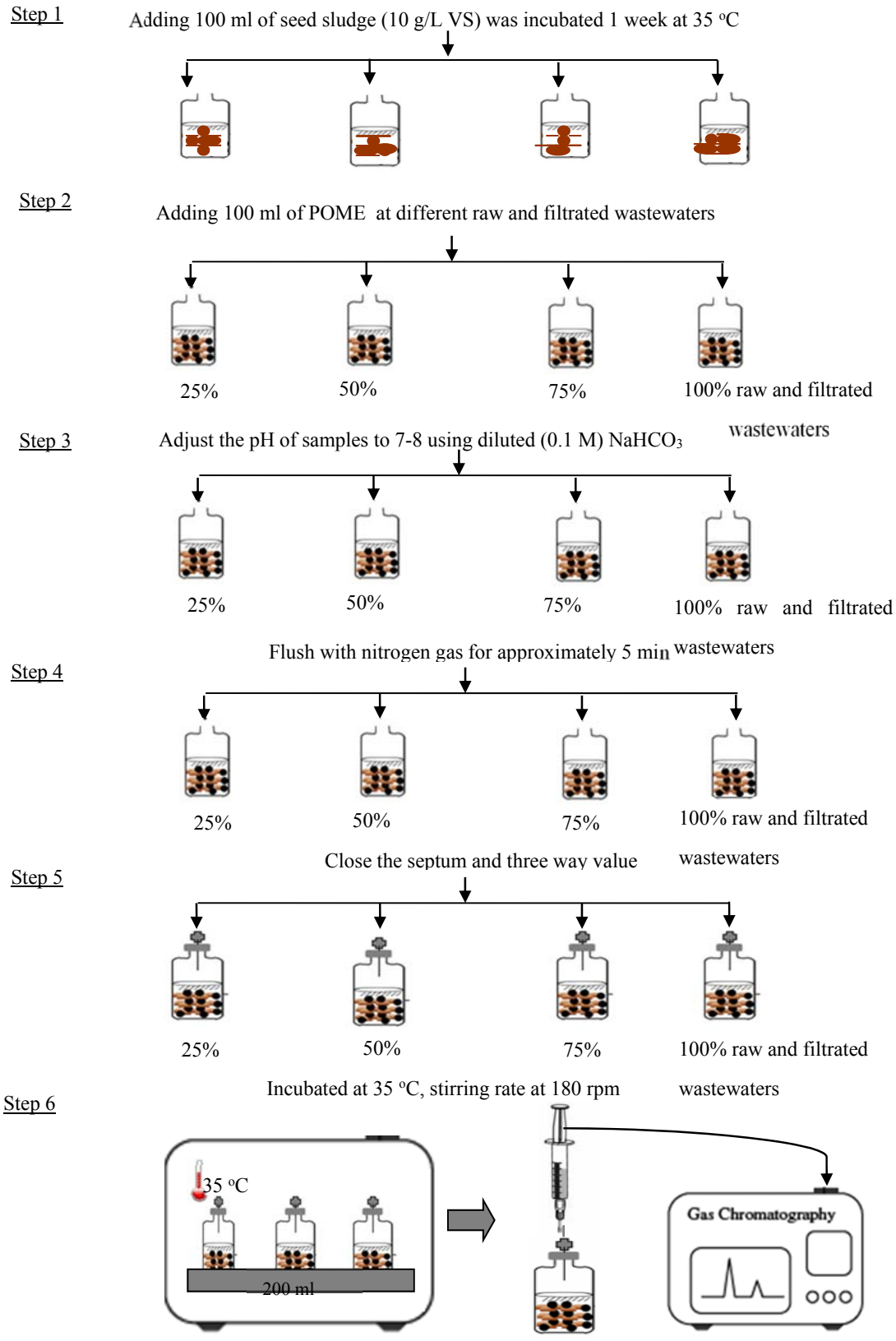



**Figure 4.2** Method of BMP assay.

## 4.4 Results and Discussion

### 4.4.1 Palm Oil Mill Effluent Characteristics of Three Industries

The raw POME appeared as an acidic wastewater with low pH value 3.9-4.3, high biochemical oxygen demand (30.87 ±9.25 g/l), chemical oxygen demand (64±10.24 g/l), oil and grease (21.99±6.75g/l), total solids (46.47±7.63 g/l), nitrogen content (0.85±0.21 g/L) as total nitrogen and discharge temperature of 80–90 $^{o}$C. Nevertheless, though it was complex, it appeared biodegradable with a BOD/COD ratio close to 0.5 [208]. Moreover, the raw POME consisted of varying amounts of LCFAs such as Palmitic acid (16:0) 32.18%, Oleic acid (18:1) 35.4 %, Heptadecanoic acid (17:0) 17.49 % and Linoleic acid (18:2) 10.62 %.

### 4.4.2 Selection of Active Inoculums

This experiment was a primary test to determine and select the best active inoculums from the three industries in which SMA assay was used as the method selection for the best active inoculums. The results of this experiment are shown in Figure 4.3.

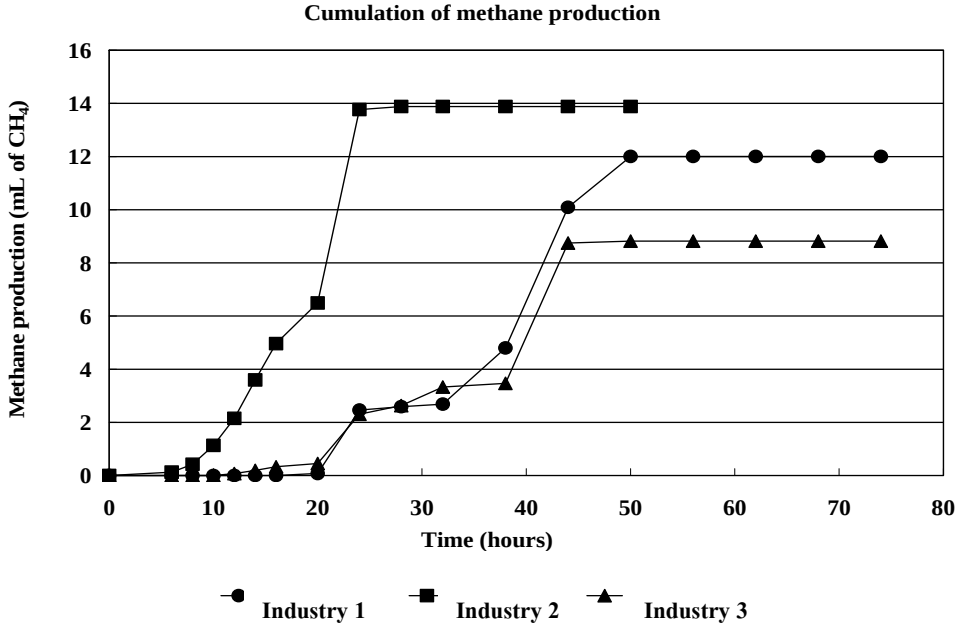



**Figure 4.3** Cumulative methane productions during SMA assay.

Figure 4.3 shows the cumulative methane production on the degradation of acetate acid. It was found that the seed sludge from the biogas plant of industry 1 had initial methane productions higher than values from industries 2 and 3. Moreover, the seed sludge from industry 1 also had a maximum methane production (39.05±0.17 mL of STP $CH_4$) higher than the other industries (industries 2 and 3 were 31.74±0.21 and 29.06±0.16 mL of STP $CH_4$, respectively). This was due to seed sludge from industry 1 having a VSS/SS ratio 0.78 (Table 4.2), which was higher than seed sludge from industries 2 and 3 (0.71 and 0.69, respectively). In theory, the VSS/SS ratio implied the elementary quantity of microorganisms. If VSS/SS ratio is high, the biomass in the system has more concentration.This could degrade the acetic acis as substrate when SMA was tested. This indicated that the seed sludge from industry1 had a good SMA. Therefore, the seed sludge from industry 1 was selected to evaluate the inhibition POME concentration by BMP assay, as in section 4.4.3.

### 4.4.3 Evolution of COD Removal Efficiency

In this study, the final values and removal efficiency of COD in different concentration levels of raw POME and filtrated raw POME were measured and summarized in Table 4.3. It was observed that the COD removal efficiency increased when increasing COD concentrations in raw POME from 62 to 70 % and filtrated raw POME from 70 to 83% which indicates that methanogenic bacteria is very effective. The filtrated raw POME had a higher COD removal efficiency than the raw POME tested because the biofibers were removed in the filtration process which was more recalcitrant in raw POME**.**

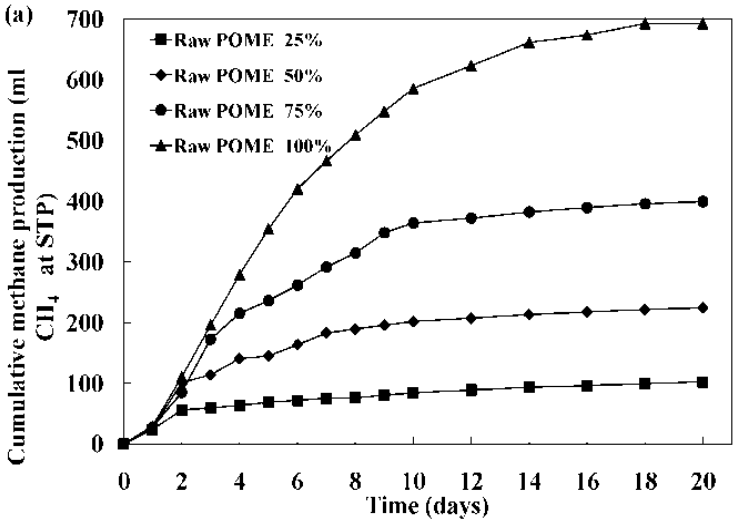
Labutut et al [62] reported that methane productivity based on the amount of COD removed should be in conjunction with substrate (COD) removal. Therefore, the filtrated raw POME was able to provide a higher methane yield than did the raw POME.

**Table 4.3** COD removal efficiency at different concentration levels of raw and filtrated POME.

| COD | Concentration levels | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Raw POME | | | | Filtrated raw POME | | | |
| | 25% | 50% | 75% | 100% | 25% | 50% | 75% | 100% |
| Influent (g/L) | 19.08 ±1.56 | 25.20 ±1.72 | 41.76 ±3.45 | 63.36 ±5.23 | 10.10 ±1.46 | 15.84 ±1.15 | 19.44 ±1.37 | 29.52 ±1.93 |
| Effluent (g/L) | 7.25 ±0.56 | 9.24 ±0.71 | 14.15 ±0.83 | 18.96 ±0.95 | 3.25 ±0.28 | 3.95 ±0.32 | 4.50 ±0.24 | 4.90 ±0.37 |
| % removal | 62 | 63 | 66 | 70 | 70 | 75 | 77 | 83 |

### 4.4.4 Methane Production

Figure 4.4 shows the cumulative methane production as a function of time at different concentration levels (25%, 50%, 75% and 100%) of raw POME and filtrated raw POME. The peaks of raw POME and filtrated raw POME at concentration levels higher to 50% demonstrated that methane was produced immediately during the first week of the digestion time. After that, the peaking trend was stable or had slightly changed with time. This can be explained and linked to the activity of microorganisms which could adapt very fast and easily degraded soluble organic fraction in POME at the beginning of the fermentation period.



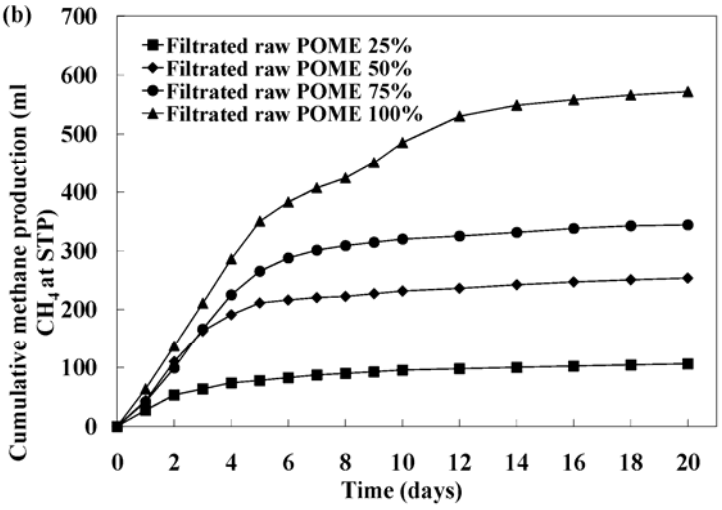



**Figure 4.4** Methane rate evolution with time: (a) raw POME and (b) filtrated raw POME.

During batch fermentation, it was found that up to 75% of raw POME concentrations were able to provide higher methane production than could the filtrated raw POME for all concentration levels. Similar results from Liu et al [57] reported higher methane production at high substrate concentration than that obtained from low substrate concentration due to the difference in the chemical composition (organic carbon, nitrogen, and oil and grease). In addition, the volume of cumulative methane production after 10 days of digestion increased with rising substrate concentrations. The maximum cumulative methane production, 692 ml. $CH_4$ STP, was obtained at concentration levels of 100% of raw POME. This result was similar to the results obtained by Raposo et al [55] who showed that an increase of the maximum methane production was observed when substrate loading increased in the system.

### 4.4.5 Methane Yield

Figure 4.5 shows the methane yield (at STP) during BMP assays with raw and filtered raw POME. Methane yield is described as the amount of methane produced for a given quantity of organic matter removed. During the degradation of POME, methane yield rapidly increased when POME concentration was up to 50% for raw and filtrated wastewater. Indeed, high dilution of wastewater might have had an effect on the chemical composition (organic carbon, nitrogen, and oil and grease), which did not appear efficient

according to microorganism requirements. Similar results were reported by Liu et al. [57] when higher methane yields were observed at high substrate concentrations due to the differences in the chemical composition (organic carbon, nitrogen, and oil and grease). Conversely, Raposo et al. [55] indicated that methane production yields decreased with increasing substrate concentrations. Altamira et al. [205] found that wastewater concentration up to 50% had a positive effect on methane yield but a negative one when wastewaters were undiluted during fermentation.

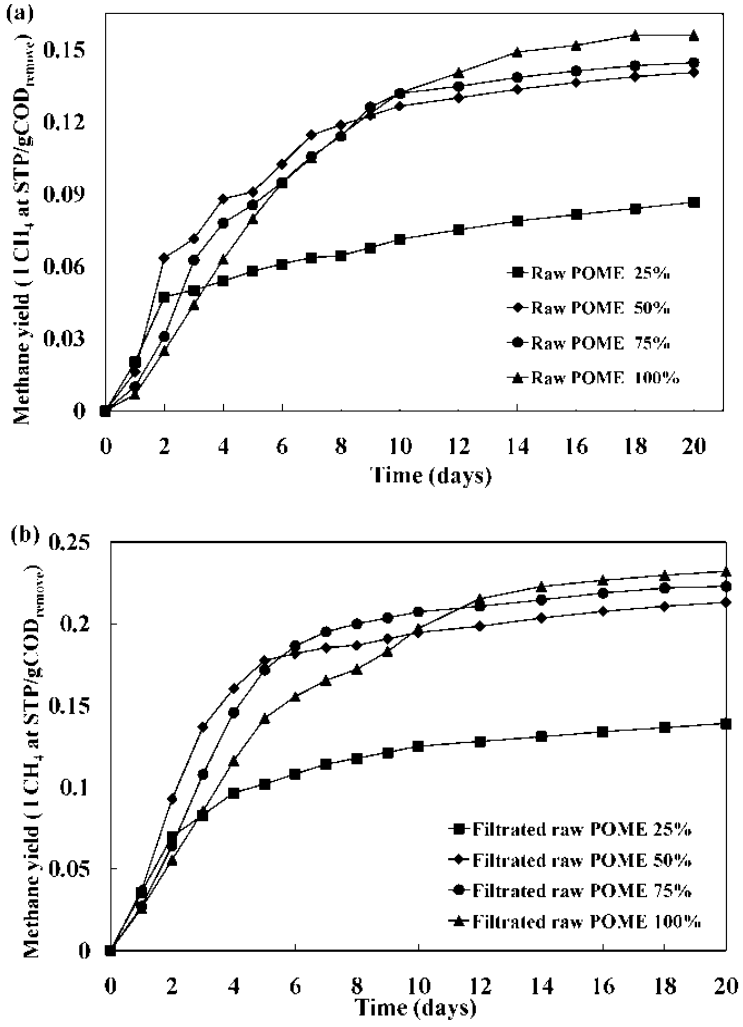



**Figure 4.5** Methane yield evolution with time: (a) raw POME wastewater concentrations and (b) filtrated raw POME.

In this study, the highest methane yield was found at concentration levels of 100% filtrated raw POME with a value of about 0.23 LSTP $CH_4$/g COD $_{removed}$. However, the methane yield of filtrated raw POME present had a higher methane yield as compared to raw POME (0.15 L STP $CH_4$/g COD $_{removed}$), because the biofibers had been removed in the filtration process, which were more recalcitrant in raw POME**.** In addition, this was because the microorganisms prefer to consume most of the soluble organic fractions in filtered raw POME while some of the insoluble fractions in it could contain recalcitrant organics such as long chain fatty acids (LCFA) [69]. In this study, the oleic (C18:1) and palmitic (C16:0) acids were the main LCFA in the raw POME used. Lalman and Bagley [209] reported that 0.2 g/L of oleic acid and 0.3 g/L stearic acid had some acute toxic effect on anaerobic microbial activity affecting both aceticlastic and hydrogenotrophic methanogens. The hydrogenotrophic methane-forming bacteria were inhibited and the hydrogen pressure to increase in the anaerobic digester. The increase in hydrogen pressure inhibited acetogenic bacteria. This resulted in a decrease in acetate production and consequently a decrease in methane production by aceticlastic methanogens [209].

In theory, each gram of removed COD induced about 0.35 L STP $CH_4$/g COD $_{removed}$ and generally methane constituted approximately 70% of the biogas due to its low solubility in water as compared to $CO_2$. In our experiment, the methane content in biogas was approximately 40-50%. Consequently, the methane yield was very low (0.08-0.16 and 0.14-0.23 L STP $CH_4$/g COD $_{removed}$ of raw POME and filtered raw POME respectively) compared to the theoretical methane yield (0.35 L STP $CH_4$/g COD $_{removed}$). This could have been due to: (1) the high degree of alkalinity chosen in this study that was able to generate some $CO_2$ inhibition due to the excess of $CO_2$ in the mixed liquor, and (2) the specific characteristics of POME. Indeed, the POME composition showed high cellulose, hemicelluloses and LCFA concentrations which could inhibit methanogenic bacteria activity. This led to reduced methane production even though operating at high COD removal. (3) better performances for filtrated raw POME. Their characteristics showed that oil and grease content was less in filtrated raw POME compared to raw POME as indicated (for a concentration coefficient of 100%). In addition concentration of lipids up to 5% (w/v) inhibited the dynamics of hydrolysis of microorganisms [210], and the oil and grease fraction in raw wastewater was 4 times higher than in the filtrated raw POME fraction.

The maximum methane yield appeared to be four times lower than the value reported by Fang et al [69]. This could have been due to using BMP under thermophilic conditions, which presented reaction rates much faster than under mesophilic conditions, enabling increased bacteria growth rates and activity [105]. Moreover, a number of papers reported that higher methane yield was observed in large scale anaerobic processes under continuous operation when using POME as substrate. Najafpour et al [34] showed that using upflow anaerobic sludge-fixed film (UASFF) treating POME could produce a methane yield of 0.34 L $CH_4$/g $COD_{removed}$. Faisal and Unno also reported high methane yields (0.32-0.42 L $CH_4$/g $COD_{removed}$) when using a modified anaerobic baffled bioreactor (MABR) treatment. In SAnMBR treating various wastewaters, such as domestic, slaughter house and landfill leachate, the methan productions that revealed in previous studies were in range 0.2–0.4 L $CH_4$/g $COD_{removed}$ [17-28]. Therefore, methane yield can also be enhanced in full scale operation when operating in a Submerged Anaerobic Membrane Bioreactor (SAnMBR). In addition, the methane production and yield from BMP test was also to compare with the results from SAnMBR treating POME, as shown chapter 6, topic 6.4.2.7.

### 4.4.6 Kinetic Modeling of Cumulative Methane Production in BMP Assay

In order to evaluate the effects of different concentration levels (25%, 50%, 75% and 100%) of raw POME and filtrated raw POME, the experimental data obtained from cumulative $CH_4$ production at different COD concentrations according to different concentrations of feed sample were fitted to a modified gompertz equation, as shown in (Figure 4.6) which illustrates methane production for the different experiments. The cumulative methane production appears as a function of time at different concentration levels (25%, 50%, 75% and 100%) of raw POME and filtrated raw POME.

This methane production appeared proportional to the dilution coefficient and no significant differences appeared between the raw POME and filtrated raw POME that was studied. The volume of cumulative methane production after 10 days of digestion increased with an increasing of substrate concentrations. The maximum cumulative methane production, 556.32 mL $CH_4$ STP, was obtained at a concentration level of 100% of raw POME. This result is similar to the results obtained [55].

The use of the gompertz equation to analyze the results of cumulative methane production is illustrated in Figure 4.6 and Table 4.4. The relation between theoretical modeling and experimental data was well fitted for the different assays ($R^2$>0.95). Nopharatana et al [211] reported that the parameter values (P, λ and $R_m$) from the gompertz equation was significant at a confident interval of 95%. For this study the modified gompertz equation appeared acceptable for simulating cumulative methane production.

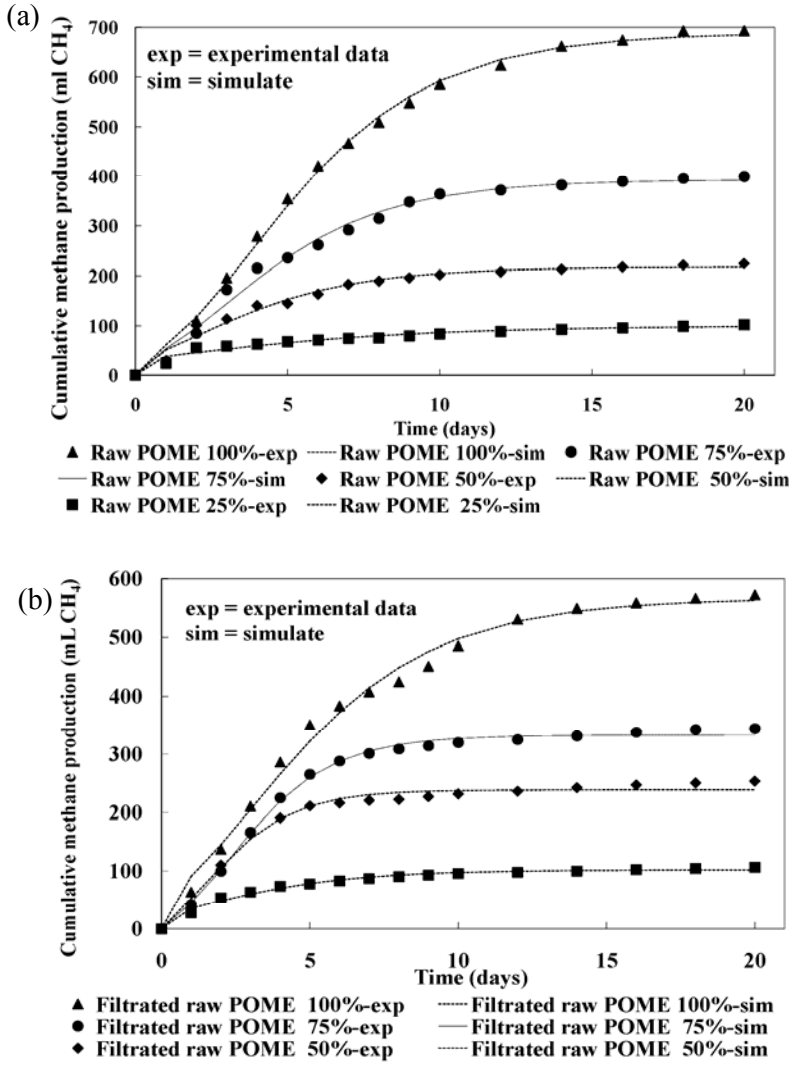



**Figure 4.6** Cumulative methane production estimated by the modified gompertz model; (a) raw POME and (b) filtrated raw POME.

**Table 4.4** Kinetic parameters of cumulative methane production at different concentration levels of raw POME and filtrated raw POME estimated by the modified gompertz model.

| Parameters | Concentration and levels | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Raw POME | | | | Filtrated raw POME | | | |
| | 25% | 50% | 75% | 100% | 25% | 50% | 75% | 100% |
| $P_{CH4}(t)$ ($mLCH_4$) | 102.45 | 224.30 | 399.32 | 692.71 | 106.34 | 253.31 | 344.16 | 571.86 |
| $P_{Max}$ (mL $CH_4$) | 100.33 | 217.50 | 393.26 | 689.46 | 101.98 | 238.47 | 332.89 | 566.25 |
| $R_{Max}$ (mL $CH_4$/day) | 7.67 | 27.36 | 49.38 | 77.96 | 13.04 | 55.07 | 59.68 | 61.37 |
| λ (day) | 0.28 | 0.51 | 1.83 | 4.04 | 0.07 | 0.15 | 0.21 | 0.38 |
| COD concentration (g/L) | 19.08 ±1.56 | 25.20 ±1.72 | 41.76 ±3.45 | 63.36 ±5.23 | 10.10 ±1.46 | 15.84 ±1.15 | 19.44 ±1.37 | 29.52 ±1.93 |

The kinetic parameters obtained from this modeling analysis are summarized in Table 4.4. The results showed that all criteria described bacterial activity had increased with the organic carbon concentration in the wastewater studied. In terms of maximum methane potential, it increased approximately seven-times (from 100.33 to 689.46 mL $CH_4$) and about ten-times in terms of the maximum methane rate (from 7.67 – 77.96 mL $CH_4$/d) when the concentration of raw POME increased from 19.08 to 63.360 g/L (the concentration level ofraw POME increased from 25 to 100%) . Similarly, a six-times increase of the maximum methane potential (from 101.98 to 566.25 ml $CH_4$) and a five-times increase in terms of the maximum methane rate were also found (from 13.04- 61.37 mL $CH_4$/d) when the concentration of filtrated raw POME was increased from 10.10 to 29.52 g/L (the concentration level offiltrated raw POME increased from 25 to 100%) . The above-mentioned results, as well as other related research reports showed that the methane potential (P) and maximum methane rate ($R_m$) increased with increasing COD concentrations in the substrate [211-213]. This implied that the quantity of substrate loaded was not limited by microbial activity or a specific rate of methane production in these experiments. This information obtained illustrated that microbial activity was capable of tolerating high COD concentrations (63.36 g/L) in batch tests. Therefore, follow-up

studies could be employed in the high OLR of continuous flow in a larger scale anaerobic digester for the treatment of palm oil mill effluent (POME) without affecting methanohenic activity.

Moreover, when considering the lag phase times (depending on the anaerobic-microorganisms adaptation to substrate concentration and environmental factors), the small values indicate that the bacteria had some good adaptations to the substrate. In this study, all experiments had a short lag phase time (5-12 hr) for concentration levels of 75 and 100%. These results were similar to the previous report of O-thong et al [61], which found and described the lag phase time of anaerobic digestion sludge from palm oil mill plants in the range of 5-10 hr. The concentration levels at 25 and 50% of raw POME and filtrated raw POME experiments presented negative values for lag phase time. Such results indicated that anaerobic microorganisms did not need acclimatization with substrate at low substrate concentrations (10.10-25.20 g/L). In addition, all dilutions of filtrated raw POME had a shorter lag phase time than raw POME samples. These results demonstrated that microorganisms presented more facility to utilize the substrate in filtrated wastewater than in raw wastewater.

### 4.5 Conclusion

Anaerobic fermentation of POME was analyzed in batch fermentation using different dilutions of the raw and the filtrated raw POME. The data obtained by BMP assays show the biodegradability of such wastewater. In addition, this data shows the performance of anaerobic digestion to remove organic matter and protect the environment but also to produce biogas. The use of the gompertz equation allowed the calculation of kinetics parameters. Nevertheless, the experimental conditions did not allow the obtaining of high ratios of methane production in comparison with COD removal efficiency. This is due to POME characteristic with high strength organic wastewater such as suspended solid, oil & grease and TCOD resulting in long sludge retention time to degrade organic matter. This point must be improved in the future research in a bench scale or full scale anaerobic membrane bioreactor (AnMBR). Furthermore, AnMBR converts the organic matter to methane gas that could be used as energy source. On the other hand, since it is

coupled with membrane separation technology, AnMBR retains sludge perfectly in the reactor and produces high quality effluent without suspended solids, which is impossible to achieve in conventional anaerobic biological treatment processes [25].

# CHAPTER 5
# INFLUENCE OF RELAXATION FREQUENCY ON MEMBRANE FOULING CONTROL IN SUBMERGED ANAEROBIC MEMBRANE BIOREACTOR (SAnMBR)

This chapter presents the effects of different intermittent filtration modes on membrane fouling, which was investigated in a submerged anaerobic membrane bioreactor (SAnMBR) treating palm oil mill effluent (POME). The filtration was operated in the range of supra critical conditions (permeate flux equal to 20 $L/m^2h$), and the submerged membranes were continuously cleaned by gas injection and intermittent periods of relaxation. Four conditions of relaxation (S1: 240 s filtration /30 s relaxation, S2: 480 s filtration /30 s relaxation, S3: 720 s filtration /30 s relaxation and S4: 960 s filtration /30 s relaxation) were analyzed by comparing the trans-membrane pressure TMP evolution rates, the main fouling origins in terms of proteins and carbohydrates.

## 5.1 Introduction

In recent years, submerged anaerobic membrane bioreactors (SAnMBRs) have appeared as an increasingly interesting solution for the treatment of municipal, as well as many kinds of industrial wastewaters, because they present numerous advantages over the conventional anaerobic treatment processes. The characteristics of POME are high concentrations of oil and grease, organic matter, suspended solids (SS), protein and polysaccharide [1]. This would induce the cake layer, which is the dominant effect on membrane permeability variations. It was then proposed (i) to study the influence of intermittent filtration frequency on filtration step control, and (ii) to quantify the main origin of membrane fouling.

Indeed the use of membranes to separate the biomass from the effluent can maintain a high concentration of microorganisms in SAnMBR, resulting in a highly efficient removal (more than 90% of biodegradable organic matter and total removal of suspended solids SS), including the recovery of renewable energy sources (0.37l $CH_4$/g $COD_{removed}$). Moreover, the solid liquid phase separation by porous membranes avoids any

wash out of biomass even under extreme conditions (elevated temperatures, pH, high content of organic pollutants and salt) as indicated by Jeison *et al,* (2008)[25], Gao *et al.*(2010)[85] and Abdurahman *et al.*(2011) [27]. Thus SAnMBR can maintain a high solid retention time (>100 d) coupled with high loading rates, which results in less sludge production and HRT shortening [214, 215].

In addition, the filtration through porous membranes not only produces better effluent quality, including disinfection in relation to water reuse applications, such as washing, agricultural irrigation and power plant cooling, but also eliminates the necessity of large clarifying basins to settle out the biomass [71, 216, 217]. Therefore, SAnMBR appears as an intensive process with regard to conventional anaerobic digesters [218].

However, the control of membrane fouling during operations remains the main challenge with SAnMBRs. In filtration, many compounds present in suspension are retained by the membrane and interact with the membrane material, then membrane permeability is drastically modified obliging the use of energy to maintain it at a sustainable level (gas bubbling, backwashing, chemical consumption) and the intensity and frequency of membrane cleaning can significantly affect membrane life-time[115, 219, 220]. Membrane fouling can be attributed to different reversible and irreversible mechanisms such as concentration of polarization, cake formation, pore blocking and adsorption of foulants [221]. For SAnMBR applications, the cake layer formation has been identified as the predominant origin of fouling [222]. According to the filtration conditions, sub or supra critical conditions, the cake layer formation is mainly due to (i) EPS adsorption and individual bacteria deposition onto the membrane surface, after which the cells multiply and form a cake layer as biofilm [222] and (ii) accumulation of suspended solids until a structured deposit is formed which can be progressively compressed, dewatered and made denser causing some high hydraulic resistance and rapid flux decline or trans-membrane pressure increase [204, 222, 223].

Various techniques are used to reduce fouling. Basically, the fouling of membranes in MBR systems can be minimized by the reduction of flux, promotion of turbulence to limit the thickness of the boundary layer, and/or periodical application of cleaning measures to remove the cake layer and foulants [31]. At present, the operating with intermittent filtration mode (filtration-relaxing cycle) has been further attention in term of

retarding fouling and reducing energy consumption [233-234]. It has been reported that the filtration and relaxation times have strongly effects on retarding TMP increase and reducing fouling resistance, respectively [224]. In filtration time, the previous researches recommended that should not be short than 440 s [31], while relaxation time was in range of 30-60 s [225]. However, Wu et al. [31] concluded that long and frequent relaxation would cause severse fouling due to the relactively high instantaneous flux. Thus, the intermittent modes in this study were operated at various filtration times with relaxation time at 30 s.

However, this strategy cannot directly be applied, and still has some limits. This due to several differences between anaerobic and aerobic biomass such as the high mixed liquor suspended solid (MLSS) concentration in the reactor, the size reduction of the biomass and the size distribution of bio-solid particles. These biomass characteristics affect the mechanical sheer stress, the operating conditions, and the inorganic precipitates generated during anaerobic digestion, which is a different mechanism of membrane fouling [215]. There is a need of more research to investigate and apply intermittent filtration technique influencing membrane fouling in SAnMBR. While, the development of design and operation strategies should be answered for fouling control in SAnMBR.

To minimize cake structuring during the operation, complementary techniques were developed in SAnMBRs, such as biogas injection or biogas recirculation, as a replacement for air bubbling in the aerobic SMBR [219, 226]. Also, since continuous filtration could induce a denser and more compressed cake layer, the combination between biogas injection and intermittent permeation mode appeared of great interest as it offered a potential solution to favor membrane fouling reduction and energy saving [18, 19, 30, 83, 227].

## 5.2 Research Objectives

1. The influence of intermittent filtration frequency on the filtration step control in SAnMBR.
2. To quantify the main origin of membrane fouling.

## 5.3 Materials and Methods

### 5.3.1 Experimental Set-up

The lab scale SAnMBR reactor used was composed of a 5 L chamber equipped with an immersed hollow fiber module, as shown in Figure 5.1. The reactor was filled with a biological suspension coming from a full-scale biogas plant in industry1. After collecting the biological suspension, it was incubated at 35$^{o}$C for approximately 1 week in order to reduce the biodegradable content of the sludge before experiments. The characteristics of the suspension are given in Table 5.1.

**Table 5.1** Characteristics of the anaerobic suspension in the SAnMBR

| Parameter | Range and Macromolecular types | | |
|---|---|---|---|
| MLSS (g/L) | 16.7 ± 2 | | |
| MLVSS (g/L) | 10.5 ± 2 | | |
| Macromolecular in Sample | Protein | Carbohydrate | Protein/ Carbohydrate (PN/PC) |
| Mix liquor (mg/L) | 547.6±55 | 293.4±65 | 1.9 |
| SMP (mg/L) | 130.2±4.3 | 63.9±2.5 | 2.0 |
| EPS (mg/L) | 190.4±16.4 | 92.3±3.7 | 2.1 |

The membrane module equipped with the PVDF hollow fibers was located at the upper part of the reactor. The hydrophilic polyvinylidene fluoride (PVDF) membranes had a nominal pore size of 0.1 μm (obtained from Shanghai Jofur Advanced Materials Co., Ltd, China). The effective filtration area was 0.1 m$^2$. A diffuser providing nitrogen bubbles was set up at the bottom of the membrane module. Permeate was collected from the SAnMBR at a constant flux by using a peristaltic pump and it was recycled into the bioreactor to work at constant MLVSS concentration. The trans-membrane pressure was continuously monitored by a pressure transducer set up on the permeate side of the membrane module. A computer with LabView (National Instruments, Austin, USA) was used to record the TMP data in real time.

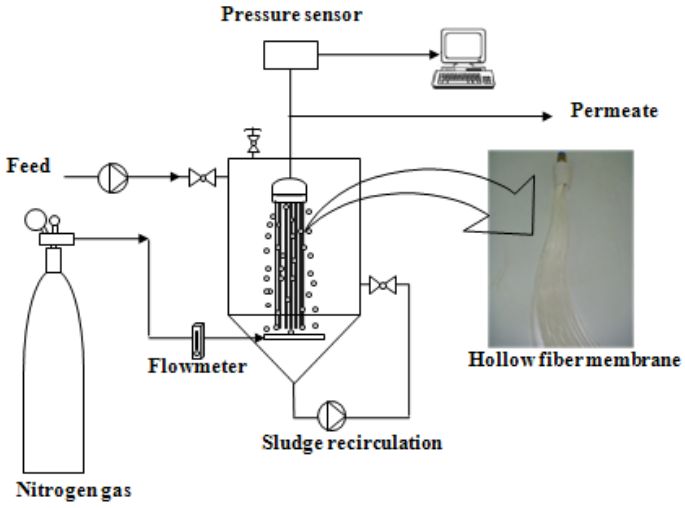



**Figure 5.1.**Schematic diagram of the laboratory scale submerged anaerobic membrane bioreactor.

### 5.3.2 Operating Conditions

To highlight the importance of the cake deposit on membrane fouling rates, the membrane module was operated in the range of supra-critical conditions (about 20 $L/m^2h$) according to Jeison and van Lier et al.[20].The filtration was shut down when the TMP reached a value close to 250 mbar (25kPa), and the membrane module was then taken off from the SAnMBR and cleaned according to a specific procedure (see analytical methods) to identify the main origins of membrane fouling.

In order to minimize membrane fouling, experiments were operated for four short-term intermittent filtration modes, including a relaxation period of only 30 s (without anybackwashing): S1: 240 s filtration /30 s relaxation, S2: 480 s filtration /30 s relaxation and S3: 720 s filtration /30 s relaxation and S4: 960 s filtration /30 s relaxation.In each case, the nitrogen gas injection flow rate close to the membrane surface was 1.8 $m^3/h/m^2$.

### 5.3.3 Analytical methods

#### 5.3.3.1 Hydraulic resistances

The membrane fouling was identified as being caused by several resistances in a series based on Darcy's Law in Equations 5.1:

$$R_t = R_m + R_{cake\ deposit} + R_{pore\ blocking} + R_{adsorption} = TMP/\mu J \quad (5.1)$$

where $R_t$ is the total membrane resistance at the time of cleaning ($m^{-1}$), $R_m$ is the clean membrane resistance ($m^{-1}$), $R_{cake\ deposit}$ is the cake layer resistance ($m^{-1}$), $R_{pore\ blocking}$ is the pore blocking resistance ($m^{-1}$), $R_{adsorption}$ is the resistance due to compounds adsorbed in pores, and only removable by chemical cleaning ($m^{-1}$), J is the membrane flux ($m^3/m^2 \cdot s$), TMP is the trans-membrane pressure (Pa), and $\mu$ is the dynamic viscosity of permeate considered as water ($0.81 \cdot 10^{-3}$ Pa.s at 30°C).

The quantification of each resistance was done step by step. $R_m$ was determined by measuring the water flux when filtering DI water with a new membrane ($R_m$ was found equal to 2.5 $x10^{11}$ $m^{-1}$ in this study), and $R_t$ was calculated at the final time of operation before membrane cleaning. It corresponded to the maximal value of TMP at the end of each experiment.

To evaluate each fouling resistance, a specific cleaning procedure was carried out. When the TMP reached a value close to 250 mbar, the membrane module was taken off from the reactor, and then three successive steps of cleaning were practiced:

1. A physical cleaning was carried out by scraping off the cake layer from the membrane surface by carefully using a plastic sheet. The membrane surface was then rinsed with DI water to remove any sludge entrapped in the module. Then, the membrane resistance was measured by filtering DI water. It corresponded to $R_1$.
2. A back-washing with DI water was then carried out for 2 h to remove any compounds, inducing pore blocking. Then, the membrane resistance was measured by filtering DI water. It corresponded to $R_2$.
3. Finally, chemical cleaning was carried out by soaking the membranes (i) in a 0.5% NaOH solution for 2 h. and then after rinsing (ii) in a 0.5% NaClO solution for 2 h. After rinsing, the membrane resistance was measured by filtering DI water. It corresponded to $R_3$ .

The resistance due to each identified phenomenon could then be deduced as follows:

- $R_{cake\ deposit}$ = $R_t - R_1$ (Residual resistance after physical cleaning)
- $R_{pore\ blocking}$ = $R_1 - R_2$ (Residual resistance after backwashing with DI)
- $R_{adsorption}$ = $R_2 - R_3$ (Residual resistance after chemical cleaning)

After the last cleaning step, the residual hydraulic resistance $R_3$ was equal to $R_m$.

#### 5.3.3.2 The SMP and EPS Extraction and Analysis

Extracellular polymeric substances (EPS) and soluble microbial products (SMP) have been established as an important cause of membrane fouling by structuring the cake deposit and favoring pore size decrease. EPS and SMP typically consist of carbohydrates (PC), proteins (PN), nucleic acids, lipids and other polymeric compounds coming from bacterial growth and decay [228, 229]. The PN/PC ratio in EPS and SMP can also appear as a criterion of membrane fouling dynamics [230, 231].

SMP and EPS concentrations were normalized as the sum of protein and polysaccharide. Protein concentration was analyzed according to the colorimetric method suggested using the modified Lowry procedure of Peterson [232], which used bovine serum albumin (BSA) as a standard protein. Carbohydrate concentration was analyzed by the phenol-sulfuric acid methods with D-glucose used as a standard carbohydrate. Carbohydrate samples analyses were measured at 480 nm [233].

It was important to quantify the SMP and EPS not only in the filtered suspension, but also in cleaning solutions. The rinsing, backwashing and desorpting solutions were collected and centrifuged at 6000 rpm for 30 minutes and the centrifuged supernatant was filtrated through a membrane with a mean pore size of 0.45 μm.

## 5.4 Results and Discussion

### 5.4.1 Hydraulic Filtration Performances

The TMP evolutions for the four relaxation conditions are shown in Fig 5.2. The membrane fouling in this study was continuously monitored by observing the TMP changes while controlling flux constant at 20 $L/m^2h$ during the filtration. In Fig 5.2, it appears that the TMP had reached 250 mbar due to the increased speed of the suction pump to control the flux constant value at 20 $L/m^2h$. All curves can be divided in two parts with time. The first is a progressive increase of TMP with time until the maximal allowed

value is (second part of the curve). Only the first part of TMP evolutions has been discussed, with the highest curves corresponding to the TMP measured during filtration, the lowest corresponding to the TMP values at the end of each relaxation period. Results allow the differentiation of S1 and S2 from S3 and S4.

For S1 and S2 (frequent relaxation periods), relaxation allowed a net decrease of TMP when comparing the TMP at the end of each filtration period, and its value at the end of the following relaxation period. Even with a progressive TMP evolution when filtration was operating, relaxation was able to regenerate partially the membrane permeability during more than 5 to 6h with an average TMP evolution rate of about 0.6 to 0.8 mbar/min. After this first period, it was obvious that relaxation was not sufficient to maintain the membrane fouling dynamics.

On the other hand, for S3 and S4, too long a time between the two relaxation periods did not allow any control of TMP evolution. The maximal value was reached in hardly 30 minutes, about ten times lower than observed in S1 and S2 conditions (the average TMP evolution rate was close to 8 mbar/min). This point was soon noticed by other authors (Bae et al., 2005) [234].

The relaxation frequency appeared to favour the control of TMP evolution. As discussed by Huang et al [235] and Wang et al [236], the slow increase of TMP in the early stage was related to the formation of a biogel layer due to ESP and SMP adsorption. Biogel layer makes easier the attachment of small flocs and/or bacterial clusters on the membrane surface to induce sludging cake formation. During relaxation, the loosely attached compounds were removed from the deposit. The high relaxation frequency avoided any rapid structuring of the cake deposit but it appeared not sufficient in these experiments to limit TMP increase during filtration. This could be due to:

1. A progressive compression of the deposit becoming less breakable. Moreover, with time, the deposit structure became comparable to a dense biofilm composed by mucilaginous biomass presenting low water permeability.
2. The other origins of fouling. If relaxation can limit the deposit development, it is inefficient for pore blocking and the adsorption of solutes in the pores. According to the intensity of these phenomena, relaxation can then appear as insufficient with time.

The cleaning procedure has confirmed this analysis. Compounds deposited on the membrane surface were collected after the initial cleaning of the membranes for each condition. The intermittent filtration 960 s/30s relaxation (S4) showed the highest foulant content of 1173 mg/m$^2$, while the lowest biofoulant content was observed in the intermittent filtration 480 s/30s relaxation (S2) (593 mg/m$^2$). This indicated that membrane fouling could be dominated by cake layer formation when operating at a low relaxation period, whereas high relaxation frequency may also be influenced by pore-blocking and adsorption.

As expected, the optimal relaxation frequency was dependent on the suspension characteristics and filtration conditions (permeate flux in regard with local shear stresses). In this experiment, the permeate flux value chosen was very high (for SAnMBRs) to highlight the role of relaxation as a simple means of membrane fouling control, relaxation being an economic cleaning procedure. No loss of filtered water and energy was necessary with backwashing).

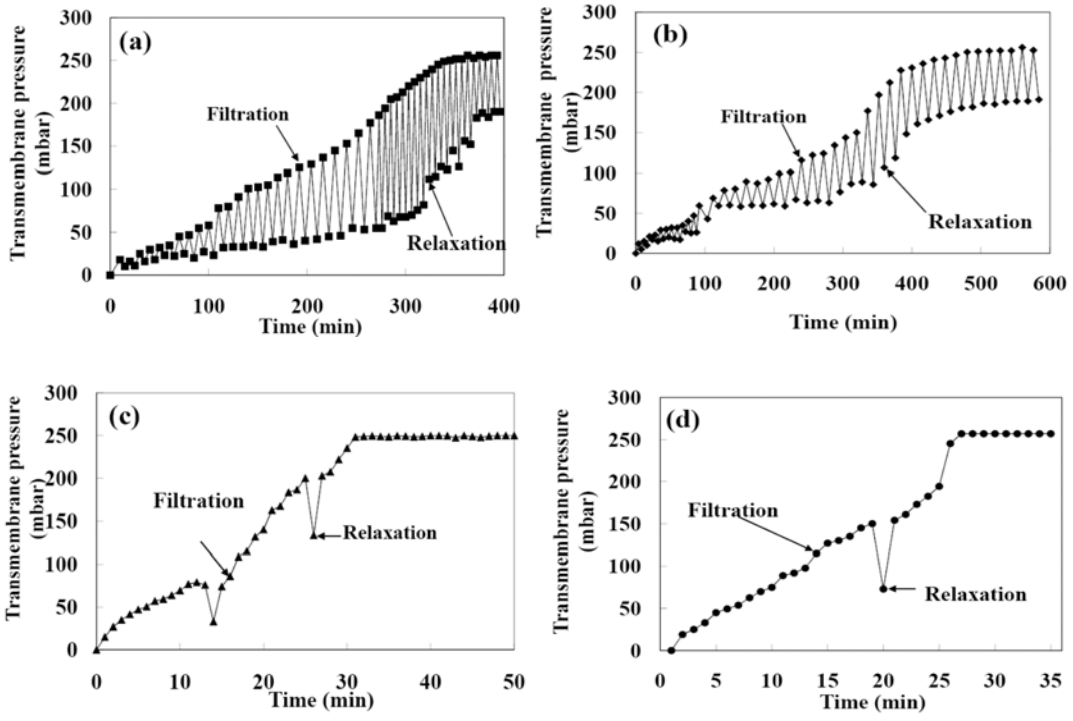



**Figure. 5.2.** Variations of TMP under different intermittent permeate filtration mode: (a) 240 s filtration /30 s relaxation, (b) 480 s filtration /30 s relaxation, (c) S3: 720 s filtration /30 s relaxation and (d) S4: 960 s filtration /30 s relaxation

### 5.4.2 Hydraulic Resistances of the Different Intermittent Filtration Modes

Figure 5.3 presents the hydraulic resistances due to the main causes of fouling as defined in "Materials and methods". Whatever the relaxation frequency, the external deposit appeared as the more influent phenomenon on total hydraulic resistance (about 50%). The two other origins of fouling were of the same order of magnitude, and even the pore blocking seemed slightly higher. Thus, the role of the deposit remained important, though the external cleaning by relaxation and gas bubbling under working in supra-critical conditions. Pore blocking appeared also as an important phenomenon of fouling. A combination of relaxation and backwashing could then offer a better control of fouling dynamics by letting only molecule adsorption in the membrane pores as the limiting step.

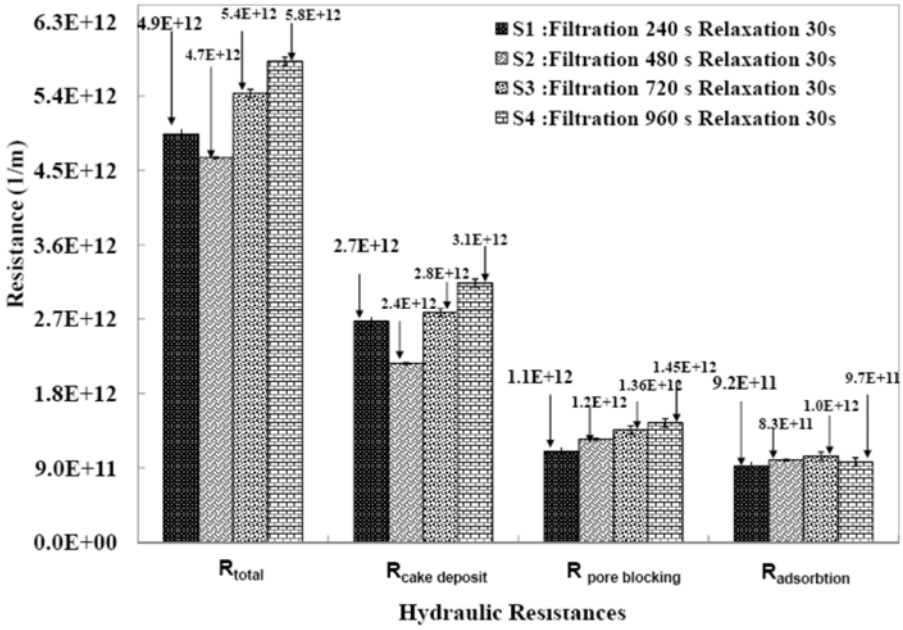



**Figure 5.3.** Hydraulic resistances of different intermittent permeate filtration modes.

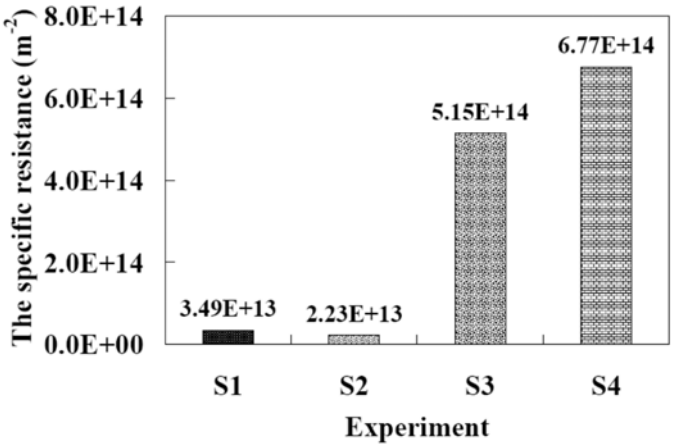



**Figure 5.4** the different specific resistance R*.

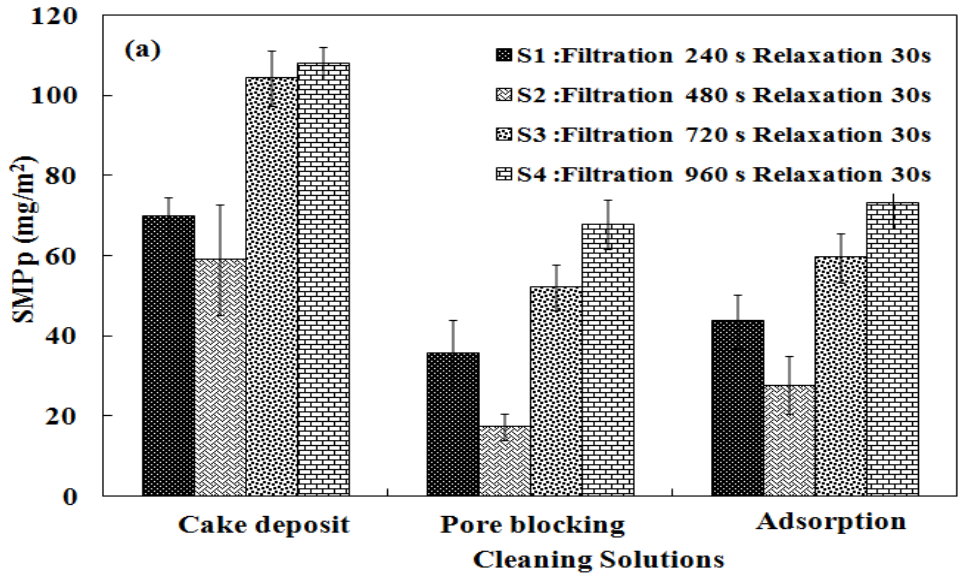
The conditions of S1-S4 actually produced different quantities of water within the same long time operation, while the same membrane flux value was applied. It might be unfair for the runs where low relaxation frequency was applied (S3 and S4). To compare all of study conditions the specific cumulated permeate volume was calculated. It is the ratio between the cumulated permeates volume and the membrane surface of filtration. This obtained specific resistance R* (in $m^{-2}$) can be compared to other experiments whatever the time of filtration and the permeate volume recovered. Figure 5.4 presents the hydraulic resistances divided by the specific cumulated permeate volume obtained at the end of each run before membrane cleaning. The result obtained clearly confirms the interest of working in conditions S1 and S2. The specific resistance R* of S4 was about 50 times higher than that of S2 respectively. The results indicated that the specific resistance drastically increased with low relaxation frequency.

### 5.4.3 Roles of SMP and EPS in Fouling

At the end of each experiment, the first step of membrane cleaning allowed the recovery of sludge flocs, biogel and mixed liquor entrapped on the membrane surface, and in the capillary fiber network. Then, for these first rinsing solutions, it was possible to quantify the concentration of linked EPS and soluble biopolymers as indicated in the materials and methods.

Regarding the soluble compounds (SMP) more specifically, the three cleaning solutions (rinsing, backwashing and desorbing solutions) were characterized in terms of

protein and carbohydrate concentrations (as shown in Figures 5 and 6). The presence of soluble protein SMPp appeared dominant in the cake deposit, about two times higher than in pore blocking and adsorption. The lower concentrations of SMPp and SMPc observed in Figure 5.5a-b confirmed the interests of these working conditions. Low relaxation (S3 and S4) present SMPp and SMPc than high relaxation (S1 and S2). So, the decrease of relaxation frequency cause increased the retention of SMP onto the membrane surface and in pores [31].



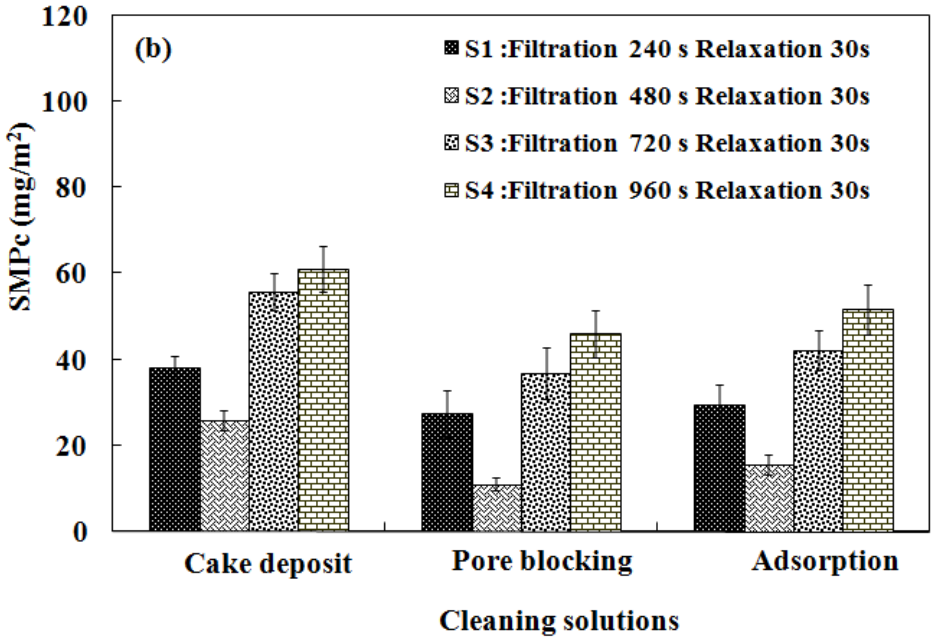



**Figure 5.5** SMP content (protein + carbohydrate) in different cleaning methods

Table 5.2 shows the PN/PC ratio in the three cleaning solutions. This ratio appeared higher in the first cleaning solution (cake deposit removal) corresponding to the higher fouling effect, which could have been due to the cake deposit capacity to retain/adsorb a part of soluble compounds [3]. Nevertheless no actual influence of the relaxation frequency could be noticed. Figure 6 presents the linked EPS content (protein (PN) and carbohydrate (PC) and PN/PC ratio in the first rinsing method (cake deposit recovery) for the four studied filtration modes.

**Table 5.2** The PN/PC ratio of SMP in different cleaning methods after practicing cleaning steps.

| Experiment | Samples | | |
|---|---|---|---|
| | Cake deposit | Pore blocking | Adsorption |
| S1 | 1.8±0.1 | 1.3±0.5 | 1.5±0.3 |
| S2 | 2.3±0.2 | 1.6±0.3 | 1.8±0.4 |
| S3 | 1.9±0.2 | 1.4±0.4 | 1.4±0.4 |
| S4 | 1.8±0.2 | 1.5±0.3 | 1.4±0.3 |

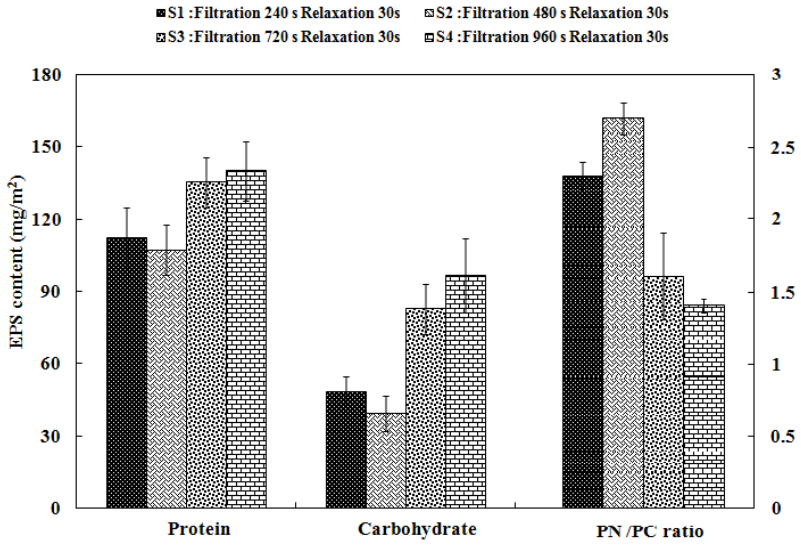



**Figure 5.6** EPS content (protein and carbohydrate) and PN/PC ratio in intermittent permeate filtration modes.

Protein was found to be the major component in the EPS rather than carbohydrate. These results confirmed the results of Metzger et al [223] and Gao et al [85]. It can also be observed that the most favorable conditions of filtration corresponded to the highest ratios the PN/PC as shown in Figure 5.6. It can be analyzed in comparison with observations of Liao et al [237] and Yao et al. [231], demonstrating that a decrease in the PN/PC ratio ($\leq 2$) could induce a decrease in floc hydrophobicity, resulting in higher resistance from cake formation rather than pore adsorption. Furthermore, Yamato et al [238] reported that the most irreversible fouling on PVDF membrane was caused by dissolved EPS and SMP, which was detected when the PN/PC ratio was close to 1.4. The filtration modes S3 and S4 showed the PN/PC ratio in a range of 1.4-1.6. Then for linked EPS, the ratio PN/PC can be a relevant criterion to qualify the fouling potential of a cake deposit.

This finding indicated that the different relaxation frequency also greatly influenced the ratio of protein and polysaccharide. The filtration mode S2 featured so different ratio of PN/PC from other runs. This can be explained by some review and discussion dealing with the instantaneous flux of Wu et al [30] that reported the interval relaxation 440s a more efficient effect on TMP control higher than 220 s filtration. In this study, the interval relaxation of S2 (480 s filtration /30 s relaxation) showed the lowest fouling rate at the end

of filtration with its instantaneous flux lower than the other relaxation runs and it was suitable operation for the membrane fouling control in SAnMBR treating POME.

In addition, the interval relaxation has strongly effect on fouling characteristics (ie. protien, carbohydrate and PN/PC ratio). The previous research was performed by Braak et al [226] that reported also the interval relaxation should be moderate rather than too long or too short because of the high instantaneous fluxes needed to maintain water production. If the operation with too long, foulant (protein and carbohydrate) was induced to attach on the membrane surface [227]. While the operation with too short, it has high instantaneous flux and resulting in the deposit became denser [226].

In this study the interval relaxation of S2 (480 s filtration /30 s relaxation) presented the lowest in PC and PN and the highest PN/PC ratio. Similar result has been also reported that proteins have higher potential for deposition/adsorption directly on the membrane surface and appear to be more strongly attached to the membrane than carbohydrates during filtration [204]. Therefore, the highest PN/PC ratio in S2 was observed due to this condition containing the loose carbohydrates, which were easily removed during relaxation time.

## 5.5 Conclusions

This study investigated the impacts of different intermittent filtration modes based on different relaxation times on the hydraulic resistance evolutions. The experiments were carried out to analyze the filtering steps of a submersed anaerobic membrane bioreactor (SAnMBR). When filtering in supra-critical conditions, the results pointed out the following elements:

1. The dominant effect of fouling was the cake deposit (as it is currently observed when filtering in supra-critical conditions), representing about 50% of the total hydraulic resistance. The other origins of fouling were pore blocking and adsorption.
2. The highest relaxation frequencies, conditions S1 and S2 allowed an operating time 10 times longer than when working with low relaxation frequencies. The specific

resistance of the fouling phenomena then appeared 50 times lower than when working with the lowest relaxation frequencies.

3. Proteins and carbohydrates appeared as the main organic components present in the cleaning methods. They were observed both in soluble and particulate fractions. The Protein/Carbohydrate ratio in linked EPS present in the cake deposit appeared lower when operating at a lower relaxation frequency, which also corresponded to a higher fouling property of the cake deposit.

# CHAPTER 6
# INFLUENCE OF SRT ON TWO-STAGE SAnMBR EEFFICIENCY WHEN TREATING POME

This chapter presented two main parts: the treatment efficiency and methane production (1) during start-up and (2) SAnMBR, which the seed sludge and raw POME 100% concentration obtained from industry 1 in chapter 4 was used for this investigation. At start-up period, OLR was progressively increased by decreasing the daily hydraulic retention time (HRT). After the start-up period, a third tank containing porous membranes was set up downstream of the methanogenic bioreactor to constitute a selective barrier to separate solid and soluble phases. This stage (SAnMBR) was operated at various SRTs of 15, 30 and 60 d and HRT of 2 d.

## 6.1 Introduction

In the past decades, anaerobic biological processes have been successfully applied for treating palm oil mill effluents (POME), because they offer a good method in terms of (i) renewable energy recovery through biogas production, and (ii) organic matter removal efficiency [1]. The success of processes can be attributed to an efficient solids retention time (SRT), defined as a key parameter in the anaerobic digestion of wastewater [239, 240]. Many studies focused on the effect of SRT on reactor performance, such as biogas production and volatile solids (VS) destruction. In general, longer SRT was favorable to the organic carbon removal, but not always for the energy production [241, 242]. The study of Miron et al. [243] revealed that the hydrolysis of lipids and carbohydrates of primary sludge increased with SRT. Similarly, the results of Zhang et al [244] showed that the SRT increase from 3 d to 18 d had a significant influence on the hydrolysis of proteins, carbohydrates lipids and methane production. De la Rubia et al [241] found that SRT has a considerable effect on the population levels of methanogens, and the total VFA increased when SRT decreased. Although, conventional anaerobic digesters are widely used, they have the disadvantages of operating with long hydraulic retention time, risking sludge washout, and requiring a large capacity tank. High rate anaerobic processes are efficient

for the treatment of various industrial wastewater but they face the problem of biomass retention when treating wastewaters having high concentrations of suspended solids [245].

To improve the quality of the effluent and stablility of the system, the use of a filtration process with porous membranes has recently been proposed [242, 243, 245, 246]. Due to their high selectivity and high potential of bacterial retention, porous membranes favour the retention of any microbial communities able to degrade specific pollutants present in wastewater [74]. Therefore, this technology presents an attractive option to treat industrial wastewaters at extreme conditions, such as high salinity, temperatures, and concentrations of suspended solids (SS) even in the presence of toxics that hamper granulation and biomass retention by conventional systems [27, 247].

Anaerobic membrane bioreactors (AnMBRs) can operate at high biomass concentrations, high SRT and OLR and relatively short HRT, independently of the sludge flocculation state. Nevertheless, the weak point of AnMBR remains the membrane fouling and the presence in the suspension of high sludge concentrations and/or high colloidal matter concentrations which can drastically limit AnMBRs development despite using some original samples to reduce fouling such as high cross-flow velocity[106], ultrasonic irradiation [111], gas sparging and addition of activated carbon [247].

The choice of SRT value directly influences the system performances. Huang et al [101] reported that the effect of SRT on the treatment performance of SAnMBR in synthetic wastewater was significant when the SRT was short, e.g. 30 d. However, with a prolonged SRT, especially longer than 60 d, the effect became less pronounced. A longer SRT operation achieved a better treatment performance and more biogas generation. However, for a SAnMBR treating real wastewater, the impacts different SRTs on treatment performance and methane production are still unclear.

## 6.2 Research Objective

The aim of this chapter was to present experimental results describing the SRT influence on treatment efficiency of a two-stage SAnMBR treating a high-strength wastewater from palm oil mill effluent under mesophilic conditions.

## 6.3 Materials and Methods

### 6.3.1 Experimental Set-up and Analytical Methods

The experimental setup and analytical methods was conducted using the laboratory scale system, as described in Chapter 3. During SAnMBR the start-up samples were collected daily from influent and effluents of acidogenic, methanogenic reactor, and analyzed through the measurements of pH, TCOD, SCOD, MLVSS, MLSS, VFA, alkalinity and biogas composition, according to Standard Methods for the Examination of Water and Wastewater [206].

Hydrolysis yield coefficient of organic matter was calculated according to equation 6.1 [248].

$$\text{Hydrolysis yield coefficient} = \frac{\text{SCOD effluent} - \text{SCOD influent}}{\text{PCOD influent}} \text{x} 100 \qquad (6.1)$$

Where SCOD is the soluble chemical oxygen demand in the influent and the effluent and PCOD is the particulate chemical oxygen demand in the influent, which could be considered stable during the study.

### 6.3.2 POME Characteristics from Industry 1

SAnMBR influent was taken from the clarification tank of a palm oil mill plant in Southern Thailand from industry 1.Generally, the characteristics of POME appear as an acid wastewater with a low pH value of 3.9-4.3, high biochemical oxygen demand (28-30g$BOD_5$/L), chemical oxygen demand (55-60 gCOD/L) and total suspended solids (40-46 g/L), the presence of oil and grease (22-31g/L), relatively low nitrogen content (0.76-0.85 gN/L) and a high discharge temperature of 80–90 $^oC$.

### 6.3.3 Start-up of SAnMBR

The start-up is an important step for the smooth operation process of an anaerobic bioreactor. The purpose of the start-up of SAnMBR is to grow, build up and retain a sufficient concentration of active and well balanced biomass able to treat POME influent. The two-SAnMBR stages consist of an acidogenic reactor (working volume of 5 L) upstream a methanogenic reactor (working volume of 10 L). The reactors were initially seeded with sludge taken from a full-scale ASBR reactor treating a palm oil industry

effluent in the southern part of Thailand (also composed by two reactors (i) the acid reactor and (ii) methanogenic reactor).

During start-up, the lab scale reactors worked in mesophilic conditions (35 ± 3 $^{o}$C) and were operated continuously. No liquid – solid phase separation was operated downstream reactors and HRT was then equal to SRT. A first organic loading rate OLR was imposed on the system and according to the biomass acclimatization, the daily OLR was progressively increased by decreasing the daily hydraulic retention time HRT, from higher to lower values (12 to 10, 8, 6, 3, and 2 days) which corresponded to average daily flow-rates from 1.25 to 1.5, 1.87, 2.5, 5 and 7.5 liters of raw POME per day (Tables 6.1). During this start-up period samples were taken from each reactor three times a week to evaluate biogas production, changes in pH, MLVSS, MLSS, VFA concentrations and COD removal. The attainment of "steady-state" conditions was verified after a period equivalent to 2–3 times the final HRT by checking whether constant effluent characteristic values (TCOD removal and biogas production levels) were achieved. The sampling during each steady-state period was performed for five consecutive days.

**Table 6.1** Functioning conditions during the acclimatization period.

| OLR (g COD/L·d) | Feed concentration (gCOD/L) | Flow rate (L/d) | HRT (d) |
|---|---|---|---|
| 4.56-4.98 (4.79) | 55±11 | 1.25 | 12 |
| 5.39-5.98 (5.70) | 55±15 | 1.5 | 10 |
| 6.72-7.42 (7.08) | 55±7 | 1.87 | 8 |
| 9.12-9.98 (9.73) | 55±12 | 2.5 | 6 |
| 17.97-19.83 (19.18) | 55±9 | 5 | 3 |
| 27.35-29.95 (28.59) | 55±9 | 7.5 | 2 |

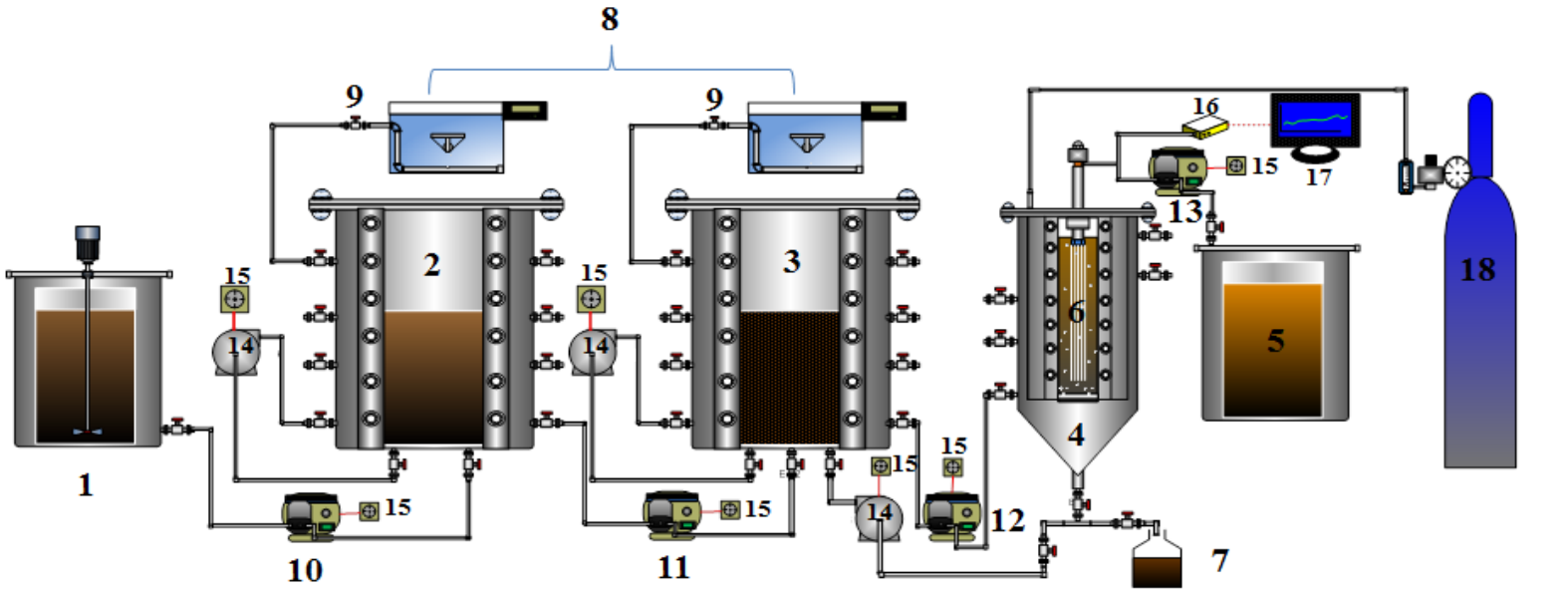
### 6.3.4 Operating Conditions of SAnMBR

After the start-up period, a third tank containing porous membranes was set up downstream of the methanogenic bioreactor to constitute a selective barrier to separate the solid and soluble phases, and constitut the Sequencing Anaerobic Membrane Bioreactor SAnMBR, as shown in Figure 6.1. The bioreactors were then operated in sequencing batch conditions. The filtration on membrane was operated in a semi continuous mode.

#### 6.3.4.1 Description of Submerged Anaerobic Membrane Bioreactor

The experimental setup was conducted using a laboratory scale system, as shown in Figure 6.1, composed of 5 tanks. The first worked as POME storage, it was filled every day. The next three tanks consisted of SAnMBRs. The fifth tank served as permeate storage. The SAnMBRs system was then composed of three tanks working in mesophilic conditions (35°C):(i) the first of the three tanks was a 5L acidogenic reactor where the pH was maintained in the range of 5 to 5.5, (ii) the effluent from the acidogenic tank was pumped towards the second 10L methanogenic tank where the pH was maintained in the range of 6.9 to 7.2 and the effluent of the methanogenic tank was pumped towards the third 5L tank where a module of hollow fiber porous membranes was submerged to separate permeate from biological suspension. The permeate was extracted by pumping and sent into the fifth tank for storage.

A large part of the circulating suspension in the membrane tank was recycled towards the methanogen tank; a small part (including sampling) was extracted from the system according to the chosen SRT value. Biogas production and its composition were continuously quantified. Hollow fiber membranes were made with polymeric material (PVDF), and presented an average pore size of 0.1 μm, an effective filtration area of 0.1 $m^2$/module and an initial (clean membrane) hydraulic resistance $R_m$ of $1.1x10^{11}$ $m^{-1}$. The trans-membrane pressure (TMP) was continuously monitored by a pressure transducer at the permeate side of the membrane module. TMP was determined measuring the pressure in the membrane module, by a pressure sensor (National Instruments, Austin, USA).




1. Influent tank
2. Acidogenic reactor
3. Methanogenic reactor
4. Submerged membrane reactor
5. Permeate tank
6. Hollow fiber membrane
7. Sludge drain out
8. Biogas collector
9. Biogas sampling port
10. Influent pump
11. Effluent acidogenic pump
12. Effluent methanogenic pump
13. Permeate pump
14. Recirculation pump
15. Timer control
16. Pressure sensor
17. Labview 10.0
18. Nitrogen gas

**Figure 6.1** Schematic diagram of two-stage SAnMBR.

#### 6.3.4.2 Sequencing Conditions in Bioreactors

The acidogenic reactor was fed during 10 minutes with a defined volume (close to 2.5 L) of POME effluent. It was then operated during 5h before a 1h settling step allowing a liquid solid phase separation. An equivalent volume of liquid phase was then extracted from acedogenic reactor to be added into methanogenic reactor, and a part of settled sludge was extracted from acetogenic reactor according to the imposed SRT. The functioning of the methanogenic reactor was of course similar, 10 minutes feeding with the liquid phase extracted from acedogenic reactor, 5 hours of operation, and 1 hour of settling. At the end of the settling phase, the defined volume of liquid phase was extracted from anaerobic reactor to be added into the membrane tank (that worked in methanogenic conditions and could be considered as a part of methanogenic reactor). As for acidogenic reactor, a defined volume of sludge was also extracted from methanogenic reactor in accordance with the imposed SRT. Both reactors were functioning at the same chosen SRT by imposing the daily adequate sludge volume extraction from each reactor (including sludge extraction for sampling and analyses). At the beginning of the experiment, when the reactor was set up, Nitrogen gas was used to expelthe air from the head space of reactor.

#### 6.3.4.3 Filtration Mode in Membrane Tank

To minimize membrane fouling, intermittent filtration was operated with a hollow fiber membrane module: a 6 minute period of filtration was followed by 4 minutes of relaxation (no filtration). During the 6 minutes of filtration, nitrogen was injected (1L/min corresponding to $0.6 m^3_{gas}/m^2_{membrane}/h$) in the membrane tank close to the membrane surface, and the permeate flux was constant and equal to 4 $L/m^2 \cdot h$. When including the relaxation period, the corresponding daily average permeate flux value was then equal to 2.4 $L/m^2 \cdot h$ [249].

The SRT influence was then investigated during the 3 experiments for 15, 30 and 60 days. For each experiment, the MLSS concentration in the suspension present in both reactors was started up at approximately 10 g/L. Table 6.2 gives the experimental conditions during steady state conditions.

**Table 6.2** Operating conditions of a two-stage SAnMBR system.

| Operating conditions | Values |
|---|---|
| Total SAnMBR working volume (L) | 20 |
| COD concentration in influent (g/L) | 55-60 |
| SRT (d) | 15, 30, 60 |
| HRT in Acidogenic tank (d) | 0.5 |
| HRT in Methanogenic and membrane reactor (d) | 1.5 |
| Initial MLSS concentration (g/L) | 10 |
| Temperature ($^{O}$C) | 35 |
| pH in Acidogenic rector | 5-5.5 |
| pH in Methanogenic and HF reactors | 6.9-7.2 |
| Daily average permeate flux (L/m$^2$·h) | 2.4 |

## 6.4 Results and Discussion

### 6.4.1 Performances During Start-up of Bioreactor

6.4.1.1 Effect of OLR Increase on TCOD and SCOD Removal Efficiency

Figure 6.2 shows the evolution of TCOD and SCOD concentrations in the acidogenic and methanogenic reactors (Figures 6.2a and 6.2b respectively) when increasing the OLR value. For acidogenic reactor, the influent characteristics did not changed with time, the initial part of soluble organic fraction represented about 50% of the total organic matter in the suspension to be treated that means that the particulate organic fraction also represented 50% of TCOD.

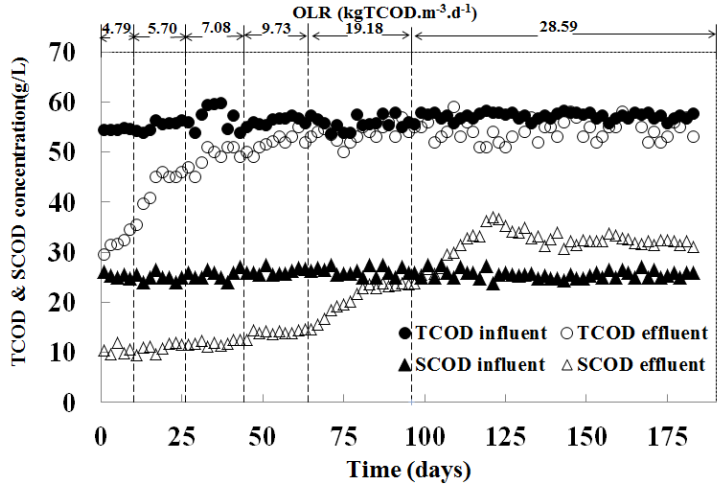
The effluent, the TCOD and SCOD concentrations were lower than the influent values when working at the lowest OLR. Nevertheless TCOD concentration increased continuously with OLR increase and became constant as soon as OLR reached 10 kgTCOD/m$^3$/d.

The soluble fraction SCOD in the effluent should be higher than in the influent due to the hydrolysis of the particulate fraction in the acidogenic reactor. It was not the case still OLR reached 20 kgTCOD/m$^3$/d. For lower OLR such behaviour can be explain by biological ways that favour:

- Some degradation of soluble COD allowed significant biomass growth (particulate COD in the acidogenic reactor increased for OLR varying in the range of 5 to 10 kgTCOD/$m^3$/d. It was the same as in the influent when OLR equal at 20 kgTCOD/$m^3$/dand lower for the highest OLR value).
- Some transformation of a part of the organic matter in the carbonate ions during the acidogenic phase (that also contributes to decrease SCOD and TCOD in effluent).

When working at the highest OLR, the apparent hydrolysis yield of the particulate fraction was close to 30%. Such a result appears in the range of results obtained by Bouallagui et al. [249]with an apparent hydrolysis ratio closed to 50% when treating fruit and vegetable wastes (operating at an HRT of 3 days and OLRs of between 3.7 and 10.1 kgTCOD/$m^3$/d) and results obtained by D'Addario, et al. [250]with an apparent hydrolysis ratio of 10% when treating organic fraction of municipal solid wastes (OLR was about 12.5 kg TSS/$m^3$/d).

(a)



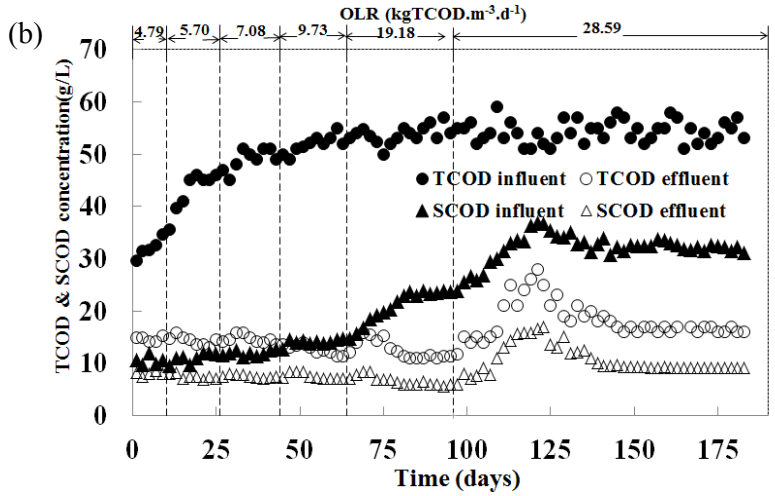



**Figure 6.2**Variations versus time of TCOD and SCOD concentrations in (a) acidogenic: and (b) methanogenic reactor at different OLR.

In the methanogenic reactor, the TCOD and SCOD concentrations in the effluent were close to 15 and 8 g/l respectively during the first 5 periods. According to the TCOD concentration in the influent of the acidogenic reactor, such values correspond to a TCOD removal efficiency of the total system (acidogenic and methanogenic reactor) of about 73 and 85% taking into account the TCOD and SCOD concentration in methanogenic effluent respectively.

When comparing the particulate COD concentrations (difference between TCOD and SCOD) in methanogenic influent (or acidogenic effluent) and methanogenic effluent, it was obvious that a large part of this particulate fraction was still hydrolysed in the methanogenic reactor. The fact that the SCOD in the methanogenic effluent remained low proves that the solubilized fractions were largely transformed to biogas in the methanogenic reactor.

It can be noticed that at the beginning of the sixth period, the important OLR modification generated a significant decrease of efficiency during 25 days. The TCOD and SCOD concentrations were then about twice higher than in precedent periods. Nevertheless, these values progressively decreased till reaching the precedent levels after a period of about 30 days. Such a modification of system performances was linked to the rough appearance of high VFA concentrations in acidogenic phase and corresponded to punctual pH decrease in methanogenic reactor before adding of soda solution.

Figure 6.3 shows the relation between the VFA and OLR evolutions. In acidogenic reactor, VFA concentration was continuously increased with OLR increase, this evolution can be compared with SCOD evolution even no relation of proportionality cannot be noticed. Such behaviour proved the hydrolysis capacity of acidogenic bacteria to fit with such OLR increases. Nevertheless during the last period of methanogenic reactor the improving of VFA concentration was not important showing a beginning of saturation of biomass due to the increase of OLR without initial pH adjustment resulted in highest cumulativeVFA production. The system then necessitated 50 days to be stabilized again operational control of pH by soda addition.

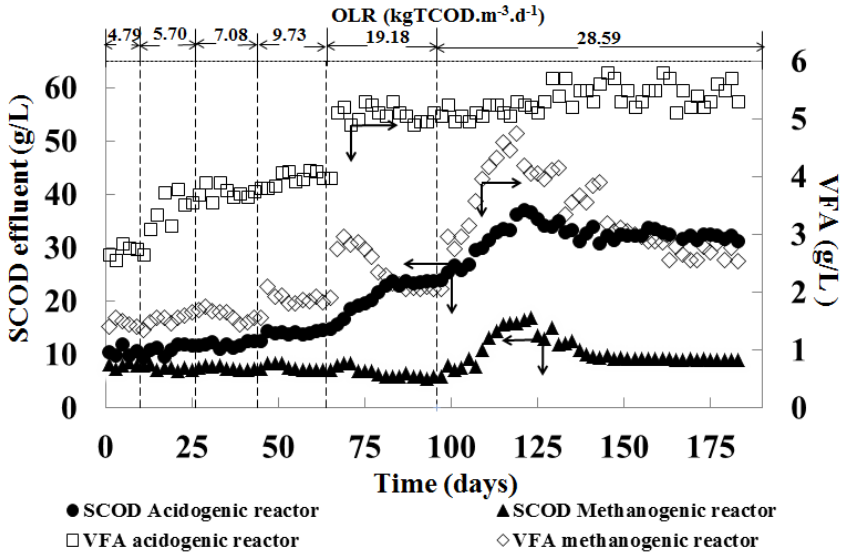



**Figure 6.3** Variations versus time of SCOD and VFA concentrations at different OLR in both bioreactors.

In the methanogenic reactor, the VFA concentration was still constant (1.5 g/l) when working at the OLR range varying between 4.79 and 7.08 kgTCOD/$m^3$/d. For higher OLR values, VFA value increased roughly at the beginning of each new OLR increase (with a maximum value closed to 5 g/l for the highest OLR value) to progressively reach a stabilized value (2.5 g/l for the highest OLR value). The difference between VFA concentrations in acidogenic and methanogenic reactors and the progressive reaching of "steady state" conditions whatever OLR confirms the adaptability of methanogenic bacteria to such operational conditions even if the time of adaptation appeared largely

depending on OLR value. Indeed, 10 days were necessary to stabilize the VFA concentration in methanogenic reactor when doubling $OLR_{influent}$ at the beginning of the 5$^{th}$ period. During this last period, the VFA concentration was not modified in effluent of acidogenic reactor but VFA concentration rapidly increased in the methanogenic system proving a beginning of the saturation of the methanogen population. The system then necessitated 50 days to be stabilized again including operational control of pH by soda addition.

Nevertheless, such bacterial behaviour in both reactors illustrated the self-regulation capability inherent of the biological system, making it possible for the microbial consortium to acclimate itself to defined OLR increase.

#### 6.4.1.2 Effect of VFA/alkalinity Ratio

Figure 6.4 points out the evolution of the VFA and VFA/alkalinity ratio. VFA concentration in acidogenic reactor appeared lower than in other studies describing similar treatment of highly biodegradable wastewater. Saddoud et al.[73]have investigated a two-phase anaerobic digestion of cheese whey and found VFA up to 5 g/L in acidogenic reactor. Similar observations were also found by Wijekoon et al.[181]when treating high strength molasses-based synthetic wastewater with VFA concentration closed to 7 g/L. Moreover, except at the beginning of the 6$^{th}$ period, the VFA/Alkalinity ratio was found between 1.5-2.4 within the optimum range of TVFA/ Alkalinity ratio 1.5–2.5 required for a stable acidogenic fermentation [251]. At the beginning of period 6, the ratio VFA/alkalinity reaching a value of 4, appeared significantly higher than the recommended values explaining the punctual saturation of acidogenic bacteria activity.

The VFA concentration in the methanogenic reactor remained relatively low (lower than 2 g/L except at the beginning of period 6, the value then reached 5g/l). The ratio VFA/alkalinity was also lower than 0.3-0.4 (except at the beginning of periods 5 and 6). The optimal range of such a ratio, as indicated by Borja et al [252] are stable conditions for the anaerobic reactor. In opposite, the VFA/Alkalinity ratio appeared close to 0.5 and 0.7 at the beginning of periods 5 and 6 respectively, explaining the punctual accumulation of VFA in anaerobic reactor because of reaching the failure limit values.

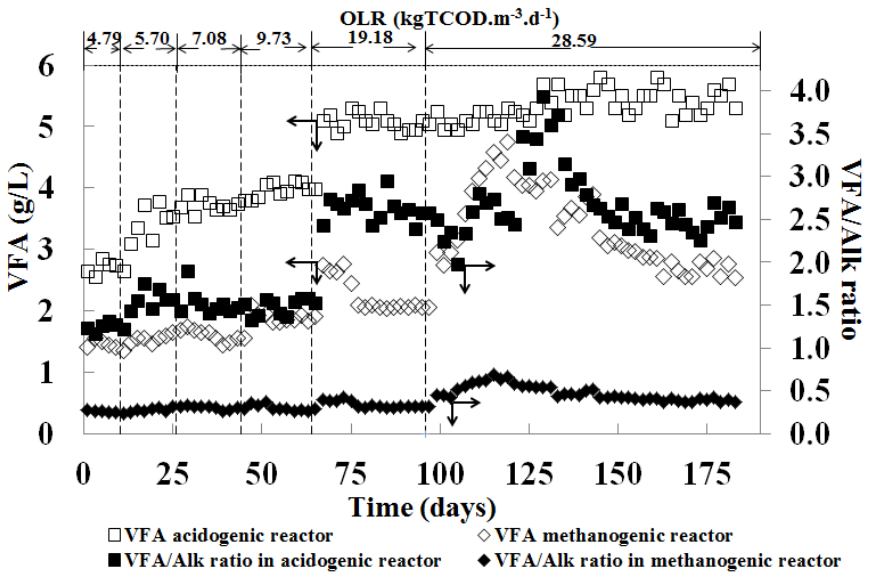



**Figure 6.4** Variations versus times of VFA concentration and VFA/Alk ratio in acidogenic and methanogenic reactors at different OLR.

#### 6.4.1.3 Biogas Production and Yield

The most important parameters of the biogas produced are its composition and amount. Both parameters were influenced by the OLR applied and the influent characteristics [253]. Figure 6.5 illustrates the biogas and methane productions rate and the methane gas percentages. As indicated, the methane content in biogas was in the range of 61-69%. The biogas and methane production increased linearly with COD loading rates from 8.04 to 57.34 L/d and 5.76 to 39.61 L/d respectively as the OLR increased from 4.79 to 19.18 kgTCOD.$m^{-3}$.$d^{-1}$. A similar result was reported by Han et al [254] when increasing OLR step by step.

However, the OLR increase up to 28.59 kgTCOD/$m^3$/d resulted in some methane production decrease (35.23 L/d). These results suggest that the methanogenic process was then the rate-limiting step, too high OLR levels can cause disadvantageous circumstances for methanogenic bacteria. Afterwards the methane production also decreased because of too important VFA accumulation and pH reduction (Figure 6.4). Moreover, the TCOD concentration in methanogenic effluent also increased sharply due to biomass growth and probably presence of gas in biomass flocs inducing easier washout of biomass from reactor when working at short HRT and high OLR. Moereover, asindicated by Zinatizadeh et

al.[255] the carbon dioxide percentage in gas increased due to insufficient HRT durations for such corresponding OLR values [256].

Table 6.3 gives a mass balance between TCOD removal in both the reactor and the expected methane production. With respect to the maximum methane yield presented 0.28 $LCH_4$/g COD removedcorresponded to OLR equal to 10 kgTCOD/$m^3$/d(3rd period). This value accounts for only 80% of the maximal theoretical methane yield of 0.4 $LCH_4$/g COD removed (1 g of COD is equivalent to 0.4 L of methane at 35 $^oC$) [267]. The maximal theoretical methane yields determine the COD equivalence of methane, the amount of oxygen required to completely oxidize 1 mole of $CH_4$at STP is calculated. The balanced reaction is equation 6.2:

$$\underset{16}{CH_4} + \underset{64}{2O_2} \rightarrow \underset{44}{CO_2} + \underset{36}{2H_2O} \qquad (6.2)$$

The COD of methane is 64 gCOD/16 $gCH_4$ or 4 gCOD/$gCH_4$. The complete metabolism of 1 kg of COD produce 0.25 kg of $CH_4$. The number of moles of $CH_4$ produced will be 250 g/16 g = 15.6 moles. The volume of 1 mole of gas is 22.4 L. The total volume of gas produced per kg COD converted is then 22.4 L/mole × 15.6 moles = 349 L = 0.35 $LCH_4$/g COD removedat (0 $^oC$ and 1 atm). However, the methane production of the experiment is operated at 35 $^oC$ to determine the volume of gas occupied by one mole of $CH_4$ at temperatures of 35 $^oC$ as shown in Equation 6.3

$$P = \frac{nRT}{T} \qquad (6.3)$$

Where V= volume occupied by the gas, L

$n$ = moles of gas, mole

R = universal gas law constant, 0.082057 $atm \cdot \frac{L}{mole} \cdot K$

T = temperature, K (273.15 + $^oC$)

P = absolute pressure, atm

Thus, at 35 $^oC$, the volume occupied by one mole of methane is

$$V = \frac{(1\ mole)\left(0.082057\ atm \cdot \frac{L}{mole} \cdot K\right)[(273.15 + 35)K]}{1\ atm}$$

$$= \quad 25.29 \text{ L}$$

Since the COD of one mole of $CH_4$ is equal to 64 g, the amount of $CH_4$ produced per unit of COD converted under anaerobic conditions is equal to 0.4 $LCH_4$/g COD as determined below.

(25.29 L) / (64 gCOD/mole $CH_4$) = 0.4 $LCH_4$/g COD

If the composition of the waste is known, and neglecting the amount of the constituent used for synthesis, the following relationship, first proposed by Buswell and Boruff [253] can be used to estimate the amount of methane ($CH_4$), carbon dioxide ($CO_2$), ammonia ($NH_3$) and hydrogen sulfide ($H_2S$) produced under anaerobic conditions, as shown in Equation 6.4.

$$C_v H_w O_x N_y S_z + \left(v - \frac{w}{4} + \frac{x}{2} + \frac{3y}{4} + \frac{z}{2}\right) H_2O \rightarrow \left(\frac{v}{2} + \frac{w}{8} + \frac{x}{4} + \frac{3y}{8} + \frac{z}{4}\right) CH_4 + \left(\frac{v}{2} + \frac{w}{8} + \frac{x}{4} + \frac{3y}{8} + \frac{z}{4}\right) CO_2 + yNH_3 + zH_2S \quad (6.4)$$

The maximum methane yield value obtained in the present work, which is still lower than the theoretical value, indicates that particulates or soluble organics were not completely degraded. This could be explained by the presence of important of hemicelluloses and lignin is entirely organic in nature. It is known that hemicelluloses and lignin contain a substantial non-biodegradable part particularly difficult to biodegrade.

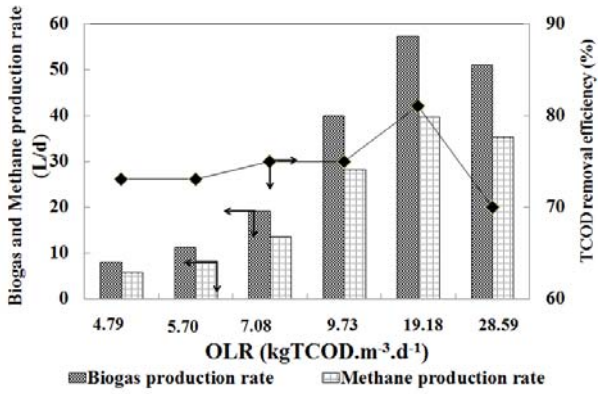



**Figure 6.5** Effect of OLRs on the biogas production rates and methane percentages.

**Table 6.3** Total performances of bioreactors during start-up periods in steady-state conditions for different HRT and OLR.

| Days | OLR ($kgTCOD.m^{-3}.d^{-1}$) | HRT (d) | Q (L/d) | COD concentration in Influent, Effluent (g/L) and [Removal efficiency %] | | $VFA_{effluent}$ (g/L as acetic acid) | Alkalinity In effluent (g/L as $CaCO_3$) |
|---|---|---|---|---|---|---|---|
| | | | | TCOD | SCOD | | |
| 0-11 | 4.56-4.98 (4.79) | 12 | 1.2 | 14.58 ±0.49 [73 ±1.71] | 8.05 ±0.66 [69 ±2.68] | 1.44± 0.07 | 5.61±0.35 |
| 12-27 | 5.39-5.98 (5.70) | 10 | 1.5 | 13.87 ±0.59 [73 ±1.16] | 7.23 ±0.29 [71 ±1.74] | 1.57±0.08 | 5.54±0.37 |
| 28-45 | 6.72-7.42 (7.08) | 8 | 1.87 | 14.07 ±0.56 [75 ±0.68] | 7.20 ±0.55 [72 ±1.60] | 1.54±0.07 | 5.28±0.08 |
| 46-65 | 9.12-9.98 (9.73) | 6 | 2.5 | 12.10±0.60 [75 ±0.94] | 7.04 ±0.53 [73 ±2.14] | 1.85±0.23 | 6.55±0.29 |
| 66-97 | 17.97-19.83 (19.18) | 3 | 5 | 11.31 ±0.66 [81 ±1.28] | 5.91 ±0.29 [77 ±1.30] | 2.06±0.11 | 6.55±0.07 |
| 98-183 | 27.35-29.95 (28.59) | 2 | 7.5 | 17.71 ±0.61 [70±1.72] | 9.12 ±0.68 [64±2.72] | 2.92±0.15 | 6.91±0.12 |

**Table 6.3** Total performances of bioreactors during start-up periods in steady-state conditions for different HRT and OLR (cont').

| Days | OLR (kgTCOD. $m^{-3}.d^{-1}$) | HRT (d) | Q (L/d) | VFA/Alkalinity in effluent | Theoretical methane production ($L_{CH4}$/d) | Experimental methane production ($L_{CH4}$/d) | Methane yield ($L\ CH_4/g\ COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0-11 | 4.56-4.98 (4.79) | 12 | 1.2 | 0.25 | 17.5 | 6 | 0.12 |
| 12-27 | 5.39-5.98 (5.70) | 10 | 1.5 | 0.28 | 21 | 8.5 | 0.14 |
| 28-45 | 6.72-7.42 (7.08) | 8 | 1.87 | 0.29 | 26 | 13 | 0.17 |
| 46-65 | 9.12-9.98 (9.73) | 6 | 2.5 | 0.28 | 35 | 28 | 0.28 |
| 66-97 | 17.97-19.83 (19.18) | 3 | 5 | 0.31 | 70 | 40 | 0.2 |
| 98-183 | 27.35-29.95 (28.59) | 2 | 7.5 | 0.39 | 92 | 35 | 0.13 |

### 6.4.2 Treatment Performances of SAnMBR

After the precedent periods corresponding to the start-up of the experimental work, the membrane separation step was added downstream in the methanogenic reactor. The functioning conditions were described in section 6.3.4.2: the bioreactors were working as sequencing batch reactors, the membrane filtration were operated by alternating 6 minutes of filtration and 4 minutes of relaxation.

In all experiments, the OLR value was constant and equal to 28.5 kgTCOD/$m^3$/d, the TCOD concentration in influent was closed to 57 kgTCOD/$m^3$/d and the total HRT was closed to 2d. The only studied variable was the sludge retention time SRT, 3 values were chosen (15, 30 and 60 d) to analyse the response of the SAnMBR to such different functioning conditions. Results are presented as following.

#### 6.4.2.1 Evolution of COD Fractions and Suspended Solids with SRT

Figure 6.6 shows the COD fractions evolution with different SRTs in the acidogenic and methanogenic reactors, respectively.

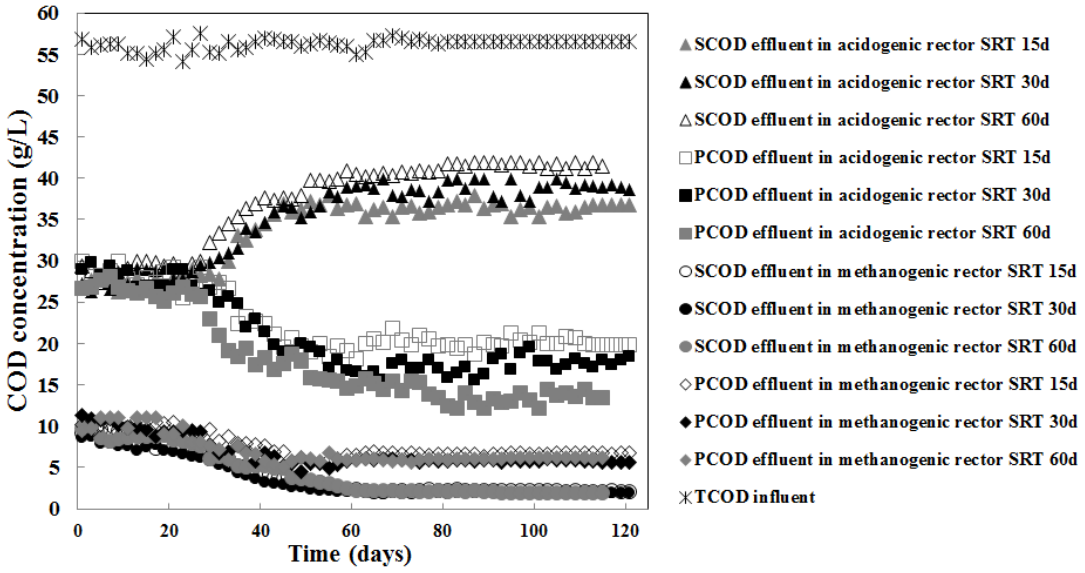



**Figure 6.6** Evolution of COD fractions with time in acidogenic and methanogenic reactors for each SRT.

The results show that the TCOD concentration in the influent was not modified regardless of SRT (each particulate and soluble fraction represented 50% of TCOD). In the

acidogenic reactor, a duration time of about 30 days was necessary to observe a significant hydrolysis of the particulate organic fraction regardless of SRT. Such behaviour corresponded to a simultaneous increase of the soluble organic fraction SCOD. The particulate fraction concentration was calculated as the difference between TCOD in influent minus SCOD in effluent. The SRT increase allowed a significant increase of particulate fraction hydrolysis from 30% at 15d SRT to 60% at 60d SRT. Such a degree of solubilisation, COD yield, can be compared with the values reported by Ucisik and Henze[257] for primary sludge (19.1%), in semi-continuous experiments operated at SRT of 5d and temperature of 37 $^{o}$C, and Chen et al. [258]for waste activated sludge (13.8%) in 1.5 L batch reactors operated at 21±1 $^{o}$C.

The comparison between acidogenic and methanogenic effluents points out an important removal of the different fractions of COD:

- The soluble COD fraction decreased from about 40 to 2 g COD/l regardless of SRT, proving the formation of an important biogas.
- The particulate COD fraction decreases from about 17 to 8 g COD/l regardless of SRT. This reduction can be due to an hydrolysis of this fraction but probably it was also due to the settling step at the end of each sequencing batch functioning that retained the settleable part of the particulate fraction avoiding its presence in influent of methanogenic reactor.

A total TCOD balance between raw POME influent and the methanogenic reactor effluent shows the important removal of organic matter, more than 80%, with a decrease of TCOD from 57 to 10 gTCOD/l respectively, regardless of SRT. A residual part of particulate COD could be observed proving that a part of this fraction was not hydrolyzed. Indeed, hemicelluloses and lignin contain a substantial non-biodegradable part particularly difficult to biodegrade by conventional biological processes such as anaerobic digestion, anaerobic lagoons, or activated sludge processes [69]. Moreover it is obvious that a part of particulate COD was also due to the wash out of small biomass from the reactor caused high concentrations of suspended solids presence in the mixed liquor [5].

Figures 6.7 present the difference between SCOD in the supernatant of the methanogenic reactor and in permeate.

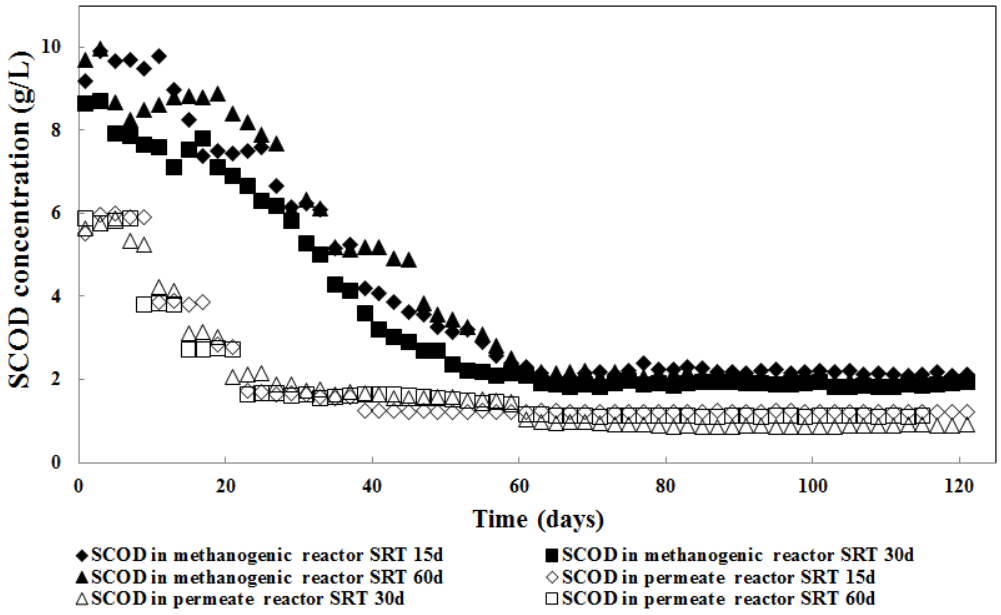



**Figure 6.7** Comparison between SCOD concentrations in the methanogenic reactor and in the permeate.

The presence of the porous membrane barrier allows a supplementary COD removal (about twice lower in permeate) mainly due to the retention of large soluble organic matter (organic polymeric substances) by the membrane. Taking into account such performances, the use of the SAnMBR allowed the obtaining of final water that contained hardly 1g COD/l, which means a total COD removal efficiency was higher in the range of 97 to 99% with a SCOD in permeate lower than 1g/l. Such a low value is favorable to the presence of a tertiary treatment to achieve a very high level of treated water quality and encourage treated water reuse.

#### 6.4.2.2 Effect of SRT on VFA Concentrations

Figure 6.8 shows the influence of SRT on SCOD and VFA concentrations in acidogenic, methanogenic and membrane tanks. If the evolution of SCOD in an acidogenic reactor was a significant indicator of the hydrolysis ability of biomass, the VFA production shows the corresponding acidification of the hydrolysed products.

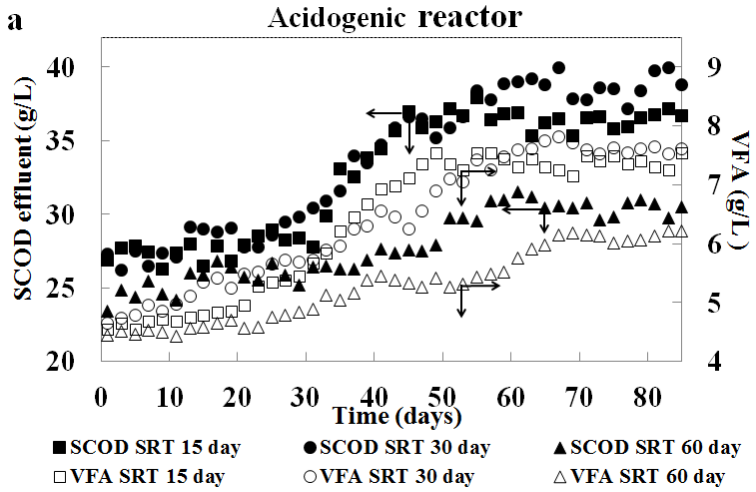



**Figure 6.8** SCOD and VFA evolutions in the acidogenic reactor for different SRT.

As in an acidogenic reactor, SCOD was slightly lower for the highest SRT values, but VFA concentrations did not appear to depend on SRT as soon as the time of operation was higher than 50d. The lower values of SCOD and VFA were observed when the time of experiment was higher than 50d proving the slow adaptation of the system to reach steady state conditions.

It can be observed that a similitude of the concentration evolutions in the reactor proving the link between the hydrolysis (increase of SCOD) and the acidogenic step with the simultaneous transformation of hydrolysed products in VFA without modifying the instantaneous apparent SCOD concentration. Such results prove that the acidogenic phase works as soon as hydrolysed products appear. The hydrolysis process was optimal after an experimental time higher than 50d whatever SRT.

The SRT increase induced a reduction of SCOD and VFA concentrations in the reactor to notify that a part of VFA could be transformed into biogas (some gas was detected at 60d SRT in acidogenic reactor) or directly used for biomass growth. Such a result should prove that it is not necessary to develop too high SRT in AnSMBR for an optimisation of VFA production in acidogenic reactor.

On the other hand, SCOD and VFA concentrations decreased in the methanogenic reactor (Figure 6.9) due the transformation of VFA in biogas. The SRT increase induced a hardly higher reduction of SCOD. The residual VFA concentration did not appear as depending on SRT in methanogenic reactor.

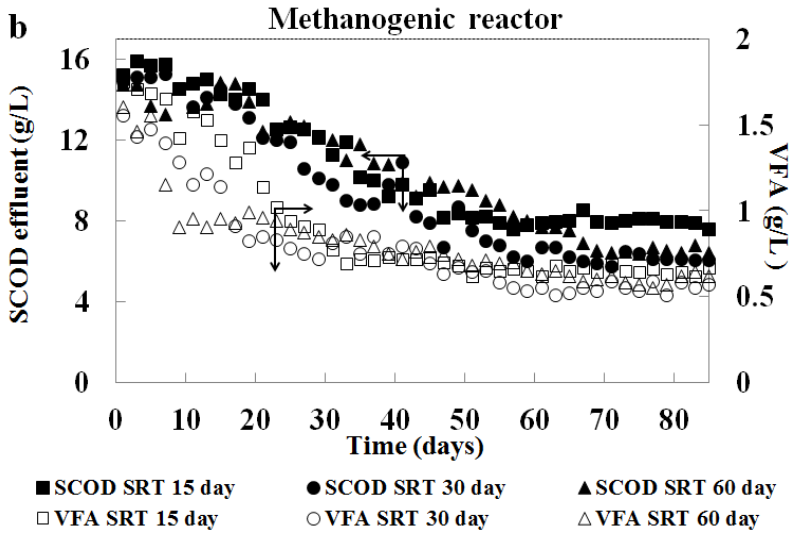



**Figure 6.9** SCOD and VFA evolutions in the methanogenic reactor for different SRT.

#### 6.4.2.3 Effect of SRT on VFA Yield and Composition in Bioreactors

##### 6.4.2.3.1 VFA yield at Different SRT

Figure 6.10 gives the evolution of VFA yield (defined as $VFA_{produced}/TCOD_{removed}$) in the acidogenic reactor according to SRT value. During the first 20 days, VFA yields were quite low in the range of 0.08–0.09 gVFA/gCOD. It increased gradually along with time of operation. The VFA production did not seem depending on SRT but the final level appeared lower for the highest SRT value. VFA yield obtained in this study was comparable to values notified in previous works, 0.095–0.19 gVFA/gTCODfor Ubay-Cokgor et al.[259], 0.058–0.14 gVFA/gTCOD, as reported by Yuan et al. [240].

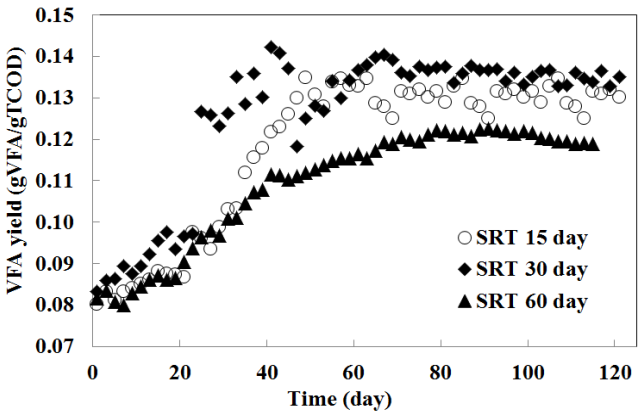



**Figure 6.10** Effect of SRT on VFA yield in the acidogenic reactor.

#### 6.4.2.3.2 VFA Composition at Different SRT

The produced VFA composition is important in assessing the effectiveness of the degree of hydrolysis and fermentation, and in selecting a suitable carbon source in the subsequent carbon removal process. The multiple factors could influence not only the VFA production but also their individual percentages. Thus, the distribution of six VFAs responsible for acetic, propionic, isobutyric, n-butyric, isovaleric, and n-valeric acids was investigated in all the experiments. Acetic, propionic, and n-butyric acids were found to be the dominant of the VFAs in the effluents of acidogenic reactor as shown in Table 6.4. Iso-butyric, n-valeric, and iso-valeric acids were also present but in relatively lower quantities. This result is similar to the general observation reported by other researchers that short-chain VFAs are the main acidification products in low-strength wastewaters [260]. This finding may be attributed tothe direct formation of acetic, propionic, and n-butyric acids fromthe anaerobic acidogenesis of carbohydrates (sucrose in this experiment). However, the higher molecular-weight VFAs, such as n-valeric and isovaleric acids, are partially associated with the fermentation of proteins, which was quite low in the influent.

**Table 6.4** The VFA composition in the acidogenic reactor.

| SRT | VFA composition (g/L) | | | | | |
|---|---|---|---|---|---|---|
| | Acetic | propionic | n-butyric | iso-butyric | isovaleric | n-valeric |
| 15 | 4.282±0.353 | 0.852±0.023 | 1.523±0.033 | 0.261±0.061 | 0.142±0.023 | 0.043±0.012 |
| 30 | 4.664±0.091 | 0.344±0.015 | 1.392±0.032 | 1.051±0.023 | 0.114±0.022 | 0.035±0.011 |
| 60 | 3.904±0.072 | 0.377±0.027 | 1.302±0.061 | 0.983±0.052 | 0.125±0.023 | 0.045±0.011 |

Table 6.5 shows the results from the methanogenic and membrane reactors. The effluent of the methanogenic and membrane reactors also showed predominant volatile fatty acids of acetic and propionic acids, with concentrations higher than other acids (butyric acid, iso-butyric acid, valeric acid and iso-valeric acid). Acetic, propionic, butyric acid, iso-butyric acid, valeric acid and iso-valeric acid concentrations in permeate were always under the inhibition limit (0.1 g/L) [253].It can be noticed that the maximum acetic acid concentration was between 0.28 - 0.31 g/L whatever SRT. This concentration was lower than the inhibitory acetic acid concentrations reported in the bibliography, where it was shown that concentrations higher than 0.78 g/L caused failure in the process and low stability [143]. The propionic and valeric acid concentrations reported in previous works for a correct working process in anaerobic reactors were below 0.74 g/L and 1.02 g/L, respectively [261]. In the present methanogenic reactor a maximum concentration of propionic acid of 0.21 g/L was achieved at the highest SRT studied, and this concentration was always below the failure limit value mentioned in the literature. This indicates the possible growth of aceticlastic methanogens actively utilizing the HAc produced [241]. SRT can then govern the selection of predominant microbial species in the reactor [39].

**Table 6.5** The VFA composition in the methanogenic reactor.

| SRT | VFA composition (g/L) | | | | | |
|---|---|---|---|---|---|---|
| | Acetic | propionic | n-butyric | iso-butyric | isovaleric | n-valeric |
| 15 | 0.314±0.061 | 0.179±0.042 | 0.144±0.022 | 0.023±0.008 | 0.012±0.001 | 0.002±0.001 |
| 30 | 0.281±0.063 | 0.204±0.053 | 0.139±0.021 | 0.018±0.004 | 0.011±0.002 | 0.002±0.001 |
| 60 | 0.299±0.092 | 0.214±0.032 | 0.128±0.023 | 0.019±0.009 | 0.010±0.001 | 0.002±0.001 |

The VFA composition of the acidogenic reactor showed the dominance of acetic acid (n-butyric acids > propionic acid > iso-butyric acid > n-valeric acid >isovaleric) regardless of SRT (Figure 6.11). This indicates the presence of acidogens utilizing the organic compounds in the POME wastewater to generate VFAs. However, when working with the highestSRT (60 d) it resulted in a lower production ofVFA probably consumed by methanogens. Similar observation was foundwhen treating by fermentation primary sludge with SRT higher than 10d [243].

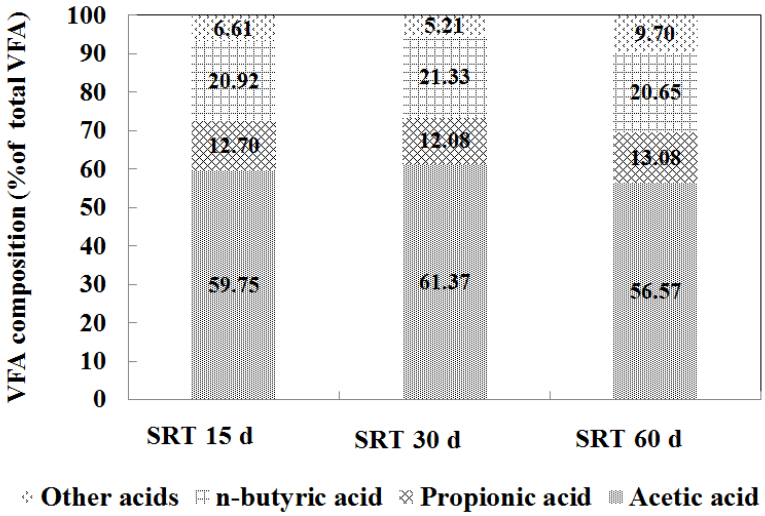



**Figure 6.11** The VFA composition in the acidogenic reactor.

On the contrary, during the methanogenic process, the composition of metabolites varied significantly. A marked variation in acetic acid (42.73-46.60%) concentration was observed along with increase in propionic acid (26.53-32.61%), n-butyric acid (20.03-21.54%), and small concentrations of other acids (isobutyric, n-valeric, and isovaleric acids) in Figure. 6.12. The variation observed in soluble metabolites concentration suggested that VFA was consumed under methanogenic environment in the process of $CH_4$ generation.

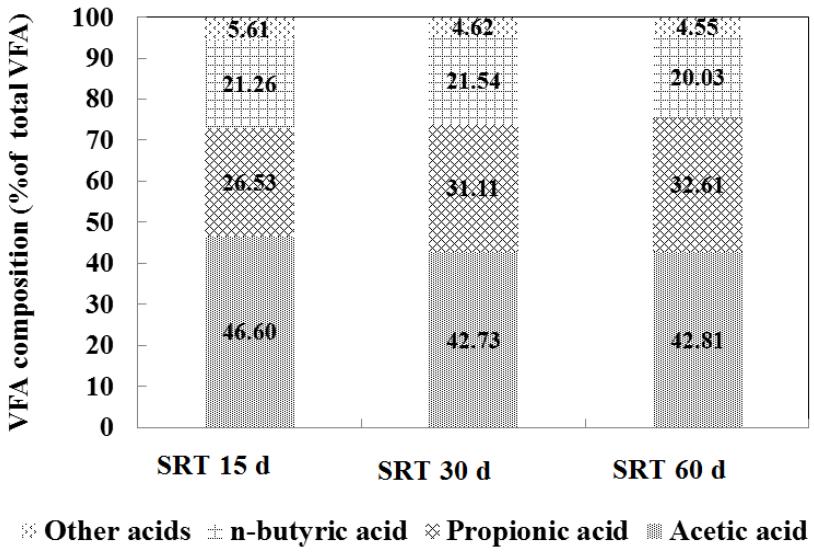



**Figure 6.12** The VFA composition in the methanogenic reactor.

#### 6.4.2.4 The pH, TVFA and Alkalinity in the Fermenter

The pH of the POME influent entering into the acidogenic reactor was adjusted in the range of 5.5–6. Inside the acidogenic reactor, the average pH variation was not significant, 5.85 -5.96 at SRT 15, 30 and 60 day. A number of studies have found acidogenic reactors to operate successfully at pH of between 5.0 and 6.0 while utilising primary sludge [48, 262]. Such pH values in effluent were in range of acidified olive and palm oil wastewater, 4.5–7.5 and 5.0–6.0 [14]. Such results prove that significant levels of acidification were achieved without the use of a pH controller, suggesting that pH control was not necessary for the acidogenic stage.

The effluent generated in the acidogenic reactor was pumped into the equalizer, where the pH was adjusted between 6.85 and 7.16 with a solution of NaOH (6 N), before entering into the methanogenic stage. The optimum range for all methane microorganisms was between 6-8, with an optimum near pH 7.0, while acid microorganism had lower pH optimum around 6.0. The average pH of effluent in the methanogenic reactor increased between 7.44-7.89. The result can be explained by the conversion of volatile fatty acids (VFA) into $CH_4$ and $CO_2$ as well as the alkalinity generated by $CO_2$ dissolution. Of course, pH in permeate did not appear different from pH in methanogenic reactor.

The VFA concentration in the acidogenic reactor appeared lower than in the other studies describing similar treatment of highly biodegradable wastewater. Saddoud et al

[78] have investigated a two-phase anaerobic digestion of chess whey and found VFA up to 5 g/L in the acedogenic reactor. Similar observations were also noticed by Wijekoon et al [181] when treating high strength molasses-based synthetic wastewater with VFA concentration closed to 7 g/L. The stability was regained, the effluent Alkalinity increased again until reaching a final value of 3.6-3.7g/L and VFA was below 0.6 g/L as resulting VFA/TA ratio was 0.15-0.2 and much lower than the failure limit value 0.3–0.4, the process is considered to operate favorably [263]. This was due to the *archaea* and bacteria species producing $CO_2$, $HCO_3^-$, and $NH_3$ [264]. Also, higher ammonium concentrations in the SAnMBR likely led to higher alkalinity concentrations compared to those of the influent.

#### 6.4.2.5 TKN and Ammonia Nitrogen Concentration

The concentration of soluble TKN in the POME influent was closed to 0.8g/l. Figure 6.13 shows the concentration of TKN (a) ammonium (b) and the ratio ammonia-nitrogen/TKN (c) in the acedogenic and methanogenic reactors and in the permeate. The comparison with the concentration of TKN in influent and acedogenic reactor shows a similitude for the lowest SRT. The increase of SRT allowed an increase of TKN proving the hydrolysis of more refractory compounds as proteins for example [265]. Xu et al.[266] supported that the high population in anaerobically digested sludge leads to a higher hydrolysis rate of protein due to *proteolytic bacteria*[267]. The concentration of TKN was not significantly different in the three tanks.

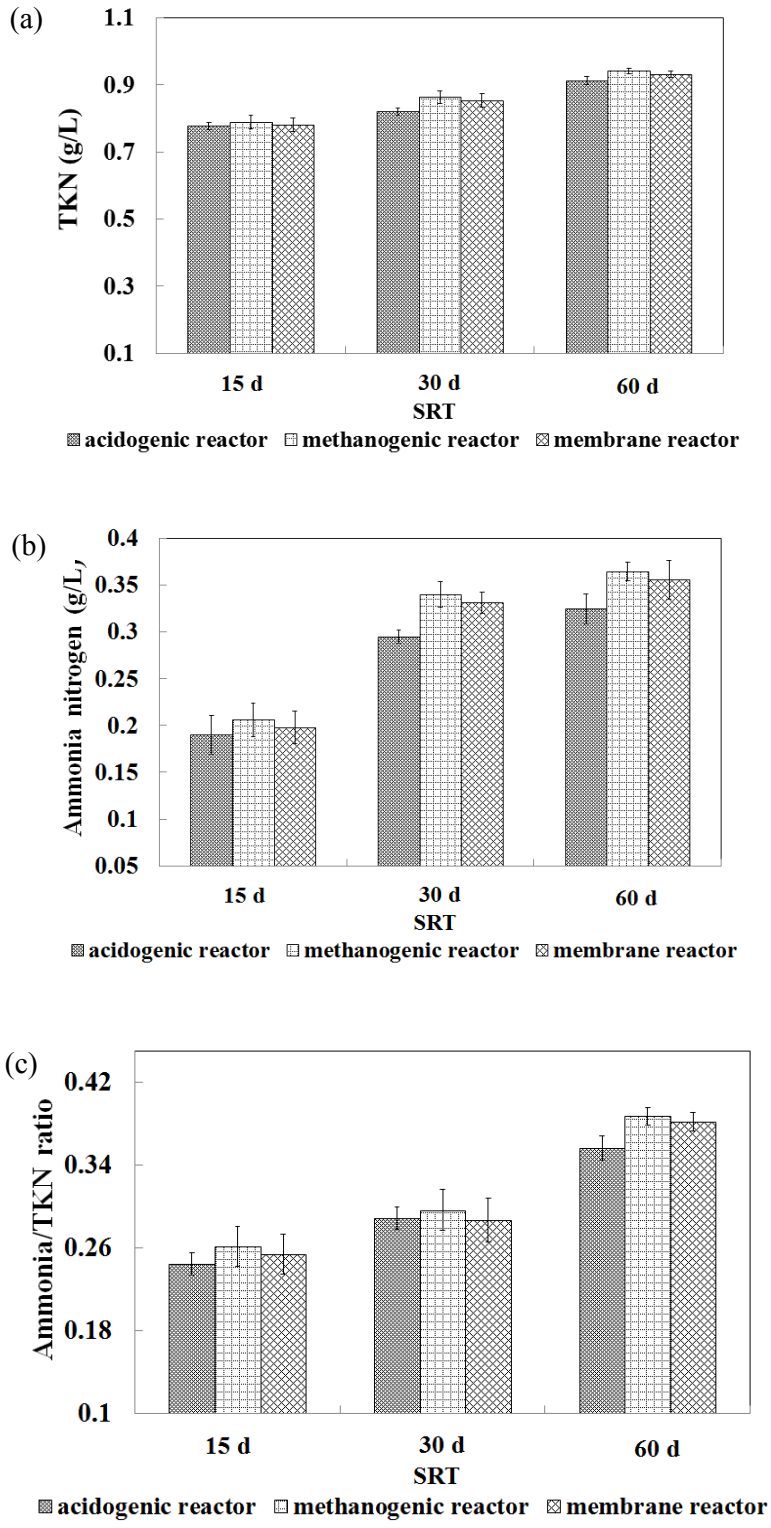



**Figure 6.13** The overall TKN and ammonia nitrogen concentrations with different SRT : (a) TKN, (b) ammonia nitrogen concentration and (c) ammonium-nitrogen/TKN ratio.

The ammonia concentration was not significantly different in the three tanks. TKN increased with SRT. Ammonium represented about 25 to 40% of TKN, its proportion relatively to TKN concentration increased with SRT increase. Nevertheless, the increase of TKN and ammonia in the reactors was not in proportion of their content in protein content of POME influent (26.39% of major constituents). Ammonia should be the main nitrogen component produced during the POME fermentation by organic Nitrogen hydrolysis and production of amino acids and then to ammonia. Garcia-Pena et al.[268] reported that acidification process promoted the subsequent production of ammonia-nitrogen. It is important to notice that Ammonia-nitrogen had a major role in the growth of microorganisms and increases the buffering capacity within the AD process. A large part of ammonia released by hydrolysis could then have been used to insure biomass growth. Ros et al. [269] underlined the importance of Ammonia-nitrogen to insure good performance and stability of AD processes.

The concentration of both ammonia nitrogen and TKN were never in a range that could adversely affect the performance and stability of the anaerobic acidogenic reactors during the whole study. McCarty [178] reported that ammonia concentrations larger than 3g N/L were expected to be toxic at any pH value. Speece [41] demonstrated that an excess of 0.04–0.07 g N/L ammonia-nitrogen must be remained in the reactor to maintain microbial activity. In this study, the bioconversion efficiency and system stability were likely due to the sufficient ammonia-nitrogen concentrations to support microbial growth and adequate buffering capacity. Also Chen et al. [270] reported that ammonia concentrations below 2 g N/L are even beneficial to anaerobic process as an essential nutrient for anaerobic microorganisms. Ammonia-nitrogen to total nitrogen ratio (ammonia-nitrogen/TKN ratio) was considered as a good indicator for estimating the percentage conversion of total nitrogen into ammonia-nitrogen during the anaerobic digestion [271]. The ammonia-nitrogen/TKN ratio slightly increased with SRT as shown in Figure 6.13 (c). The 60 day SRT presented an ammonia-nitrogen /TKN ratio 1.5 times higher than 15 day SRT. Table 6.6 gives a synthetic presentation of the main criterion values.

**Table 6.6** Performance of each reactor of the two-stage SAnMBR at different SRT under steady state conditions.

| SRT (day) | Reactor | Effluent concentration (g/L) * [Removal efficiency %] | | |
|---|---|---|---|---|
| | | pH | TCOD | TVFA |
| 15 | Acidogenic | 5.85±0.23 | 56.50±0.52 [NR] | 4.37±0.17 [NR] |
| | Methanogenic | 7.44±0.18 | 8.84±1.04 [82.99±1.82] | 1.65±0.12 [62.25±3.21] |
| | Membrane | 7.51±0.11 | 1.23±0.02 [97.84±1.48] | 1.54±0.13 [6.39±2.12] |
| 30 | Acidogenic | 5.93±0.26 | 56.03±0.49 [NR] | 5.10±0.13 [NR] |
| | Methanogenic | 7.73±0.14 | 7.79±0.32 [86.16±0.65] | 1.59±0.19 [68.81±3.62] |
| | Membrane | 7.78±0.16 | 0.89±0.02 [98.44±0.56] | 1.46±0.15 [8.31±2.85] |
| 60 | Acidogenic | 5.96±0.19 | 55.82±0.53 [NR] | 5.12±0.18 [NR] |
| | Methanogenic | 7.89±0.17 | 8.05±0.10 [84.40±0.21] | 1.64±0.13 [67.82±1.91] |
| | Membrane | 7.96±0.15 | 1.12±0.05 [98.02±0.53] | 1.51±0.17 [8.57±2.21] |

NR = No removal, *g/L of the every parameter except turbidity unit NTU

**Table 6.6** Performance of each reactor of the two-stage SAnMBR at different SRT under steady state conditions (cont').

| SRT (day) | Reactor | Effluent concentration (g/L) * [Removal efficiency %] | | | |
|---|---|---|---|---|---|
| | | Alkalinity | VFA/Alk | TSS | Turbidity |
| 15 | Acidogenic | 1.87±0.12 [39.74±4.23] | 2.33 | 11.16±0.33 [60.13±2.16] | 17,195±502 [15.41±2.50] |
| | Methanogenic | 4.76±0.25 [NR] | 0.34 | 7.72±0.32 [72.42±3.21] | 8,016±513 [60.58±4.40] |
| | Membrane | 4.54±0.23 [4.61±0.42] | 0.34 | 0.001±.0001 [99.98±0.002] | 6±0.2 [99.92±0.008] |
| 30 | Acidogenic | 2.09±0.08 [47.87±2.31] | 2.44 | 10.93±0.40 [61.24±2.11] | 12,857±443 [38.68±2.56] |
| | Methanogenic | 4.58±0.14 [NR] | 0.34 | 7.01±0.25 [74.88±2.31] | 5,887±261 [71.46±2.31] |
| | Membrane | 4.41±0.23 [3.72±2.12] | 0.33 | 0.0008±.0004 [99.98±0.001] | 6.1±0.1 [99.90±0.01] |
| 60 | Acidogenic | 2.26±0.18 [43.63±4.51] | 2.26 | 10.40±0.53 [62.84±1.83] | 15,086±610 [26.28±2.20] |
| | Methanogenic | 4.77±0.20 [NR] | 0.34 | 7.28±0.42 [74.43±3.81] | 6,498±312 [68.05±3.48] |
| | Membrane | 4.62±0.16 [3.41±1.80] | 0.32 | 0.0009±.0002 [99.98±0.003] | 6.50±0.4 [99.89±0.02] |

NR = No removal, *g/L of the every parameter except turbidity unit NTU

#### 6.4.2.6 Microbial Growth and Concentration

MLSS and MLVSS are both used as measures of the microorganism concentration in the activated sludge system. MLSS includes both the volatile and inert solids in the mixed liquor. MLVSS more closely approximates the biologically active portion of the solids in the mixed liquor, as the microbial cellular material is organic and volatilizes or burns at 550 $^{o}$C. The volatile fraction, i.e. the MLVSS/MLSS ratio observed in this experiment concurs with the typical values given by Metcalf and Eddy [272](0.85) and Woodside and Kocurek [273] (0.80).

Figure 6.14 shows the MLSS and MLVSS concentrations and MLVSS/MLSS ratio under different SRTs. In initial period, the MLSS concentrations varied between 11.52-11.94, MLVSS between 9.89-10.62 and MLVSS/MLSS ratio between 0.88-0.89. With increasing SRT from 15 to 60 days, the concentration of MLSS and MLVSS increased higher than concentration in initial period and the ratio MLVSS/MLSS decreased due to the progressive mineralisation of sludge and the presence of highest alkalinity. Nevertheless the majority of cells were in an endogenous respiration state despite the high organic loading operation condition. Hence, it resulted in an accumulation of inert and inorganic substances as a decrease of the MLVSS/MLSS at longer SRT. This result indicated that the influence of SRT on the characteristics and concentration of the sludge in the SAnMBRs was quite obvious, but its impact on the SAnMBRs performance was insignificant by measuring only COD removal and gas production.

It can be noticed that POME influent also contained many inorganic elements, such as P 94-131, K 1281-1928, Mg 254-344, Ca 276-405 and Fe 75-164 mg/L [274, 275]. Such inorganic matter was not removed significantly by biological processes. Since a large part of the organic matter had disappeared into the bioreactor, a mineralisation of sludge can be noticed in SAnMBR. Moreover, taking into account pH values in methanogenic reactor in comparison with pH values in POME influent and acidogenic reactor, a fraction of inorganic substances might precipitate in the methanogenic reactor modifying the ratio MLVSS/MLSS. MLSS ratio was found in the methanogenic reactor than in the acidogenic reactor.

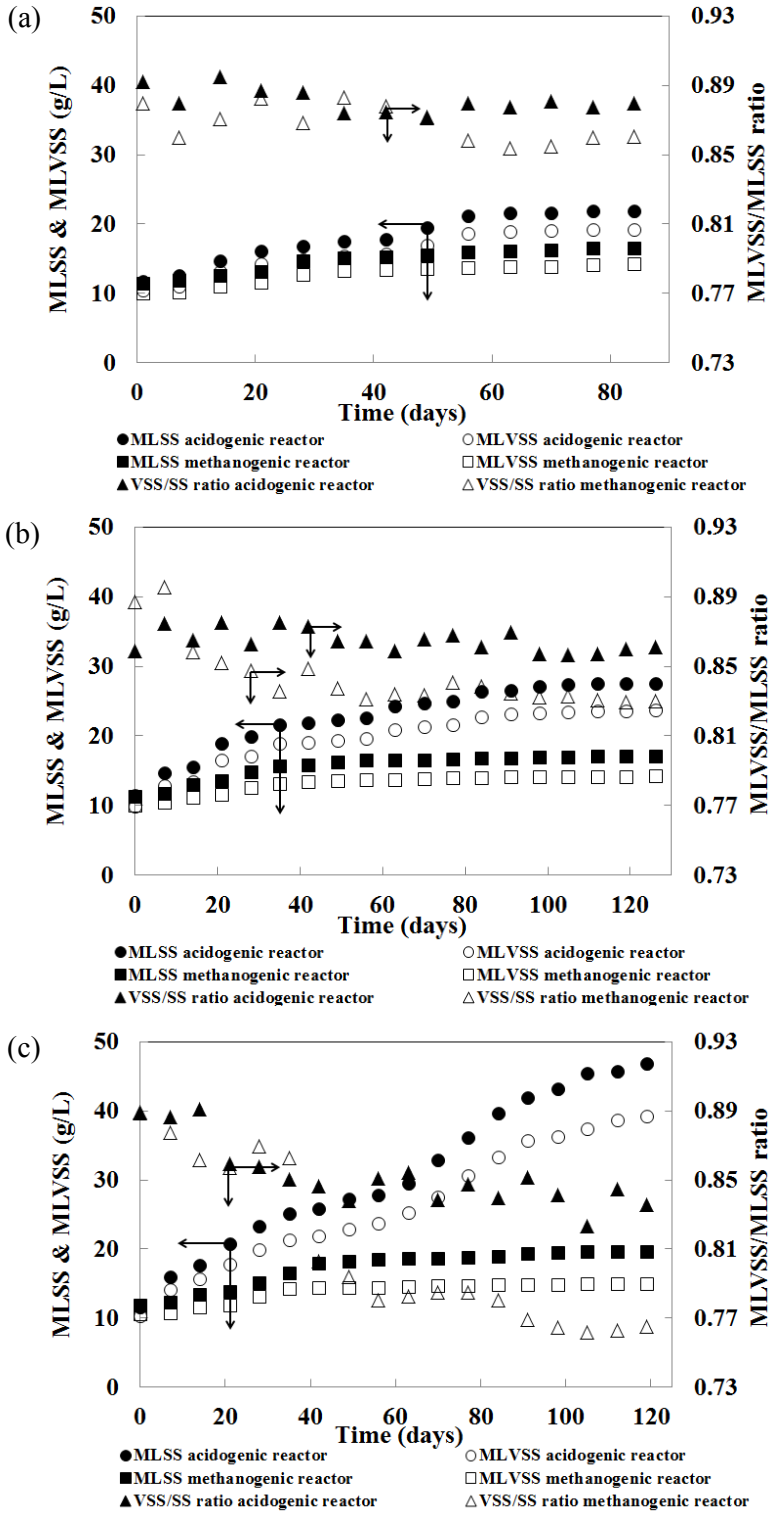



**Figure 6.14** Evolution with the times of MLSS and MLVSS concentrations and MLVSS/MLSS ratio in SAnMBRs for 3 SRTs: (a) 15d, (b) 30 d and (c) 60d.

Since each experiment began with a MLSS concentration equal to 10g/l, it is easy to analyse the biomass growth rate and apparent bioconversion yield coefficient by supposing that MLVSS corresponded to biomass. Such a hypothesis is not strictly correct because a part of MLVSS is composed by unhydrolysed particulate fractions of POME. Such kinetics coefficient values are given in Table 6.7.

**Table 6.7** Average biomass growth rates and apparent bioconversion yield.

| Biological criteria | SRT=15d | SRT=30d | SRT=60d |
|---|---|---|---|
| Apparent bioconversion Yield ($kg_{MLVSS}/kg_{CODrevoved}$) | 0.03 | 0.02 | 0.01 |

### 6.4.2.7 Methane Production and Yield

The production of biogas and its potential use as a source of energy is one of the most interesting benefits of such an anaerobic wastewater treatment. Biogas production and biogas composition were measured during the study as presented in Figure 6.15. Biogas composition and methane yield (litres of methane produced per gram COD removed and litres of methane produced per gram MLVSS) were quantified.

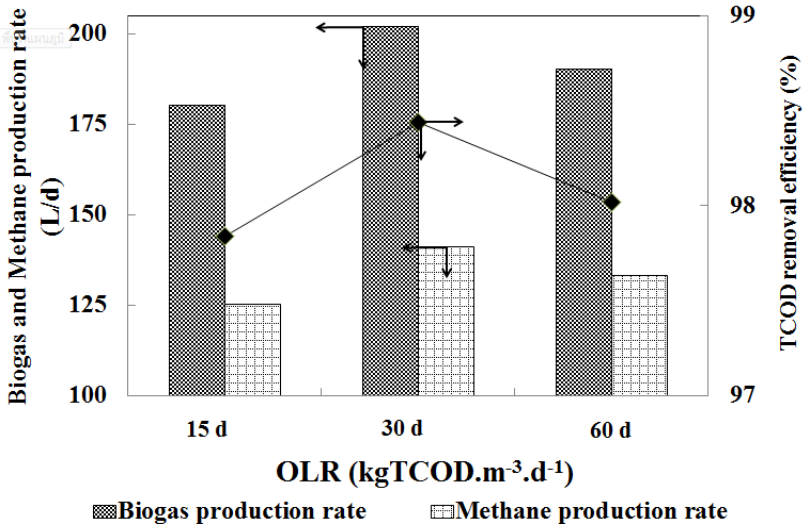



**Figure 6.15** The biogas and methane production rate and methane percentage.

The gas and methane productions increased when increasing SRT from 15 to 30 d but such productions decreased when increasing SRT from 30 to 60 d. The maximum methane yield was found average about 0.35 L $CH_4$/g$COD_{remove}$. Methane yield values found in this study are in accordance with values reported in the theoretic values of 0.35 L $CH_4$/g $COD_{removed}$ and values reported in the literature. Methane yields in the range of 0.3–0.33 L $CH_4$/g $COD_{removed}$ were measured in a UASB reactor treating POME at OLR 10.63 g/L/d [276]. In another study, methane yield from 0.3-0.34 L $CH_4$/g $COD_{removed}$ were estimated in a two-stage UASB and anaerobic digester treating POME in OLR 2.16-16 g/L/d [112, 277]. Comparison with BMP testing, it has relatively low methane yield (0.23 LSTP $CH_4$/g COD $_{removed}$), compared with SAnMBR (0.35 L $CH_4$/g$COD_{remove}$). This was because pH in BMP testing was unsuitable for growth methanogenic bacteria.

For instance, methane achieved a proportion in the biogas of 68-69%, whereas $CO_2$ decreased between 20-21%. This variation in the biogas composition can be explained by a difference in the gas solubility of these two gases. According to Henry's Law, the solubility of methane in water is 11.4 times lower than that for $CO_2$, with partial pressures of methane and $CO_2$ at 0.7 and 0.3 bar, respectively, at 20$^o$C. Thus, $CO_2$ was dissolved in a major proportion in the liquid phase of the reactor and left the reactor dissolved in the effluent.

The two-stage SAnMBR with SRT 30 d would benefit the growth of methanogenesis, and become more efficient in terms of methane production. Therefore, the role of SRT appeared as determining for biogas production, increasing with SRT probably by the adaptation time of the biomass to the different compounds present in POME. The results show that the SAnMBR achieves higher performance in terms of organic removal efficiency and methane yield at higher OLR and shorter HRT as compared to the conventional system.

#### 6.4.2.7 The Overall Performance of SAnMBR

From the COD, SS removal performance, the biomass concentration and methane production and yield of the three SAnMBR operating at different SRTs are summarized in Table 6.8. It was shown that the SAnMBR could achieve excellent treatment performance in terms of COD removal and biogas production for treating POME. Such results are mainly due to the total control of biomass concentration in bioreactors (notably in the most sensitive methanogenic reactor) by the high retention capacity of the porous membrane in regard with microbial cells. SAnMBR has high

performance in treating POME with high level of COD and SS removal more than 97%, but it slightly changed when operated under different SRTs due to the high quality of permeates produced by the MF membranes.

Meanwhile, the methane production and biomass concentration show significant differences under different operating SRTs. This suggested that the effect of SRT on methane production of SAnMBR was significant when the SRT was short, e.g. 15 d. This is due to a lowest methanogenic activity was occurring in SRT 15d than the other two SAnMBRs SRT 30 and 60 d. The increased as SRT would benefit increased concentration of slow-growing methanogenesis and become more efficient in terms of methane production [100].

However, a slight decrease methane production was observed at the longest investigation of SRT in 60 days. The results are consistent with those reported by Rubia et al., 2006. With respect to organic decomposition, sludge specific activity slightly decreased with prolonged SRT. This might be explained by: (1) the decline of MLVSS/MLSS was mainly due to accumulation of inert biomass, which was metabolic products of the endogenous respiration, in the membrane bioreactor; and (2) impeded transfer of substrate from the outside to the inside of activated sludge flocs owing to an increase of the sludge concentration at long SRT. SAnMBR with SRT 30 d would long enough to provide sufficient retention time for contact of biomass and the growth of methanogen and become achieved a better treatment performance and lead to more biogas generation. Therefore, this result indicated the influence of SRT was not slightly impact performance treatment whereas it was quite obvious on biomass concentration and biogas production rate.

Such performances can be compared with other works (Table 6.9) developing POME treatment by other intensive anaerobic treatments, such as UASB, AFFR, CSTR and anaerobic digester [15, 112, 138, 277], which attained overall COD removals in the range of 85–95% at a much lower OLR ( 4.5 kg COD/$m^3$/d). Moreover, the high concentration of biomass in SAnBMR related with the biogas production and energy conversion. SAnMBR treating 1 $m^3$ POME have been estimated to generate electricity energy approximately 42 kW/h (based on 1 $m^3$ biogas could be generated the electricity approximately 1.2 kW/h) [278], which was higher than treatment by a covered lagoon type of anaerobic biodigester (average 2.185 kW/h) [279].

**Table 6.8** Performance of the overall two-stage SAnMBR reactor at different SRT**.**

| Parameters | SRT (days) | | |
|---|---|---|---|
| | 15 | 30 | 60 |
| COD removal (%) | 97.25±0.04 | 98.05±0.04 | 98.41±0.07 |
| SS removal (%) | 99.99±0.01 | 99.99±0.01 | 99.99±0.01 |
| MLSS (g/L) | 20.38±1.83 [a]<br>15.91±0.56 [m] | 26.88±0.88 [a]<br>16.94±0.18 [m] | 42.70±3.82<br>19.33±0.36 [m] |
| MLVSS (g/L) | 17.87±1.65 [a]<br>13.76±0.34 [m] | 23.15±0.68 [a]<br>14.12±0.08 [m] | 35.87±3.04 [a]<br>14.87±0.11[m] |
| MLVSS/MLSS | 0.88±0.01 [a]<br>0.87±0.01 [m] | 0.86±0.01 [a]<br>0.83±0.01 [m] | 0.84±0.01 [a]<br>0.77±0.01 [m] |
| Methane production (L/day) | 125±0.02 | 142±0.01 | 134±0.05 |
| Methane yield (L $CH_4$/g $COD_{remove}$) | 0.31±0.01 | 0.35±0.03 | 0.33±0.05 |
| Specific methane yield (L $CH_4$/g VSS) | 1.10±0.06 | 1.16±0.03 | 1.13±0.02 |
| Apparent bioconversion Yield ($kg_{MLVSS}/kg_{CODrevoved}$) | 0.03 | 0.02 | 0.01 |

[a] acidogenic reactor [m] methanogenic reactor.

**Table 6.9** Comparison of AnMBR with other anaerobic treatment performance of POME.

| Processes | OLR (g COD /L day) | HRT (days) | Methane composition (%) | Methane yield (L $CH_4$/g COD $_{remove}$) | COD removal efficiency (%) | Ref. |
|---|---|---|---|---|---|---|
| Two-stage SAnMBR | 27.35-29.95 | 2 | 68-70 | 0.35 | >98 | This study |
| One-stage AnMBR | 1-11 | 6.8-600 | 67-72 | 0.25-0.57 | 96-99 | [40] |
| AFFR | 1.5-11 | N/A | 65-70 | 0.3-0.5 | 60-70 | [26] |
| UASB | 10.63 | 4 | 54.2 | 0.3-0.33 | 98.4 | [79] |
| UASFF | 1.75-23.15 | 3 | 62-71.9 | 0.34 | 89.5-97.5 | [33] |
| CSTR | 3.33 | 18 | 62.5 | N/A | 80 | [80] |
| Fluidized bed | 40 | 0.25 | N/A | N/A | 78 | [81] |
| Anaerobic digester | 2.16 | 20 | 36 | 0.3-0.34 | 80.7 | [82] |
| Two-stage UASB | 16.6 | 6.5 | 63 | 0.3-0.33 | 90 | [83] |

## 6.5 Conclusion

The results presented in this chapter confirm the potential of SAnMBR to treat POME wastewater.

- SAnMBR has high performance in treating POME with high level of COD and SS removal of more than 97%.
- The methane production and biomass concentration shows significant differences under different operating SRTs.

- Nevertheless, biological performance analyses shows the important role of SRT with an optimal SRT closed to 30d for the chosen OLR in term of COD removal and gas production. Moreover, SRT let also appears determining in term of final separation step by porous membranes.
- Indeed this separation step if often the weak point of submerged MBR due to the difficulty to control membrane fouling during operation. Due to the importance of biomass concentration and soluble fraction in methanogenic reactor, such a control should be the limiting point of AnMBR development. The importance of membrane to get a so important total retention of COD confirms the risk of accumulation of large amount of matter onto the membrane surface when filtering such complex suspension. It is then important to analyse specifically the membrane separation step to have a better idea of the SAnMBR challenge to treat POME effluent.

# CHAPTER 7
# EFFECT OF SRT ON MEMBRANE FOULING INTENSITY IN TWO-STAGE SAnMBRs TREATING PALM OIL MILL EFFLUENT

Submerged anaerobic membrane bioreactors (SAnMBRs) treating palm oil mill effluents (POME) were analysed in terms of membrane fouling when working at three different sludge retention times (SRTs of 15, 30 and 60 d).The average permeate flux was fixed at 2.4 L/m$^2$·h. During operation, the membrane was regenerated by using two steps: membrane rinsing during each experiment as soon as trans-membrane pressure (TMP) reached 125-130 mbars, and complete membrane cleaning including backwash and chemical cleaning at the end of each experiment when analysing the membrane surface and foulant material.

## 7.1 Introduction

In the past decades, anaerobic biological processes have been successfully applied for treating palm oil mill effluents (POME), because they offer advantage in terms of renewable energy recovery through biogas production and organic matter removal efficiency [280]. Nevertheless, the complexity of POME compositions (e.g. suspended solids (SS), more or less biodegradable organic matter, oil and grease that generate scum formation), and the slow growth rates of anaerobic microorganisms have obliged the development of large reactor sizes to treat such wastewater. However, the intensification of anaerobic processes can be obtained when the sludge retention time (SRT) is dissociated from the hydraulic retention time (HRT) by specific reactor configuration [119]. The reactor model for such success is the Up-flow Anaerobic Sludge Blanket (UASB). The concentration of sludge in such reactors is important and large OLR over 15.1 kg COD·m$^{-3}$·d$^{-1}$ can be applied [5].

The only default of such a system is the possibility of degradation of the sludge pellet settle-ability that induces reactor unsteadiness. To improve effluent quality and system steadiness, the use of a final filtration on porous membranes was proposed in the last decade [19, 20, 22, 281]. Due to their high potential of bacterial retention, porous membranes favour the retention of any microbial communities able to degrade specific pollutants present in wastewater [282]. Therefore, this technology presents an attractive

option to treat industrial wastewaters at extreme conditions, such as high salinity, temperatures, and concentrations of suspended solids (SS), even in the presence of toxics that hamper granulation and biomass retention by conventional systems [24, 27]. Anaerobic membrane bioreactors (AnMBRs) can operate at high biomass concentrations, high SRT and OLR and relatively short HRT, independently of the sludge flocculation state. Nevertheless, the weak points of MBRs remain in the membrane fouling and the presence in the suspension of high sludge concentrations and/or high colloidal matter concentrations can drastically limit AnMBRs development, despite using some methods, such as high cross-flow velocity [105], ultrasonic irradiation [111], gas sparging and the addition of activated carbon [24].

The choice of SRT value directly influences the system performance, but also the biological characteristics of the suspension through the values of biomass concentration, extracellular polymeric substances and soluble microbial products (EPS, SMP). Moreover, the degree of local shear stresses favourable to mass transfer also influences the particle size distribution, and such behaviours modify suspension filterability [235]. When treating a municipal wastewater, the analysis of membrane fouling in SAnMBRs showed the determining influence of solids deposit on the membrane surface, which formed a layer whose permeability was continuously decreasing over operation time [129].Such a layer also plays the role of a membrane through its contribution to the retention of soluble and colloidal contents such as SMP and EPS during filtration that improves permeate quality, but such retention also has a drastic negative effect on deposit permeability [220]. Hence, the EPS/SMP concentration in the mixed liquor supernatant plays an important role in membrane fouling, notably by generating a layer including biofilm development. Then longer SRTs combined with shorter HRTs lead to both higher mixed liquor suspended solids (MLSS) and SMP concentrations speeding up cake and biofilm formation and a high degree of fouling. Since membrane fouling is one of the most important problems of SAnMBRs processes, the influence of SRT on membrane fouling needs to be investigated.

Therefore, SRT appears as the determining criterion in AnMBRs, not only for the bacterial capacity to transform organic matter in volatile fatty acids (VFA) and biogas, but also for its influence on the filterability of the suspension through two main criteria: solids in suspension and exo-polymeric substance concentrations, and their roles in fouling dynamics.

## 7.2 Research objective

This work is to investigate the role of SRT on membrane separation and membrane fouling when treating POME.

## 7.3 Materials and Methods

### 7.3.1 Experimental Set-up

The experimental setup and POME characteristics were conducted using a laboratory scale system as explained in sections 6.3.2-6.3.4.

### 7.3.2 Operating Conditions

The SRT influence was investigated by 3 experiments through three values of 15, 30 and 60 days. For each experiment, the MLSS concentration in the suspension was started up at approximately 10 g/L. Table 1 gives the experimental conditions. To minimize membrane fouling, intermittent filtration was operated with a hollow fiber membrane module: a 6 minutes period of filtration was followed by 4 minutes of relaxation (no filtration). During the 6 minutes of filtration gaseous nitrogen was injected (1L/min corresponding to $0.6 m^3_{gas}/m^2_{membrane}/h$) in the membrane tank close to the membrane surface and the permeate flux was constant and equal to 4 $L/h/m^2$. The corresponding daily average permeate flux value was 2.4 $L/h/m^2$when including the relaxation period.

**Table7.1** Operating conditions of two stage SAnMBRs system.

| Operating conditions | Values |
|---|---|
| Total SAnMBR working volume (L) | 20 |
| COD concentration in influent (g/L) | 55-60 |
| SRT (d) | 15, 30, 60 |
| HRT in acidogenic tank (d) | 0.5 |
| HRT in methanogenic and membrane reactor (d) | 1.5 |
| Initial MLSS concentration (g/L) | 10 |

The duration time for each experiment was 90, 130 and 125 days for the three SRT values (15, 30 and 60 days), respectively.

### 7.3.3 Analytical Methods

Membrane fouling as analysed in terms of hydraulic resistances, observations of membrane surface (SEM, EDX, AFM and FTIR) spectroscopy, as explained in section 3.4.1 -3.4.4 and biological suspension characteristics (SMP and EPS analysis), as shown in Figure 3.2.

## 7.4 Results and Discussion

### 7.4.1 Filtration Performances

Figure 7.1 points out some main observations:

1) The average TMP continuously increased during a long period of working despite intermittent relaxation periods and nitrogen sparging. TMP increase can be divided into two periods representing different rates of evolution (dTMP/dt = P1 and P2 respectively, as presented in Figure 7.1a-c). The second period presented a higher rate of evolution, probably due to higher values of TMP, that could have an impact on deposit compressing. It could also be due to biofilm structuring with a lower permeability than only compound accumulation onto the membrane surface.
2) Membrane rinsing (step 1 of the cleaning procedure) was not sufficient to recover the initial membrane permeability, because of internal membrane fouling, whose evolution intensity over time can be quantified by the slope P3, representing the evolution of the TMP value obtained after each membrane rinsing all along each experiment (P3 is also presented in Figures 4a-c). It can be noticed that P1 period could disappear when the internal fouling became significant (second period of the third experiment at SRT equal to 60d)
3) At the end of each experiment, the total membrane cleaning procedure allowed the total recovery of membrane permeability.

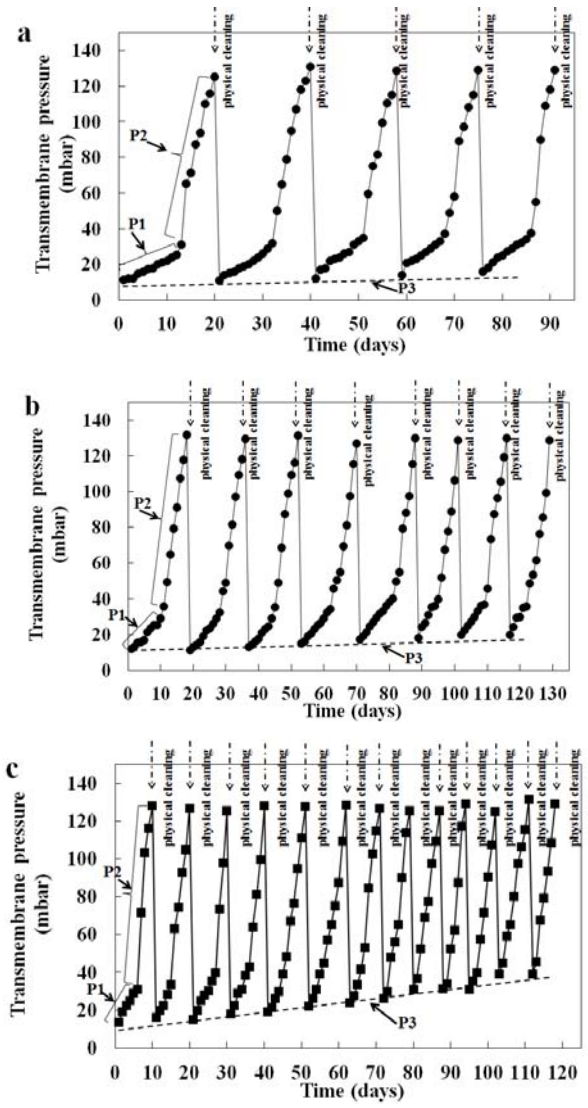



**Figure7.1** Average TMP evolution versus time for (a) 15d SRT, (b) 30d SRT and (c) 60d SRT.
(each verticle arrow represents a wipe cleaning of membranes)

Table 7.2 displays the values of P1, P2 and P3 for the three tested SRTs. It can be observed that P2 is more than 3 times higher than P1. In contrast P3 appears very low in comparison with P1 (25 times lower), confirming the dominant role of cake deposit in comparison with internal fouling phenomena. The values of P1, P2 and P3 increased with SRT in agreement with previous studies [235].

**Table 7.2** Evolution rates of TMP versus time in accordance with SRT.

| SRT (day) | P1 (mbar/d) | P2 (mbar/d) | P3 (mbar/d) |
|---|---|---|---|
| 15 | 1.99± 0.57 | 13.35± 1.39 | 0.065 |
| 30 | 2.66± 0.79 | 13.89± 1.94 | 0.068 |
| 60 | 5.43± 1.03 | 19.33± 4.23 | 0.223 |

The complete regeneration of the membrane was carried out at the end of each experiment. In accordance with the three cleaning steps, the hydraulic resistance due to each defined origin of fouling could be calculated by using Equations 5.1. Figure 7.2 illustrates the respective values of each resistance at the end of each experiment in accordance with SRT values.

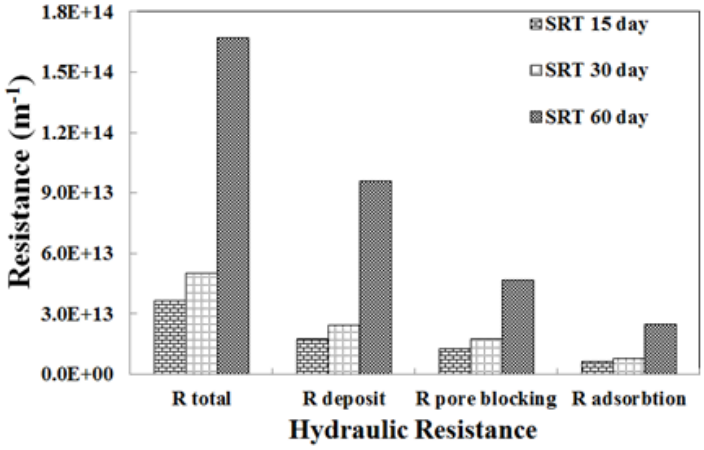



**Figure7.2** Total and specific hydraulic resistance values versus SRT.
(Virgin membrane resistance: $R_m$ equal to $1.1.10^{11}$1/m)

Figure 7.2 clearly confirms the dominant contribution of cake deposit on the membrane fouling. Taking into account that the internal fouling was only removed when each SRT experiment was achieved (in comparison, external fouling was more frequently removed, 5, 8 and 13 times for 15, 30 and 60d SRT experiment, respectively), the internal fouling appeared very slow in comparison with external fouling ones. The role of SRT on these resistances appeared determining as its increase had drastically increased the values

of each type of resistance. Its influence on deposit seems obvious in accordance with its influence on sludge concentration (Figure7.2).

### 7.4.2 Membrane and Foulants Characterization

#### 7.4.2.1 SEM

SEM images were taken to investigate the surface morphology for both virgin and fouled membranes, as shown in Figure 7.3. Virgin PVDF membranes (Figure 7.3a) show a quite smooth and clean surface free of particles. In contrast, the fouled membrane surface (Figures 7.3b-d) was covered by deposited particles presenting diverse sizes and shapes. The fouled membrane at the shortest SRT (15 d) shows some slime colloid and rod shaped bacteria cells. At 30d SRT, a more compact deposit was presented showing the presence of filamentous shaped bacteria cells. Similar observations at 60d SRT can be found with an improvement in cell density in deposit. A longer SRT corresponded to higher biomass concentration in the reactor that decreased the filterability of the suspension and also the organic loading rates applied on biomass (F/M ratio decreased from 0.44 to 0.11 kg COD/kgMLVSS/dwhen SRT increased from15 to 60d respectively). The deposit thickness clearly increased with SRT increase (1.39-2.84 µm, 3.10-3.56 µm and 3.76-4.64 µm for SRT of 15, 30 and 60d, respectively). Consequently, the deposit resistance rapidly increased, inducing a higher TMP value and more severe membrane fouling.

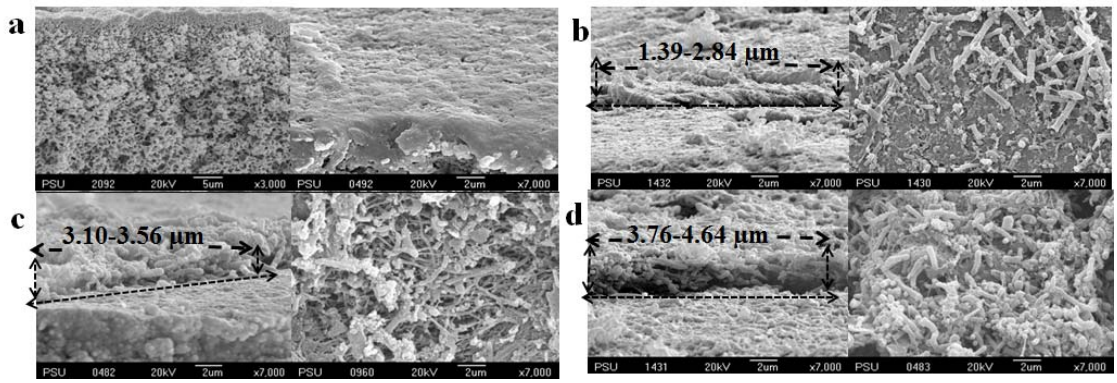



**Figure 7.3** SEM photographs of (a) virgin membrane surface and (b), (c) and (d) fouled membrane surfaces at SRT 15, 30 and 60d.

#### 7.4.2.2 AFM

Surface roughness may influence foulant interactions with the membrane surface. AFM images of fouled membranes were significantly different from the surface morphologies of the virgin membrane, as reflected by Ra and Rms.The AFM data in term $R_a$, $R_{rms}$ and $R_z$ for virgin and fouled membranes at the end of each SRT 15, 30 and 60 days are presented in Table 7.3and Figure 7.4a-d.

The virgin membrane exhibited $R_a$ and $R_{rms}$ values significantly lower than the values for fouled membranes. SRT had a direct impact on surface roughness and higher SRT corresponded to lower roughness but more compact deposits presenting lower permeability as indicated by other authors [283].The surface morphology of fouled membrane showed Ra and Rms values four times higher than that of the virgin membrane, and exhibiting a surface coverage with a ‘‘higher topography’’. The increase in membrane surface roughness might be attributed to the surface enrichment of molecules due to fouling caused by pore blocking or surface adsorption.

Figures 7.4b-d show the overall AFM images of the outer surface of the fouled membrane at SRT 15, 30 and 60 days, which are presented in 25 μm× 25 μm. It can be seen that the surface of the membranes is not smooth but consists of a mass of peaks (bright region) and valleys (dark region). Moreover, the change in surface roughness indicates the deposition of foulants on membranes and uneven distribution of foulants. Table 7.3 showed the Ra and Rms of fouled membrane decrease from 305.5 to 106.17 nm and from 416.14 to 159.12 nm, respectively with increasing SRT from 15 to 60 day. The SRT 60 day had a lower surface roughness. A smaller roughness same as the previous study that attributed a low roughness usually corresponds to a compact structure; therefore, a high roughness of fouling layer would be helpful to obtain better filtration performance [283]. These results are in good agreement with the research by Lee et al [71], who reported that the fouling layer formed with suspended growth microorganisms (87 nm) had higher roughness than that formed with attached growth microorganisms (34 nm) in MBR. A more recent study also suggested that the cake layers formed with thermophilic (52 nm) smaller surface roughness mesophilic sludge (26 nm) [22]. Therefore, the smaller roughness with prolong SRT responded to a more compact structure of foulant deposition on membrane surface.

**Table 7.3** AFM data in term of $R_a$ and $R_{rms}$ for virgin and fouled membranes.

| Experiments | Mean roughness, Ra (nm) | RMS roughness (nm) | Peak-to-valley height Rz nm) |
|---|---|---|---|
| Virgin membrane | 77.6 | 104.8 | - |
| SRT 15 day | 305.5 | 416.14 | 3950.6 |
| SRT 30 day | 291.30 | 397.10 | 3720.9 |
| SRT 60 day | 106.17 | 159.12 | 1695.8 |

Virgin membrane exhibits $R_a$ and $R_{rms}$ values significantly lower than the values for fouled membranes. SRT had a direct impact on surface roughness, and higher SRT corresponded to lower roughness, but more compact deposits presenting lower permeability as indicated by other authors [284]. After that, the virgin and fouled membrane surface at the end of SRT 15, 30 and 60 days was taken out to analyze the major membrane fouling, such as the presence of inorganic fouling using EDS analysis, FTIR technique and coupled with SMP and EPS methods to identify other types of membrane fouling, such as organic or bio-fouling.

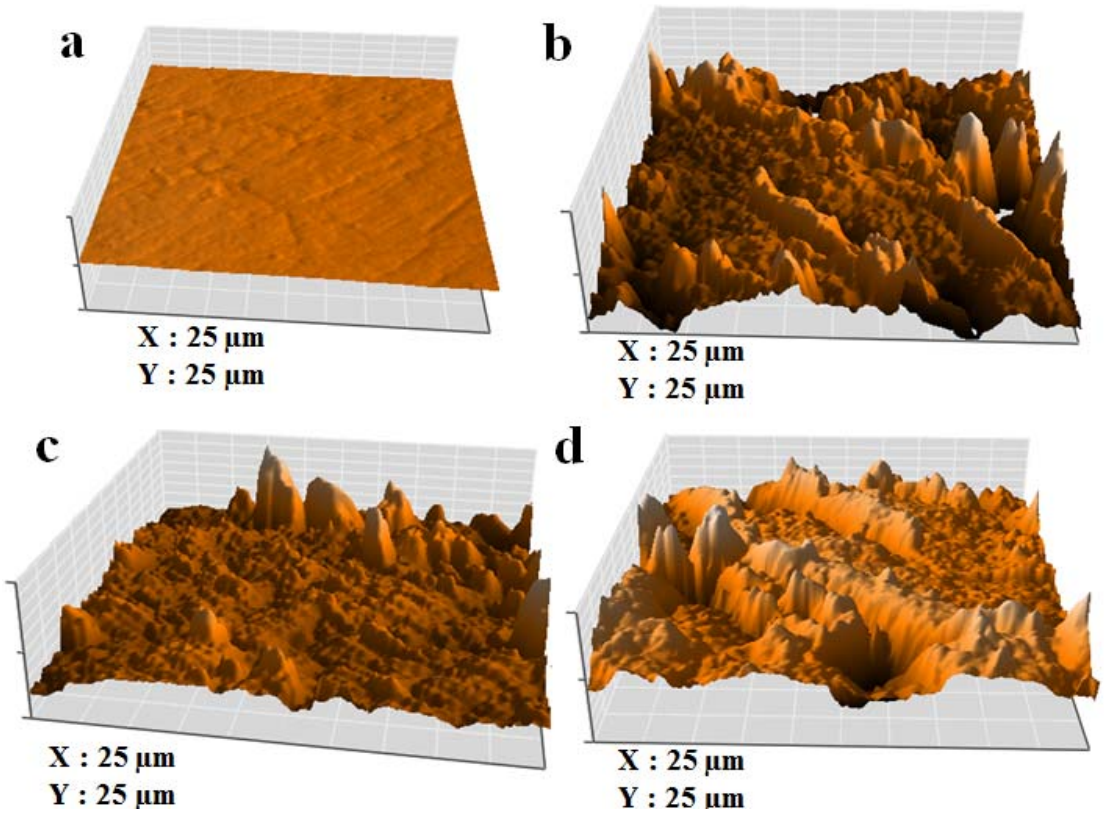



**Figure 7.4** AFM images of virgin and fouled membranes at different SRT; (a) clean membrane,(b) SRT 15 day,(c) SRT 30 day,(d) SRT 60 day.

#### 7.4.2.3 EDX

Element analysis was performed to quantify the adsorption of major chemical components during filtration. Figure 7.5 illustrates such results. The major peaks of C, F and O (Figure 7a) correspond to the components of the virgin membrane (PVDF material), as indicated by Lee and Kim [284]. After filtration, the EDX analysis of the fouled membrane (Figures 7b-d) showed the presence of Mg, Al, Si, P, S, K, Ca and Fe. Such elements are contained in POME feed water (94<P<131 mg/L, 1281<K<1928mg/L, 254<Mg<344mg/L, 276<Ca<405mg/L and 75<Fe<164 mg/L as indicated by Wong et al. [275]. Wang et al.[236] reported that inorganic elements such Mg, Al, Si, Fe, and Ca have some significant role in the development of gel and cake layer. You et al.[285] found that they induced more severe membrane fouling with greater difficulty to recover initial membrane permeability even by chemical cleaning. Peak intensity increased significantly with SRT increase (Figures 7b-d) and could contribute to more drastic membrane fouling

when working at 60d SRT. Moreover, the biopolymers from microorganisms containing ion groups, such as $COO^{-}$, $CO_3^{2-}$, $SO_4^{2-}$, $PO_4^{3-}$, and $OH^{-}$, could induce biological precipitation from captured metal ions [286]. Chemical and biological precipitations might then occur by means of local conditions inside the deposit [285]. In addition, inter-bridging between deposited inorganic precipitation and organic foulants would enhance a dense cake layer formation and thus cause more intensive fouling behaviour [287]. Such phenomena confirmed the negative contribution of high SRT on fouling intensity.

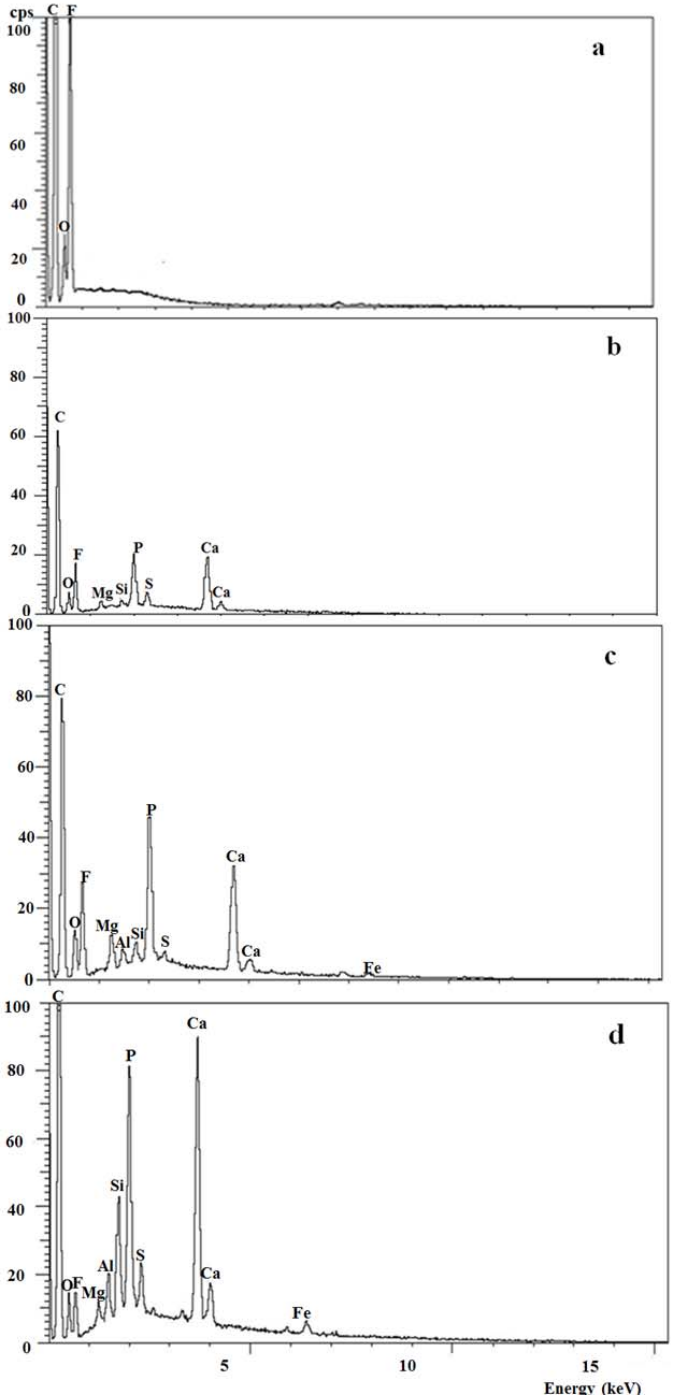



**Figure 7.5** EDX of (a) original membrane: and fouled membrane surface (b) 15d SRT, (c) 30d SRT and (d) 60d SRT.

#### 7.4.2.4 FTIR

FTIR has been used to characterize functional groups contained in organic compounds, such as proteins and polysaccharides. FTIR has been used to characterize functional groups contained in organic matter molecules such as proteins and polysaccharides adsorbed on the surfaces of membranes [288, 289]. The FTIR spectra of the virgin and fouled membrane surface at the end of each SRT 15, 30 and 60 days are shown in Figure 7.6.

The FTIR spectrum for SRT 15, 30 and 60 days presented polysaccharide and protein in as shows in Figure 7.6. They are similar in profile, but significantly different in the adsorption intensity. There are characteristic peaks around 1000–1200cm$^{-1}$, which is due to C-O bond is associated with polysaccharides [290]. Also, there are three peaks around 1600–1700 cm$^{-1}$, 1500–1600 and 1000-1350 cm$^{-1}$ in the spectrum which are unique to the protein secondary structure, called amides I (C=O), II (N-H in plane) and III (C-N stretching), respectively [291, 292]. The amide I is the stretching vibration bands associated primarily with the peptide carbonyls (C=O), and the amide II bands is due to the interaction between the N–H bonding and the C–N stretching of the C–N–H group [293]. Thus, these results indicated that there were proteins in the membrane foulants. Other peaks, Peaks in the vicinity around 2850- 2920 cm$^{-1}$ are indicative of aliphatic C-H stretching [81]. There is a broad region of absorption at 3293-3432.42 cm$^{-1}$, which is due to the stretching of the O–H bond in hydroxyl groups [198, 286].

The FTIR spectra of the fouled membrane measured at the end of each experiment (SRT 15, 30 and 60d respectively) are shown in Figure 7.6. Peaking around 1000–1200cm$^{-1}$ is due to C-O bonds associated with polysaccharides [22]. The three peaks around 1600–1700 cm$^{-1}$, 1500–1600 and 1000-1350 cm$^{-1}$ are unique to the protein secondary structure, called amides I (C=O), II (N-H in plane) and III (C-N stretching), respectively [22]. The major components of foulants found in this study were then identified as proteins and polysaccharides. If the spectra are similar in their profiles, they present a significant difference in their adsorption intensities at a wavelength of around 1000–1200 cm$^{-1}$ and 1350-1700 cm$^{-1}$. The higher peak corresponds to 60d SRT. Such higher concentrations were observed when the cake layer induced faster membrane fouling [101].

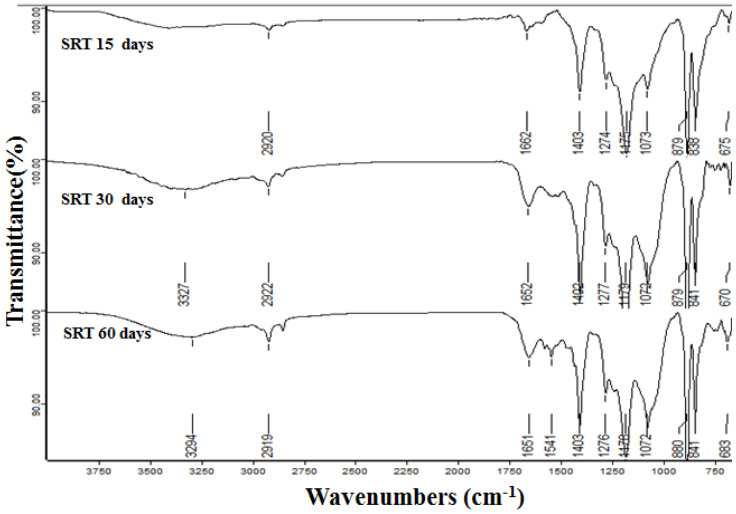



**Figure 7.6** FTIR spectra of fouled membrane surface at SRT 15, 30 and 60d.

#### 7.4.2.5 EPS/SMP Quantification in Fouling

This section describes the significant differences of fouling propensity due to the different soluble organic fraction concentration present in the biofilm/cake layer. The biofilm/cake layer formation was regarded as the dominate factor blocking the membrane filterability for a long term operation [105]. SMP and EPS have often been cited as the main factors affecting fouling in submerdge anaerobic MBRs [228, 244].

Figure 7.7 gives the concentrations of the soluble organic fraction present in the cake layer when working at different SRT. SMP concentrations increased with SRT, in contrast to EPS concentrations that decreased with SRT. Proteins were the major quantified compounds both in EPS and SMP.

All SRT had the comparable ratio of protein and polysaccharide (PN/PS) ranged at 1.86-3.2 of EPS and 2.04-2.46 of SMP. It was established that proteins had a strong positive influence on the hydrophobicity of microbial flocs, while polysaccharides had no remarkable influence [127]. In relation to their hydrophobicity and surface charge, the affinity between proteins and sludge flocs should generally be greater than that between polysaccharides and flocs [197]. Therefore, sludge with high PN/PS ratio in bound EPS and SMP are usually considered to have high stickiness, and thus, favor the development of cake formation. This explained why sludge in this study had relatively high filtration resistance compared to sludge with low PN/ PS ratio in some other studies [127, 294].

Therefore, increases in the sludge age leads to an increase in the maximum specific growth and substrate utilization rates. The production of EPS and SMP is growth-associate and in direct proportion to substrate utilization. Also, endogenous microorganisms tend to produce more EPS and SMP with longer sludge age [295]. This indicated; it found longer SRT as resulted change SMP and EPS concentration in SAnMBR.

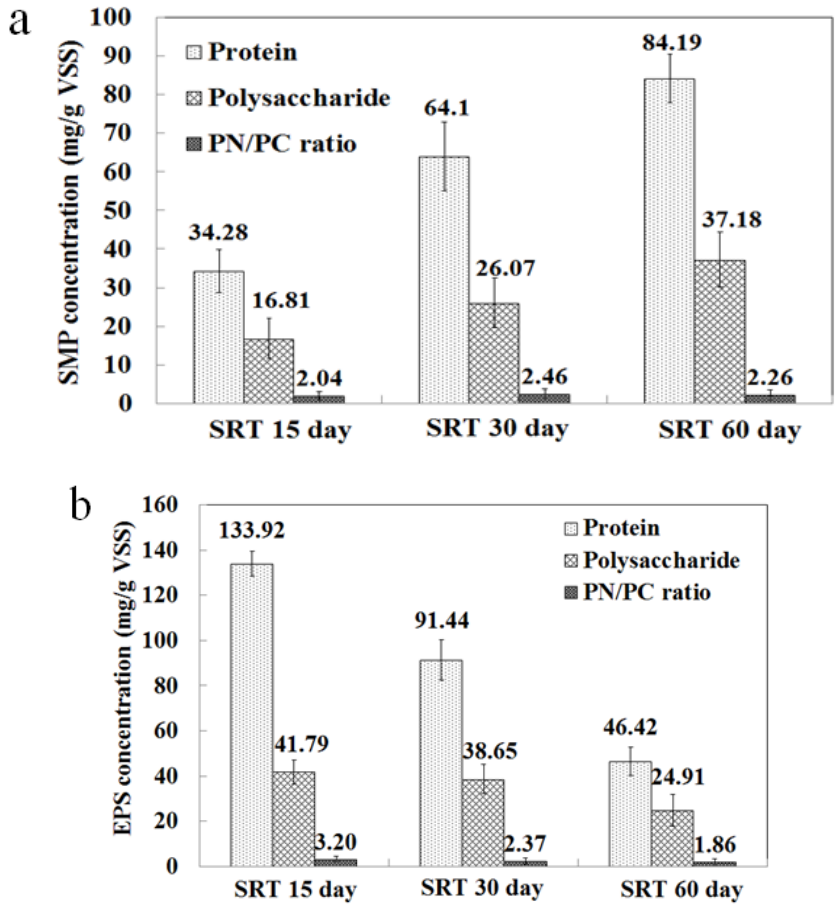



**Figure 7.7** SMP and EPS concentrationsin cake layers for different SRT: (a) SMP and (b) EPS.

## 7.5 Conclusion

The present study focused on the impacts of SRT on membrane fouling in SAnMBRs treating POME. The following major conclusions can be drawn:

1. An increasing SRT induced higher biomass concentration in the SAnMBRs and lower soluble and colloid fractions in cake deposits.

2. Since the major origin of membrane fouling was due to the external cake layer formation directly linked to suspended solid concentrations in the mixed liquor, SRT increase had a negative impact on membrane fouling intensity in SAnMBRs.
3. The role of soluble organic fractions appeared less significant even though they can play a determining role on cake layer structuring and permeability.
4. The analyses of foulant materials by SEM, EDX, AFM and FTIR confirmed the higher compactness of the cake layer in terms of mineral composition, protein and polysaccharides concentrations when working at a higher SRT.

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# CHAPTER 8
# MODELLING THE EFFECT OF OLR AND SRT ON THE PERFORMANCE DURING START-UP PERIOD AND SAnMBR TREATING POME USING GPS-X

This chapter presented the mantis model to simulate the relationship between the OLR and SRT on the biological treatment efficiency and biogas production during the start-up period and SAnMBR treating the wastewater generated from POME. The Mantis model is based in part on the ADM1 anaerobic digestion model [296]. The start-up period comprised an acidogenic and methanogenic reactor connected in series, to treat wastewater from POME. Five experimental runs were conducted at organic loading rates (OLRs) of 4.79, 5.70, 7.08, 9.73, 19.18 and 28.59 kgTCOD/$m^3$/d. Submerged anaerobic membrane bioreactors (SAnMBRs) worked three different sludge retention times (SRTs of 15, 30 and 60 d).

## 8.1 Introduction

Palm oil mill effluent (POME) is one of the industries generating the most polluted wastewater. The generated wastewater is both high strength and large in volume. The wastewater has high concentrations of chemical oxygen demand (COD) and biochemical oxygen demand (BOD), low pH, odorous, yellowish brown color, and contains substances, such as nitrogen, total suspended solid, and oil and grease, etc. which would severely affect the environment if discharged directly [5]. Since POME had high strength, the most suitable treatment process of interest is the submerged anaerobic membrane bioreactor (SAnMBR), which is capable of handling high organic loading rates and small footprints. Furthermore, the process has the positive net energy production in the form of biogas which can replace fossil fuel [27]. The SAnMBR has two main biochemical stages; the acidogenesis and methanogenesis stages. Since acidogenic and methanogenic organisms require different kinetic parameters and optimum pH for growth, two reactors were used to create suitable environment for each group of organisms [140]. In order to describe the effect of operating variables on the performance and kinetic of the degradation process of POME by using SAnMBR and enable used as a tool for the design. There are only a few studies available on the modelling of SAnMBR.

Several models describing anaerobic digestion processes have been developed over during the last three decades [141]. Early models were very simple and considered organic matter as a whole, but did not account for the composition of the feedstock [142, 143]. After that a development of models for anaerobic digestion processes considered complex feed compositions (carbohydrate, protein, volatile fatty acids (VFA) and other organics) yielding more accurate results [144, 145]. Nowadays, the increasing knowledge on anaerobic digestion and the interactions of the multiple functional species involved in it require more complex models to simulate the impact of the changing environmental conditions on complex biological treatment systems was developed. The latest developed model is the International Water Association (IWA) Anaerobic Digestion Model No. 1 (ADM1), published in 2002[140].

Anaerobic Digestion Model No. 1 (ADM1), developed by the IWA Task Group for Mathematical Modeling on Anaerobic Digestion, consisted of a number of processes to simulate all possible reactions occurring in anaerobic sludge, including not only biological reactions, such as disintegration, hydrolysis of suspended solid, uptake (growth) and decay of microorganisms, but also physicochemical reactions including ion association/dissociation and liquid–gas transfer [140]. The extended applications of ADM1 model as a basic model concept for further development for simulation of different anaerobic reactor may be applied to different forms of equations. For example, the ADM1 applying different anaerobic processes include:

1. Single-stage anaerobic process: co-substrate anaerobic digestion process of olive mill wastewater (OMW) with olive mill solid waste (OMSW) in semi-continuous tubular digester [49]; dog food and flour in anaerobic sequencing batch reactor (ASBR) [297]; opium alkaloid effluent in lab-scale upflow anaerobic sludge bed reactor (UASBR) [162]; municipal solid wastes (OFMSW) in continuous stirred-tank reactor (CSTR) and upflow sludge blanket (UASB) [148].
2. Two-stage anaerobic process: traditional Chinese medicine (TCM) wastewater [149]; olive pulp [150, 151] and grass silage [152]; acidified sorghum extract generated from a hydrogen producing bioreactor in a two-stage CSTR [153].
3. Hybrid anaerobic reactor: wastewater from wine residue after distillation in hybrid upflow anaerobic sludge filter bed (UASFB) [154].

However, the application of the ADM1 model to the two-phase anaerobic process, especially for the treatment of POME with two-phase SAnMBR wastewater remained

limited. This model aims at assessing the performances of a start-up period and SAnMBR treating POME in terms of TCOD removal and methane production.

### 8.2 Research Objective

This study was to verify if the ADM model is suitable for SAnMBR simulation.

- Compare performances of experimental results (start-up period and SAnMBR functions ) with simulations results (GPS-X).

### 8.3 System Description

The start-up of SAnMBR consist of an acidogenic reactor (working volume of 5 L) upstream of a methanogenic reactor (working volume of 10 L). During start-up, the lab scale reactors worked in mesophilic conditions (35 ± 3 $^{o}$C) and were operated in continuous conditions. No liquid – solid phase separation was operated downstream reactors and HRT was then equal to SRT. A first organic loading rate OLR was imposed on the system and according to the biomass acclimatization, the daily OLR was progressively increased by decreasing the daily hydraulic retention time HRT, from higher to lower values (12 to 10, 8, 6, 3, and 2 days) which corresponded to average daily flow-rates from 1.25 to 1.5, 1.87, 2.5, 5 and 7.5 liters of raw POME per day**.**

After the start-up period, a third tank containing porous membranes was set up downstream of the methanogenic bioreactor to constitute a selective barrier to separate solid and soluble phases, and constitutes the Sequencing Anaerobic Membrane Bioreactor SAnMBR. The SAnMBRs system was then composed of three tanks working in mesophilic conditions (35°C): (i) the first tank was a 5L acidogenic reactor where the pH was maintained in the range of 5 to 5.5, (ii) the effluent from the acidogenic tank was pumped towards the second 10L methanogenic tank where the pH was maintained in the range of 6.9 to 7.2 and the effluent of the methanogenic tank was pumped towards the third 5L tank where a module of hollow fiber porous membranes was submerged to separate the permeate from biological suspension. The experiment investigated the influence of SRT through three values of 15, 30 and 60 days.

## 8.4 System modelling

### 8.4.1 Plant flow chart during start-up and SAnMBR

In this study, GPS-X simulation platform (Hydromantis, 6.1) software was applied to test the effect of OLR and SRT during the start-up period and SAnMBR. The hydraulic operation during start-up combines the continuous-flow stirred-tank reactor (CSTR) of acidogenic and methanogenic reactor as shown in Figure 8.1. Meanwhile, the layout of the SAnMBRs units model used in this project includesan influent, effluent object, the sequencing units of acidogenic and methanogenic with connect a secondary clarifier together and anaerobic membrane unit as shown in Figure 8.2.

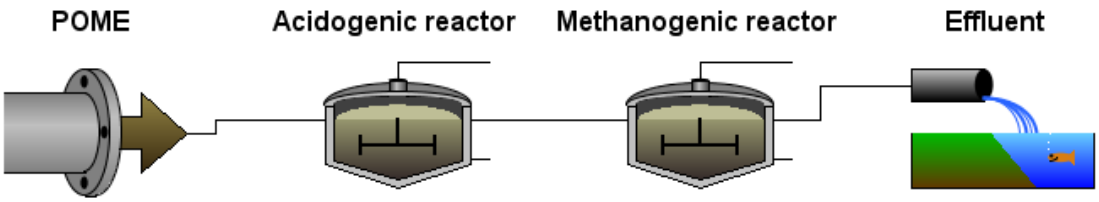


**Figure 8.1** Plant flow chart of start-up period as represented in GPS-X 6.1.

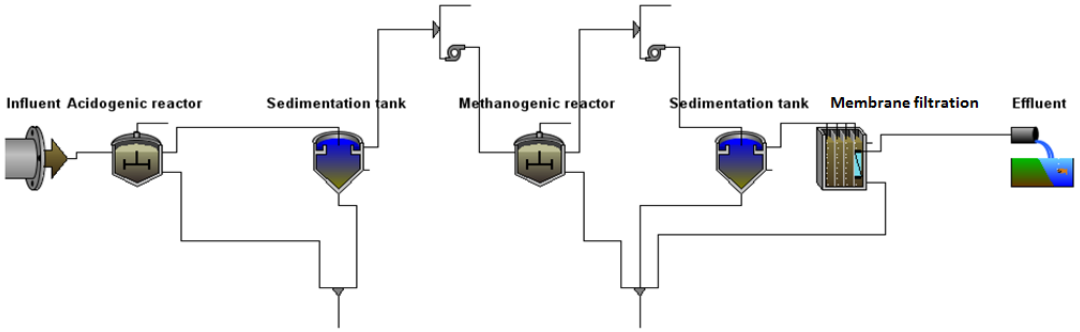


**Figure 8.2** Plant flow chart of SAnMBR as represented in GPS-X 6.1.

### 8.4.2 Modelling and Simulation ADM

Before beginning the simulation, three data sets were prepared consisting of (1) influent characterization, (2) operational condition and (3) four anaerobic microorganism population (hydrolysis, acidogenesis reactor, acetogenesis reactor and methanogenesis reactor). Tables 8.1-8.3 are the influent fractionations and operating conditions during the

start-up period and SAnMBR. These data were inserted to GPS-X software for simulating ADM model.

**Table 8.1** Influent Wastewater Characteristics.*

| Parameter | Unit | Concentration |
|---|---|---|
| Total COD | $gCOD/m^3$ | 57 |
| Total TKN | $gN/m^3$ | 0.77 |
| XCOD/VSS ratio | gCOD/gVSS | 2.5 |
| VSS/TSS ratio | gCOD/gTSS | 0.85 |
| $BOD_5$/BOD ultimate ratio | - | 0.66 |
| Readily biodegradable fraction of total COD | - | 0.45 |

Remark: * = ammonia fraction of soluble TKN 0.23

**Table 8.2** Operating conditions during acclimatization period.

| OLR (gCOD/L·d) | Feed concentration (gCOD/L) | Flow rate (L/d) | HRT (d) |
|---|---|---|---|
| 4.56-4.98 (4.79) | 55±11 | 1.25 | 12 |
| 5.39-5.98 (5.70) | 55±15 | 1.5 | 10 |
| 6.72-7.42 (7.08) | 55±7 | 1.87 | 8 |
| 9.12-9.98 (9.73) | 55±12 | 2.5 | 6 |
| 17.97-19.83 (19.18) | 55±9 | 5 | 3 |
| 27.35-29.95 (28.59) | 55±9 | 7.5 | 2 |

**Table 8.3** Operating conditions in SAnMBR

| SRT (d) | Feed concentration (gCOD/L) | Excess sludge (L/d) |
|---|---|---|
| 15 | 55±11 | 0.67 |
| 30 | 55±15 | 0.34 |
| 60 | 55±7 | 0.16 |

Experimental results of acclimatization period and SAnMBR were used for model calibration. In order to fit the model to the experimental results, the simulation was undertaken to fit the outputs to the experimental data by changing the most kinetic parameters until finding the best values. The kinetic parameter values such as maximum fermentation rate, growth rate and decay rate of microorganism were adjusted the estimated values from initial values which using temperature correlations given by Buhr and Andrews [295] for temperatures of 35°C. The Buhr and Andrews [295] temperature expressions were implemented in GPS-X. The temperature correlations were applied to the rates of hydrolysis and all of the anaerobic biomass growth and decay processes. The estimated values were adjusted until the simulations data better fit the experimental results are given in Tables 8.4-8.5.

The estimated values during the start-up period showed nearly with the initial values, but SAnMBR showed higher than two times at the start-up period. This different may possible due to the capacity of membrane coupled with anaerobic biological treatment could retain and then induce high biomass concentration concerning specific growth of slow methanogenic inside the reactor, which enhanced for biogas and methane production.

**Table 8.4** Summary of kinetic parameters for various substrates utilized acclimatization period.

| Kinetic coefficient | Unit | Initial values | Estimated values |
|---|---|---|---|
| Maximum fermentation rate | $d^{-1}$ | 3.2 | 3.63 |
| Decay rate for fermentive biomass | $d^{-1}$ | 0.04 | 0.04 |
| Maximum growth rate of acetogens | $d^{-1}$ | 0.35 | 0.38 |
| Decay rate for acetogens | $d^{-1}$ | 0.02 | 0.02 |
| Max growth rate of hydrogenotrophic methanogens | $d^{-1}$ | 0.368 | 0.415 |
| Decay rate of hydrogenotrophic methanogens | $d^{-1}$ | 0.01 | 0.01 |
| Max growth rate of acetate utilizing bacteria | $d^{-1}$ | 0.15 | 0.19 |
| Decay rate for acetoclastic methanogens | $d^{-1}$ | 0.02 | 0.02 |
| Hydrolysis rate constant | $d^{-1}$ | 3 | 3.63 |

Note the initial values adapted by Buhr and Andrews [295] for mesophilic temperatures

**Table 8.5** Summary of kinetic parameters for various substrates utilized in SAnMBR.

| Kinetic coefficient | Unit | Initial values | Estimated values |
|---|---|---|---|
| Maximum fermentation rate | $d^{-1}$ | 3.2 | 4.86 |
| Decay rate for fermentive biomass | $d^{-1}$ | 0.04 | 0.07 |
| Maximum growth rate of acetogens | $d^{-1}$ | 0.35 | 0.43 |
| Decay rate for acetogens | $d^{-1}$ | 0.02 | 0.03 |
| Max growth rate of hydrogenotrophic methanogens | $d^{-1}$ | 0.368 | 0.397 |
| Decay rate of hydrogenotrophic methanogens | $d^{-1}$ | 0.01 | 0.02 |
| Max growth rate of acetate utilizing bacteria | $d^{-1}$ | 0.15 | 0.24 |
| Decay rate for acetoclastic methanogens | $d^{-1}$ | 0.02 | 0.03 |
| Hydrolysis rate constant | $d^{-1}$ | 3 | 4.86 |

Note the initial values adapted by Buhr and Andrews [295] for mesophilic temperatures

## 8.5 Results and Discussion

### 8.5.1 Effect of OLR on TCOD

Figures 8.3 and 8.4 show the results of the model simulations performed to assess the effect of OLR and SRT on digester performances in terms of TCOD effluents of acidogenic and methanogenic reactors. To ascertain that a steady state condition had been established, the reactor was operated at all period for 100 days. An increase of OLR in acidogenic reactor results in the increase TCOD effluent of both the experimental data with the simulation results (Figure. 8.3). This is due to hydrolysis of particulate fraction in acidogenic reactor. Meanwhile, TCOD effluent in SAnMBR at different SRT was quietly stable.

Figure 8.4 compares the experimental data with the simulation results of TCOD in the effluent obtained from the methanogenic reactor during start-up and SAnMBR. The simulation results of effluent TCOD showed a good agreement with the experimental data. It found that effluent TCOD concentration in methanogenic reactor decreased continuously with OLR increase(except period 6 in start-up period).The high OLR of experiment and

simulation data for period 6 is result of washout of substrate and bacteria at higher load. Meanwhile, SAnMBR show quietly stable TCOD effluent when operating at high OLR.

Similarly, Dereli et al. [159] reported that the accuracy of the model prediction in effluent COD, biogas and methane flows decreased with the increase in the organic loading rate.

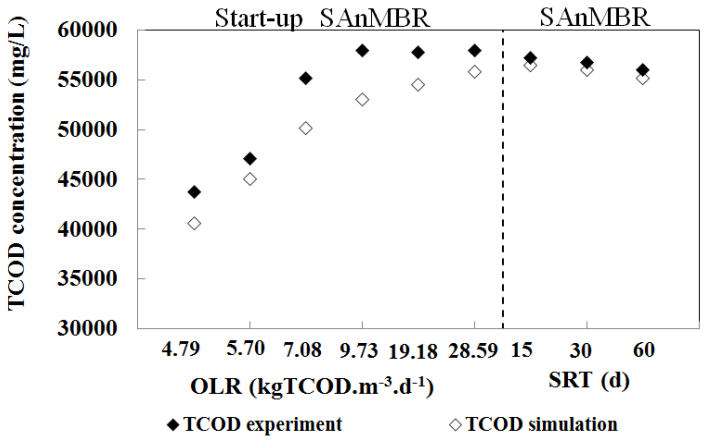


**Figure 8.3** TCOD concentration of acidogenic reactor.

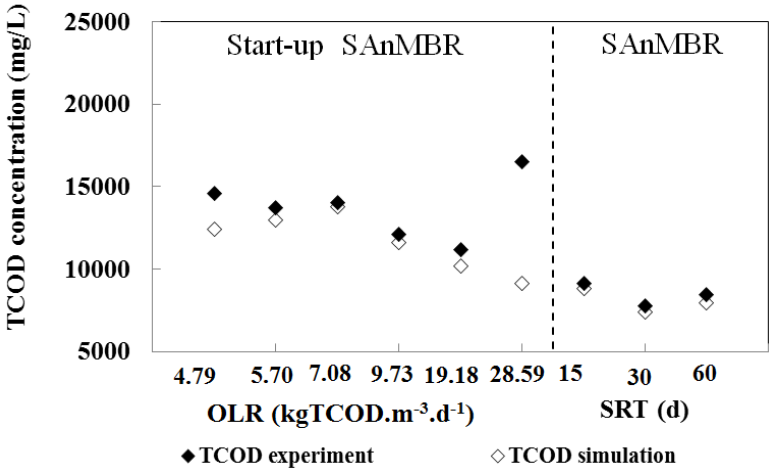


**Figure 8.4** TCOD concentration of methanogenic reactor.

### 8.5.2 Effect of OLR on Methane Gas Production

The result of methane production modeling in comparison with experimental data is shown in Figure 8.5. The methane production was converted to the COD value, which

was directly proportional to the TCOD concentration added. The simulation results with optimized parameters showed a good agreement with the experimental data of both start-up and SAnMBR. The model predicts the increment of the methane production as a response of the load increase in range 4.79-9.73 kgCOD·$m^{-1}$· $d^{-1}$. However, when OLR operating until 19.18-28.59 kgCOD·$m^{-1}$· $d^{-1}$considered the pH drop and results in the digester failure (Figure. 3.6). The pH drop affected the microbial activity, resulting in a sharp decrease of the bacterial concentration in the digester. Methane and flows were over predicted in OLR 28.59 kgCOD·$m^{-1}$· $d^{-1}$.Similarly, Dereli et al. [159] reported that the accuracy of the model prediction in methane flows decreased with the increase in the organic loading rate.It was seen that the model simulated an overload situation for methane flows. It was difficult to further calibrate the model parameters to get better simulation results, and a complete replication of experimental data by the model for all loading periods could not be obtained. This might a rise from complication of applying ADM for a lab-scale reactor which has relatively lower tolerance to changes in operating conditions in comparison to full-scale systems.

For instance, the methanogenic archea were completely washed out from the digester. The methane production from simulation and experimental result showed that SAnMBR present higher methane production than the start-up. This result indicated that membrane coupled with anaerobic digester was favorable to enhance methane recovery and treatment performance.

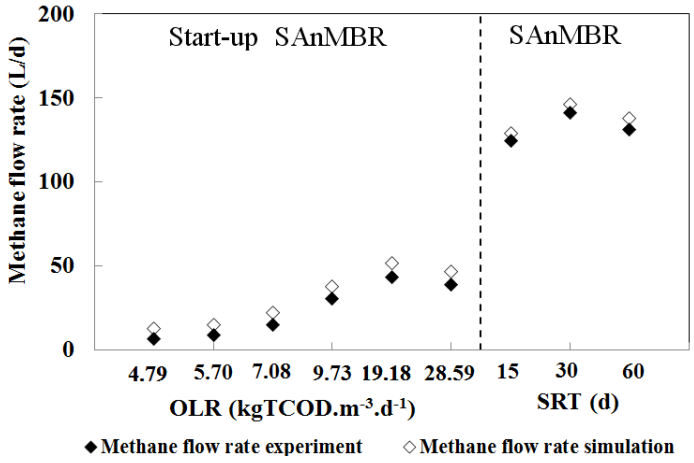


**Figure 8.5** Methane production flow rate.

### 8.5.3 Effect of SRT on TSS and VSS Concentrations

Figure 8.6 and 8.7 show simulation and experimental results of effluent TSS and VSS in acidogenic and methanogenic reactor at SRT 15, 30 and 60 d. It can be seen that the VSS in acidogenic and methanogenic reactors were well predicted by the model, whereas it was low accuracy in the TSS. This due to the TSS compounds contain highly complex of biomass concentration and minerals, such as $Ca^{2+}$, $Mg^{2+}$ and $Na^{+}$. The mineral ions, then, could be reacted with biopolymers ion groups, for example $COO^{-}$, $CO_3^{2-}$, $SO_4^{2-}$, $PO_4^{3-}$, $OH^{-}$[286], and consequently precipitated insideacidogenic and methanogenic reactors[287]. Thus, this phenomenon might be caused the relative low TSS concentration inside the reactor comparing with the simulation results, which considered mineral compounds as a parameter input.

The acidogenic reactor showed higher TSS and VSS concentrations than did the methanogenic reactor. This is due to most of suspended solids and dissolved organic solids transformed into volatile fatty acids which were volatilized during the analysis of total and volatile suspended solids. Effluent TSS and VSS concentrations in methanogenic reactor decreased. This might be caused not only by methabolism of methanogenic bacteria but also by the transformation of suspended and dissolved organic solids into soluble COD to biogas production.

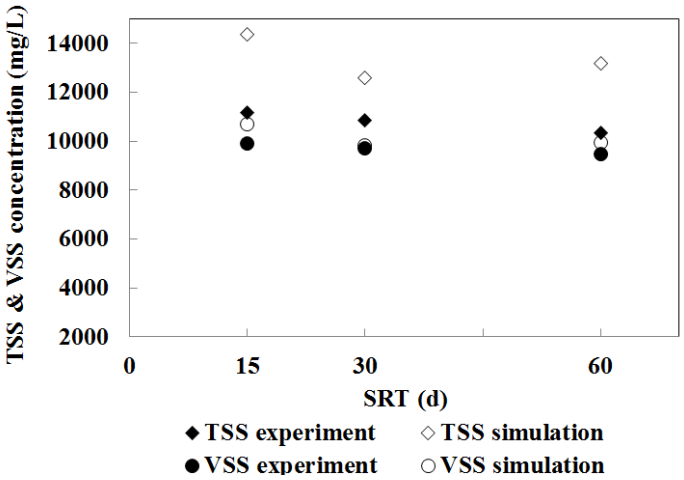


**Figure 8.6** TSS and VSS concentration of the acidogenic reactor.

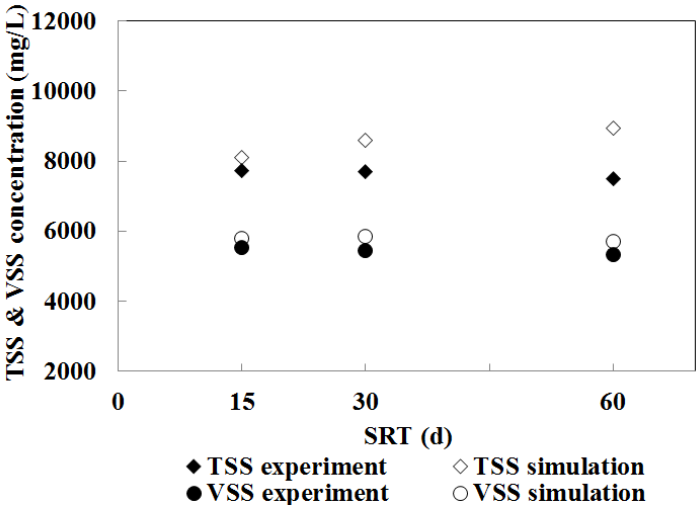


**Figure 8.7** TSS and VSS concentrations of the methanogenic reactor.

## 8.6 Conclusion

The mathematical model proposed is capable of assessing the effects of the OLR and SRT on the TCOD effluent and the methane production rate during start-up and SAnMBR treating POME.

1. The mathematical model can be used to assess the maximum OLR increase due to POME addition that an anaerobic digester can tolerate. In particulates, it can be applied to optimize the POME addition into the anaerobic digesters of SAnMBR.
2. Model simulations show as OLR excess results in methane production dropped, and thus, a digester failure.
3. SAnMBR can be a potentially promising technology for POME treatment in terms of COD removal and biogas production.

# CHAPTER 9
# CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

## 9.1 Summary

The main objective of this study was to investigate the effect of SRTs on treatment performances of two-stage SAnMBR treating palm oil mill effluent. The characteristics of sludge and microbials on filterability and biofouling were evaluated at different SRT. From the obtained results of the experiments, as the following conclusions can be drawn:

### 9.1.1 The Investigation of Biochemical Methane Potential in Palm Oil Mill Effluent (POME): Effects of Organic Fraction and Concentration.

In palm mill processes, the main by-product waste is palm oil mill effluent (POME), which also represents its characteristics as highly complex wastewater, i.e. high organic matter, SS, oil and grease, nutrients, and including acid and high temperature conditions from discharged processes. This is opened the door to develop the anaerobic digestion technology for treatment this wastewater. The most and simple method for evaluating the anaerobic treatability of any organic wastewaters is biochemical methane potential (BMP). This is because the BMP test could be determined the ultimate biodegradability (BD) of an organic substrate and methane yield during anaerobic fermentation as well as determining the maximum applicable loading rate or the inhibitory potential of specific substrates. In the present study, the BMP testfor POME was conducted for 20 d at various diluted levels 25%, 50%, 75% and 100% of both raw and filtrated of POME. After testing, the results data of both POME conditions were fitted in the modified gompertz equation to predict $CH_4$ yield.

The results indicated that POME from raw and filtrated conditions played significant roles as a substrate for $CH_4$ production. The COD removal efficiency and maximum methane production increased when increasedg COD concentrations in raw and filtrated POME. From the gompertz equation analysis, it represented the good relationship betweentheoretical modeling and experimental data ($R^2$>0.95). However, the experimental conditions did not allow the obtaining of high ratios of methane production in comparison with COD removal efficiency. The overall mechanisms in this study could be shown the direction for development or improvement in a bench scale or full scale anaerobic membrane bioreactor.

### 9.1.2 Investigation of Influence of Relaxation Frequency on Membrane Fouling Control in SAnMBR.

In recent years, submerged anaerobic membrane bioreactors (SAnMBRs) have appeared as an increasingly interesting advantage for treatment of many kinds of industrial wastewaters. This isbecause SAnMBRs present numerous advantages over the conventional anaerobic treatment processes, such as maintained high concentration of microorganism, high efficiency treatment and recovery of renewable energy sources. In this study, POME was also treated by SAnMBRs technique. However, the drawback of SAnMBRs technique is fouling in membrane reactor. Thus, the research was proposed to study the effect of filtration and relaxation times for membrane fouling control in SAnMBR treating POME, as well as the mechanisms of fouling at each filtration and relaxation time. The filtration was operated in the range of supra critical conditions (permeate flux equal to 20 $L/m^2h$), and the submersed membranes were continuously cleaned by gas injection and intermittent periods of relaxation. Four conditions of relaxation (S1: 240 s filtration /30 s relaxation, S2: 480 s filtration /30 s relaxation, S3: 720 s filtration /30 s relaxation and S4: 960 s filtration /30 s relaxation) were analyzed by comparing the trans-membrane pressure TMP evolution rates, the main fouling origins and the content of membrane cleaning methods in terms of proteins and carbohydrates.

The results showed that the highest relaxation frequencies, conditions S1 and S2 allowed longer operating at times than when working with low relaxation frequencies. The high relaxation frequency avoided any rapid structuring of the cake deposit but it appeared not sufficient in these experiments to limit TMP increase during filtration. The specific resistance (R*) values confirmed that increased with low relaxation frequency. In fouling mechanisms, the main kindof fouling was the cake deposit, which represented about 50% of the total hydraulic resistance. The other origins of fouling were pore blocking and adsorption.The decrease of relaxation frequency increased significantly the retention of SMP onto the membrane surface and in pores.

Protein was found to be the major component in the EPS rather than carbohydrate. Moreover, the different relaxation frequency also greatly influences the ratio of protein and polysaccharide. The ratio Protein/Carbohydrate in linked EPS present in the cake deposit appeared lower when operating at a lower relaxation frequency and it also corresponded to a higher fouling property of the cake deposit. From these results, the optimum intermittent filtration frequency was 480s filtration and 30 s stop which applied in the two-stage

SAnMBR. In fact, such intermittent filtration frequencies could not to be the optimum in the two-stage SAnMBR, due to an increase in OLR from 4.79 kg COD/$m^3$·d in this study to 28.59 kg COD/$m^3$·d in the two-stage SAnMBR. Therefore, the intermittent filtration of 480s filtration and 30 s stop was adjusted and obtained the new optimum at 360 s filtration and 240 s stop, which was applied in the next study.

### 9.1.3 Investigation of the Influence of SRTs on Two-stage SAnMBR Performances Efficiency When Treating POME

The previous studies showed that the concentrations of OLR and biomass in anaerobic digestion combined with membrane reactor strongly affect the COD removal, methane production and membrane fouling. In term of biomass content, it depends on SRT and HRT.In SAnMBR process, it could operate at high biomass concentrations, high SRT and OLR and relatively short HRT. Therefore, this study aimed to focus the performance of SAnMBR treating POME under different SRT of 15, 30 and 60 d, with HRT in Acidogenic, Methanogenic and membrane reactor tank of 0.5, 1.5 and 1.5 d, respectively. Results indicated that the COD removals were 97, 98 and 98% for SRT of 15, 30 and 60 d, respectively. While the methane yield forSRT of 15, 30 and 60 d found as 0.31, 0.35 and 0.33 L $CH_4$/g $COD_{remove}$, respectively. This could be concluded that the SAnMBR has high performance on treating POME with high level of COD removal (>97%), but it has slightly changed when operated under different SRT.

### 9.1.4 Effect of SRT on Membrane Fouling Intensity in Two-stage SAnMBRs Treating Palm Oil mill Effluent

Although the SAnMBR can maintain high concentrations of biomass, and consequently high COD removal, the disadvantage is membrane fouling. The previous study showed that the increase in SRT has no different on COD removal for SAnMBR treating POME. However, in this study, the introduction of various SRT has strongly effect on membrane fouling. The increase in SRT over 30 d reached the evolution rates of TMP (dTMP/dt) increased, which also responded to hydraulic resistance. The dominat of fouling that occurred was the cake deposit, especially for SRT 60 d. SEM, EDX, AFM, and FTIR analysises indicated that the fouled membrane surfaces were covered with a cake layer containing organic and inorganic elements whose concentrations were higher when working at a higher SRT. Considerable SMP and EPS quantification, the SMP increased with the increasing of SRT, whereas the EPS decreased. This effect occurred from the increase in maximum specific growth and substrate utilization rates with the further sludge

age. In term of the seed sludge size, the prolonged SRT could be reduced the particle size distribution, due to defloccution of bacteria for enhancement of mass transfer. From the overall results, including the section 7.1.3, the optimum SRT for SAnMBR treating POME was 30 d. In addition, the physical, chemical parameters in this experiment were simulated by GPS-X, which is presented in the next study.

**9.1.5 Modelling the Effect of OLR and SRT on the Performance During the Start-up Period and SAnMBR Treating POME Using GPS-X**

The ADM1 model was widely used for the simulation of different anaerobic treatment processes, such as olive mill wastewater (OMW) with olive mill solid waste (OMSW), municipal solid wastes (OFMSW), traditional Chinese medicine (TCM) wastewater, olive pulp and grass silage, many years ago. However, the application of ADM1 with the simulation of two-stage anaerobic treatment process of POME had not been undertaken before. The objective of this study was to apply ADM1 from GPS-X software to build a model to assessing the performances of a start-up period and SAnMBR treating POME in terms of TCOD removal and methane production.

The results showed that the ADM1 had been successfully implemented to simulate the start-up period and SAnMBR treating POME. The simulation results that revealed could accurately predict TCOD removal, TSS, VSS and methane production reactor. Moreover, the mathematical model can be used to determine the optimum OLR for POME addition addition into the anaerobic digesters of SAnMBR. Therefore, this fundamental of the model are generally valid and sufficient for the application in the design and operation of the full-scale system under various operating conditions in the future.

## 9.2 Recommendations

In this thesis, several limitations that occurred in the experiments can be summarized in following three topics, which could be improve further investigations in the future.

**1) POME characterization**

- POME is a very complex substrate. It is important to define easy tools for its characterization. BMP tests can be a part of the answer.
- To develop the research between microbial identification and activities such as influent characterization and engineering approach.

**2) Biological Performance**

- The measurement of proteins, carbohydrates and lipids in POME is important, as well as the definition of the associated specific kinetic parameters to better analyze the effects of POME composition on anaerobic performance.
- A further model development will require to improve the ADM performance on the prediction of biological reactions and the impact of all operating variables.

**3) Fouling Minimization**

- In this study, the relaxation frequency operated in membrane reactor represented that could be limited the fouling occurred and helpful for extend membrane operation. The next attention strategy for membrane fouling control will be the combination of relaxation frequency with backwashing, which could more optimize this operating.
- To promote new reactor configurations by combination of membrane separation with UASB for recovery biomass washout from UASB reactor and enhance the biogas production. Membrane fouling in this case can be controlled and minimized by using low energy comsumption.

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# APPENDIXES

# APPENDIX A
# BIOCHEMICAL METHANE POTENTIAL AND ESTIMATION FROM GOMPERT EQUATION RESULTS

**Table A-1** The methane potential in BMP assay at concentration 25% of raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cum (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 115 | 23.51 | 27.04 | 27.04 | 23.97 | 0.02 | 0.02 |
| 2 | 93 | 38.79 | 36.08 | 63.12 | 55.95 | 0.05 | 0.04 |
| 3 | 40 | 9.34 | 3.73 | 66.86 | 59.26 | 0.05 | 0.05 |
| 4 | 31 | 15.97 | 4.95 | 71.81 | 63.65 | 0.06 | 0.05 |
| 5 | 14 | 39.39 | 5.51 | 77.33 | 68.54 | 0.06 | 0.05 |
| 6 | 12.2 | 33.13 | 4.04 | 81.37 | 72.12 | 0.07 | 0.06 |
| 7 | 8.6 | 40.85 | 3.51 | 84.88 | 75.23 | 0.07 | 0.06 |
| 8 | 7.2 | 49.74 | 3.58 | 88.46 | 78.41 | 0.07 | 0.06 |
| 9 | 8.4 | 52.23 | 4.38 | 92.85 | 82.30 | 0.08 | 0.07 |
| 10 | 9 | 52.23 | 4.70 | 97.55 | 86.46 | 0.08 | 0.07 |
| 12 | 10 | 52.23 | 5.22 | 102.77 | 91.09 | 0.09 | 0.07 |
| 14 | 9.5 | 51.68 | 4.91 | 107.68 | 95.45 | 0.09 | 0.08 |
| 16 | 5.6 | 62.71 | 3.51 | 111.20 | 98.56 | 0.09 | 0.08 |
| 18 | 3.8 | 62.23 | 3.43 | 114.63 | 101.60 | 0.10 | 0.08 |
| 20 | 3.5 | 65.43 | 3.44 | 118.07 | 104.65 | 0.10 | 0.08 |

**Table A-2** The methane potential in BMP assay at concentration 50% of raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cu m (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 140 | 20.67 | 28.95 | 28.95 | 25.66 | 0.03 | 0.01 |
| 2 | 187 | 45.63 | 85.34 | 114.28 | 101.29 | 0.10 | 0.06 |
| 3 | 119 | 11.91 | 14.18 | 128.46 | 113.86 | 0.11 | 0.07 |
| 4 | 50 | 60.22 | 30.11 | 158.58 | 140.56 | 0.14 | 0.08 |
| 5 | 46.5 | 10.76 | 5.01 | 163.58 | 144.99 | 0.14 | 0.09 |
| 6 | 43 | 48.73 | 20.95 | 184.54 | 163.57 | 0.16 | 0.10 |
| 7 | 42 | 51.45 | 21.61 | 206.15 | 182.72 | 0.18 | 0.11 |
| 8 | 13 | 58.45 | 7.60 | 213.75 | 189.46 | 0.19 | 0.11 |
| 9 | 12 | 58.45 | 7.01 | 220.76 | 195.68 | 0.20 | 0.12 |
| 10 | 12 | 58.45 | 7.01 | 227.78 | 201.90 | 0.20 | 0.12 |
| 12 | 12 | 51.82 | 6.22 | 234.00 | 207.41 | 0.21 | 0.13 |
| 14 | 11.5 | 55.70 | 6.41 | 240.40 | 213.09 | 0.21 | 0.13 |
| 16 | 7.5 | 66.80 | 5.01 | 245.41 | 217.53 | 0.22 | 0.13 |
| 18 | 6.4 | 66.80 | 4.46 | 249.87 | 221.48 | 0.22 | 0.13 |
| 20 | 6.4 | 67.26 | 3.18 | 253.05 | 224.30 | 0.22 | 0.14 |

**Table A-3** The methane potential in BMP assay at concentration 75% of raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cum (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 155 | 19.99 | 30.99 | 30.99 | 27.47 | 0.03 | 0.00 |
| 2 | 219 | 29.70 | 65.06 | 96.04 | 85.13 | 0.09 | 0.03 |
| 3 | 198 | 49.87 | 98.75 | 194.80 | 172.66 | 0.17 | 0.06 |
| 4 | 128 | 37.53 | 48.04 | 242.84 | 215.24 | 0.22 | 0.07 |
| 5 | 47 | 49.87 | 23.44 | 266.28 | 236.02 | 0.24 | 0.08 |
| 6 | 50 | 58.63 | 29.32 | 295.59 | 262.00 | 0.26 | 0.09 |
| 7 | 57 | 58.60 | 33.41 | 329.00 | 291.61 | 0.29 | 0.10 |
| 8 | 45 | 57.51 | 25.88 | 354.88 | 314.55 | 0.31 | 0.11 |
| 9 | 66 | 57.51 | 37.96 | 392.84 | 348.20 | 0.35 | 0.12 |
| 10 | 31 | 57.51 | 17.83 | 410.67 | 364.00 | 0.36 | 0.13 |
| 12 | 20 | 44.88 | 8.98 | 419.65 | 371.96 | 0.37 | 0.13 |
| 14 | 20 | 58.16 | 11.63 | 431.28 | 382.27 | 0.38 | 0.13 |
| 16 | 12 | 68.78 | 8.25 | 439.54 | 389.59 | 0.39 | 0.14 |
| 18 | 12 | 68.78 | 6.92 | 446.46 | 395.72 | 0.40 | 0.14 |
| 20 | 16 | 68.62 | 4.06 | 450.52 | 399.32 | 0.40 | 0.14 |

**Table A-4** The methane potential in BMP assay at concentration 100% of raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cum (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 157 | 21.49 | 33.74 | 33.74 | 29.91 | 0.03 | 0.00 |
| 2 | 245 | 37.00 | 90.66 | 124.39 | 110.26 | 0.11 | 0.02 |
| 3 | 210 | 45.88 | 96.35 | 220.74 | 195.66 | 0.20 | 0.04 |
| 4 | 190 | 49.43 | 93.93 | 314.67 | 278.91 | 0.28 | 0.06 |
| 5 | 137 | 61.87 | 84.77 | 399.45 | 354.05 | 0.35 | 0.07 |
| 6 | 129 | 58.07 | 74.91 | 474.36 | 420.46 | 0.42 | 0.09 |
| 7 | 104 | 49.66 | 51.66 | 526.02 | 466.24 | 0.47 | 0.10 |
| 8 | 86 | 55.93 | 48.10 | 574.12 | 508.88 | 0.51 | 0.11 |
| 9 | 79 | 55.93 | 44.19 | 618.30 | 548.04 | 0.55 | 0.12 |
| 10 | 65 | 65.93 | 42.86 | 661.16 | 586.03 | 0.59 | 0.13 |
| 12 | 65 | 64.57 | 41.97 | 703.13 | 623.23 | 0.62 | 0.14 |
| 14 | 65 | 66.38 | 43.15 | 746.29 | 661.48 | 0.66 | 0.14 |
| 16 | 21 | 66.11 | 13.88 | 760.17 | 673.79 | 0.67 | 0.15 |
| 18 | 30 | 71.16 | 21.35 | 781.52 | 692.71 | 0.69 | 0.15 |
| 20 | 30 | 71.16 | 21.35 | 781.52 | 692.71 | 0.69 | 0.15 |

**Table A-5** The methane potential in BMP assay at concentration 25% of filtrated raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cum (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 128 | 24.10 | 30.85 | 30.85 | 27.35 | 0.03 | 0.03 |
| 2 | 101 | 29.06 | 29.35 | 60.21 | 53.37 | 0.05 | 0.06 |
| 3 | 45 | 25.39 | 11.43 | 71.64 | 63.50 | 0.06 | 0.08 |
| 4 | 25 | 46.84 | 11.71 | 83.35 | 73.88 | 0.07 | 0.09 |
| 5 | 10 | 45.67 | 4.57 | 87.91 | 77.92 | 0.08 | 0.10 |
| 6 | 11 | 48.81 | 5.37 | 93.28 | 82.68 | 0.08 | 0.10 |
| 7 | 10.5 | 48.85 | 5.13 | 98.41 | 87.23 | 0.09 | 0.11 |
| 8 | 6.5 | 49.00 | 3.19 | 101.60 | 90.05 | 0.09 | 0.11 |
| 9 | 6 | 50.70 | 3.04 | 104.64 | 92.75 | 0.09 | 0.12 |
| 10 | 6.5 | 50.33 | 3.30 | 107.94 | 95.67 | 0.10 | 0.12 |
| 12 | 5 | 50.70 | 2.54 | 110.47 | 97.92 | 0.10 | 0.12 |
| 14 | 5 | 48.43 | 2.42 | 112.89 | 100.07 | 0.10 | 0.13 |
| 16 | 5 | 53.23 | 2.66 | 115.56 | 102.43 | 0.10 | 0.13 |
| 18 | 5 | 53.23 | 2.21 | 117.77 | 104.38 | 0.10 | 0.13 |
| 20 | 5 | 65.05 | 2.21 | 119.98 | 106.34 | 0.11 | 0.13 |

**Table A-6** The methane potential in BMP assay at concentration 50% of filtrated raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cu m (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 210 | 23.43 | 49.21 | 49.21 | 43.62 | 0.04 | 0.03 |
| 2 | 185 | 40.67 | 75.25 | 124.46 | 110.31 | 0.11 | 0.09 |
| 3 | 123 | 47.92 | 58.94 | 183.40 | 162.56 | 0.16 | 0.13 |
| 4 | 56 | 56.70 | 31.76 | 215.15 | 190.70 | 0.19 | 0.16 |
| 5 | 44 | 52.19 | 22.96 | 238.12 | 211.06 | 0.21 | 0.17 |
| 6 | 11 | 51.11 | 5.62 | 243.74 | 216.04 | 0.22 | 0.18 |
| 7 | 9 | 53.59 | 4.82 | 248.56 | 220.32 | 0.22 | 0.18 |
| 8 | 9 | 22.74 | 2.05 | 250.61 | 222.13 | 0.22 | 0.18 |
| 9 | 11.5 | 45.76 | 5.26 | 255.87 | 226.80 | 0.23 | 0.19 |
| 10 | 11.5 | 45.76 | 5.26 | 261.14 | 231.46 | 0.23 | 0.19 |
| 12 | 11 | 45.76 | 5.03 | 266.17 | 235.92 | 0.24 | 0.19 |
| 14 | 10 | 68.28 | 6.83 | 273.00 | 241.98 | 0.24 | 0.20 |
| 16 | 8.2 | 66.39 | 5.44 | 278.44 | 246.80 | 0.25 | 0.20 |
| 18 | 7 | 66.39 | 4.12 | 282.56 | 250.45 | 0.25 | 0.21 |
| 20 | 11 | 66.72 | 3.22 | 285.78 | 253.31 | 0.25 | 0.21 |

**Table A-7** Methane production and methane yield in BMP assay at concentration 75% of filtrated raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cum (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 225 | 20.96 | 47.18 | 47.18 | 41.81 | 0.04 | 0.02 |
| 2 | 205 | 31.59 | 64.77 | 111.94 | 99.22 | 0.10 | 0.06 |
| 3 | 196 | 38.56 | 75.58 | 187.53 | 166.22 | 0.17 | 0.10 |
| 4 | 103 | 64.31 | 66.25 | 253.77 | 224.93 | 0.22 | 0.14 |
| 5 | 87 | 52.23 | 45.44 | 299.21 | 265.21 | 0.27 | 0.17 |
| 6 | 52 | 49.13 | 25.55 | 324.76 | 287.86 | 0.29 | 0.18 |
| 7 | 28 | 53.26 | 14.91 | 339.68 | 301.08 | 0.30 | 0.19 |
| 8 | 16 | 53.72 | 8.60 | 348.27 | 308.69 | 0.31 | 0.19 |
| 9 | 12 | 53.72 | 6.45 | 354.72 | 314.41 | 0.31 | 0.20 |
| 10 | 11.5 | 53.72 | 6.18 | 360.90 | 319.89 | 0.32 | 0.20 |
| 12 | 11 | 54.46 | 5.99 | 366.89 | 325.20 | 0.33 | 0.21 |
| 14 | 11 | 62.63 | 6.89 | 373.78 | 331.30 | 0.33 | 0.21 |
| 16 | 11 | 67.37 | 7.41 | 381.19 | 337.87 | 0.34 | 0.21 |
| 18 | 11 | 67.37 | 5.26 | 386.45 | 342.53 | 0.34 | 0.22 |
| 20 | 10.5 | 67.57 | 1.84 | 388.29 | 344.16 | 0.34 | 0.22 |

**Table A-8** The methane potential in BMP assay at concentration 100% of filtrated raw POME.

| Day | Biogas volume (mL) | % $CH_4$ | $CH_4$ daily (mL) | $CH_4$ cum (mL) | $CH_4$ at STP (mL) | $CH_4$ at STP (L) | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 255 | 28.09 | 71.65 | 71.65 | 63.51 | 0.06 | 0.02 |
| 2 | 232 | 35.65 | 82.71 | 154.36 | 136.82 | 0.14 | 0.05 |
| 3 | 202 | 41.04 | 82.90 | 237.26 | 210.30 | 0.21 | 0.08 |
| 4 | 149 | 57.34 | 85.45 | 322.71 | 286.04 | 0.29 | 0.11 |
| 5 | 124 | 58.47 | 72.51 | 395.22 | 350.31 | 0.35 | 0.14 |
| 6 | 64 | 57.66 | 36.91 | 432.13 | 383.02 | 0.38 | 0.15 |
| 7 | 49 | 55.92 | 27.40 | 459.54 | 407.32 | 0.41 | 0.16 |
| 8 | 34 | 55.76 | 18.96 | 478.49 | 424.12 | 0.42 | 0.17 |
| 9 | 47 | 64.34 | 30.24 | 508.74 | 450.92 | 0.45 | 0.18 |
| 10 | 58 | 67.23 | 39.00 | 547.73 | 485.49 | 0.49 | 0.19 |
| 12 | 74 | 68.46 | 50.67 | 598.40 | 530.40 | 0.53 | 0.21 |
| 14 | 29 | 72.11 | 20.91 | 619.31 | 548.94 | 0.55 | 0.22 |
| 16 | 15 | 70.19 | 10.53 | 629.84 | 558.27 | 0.56 | 0.22 |
| 18 | 15 | 70.19 | 8.78 | 638.62 | 566.05 | 0.57 | 0.22 |
| 20 | 22 | 69.66 | 6.55 | 645.17 | 571.86 | 0.57 | 0.23 |

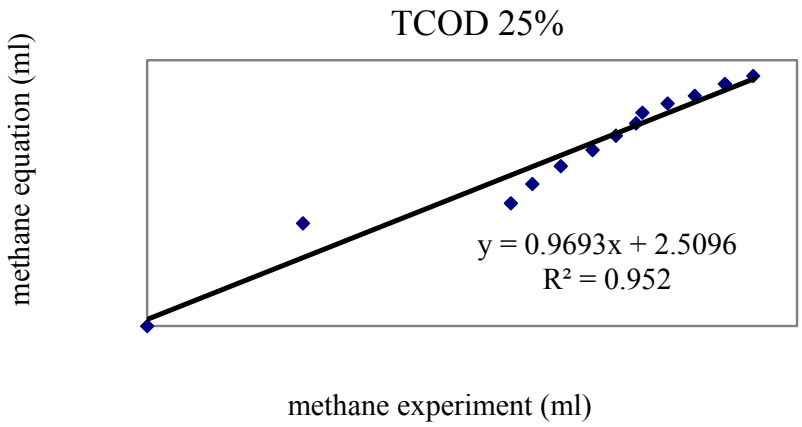



**Figure A-1** Kinetic modeling of cumulative methane production in BMP Assay atconcentration 25% of raw POME.

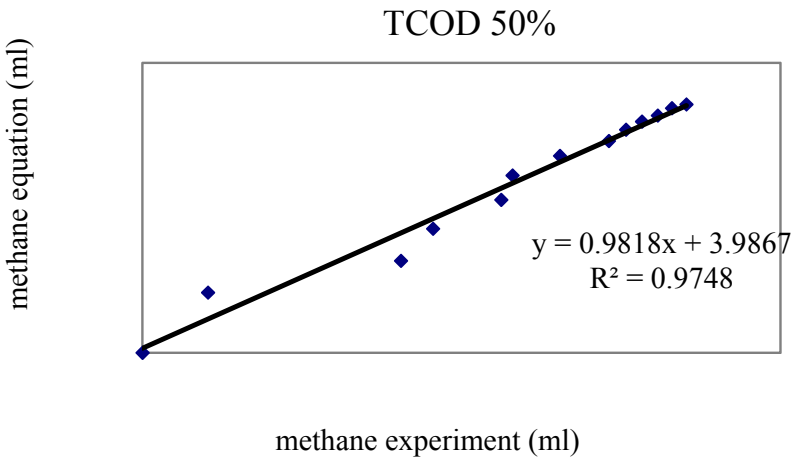



**Figure A-2** Kinetic modeling of cumulative methane production in BMP Assay at concentration 50% of raw POME.

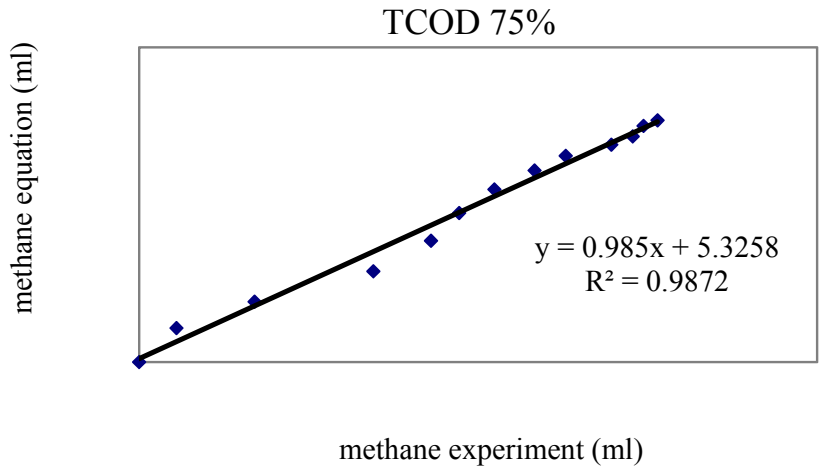



**Figure A-3** Kinetic modeling of cumulative methane production in BMP Assay atconcentration 75% of raw POME.

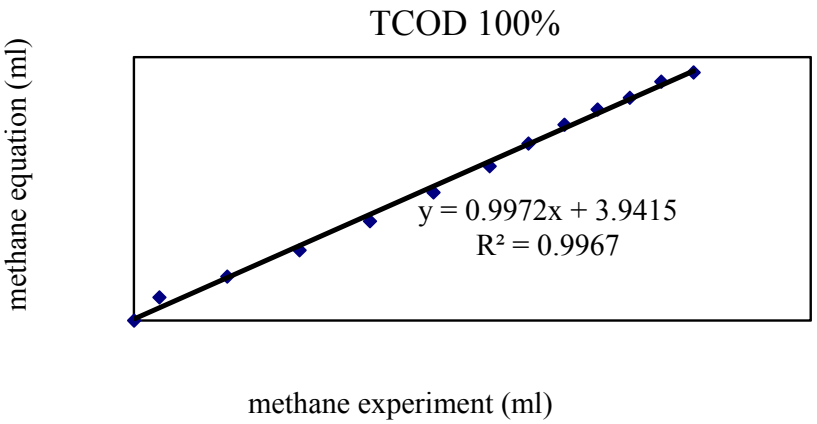



**Figure A-4** Kinetic modeling of cumulative methane production in BMP Assay at concentration 100% of raw POME.

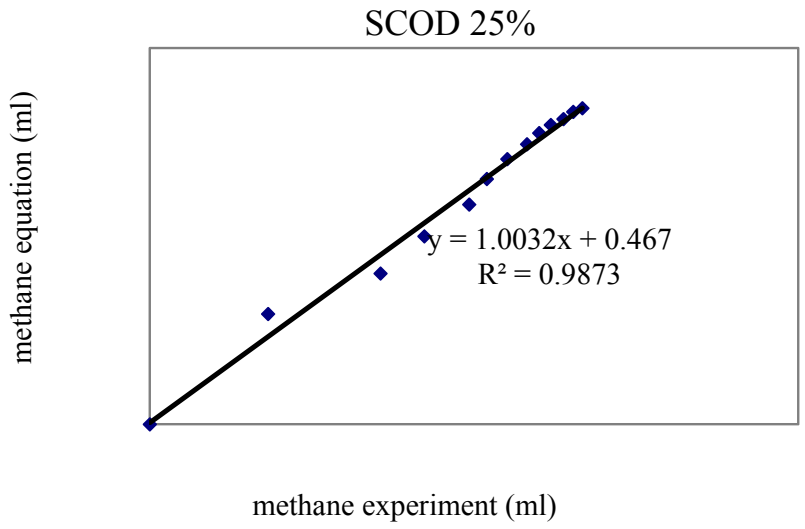



**Figure A-5** Kinetic modeling of cumulative methane production in BMP Assay at concentration 25% of filtrated raw POME.

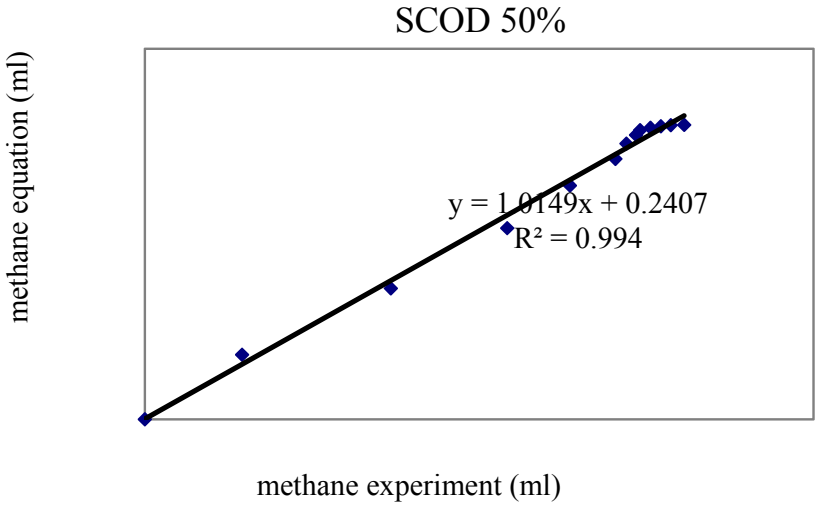



**Figure A-6** Kinetic modeling of cumulative methane production in BMP Assay at concentration 50% of filtrated raw POME.

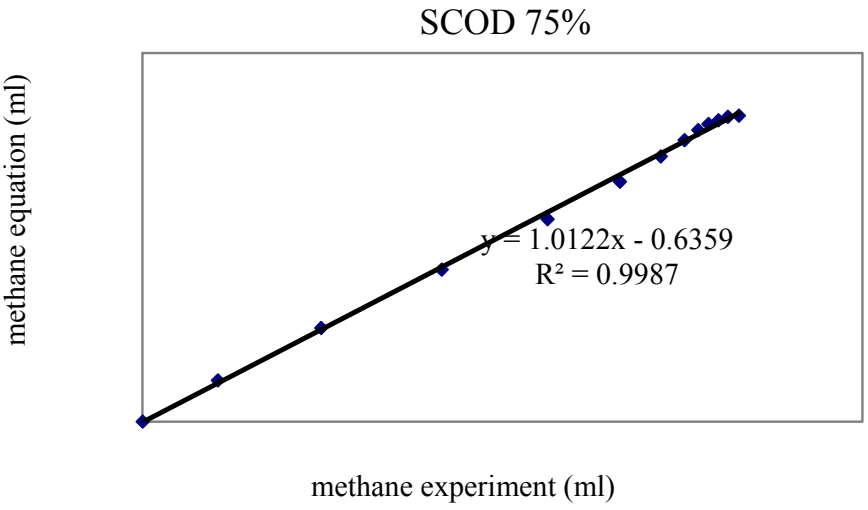



**Figure A-7** Kinetic modeling of cumulative methane production in BMP Assay atconcentration 75% of filtrated raw POME.

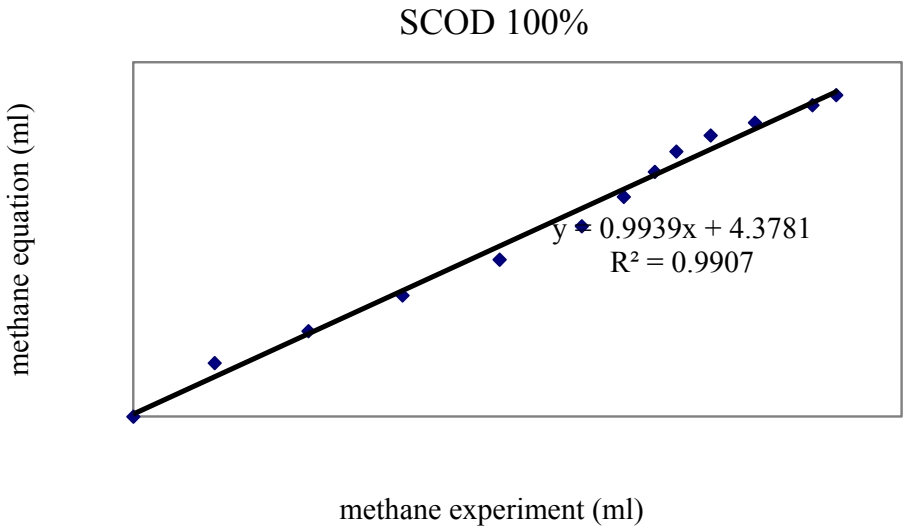



**Figure A-8** Kinetic modeling of cumulative methane production in BMP Assay atconcentration 100% of filtrated raw POME.

# APPENDIX B

## SMP AND EPS ANALYSIS AND CALIBRATION CURVES OF PROTEIN AND CARBOHYDRATE OF DIFFERENT INTERMITTENT FILTRATION MODES

- SMP and EPS Analysis

SMP and EPS were normalized as the sum of proteins (PN) and polysaccharides (PS). The sludge cake layer on the membrane surface was rinsed with DI water and centrifuged for 30 min at 6000rpm and then the extracted supernatant was filtrated through a membrane with a mean pore size of 0.45 µm. The filtrate of the centrifuged supernatant represented the concentration of SMP. The remaining pellet was washed and suspended again with saline water (0.9% NaCl solution). The sludge cake layer was then subjected to heat treatment (100$^{o}$C, 1 h) and centrifuged again under the same operating conditions. The centrifuged supernatant was clarified as EPS solution, as shown in Figure B-1.

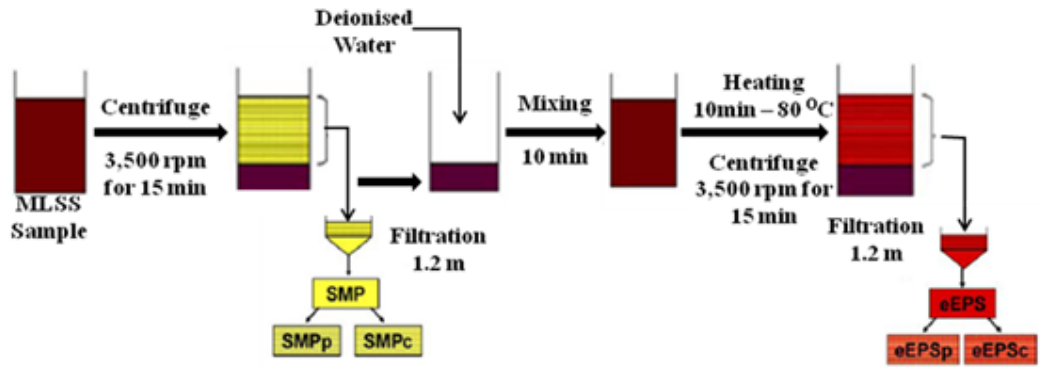



**Figure B-1** Method for EPS and SMP extractions [189].

- Protein Measurement

Step 1: Prepare the stock bovine serum albumin (BSA) solution and dilutions for the standard curve:

1. Weigh 0.05 g of BSA and add to a 500 ml volumetric flask containing DI water.
2. Stir well to dissolve and adjust the volume to 500 ml with DI water and final concentration of the stock is 100 mg BSA/L
3. Prepare dilutions in 15 ml tubes, following the recipe in table B-1

**Table B-1** Dilutions from the BSA stock solution (100 mg/L) for the standard curve.

| Volume of DI water (ml) | Volume of stock BSA solution (mL) | Final concentration (mg/L) |
|---|---|---|
| 10 | 0 | 0 |
| 8 | 2 | 20 |
| 6 | 4 | 40 |
| 4 | 6 | 60 |
| 2 | 8 | 80 |
| 0 | 10 | 100 |

Step 2: Prepare the Lowry solution;

Folin-ciocalteu protein measurement method was used (Lowry et al. [232]). In this method, the reagents given below were used:

4. Reagent A : 2 % W/V sodium carbonate in 0.1 N NaOH.
5. Reagent B : 1 % W/V sodium potassium tartarate in 0.5 % W/V cupric sulphate.
6. Reagent C : 1 mL of Reagent B + 49 mL of Reagent A.
7. Reagent D : Folin-Ciocalteu's phenol reagent (Diluted by the ratio of 10:9 with distilled water).

Step 3: Protein measurement procedure of sample/dilution standard:

1. 3 mL of reagent C was added to the solution having a volume of 0.5 mL of sample or dilution standard protein, and mixed and stood at room temperature for 10 minutes.
2. 0.3 mL of Reagent D was added and mixed well immediately. Then, it was allowed to stand for 30 minutes.
3. Transferring sample or dilution standard protein to UV-VIS spectrometer that it used the intensity at 750 nm and the protein calibration curves are given in B-2.

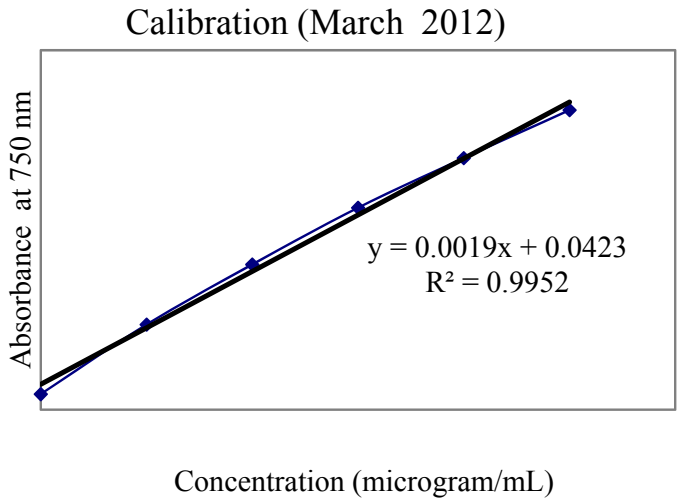



**Figure B-2** Calibration curve for protein measurement in March 2012.

- Carbohydrate Measurement

  Step 1: Prepare the glucose solution and dilutions for the standard curve:

1. Weigh 0.05 g of glucose and add to a 500 ml volumetric flask containing DI water.
2. Stir well to dissolve and adjust the volume to 500 ml with DI water and final concentration of the stock is 100 mg glucose/L
3. Prepare dilutions in 15 ml tubes, following the recipe in Table B-2.

**Table B-2** Dilutions from the glucose stock solution (100 mg/l) for the standard curve.

| Volume of DI water (ml) | Volume of stock glucose solution (ml) | Final concentration (mg/l) |
|---|---|---|
| 10 | 0 | 0 |
| 8 | 2 | 20 |
| 6 | 4 | 40 |
| 4 | 6 | 60 |
| 2 | 8 | 80 |
| 0 | 10 | 100 |

Step 2: Prepare the Carbohydrate solution:

1. Phenol 5%
2. Sulfuric acid solution

Step 3: Carbohydrate measurement procedure of sample/dilution standard:

1. Transferring 0.5 mL all of sample or dilution standard carbohydrate to the COD tubes
2. Add to 0.5 mL of 5% Phenol solution and 5 ml sulfuric acid solution to tubes, and mixed well immediately. Then, it was stood at room temperature for 10 minutes.
3. Cap and the speed of the vortex your sample or dilution standard carbohydrate for 30 minutes.
4. Place the COD tubes on the heating-block and boil at 100 $^{o}$C for 15 minutes.
5. Remove the tubes from the heating-block carefully and cool down to room temperature.
6. Transferring sample or dilution standard carbohydrates to UV-VIS spectrometer that it used the intensity at 490 nm and the carbohydrate calibration curves are given in B-3.

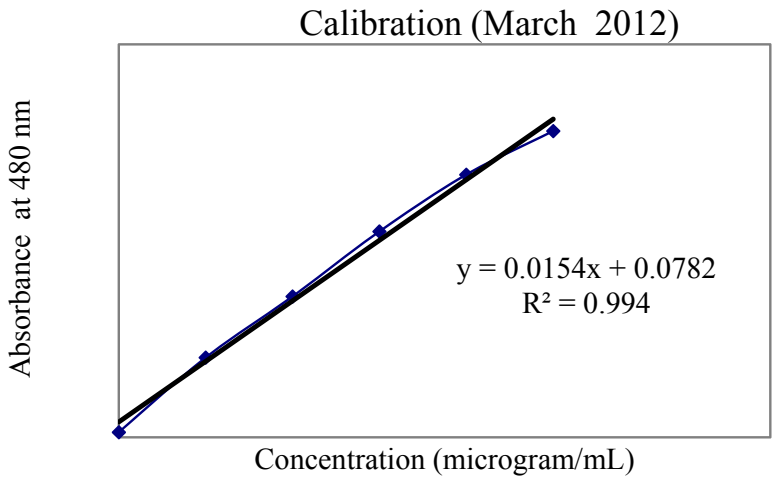



**Figure B-3** Calibration curve for carbohydrate measurement in March 2012.

# APPENDIX C
# CALIBRATION CURVES OF PROTEIN AND CARBOHYDRATE OF SAnMBR

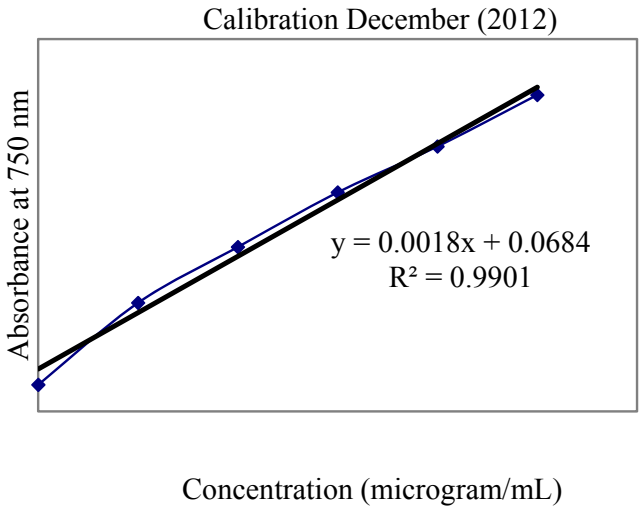



**Figure C-1** Calibration curve for protein measurement in December 2012.

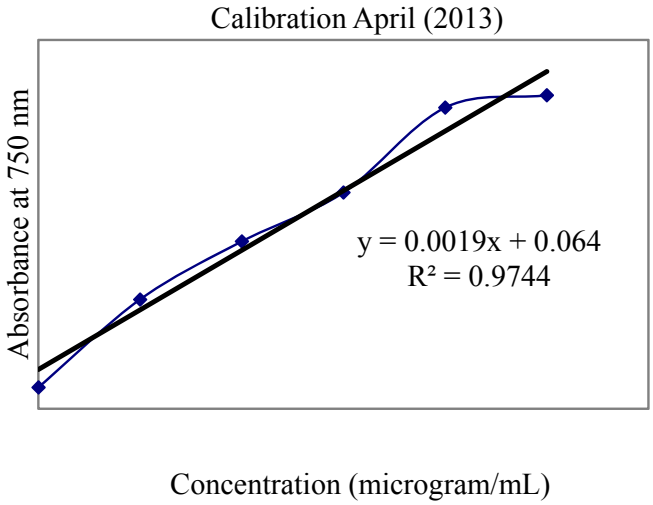



**Figure C-2** Calibration curve for protein measurement in April 2013

.

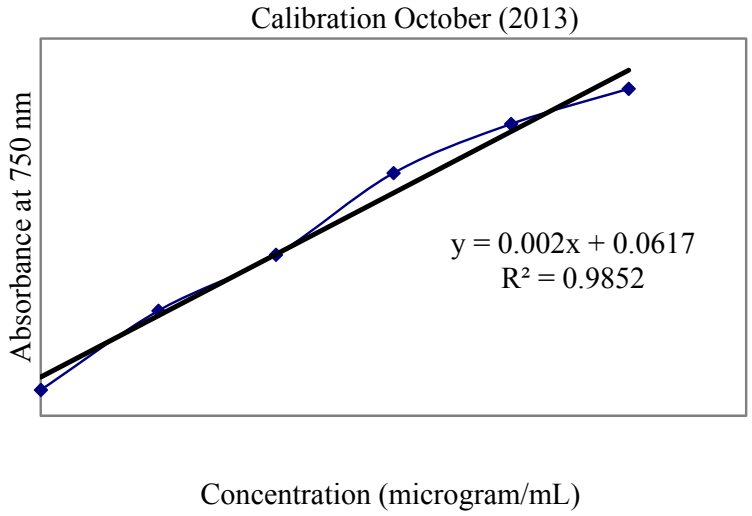



**Figure C-3** Calibration curve for protein measurement in October 2013.

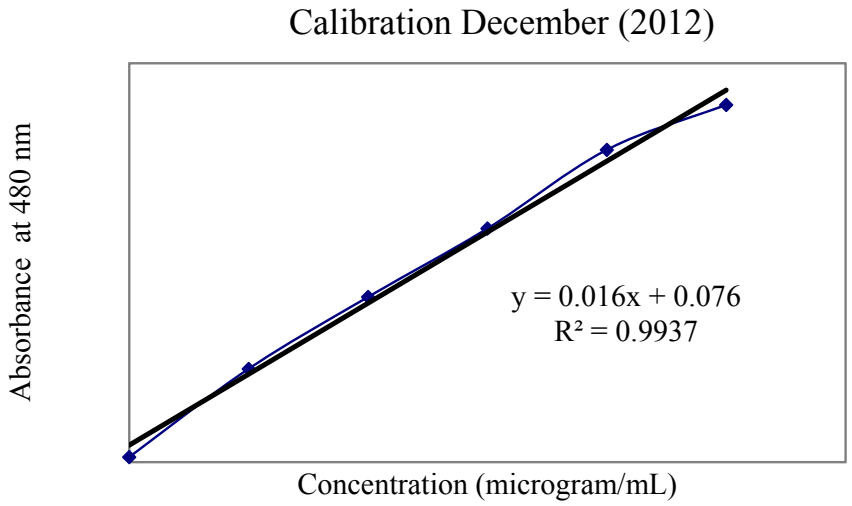



**Figure C-4** Calibration curve for carbohydrate measurement in December 2012.

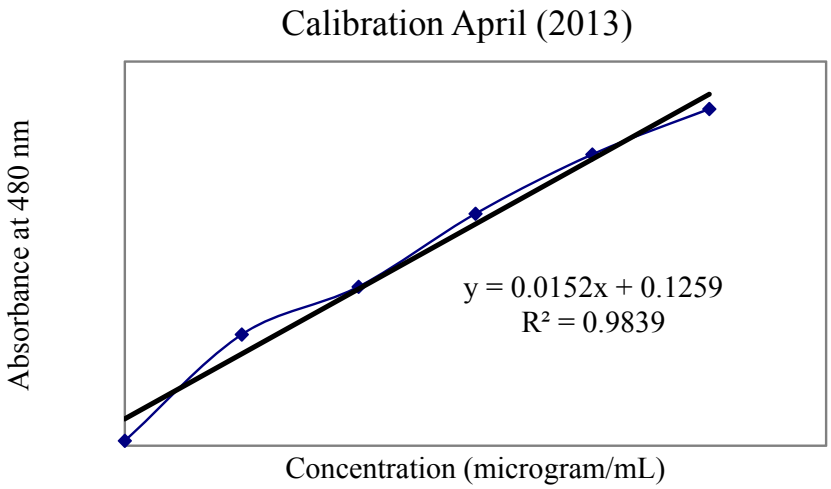



**Figure C-5** Calibration curve for carbohydrate measurement in April 2013.

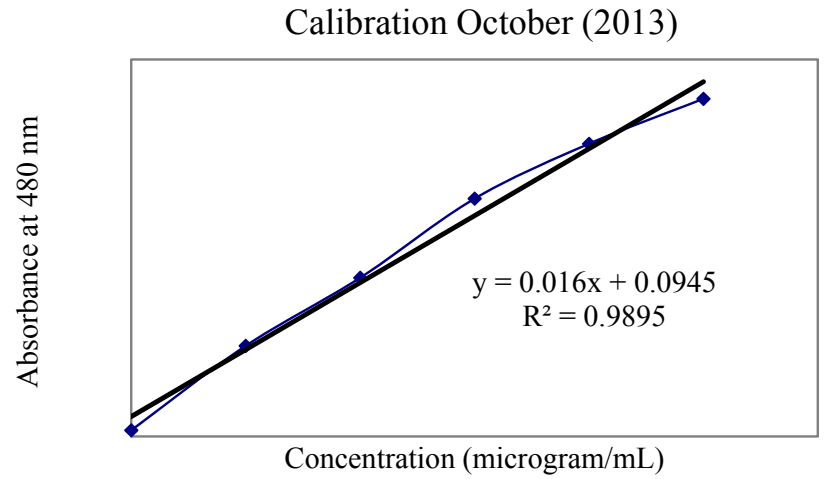



**Figure C-6** Calibration curve for carbohydrate measurement in October 2013.

# APPENDIX D
# SAnMBR ANALYTICAL METHOD

- pH

pH was measured with a pH meter (HI 8314, Hanna Instruments) and a pH probe (HI 1230, Hanna Instruments).

- Total and Soluble Chemical Oxygen Demand

TCOD and SCOD were analyzed as described in standard methods (5220 B. Close Reflux Method) (APHA 2005). Prior to analyses, samples were filtered through 0.45 μm pore size filters (Millipore). Then, SCOD determinations were carried out, as described in standard methods (5220 B. Close Reflux Method) (APHA 2005).

- Ammonium Nitrogen

Ammoniaum Nitrogen was measured according to the procedure described in standard methods (4500-Norg B. Macro-Kjeldahl Method) (APHA 2005).

- Total Kjeldahl Nitrogen

TKN was measured according to the procedure described in standard methods (4500-Norg B. Macro-Kjeldahl Method) (APHA 2005).

- Alkalinity

Alkalinity was measured according to standard methods (2320-B Titration Method) (APHA 2005).

- Volatile Fatty Acids

The gas chromatograph (Thermo Electron Co.) used for biogas composition determinations was also used for the periodical VFA measurements. However, the column, the detector and the operational conditions were different: Nukol column (Model 25326, 15 m × 0.53 mm) was used to separate VFAs (acetic, propionic, nbutyric, iso-butyric, n-valeric, iso-valeric, n-caproic, iso-caproic and n-heptanoic acids). Flame ionization detector (FID) was adjusted to 280 $^{o}$C. Helium was used as carrier gas with a constant flow rate of 6 mL/min and the inlet temperature was kept at 250 $^{o}$C. Oven temperature was initially set to 100 $^{o}$C with 2 min holding time and then increased up to 200 $^{o}$C with 8 $^{o}$C /min ramping.

Prior to the gas chromatography injections, a series of pretreatments were conducted for VFA measurements. First, samples were filtered through 0.22 μm pore-size filters. Then the samples were diluted with deionized water to assure the VFA

concentration of the sample to be in the range of pure VFA calibration of gas chromatograph. After filtering and dilution, the samples were acidified with 98% formic acid to a pH less than 2.5 in order to convert the fatty acids to their undissociated forms (i.e. molecular forms).

- Suspended Solids and Volatile Suspended Solids

SS and VSS determinations were carried out as described in standard methods (2540 D. Total Suspended Solids Dried at 103–105 $^{o}$C and 550 $^{o}$C ) (APHA 2005).

- Biogas Production

Biogas productions (in acidogenic and methanogenic reactors) were measured by using a graduated water reservoir (2000 mL) connected directly to the reactor headspace. Acid brine (10% NaCl w/v, 2% $H_2SO_4$ v/v) was used as displaced water in order to eliminate the solubilization of the biogas (Tezel et al. 2007).

- Biogas Composition

Biogas compositions were determined with a gas chromatograph (Thermo Electron Co.) equipped with a thermal conductivity detector (TCD). Produced biogases were separated as hydrogen ($H_2$), carbon dioxide ($CO_2$), methane ($CH_4$) and nitrogen ($N_2$) by using parallel connected columns (CP-Moliseve 5A and CPP orabond Q) at a fixed oven temperature of 45 $^{o}$C. Helium was used as carrier gas at a 100 kPa constant pressure. The inlet and detector temperatures were set to 50 $^{o}$C and 80 $^{o}$C, respectively.

## APPENDIX E

## THE METHANE POTENTIAL AND EFFICIENCY OF SAnMBR AT 3 SRTS

**Table E-1** The methane potential results of two-stage SAnMBR at SRT 15 day.

| Date | Biogas volume (mL) | | | Biogas volume at STP (mL) | | | g TCOD removed | (%) of Biogas | | | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_2$ | $CO_2$ | $CH_4$ | $N_2$ | $CO_2$ | $CH_4$ | | $N_2$ | $CO_2$ | $CH_4$ | |
| 1 | 14 | 36 | 98 | 13 | 33 | 90 | 48 | 9.38 | 65.98 | 24.44 | 0.25 |
| 6 | 15 | 36 | 98 | 13 | 33 | 90 | 48 | 9.76 | 66.12 | 24.12 | 0.25 |
| 11 | 14 | 38 | 104 | 13 | 35 | 96 | 48 | 8.84 | 66.85 | 24.31 | 0.27 |
| 16 | 14 | 37 | 107 | 13 | 34 | 98 | 48 | 8.75 | 67.74 | 23.52 | 0.27 |
| 21 | 16 | 36 | 110 | 15 | 33 | 100 | 46 | 9.93 | 67.98 | 22.29 | 0.29 |
| 25 | 15 | 37 | 112 | 13 | 34 | 103 | 46 | 8.92 | 68.38 | 22.70 | 0.30 |
| 29 | 17 | 35 | 114 | 15 | 32 | 105 | 46 | 10.03 | 68.97 | 21.00 | 0.30 |
| 33 | 16 | 37 | 116 | 14 | 34 | 107 | 47 | 9.24 | 68.98 | 21.78 | 0.30 |
| 37 | 17 | 37 | 118 | 16 | 34 | 108 | 48 | 10.15 | 68.41 | 21.43 | 0.30 |
| 41 | 17 | 36 | 120 | 16 | 33 | 110 | 48 | 9.96 | 69.33 | 20.71 | 0.31 |
| 45 | 17 | 37 | 121 | 16 | 34 | 111 | 48 | 9.65 | 69.20 | 21.15 | 0.31 |
| 49 | 16 | 38 | 124 | 15 | 35 | 114 | 48 | 9.05 | 69.53 | 21.42 | 0.32 |
| 53 | 18 | 37 | 125 | 16 | 34 | 115 | 48 | 9.91 | 69.63 | 20.46 | 0.32 |

**Table E -1** The methane potential results of two-stage SAnMBR at SRT 15 days (cont').

| Date | Biogas volume (mL) | | | Biogas volume at STP (mL) | | | g TCOD removed | (%) of Biogas | | | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_2$ | $CO_2$ | $CH_4$ | $N_2$ | $CO_2$ | $CH_4$ | | $N_2$ | $CO_2$ | $CH_4$ | |
| 57 | 16 | 39 | 125 | 14 | 36 | 115 | 48 | 8.76 | 69.64 | 21.61 | 0.32 |
| 61 | 15 | 39 | 125 | 14 | 36 | 115 | 49 | 8.46 | 69.83 | 21.71 | 0.32 |
| 65 | 17 | 39 | 124 | 16 | 36 | 113 | 49 | 9.47 | 68.80 | 21.73 | 0.31 |
| 69 | 16 | 38 | 126 | 15 | 35 | 115 | 49 | 8.84 | 69.92 | 21.23 | 0.32 |
| 73 | 16 | 38 | 126 | 15 | 35 | 115 | 49 | 9.04 | 69.94 | 21.02 | 0.32 |
| 77 | 17 | 38 | 126 | 15 | 35 | 115 | 49 | 9.27 | 69.86 | 20.98 | 0.32 |
| 81 | 16 | 39 | 125 | 14 | 36 | 114 | 49 | 8.75 | 69.31 | 21.95 | 0.31 |

**Table E -2** The methane potential results of two-stage SAnMBR at SRT 30 days.

| Date | Biogas volume (mL) | | | Biogas volume at STP (mL) | | | g TCOD removed | (%) of Biogas | | | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_2$ | $CO_2$ | $CH_4$ | $N_2$ | $CO_2$ | $CH_4$ | | $N_2$ | $CO_2$ | $CH_4$ | |
| 1 | 15 | 41 | 108 | 13 | 38 | 99 | 49 | 8.93 | 65.98 | 25.29 | 0.27 |
| 6 | 17 | 38 | 113 | 15 | 35 | 103 | 49 | 9.92 | 67.38 | 22.70 | 0.28 |
| 11 | 14 | 42 | 113 | 12 | 39 | 103 | 49 | 8.03 | 66.97 | 25.00 | 0.28 |
| 16 | 13 | 43 | 117 | 11 | 39 | 108 | 49 | 7.24 | 67.98 | 24.78 | 0.29 |
| 21 | 14 | 42 | 121 | 13 | 38 | 111 | 49 | 7.95 | 68.33 | 23.43 | 0.30 |
| 25 | 14 | 41 | 124 | 13 | 38 | 114 | 49 | 7.96 | 68.85 | 22.91 | 0.31 |
| 29 | 14 | 40 | 128 | 13 | 37 | 117 | 49 | 7.84 | 69.94 | 21.81 | 0.32 |
| 33 | 14 | 43 | 127 | 13 | 40 | 116 | 50 | 7.75 | 68.90 | 23.52 | 0.31 |
| 37 | 18 | 39 | 127 | 16 | 36 | 116 | 49 | 9.65 | 68.93 | 21.15 | 0.32 |
| 41 | 16 | 44 | 142 | 15 | 41 | 130 | 49 | 8.05 | 69.93 | 21.85 | 0.35 |
| 45 | 18 | 44 | 141 | 17 | 40 | 129 | 49 | 8.91 | 69.41 | 21.46 | 0.35 |
| 49 | 18 | 44 | 141 | 16 | 40 | 129 | 49 | 8.76 | 69.64 | 21.61 | 0.35 |
| 53 | 17 | 44 | 141 | 16 | 40 | 129 | 48 | 8.46 | 69.83 | 21.71 | 0.35 |
| 57 | 17 | 44 | 141 | 16 | 40 | 129 | 49 | 8.47 | 69.80 | 21.73 | 0.35 |
| 61 | 18 | 43 | 141 | 16 | 39 | 129 | 49 | 8.84 | 69.92 | 21.23 | 0.35 |
| 65 | 18 | 42 | 141 | 17 | 38 | 129 | 49 | 9.04 | 69.94 | 20.72 | 0.35 |

**Table E -2** The methane potential result of two-stage SAnMBR at SRT 30 day (cont').

| Date | Biogas volume (mL) | | | Biogas volume at STP (mL) | | | g TCOD removed | (%) of Biogas | | | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_2$ | $CO_2$ | $CH_4$ | $N_2$ | $CO_2$ | $CH_4$ | | $N_2$ | $CO_2$ | $CH_4$ | |
| 69 | 18 | 42 | 141 | 17 | 39 | 129 | 49 | 8.97 | 69.86 | 20.88 | 0.35 |
| 73 | 18 | 47 | 139 | 16 | 43 | 127 | 49 | 8.75 | 68.31 | 22.95 | 0.35 |
| 77 | 18 | 44 | 140 | 16 | 41 | 128 | 49 | 8.76 | 69.12 | 21.82 | 0.35 |
| 85 | 17 | 43 | 141 | 16 | 40 | 129 | 49 | 8.38 | 69.88 | 21.42 | 0.35 |
| 89 | 19 | 41 | 141 | 18 | 38 | 129 | 49 | 9.51 | 69.83 | 20.46 | 0.35 |
| 93 | 18 | 44 | 141 | 16 | 40 | 129 | 49 | 8.67 | 69.31 | 21.61 | 0.35 |
| 97 | 17 | 44 | 140 | 16 | 40 | 129 | 49 | 8.64 | 69.31 | 21.71 | 0.35 |
| 101 | 17 | 44 | 141 | 16 | 40 | 129 | 49 | 8.55 | 69.52 | 21.73 | 0.35 |
| 105 | 18 | 44 | 141 | 16 | 40 | 129 | 49 | 8.85 | 69.81 | 21.83 | 0.35 |
| 109 | 18 | 42 | 141 | 17 | 39 | 129 | 49 | 9.00 | 69.89 | 21.02 | 0.35 |
| 113 | 19 | 42 | 141 | 17 | 39 | 129 | 49 | 9.38 | 69.79 | 20.98 | 0.35 |
| 117 | 16 | 46 | 140 | 14 | 43 | 129 | 49 | 7.73 | 69.41 | 22.95 | 0.35 |
| 121 | 16 | 45 | 141 | 14 | 41 | 129 | 49 | 7.73 | 69.93 | 22.12 | 0.35 |

**Table E -3** The methane potential results of two-stage SAnMBR at SRT 60 days.

| Date | Biogas volume (mL) | | | Biogas volume at STP (mL) | | | g TCOD removed | (%) of Biogas | | | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_2$ | $CO_2$ | $CH_4$ | $N_2$ | $CO_2$ | $CH_4$ | | $N_2$ | $CO_2$ | $CH_4$ | |
| 1 | 14 | 40 | 105 | 13 | 37 | 96 | 48 | 8.96 | 24.93 | 65.61 | 0.27 |
| 6 | 16 | 39 | 105 | 15 | 35 | 96 | 48 | 9.94 | 24.16 | 65.60 | 0.27 |
| 11 | 15 | 40 | 110 | 14 | 36 | 101 | 48 | 9.22 | 24.08 | 66.70 | 0.28 |
| 16 | 15 | 40 | 112 | 14 | 36 | 103 | 48 | 8.96 | 23.70 | 67.05 | 0.29 |
| 21 | 17 | 42 | 118 | 16 | 38 | 108 | 48 | 9.81 | 23.59 | 66.70 | 0.30 |
| 25 | 17 | 39 | 121 | 16 | 36 | 111 | 48 | 9.71 | 21.90 | 67.89 | 0.31 |
| 29 | 17 | 39 | 126 | 16 | 36 | 115 | 48 | 9.49 | 21.60 | 68.91 | 0.32 |
| 33 | 18 | 40 | 126 | 17 | 37 | 115 | 49 | 9.78 | 21.90 | 68.32 | 0.31 |
| 37 | 18 | 39 | 129 | 16 | 36 | 118 | 49 | 9.57 | 20.98 | 69.45 | 0.32 |
| 41 | 17 | 41 | 129 | 15 | 38 | 118 | 49 | 9.01 | 21.95 | 69.04 | 0.32 |
| 45 | 20 | 45 | 150 | 18 | 41 | 137 | 49 | 9.20 | 21.03 | 69.77 | 0.37 |
| 49 | 16 | 43 | 131 | 15 | 39 | 120 | 48 | 8.36 | 22.60 | 69.05 | 0.33 |
| 53 | 17 | 41 | 132 | 16 | 37 | 121 | 49 | 9.19 | 21.50 | 69.31 | 0.33 |
| 57 | 21 | 39 | 130 | 19 | 36 | 119 | 49 | 10.85 | 20.47 | 68.67 | 0.32 |
| 61 | 20 | 40 | 131 | 18 | 36 | 120 | 49 | 10.32 | 20.86 | 68.82 | 0.33 |
| 65 | 20 | 38 | 131 | 18 | 35 | 120 | 49 | 10.55 | 20.06 | 69.39 | 0.33 |

**Table E -3** The methane potential results of two-stage SAnMBR at SRT 60 days (cont').

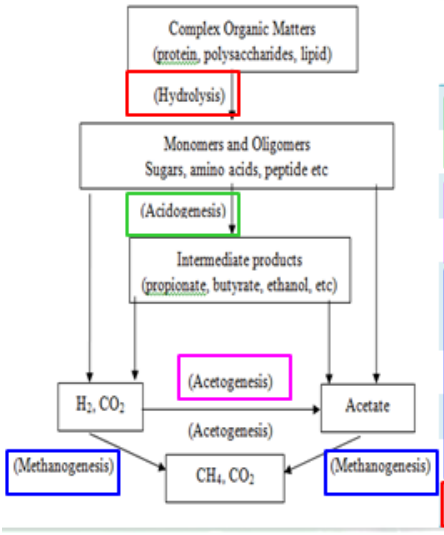
| Date | Biogas volume (mL) | | | Biogas volume at STP (mL) | | | g TCOD removed | (%) of Biogas | | | Methane yield ($L/COD_{remove}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_2$ | $CO_2$ | $CH_4$ | $N_2$ | $CO_2$ | $CH_4$ | | $N_2$ | $CO_2$ | $CH_4$ | |
| 69 | 19 | 41 | 130 | 17 | 38 | 119 | 49 | 9.77 | 21.64 | 68.59 | 0.32 |
| 73 | 18 | 42 | 131 | 17 | 38 | 120 | 49 | 9.51 | 21.84 | 68.65 | 0.33 |
| 77 | 20 | 39 | 131 | 19 | 35 | 120 | 48 | 10.67 | 20.26 | 69.07 | 0.33 |
| 85 | 18 | 40 | 132 | 17 | 37 | 121 | 49 | 9.64 | 21.03 | 69.41 | 0.33 |
| 89 | 18 | 40 | 133 | 17 | 37 | 122 | 49 | 9.55 | 21.14 | 69.64 | 0.33 |
| 93 | 18 | 40 | 133 | 17 | 36 | 122 | 49 | 9.55 | 20.83 | 69.83 | 0.33 |
| 97 | 17 | 40 | 133 | 16 | 36 | 122 | 49 | 9.10 | 20.88 | 69.80 | 0.33 |
| 101 | 16 | 41 | 133 | 15 | 38 | 122 | 49 | 8.38 | 21.51 | 69.92 | 0.33 |
| 105 | 17 | 40 | 133 | 15 | 37 | 122 | 49 | 8.73 | 21.08 | 69.94 | 0.33 |
| 109 | 17 | 42 | 133 | 15 | 38 | 122 | 49 | 8.73 | 21.98 | 69.56 | 0.33 |
| 113 | 18 | 40 | 133 | 16 | 37 | 122 | 49 | 9.31 | 20.97 | 69.81 | 0.33 |
| 117 | 16 | 41 | 133 | 15 | 38 | 122 | 49 | 8.36 | 21.71 | 69.92 | 0.33 |

# APPENDIX F
# MODELLING AND SIMULATION ADM

**F-1** The data input for ADM model

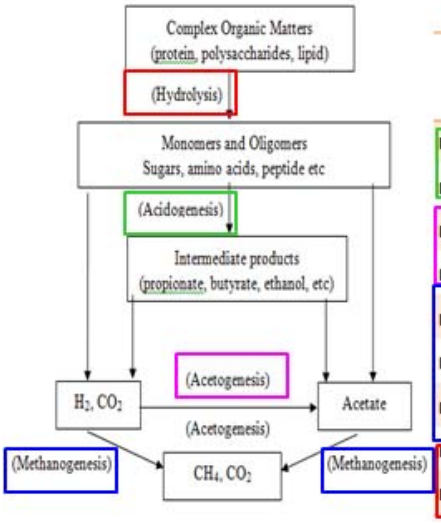
Before beginning simulation and/or reactor design

- Influent characterization
- Process configuration and operational conditions
- Dynamics of population
  - Hydrolysis
  - Acidogenesis reactor
  - Acetogenesis reactor
  - Methanogenesis reactor




| Kinetic coefficient | Unit | Initial values | Estimated values |
|---|---|---|---|
| Maximum fermentation rate | 1/d | 3.2 | 3.63 |
| Decay rate for fermentive biomass | 1/d | 0.04 | 0.04 |
| Maximum growth rate of acetogens | 1/d | 0.35 | 0.38 |
| Decay rate for acetogens | 1/d | 0.02 | 0.02 |
| Max growth rate of hydrogenotrophic methanogens | 1/d | 0.388 | 0.415 |
| Decay rate of hydrogenotrophic methanogens | 1/d | 0.01 | 0.01 |
| Max growth rate of acetate utilizing bacteria | 1/d | 0.15 | 0.19 |
| Decay rate for acetoclastic methanogens | 1/d | 0.02 | 0.02 |
| Hydrolysis rate constant | 1/d | 3 | 3.63 |

**F-2** Summary of Kinetic Parameters for Various Substrates Utilized in Acclimatization Period




| Kinetic coefficient | Unit | Initial values | Estimated values |
|---|---|---|---|
| Maximum fermentation rate | 1/d | 3.2 | 4.86 |
| Decay rate for fermentive biomass | 1/d | 0.04 | 0.07 |
| Maximum growth rate of acetogens | 1/d | 0.35 | 0.43 |
| Decay rate for acetogens | 1/d | 0.02 | 0.03 |
| Max growth rate of hydrogenotrophic methanogens | 1/d | 0.368 | 0.397 |
| Decay rate of hydrogenotrophic methanogens | 1/d | 0.01 | 0.02 |
| Max growth rate of acetate utilizing bacteria | 1/d | 0.15 | 0.24 |
| Decay rate for acetoclastic methanogens | 1/d | 0.02 | 0.03 |
| Hydrolysis rate constant | 1/d | 3 | 4.86 |

**F-3** Summary of Kinetic Parameters for Various Substrates Utilized in SAnMBR