Effect of PVA-Gel on Performance Improvement of a Two Stage Thermophilic Anaerobic Membrane Bioreactor

by

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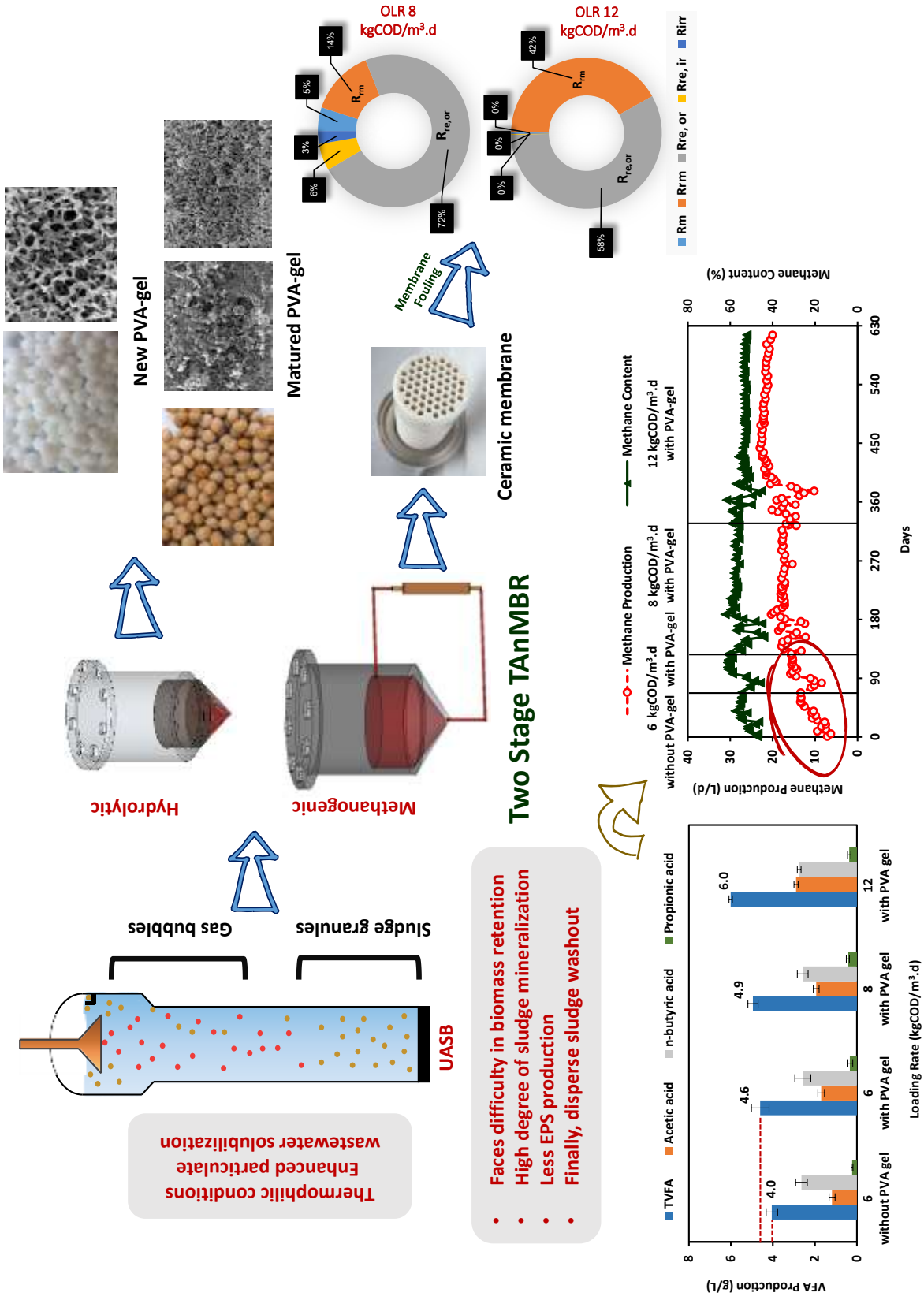
Graphical Abstract



- Faces difficulty in biomass retention
- High degree of sludge mineralization
- Less EPS production
- Finally, disperse sludge washout

Abstract

A two stage thermophillic anaerobic membrane bioreactor (TAnMBR) was used for treating high strength particulate wastewater (tapioca starch) with enhanced biodegradation rates and low biomass generation. Operating the reactor under thermophillic condition offers benefit like higher organic removal rate with higher growth rate and better biodegradation efficiency. Thus leading to shorter hydraulic retention time (HRT). It also provides a more complete pathogenic microorganism destruction, lower biomass yield, elimination of cooling needs for wastewater discharge at high temperature. On the contrary, thermophillic condition causes poor sludge granulation and sludge settleability due to high sludge deflocculation and deterioration of settling properties. This negatively affects the biomass to produce extracellular polymeric substances (EPS) which promotes dense and firm sludge granulation. Therefore, resulting in sludge washout due to highly sludge degranulation and dispersed sludge formation which subsequently deteriorated the quality of effluent.

In this study, two stage TAnMBR which consists of a hydrolytic reactor followed by a methanogenic reactor and a microfilter (0.1 µm) was operated under external semi dead-end mode at thermophillic condition (55°C). This assured complete biomass retention; consequently ensure handling high loading conditions. Two stage TAnMBR ensures the optimum growth conditions for hydrolytic/acidogenic bacteria and methanogenic archaea. This enhances the biological activity, consequently increases methane production. Initially, anaerobic seed sludge enrichment and acclimatization was done in sequencing batch reactor (SBR). After that a two stage TAnMBR was operated at three different loading conditions. The reactor was first operated at loading rate 6 kgCOD/m^3.d, and then PVA-gel was added to compare the performance of hydrolytic reactor. Similar performance evaluations were conducted at loading rate 8 and 12 kgCOD/m^3.d, respectively.

At loading rate 6 kgCOD/m^3.d, hydrolytic reactor operated at 9.6±0.5 g/L of volatile suspended solid (VSS) concentration in order to study the performance of hydrolytic reactor with and without PVA-gel addition on volatile fatty acid (VFA) concentration. The results showed that hydrolytic reactor with PVA-gel addition significantly increased VFA concentration and enhances methane productivity at loading rate of 6 kgCOD/m^3.d ($p < 0.05$). The VFA production in hydrolytic reactor significantly increased from 4.0±0.2 to 4.6±0.5 g/L with PVA-gel addition at OLR 6 kgCOD/m^3.d ($p < 0.05$). Once the loading rate was increased to 8 and 12 kgCOD/m^3.d, VFA production also significantly increased to 4.9±0.2 and 6.0±0.1 g/L ($p < 0.05$), respectively. The increase in VFA concentration could be attributed to an increase in biological activity with PVA-gel addition. Furthermore, methane productivity had also significantly increased from 1.4 to 1.7 $L_{methane}/L_{reactor}$.d ($p < 0.05$). This was due to an increasing in VFA concentration in hydrolytic effluent. Similarly with an increase in loading rate to 8 and 12 kgCOD/m^3.d methane productivity further significantly increased to 1.9 and 2.4 $L_{methane}/L_{reactor}$.d ($p < 0.05$), respectively. Two stage TAnMBR achieved organic removal rate (ORR) of 5.3 to 10.1 kgCOD/m^3.d with organic removal efficiency of 84-92%. However, membrane fouling was one of the limiting factors in membrane application. Membrane fouling investigations indicated that the predominant fouling in TAnMBR was organic reversible fouling caused by bound extracellular polymeric substances (EPS). Bound EPS was observed to be increased at loading rate 12 kgCOD/m^3.d as compared to 8 kgCOD/m^3.d. Furthermore, fouling investigation at both loading conditions showed that filtration resistance was due to the presence of higher bound EPS at higher loading rate, which lead to sticky sludge and thus favor to develop cake/gel formation on membrane surface or inside the pore of membrane.

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List of Abbreviations

AF	Anaerobic Filter
AFB	Anaerobic Fluidized Bed
AnMBR	Anaerobic Membrane Bioreactor
atm	Atmospheric Pressure
BOD	Biochemical Oxygen Demand
BSA	Bovine Serum Albumin
CER	Cation Exchange Resin
CO_2-eq	Carbon Dioxide Equivalent
COD	Chemical Oxygen Demand
COD_r	Chemical Oxygen Demand Removed
CSTR	Continuous Stirring Tank Reactor
d	Day
EPS	Extracellular Polymeric Substances
FID	Flame Ionization Detector
g	Gram
GAC	Granular Activated Carbon
GC	Gas Chromatography
h	Hour
HRT	Hydraulic Retention Time
J	Permeate Flux
kg	Kilogram
kPa	Kilopascal
kWh	Kilowatt Hour
L	Litre
M	Molar
m^3	Cubic Metre
MF	Microfiltration
mg	Milligram
mL	Millilitre
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
mV	Millivolt
OLR	Organic Loading Rate
ORP	Oxidation Reduction Potential
ORR	Organic Removal Rate
PAC	Powder Activated Carbon
PCOD	Particulate Chemical Oxygen Demand
PE	Polyethylene
PEI	Polyetherimide
PN	Protein
PP	Polypropylene
PS	Polysaccharide or Carbohydrate

PTFE	Polytetrafluoroethylene
PVA-gel	Polyvinyl Alcohol Hydrogel
PVDF	Polyvinylidene Fluoride
R_{irr}	Irreversible Fouling Resistance
R_m	Intrinsic Membrane Resistance
R_{re}	Reversible Fouling Resistance
R_{rm}	Removable Fouling Resistance
R_t	Total Membrane Resistance
SAnMBR	Submerge Anaerobic Membrane Bioreactor
SCOD	Soluble Chemical Oxygen Demand
SEM	Scanning Electron Microscopic
SRT	Sludge Retention Time
SS	Suspended Solid
SVI	Sludge Volume Index
T	Temperature
TAnMBR	Thermophilic Anaerobic Membrane Bioreactor
TCD	Thermal Conductive Detector
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjedhal Nitrogen
TMP	Trans-membrane Pressure
TP	Total Phosphorus
TS	Total Solid
TVFA	Total Volatile Fatty Acids
UASB	Upflow Anaerobic Sludge Blanket Reactor
v/v	Volume to Volume Ratio
V	Volume
VFA	Volatile Fatty Acids
VS	Volatile Solid

Chapter 1

Introduction

1.1 Background

Anaerobic processes are considered as cost effective and sustainable technology for wastewater treatment due to low biomass production, less energy requirements and reduce greenhouse gas emission through utilization of methane gas. Nowadays, it is widely used in the industrial sector to treat wastewater with low to high strength wastewater. The treatment performance depends on the activity of a wide range of microorganisms, converting complex organic matters present in wastewater. The treatment capacity is also directly related to biological activity and biomass concentration that can be effectively retained in the system. Normally, biomass retention can be achieved by sludge granulation, biofilm formation and sludge immobilization. This is very important in anaerobic wastewater treatment systems since they ensure an effective uncoupling of solids retention time (SRT) from hydraulic retention time (HRT), enabling high loading potentials at short HRT.

The major difficulty in anaerobic wastewater treatment is the retention of sufficient quantity of active microorganisms due to differences in their growth rates and metabolic characteristics between acidogenic bacteria and methanogenic archaea. The instability of a system is usually associated with an imbalance between volatile fatty acids (VFA) production and utilization. Acidogenic bacteria have the highest growth rate among microorganisms consortia and are more tolerant to environmental perturbations and stress conditions than syntrophic acetogenic bacteria and methanogenic archaea (Ke et al., 2005). Numerous studies showed that hydrolysis is the rate limiting step in degradation of complex particulate matters, whereas methanogenesis is considered as a rate limiting step for fermentation of soluble substrates (Yu et al., 2003; Vavilin et al., 2008; Ferrer et al., 2010). Two stage anaerobic process is considered to be effective when the hydrolysis is the rate limiting step in the degradation of particulate compounds (Shin et al., 2001; Ponsá et al., 2008). Therefore separate reaction of these two distinctive microorganisms (acid forming bacteria and methanogenic archaea) facilitates higher performance in terms of organic removal with reduction of propionic acid accumulation which can be achieved in two stage anaerobic process (Ke et al., 2005; Mota et al., 2013).

Two stage thermophilic anaerobic wastewater treatment is one of the advanced wastewater treatment technology for treating high strength particulate wastewater. It also offers several advantages such as accelerated biodegradation rate, increased biogas generation, low sludge yield, enhanced solubility of low soluble substrates, inactivation of pathogenic microorganisms and elimination of cooling requirements when wastewater is discharged at high temperature. However, higher biogas production negatively affects sludge settleability due to excessive biomass carryover and washout. This phenomenon could be largely overcome by biofilm or granule formation.

Nevertheless, sludge granulation is adversely affected by elevated temperature (observed in thermophilic reactors) due to high degree of sludge mineralization (Soto et al., 1992). Mineralization negatively effects extracellular polymeric substances (EPS) production which ultimately restricts formation of dense and firm sludge granules. This results in, low or no granulation or even de-granulation when mesophilic seed sludge are used as inoculum (Jeison et al., 2009a). Therefore, lower treatment performances are expected compared with

systems where high biomass concentrations are achieved. Van Lier (1996) reported that both fixed film and suspended growth reactors faced this problem at thermophilic conditions. Thus limiting the use of thermophilic anaerobic reactor in industrial applications due to excessive biomass washout resulting in poor treatment performances and effluent quality.

Application of thermophilic anaerobic membrane bioreactor (TAnMBR) effectively retains biomass within the system assures no biomass washout from the system, hence no risk of biomass washout and system performance is apparently independent on biofilm or granule formation. Typically, TAnMBR studies have been performed with cross-flow configuration because of less membrane fouling (Liao et al., 2006; Visvanathan and Abeynayaka, 2012). However, biomass recirculation through pumps and valves has negative effect on the biological activity. This is due to high shear intensities during biomass recirculation, disrupting the syntrophic association and prevents interspecies hydrogen transfer, resulting in low biological activity (Brockmann and Seyfried, 1997; Lin et al., 2009; Wijekoon et al., 2011). Therefore it is important to study the alternative strategy to operate TAnMBR in external semi dead-end configuration to decrease the frequency of biomass recirculation through pumps and valves by combining cross-flow and dead-end configuration to the system. Due to limited studies on membrane fouling in thermophilic anaerobic condition, there was a necessity for assessing membrane fouling under thermophilic conditions.

A few studies reported the effect of biocarrier on the performance of hydrolytic reactor especially VFA production such as hiflow ring, granular activated carbon (GAC) and ceramic filters (Saddoud and Sayadi, 2007; Bertin et al., 2010). However, no efforts to optimize the hydrolytic reactor in terms of VFA concentration and composition were made in these studies. Polyvinyl alcohol hydrogel (PVA-gel) beads, which are readily available, low cost polymeric gel, have demonstrated effectiveness as biocarrier in upflow anaerobic sludge blanket (UASB) reactor and anaerobic fluidized bed (AFB) reactor to treat high strength wastewater (Rouse et al., 2007; Wenjie et al., 2008; Zhang et al., 2009; Khanh et al., 2011). PVA-gel has a suitable microporous structure in which microbes could be retained and an alternative biocarrier for the hydrolytic reactor of a two stage TAnMBR. Although, the PVA-gel has already been assessed for their performance with high rate anaerobic reactor, there is still a lack of understanding especially VFA production and its composition in hydrolytic reactor under thermophilic conditions. Therefore, by incorporating PVA-gel as biocarrier to hydrolytic reactor with the aim to ferment particulate wastewater to generate an overflow which was VFA rich effluent to the methanogenic reactor. Then, methane productivity from the system increased with an increase in VFA in the hydrolytic reactor's effluent.

Thus the overarching goal of this study was to investigate the effects of PVA-gel on VFA production and its composition, organic removal rate and methane production of two stage TAnMBR treating tapioca starch based synthetic wastewater. This was carried out to simulate high strength particulate wastewater (tapioca starch wastewater) discharge at high temperature. Furthermore, membrane fouling characteristics of two stage TAnMBR were also discussed.

1.2 Objectives of Study

The main objectives of this study are the following:

1.2.1 To investigate the effect of PVA-gel as biocarrier on total VFA concentration and methane production of two stage thermophilic anaerobic membrane bioreactor.
1.2.2 To study the performance at optimized two stage thermophilic anaerobic membrane bioreactor in different loading rates.
1.2.3 To investigate the fouling characteristics of two stage thermophilic anaerobic membrane bioreactor.

1.3 Hypothesis of Study

The hypothesis of this study is that the total VFA concentration and methane production efficiency could be increased by the addition of PVA-gel as biocarrier into hydrolytic reactor, under thermophilic condition. Moreover, membrane fouling should be minimized by operating membrane in an external semi dead-end configuration by combining cross-flow and dead-end configuration to single unit as two stage TAnMBR.

1.4 Scope of Study

This study was carried out with following considerations and limitations in order to achieve the above objectives:

1.3.1 Two stage TAnMBR was operated in external semi dead-end configuration with suction pressure.
1.3.2 The study was carried out in bench scale with tapioca starch as high strength particulate synthetic wastewater as influent.
1.3.3 The system was operated at thermophilic temperature of 55°C at three loading rates of 6, 8 and 12 kgCOD/m^3.d.
1.3.4 The system optimization was done at loading rate 6 kgCOD/m^3.d using PVA-gel as biocarrier in hydrolytic reactor.
1.3.5 The system performance was evaluated in terms of VFA production and methane productivity.
1.3.6 Fouling characteristics were studied at loading rates 8 and 12 kgCOD/m^3.d.

Chapter 2

Literature Review

2.1 Principle of Anaerobic Wastewater Treatment Process

Anaerobic process takes place through three metabolic pathways namely; hydrolysis, acidogenesis and methanogenesis to accomplish in sequence the complex ecological interactions of microorganisms (Lee et al., 2001). First, complex organic matters such as polysaccharides and proteins are hydrolyzed (hydrolysis), next the hydrolyzed products are degraded to volatile fatty acids (VFAs), hydrogen (H_2) and carbon dioxide (CO_2) (acidogenesis), after that methane (CH_4) is produced from acetic acid or H_2/CO_2 (methanogenesis).

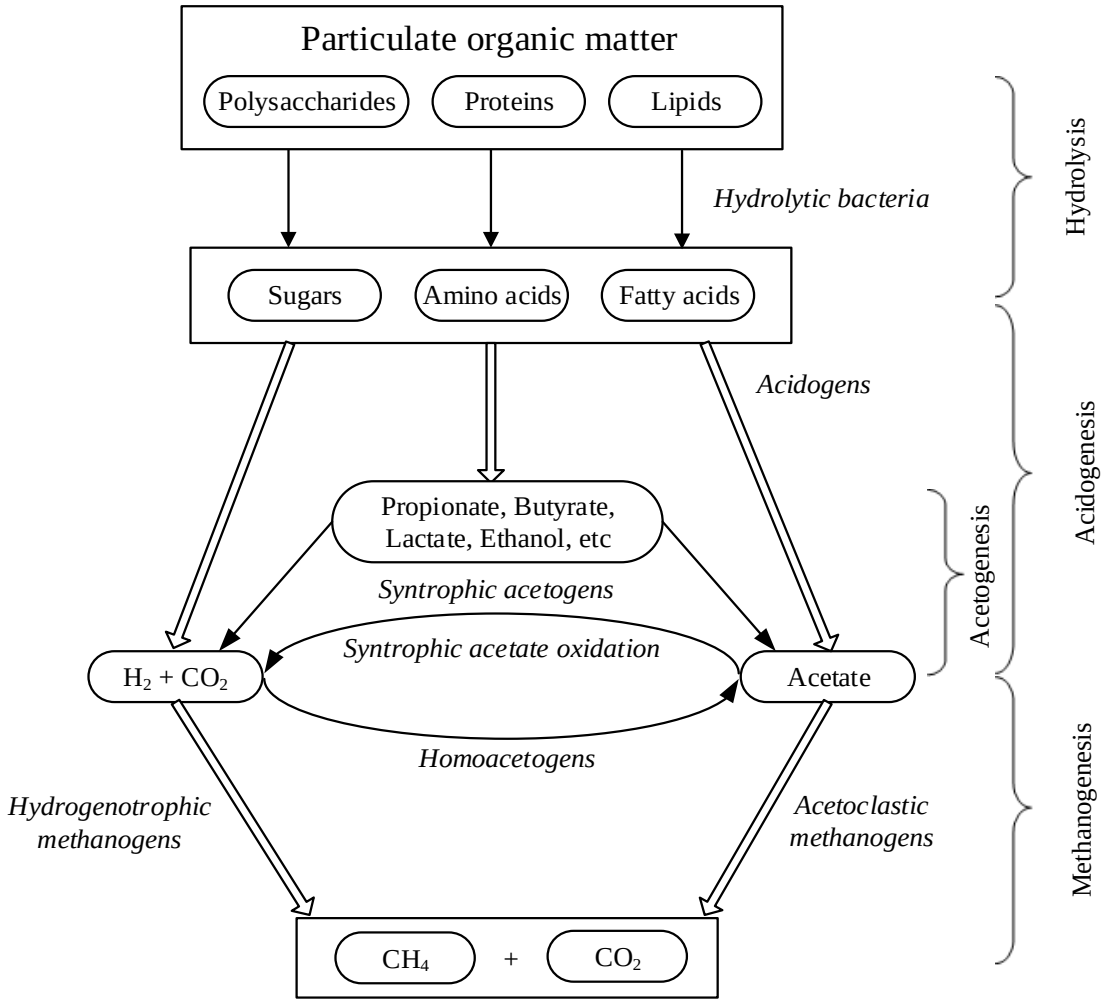


Figure 2.1 The metabolic pathway of anaerobic degradation

Acetic acid has been reported as a key intermediate metabolite during methanogenesis, and the utilization of acetic acid is identified as rate limiting step of overall anaerobic process (Sasaki et al., 2011). Yet, this is mainly for highly soluble wastewater while the rate limiting step in degradation of particulate wastewater is the hydrolysis of complex organic matters. Further degradation of acetic acid can follow two separate methanogenic pathways. One is direct methanogenesis by acetoclastic methanogens, where the carboxyl and methyl groups

in acetic acid convert to CH_4 and CO_2. Another is hydrogenotrophic methanogens, here acetic acid is first oxidized to CO_2 and H_2, and then the produced CO_2 is reduced to CH_4 using H_2 as an electron donor. The acetic acid decomposition pathway is affected by temperature, organic matters composition, reactor configuration and loading rate (Hattori, 2008). According to Khanal (2008) and Wang et al. (2013a), the acetoclastic pathway is the major metabolic process contributing up to 72-77% of methane production. The metabolic pathway in anaerobic degradation is illustrated in Figure 2.1.

2.2 Importance of Interspecies Hydrogen Transfer

The syntrophic relationships are usually associated with interspecies hydrogen transfer. It is an essential intermediate step in anaerobic degradation of organic matter to methane. Here the hydrogen produced by hydrolysis/acidogenesis is utilized by methanogenic archaea to form methane, which is known as interspecies hydrogen transfer.

As shown in Figure 2.2, butyrate and propionate are the two important intermediate products in the mineralization of organic matter. Then, these organic acids are utilized by syntrophic relationships between hydrogen producing acetogenic bacteria and hydrogen consuming methanogenic archaea. Complete conversion of butyrate and propionate requires three metabolic stages as presented in Section 2.1. At low hydrogen partial pressures, butyrate and propionate oxidation become thermodynamically favorable (negative Gibbs free energy) and there is a shift in fermentation to the reaction products such as acetate and methane. The oxidation of butyrate and propionate to acetate becomes thermodynamically favorable at hydrogen partial pressure below 101.3 Pa (10^{-3} atm) and 10.13 Pa (10^{-4} atm), respectively (Khanal, 2008). McCarty and Smith (1986) also reported that the conversion of propionate to acetate requires hydrogen partial pressure between 0.1 to 10.13 Pa (10^{-6} to 10^{-4} atm).

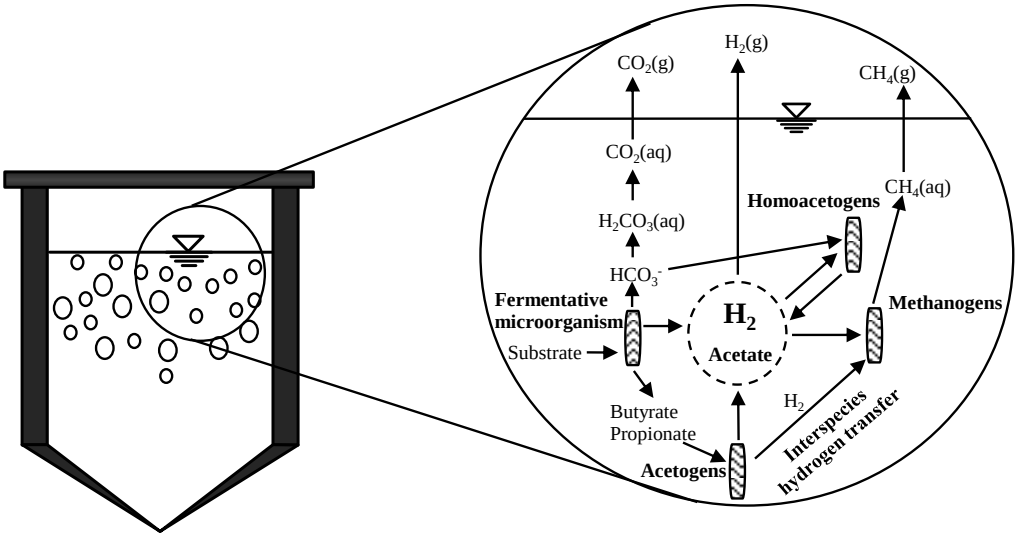


Figure 2.2 Schematic diagram of interspecies hydrogen transfer (Modified from Clark et al. (2012))

Methanogenic archaea and homoacetogens are the main hydrogen consumers. At low hydrogen partial pressures, hydrogen forming reactions become thermodynamically favorable and there is a shift in fermentation toward the production of acetate and away from butyrate and ethanol. However, homoacetogens become increasingly important for removing hydrogen especially when methanogenic archaea is inhibited. An Increased in

acetic acid in the first stage reactors enhance acetoclastic methanogenesis in second stage reactors. Moreover, low and high hydrogen partial pressure maintained by hydrogenotrophic methanogens (< 2 Pa) and homoacetogens (< 200 Pa) are a key requirement for thermodynamic feasibility of reaction products (Kotsyurbenko et al., 2001; Paulo et al., 2003). Hence, high H_2 partial pressure will be favored with the formation of VFA having more than two carbon atoms (butyric acid and propionic acid) while low H_2 partial pressure, acetic acid will predominate. It is also related with the inhibition of propionic acid because it has the lowest hydrogen partial pressure requirement. It is apparent that the propionic acid oxidation to acetate is the slowest process among VFA. Furthermore, propionic acid is considered as the most toxic among all the VFA species. Methanogenic archaea have been reported to be inhibited at propionic acid concentration of 1 g/L, on the other hand the same archaea have been reported to tolerate acetate and butyrate up to 10 g/L (Inanc et al., 1999).

In addition at elevated temperature of 55°C, the thermodynamics for acetogenic conversions are more favorable as shown in Table 2.1 (less Gibbs free energy is required when elevated temperature). Nevertheless, Speece et al. (2006) demonstrated that propionic acid accumulation is more critical at thermophilic conditions as it is the slowest to oxidize to acetate which compared with other intermediate products such as butyric acid (free energy of +62.3 kJ/mol is needed for propionate oxidation to acetate, whereas for butyrate oxidation to acetate only +37.9 kJ/mol is required). Hence a rapid utilization of hydrogen produced during hydrolysis/acidogenesis should be a greater concern at elevated temperature. In this regard, high rate anaerobic reactor such as upflow anaerobic sludge blanket reactor (UASB) or anaerobic membrane bioreactor (AnMBR) could maintain low hydrogen partial pressure as it can provide the opportunity for close proximity of anaerobic microorganisms (McCarty and Smith, 1986; Khanal, 2008).

Table 2.1 Reactions Involved in VFA Oxidation with Free Energy at 25°C and 55°C

Reaction	$\Delta G^{o\prime}$ (kJ/mol)	
	25°C	**55°C**
Butyrate to Acetate		
$CH_3CH_2CH_2COO^- + 2H_2O \longrightarrow 2CH_3COO^- + H^+ + 2H_2$	+48.1	+37.9
$CH_3CH_2CH_2COO^- + 2HCO_3^- \longrightarrow 2CH_3COO^- + H^+ + 2HCOO^-$	+45.5	+36.1
Propionate to Acetate		
$CH_3CH_2COO^- + 3H_2O \longrightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1	+62.3
$CH_3CH_2COO^- + 2HCO_3^- \longrightarrow CH_3COO^- + H^+ + 3HCOO^-$	+72.2	+59.7
Acetate to Methane		
$CH_3COO^- + H_2O \longrightarrow HCO_3^- + CH_4$	-31.0	-34.7
Hydrogen to Methane		
$HCO_3^- + H^+ + 4H_2 \longrightarrow CH_4 + 3H_2O$	-135.6	-122.5
Formate to Methane		
$4HCOO^- + H^+ + H_2O \longrightarrow CH_4 + 3HCO^-$	-130.4	-118.9

Source: Schmidt and Ahring (1993); Van Lier et al. (1993)

2.3 Reactor Design for Anaerobic Wastewater Treatment

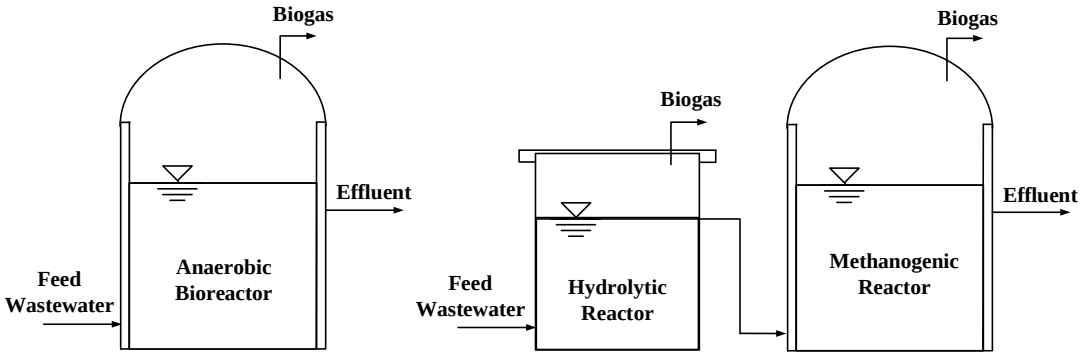
2.3.1 Single stage and two stage anaerobic reactor

Anaerobic wastewater treatment can be carried out in two type of reactor configurations such as single stage and two stage reactor. Single stage reactor comprises of one basic reactor

where all metabolic pathways of anaerobic degradation take place. The production of VFA from anaerobic degradation of organic matters may lead to accumulation of VFA resulting in pH drop and subsequent inhibition of the methanogenesis process. Furthermore, acidogenic bacteria have the highest growth rates among microbial consortia and are more tolerant to environmental perturbations and stress conditions than methanogenic archaea. To overcome this situation, several studies have focused on two stage configuration as it allowed a separate optimization of both reactors which leads to higher process stability. Since acidogenic bacteria and methanogenic archaea are kept in two separate reactors, it can maintain optimum conditions for each group of microorganisms involved in each stage of anaerobic reactor (Guerrero et al., 1999; Kim et al., 2002; Ke et al., 2005; Wijekoon et al., 2011; Mota et al., 2013). As a result, higher loading potentials and higher organic removal rates could be achieved in two stage reactor configuration (De Gioannis et al., 2008). The two reactor configurations are illustrated in Figure 2.3, and the advantage of two stage anaerobic reactor is presented in Table 2.2.

Table 2.2 The Advantage and Disadvantage of Two Stage Anaerobic Reactor

Advantages	**References**
Higher methanogenic activity	Alexiou et al. (1994); Yeoh (1997); Guerrero et al. (1999)
Higher methane content in biogas	Lun et al. (1995); Yeoh (1997)
Reduction of the inhibitory effects of toxic substances on methanogenic archaea	Beccari et al. (1996)
Improvement in treatment efficiency and process stability as well as reduced risk of digester overloading	Ince (1998); Yilmazer and Yenigün (1999); Alkaya and Demirer (2011); Cui et al. (2011); Mota et al. (2013)
Higher suspended solids removal efficiency	Guerrero et al. (1999)
Smaller reactor volumes	Guerrero et al. (1999); Alkaya and Demirer (2011)
Larger organic degradation rates and biogas yield	Blonskaja et al. (2003); Liu et al. (2006); Cui et al. (2011)
Decreasing hydrogen sulfide toxicity because sulfur compounds can be removed in the first stage	Khanal and Huang (2003); Deublein and Steinhauser (2011)
Reduction in propionic acid accumulation	Speece et al. (2006); Mota et al. (2013)
Allows selection and enrichment of different bacteria in each stage by independently controlling reactor operating conditions	Alkaya and Demirer (2011); Cui et al. (2011); Mota et al. (2013)
Capable of handling greater loading rates	Alkaya and Demirer (2011); Mota et al. (2013)
Disadvantages	**References**
Higher investment and operating cost	Lettinga and Hulshoff Pol (1991)
More complex system	



(a) Single Stage (b) Two Stage

Figure 2.3 Single stage and two stage anaerobic reactor configurations

2.3.2 Mesophilic and thermophilic

The important factor affecting microbial activity is operating temperature. It can be operated in either mesophilic (preferably 35°C) or thermophilic condition (preferably 55°C). Operating the reactor under thermophilic condition has many benefits, for example, higher organic matters removal due to higher growth rate and degradation efficiency (Zbransk et al., 2000). Thus, a shorter hydraulic retention time (HRT) is required. It also provides a more complete pathogenic destruction, lower sludge yield and high amount of methane production. Borja et al. (1995) and Khanal (2008) have reported that the methane production rate in thermophilic condition is 25-50% higher than mesophilic condition. Furthermore, elevated temperatures have a positive effect on solubilization and/or hydrolysis/acidogenesis process (Bouallagui et al., 2004; Komemoto et al., 2009; Lv et al., 2010). This will increase SCOD, VFA and biogas production. Since the solubilization/hydrolysis of particulate organic compounds to soluble substances is a rate limiting step in anaerobic degradation, it is crucial to study the effect of PVA-gel as biocarrier on total VFA concentration of hydrolytic reactor. Nevertheless, thermophilic treatment has some drawbacks such as less stability and poor sludge granulation, restraining the application of thermophilic condition in industrial wastewater treatment processes. Therefore, using membrane technology for thermophilic wastewater treatment is attractive approach to retain biomass within the system.

2.4 Application of PVA-gel as Biocarrier for Wastewater Treatment

Immobilization of bacteria has received increasing interest in wastewater treatment. It offers a promising approach for increasing the process efficiency. Comparing with free cells, immobilization of microorganisms have several advantages such as it increase biodegradation rate and the system can be operated more easily (Zhang et al., 2007a). Either synthetic polymers (polyvinyl alcohol, polyether) or natural biopolymers (polysaccharides such as alginate or protein such as gelatin) can be used to enhance granulation or immobilization. Recently, polyvinyl alcohol hydrogel (PVA-gel) which is low cost polymeric gel and nontoxic synthetic polymer has been wildly used for immobilization of microorganisms (Zhang et al., 2007b; Zhang et al., 2009). With attractive properties such as hydrophilic surface and resistance to oxidation, PVA-gel is a potential biocarrier which can be applied in food industry, fermentation industry etc. Table 2.3 illustrated the recent bench

scale work on application of PVA-gel as biocarrier. In addition, Zhang et al. (2008) achieved a high loading rate (22.5 kgCOD/m^3.d) treating high strength particulate wastewater (corn steep liquor) obtaining about 87% of COD removal at HRT of only 12 h. Furthermore, Zhang et al. (2009) reported 88-90% of COD removal for loading rate 5.4-27.5 kgCOD/m^3.d. As shown in Table 2.3, there is limited studies done on PVA-gel application by high rate anaerobic reactor and all of the studies operated under mesophilic condition. Therefore, there is a growing need to study the effect of PVA-gel on thermophilic anaerobic process.

Table 2.3 Application of PVA-gel as Biocarrier in Anaerobic Wastewater Treatment

Parameters	**Zhang et al. (2008)**	**Zhang (2008)**	**Zhang et al. (2009)**	**Khanh et al. (2011)**		**Zhang et al. (2011)**	**Chaikasem et al. (2014a)**	**Chaikasem et al. (2014b)**
Wastewater	Corn steep liquor	Ethylene glycol	Corn steep liquor	Synthetic (Bonito extract)		Ethylene glycol	Synthetic (Tapioca starch)	Synthetic (Tapioca starch)
T (°C)	35	35	35	15 25 35		35	55	55
Volume (L)	12.5	12.5	12.5	3.9	3.9	12.5	9	9
Reactor type	UASB	UASB	UASB	AFB	UASB	UASB	Two stage TAnMBR	Two stage TAnMBR
OLR (kgCOD/m^3.d)	0.4-22.5	1.0-11	-	5.4-27.54	6.4-35.5 6.3-37 5.2-47	1-11.2	8	6 8
ORR (kgCOD/m^3.d)	20.5 (91.1%)	10.7 (97.3%)	-	4.86-24.23 (88-90%)	12 16 25	0.74-9.9 (74-90%)	6.4 (80%)	5.52-7.2 (90-92%)
HRT (h)	10-48	8.0-14.4	-	6-10	0.28-1.56 0.28-1.56 0.22-2.00	8, 14.4	48 (16 for hydrolytic reactor)	58.37 (19.45 for hydrolytic reactor)
Packing ratio (%)	8	12	-	20	20 20 20	12	15	30
Activity (kgCOD/m^3 PVA-gel.d)	154	78	60 (No bacteria growth inside PVA-gel)	130 (Have bacteria growth inside PVA-gel)	60 81 119	30.6 (L CH_4/L PVA-gel.d)	35 (gVFA/L.PVA-gel.d)	18.9 20.3 (g VFA/L.PVA-gel.d)
Settling velocity (m/h)	200	322	200	281	201 199 194	322	-	228

2.5 Factors Affecting Anaerobic Wastewater Treatment

Anaerobic biological activity is suppressed by numerous factors affecting the conversion of organic matters in anaerobic wastewater treatment process. The operating parameters of the system should be controlled to enhance the anaerobic biological activity and improve the anaerobic degradation within the system. The control parameters are discussed in following section.

2.5.1 Temperature

Temperature is the most critical parameter on anaerobic wastewater treatment process, as the degradation rate and anaerobic microbial activity strongly depends on it. Yet, anaerobic microbial activity doubles in every 10°C increment in temperature within the optimum temperature range. There are two temperature ranges which provide the optimum condition for methane generation such as mesophilic (30-35°C) and thermophilic range (50-65°C). It has been observed that higher operating temperature decrease the required retention time due to rapid growth rate in microbial consortia. However, thermophilic microorganisms are more sensitive than mesophilic microorganisms. There is recommended that the temperature variation in anaerobic reactors should not exceed 0.6-1.2°C per day as methanogenic archaea are very sensitive to environmental changes (Visvanathan and Abeynayaka, 2012). However, anaerobic microbial activity can recover immediately once the operating temperature returns to the optimum value (Khanal, 2008).

2.5.2 pH and alkalinity

Even though, VFA and pH are related to each other but their relation depends on characteristic of substrate that may differ from type of substrate and environmental conditions of anaerobic wastewater treatment process. It has been determined that the optimum pH range for acidogenic bacteria is 5.5-7.2, while the optimum pH value for methanogenic archaea is 6.6-7.6 (Visvanathan and Abeynayaka, 2012). The decreasing in pH is often caused by VFA accumulation and excessive production of carbon dioxide due to reactor overload. Anaerobic treatment processes require sufficient buffering capacity (alkalinity) to mitigate pH variations. Typically, pH of anaerobic system is fundamentally maintained by natural alkalinity or self-producing alkalinity. Excessive VFA production can suppress the methanogenic archaea. Reduction in pH can be controlled by the addition of sodium bicarbonate, sodium hydroxide or lime. Sodium bicarbonate is preferred due to its long lasting impact, low toxicity and less pH fluctuations while lime can cause scaling problem in the reactor with $CaCO_3$ precipitation. The alkalinity should maintain within the range of 1-5 g/L as $CaCO_3$ (Metcalf and Eddy, 2003). The proper pH control is important factor in anaerobic system as it directly affects enzyme activity in microbial consortia. Nevertheless, De Gioannis et al. (2008) reported that anaerobic microbial consortia are capable to adapt to adverse condition when adequate time is given.

2.5.3 Nutrient

Anaerobic microorganisms require both macronutrients (nitrogen and phosphorus) and micronutrient (trace elements) to support the new biomass synthesis. Typically, industrial wastewater may lack sufficient nutrients. Addition of nitrogen and phosphorus are required to maintain C:N:P at sufficient ratios. The suitable C:N:P ratio of about 100:5:1 and 100:1.8:0.28 have reported for anaerobic microorganisms (Gonzalez et al., 1998; Yilmaz et al., 2008). Besides nitrogen and phosphorus, other trace elements are essential at low concentration stimulating the activity of anaerobic microorganisms. However, the trace elements can have toxic effects at higher concentration. The minimum requirement of heavy metals as trace element and toxic concentration are presented in Table 2.4.

Table 2.4 Minimum Requirement of Trace Elements and Toxic Concentration

Substances	Minimum requirement of trace element (mg/L)	Inhibition concentration start (mg/L)		Toxicity (mg/L)
		Free ions	As carbonate	
Cr	0.005-50	28-300	530	3 (Cr^{+3}), 500 (Cr^{+6})
Fe	1-10	-	1,750	-
Ni	0.005-0.5	10-300	-	30-1,000
Cu	-	40-300	170	170-300
Mg	-	1,000-2,400	-	3,000
Zn	-	400	160	250-600
Cd	-	70-600	180	20-600
Pb	0.02-200	9-340	-	340
Co	0.003-0.06	-	-	-
Mo	0.005-0.05	-	-	-
Mn	0.005-50	1,500	-	-
Na	-	3,500-30,000	-	60,000
K	-	2,500-5,000	-	12,000
Ca	-	2,500-7,000	-	8,000

Source: Polprasert (2007); Chen et al. (2008); Deublein and Steinhauser (2011)

2.5.4 Redox potential

The redox potential (ORP) should be maintained in the range of -200 to -350 mV at neutral pH. Khanal (2008) reported that the ORP value is an important parameter for methanogenic archaea. It is well established that methanogenic archaea require ORP as low as -400 mV.

2.5.5 Volatile fatty acids distribution

VFA distribution is an important parameter which affects anaerobic process. It is an important intermediate products in metabolic pathway of methane formation. The intermediate compounds present during anaerobic degradation of organic matter are mostly acetic acid, butyric acid, propionic acid and valeric acid. Amongst these, acetic acid and butyric acid are the predominant VFA and their concentration provide a useful measure of reactor performance. Hu and Yu (2006) reported that acetic acid concentration is increased slightly with increasing pH, while butyric acid concentration is increased with decreasing pH. However, propionic acid concentration was observed unrelated to pH. Individual VFA also indicate the metabolic stage of hydrogen producing acetogens and acetoclastic methanogens, which are the most delicate microorganism groups (Buyukkamaci and Filibeli, 2004). Therefore, VFA species can be considered as control parameters in anaerobic reactor.

2.5.6 Toxic materials

The microbial activity can be inhibited by anaerobic inhibitors present in wastewater or by products from metabolic activities of anaerobic microorganisms. Furthermore, anaerobic inhibitors is largely depends on wastewater characteristics. A description of anaerobic inhibitors observed in different type of wastewater are listed in Table 2.5. Ammonia, heavy metals, phenol and halogenated compounds are the examples for toxic materials of anaerobic microorganisms. Typically, the toxicity levels of each toxic materials have reported differently in different researches. This could be due to the difference in reactor

configurations and seed sludge acclimatization. Table 2.6 illustrates the inhibiting concentrations of organic and inorganic compounds to anaerobic microorganisms.

Table 2.5 Details on Anaerobic Inhibitors in Different Type of Wastewaters

Wastewater	Anaerobic inhibitors	Control/Preventive measures
Seafood industry Vegetable oil	High salinity (Na^+, Cl^-, SO_4^{-2})	Anaerobic codigestion to improve C:N ratio and dilute inhibitory compounds
Dairy processing	High salinity (Na^+, Cl^-, SO_4^{-2}) High ammonia Long chain fatty acids (LCFA)	
Meat processing	High ammonia Long chain fatty acids (LCFA) Biocides and detergents	
Pulp and paper industry	Sulfides, tannins and halogenated compounds	Sulfide remove by stripping, coagulation, oxidation and precipitation
Petrochemical industry	Aromatic hydrocarbons Aliphatic compounds (alkanes)	Long term acclimatization

Source: Chen et al. (2008)

Table 2.6 Inhibiting Concentrations of Organic and Inorganic Compounds to Anaerobic Microorganisms

Parameter	Inhibiting concentration (mg/L)
Individual VFA	> 10,000 (acetic acid and butyric acid) > 1,000 (propionic acid)
Total ammonia nitrogen	1,500-3,000 (at pH > 7.6)
Free ammonia	600-800
Sulfide	> 100 (as soluble sulfide)
Sulfide	250 (as H_2S at pH 6.5-7.2) 90 (as H_2S at pH 7.8-8.0)
Calcium (Ca^{+2})	2,500-4,500 8,000 (strongly inhibitory)
Magnesium (Mg^{+2})	1,000-1,500 3,000 (strongly inhibitory)
Potassium (K^+)	2,500-4,500 12,000 (strongly inhibitory)
Sodium (Na^+)	3,500-5,500 8,000 (strongly inhibitory)
Heavy metals Copper (Cu) Cadmium (Cd) Iron (Fe) Chromium (Cr^{+3}) Chromium (Cr^{+6})	 170-300 20-600 1,750 3 500

Source: Inanc et al. (1999); Polprasert (2007); Chen et al. (2008); Deublein and Steinhauser (2011)

2.6 Consideration for Thermophilic High Rate Anaerobic Wastewater Treatment

Anaerobic wastewater treatment can be operated at low rate anaerobic reactors or high rate anaerobic reactors. High rate anaerobic reactor can be further classified as suspended growth, attached growth and others. High rate anaerobic processes have been proven to perform well in treating soluble organic wastewater, especially industrial wastewater containing carbohydrate. High rate anaerobic reactors (UASB) provide straightforward methane collection facilities as compared with conventional anaerobic treatment (anaerobic pond). Nevertheless, their applications in complex wastewater, for example, particulate wastewater is limited. Most of high strength wastewater contains large amount of particulate compounds such as wastewater from palm oil mill, starch industry, slaughterhouses, vermicelli industry and tanneries. These complex wastewater is complicated to biodegrade since hydrolysis of particulate matters poses certain degree of difficult. Furthermore, degradation kinetics of particulate compounds is slow and the growth of granular sludge is also slow. In addition, the settling velocity of anaerobic sludge is very poor since their diffusible and filamentous in nature (Choo and Lee, 1996a). This causes poor performances of high rate anaerobic reactors treating particulate wastewater. Kayhanian (1994) reported that anaerobic microorganisms (especially acidogenic bacteria) are easily washed out from the system due to poor granule formation. Mota et al. (2013) also stated that the acidogenic bacteria grow mostly as individual cells rather than bioflocs. However, high rate anaerobic reactors are widely used in industrial sector. They have been in operation successfully in terms of organic removal rate and methane production rate, but the quality of effluent is still not satisfying in terms of solid removal. The selected experimental results of high rate anaerobic reactor performance is illustrated in Table 2.7. High rate anaerobic reactor classification is shown in Table 2.8.

For particulate wastewater biodegradation, higher retention time is required. The effect of retention time in anaerobic wastewater treatment are discussed in detailed by Zinatizadeh et al. (2006), where the decreasing in retention time leads to VFA accumulation in the system and reducing organic removal rate and methane production. This effect has increased at high loading rate and high influent COD concentration. Furthermore, this observation has elucidated the unbalance between VFA production and methane formation in anaerobic reactor operates in higher loading rate at low retention time.

Yeoh (1997) presented that more methane production was possible in a two stage reactor as posed to a single stage reactor for treating sugar cane molasses. Furthermore, high rate anaerobic reactors operated at thermophilic condition have given more methane production than mesophilic condition (Bouallagui et al., 2004; Parawira et al., 2007; Ramakrishnan and Surampalli, 2013; Jeong et al., 2014). In addition, thermophilic reactor exhibits other advantages, for example, higher metabolic rates, effective pathogenic microorganisms removal and elimination of cooling requirements when wastewater is discharged at high temperature. The elevated temperature has particularly positive affected on hydrolysis process. Bouallagui et al. (2004) reported that thermophilic hydrolysis rate of cellulose is higher than mesophilic hydrolysis rate around 5-6 times. Considering these advantage of two stage anaerobic reactor under thermophilic condition, further research should be considered towards study two stage thermophilic anaerobic reactor for treating particulate wastewater.

Table 2.7 Selected Research on High Rate Anaerobic Reactors and Their Performances

Wastewater	Reactor type	T (°C)	Volume (L)	HRT (d)	OLR ($kgCOD/m^3.d$)	Efficiency (%)	Methane yield ($m^3CH_4/kgCOD_r$)	References
Sugar cane molasses	Single stage CSTR	55	5	9-36	3.45-14.5	-	0.055	Yeoh (1997)
	Two stage CSTR	55	3.6, 5	5.6-32.7	4.65-20.02	65	0.168	
Fruit and vegetable wastes	Two phase ASBR (Acidogenic reactor)	35	-	3 h	3.7	-	0.32	Bouallagui et al. (2004)
	Two phase ASBR (Methanogenic reactor)	55	-	3 h			0.45	
Winery wastewater	Multi-fed UAF	19-27	3	8 h	Up to 37.68	> 82	0.3-0.35	Yu et al. (2006)
Potato processing	Two stage USAB	35	-	-	11	-	0.41	Parawira et al. (2007)
		55			36		0.49	
Paper mill wastewater	Anaerobic filter	35	1.77	0.25-1	1.08-11.38	-	0.205-0.295	Yilmaz et al. (2008)
		55			1.07-12.25		0.188-0.317	
Synthetic domestic wastewater (high fraction of particulate matter)	Two phase anaerobic sequencing bath reactor (ASBR)	35	5 (Overall)	-	0.63	69 (TCOD)	-	Donoso-Bravo et al. (2009)
					1.22	50 (TCOD)		
Molasses based synthetic wastewater	Multistage anaerobic biofilm reactor	35	54	8, 16, 24 h	3, 4, 6.75, 9	88.3-91.6	-	Ghaniyari-Benis et al. (2009)
Winery wastewater	UAFB reactor	35	10	0.476	Up to 42	80	-	Ganesh et al. (2010)
Slaughterhouse wastewater	Hybrid UASB with pleated PVC rings	29-35	5.4	8-36 h	Up to 19	80-92	1.1-5.2 $m^3/m^3.d$ 0.19-0.32	Rajakumar et al. (2012)
Palm oil mill effluent	Combined high rate anaerobic reactors	36	20, 10	0.7-2.4	0.91-23	88-95.6	0.171-0.269	Choi et al. (2013)
Coal wastewater	Anaerobic hybrid reactor (AHR)	35	13.5	0.5-3.0	1.13-8.22	88	0.325	Ramakrishnan and Surampalli (2013)
		55		0.6-3.12	1.13-8.22	92	0.340	
Palm oil mill effluent	Anaerobic hybrid reactor and anaerobic baffled filter	37	3, 1.5	5.8, 6.2	2-15	90-93	13.5 L/d	Jeong et al. (2014)
		55			2-15	93-95	20 L/d	
	UASB reactor	55	5	1.5	4.28-27.65	> 88	7.352-36.76 L/d	Poh and Chong (2014)

Table 2.8 Anaerobic Reactors Classification

Low rate anaerobic reactors	High rate anaerobic reactors		
	Suspended growth	**Attached growth**	**Others**
• Anaerobic pond, • Septic tank	• Upflow anaerobic sludge blanket (UASB), • Anaerobic sequencing batch reactor (ASBR) • Continuous stirring tank reactor (CSTR)	• Anaerobic filter (AF) • Fluidized/Expended bed reactor	• Anaerobic membrane bioreactor (AnMBR)

2.6.1 Biological aspects of thermophilic anaerobic reactor

Thermophilic microorganisms can be divided into 2 groups, thermophiles (temperature above 45°C) and hyperthermophile (temperature above 80°C). Metcalf and Eddy (2003) mentioned that the optimum temperature range of thermophiles is 55-65°C. Thermophilic microorganisms are also found in naturally such as thermal springs, tropical soils and compost heaps. Thermophilic bacteria comprised of eukaryotes and prokaryotes. Both eukaryotes (protozoa, algae and fungi) and prokaryotes (bacteria and archaea), thrive in ambient temperature and have a potential to adapt and survive in thermophilic conditions. Nevertheless, there are upper limits to the temperatures at which biological reactions occur. Prokaryotic microorganisms are better at adapting to higher temperature than eukaryotes. Madigan et al. (2003) presented that the growths of eukaryotes limits around 60°C. While for prokaryotes, it is 70°C (for bacteria) and 113°C (for archaea).

2.6.2 Thermophilic anaerobic biological activity

Even though thermophilic anaerobic microorganisms are more sensitive to environmental perturbation and extreme condition than mesophilic anaerobic microorganisms but the biological activity tend to become higher. Furthermore, thermophilic condition decreases require retention time and overall volume of system. Additionally, biological reaction rates in thermophilic condition are much faster than mesophilic condition. Thermophilic conditions also has more higher loading capacity compared to mesophilic condition since the growth rate and biodegradation rate could be enhanced by elevated temperature. Nielsen et al. (2004) elucidated that the thermophilic hydrolytic/acidogenic bacteria and hydrogen consuming methanogens are in the range of 55°C to 75°C and 55°C to 70°C, respectively. Furthermore, Ahring (1994) reported that conversion of acetate, butyrate and propionate to methane had an optimum temperature range at 55°C to 60°C. Thermophilic condition is also a key factor for pathogenic inactivation. Smith et al. (2005) stated that the pathogens can be inactivated within 20-60 minutes at 55°C depending on types of bacteria. Therefore, making operations attractive at thermophilic temperatures (~55°C).

2.6.3 Thermophilic anaerobic sludge settleability

In anaerobic wastewater treatments (suspended growth), both system performance and quality of effluent depend on sludge settleability. Typically, the settling of anaerobic sludge should increase at elevated temperatures due to lower liquid viscosity. However, thermophilic anaerobic processes suffer from poor anaerobic sludge settleability due to their diffusible and filamentous behavior (Choo and Lee, 1996a). Furthermore, more biogas production at higher temperature has negatively affected on anaerobic sludge settleability. This phenomenon can overcome by sludge granulation and biofilm formation. Nevertheless,

it has adversely affected by elevated temperature due to high degree of sludge mineralization. Mineralization negatively effects extracellular polymeric substance (EPS) generation which restricts the formation of dense and firm the granulation of sludge. This results in low or no sludge granulation or even sludge degranulation when mesophilic anaerobic seed sludge are used as inoculum. Furthermore, the extreme conditions such as high salinity and high temperature obstruct the UASB reactor since the granule formation does not perform well due to dispersed sludge formation or sludge degranulation. This is an important factor to design the system since industrial wastewater may contain high organic concentration, high salinity, high temperature and high particulate compounds, for instances, fish and seafood processing, tannery industries, chemical industries and vermicelli processing. In this regard, application of membrane technology integrated with high rate anaerobic would provide important solution for anaerobic sludge degranulation and dispersed sludge formation induced by extreme conditions.

2.7 Membrane Filtration Technology

2.7.1 Introduction to membrane processes

Membrane filtration technique involves the separation of both particulate and dissolved organic matter from liquid. The role of the membrane is to serve as a selective barrier which allow the passage of certain constituents, and while retaining others. The influent to the membrane is known as feed. While liquid pass through the membrane is known as permeate and liquid containing retained constituents is known as concentrate or retentate. The rate at which the permeate flow through the membrane is known as permeate flux. Its apparent benefits over other wastewater treatments include continuous separation, solid free effluent, easy combination with other existing techniques and low chemical costs. Membrane technology is being currently applied in various industrial sectors such as food and beverage, pulp and paper, metallurgy, textiles, pharmaceutical, automotive, dairy, chemical industry, power plant, water treatment, wastewater treatment and chemical industry.

Membrane can be produced from a variety of materials. The major of the materials are organic or inorganic membrane. The inorganic membranes can be distinguished into four groups such as ceramic, metallic, glass and zeolite membranes. Their advantages of these inorganic membranes are high mechanical, chemical, thermal stability and long lifetime. Nevertheless, they are fragile and expensive. Organic membranes (cellulose and synthetic polymer), are also widely used in wastewater treatments since they are more flexible and can install in compact modules. As membrane used for the separation of solid from liquid, membrane processes can be classified into four types according to membrane pore size (rejected particle size) such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis. Microfiltration membrane is widely applied for wastewater treatment. The typical range for the pore size and operating pressure vary widely between 100-1,000 nm and 0.1-4 bar, respectively. Typically, it is mainly used to separate suspended solids and colloidal particles by sieving mechanism. The membrane processes with respect to membrane pore size and operating pressure is illustrated in Figure 2.4.

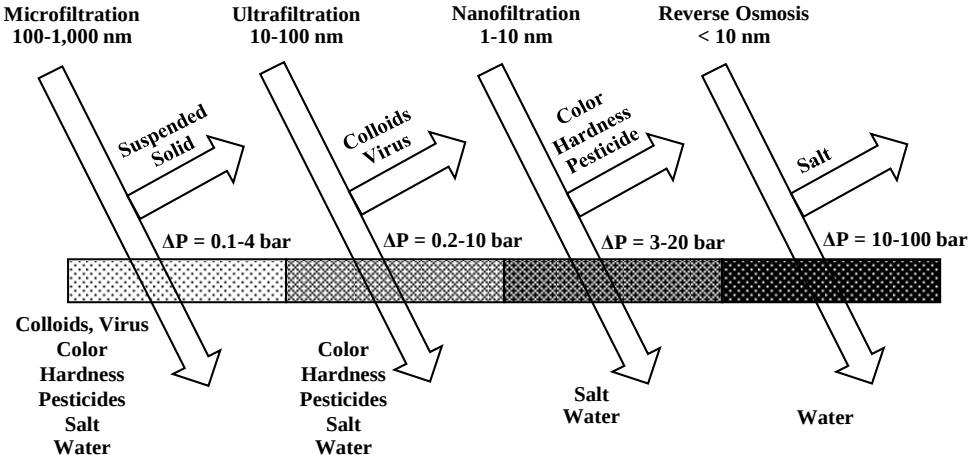


Figure 2.4 Membrane processes

2.7.2 Membrane operational configurations

Typically, membrane operational configuration can be classified into two group such as dead-end and cross-flow filtration. The schematic diagrams of two membrane operational configurations are illustrated in Figure 2.5. In dead-end configuration, feed is pumped perpendicular through membrane surface, rejected particle are gradually accumulated on membrane surfaces. While cross-flow operation feed is pumped tangential to membrane surface and rejected particles can be recirculated back to the system again. Cross-flow with its higher flow velocity, less membrane fouling occur due to less cake layer formation. Therefore, less membrane fouling in cross-flow filtration as well as smaller permeate flux decline (and high permeate flux) could obtain than in dead-end filtration. However, dead-end configuration has lower pumping requirement as well as less effect on biological activity.

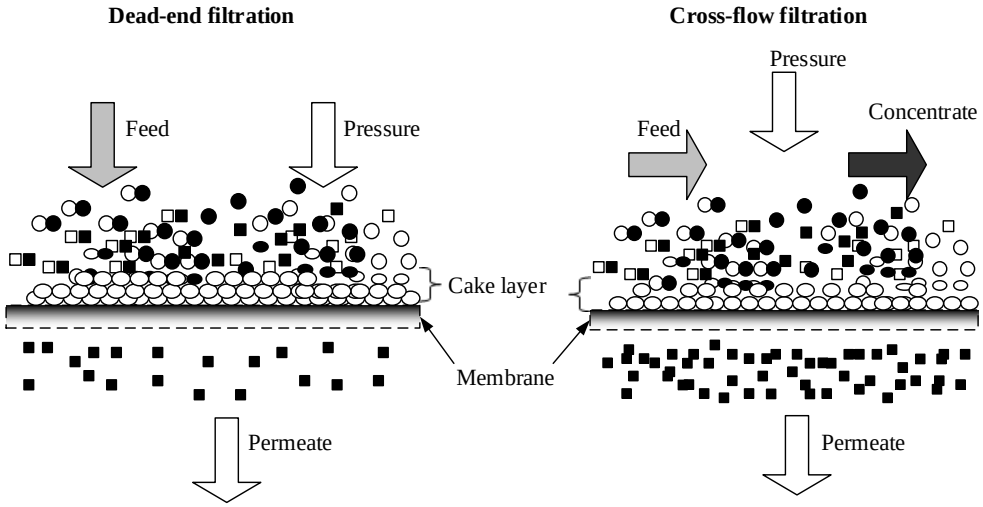


Figure 2.5 Membrane operational configurations

2.7.3 Membrane operational parameters

Trans-membrane pressure, permeate flux and filtration resistance are important parameters in membrane operation. The relationship between these operational parameters is given in the following equation.

$$J = \frac{TMP}{\mu R_t} \qquad \text{Equation 2.1}$$

$$R_t = R_m + R_c + R_f \qquad \text{Equation 2.2}$$

Where:

J	=	Permeate flux ($L/m^2.h$)
TMP	=	Trans-membrane pressure (kPa)
μ	=	Permeate viscosity (Pa.s)
R_t	=	Total membrane resistance (1/m)
R_m	=	Intrinsic membrane resistance (1/m)
R_c	=	Cake resistance (1/m)
R_f	=	Membrane resistance caused by adsorption of solute (1/m)

Filtration resistance calculations are carried out by data using a series of membrane filtration tests for pure water filtration, filtration of sludge and pure water filtration in between every step of membrane cleaning.

2.8 Application of Membrane Bioreactor in Anaerobic Wastewater Treatment

The anaerobic membrane bioreactor (AnMBR) concept was firstly reported by Grethlein (1978) for treating septic tank effluent by external cross-flow membrane configuration. Higher biomass concentration was achieved with 85-95% of biochemical oxygen demand (BOD) removal efficiency, 72% nitrate removal and 24-85% orthophosphate reduction. The development of commercially-available AnMBR was developed in early 1980s and was known as the "Membrane Anaerobic Reactor System (MARS)". MARS system has been tested in pilot scale which consisted of complete mixed suspended growth anaerobic reactor and external cross-flow membrane configuration. In 1987, a system known as "Anaerobic Digestion Ultrafiltration (ADUF)" was developed in South Africa for industrial wastewater treatment, which effectively retention sludge in the system. A significant research development was initiated by the project "Aqua Renaissance' 90 of japan government for sewage treatment and industrial wastewater. This project led to development of various membrane configuration of AnMBR system, mostly done on external cross-flow configuration (Kimura, 1991; Minami et al., 1991; Minami, 1994). By the 1990s, research studies of AnMBR increased and focused on characterization of membrane foulants, development of membrane cleaning and fouling control, filtration characteristics and system performance.

The success of submerged aerobic membrane bioreactor in 1989 was introduced by Prof. Yamamoto and his coworkers at Asian Institute of Technology (AIT), Thailand. It has highly stimulated the development of submerged AnMBR for wastewater treatment. In the last decade, the full scale application on submerged AnMBR was developed by Kubota Corporation known as "Kubota Submerged Anaerobic Membrane Bioreactor Process (KSAMBR)", which has been successfully applied in full scale food and beverages industries (Kanai et al., 2010). Later in the 2010s, the significant research in submerged

AnMBR effort to improve energy efficiency, extend the scope of system application and membrane fouling management. The history development on AnMBR is illustrated in Figure 2.6.

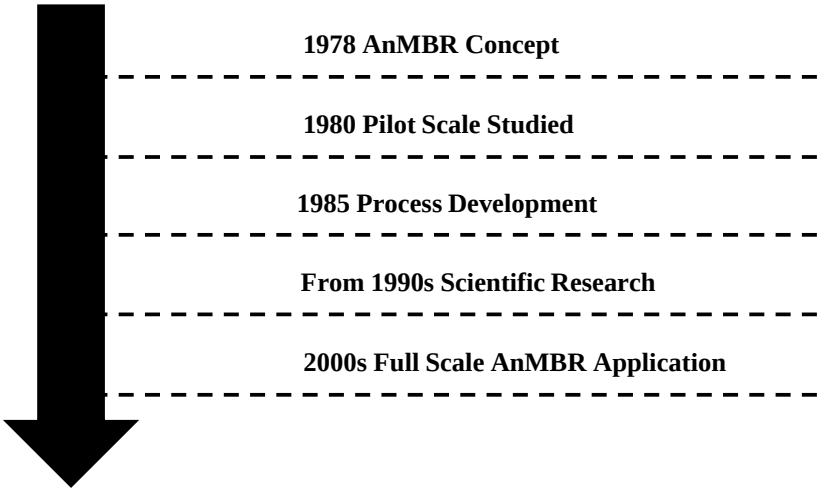


Figure 2.6 History development of AnMBR

Furthermore, the trends of the journal publications during last 15 years (2000-2014) is a good indicator of an increased interests in AnMBR applications for both mesophilic and thermophilic conditions. It indicates a huge increment of AnMBR studies in mesophilic condition. At the same time, limited reports were observed for AnMBR at thermophilic condition. The number of published articles in journals on AnMBR is presented in Figure 2.7 (Scopus, 2015).

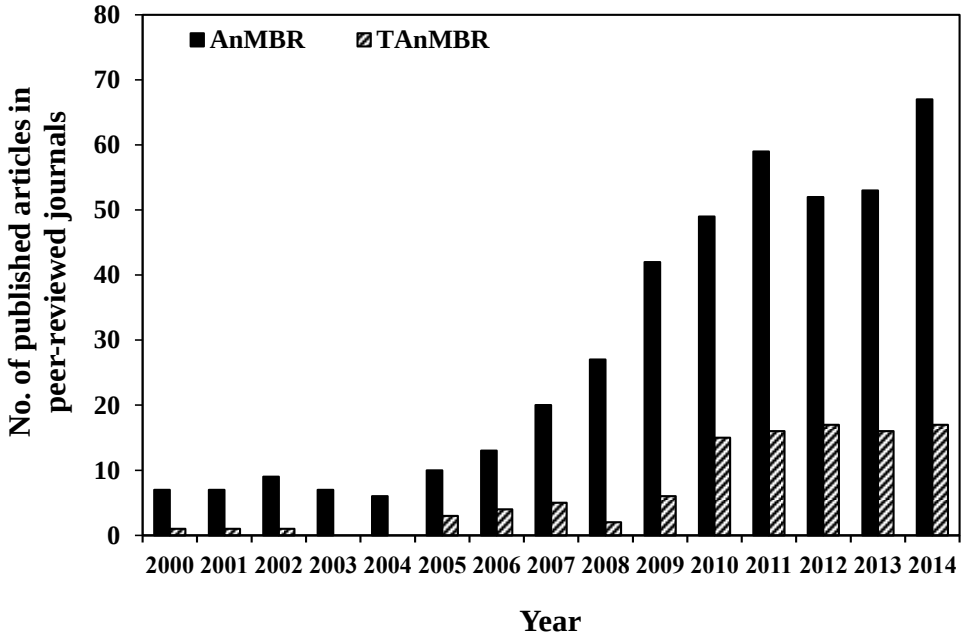


Figure 2.7 Number of published articles in journals on AnMBR
Source: Scopus (2015)

2.9 Anaerobic Membrane Bioreactor Configurations

Combining high rate anaerobic reactors and membrane can be operated in four different configurations such as pressure driven external cross-flow configuration, vacuum driven submerged membrane configuration, external gas-lift membrane reactor and sequential membrane reactor configuration. In the first approach, the membrane is separated from the reactor which is convenience in terms of membrane replacement and membrane cleaning. However, it involves external pump to recirculate biomass at high cross-flow velocity (1-5 m/s) for scouring membrane surface to minimize membrane fouling (Figure 2.8a). Typically, high cross-flow velocity can disrupt bioflocs, producing smaller particle sized which negatively affect membrane filtration. Furthermore, high cross-flow velocity resulting in high shear intensity can negatively effect on biological activity. The loss in microbial activity was due to a reduction in biofloc size which in turn interrupted the syntrophic association between acidogenic bacteria and methanogenic archaea (Brockmann and Seyfried, 1997; Choo and Lee, 1998; Stroot et al., 2001).

The second approach of operating high rate anaerobic reactor is to use a vacuum to withdraw the permeate through membrane (Figure 2.8b). In this configuration, suction pump is required to withdraw permeate from membrane. Furthermore, the biogas produced can be recirculated and used to decrease cake layer formation on membrane surface. However, anaerobic microorganisms get disturbed when membrane cleaning and maintenance is done. This problem mainly occurs in membrane install inside a close reactor. This will lead to develop high rate anaerobic reactor followed by membrane tank to minimize disturb anaerobic microorganisms.

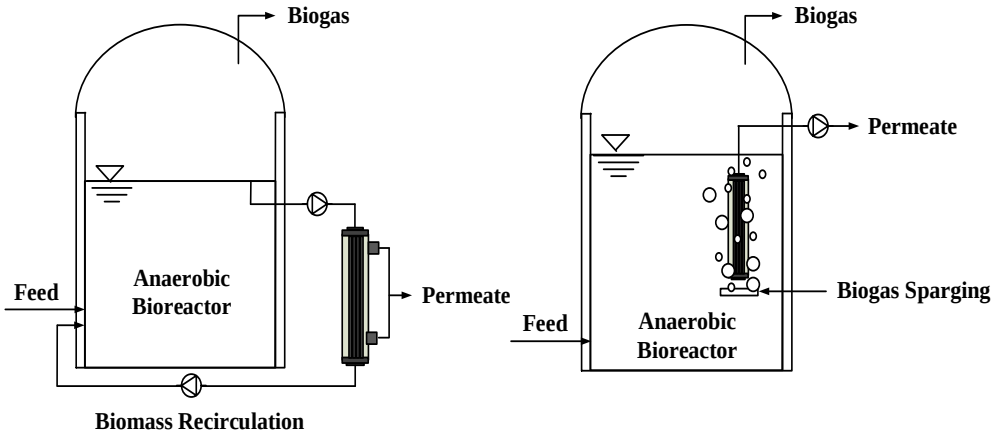
Table 2.9 Filtration Comparison between External Cross-flow and Submerged Membrane Configuration for AnMBR

Parameter	Unit	External cross-flow configuration	Submerged configuration
Design flux	$L/m^2.h$	10-40	15
Applied pressure	kPa	150-450	15-50
Cross-flow velocity	m/s	1-5	-
Energy consumption	kWh/m^3	3-7.3	0.25-1.0
Energy production	kWh/m^3	5-20	-
Temperature	°C	20-50	20-50
Size of microorganisms	µm	0.1-0.4	50-500
Effect on microbial activity	L CH_4/g MLVSS.h	Higher	Lower
Membrane fouling potential	-	Lower	Higher
Reduction in floc size	-	Higher	Lower

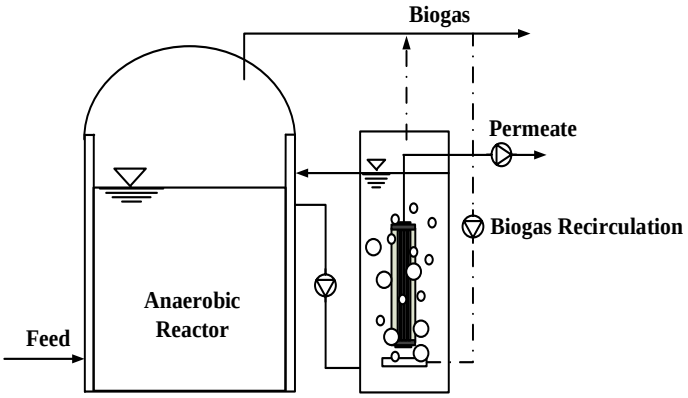
Source: Brockmann and Seyfried (1997); Fuchs et al. (2003); Berube et al. (2006); Liao et al. (2006); Visvanathan and Abeynayaka (2012)

Furthermore, membrane can either be immersed directly into the reactor or in a separate tank, which requires a pump to recirculate biomass back to the anaerobic reactor. The third membrane configuration is called external gas-lift membrane bioreactor (Figure 2.8c). This type of setup is easier to clean without disturbing the system. The advantage of having membrane immersed in separate reactor is the low energy requirement (the biomass flow is not pass through membrane module), although biogas needs to recirculate from headspace of reactor to below membrane to provide biogas bubble shear to reduce membrane fouling (Vyrides and Stuckey, 2009). Recently, several researchers have used this concept to

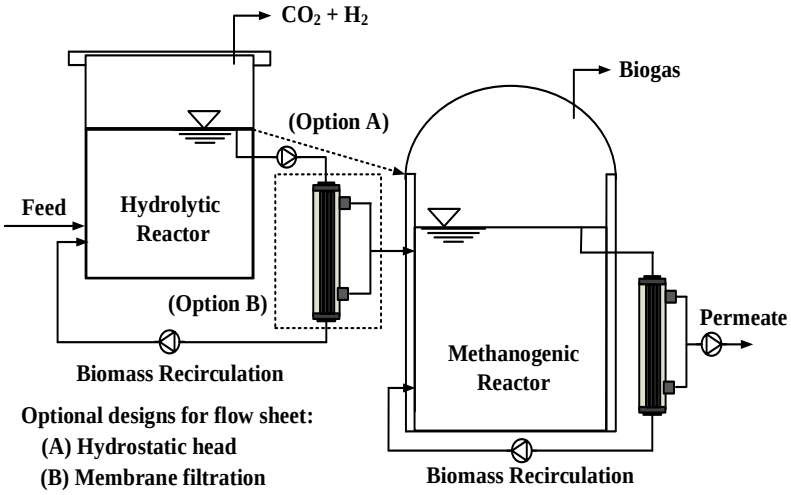
submerged membrane in separate tank treating snack food wastewater with high oil and grease (Diez et al., 2012) and bamboo industry wastewater (Wang et al., 2013b). Another advantage of this membrane configuration is minimal affected on microbial activity due to lower shear intensity application than in a cross-flow membrane configuration. The filtration comparison between external cross-flow and submerged membrane configuration is illustrated in Table 2.9.



(a) External cross-flow (b) Submerged



(c) External gas-lift membrane bioreactor



(d) Sequential Membrane Bioreactor (Two Stage)

Figure 2.8 Different AnMBR configurations

The final configuration (Figure 2.8d), which has been developed recently, is sequential membrane bioreactor (two stage) where the effluent from one bioreactor (with coarse or large pore size) is treated by the next bioreactor (with none or smaller pore size). Several researches have been used this flow sheet treating slaughterhouse wastewater and organic fraction of municipal solid waste in two stage AnMBR where hydrolytic reactor contains a course membrane and effluent containing fine particles is treated in either AnMBR (Saddoud and Sayadi, 2007; Trzcinski and Stuckey, 2009) or anaerobic filter (Walker et al., 2009) or high rate anaerobic reactor (Lee et al., 2001) or used the height difference (hydrostatic head or gravity flow) between hydrolytic and methanogenic reactor (Wijekoon et al., 2011; Chaikasem et al., 2014a; Chaikasem et al., 2014b). By placing membrane after hydrolytic reactor, it could be achieved higher SRT which increased the solubilization of particulate matters leading to increase SCOD in hydrolytic effluent as well as VFA and ultimately to biogas.

2.10 Anaerobic Membrane Bioreactor Performances

It is apparent that anaerobic wastewater treatment is very suitable for treating high concentration of organic matters and/or high loading rates. With the advantages of membrane technology, excellent biomass retention could be achieved using a high rate anaerobic reactor operation coupled with membrane so that it is able to perform at higher loading conditions. Considering the advantage of membrane process with complete biomass retention, high rate anaerobic reactor could be operated at extreme condition, where extreme conditions always cause system failure due to biomass washout. Therefore, AnMBR will be able to facilitate this because it is able to retain high biomass concentration and adaptation to extreme conditions such as high temperature wastewater, high salinity and high particulate wastewater (Brockmann and Seyfried, 1997; Fuchs et al., 2003; Vallero et al., 2005; Roh et al., 2006; Wijekoon et al., 2011; Meabe et al., 2013).

AnMBR could be operated in wide range of temperature and wastewater characteristics such as low to high organic concentration as well as low to high temperature. The select reports in literature on AnMBR performance are given in Table 2.10. Many AnMBR research studies done on external cross-flow configuration since it is easy to clean the membrane. Both external and submerged configuration could be used the height difference between anaerobic reactor and membrane module provided enough TMP for membrane filtration. Lew et al. (2009) and Wang et al. (2013b) have studied using these concepts to reduce energy consumption.

Most of the research on AnMBR have conducted on synthetic wastewater since it is easy to maintain the quality of feed. The majority of feed in the literature are VFA mixture with high salinity, molasses and synthetic municipal wastewater. The results of the studied shown good COD removal efficiency higher than 80% even treating high salinity wastewater (Vallero et al., 2005). Jeison and van Lier (2008a) compared UASB reactor and UASB coupled with membrane treating high salinity wastewater. The results show that AnMBR has higher system performance than stand alone UASB. The similar observation by Liu et al. (2013) reported that UASB reactor performance could be increased from 50% to 90% by coupling with membrane to operate it as AnMBR. The results implied the important of anaerobic reactor coupled with membrane for the treatment of wastewater under extreme conditions. An increasing in system performance can be attributed to complete biomass retention and higher biomass concentration in AnMBR.

Even though membrane coupled with high rate anaerobic reactor has better performance over only high rate anaerobic reactor. A decrease in system performance has been observed in external cross-flow membrane configuration. This was due to high shear or high cross-flow velocity (> 5 m/s) applied during biomass recirculation through pump and valve, disturbed syntrophic relationship between hydrolytic/acidogenic bacteria and methanogenic archaea resulting in low microbial activity (Choo and Lee, 1996a; Brockmann and Seyfried, 1997; Lin et al., 2009). Considering these factors, it is important to operating membrane intermittently under semi dead-end configuration to minimize the pumping cost and reduce the effects on biological activity.

In addition, the application of two stage submerged AnMBR (Flat sheet Kubota) in full scale plant had been successfully reported by Kanai et al. (2010) treating distillery wastewater at thermophilic condition with 75-92% of COD removal efficiency and 60% of methane content. Operating at thermophilic condition is very attractive since it could be operated at higher loading rate and higher methane production. Several researchers have reported the comparison on high rate anaerobic reactor and AnMBR operation in thermophilic and mesophilic conditions. These results indicated the ability of achieving higher loading rate and higher methane production in thermophilic condition (Meabe et al., 2013; Ramakrishnan and Surampalli, 2013; Jeong et al., 2014).

Table 2.10 Selected Experimental Details of AnMBR for Wastewater Treatment

Wastewater	T (°C)	Volume (L)	Membrane module	Membrane area (m^2)	HRT (h)	MLSS (kg/m^3)	OLR ($kg/m^3.d$)	Flux ($L/m^2.h$)	TMP (bar)	v (m/s)	Efficiency (%)	References
Synthetic wastewater	30	-	Cross-flow MF (Woven polyester tube)	-	14.4	-	8.3	20	2-2.5	2.2-3.6	98-99	Bailey et al. (1994)
Brewery wastewater	35	120	Cross-flow UF (MWCO 10 kDa)	-	87-96	31.5-38.3	12-20	-	1-15	-	96-98	Fakhru'l-Razi (1994)
Synthetic (Particulate wastewater)	35	10	Cross-flow UF (Flatplate, polysulfone, MWCO $3x10^3$ kDa)	0.02	120, 80, 48	15	1, 1.5, 2.5	-	0.5	0.8	> 98	Harada et al. (1994)
Synthetic (Acetate)	35	7	Cross-flow MF (Ceramic, 0.2 µm)	0.2	24	30	0.8-1.2	18-126	25-150	0-3.5	65-95	Beaubien et al. (1996)
Alcohol distillery	55	4	Cross-flow UF (Plate and flame, MWCO 20 kDa)	0.0336	360	3 (MLVSS)	1.5	< 10	2	0.24-0.95	97	Choo and Lee (1996a)
Potato starch wastewater	-	4,000	Cross-flow MF (Tubular)	-	-	> 15	> 6	-	-	-	-	Brockmann and Seyfried (1997)
Alcohol distillery	55	4	Cross-flow UF (Fluoropolymer, MWCO 20 kDa)	0.0168	-	1-3.2	1.5	-	0.5-3	0.5-1.25	-	Choo and Lee (1998)
Synthetic (Acetic acid)	35	10	Cross-flow MF, UF (Zirconia, 0.2, 0.14, 0.05, 0.08 µm)	-	-	-	-	120	0.5	3.5	-	Elmaleh and Abdelmoumni (1998)
Palm oil mill	35	50	Cross-flow UF (MWCO 200 kDa)	-	68-76	50-57	15-20	2	1.5	2.3	92-94	Fakhru'l-Razi and Noor (1999)
Brewery wastewater	35	120	Cross-flow UF (Fluoropolymer, MWCO 200 kDa)	0.024	60-100	10-50	7-28.5	-	-	-	97-99	Ince et al. (2000)
Alcohol distillery	55	5	Cross-flow MF (Hydrophobic PP, 0.2 µm)	0.0129	312	2	3-3.5	300-400	0.6	3	> 90	Kang et al. (2002)
			Cross-flow MF (Zirconia, 0.14 µm)	0.0113				140-180				

Table 2.10 Selected Experimental Details of AnMBR for Wastewater Treatment (Con't)

Wastewater	T (°C)	Volume (L)	Membrane module	Membrane area (m^2)	HRT (h)	MLSS (kg/m^3)	OLR ($kg/m^3.d$)	Flux ($L/m^2.h$)	TMP (bar)	v (m/s)	Efficiency (%)	References
Synthetic (High organic content)	30	7	Cross-flow MF (Ceramic Al_2O_3, 0.2 µm)	0.126	-	-	20	-	-	-	> 90	Fuchs et al. (2003)
Vegetable processing							8					
Slaughterhouse							6-8					
Food wastewater (Flour processing)	37	400	Cross-flow (Flat plate PES, MWCO 20-70 kDa)	0.32	60	6-8	0.88-4.83	13.1-18.9	2	1.02-1.09	81-94	He et al. (2005)
Synthetic (High salinity)	33	6	Submerged (Polysulfone, 0.2 µm)	0.07	24	-	14	4.7	< 0.15	-	80	Vallero et al. (2005)
Synthetic (Low organic content)	35	3	Submerged MF (Flat sheet PE, 0.4 µm)	0.1	6 h	2.62	-	10	-	-	95	Aquino et al. (2006)
Synthetic (VFA mixture)	30	3.7	Gas-sparged submerged (Tubular polysulfone, MF, 0.2 µm)	0.042	8	25-50 (gTSS/L)	15	5-21		70 m/h (gas)	-	Jeison and van Lier (2006)
	55				6		20	16-23				
Tapioca starch wastewater	30	1	Cross-flow UF (Hollow fiber, 0.03-0.15 µm	0.17	1.5-10 d	-	1.76	-	138	3	80-95	Roh et al. (2006)
Synthetic municipal wastewater	25	-	Cross-flow (Polypropylene, non-woven filter, 12 µm)	0.015	-	9.6-12.5	-	120	0.07-0.2	0.1-0.2	94-97	Ho et al. (2007)
			Cross-flow (PTFE composite, non-woven filter, 10 µm)									

Table 2.10 Selected Experimental Details of AnMBR for Wastewater Treatment (Con't)

Wastewater	T (°C)	Volume (L)	Membrane module	Membrane area (m^2)	HRT (h)	MLSS (kg/m^3)	OLR ($kg/m^3.d$)	Flux ($L/m^2.h$)	TMP (bar)	v (m/s)	Efficiency (%)	References
Cheese whey	37	5	Cross-flow, MF (Ceramic, 0.2 µm) (Hydrolytic reactor)	0.4	24	-	2.86	-	1.25, 1.75, 2.25	5	98.5	Saddoud et al. (2007)
		15	Cross-flow MF (Ceramic, 0.2 µm) (Methanogenic reactor)		96	6.44	3-19.78	-				
Synthetic (Sucrose)	35	3	Submerged MF (Kubota, PE, 0.4 µm)	0.1	6-15	11-16	Up to 16	2-9	0.2	-	98	Akram and Stuckey (2008)
Synthetic (High salinity and VFA mixture	30	5	-	-	-	30 gVSS/L	-	-	-	-	Lower	Jeison et al. (2008)
			Cross-flow (Tubular MF, 0.2 µm)	0.022				10-15		0.5-0.7 (m/h)	Higher	
Synthetic (VFA mixture)	55	5	Cross-flow MF (Tubular ceramic Al_2O_3, 0.2 µm)	0.022	-	< 21	10-55	20-40	-	Up to 1.5	-	Jeison et al. (2009b)
Domestic wastewater	25	180	Cross-flow MF (Hollow fiber, 0.2 µm, dead-end)	4	4.5, 6, 12	14 gTSS/L	1.08, 2.16, 4.32	3.75, 7.5, 11.25	-	-	88	Lew et al. (2009)
Synthetic (VFA + glucose + maltose)	35	-	Submerged (Hollow fiber, PP, 0.45 µm)	0.003	14	19.5	2.5	-	-	-	95	Jeong et al. (2010)
Thermomechanical pulping whitewater	37	10	Submerged (Flat sheet, PVDF, MWCO 70 kDa)	0.03	-	6.7-11.3	2.6-4.8	4.8-9.1	< 0.4	0.75 L/min (gas)	90	Lin et al. (2011)
Brewery wastewater and surplus yeast	30	4.5	External gas lift (Ceramic tubular, 0.2 µm)	-	-	12 gVSS/L	12	4-20	-	0.2-0.35 (gas)	> 97	Torres et al. (2011)
Synthetic wastewater (Molasses)	55	9	Cross-flow MF (Tubular ceramic, 0.1 µm)	0.18	16 32	2 12	5, 8, 12	1.04	-	-	61-81	Wijekoon et al. (2011)

Table 2.10 Selected Experimental Details of AnMBR for Wastewater Treatment (Con't)

Wastewater	T (°C)	Volume (L)	Membrane module	Membrane area (m^2)	HRT (h)	MLSS (kg/m^3)	OLR ($kg/m^3.d$)	Flux ($L/m^2.h$)	TMP (bar)	v (m/s)	Efficiency (%)	References
Snacks production (High O&G)	35-36	760 (180)	Submerged (Hollow fiber, PVDF, 0.4 µm)	2	4.3 d	28 (7.9-10.4)	5.1	8.02-8.74	1.98-3.79	17.6 m/h (gas)	97	Diez et al. (2012)
Municipal wastewater	20	350	Submerged UF (Flat sheet, PES, 38 nm)	3.5	41-62	9.5-17.3 TSS/L	0.52-0.81	7-12	-	94 m/h	84-94	Martinez-Sosa et al. (2012)
Synthetic (Glucose)	30	10	-	-	2	2.4	6	-	-	-	< 50 (TOC)	Liu et al. (2013)
			Submerged MF (Hollow fiber, PVDF, 0.1 µm)	1		1.6		5		-	90 (TOC)	
Sewage sludge	35	25	Cross-flow UF (ceramic membrane, MWCO 300 kDa)	0.0226	7 d	39.8	4.6	7	-	3	99.1	Meabe et al. (2013)
	55					35.8	6.4				92.9	
Domestic wastewater	15	5	Submerged MF (Polyethersulfone, flat sheet, 0.2 µm)	0.0387	16 24	11	0.44 0.66	5.38 8.07	-	4.67 L/min (gas)	92	Smith et al. (2013)
Bamboo industry wastewater	28-30	5 (1.5)	Submerger UF (Hollow fiber, PVDF, 0.02 µm	0.07	2 5 7 10	8	11 4.4 3.1 2.2	2.23 0.89 0.64 0.45	-	-	85-90	Wang et al. (2013b)
Domestic wastewater	15 25 35	5.8	Submerged MF (Hollow fiber, 0.4 µm)	0.19	6	20.5	1.44 1.21 1.29	7.1	-	-	51.1 67.1 74.0	Gao et al. (2014a)
Domestic wastewater	35	5.8	Submerged MF (Hollow fiber, 0.4 µm)	0.19	4 6 8	31.5	1.8 1.2 0.9	7.63 5.05 3.82	-	-	54.1 73.6 75.8	Gao et al. (2014b)
Municipal wastewater	35	2	Cross-flow UF (Hollow fiber, PVDF, 30 nm)	0.031	6-12	4.9-13.3	0.8-10	6	< 5 kPa	0.1-0.3	> 97	Wei et al. (2014)

2.11 Membrane Fouling Issues

Membrane fouling continues to be a substantial challenge in AnMBR application, considering membrane costs, material and energy consumption associated with the prevention of membrane fouling. Membrane fouling caused by the accumulation of inorganic and organic foulants externally on membrane surface and internally in membrane pores, which decreased permeate flux, increased TMP and potentially required chemical cleaning or membrane replacement. Fouling in AnMBR is composite which can be classified into three categories such as biofouling (suspended biomass, colloidal solids), organic fouling (EPS) and inorganic fouling (struvite, $MgNH_4PO_4.6H_2O$). All these different types of fouling take place simultaneously, although the fouling nature depends on membrane type, sludge characteristics, hydrodynamic conditions, reactor operating conditions and reactor configurations. Factors influencing membrane fouling in AnMBR are illustrated in Figure 2.9.

Furthermore, AnMBR fouling can be removable, reversible and irreversible fouling depends on the particles attachment strength to membrane surface and its possibility to remove. As illustrated in Figure 2.10, removable fouling can be easily removed by physical cleaning while reversible fouling requires chemical cleaning to be removed. Furthermore, strong matrix of fouling layer formation with solute during long term continuous filtration can transform removable fouling into reversible fouling layer. The removable fouling is caused by loosely attached foulants, while reversible fouling is caused by pore blocking and strongly attachment of foulants during filtration, which are very difficult to remove by physical cleaning. But it is easily removed by only chemical cleaning. Furthermore, irreversible fouling is a permanent fouling, which is impossible to be removed by any cleaning approach.

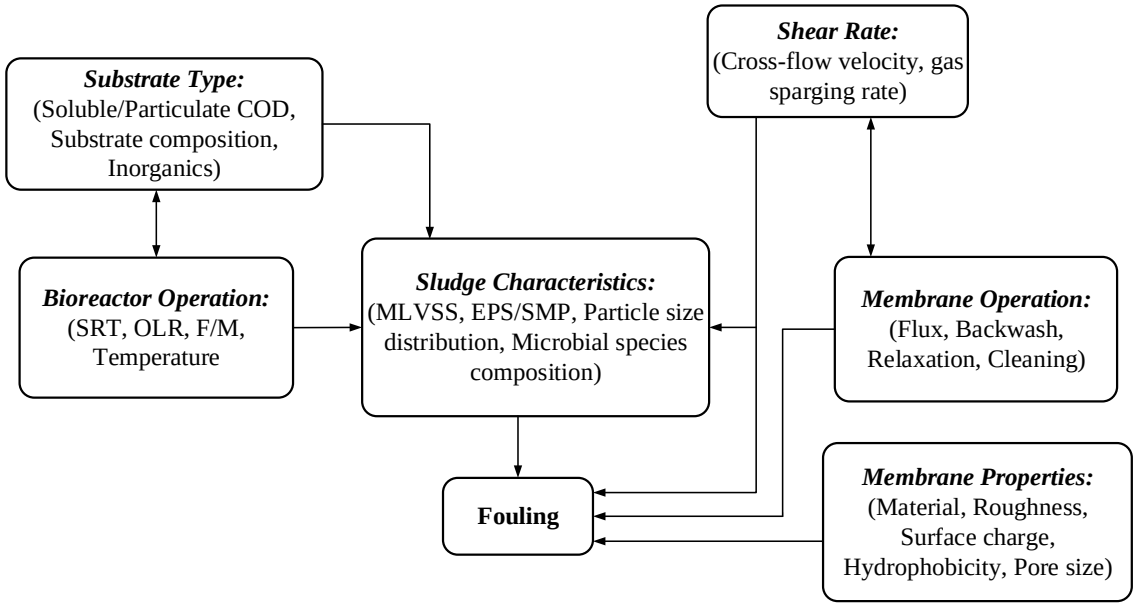


Figure 2.9 Factors influencing membrane fouling in AnMBR (modified from Dereli et al. (2012))

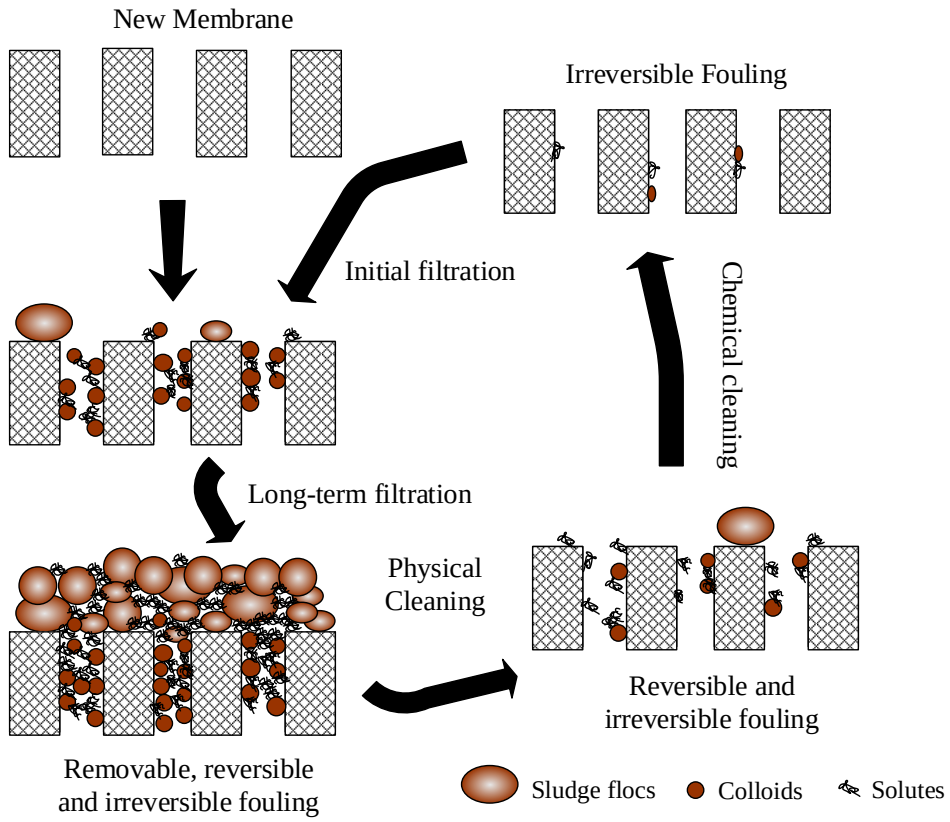


Figure 2.10 Schematic diagram of formation and removal of removable, reversible and irreversible fouling
(modified from (Meng et al., 2009))

2.11.1 Biofouling

Biofouling is caused by the interactions between membrane surface and biological components. Pore clogging, cake layer formation and adsorption of EPS are considered as biofouling. Pore blocking is clogging of the membrane pores by cell debris and sludge particles with similar or smaller pore sizes. Thus it is easily accumulate on membrane surface and decrease the surface area for filtration. Choo and Lee (1996b) reported that the colloidal particles are the major foulants in both microfiltration and ultrafiltration membranes. Furthermore, pore clogging has higher potential of membrane fouling in external cross-flow membrane configuration. This is due to the mechanical shear stress (to maintain cross-flow velocity) applied on sludge during biomass recirculation decreases the size of bioflocs which will increase membrane fouling potential.

Sludge cake formation usually occurs if the mechanical shear stress is not adequate to remove the accumulate sludge particle on membrane surface. Membrane fouling in submerged membrane configuration is mainly caused by cake layer formation on membrane surfaces which includes sludge particles and biopolymers such as polysaccharides and protein. The sludge particles/biomass are the main biofouling over biopolymer. Lin et al. (2011) reported that the smaller bioflocs size have higher filtration resistance than bulk sludge because of higher bound EPS in smaller bioflocs.

2.11.2 Organic fouling and inorganic fouling

Organic fouling refers to the accumulation and adsorption of organic constituents (EPS) on membrane surface. EPS comprised of various organic substances such as polysaccharide, protein, humic substances, nucleic acids and lipid. EPS in sludge can be classified as soluble EPS and bound EPS. The bound EPS corresponds to the polymeric substances adhering with each other and microorganisms, which contributes to reversible fouling. At higher bound EPS, stronger cake layer is formed on the membrane surface. Soluble EPS indicates the microbial products, which are produced by microorganisms and present in sludge in soluble form. This is related to irreversible fouling. Mota et al. (2013) studied the AnMBR fouling mechanism using hollow fiber membrane in submerged configuration. They reported that the cake layer formation (removable fouling) was the main fouling affecting filtration performance. Regarding EPS, the most relevant effect on filtration resistance was protein substances. Lee et al. (2008) also reported that the AnMBR fouling caused by protein substances in EPS was directly deposited on membrane surface or inside membrane pore, which resulted in clogging of membrane.

The constituents causing inorganic fouling were identified as struvite ($MgNH_4PO_4.6H_2O$). Struvite formation occurs when, magnesium (Mg^{2+}), ammonium (NH_4^+) and phosphate (PO_4^{3-}) ions exist in the feed solution. The ions in struvite may come from degradation of EPS or biomass in anaerobic condition, releasing Ca^+, Mg^{2+} and PO_4^{3-}. Furthermore, precipitation of calcium carbonate ($CaCO_3$) can also cause inorganic fouling when the wastewater consists of calcium (Ca^+) in high alkaline anaerobic reactor (pH 8-9). Moreover, inorganic foulant could interact with organic foulant forming thick cake layer which composed of sludge particles and struvite on membrane surface. Lyko et al. (2007) reported that the organic foulants coupled with inorganic foulants increase the cake layer formation, which are more difficult to remove. Kang et al. (2002) and He et al. (2005) reported that inorganic precipitation (struvite) in the membrane pore was the major factor for membrane fouling (inorganic membrane). As described above, struvite formation becomes a critical issue as it could deposit together with biomass and make a strong cake layer.

2.11.3 Membrane fouling control

Membrane fouling control has been studied through many strategies, which are related with membrane configuration. In external cross-flow membrane configuration, high cross-flow velocity is used to limit membrane fouling caused by organic and inorganic foulant accumulate on membrane surface (Kang et al., 2002; Wijekoon et al., 2011). Furthermore, the approaches to control membrane fouling in submerged membrane configuration is normally achieved by intermittent operation, biogas sparging, sub-critical flux operation, periodic physical cleaning and chemical cleaning (Vallero et al., 2005; Hu and Stuckey, 2006; Herrera-Robledo et al., 2011). The operational parameters and membrane fouling control in AnMBR is presented in Table 2.11. Typically, AnMBR has lower permeate flux than MBR due to less sludge flocculation as well as increased fine particulate and colloidal solids concentration at membrane surface. As a result, less membrane fouling propensity or similar membrane fouling intensity would be observed in AnMBR (Baek and Pagilla, 2006; Achilli et al., 2011).

Table 2.11 Operational Parameters and Membrane Fouling Control in AnMBR

Wastewater	T (°C)	OLR (kgCOD/ m^3.d)	MLVSS (g/L)	SRT (d)	HRT (h)	AnMBR configuration	TMP (bar)	Foulants	Fouling control	References
Alcohol distillery	55	2.06 1.50	3	-	240 360	CSTR, 20 kDa, external, plate and flame	1	External fouling caused by sludge cake layer and inorganic precipitation, polarization layer caused by concentration gradient	Cross-flow, physical cleaning (depressurization)	Choo and Lee (1996a)
Alcohol distillery	55	3-3.5	2	-	250	Tubular, 0.2 µm, hydrophobic PP and tubular 0.14 µm, zirconia skinned inorganic membrane	0.6	Thick cake layer composed of biomass and struvite formed on organic membrane surface. Struvite was accumulated inside membrane pore and play a key role in flux decline	Cross-flow (3 m/s), periodically backflushing with acidic (pH 2.0, 1 bar) and backfeeding	Kang et al. (2002)
Synthetic (High salinity)	33	Up to 14	0.85	-	8-36	SAnMBR, 0.2 µm, cylindrical polysulfone membranes	0.15	Physical cleaning by gentle remove cake layer is not effective for recovery of membrane permeability (compared to chemical cleaning)	Intermittent operation, frequent back flush (1 min each 10 min), flux below critical flux	Vallero et al. (2005)
Synthetic (Low strength)	35	460 (mg/L)	3	∞	3,6,12,24,48	SAnMBR, 0.4 µm, hollow fiber and polyethylene chloride, flat sheet, 0.4 µm	0.12 0.11 0.07	Fine particle (0.15-0.4 µm) and gel layer (secondary membrane, enhanced effluent quality)	Biogas sparging (5 L/min)	Hu and Stuckey (2006)
Synthetic (VFA mixture)	30	15	40 gTSS/L	-	8	SAnMBR, 0.2 µm, tubular polysulphone	2	Higher cake layer formation	Gas sparging (required lower in thermophilic), gas-lift, cycle (10 min filtration and 30 sec back-flush), critical flux concept	Jeison and van Lier (2006)
	55	20		-	6			Less cake layer formation		
Cheese whey	37	3-19.78	8.5	29.7-78.6	96	Two stage CSTR, 0.2 µm, external, ceramic	1.25 1.75 2.25	Formation and compaction of cake layer on membrane surface or by partial breakdown of cake layer and continuous infiltration of particulate matter inside porous membrane	Cross-flow (5 m/s)	Saddoud et al. (2007)
Synthetic (VFA mixture)	55	Up to 40 (10-100 g/L)	27	-	-	SAnMBR, 0.2 µm, tubular polysulphone	1.50	Cake layer formation (reversible by back flushing), low irreversible fouling, low internal fouling, a decrease in particle size caused a reduction in critical flux	Biogas sparging (required lower in thermophilic), gas-lift, cycle (10 min filtration and 30 sec back-flush), critical flux concept	Jeison and van Lier (2008b)

Table 2.11 Operational Parameters and Membrane Fouling Control in AnMBR (Con't)

Wastewater	T (°C)	OLR (kgCOD/ m^3.d)	MLVSS (g/L)	SRT (d)	HRT (h)	AnMBR configuration	TMP (bar)	Foulants	Fouling control	References
Synthetic (Glucose)	35	8.8 (TOC load)	9.5	450	9	SAnMBR in separate tank, 0.45 µm, polyolefin	-	EPS adsorbed onto polymeric membrane surface. Protein EPS have increased due to combination of polymeric matter produced from cell lysis with longer SRT and low F/M. EPS is directly deposited onto membrane surface or interior of membrane pore	Biogas sparging (5 L/min), chemical cleaning with 0.5% NaOCl	Lee et al. (2008)
Kraft evaporator condensate	33	12.2	8	230	19.68	UASB, SAnMBR, 70 kDa, PVDF	0.3	Lower filtration resistance caused by sludge cake formation with minimal pore plugging and adsorption, lower PN/PS	Biogas sparging (0.75 L/min), Intermittent filtration (4 min on and 1 min off)	Lin et al. (2009)
	55	3.1			77.28			Higher filtration resistance caused by sludge cake formation with minimal pore plugging and adsorption, higher PN/PS		
Synthetic (Organic particulate)	30	5.1 (500 mg/L)	-	50	24	Upflow anaerobic reactor, 100 kDa external cPVDF and 30 kDa external PEI, flat sheet	-	EPS was predominately proteinaceous. EPS and microbial cells of foulant layer contributed to membrane fouling. Fouling of PEI was faster than cPVDF (important of membrane material)	Cross-flow	Gao et al. (2010)
Municipal sewage	22	445 (mg/L)	-	180	6	UASB, 100 kDa, PVDF, external tubular UF	0.87	SMP and EPS were identified as significant for fouling because they are adsorb on membrane surface or inside membrane pore	Cross-flow, cleaning daily after 8 h of filtration (NaOCl at 300 mg/L, or 0.03%, for 30 min)	Herrera-Robledo et al. (2011)
Synthetic (Glucose, C:N:P 100:5:1)	25-30	0.84-1.1 0.84-1.32 0.84-1.65	5.1-6.5 5.2-7.9 5.4-9.4	30 60 ∞	8,10,12 8,10,12 8,10,12	CSTR, SAnMBR, 0.45 µm, PES, plate and flame	0.3	Longer SRT and lower HRT would cause higher SMP and lower EPS production, which induced more pore blocking and enhanced biofilm/cake layer formation. Lower PN and PS in EPS would be observed in prolong SRT resulted in less flocculation and smaller particle size (accelerate fouling at longer SRT)	External gas-lift (suction cycle 8 min on and 2 min off), soaked in 0.5% NaOCl	Huang et al. (2011)

Table 2.11 Operational Parameters and Membrane Fouling Control in AnMBR (Con't)

Wastewater	T (°C)	OLR (kgCOD/ m^3.d)	MLVSS (g/L)	SRT (d)	HRT (h)	AnMBR configuration	TMP (bar)	Foulants	Fouling control	References
Synthetic (Municipal)	-	350 (mg/L)	-	∞	4 8 12	UASB, 100 kDa external UF tubular membrane, PVDF	1	HRT reduction caused increase EPS, SMP, smaller particle size due to high shear at low HRT	Cross-flow (2 m/s), NaOCl (300 mg/L) cleaning every 6 h for 20 min	Salazar-Peláez et al. (2011)
Municipal wastewater	-	-	8-12	10-15	-	Pilot scale SAnMBR, 0.4 µm, hollow fiber, PVDF	0.2	Gel layer and pore blockage fouling, mainly caused by colloidal, EPS, SMP and inorganic substances in long term operation even under sub critical flux	Sub-critical flux and CIP with NaOCl in both TMP and time control	Wei et al. (2011)
Synthetic (Molasses)	55	5 8 12	10-12	∞	2	Two stage AnMBR, 0.1 µm, tubular ceramic	-	-	External semi dead-end	Wijekoon et al. (2011)
Snacks production (high O&G)	35	5.1	28	∞	103.2	SAnMBR in separate membrane tank 0.4 µm, hollow fiber, PVDF	1.98-3.79	Filtration resistance increase with filtration time	Intensive backwashing cycles and extended relaxation mode and different cleaning methods	Diez et al. (2012)
Domestic wastewater	25-30	1.02 (426.8 mg/L)	8 12.6 13.6	30 60 90	10	SAnMBR, 0.45 µm, polyethersulfone, plate and flame	0.3	SRT decrease lead to membrane fouling by SMP accumulation. SRT increase, higher biomass concentration caused more particle deposition on membrane surface, while SMP was attributed to more metabolism products generation	Gas-lift, soaked in 0.5% NaOCl and followed by thorough flushing with DI water	Huang et al. (2013)
Bamboo industry wastewater	28-30	2.2,3.1, 4.4,11 (22 g/L)	8	∞	2,5,7,10	SAnMBR in separate tank, 0.02 µm, hollow fiber, PVDF	0.9	Dense cake layer, biofouling	Periodically N_2 gas sparging to remove loosely attached fouling layer	Wang et al. (2013b)
Domestic wastewater	35	1,8 1.2 0.9	31.5	∞	4 6 8	AnMBR with PAC, 0.4 µm, hollow fiber	0.3	Membrane fouling rate was enhanced at short HRT. GAC decreased protein content in cake layer	GAC fluidization, backflushing	Gao et al. (2014b)
Synthetic (Municipal)	35	0.8-10	4.9-13.3	∞	6-12	CSTR, 0.03 µm, hollow fiber PVDF	< 5 kPa	Hydraulically reversible fouling (mainly cake layer)	Superficial cross-flow velocity of 0.1-0.3 m/s	Wei et al. (2014)

Considering the treatment of dilute wastewater or low strength wastewater with low methane production, minimizing energy consumptions related with membrane fouling is necessary by maximizing the energy recovery through methane gas. This lead to the development of novel AnMBR by Hu and Stuckey (2007) using powder or granular activated carbon (PAC or GAC) in submerged AnMBR to decrease membrane fouling in conjunction with biogas sparging. PAC or GAC were used for scouring membrane surface by biogas sparging not used for adsorption. Therefore, the regenerated or replaced of PAC or GAC would not be necessary. Recently, Gao et al. (2014b) have studied anaerobic fluidized-bed membrane bioreactor for domestic wastewater. The authors reported that the use of PAC or GAC in suspension decreased filtration resistance by producing extra shearing effect onto membrane surface and decreasing the deposition of particles membrane surface by scouring effect. Furthermore, PAC or GAC addition in AnMBR reduced protein content in cake layer and the reduction of protein in cake layer improved the filtration performance.

2.12 AnMBR Application for Wastewater Treatment

Wastewater characteristics can be illustrated in two aspects namely its concentration and its particulate nature of wastewater. According to that wastewater, it can categorized in to four types namely (a) high strength high particulate wastewater (b) low strength high particulate wastewater (c) low strength high soluble wastewater and (d) high strength high soluble wastewater. The AnMBR application to different kinds of wastewater is illustrated in Figure 2.11. Among that low to high strength soluble wastewater are currently treating very well in conventional high rate anaerobic reactor such as UASB. Therefore, application of AnMBR is very attractive when higher solids removal is needed for secondary purposes.

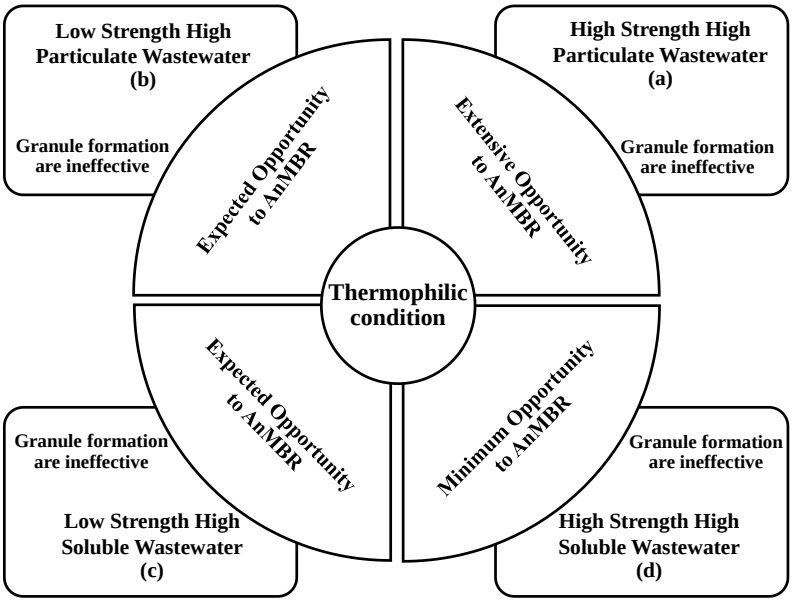


Figure 2.11 AnMBR application to different types of wastewater (modified from (Liao et al., 2006)

Typically, high particulate wastewater required higher SRT as well as compact system with high amount of biomass concentration for complete solubilization/hydrolysis of slowly degrading particulate matters. In this case, UASB reactor coupled with membrane provide an excellent approach for degradation of particulate wastewater. Therefore, this is an extensive opportunity to operate AnMBR in particulate wastewater such as distillery

wastewater, brewery wastewater, vermicelli processing, palm oil mill effluent and slaughterhouse wastewater.

Furthermore, there is high opportunity of application AnMBR for the low strength wastewater with high particulate matters or low strength completely soluble wastewater with respect to biomass retention. Energy recovery and treated water reuse application are major considerations of AnMBR treating low strength wastewater. Typically, anaerobic processes have low nutrient removal and free solid effluent. Therefore, the effluent will contain high amount of nutrient. This will lead the used of treated wastewater for agriculture. In addition, AnMBR application treating high strength wastewater under extreme conditions such as high temperature, high salinity and toxicity is highly recommended. As extreme conditions cause system failure because of excessive biomass washout. AnMBR's can facilitate this issue due to its ability to retain biomass and its ability to acclimatize to extreme conditions.

2.13 Research Directions and Future Research Needs

Thermophilic anaerobic wastewater treatment has been investigated, where the system can successfully treat high strength particulate wastewater with high biogas production and organic removal efficiency. Furthermore, anaerobic wastewater treatment operated at thermophilic condition achieves higher biodegradation rate, low sludge yield, capability of performing well under higher loading rates and pathogen inactivation. Due to the difference in growth rates and pH tolerance for acidogenic and methanogenic consortia, it becomes necessary to have two stage reactor for superior performance. Optimizing each stage separately facilitates process stability, overall reaction rate, solid removal and biogas production. However despite the advantages, two stage thermophilic anaerobic reactor faces difficulty in biomass retention due to high degree of sludge mineralization which results in less amount of EPS production and obstructs the dense and firm of sludge granulation. Finally, this is ended up in dispersed sludge and washout from the system.

To counter the above adherent problems with two stage anaerobic reactor, addition of polyvinyl alcohol hydrogel (PVA-gel) beads to the hydrolytic reactor and membrane based separation to methanogenic reactor can provide a potential solution. PVA-gel beads have been reported to be effective biocarrier in UASB reactor for treating low to high strength wastewater. However, the use of PVA-gel beads as a biocarrier in the hydrolytic reactor with high strength particulate wastewater has not been studied extensively. Similarly to overcome the difficulty in biomass separation in methanogenic reactor, ceramic membrane have been applied for effective biomass retention.

Nevertheless, membrane fouling is the major problems in AnMBR application. Leading to flux decline and higher frequency of membrane cleaning. Thus fouling needs to be studied as it is one of the limiting step for widespread application of AnMBR for industrial wastewater treatment.

Chapter 3

Methodology

3.1 Introduction

The two stage TAnMBR was designed for treating tapioca starch based synthetic high strength particulate wastewater. Operations of the system with and without PVA-gel as biocarrier were done to study the effect of PVA-gel on hydrolytic reactor performance of two stage TAnMBR. Furthermore, the system performance and membrane fouling characteristics were investigated and evaluated in different loading conditions. Figure 3.1 illustrates the overall framework of this study.

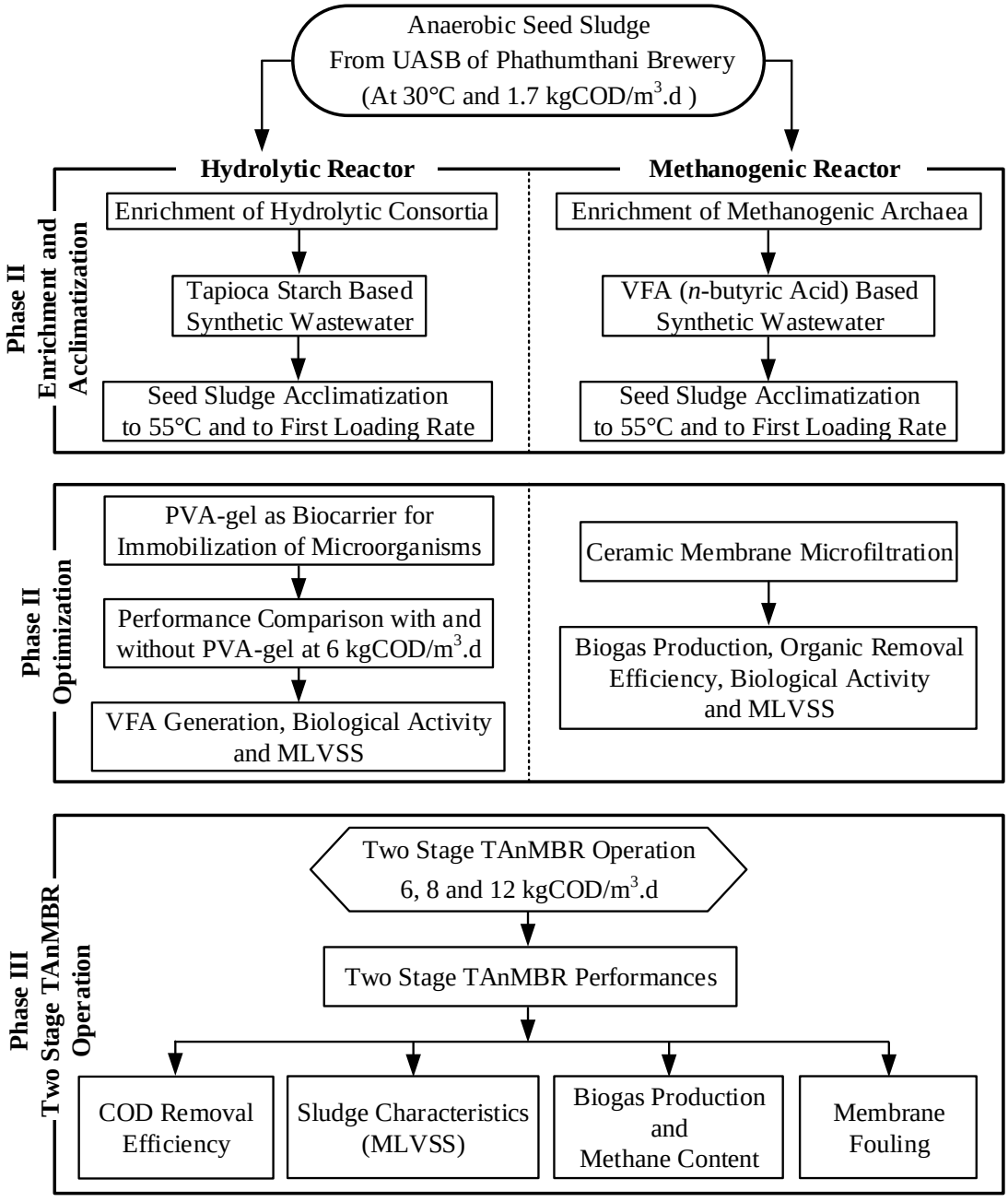


Figure 3.1 Overall study framework

3.2 Anaerobic Seed Sludge Preparation and Characterization

The anaerobic seed sludge was taken from UASB reactor of Phathumthani Brewery Co., Ltd. It has black color with spherical shape and 1-2 mm diameter. Sludge volume index (SVI) was observed as 183 mL/gVS. This was due to anaerobic seed sludge was mainly associated with filamentous growth and diffusible in nature resulting in poor settleability. Furthermore, sludge activity of anaerobic seed sludge was observed as 0.44 $kgCOD_r/kgVS.d$, indicating that anaerobic seed sludge is active for using as inoculum to start up two stage TAnMBR. The anaerobic seed sludge characteristics were shown in Table 3.1.

Table 3.1 Anaerobic Seed Sludge Characteristics

Characteristic	Unit	Value
Average temperature at source	°C	30
pH	-	7.3±0.2
Operating loading rate at source	$kgCOD/m^3.d$	1.7
Total solids (TS)	g/L	3.6±0.3
Volatile solids (VS)	g/L	1.9±0.2
SVI	mL/gVS	183
Sludge activity	$kgCOD_r/kgVS.d$	0.44

The collected sludge was allowed to settle at 4°C for 24 h and the supernatant was removed, leaving the concentrated sludge at the bottom. Next the thickened sludge was brought to room temperature, TS and VS were analyzed. The concentrated sludge had a TS of about 34.1 g/L and a VS of around 20.4 g/L.

3.3 Synthetic Wastewater Preparation

Tapioca starch based synthetic wastewater was heated at 55±3°C to dissolve the particulate matters before feeding to the system. This was done to simulate high strength particulate wastewater (tapioca starch wastewater) discharge at high temperature (Annachhatre and Amatya, 2000). Tapioca starch was used as sole carbon source. NH_4HCO_3 and KH_2PO_4 were added as nutrient to maintain COD:N:P ratio at 100:5:1. Synthetic wastewater constituents is shown in Table 3.2. Similar COD:N:P ratio has used in Yilmaz et al. (2008) for treating papermill wastewater at thermophilic condition. Even smaller or higher COD:N:P ratio such as 100:2:0.2 or 100:7:2.5 could be used in anaerobic wastewater treatment applications. The required amount of heated tapioca starch was mixed with deionized (DI) water and nutrients to obtain the required feed COD concentration of the synthetic wastewater. The characteristics of synthetic wastewater is presented in Table 3.3.

Table 3.2 Constituents of Synthetic Wastewater

Constituents	Amount per 1 L of feed wastewater in each COD concentration			Remark
	15 g/L	20 g/L	24 g/L	
Tapioca starch (g)	15	20	24	To maintain loading rate at 6, 8 and 12 kgCOD/m^3.d
NH_4HCO_3 (g)	4.5	5.6	6.8	To maintain COD:N ratio = 100:5
KH_2PO_4 (g)	0.7	0.9	1.1	To maintain COD:N:P ratio = 100:5:1

Table 3.3 Characteristics of Synthetic Wastewater

Parameters	Loading rate (kgCOD/m^3.d)		
	6	8	12
pH	7.3±0.2	7.5±0.1	7.8±0.3
TCOD (g/L)	14.5±1.4	20.6±1.2	23.9±0.8
NSCOD (g/L)	8.8±2.2	15.1±1.1	15.9±3.2
TS (g/L)	11.9±1.8	15.6±1.1	20.3±1.1
SS (g/L)	9.8±1.7	13.6±2.0	16.8±0.8
TKN (mg/L)	775±20	950±50	1,100±40
NH_4^+-N (mg/L)	600±24	680±40	807±53
TP (mg/L)	165±10	210±15	230±22

Note: total chemical oxygen demand (TCOD); soluble chemical oxygen demand (SCOD); Non-soluble chemical oxygen demand (NSCOD)

3.4 Biocarrier

In this study, polyvinyl alcohol hydrogel (PVA-gel) beads were chosen for immobilization of microorganism in hydrolytic reactor, having previously demonstrated effectiveness as biocarrier (Rouse et al., 2007; Wenjie et al., 2008; Zhang et al., 2009; Khanh et al., 2011). PVA-gel beads were supplied from Kuraray Company (Tokyo, Japan). The characteristics of PVA-gel beads are shown in Table 3.4. After the system operated with loading rate of 6 kgCOD/m^3.d was completed, PVA-gel beads were added to hydrolytic reactor of two stage TAnMBR operation at the same loading rate. PVA-gel beads were added to achieve 30% (0.9 L of PVA-gel) of hydrolytic reactor working volume and thoroughly mixed to inoculate hydrolytic bacteria on their surface.

Table 3.4 Characteristics of PVA-gel beads

Descriptions	Characteristics
Media	Polyvinyl Alcohol Hydrogel (PVA-gel) beads
Shape	Sphere
Size	Ø 3-4 mm
Specific gravity	1.025±0.01
Volumetric packing ratio	30 %
Specific surface area	2,500 m^2/m^3

3.5 Membrane Specifications

In this study, ceramic membrane was used for filtration, which could be operated at high temperature. The ceramic membrane specifications are given in Table 3.5.

Table 3.5 Ceramic Membrane Specifications

Parameters	Values/Specifications
Membrane manufacturer	NGK Insulator, Japan
Membrane material	Ceramic
Membrane type	Microfiltration
Module configuration	Tubular (multi-channel)
Channel number	55
Effective surface area	0.18 m^2
Pore size	0.1 µm
Maximum flux	87.5 $L/m^2.h$
Dimensions	Diameter-30 mm, Length-450 mm
Configuration	Inside-Out
Operating pressure range	20-90 kPa
Maximum operating temperature	300°C

3.6 Operating Conditions of Ceramic Membrane

Ceramic membrane was operated in external semi dead-end configuration in order to minimize the effect of high shear intensities on biological activity. The filtration cycle was adjusted to maintain required permeate flux in each loading rate. The ceramic membrane operating conditions are presented in Table 3.6.

Table 3.6 Ceramic Membrane Operating Conditions

Descriptions	Characteristics
Temperature	55°C
pH	6.8-7.2
Maximum operating pressure	90 kPa
Filtration method	Suction mode
Filtration cycle	4 min filtration and 1 min biomass recirculation (6, 8 $kgCOD/m^3.d$) 6 min filtration and 2 min biomass recirculation (12 $kgCOD/m^3.d$)
Permeate flux	0.86 $L/m^2.h$ (6, 8 $kgCOD/m^3.d$) 1.04 $L/m^2.h$ (12 $kgCOD/m^3.d$)

3.7 Experimental Set Up and Operating Conditions

This experimental study consisted of three phases, namely phase I: anaerobic seed sludge enrichment and acclimatization, phase II: two stage TAnMBR optimization and phase III: the two stage TAnMBR operated with different loading conditions.

3.7.1 Automated two stage TAnMBR

The two stage TAnMBR was designed to be an automated system. It was designed for phase II and III of this study and the function has discussed following. The system was constructed with a working volume of 3 L and 6 L for hydrolytic and methanogenic reactors using stainless steel, respectively. The system was operated in two stage, namely hydrolytic reactor followed by methanogenic reactor and ceramic membrane filtration. Biogas recirculation was used in order to achieve good mixing condition (10 min mixing and 2 min non-mixing) in both reactors. Synthetic wastewater was fed to hydrolytic reactor by peristaltic pump (Masterflex L/S drives, 6-600 rpm) with intermittent feeding at a controlled feed flow rate by an automatic level sensor immersed in methanogenic reactor. Once the effluent overflowed from hydrolytic reactor, it was fed into methanogenic reactor by gravity flow. When the methanogenic reactor was filled up to required level, the feed pump was stopped through a relay unit integrated with level sensor.

The final biomass separation from effluent was carried out using a ceramic microfiltration membrane. The membrane module was operated in an external semi dead-end configuration. Furthermore, it was operated under suction mode to withdraw constant flux. The filtration cycle was adjusted to increase suction pressure to obtain constant permeate flow rate. Filtration cycle in the first and second loading rate was 4 min filtration and 1 min biomass recirculation, and this was adjusted to 6 min filtration and 2 min biomass recirculation to the methanogenic reactor as required to get the required permeate flux.

Furthermore, biogas production in both hydrolytic and methanogenic reactors was collected separately in tedlar bag, and biogas composition was analyzed by gas chromatography. The experimental setup of two stage TAnMBR is illustrated in Figure 3.2 and Appendix A.

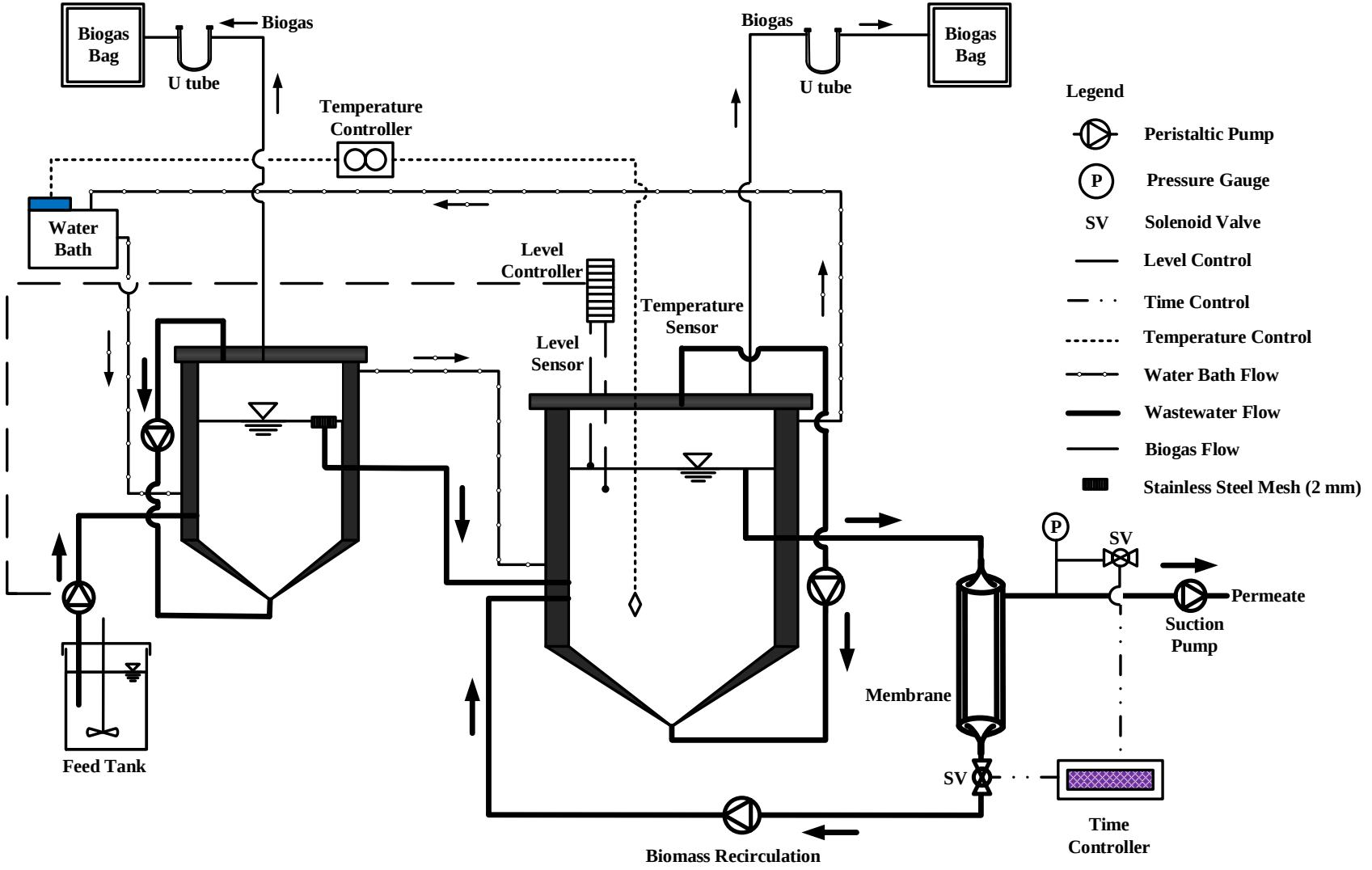


Figure 3.2 Experimental set up of two stage TAnMBR

3.7.2 Two stage TAnMBR operating conditions

Both hydrolytic and methanogenic reactors were operated in thermophilic conditions in order to evaluate the performances of two stage TAnMBR. Table 3.7 illustrates the detail operating conditions of this experiment. The two stage TAnMBR was operated in three loading rates by changing flow rate and organic concentration of feed.

Table 3.7 Operating Conditions of Two Stage TAnMBR

Parameters	Unit	Hydrolytic Reactor	Methanogenic Reactor	Overall
pH	-	5.4±0.5	7.2±0.3	-
Temperature	°C	55	55	55
Influent COD	g/L	15 20 24	12 16 19.2 (Calculated)	15 20 24
Loading rates	kgCOD/m^3.d	18.67 23.33 36	7.46 9.33 14.4	6 8 12
HRT	h	19.45 16	38.92 32	58.37 (6, 8 kgCOD/m^3.d) 48 (12 kgCOD/m^3.d)
SRT	d	0.81 0.67	∞	-
Flow rate	L/d	3.7 4.5	3.7 4.5	3.7 (6, 8 kgCOD/m^3.d) 4.5 (12 kgCOD/m^3.d)
Working volume	L	3	6	9
Biomass retention	-	PVA-gel (30% v/v)	Ceramic Membrane	-
Permeate flux	L/m^2.h	0.86 (6, 8 kgCOD/m^3.d) 1.04 (12 kgCOD/m^3.d)		

3.7.3 Phase I: Anaerobic seed sludge enrichment and acclimatization

To enrich hydrolytic and methanogenic consortia of anaerobic microorganisms, two separate batch reactor of 3 L and 6 L were fed with anaerobic seed sludge from UASB reactor. It was obtained from a beer industry's UASB reactor, which was operated under mesophilic condition (30±3°C). This separation in two stage allows the enrichment of the different populations of microorganisms by means of the control of the operational parameters.

As per required in two stage TAnMBR operations, hydrolytic and methanogenic consortia enrichment was done by providing different feeds and environmental conditions. Along with

the enrichment of microorganisms, the acclimatization of sludge to 55°C and to initial loading rate was taken place. During the acclimatization process of both hydrolytic and methanogenic reactors, the temperature increase in steps wise such as 2°C. Loading condition of hydrolytic reactor was increased by 2 kgCOD/m^3.d per time, with observing the reactor performance. Furthermore, methanogenic reactor loading rate was increased by 0.5 kgCOD/m^3.d per time, with observing the performance of the reactor. The acclimatization phase was carried out in the two separate reactors in two stages. The experimental set up of enrichment and acclimatization process is shown in Figure 3.3. After that ceramic membrane was connected to methanogenic reactor and acclimatization was continued. The anaerobic seed sludge enrichment and acclimatization process was conducted using the method by Wijekoon et al. (2011) and its discussed below.

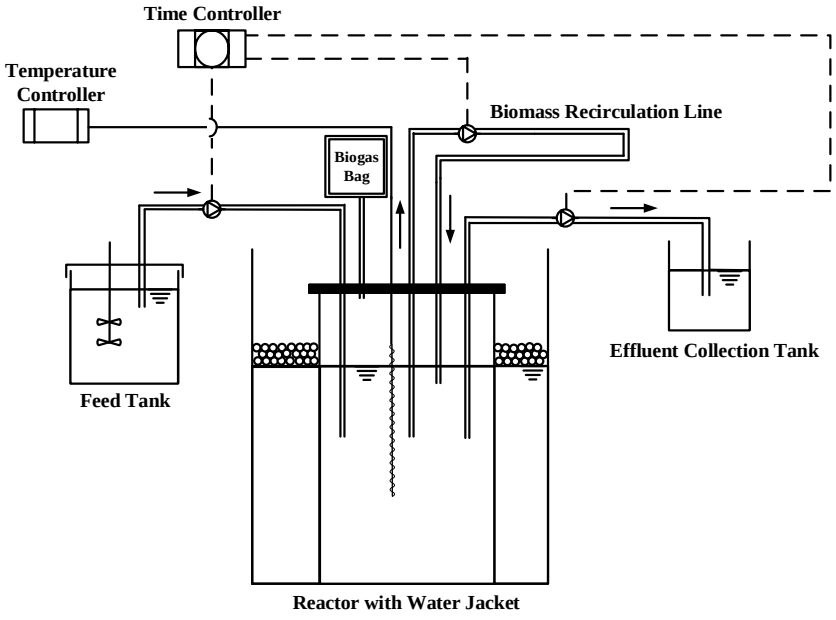


Figure 3.3 Experimental set up of enrichment and acclimatization process

3.7.3.1 Hydrolytic reactor seed sludge acclimatization

Tapioca starch was used as sole carbon source for feeding to hydrolytic reactor, while NH_4HCO_3 and KH_2PO_4 were used as nitrogen and phosphorus source, respectively. The synthetic wastewater was maintained at COD:N:P ratio of 100:5:1. At the beginning, anaerobic seed sludge was mixed with synthetic wastewater in proportion of 1:1 v/v, and it was added to the reactor. Loading rate of hydrolytic reactor was gradually increased by increasing 2 kgCOD/m^3.d at a time. Once a system became stable (total VFA and MLVSS concentration), loading rate was increased to the next higher step. Concentration of COD in feed tank and flow rate was increased to elevate loading condition for the study. Meanwhile, temperature was increased from 35°C to 55°C in steps of 2°C together with increasing loading conditions. The pH in hydrolytic reactor was controlled in the proper range of 5.5±0.5 and HCl was used for pH adjustment in this purpose.

3.7.3.2 Methanogenic reactor seed sludge acclimatization

n-Butyric acid was used as sole carbon source of feeding to methanogenic reactor. This was used to enrich methanogenic archaea of mixed culture of anaerobic seed sludge. Moreover,

n-butyric acid is an intermediary products of VFA. This product is converted further to methanogenic substrate such as acetic acid, H_2 and CO_2 by the acetogenic bacteria through the acetogenic dehydrogenation reaction occurred in methanogenic reactor. Therefore, *n*-butyric acid was used for sole carbon source to enrich the methanogenic archaea of anaerobic mixed culture during this period. Furthermore, NH_4HCO_3 and KH_2PO_4 were used as nitrogen and phosphorus source in order to maintain COD:N:P of 100:5:1. Anaerobic seed sludge was mixed with synthetic wastewater in 1:1 v/v proportion. Later, it was added to the reactor. Methanogenic reactor loading rate was gradually increased by increasing 2 kgCOD/m^3.d at a time. The reactor stability was considered by MLVSS concentration, organic removal rate and methane production. Once MLVSS concentration, organic removal rate and methane generation were stable, temperature and loading rate were increased to the next higher value. Feed flow rate and COD concentration were increased in order to increase loading condition for the study. Meanwhile, temperature was increased from 35°C to 55°C in steps of 2°C along with loading condition. Methanogenic reactor pH was controlled at 7.2±0.3 and measured amount of $NaHCO_3$ was used to maintain pH at neutral when necessary.

At the beginning of methanogenic reactor enrichment and acclimatization phase, effluent from hydrolytic reactor was mixed with *n*-butyric acid to mimic feeding more like the real feed, receiving from hydrolytic reactor of two stage anaerobic reactor. At the final stage of methanogenic reactor enrichment and acclimatization process, the influent to methanogenic reactor was converted completely to hydrolytic effluent.

3.7.4 Phase II: two stage TAnMBR optimization

The experiment was conducted in two reactors namely: (1) hydrolytic reactor and (2) methanogenic reactor. The optimization of hydrolytic reactor using PVA-gel as biocarrier and methanogenic reactor followed by membrane filtration to improve performance of two stage TAnMBR were carried out in this phase.

3.7.4.1 Optimization of hydrolytic reactor

In this phase, the application of PVA-gel as biocarrier on hydrolytic reactor of two stage TAnMBR was investigated. The purpose of this optimization phase is to investigate the effect of PVA-gel on biological activity and VFA concentration. The optimization experiments for the hydrolytic reactor was carried out using PVA-gel at loading rate of 6 kgCOD/m^3.d. By incorporating PVA-gel as biocarrier to hydrolytic reactor with the aim to ferment particulate wastewater to generate an overflow which is VFA rich effluent to methanogenic reactor. Then, methane productivity from the system should increase with increase in VFA in the hydrolytic reactor's effluent. The characteristics of PVA-gel is described in Section 3.4. The PVA-gel beads were used at volumetric packing ratio of 30% or 0.9 L of PVA-gel and thoroughly mixed with hydrolytic consortia isolated. The optimization of hydrolytic reactor with PVA-gel is illustrated in Figure 3.4. The mixing was done by biogas recirculation in an intermittent mode (10 min mixing and 2 min non mixing) to assure good fluidization of PVA-gel in hydrolytic reactor. Peristaltic pump (Masterflex L/S 6-600 rpm) was used for biogas recirculation.

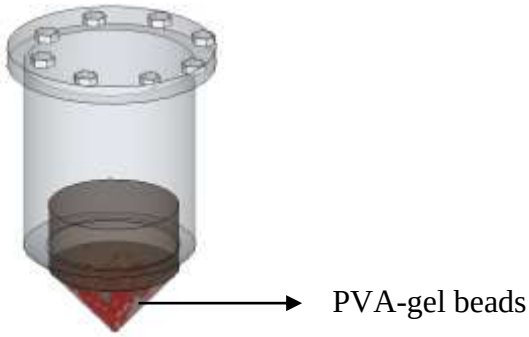


Figure 3.4 Optimization of hydrolytic reactor

3.7.4.2 Optimization of methanogenic reactor

The methanogenic reactor was connected with ceramic membrane to retain biomass because it is very difficult to separate biomass from treated water at thermophilic conditions as it presented in disperse sludge and can easily washout from the methanogenic reactor (Soto et al., 1992; Uemura and Harada, 1993). In this study, ceramic membrane with pore size of 0.1 µm was used to separate biomass from effluent as well as retains biomass and it could withstand high temperature. The purpose of this phase was to optimize methanogenic reactor and study the effect of PVA-gel on two stage TAnMBR performances in terms of methane productivity ($L_{methane}/L_{reactor}$.d) and organic removal rate. The methanogenic reactor was optimized at loading rate of 6 kgCOD/m^3.d by using ceramic membrane to retain biomass in the methanogenic reactor. Figure 3.5 shown the methanogenic reactor optimization. The mixing condition was done by biogas recirculation in an intermittent mode (10 min mixing and 2 min non mixing). Peristaltic pump (Masterflex L/S 6-600 rpm) was used for biogas recirculation. The membrane operating conditions have discussed in Section 3.6.

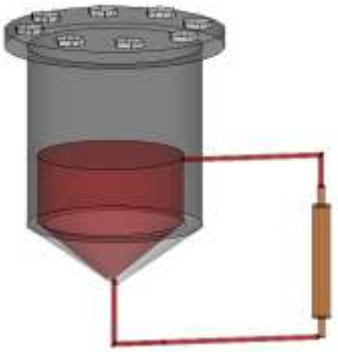


Figure 3.5 Optimization of methanogenic reactor

3.7.5 Phase III: Two stage TAnMBR operation

Once the two stage TAnMBR optimization was completed in phase II and the stable conditions were achieved, the system was operated in further high loading conditions. The purpose of this phase was to study the performance at optimized two stage TAnMBR, and membrane fouling investigation at thermophilic condition (55°C). In this phase, the performance of the system was investigated at three different loading conditions such as 6, 8 and 12 kgCOD/m^3.d. The overall loading rate of the system was increased by 2 kgCOD/m^3.d. Once loading rate was increased from 8 to 10 kgCOD/m^3.d, the system was

not faced the problem about VFA accumulated. Therefore, overall loading rate was finally increased to 12 kgCOD/m^3.d and operating at this loading rate until the system was stable. Similar to the previous phase, loading rate was gradually increased by increasing the COD concentration of feed and flow rate.

3.8 Ceramic Membrane Cleaning Procedure

Once the ceramic membrane fouling was observed, suction pump and biomass recirculation pump were stopped. Then, ceramic membrane was removed carefully into separate tank. Initially, DI water was used. After that two cleaning solutions were applied to clean membrane.

Membrane cleaning was carried out using alkaline and acid cleaning. The membrane was soaked in cleaning solution, (a) 0.5 M NaOH for 15 minutes at 75°C to remove organic fouling and (b) a dilute (5 mL/L) mixture of nitric acid (HNO_3) at 58% and phosphoric acid (H_3PO_4) at 75% for 15 minutes at 50°C to remove inorganic fouling. In between every membrane cleaning step, the ceramic membrane was rinsed with DI water until neutral solution was obtained. Furthermore, each step of membrane cleaning procedure, permeate flux was measured to evaluate the filtration resistance as described in Section 3.9.

3.9 Filtration Resistance Determination

The effect of fouling on filtration performance can be expressed in terms of hydrodynamic resistance. The resistance-in-series model was used to evaluate the filtration resistance (Choo and Lee, 1996a). According to this model, permeate flux (J) can be expressed as below:

$$J = \frac{TMP}{\mu R_t} = \frac{TMP}{\mu(R_m + R_{rm} + R_{re} + R_{ir})} \qquad \text{Equation 3.1}$$

Where TMP is trans-membrane pressure, μ is viscosity of permeate, R_t is total membrane resistance (1/m), R_m is intrinsic membrane resistance (initial membrane resistance for new membrane), R_{rm} is removable fouling, R_{re} is reversible fouling and R_{ir} is irreversible fouling. The experimental procedure to get each membrane resistance was as follows (i) R_m was tested with DI water for new membrane flux measurement; (ii) R_t was calculated from final flux and TMP; (iii) the membrane surface was then flushed with DI water. After that, tested was measured again to get the resistance of $R_m+R_{re}+R_{ir}$; (iv) membrane was clean with chemical solution. Then, pure water flux was measured again to get the resistance of $R_m+R_{ir.}$ The fouling resistance ($R_{ir,}$ $R_{re,}$ R_{rm} and R_m) was calculated from process (iv), (iii and iv), (ii, iii, iv) and (i), respectively. Figure 3.6 illustrated filtration resistance determination procedure.

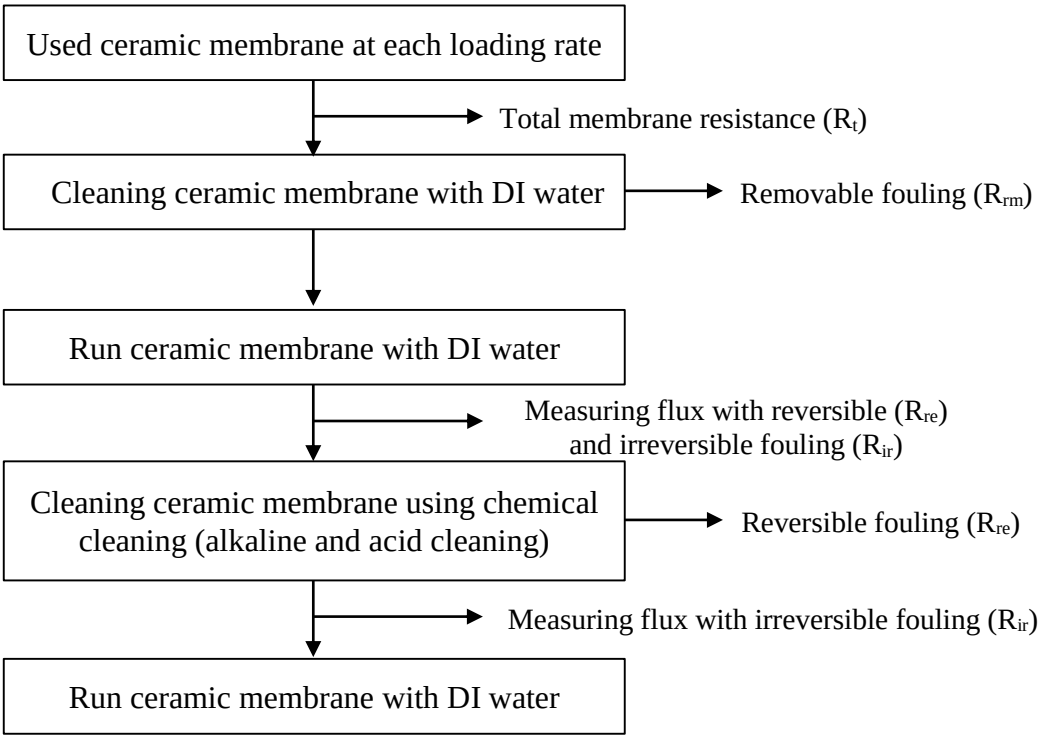


Figure 3.6 Filtration resistance determination

3.10 Analytical Methods

In order to analyze the two stage TAnMBR performances, following testing methods were carried out according to the standard methods (APHA et al., 2005). List of all parameters and their analytical methods are given in Table 3.10. Furthermore, the analytical method of EPS is described in Section 3.10.1.

3.10.1 Analysis of extracellular polymeric substances (EPS)

EPS are important materials for microbial aggregation hence it is critical factor in membrane fouling context. It consists of two main constituents such as polysaccharides (PS) and protein (PN). EPS are in two forms, namely soluble and bound EPS. Soluble EPS was extracted directly by centrifugation of bulk liquor. For measuring bound EPS, cation exchange resin (CER) method given in Frølund et al. (1996) was used. In extracting bound EPS, CER removes the cation from the sludge matrix leading to breakup of the flocs and subsequent release of EPS. Next, EPS was measure for PS and PN according to the methods given in Dubois et al. (1956) and Lowry et al. (1951), respectively. Table 3.8 provides details of CER. During the cleaning process the sludge which attach to the membrane surface was collected for EPS analysis.

Table 3.8 Cation Exchange Resin Specifications

Property	Values/Descriptions
Product	DOWEX® Marathon®
Manufacturer	Sigma-Aldrich
Type	Strong acid cation (Na^+ form)
Matrix	Styrene-DVB gel
Functional group	Sulfonic acid
Bead size distribution range	0.3-1.2 mm (20-50 mesh)
Water content	42-48 %
Maximum operating temperature	120°C (250°F)
pH range	0-14

3.10.1.1 Preparation of CER buffer solution

The CER buffer solution consists of the following constituents and respective concentration as shown in Table 3.9.

Table 3.9 Cation Exchange Resin Buffer Solution Constituents

Chemical Name	Concentration (mM)	Amount in 1 L of DI water (g)
Na_3PO_4	2	164*2/1,000 = 0.328
NaH_2PO_4	4	120*4/1,000 = 0.48
NaCl	9	58.5*9/1,000 = 0.527
KCl	1	74.6*1/1,000 = 0.075

3.10.1.2 Resin preparation

CER was washed in extraction buffer solution for 1 h prior to use. The amount of CER for the analysis was weighted based on the amount of MLVSS and soaked in buffer solution for 1 h. Then the resin was dried at room temperature for 24 h. The CER extraction procedure is presented in Figure 3.7.

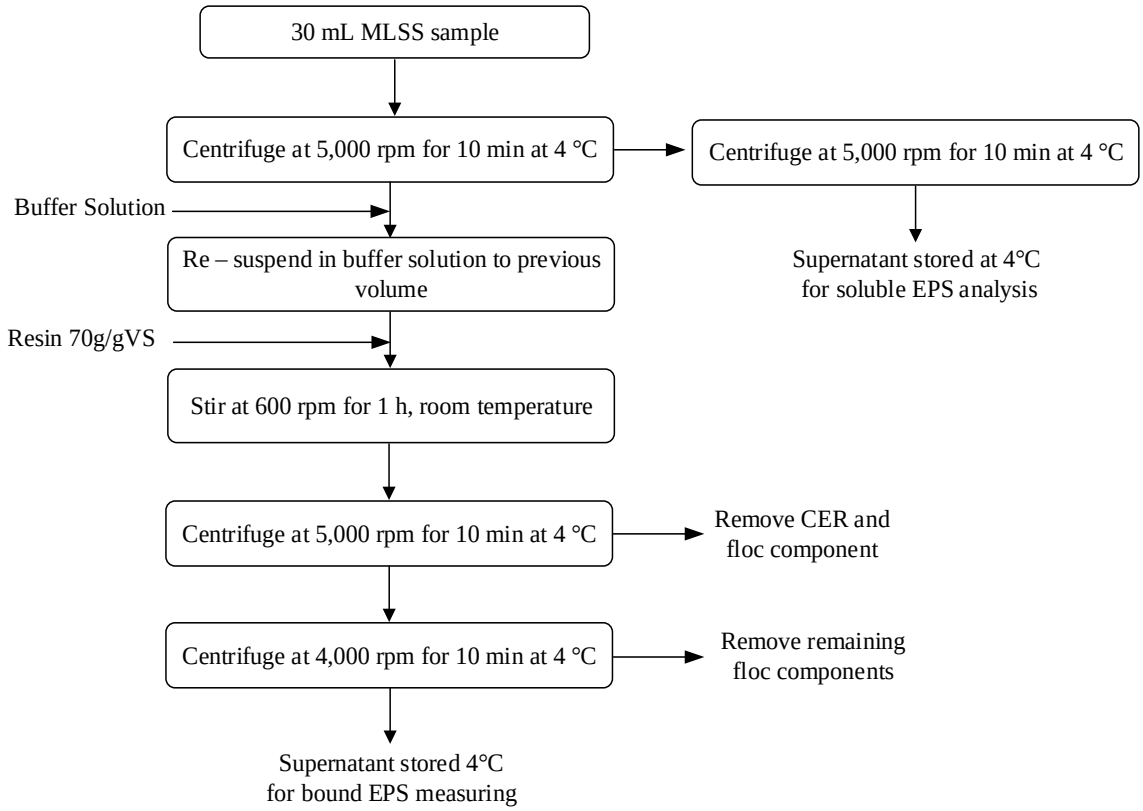


Figure 3.7 Procedure for bound EPS extraction (CER method)

3.10.1.3 Preparation of polysaccharides standard curve

The standard curve for polysaccharides was plotted using analytical glucose as the standard solution. The sugar solution containing 0, 20, 40, 60, 80 and 100 mg/L of glucose were used instead of sample, and the procedure in Figure 3.8 was followed. The standard curve of polysaccharides concentration and absorbance (490 nm) was plotted (Appendix B-1).

3.10.1.4 Polysaccharides (PS) determination (Dubois et al., 1956)

Chemical reagents:

5% phenol solution
Sulfuric acid

Calculation for soluble polysaccharides:

Polysaccharides concentration in solution = Soluble polysaccharides (mg/L)

***Calculation for bound polysaccharides*:**

Polysaccharides concentration in sample	= A mg/L
PS from EPS extracted solution X liter	= AX mg
MLVSS of the sample	= B mg/L

MLVSS if the mixed liquor volume is C liter = BC mg

$$PS(\frac{mg}{gVSS}) = \frac{PS(mg)}{MLVSS(mg)} \times 1{,}000 = \frac{AX}{BC} \times 1{,}000$$

3.10.1.5 Preparation of protein standard curve

The protein standard curve was plotted using Bovine Serum Albumin (BSA) as standard solution. The protein solution containing 0, 20, 40, 60, 80 and 100 mg/L of BSA were used instead of sample, and the procedure in Figure 3.8 was followed. The standard curve of protein concentration and absorbance (750 nm) was plotted (Appendix B-2).

3.10.1.6 Protein (PN) determination (Lowry et al., 1951)

Chemical reagents:

Solution A: 100 mL of (0.5 g $CuSO_4.5H_2O$ + 1 g $Na_3C_6H_5O_7.2H_2O$)
Solution B: 1,000 mL of (20 g $Na_2CO_3^-$ + 4 g NaOH)
Solution C: 51 mL of (1 mL of solution A + 50 mL of solution B)
Solution D: 20 mL of (10 mL of folin ciocaltaeu phenol reagent + 10 mL DI water)

Calculation for soluble protein:

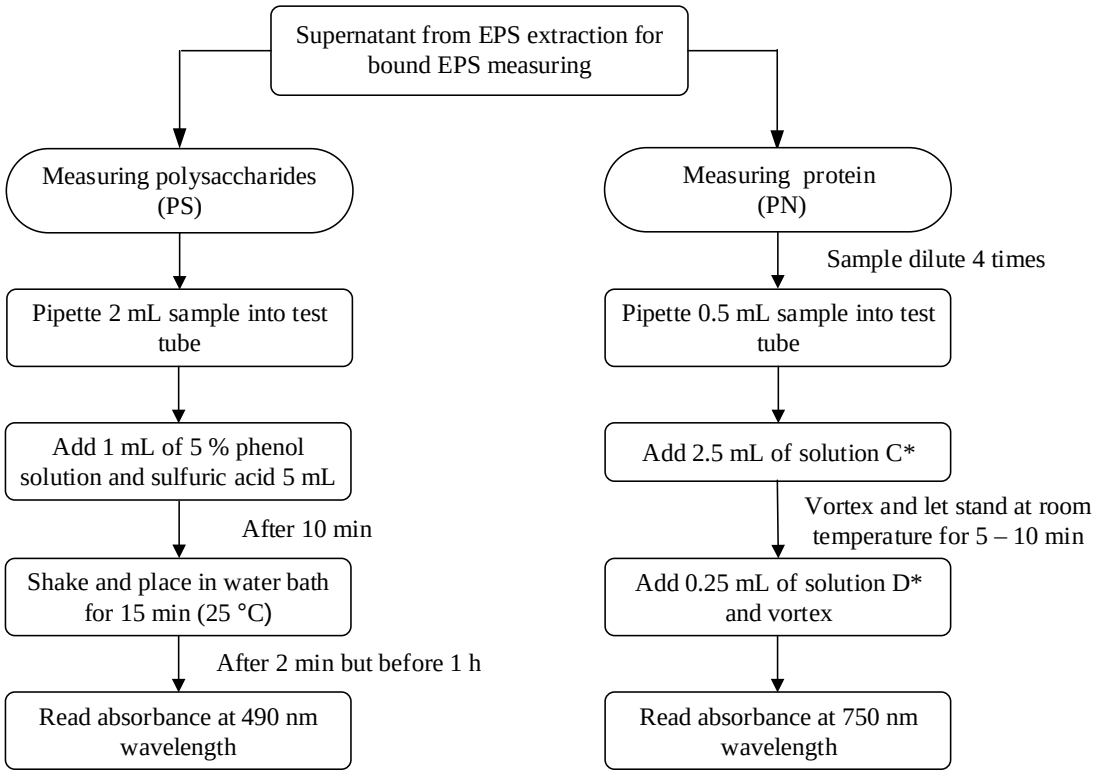
Protein concentration in solution = Soluble protein (mg/L)

Calculation for bound protein:

Protein concentration in sample = A mg/L
PN from EPS extracted solution X liter = AX mg
MLVSS of the sample = B mg/L
MLVSS if the mixed liquor volume is C liter = BC mg

$$PN(\frac{mg}{gVSS}) = \frac{PN(mg)}{MLVSS(mg)} \times 1{,}000 = \frac{AX}{BC} \times 1{,}000$$

The procedure for bound EPS (bPS+bPN) analysis is illustrated in Figure 3.8.



**** Refer to protein analysis***

Figure 3.8 Procedure of polysaccharides and protein analysis

3.10.2 Volatile fatty acids (VFA)

VFA concentration directly indicates the performances of hydrolytic reactor. VFA production was analyzed by gas chromatography (Agilent 7890A) equipped with flame ionization detector (FID) having a capillary column (DB-WAX, Length 30m, Diameter 0.32 mm, film thickness 0.25 µm and max temperature 260^{o}C). The temperature is 250°C, 250°C and 75°C for injector, detector and oven, respectively. The VFA samples in feed, hydrolytic effluent, methanogenic effluent and permeate were analyzed to assess VFA concentration and composition in each stage of the system. Three types of VFA were analyzed, namely acetic acid, propionic acid, and *n*-butyric acid.

3.10.3 Biogas composition

Methane content in biogas is good indicator of anaerobic wastewater treatment. As well as biogas composition is a rapid indicator to assure the anaerobic condition of the system. The biogas composition in both hydrolytic and methanogenic reactors were analyzed separately by gas chromatography (Agilent 7890A) equipped with thermal conductive detector (TCD) having a pack column (WG-100, SUS, Col, 1/4”O.Dx1.8 m). The temperature is 150°C, 150°C and 50°C for injector, detector and oven, respectively.

3.10.4 Methane yield

Methane yield is an indicator of anaerobic wastewater treatment. The biogas produced from hydrolytic and methanogenic reactors was collected in biogas bag (Tedlar bag) and volume of biogas was measured using wet gas meter. Methane yield of AnMBR was calculated as follows.

$$\text{Methane yield } (m^3CH_4/kgCOD_r) = \text{Volume of methane generated}/kgCOD_r$$

3.10.5 PVA-gel characteristics

The settling velocities of PVA-gel beads were determined by the method of Ghangrekar et al. (2005). The amount of biomass attached to PVA-gel (gVSS/gPVA-gel) was determined by weight difference from an average of 30 pairs of new (unused) and granulated PVA-gel beads (Wenjie et al., 2008).

3.10.6 Scanning electron microscopic (SEM)

SEM observations of the PVA-gel structure were conducted as follows: a PVA-gel beads was washed twice for 5 min each time, with 0.1 M phosphate buffer (pH 7.4). Then PVA-gel were hardened for 90 min in a 2.5% glutaraldehyde solution prepared with 0.1 M phosphate buffer. Next, PVA-gel were washed in the buffer solution three times for 10 min each and then fixed for 90 min in a 1% OsO_4 solution prepared with 0.1 M phosphate buffer. After washing samples three times for 10 min each in buffer solution, they were dewatered in serially graded solutions of ethanol at concentrations of 10%, 30%, 50%, 70%, 90% and 95% for 10 min each, and then twice at a concentration of 99.5% for 30 min each time. The samples were frozen and dried using a freeze-drier, and then sputter coated with gold for 100 s with an ion-sputtering device. Finally, the samples were observed with SEM (Wenjie et al., 2008; Khanh et al., 2011).

3.10.7 Statistical analysis

Data analysis was carried out using the SPSS 21.0 statistical package (SPSS, Chicago, IL, USA). The statistical significance of values to compare the mean obtained during each experimental condition was carried out using analysis of variance (one-way ANOVA) to test the significance of results, and $p < 0.05$ was considered to be statistically significant.

Table 3.10 Analytical Parameters and Method of Analysis

Parameters	Unit	Analytical Methods	Equipments/Techniques	Interferences/Remarks	References
Alkalinity	mg/L as $CaCO_3$	Titration method	Titration	Soap, oily matter and suspended solids	APHA et al. (2005)
Biogas production	L/d	Collect in biogas bag (Tedlar bag)	Biogas bag and measurement by wet gas meter	-	-
Methane content	%	Gas chromatography	Gas chromatography (Agilent 7890A) with TCD	-	APHA et al. (2005)
COD	mg/L	5220-C (Close reflux method)	Titration	Halide ions and nitrite (SCOD was measured by filtering the sample through 0.45 µm filter, NSCOD or PCOD was calculated by the difference between TCOD and SCOD)	APHA et al. (2005)
Bound EPS extraction	mg/gVSS	Cation exchange resin (CER method)	Centrifuge	-	Frølund et al. (1996)
Polysaccharides	mg/L	Phenolic-sulfuric acid (UV absorbance 490 nm)	Spectrophotometer (Hitachi U-2900)	-	Dubois et al. (1956)
Protein	mg/L	Lowry method (UV absorbance 750 nm)	Spectrophotometer (Hitachi U-2900)	-	Lowry et al. (1951)
MLSS	mg/L	2540-D (Dry at 103-105°C)	Filter/Oven	Mineralized water and floating material	APHA et al. (2005)
MLVSS	mg/L	2540-E (Ignite at 550°C)	Furnace	Loss of volatile inorganic salts like $(NH_4)_2CO_3$	APHA et al. (2005)
NH_4^+-N	mg/L	4500-B (Distillation method)	Titration	Nitrate > 10 mg/L, inorganic salts and solids	APHA et al. (2005)
pH	-	Glass Electrode	pH meter	Sodium if pH > 10 and temperature	-
VFA	mg/L	Gas chromatography	Gas chromatography (Agilent 7890A) with FID	Measure sample within one week to avoid VFAs evaporation	APHA et al. (2005)

Chapter 4

Results and Discussions

The results of the study are presented in five parts namely seed sludge enrichment and acclimatization, hydrolytic reactor performance, methanogenic reactor performance, system performance with comparison at different loading rates and membrane fouling. The first part discusses about the enrichment and acclimatization of hydrolytic/acidogenic bacteria and methanogenic archaea. The second and third parts presents the hydrolytic reactor performance with and without PVA-gel addition as biocarrier and methanogenic reactor performance, respectively. The fourth part discusses about the system performance with respect to VFA production, methane generation, biological activity and organic removal rates. The last section of this chapter presents the analysis and interpretation of membrane fouling in the system. Furthermore, a comparative performance of high rate anaerobic reactor, mesophilic AnMBR with the findings from this study. Finally, this chapter presents the optimum system conditions required for the treatment of high strength particulate wastewater.

4.1 Enrichment and Acclimatization of Anaerobic Seed Sludge

Mesophilic anaerobic seed sludge was collected from a UASB reactor in Phathumthani Brewery Co., Ltd. The microorganisms were enriched and acclimatized for hydrolytic and methanogenic reactor separately. Initially, anaerobic seed sludge was enriched for hydrolytic and methanogenic reactor in SBR reactor for 77 days. After that the enriched hydrolytic bacteria and methanogenic archaea were subjected to AnMBR and the acclimatization were continued until 145 days. For the first loading rate (thermophilic condition), temperature, COD concentration and flow rate were increased gradually with time to prevent shock loading in the anaerobic reactor.

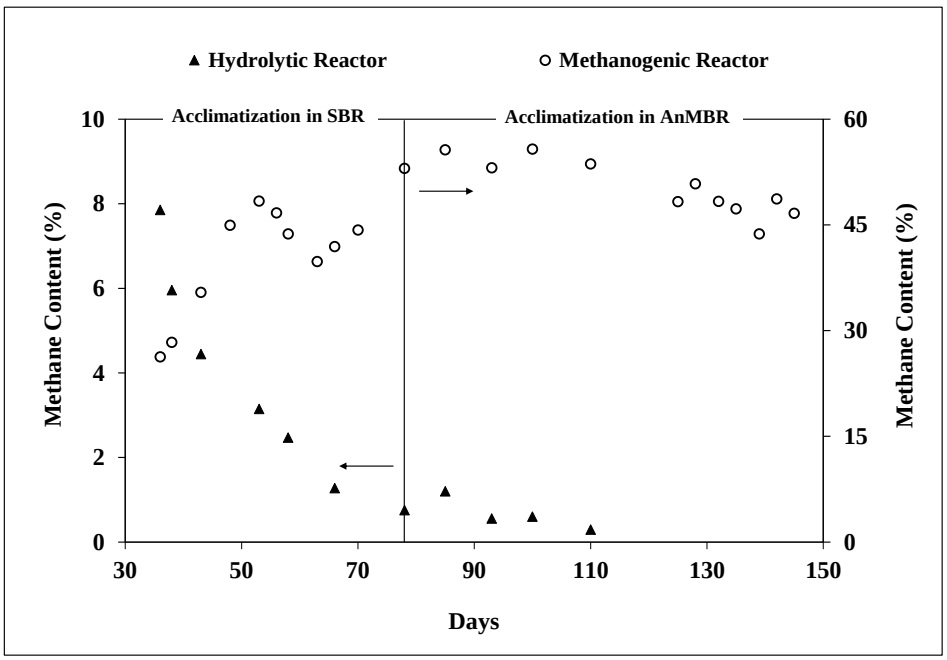


Figure 4.1 Methane content in hydrolytic and methanogenic reactors during acclimatization

During the acclimatization phase, a decrease in biomass concentration was observed in both hydrolytic and methanogenic reactors. This was due to washout and decay of the biomass during enrichment of anaerobic sludge. Furthermore, the difference in growth rate between hydrolytic/acidogenic bacteria and methanogenic archaea led to longer enrichment and acclimatization of 145 days to get the first overall loading rate of the two stage TAnMBR at 6 kgCOD/m^3.d. As illustrated in Figure 4.1, methane content in hydrolytic reactor decreased from 7.9 to 0.3 % and during the same time the methanogenic reactor methane content increased from 26.3 to 53.7%. The enrichment of hydrolytic/acidogenic bacteria and methanogenic archaea were confirmed through methane content analysis. Saddoud et al. (2007) and Mota et al. (2013) also reported similar results of no or less observable methane content as an indicator for enrichment of acidogenic bacteria in the hydrolytic reactor. Additionally, Guerrero et al. (1999) and Liu et al. (2006) also found that with lower pH and shorter HRT (6-24 h) in the hydrolytic reactor, acidogenic bacteria could be effectively enriched.

4.1.1 Hydrolytic reactor pH, temperature, OLR, VFA and MLVSS variation

Mesophilic anaerobic seed sludge for hydrolytic reactor was acclimatized in batch process by gradually increasing the loading rate and temperature simultaneously to thermophilic condition at 55°C. At the initial phase, HCl solution was mixed with sludge to bring down the pH of the hydrolytic reactor to 5.5±0.2, during the initial acclimatization phase (batch process). Tapioca starch based synthetic wastewater was used as feed, and the feed pH was maintained at 7.4±0.2 during this period. After 40 days of operation, pH adjustment was not required as the pH was found to be stable at 5.4±0.2. The temperature and pH variation of the system during acclimatization phase are presented in Figure 4.2 and the detailed experiment data is documented in Appendix C-1. Furthermore, Figure 4.3 illustrated the variation of MLVSS concentration and loading rate of the reactor during acclimatization period and detailed data are presented in Appendix C-2.

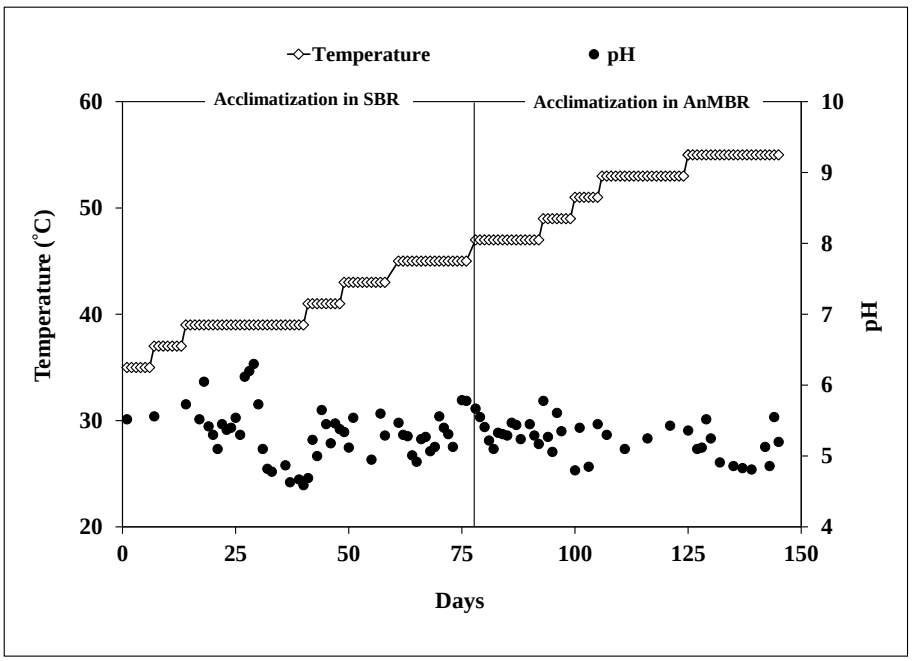


Figure 4.2 Temperature and pH variation of hydrolytic reactor during acclimatization

Rector stabilization can be evaluated by observing fairly consistent performance of hydrolytic reactor in terms of MLVSS and VFA concentration (Ferrer et al., 2010). During 77 days of acclimatization, a reduction in MLVSS from 13.7±0.6 to 5.0±0.8 g/L was observed. This was attributed to the decay in methanogenic archaea that were present in the anaerobic seed sludge and washed out as biomass. After that MLVSS concentration increased gradually and end up at 8.9±0.1 g/L. Additional increase in MLVSS concentration was observed after membrane was connected to the system in order to avoid biomass washout. Moreover, total VFA concentration of the system increased gradually from 1.8±0.4 to 2.7±0.1 g/L. Total VFA concentration during acclimatization phase is illustrated in Figure 4.4 and the detail data can be found in Appendix C-2. Nevertheless, a reduction in total VFA concentration was observed after 140 days of reactor acclimatization. This was due to a rapid increase in loading rate. The summary of the results for the hydrolytic reactor during acclimatization is given in Table 4.1.

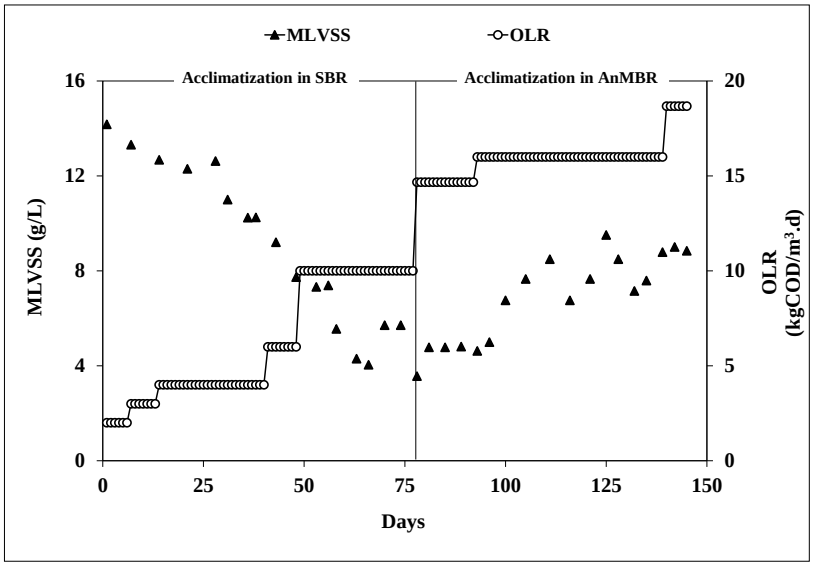


Figure 4.3 MLVSS and OLR variation of hydrolytic reactor during acclimatization

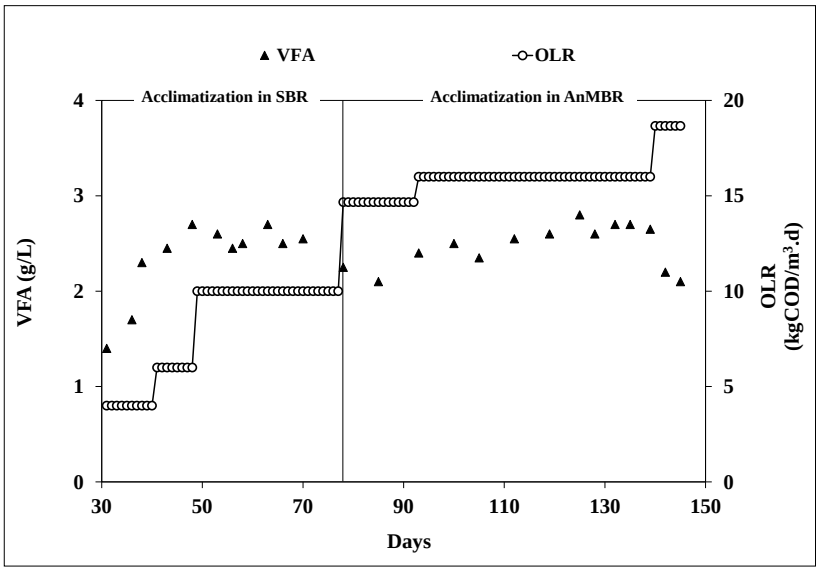


Figure 4.4 VFA and OLR variation of hydrolytic reactor during acclimatization

Table 4.1 Summary of the Results for the Hydrolytic Reactor during Acclimatization

Day (d)	OLR (kgCOD/m^3.d)	pH	VFA (g/L)	MLVSS (g/L)	Methane Content (%)	T (^{o}C)
1-6	2.0	5.5±0.2	-	13.7±0.6	-	35
7-13	3.0	5.6±0.1	-	13.0±0.5	-	37
14-40	4.0	4.7±0.1	1.8±0.4	11.0±1.1	7.9	39
41-48	6.0	5.4±0.2	2.6±0.2	8.5±1.0	5.2	41
49-58	10.0	5.5±0.3	2.5±0.1	5.0±0.8	2.8	43
59-77					1.9	45
78-92	14.67	5.3±0.1	2.2±0.1	4.8±0.6	1.0	47
93-99	16.0	5.3±0.2	2.7±0.1	8.3±0.9	0.6	49
100-105					0.6	51
106-124					0.3	53
125-139					nd	55
140-145	18.67	5.2±0.3	2.2±0.1	8.9±0.1	nd	55

4.1.2 Methanogenic reactor pH, temperature, OLR and COD removal efficiency variation

Mesophilic anaerobic sludge was acclimatized in batch operational mode, by providing butyric acid as substrate. This is led to enrich methanogenic archaea of anaerobic seed sludge. Temperature and loading rate were gradually increased to 8 kgCOD/m^3.d and 55°C (in methanogenic reactor). Temperature was gradually increased to avoid drastic drop in biological activity due to sudden temperature increment.

Methanogenic reactor pH was maintained at 7.2±0.3 during the acclimatization period by adjusting pH of anaerobic seed sludge using $NaHCO_3$. Temperature and pH profile during this phase is illustrated in Figure 4.5 and detail data is presented in Appendix C-1. The system stability was assessed by fairly stable MLVSS concentration and COD removal efficiency. The methanogenic reactor's MLVSS concentration, loading rate and COD removal efficiency are presented in Figure 4.6. The detail data is given in Appendix C-2. During the first 30 days of acclimatization, MLVSS concentration was found to be stabilized at 20.1±0.8 g/L, then started to decreased. At the end of acclimatization phase (batch operational mode), MLVSS concentration was observed at 9.2±0.4 g/L. However, once methanogenic reactor was connected with membrane, an increase in MLVSS concentration was observed to be at 12.0±0.9 g/L. Then, there were some operational and maintenance (O&M) issues on 118 days of reactor acclimatization. This led to the loss of the MLVSS concentration to 5.9±0.5 g/L. However, MLVSS concentration sharply increased from 5.9±0.5 to 15.2±1.1 g/L because leaked biomass was added back to methanogenic reactor. After that MLVSS concentration started to further increase with time and was stable at 16.6±0.9 g/L. Increasing in MLVSS concentration from 15.2±1.1 to 16.6±0.9 g/L took about 15 days, due to total biomass retention in TAnMBR. Furthermore, an accumulation of methanogenic archaea during reactor acclimatization led to decrease in start-up time of TAnMBR. Therefore, the major drawback of high rate anaerobic reactor could be avoid by simply using a microfiltration membrane. Thus suggesting the potential benefits of using TAnMBR for industrial wastewater treatment.

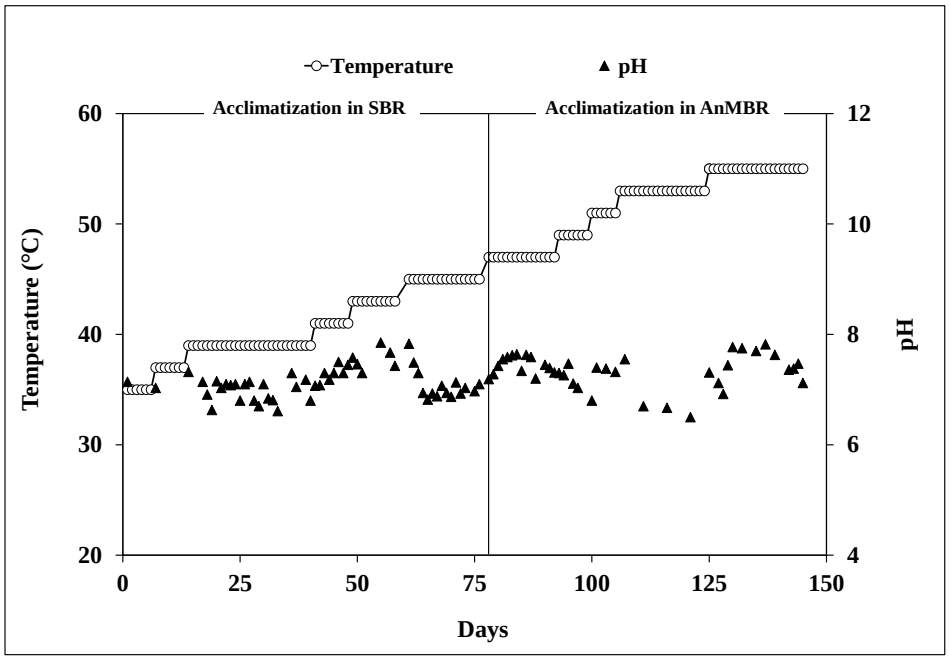


Figure 4.5 Methanogenic reactor temperature and pH variation during acclimatization

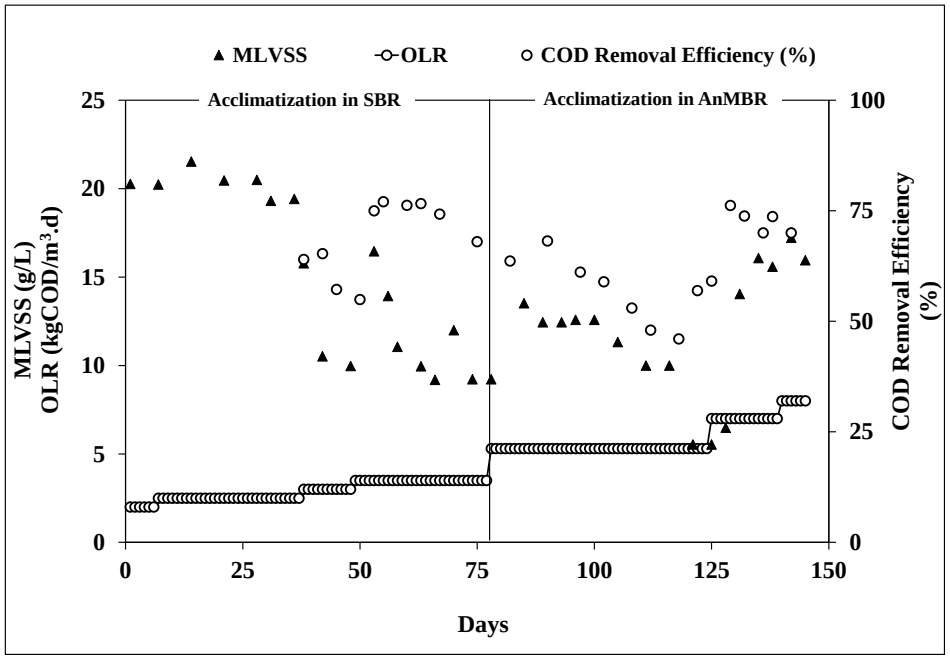


Figure 4.6 Methanogenic reactor MLVSS, OLR and COD removal efficiency variation during acclimatization

Meanwhile, COD removal efficiency was investigated to examine the overall organic removal during acclimatization. Decreasing in COD removal efficiency was observed when reactor acclimatization changed from batch to continuous operational mode. This was due to the increase in flow rate leading to a corresponding increase in loading rate. With the above mentioned O&M issue, the average COD removal efficiency was also affected and was observed as 54.0%. However after 130 days of acclimatization, the COD removal efficiency of the system increased and with corresponding increment in MLVSS

concentration (Figure 4.6). The summary of the results for the methanogenic reactor during acclimatization is given in Table 4.2.

Table 4.2 Summary of the Results for the Methanogenic Reactor during Acclimatization

Day (d)	OLR (kgCOD/m^3.d)	pH	Removal Efficiency (%)	MLVSS (g/L)	Methane Content (%)	T (^oC)
1-6	2.0	7.1±0.1	-	20.3±0.2	-	35
7-13	2.5	7.0±0.2	-	19.9±0.6	-	37
14-37			-		26.3	39
38-40	3.0	7.3±0.1	64.7	10.3±0.4	28.3	39
41-48			56.1		40.2	41
49-58	3.5	7.6±0.2	76.0	12.5±0.9	46.3	43
59-77	3.5	7.1±0.1	71.1	9.2±0.4	42.0	45
78-92	5.3	7.3±0.1	68.1	11.7±0.8	53.9	47
93-99		7.2±0.2	61.1	12.5±0.5	54.4	49
100-105		7.4±0.3	58.9	12.0±0.9	55.8	51
106-117		7.0±0.5	56.0	10.4±1.2	53.7	53
118-124		7.2±0.4	54.0	5.9±0.5	49.0	53
125-129	7.0					55
130-139		7.7±0.1	72.5	15.2±1.1	46.5	55
140-145	8.0	7.3±0.1	71.8	16.6±0.9	47.7	55

4.2 Performance Evaluation of Hydrolytic Reactor

This section shows the hydrolytic reactor performance with respect to pH, MLVSS, VFA production, organic matter and biological activity. The hydrolytic reactor performance at three difference loading rates such as 6 (with and without PVA-gel), 8 (with PVA-gel) and 12 (with PVA-gel) kgCOD/m^3.d are discussed and compared.

4.2.1 pH, VFA and MLVSS variation in hydrolytic reactor

As mentioned in Section 4.1 (after acclimatization), the hydrolytic reactor pH was stable at 5.4±0.5 and continued to be the same during three loading conditions. Further pH adjustment was not required during the entire duration of this study. Thus indicating system stability with respect to the hydrolytic reactor.

At the first loading rate (6 kgCOD/m^3.d with and without PVA-gel), MLVSS concentration was maintained at 9.6±0.5 g/L in order to study the hydrolytic reactor performance with and without PVA-gel addition on VFA production. Nevertheless, MLVSS started to decrease to 4.4±0.7 and 3.8±0.4 g/L at loading rate 8 and 12 kgCOD/m^3.d, respectively. At steady operating conditions of 8 and 12 kgCOD/m^3.d loading rate, MLVSS concentration exhibited a little changed indicating a balance between the degradation of old biomass and fresh biomass accumulation. Even though this value may be considered low, but similar observation were reported by various researchers studying anaerobic condition (Vavilin et al., 2008; Trzcinski and Stuckey, 2009; Wijekoon et al., 2011). Nonetheless even with low MLVSS concentration, hydrolytic reactor showed good performances in terms of VFA

production. The variation in VFA production and MLVSS concentration during this period is illustrated in Figure 4.7 and the detail can be found in Appendix D-1.

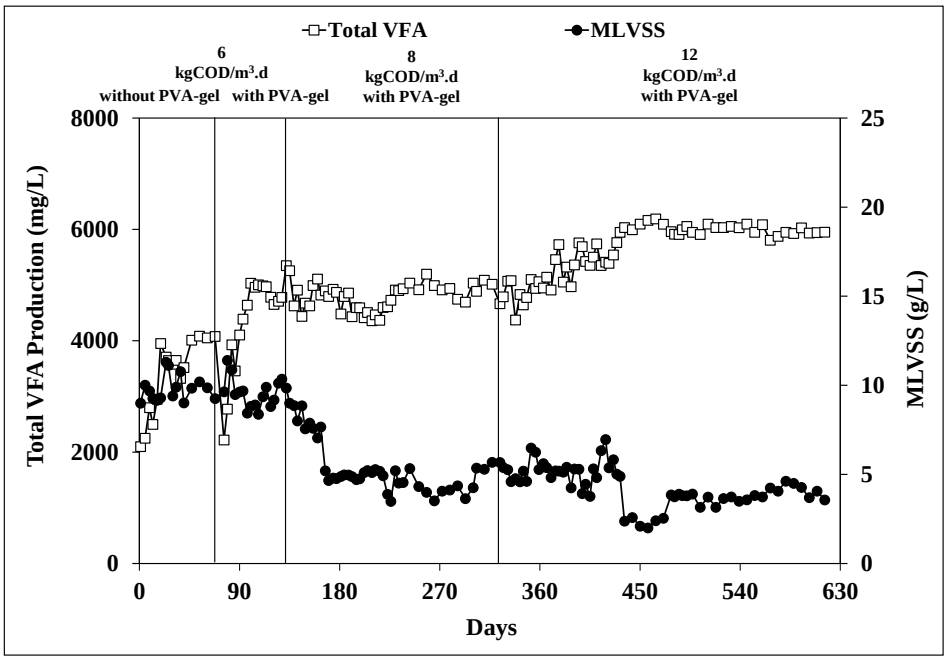


Figure 4.7 VFA production and MLVSS concentration variation of hydrolytic reactor

VFA production is considered as an important indicator for hydrolytic reactor performance. The VFA production is the difference between VFA in hydrolytic effluent and VFA in feed, and it was analyzed for all loading rates. For this purpose three main types of VFA were analyzed namely acetic acid, *n*-butyric acid and propionic acid by gas chromatography (GC) (Section 3.10.2). It was observed that VFA production in the hydrolytic reactor significantly increased from 4.0±0.2 to 4.6±0.5 g/L with PVA-gel addition at OLR 6 kgCOD/m^3.d ($p < 0.05$). Once the loading rate has increased to 8 and 12 kgCOD/m^3.d, VFA production also significantly increased to 4.9±0.2 and 6.0±0.1 g/L ($p < 0.05$), respectively. These results illustrated the hydrolytic reactor performance with PVA-gel addition increased in terms of VFA production. The increase in VFA can be directly correlated to an increase in biological activity with PVA-gel addition and concentration of organic matter in feed. The total VFA concentration comparison of this study and reported studied are illustrated in Table 4.3.

Considering individual VFA production, *n*-butyric acid and acetic acid were observed to be the predominant VFA species for all operating conditions. Furthermore, propionic acid was also observed but in very low concentration as compared with *n*-butyric acid and acetic acid. VFA species distribution for all three loading rates are illustrated in Figure 4.8 and the detail data is given in Appendix D-2.

Additionally it was observed that acetic acid concentration also increased from 29.2% to 36.9% with PVA-gel addition at loading rate of 6 kgCOD/m^3.d. Once loading rate was increased, the percentage of acetic acid increased to 39.1% and 47.8% at loading rate 8 and 12 kgCOD/m^3.d, respectively. Nevertheless, *n*-butyric acid was observed to be decreasing state from 65.2% to 55.9% with PVA-gel addition at 6 kgCOD/m^3.d loading rate. It was also decreased to 52.2% and 45.4% with increase in OLR to 8 and 12 kgCOD/m^3.d, respectively. Even though *n*-butyric acid and acetic acid are the main products of hydrolysis/acidogenesis from organic matters, but the VFA composition can be affected by many operational

parameters such as substrate characteristics, HRT, OLR, temperature, pH and reactor configuration. Jiang et al. (2013) also reported that butyric acid was the major VFA composition at 55°C followed by acetic acid. Nevertheless, acetic acid increased when loading rate increased, while butyric acid tend to decrease at higher loading conditions. The similar observations were also reported by Lim et al. (2008). Furthermore, very low propionic acid was observed in this study as the feeding contains less amount of lactic acid, which could be subsequently converted to propionic acid (Wang et al., 2009a).

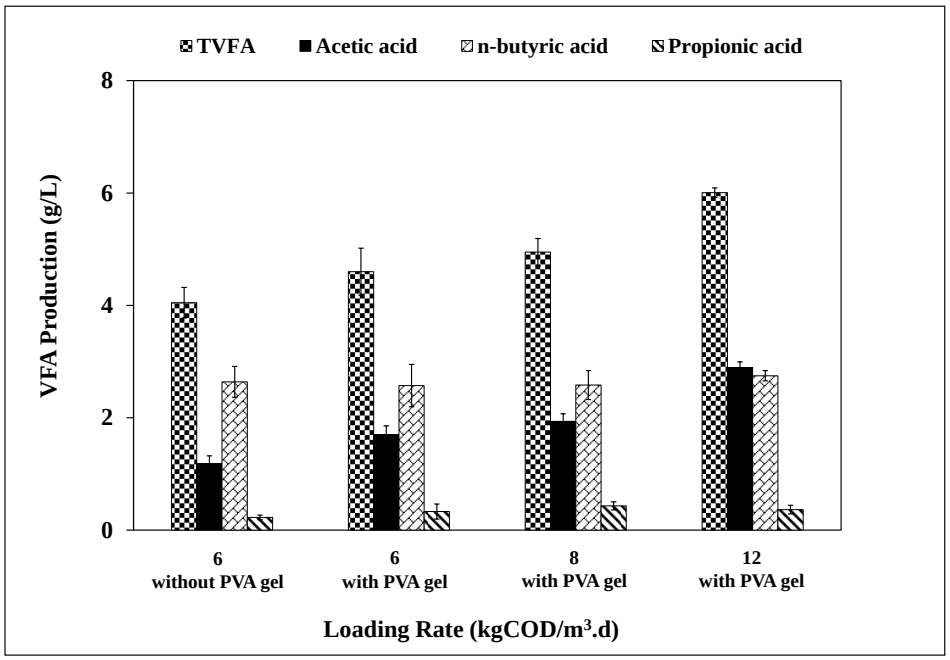


Figure 4.8 Total VFA production and VFA species variation

An increment in acetic acid (%), while decreasing *n*-butyric acid (%) was observed in all three loading rates with PVA-gel addition. This was due to an increase in conversion rate of *n*-butyric acid to acetic acid by acetogenic bacteria. The VFA species distribution finding in this study were favorable due to high acetic acid and butyric acid in total VFA. As there is a positive correlation between methane production and acetic acid (%). Therefore, optimization of hydrolytic reactor with PVA-gel addition to achieve higher acetic acid and *n*-butyric acid would increase methane production.

The specific VFA production rate is used to indicate the ability of microbes to produce VFA from organic matters of particular wastewater per unit volume of reactor or per unit dried weight of biomass, which is very useful for scaling up a reactor. The specific VFA production rate increased with PVA-gel addition at similar loading rate of 6 kgCOD/m^3.d. It was observed as 5.0 gVFA/L.d and 0.50 gVFA/gMLVSS.d (without PVA-gel), 5.7 gVFA/L.d and 0.61 gVFA/gMLVSS.d (with PVA-gel). The specific VFA production rate also increased with increasing loading rate from 6 kgCOD/m^3.d to 12 kgCOD/m^3.d (with PVA-gel). It was observed as 5.7 gVFA/L.d and 0.61 gVFA/gMLVSS.d, 6.1 gVFA/L.d and 1.11 gVFA/gMLVSS.d, and 9.0 gVFA/L.d and 2.27 gVFA/gMLVSS.d at loading rate of 6, 8 and 12 kgCOD/m^3.d (with PVA-gel), respectively.

Table 4.3 Comparison of Total VFA Concentration between This Study and Literature

Wastewater	Reactor	T (°C)	OLR (kgCOD/m^3.d)	VFA (g/L)	Reference
Synthetic (Molasses)	Multistage biofilm	35	5-9	1.5-3.7	Ghaniyari-Benis et al. (2009)
Synthetic (Molasses)	Two stage TAnMBR	55	5-12	2.5-6.9	Wijekoon et al. (2011)
Cassava wastewater	Two stage UASB	55	5-15	5.0-13.0	Intanoo et al. (2014)
Synthetic (Tapioca starch)	Two stage TAnMBR	55	6-12	4.0-6.0	This study

4.2.2 Evaluation of total, soluble and particulate COD (TCOD, SCOD and PCOD) in hydrolytic reactor

The organic content of substrate was measured in terms of COD. The difference between TCOD and PCOD showed the effectiveness of hydrolysis process in terms of SCOD. Variation of TCOD, SCOD and PCOD in feed and hydrolytic effluent is illustrated in Figure 4.9. The detail data is given in Appendix D-3. TCOD in hydrolytic effluent for all loading conditions was observed to be more than in feed. This was due to biomass washout from hydrolytic reactor. The similar observation was reported by Kayhanian (1994) for biomass washout from hydrolytic reactor in two stage anaerobic reactor. The TCOD removal efficiency comparison of this study and reported studied are illustrated in Table 4.4.

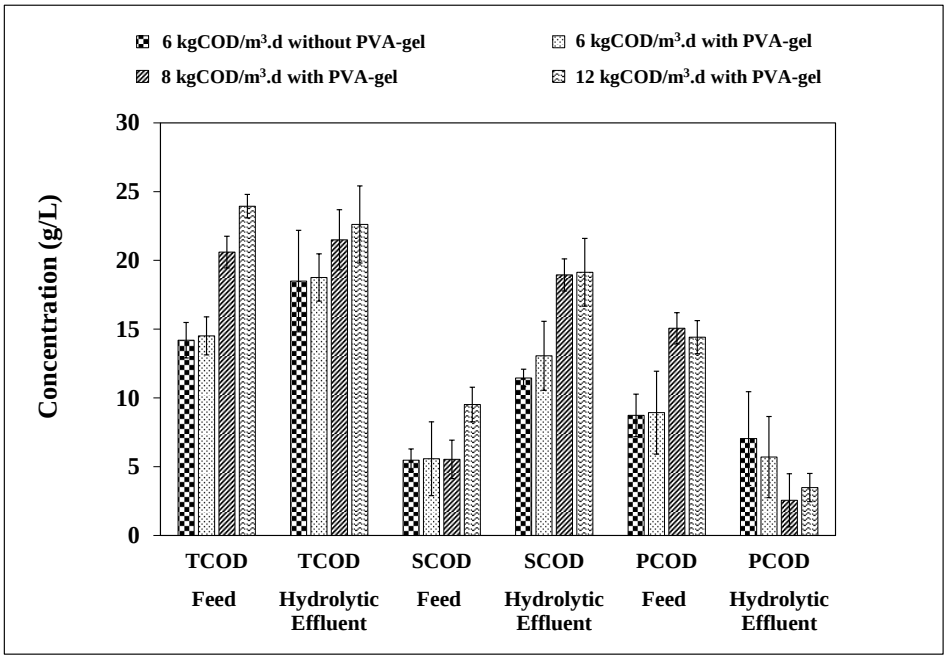


Figure 4.9 Variation of TCOD, SCOD and PCOD of hydrolytic reactor

As illustrated in Figure 4.9, PCOD decreased from 7.0±3.4 g/L to 5.7±2.9 g/L at loading rate 6 kgCOD/m^3.d after PVA-gel was added, which further decreased to 2.6±1.9 g/L at loading rate 8 kgCOD/m^3.d. Nevertheless, PCOD was observed to have increase to 3.5±1.1 g/L when OLR was increased to 12 kgCOD/m^3.d. On the contrary, SCOD significantly increased from 11.5±0.6 g/L (without PVA-gel addition) to 13.1±2.5 g/L (with PVA-gel addition), while no

significantly increased to 18.9±1.2 and 19.1±2.5 g/L ($p < 0.05$) when loading rate has increased to 8 and 12 kgCOD/m^3.d with PVA-gel addition, respectively.

Table 4.4 TCOD Removal Efficiency Comparison of This Study and Literature

Wastewater	Reactor	T (°C)	$TCOD_{inf}$ (g/L)	$TCOD_{eff}$ (g/L)	Removal Efficiency (%)	Reference
Cheese whey	Two stage AnMBR	37	68.6	65.6-56.6	18	Saddoud et al. (2007)
Cassava Wastewater	Two stage UASB	55	15	9.75-12	20-35	Intanoo et al. (2014)
Synthetic (Tapioca starch)	Two stage TAnMBR	55	15-24	13.0-19.1	8-20	This study

Suspended solids (SS) in hydrolytic reactor decreased from 10.8±1.4 to 5.8±1.0 and 4.3±0.4 g/L with an increase in total VFA production from 4.6±0.5 to 4.9±0.2 and 6.0±0.1 g/L at OLR 6, 8 and 12 kgCOD/m^3.d with PVA-gel, respectively. Implying that SS in hydrolytic reactor was being utilized by acidogens to produce SCOD and VFA found in hydrolytic effluent. Similar observation with respect to decrease in SS and increase in SCOD and VFA was reported by Komemoto et al. (2009), who examined the effect of temperature on anaerobic solubilization of food waste.

4.2.3 Biological activity of hydrolytic reactor

New PVA-gel beads have a ceramic-white color, while the biomass in hydrolytic reactor was observed to be brown in color. By the end of the study, PVA-gel had turned to a light brown color, while the surrounding biomass in hydrolytic reactor remained the same. This color change on the PVA-gel surface could be attributed to the physical attachment of the bacterial species on to the gel surface. The matured (brown color) with a biomass attachment of 0.5 gVSS/gPVA-gel had a settling velocity 228 m/h which was more than that observed for new PVA-gel, i.e. 143 m/h.

In order to observe the morphology of the biomass attached on PVA-gel, Scanning Electron Microscopic (SEM) analysis were carried out. The regular (1x magnification) and SEM images original PVA-gel and matured PVA-gel are presented in Figure 4.10 (a, c). As observed in Figure 4.10 (b), PVA-gel had a porous and reticulate macrostructure that could potentially trap and carry hydrolytic/acidogenic bacteria. As illustrated in Figure, 4.10 (d), matured PVA-gel mainly composed of hydrolytic/acidogenic cocci and bacilli. This indicated that the bacteria could effectively attach on PVA-gel surface. An increase in VFA production in hydrolytic reactor with constant biomass concentration could be directly attributed to an increment in biological activity with PVA-gel addition. This was observed by an increase in biological activity from 0.50 to 0.61 gVFA/gMLVSS.d at OLR 6 kgCOD/m^3.d once PVA-gel was added. These observations inferred the effectiveness of using PVA-gel as biocarrier in hydrolytic reactor to increase VFA production and biological activity. Furthermore, biological activity of hydrolytic reactor with PVA-gel addition increased with an increase in loading rates. Biological activity was observed to be 18.9, 20.3 and 29.9 $gVFA_{production}$/L of PVA-gel.d at 6, 8 and 12 kgCOD/m^3.d, respectively. The increment in VFA production and biological activity of hydrolytic reactor with PVA-gel addition at difference loading conditions are illustrated in Figure 4.11.

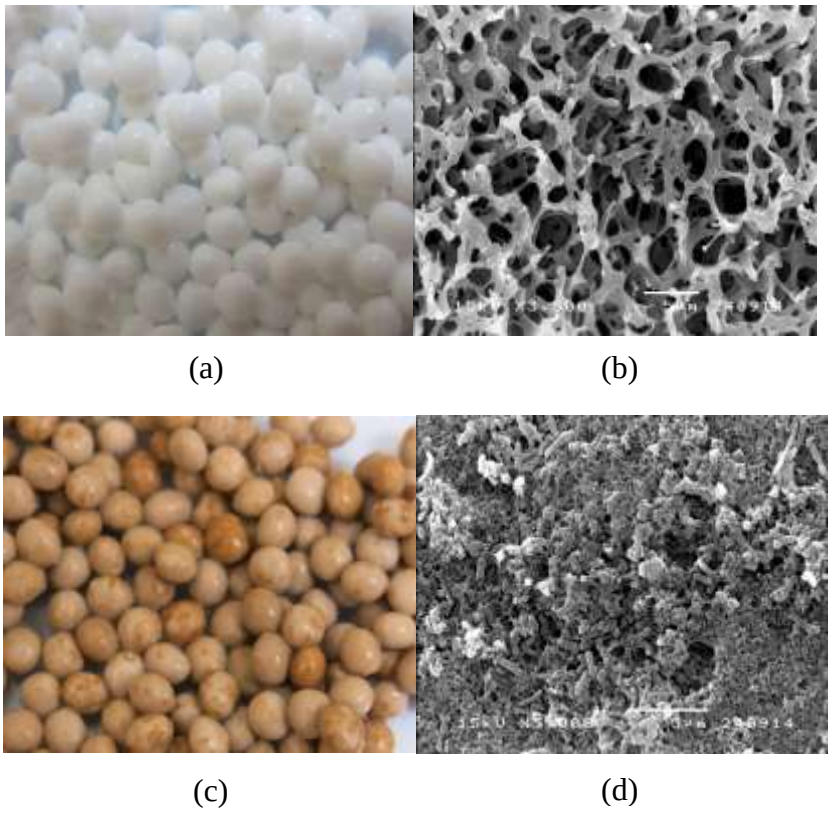


Figure 4.10 SEM of PVA-gel. (a) Blank PVA-gel, (b) Macrostructure of blank PVA-gel, (c) Cultivated PVA-gel, (d) Macrostructure of cultivated PVA-gel

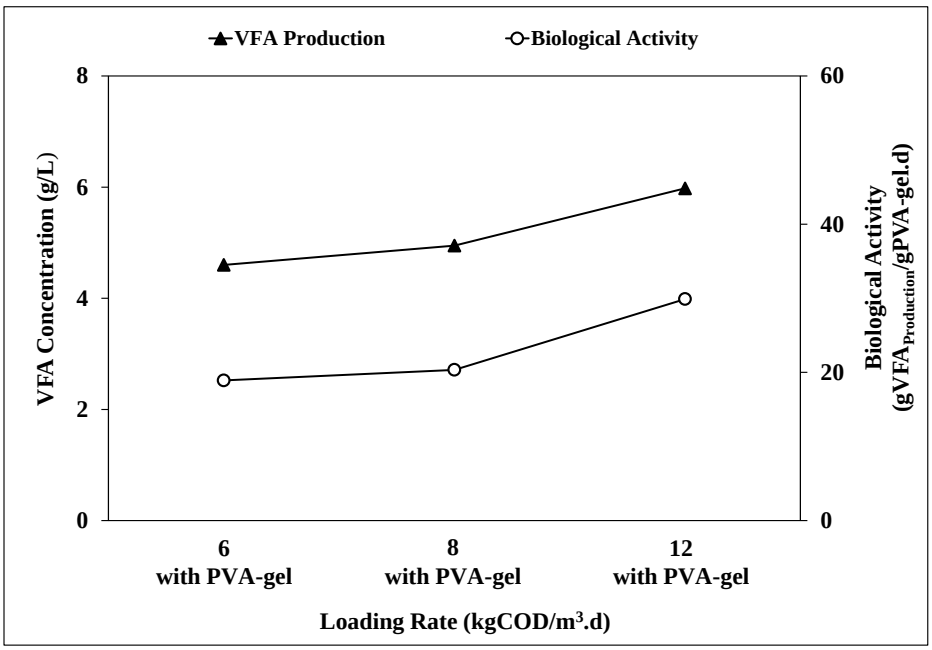


Figure 4.11 VFA production and biological activity of hydrolytic reactor with PVA-gel addition

4.3 Performance Evaluation of Methanogenic Reactor

This section discusses the performance evaluation of methanogenic reactor with respect to methane production, methane content, MLVSS, ammonium nitrogen and alkalinity. Furthermore, the performance evaluation at three loading conditions namely 6, 8 and 12 kgCOD/m^3.d were compared here.

4.3.1 Methane production and methane content

Average methane production and methane content of the system at loading rate of 6 kgCOD/m^3.d without (early stage) and with (later stage) PVA-gel addition were observed as 13 and 15 L/d, respectively. Interestingly, the system showed an increasing in methane production with PVA-gel addition at 6 kgCOD/m^3.d. Furthermore, significant increment in methane production was observed with increasing in loading rates. A significant increasing in methane production in the system during loading rates of 6, 8 and 12 kgCOD/m^3.d with PVA-gel addition in hydrolytic reactor were observed as 15, 17 and 21 L/d ($p < 0.05$), respectively. This increment in the methane production with different loading rates was due to an increase in VFA concentration in hydrolytic effluent with PVA-gel addition as discussed in Section 4.2. Similar observations of an increase in methane production with progressively increasing loading rates have been extensively reported by various authors (Yeoh, 1997; Wijekoon et al., 2011). The variation of methane production and methane content during reactor operation is presented in Figure 4.12. A detailed dataset for the same is presented in Appendix D-4.

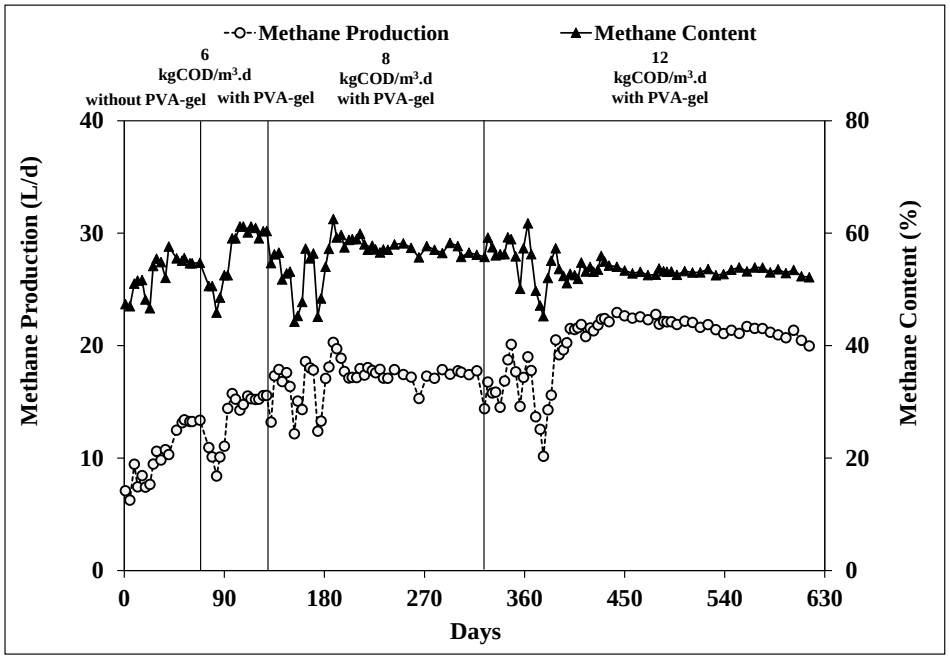


Figure 4.12 Variation of methane production and methane content during reactor operation

At steady stage condition, methane content in biogas produced from the system was observed to be in the range 53-60% for all loading conditions. As one of the prominent indicators of methanogenic reactor performance was methane productivity (methane production per unit volume of reactor). It was observed that after PVA-gel addition at loading rate 6 kgCOD/m^3.d, methane productivity of the system significantly increased from 1.4 to 1.7

$L_{methane}/L_{reactor}$.d (p <0.05). Similarly with PVA-gel addition at OLR 8 and 12 kgCOD/m^3.d methane productivity further significantly increased to 1.9 and 2.4 $L_{methane}/L_{reactor}$.d (p < 0.05), respectively. It can be observed that methane productivity at loading rate 12 kgCOD/m^3.d was 2.4 times of reactor volume. A comparative study with the literature (high strength wastewater) also presents that the methane productivity observation in this study was in the similar range as presented in Table 4.5. But it should be noted that high strength particulate wastewater is not feasible to operate with UASB and other conventional systems.

Table 4.5 Methane Productivity Comparison

Wastewater	Reactor	T (°C)	OLR (kgCOD/m^3.d)	Methane Productivity ($L_{methane}/L_{reactor}$.d)	Reference
Papermill wastewater	Anaerobic Filter	55	5 8 12	1.0 2.2 2.8	Yilmaz et al. (2008)
Synthetic (Molasses)	AnMBR	55	5 8 12	1.7 2.5 3.5	Wijekoon et al. (2011)
Synthetic (Tapioca starch)	Two Stage TAnMBR	55	6 (without PVA-gel) 6 8 12	1.4 1.7 1.9 2.4	This study

4.3.2 Ammonium nitrogen concentration, alkalinity and pH

The ammonium nitrogen concentration (NH_4^+-N) increased with an increase in loading rate. An average ammonium nitrogen concentration in methanogenic reactor was observed to be 664, 900 and 1,100 mg/L at loading rate 6, 8 and 12 kgCOD/m^3.d, respectively. Thus if higher loading rates were applied ammonium nitrogen concentration can be expected to be higher. Alkalinity of the methanogenic reactor was identified as 2,590, 3,480, 3,695 and 4,747 mg/L at loading rate 6 (without and with PVA-gel addition), 8 and 12 kgCOD/m^3.d with PVA-gel addition in hydrolytic reactor, respectively. The methanogenic reactor's alkalinity increased proportionally with an increase in VFA concentration in the hydrolytic effluent. This could be observed by neutral pH in the methanogenic reactor even though hydrolytic reactor effluent was acidic. In addition, the methanogenic reactor pH was maintained at 7.2±0.3 over the operational period. However, once the methanogenic reactor pH decreased to below 6.9, $NaHCO_3$ was added directly to methanogenic reactor for increasing pH to 7.2, which was suitable pH for methanogenic archaea. Variation of ammonium nitrogen concentration, alkalinity and pH of methanogenic reactor during reactor operation are presented in Figure 4.13 and the detail data is given in Appendix D-5.

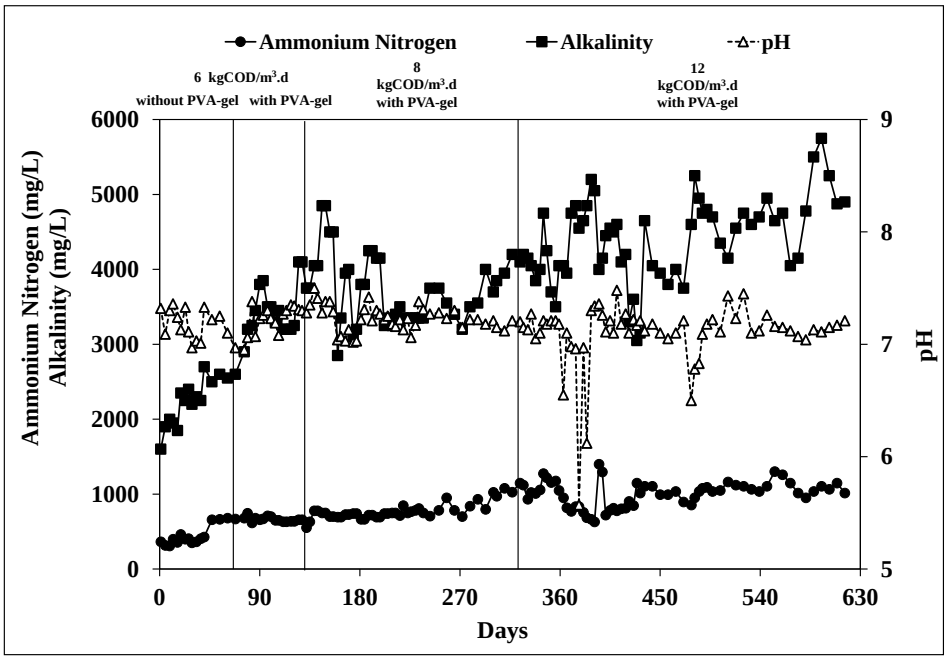


Figure 4.13 Ammonium nitrogen concentration, alkalinity and pH variation of methanogenic reactor during reactor operation

4.4 Performance Comparison of Two Stage TAnMBR at three Different Loading Conditions

This section discusses the performance of two stage TAnMBR at three different loading conditions with previous reported (literature) high rate anaerobic wastewater treatment systems. The first part presents about organic removal rate, organic removal efficiency, biological activity and methane yield of the system. The second section discusses about the VFA distribution of two stage TAnMBR.

4.4.1 System performance investigation under different organic loading rate (OLR), organic removal rate (ORR) and organic removal efficiency

The results of performance investigations of two stage TAnMBR under three different loading rates are presented in Figure 4.14. This graph illustrates the performance of the system at loading rate 6 (with and without PVA-gel as biocarrier), 8 and 12 kgCOD/m^3.d with PVA-gel addition in hydrolytic reactor. The detail data is presented in Appendix D-5.

On the 68th day of system operation at loading rate 6 kgCOD/m^3.d (without PVA-gel addition), ORR was observed as 5.3 kgCOD/m^3.d with an average TCOD removal efficiency 89%. After that PVA-gel as biocarrier were added in hydrolytic reactor. The ORR was observed as 5.5 kgCOD/m^3.d with TCOD removal efficiency 92%. There were no statistically significant differences between ORR at loading rate 6 kgCOD/m^3.d without and with PVA-gel addition ($p < 0.05$). At 132nd day of reactor operation, loading condition was increased to 8 kgCOD/m^3.d. It was observed that during this period there was a slight reduction in TCOD removal efficiency to 90% with ORR 7.6 kgCOD/m^3.d. Once the loading rate increased to 12 kgCOD/m^3.d, ORR has significantly increased to 10.1 kgCOD/m^3.d with TCOD removal efficiency 84% ($p < 0.05$). As presented in the above figure, ORR also increased with an increase in loading rate. This increasing in ORR indicated more organic

matter removal at higher loading rates. As a results, methane production was observed to be higher with increase OLR. Table 4.6 presents the comparison of removal rate between this study and studies reported in literature.

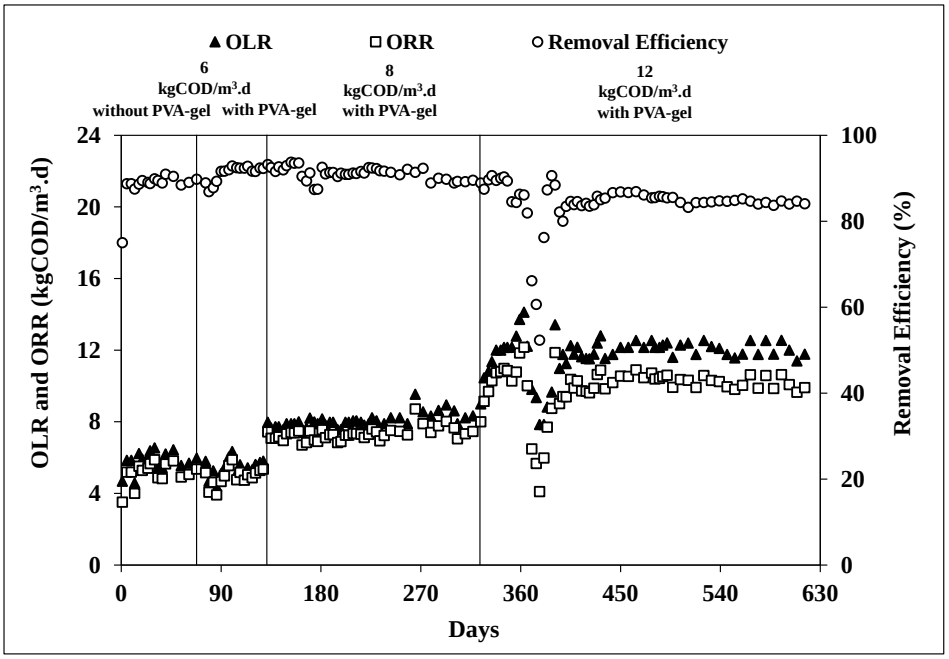


Figure 4.14 Variation of OLR, ORR and organic removal efficiency during reactor operation

Table 4.6 Comparison of Removal Rate between This Study and Literature

Wastewater	T (°C)	Reactor Configuration	Removal Efficiency (%)	OLR (kgCOD/m^3.d)	ORR (kgCOD/m^3.d)	Reference
Synthetic (Starch based)	55	Anaerobic upflow filters	88-93	4.7-17.2	4.4-16.0	Ahn and Forster (2000)
Slaughter house	37	AnMBR	90-96	4.4-8.2	4.2-7.4	Saddoud and Sayadi (2007)
Synthetic (VFA mixture)	55	Submerged gas lift	98	10-50	9.8-49.0	Jeison and van Lier (2008b)
Dairy wastewater	50	Anaerobic SBR	68-95	1.6-12.8	1.5-8.7	Göblös et al. (2008)
Synthetic (Starch based)	55	Two Stage TAnMBR	84-92	6-12	5.3-10.1	This study

4.4.2 Organic removal efficiency, biological activity, methane productivity, methane yield and MLVSS

Organic removal efficiency of the two stage TAnMBR was analyzed over the reactor operation. The organic removal efficiency of the system increased from 89% at loading rate 6 kgCOD/m^3.d. After that PVA-gel was added in hydrolytic reactor at similar loading condition, COD removal efficiency slightly increased to 92%. Then, loading rate was increased to 8 and 12 kgCOD/m^3.d with PVA-gel addition in hydrolytic reactor. This was observed as TCOD removal efficiency decreased to 90% and 84%, respectively. Furthermore, biological activity of the system was also observed to be between 0.24 and 0.30 kgCOD$_r$/kgMLVSS.d. This indicated the balance between organic removal rate and

biomass concentration during experimental period. The biological activity comparison of this study and reported studies are illustrated in Table 4.7.

Table 4.7 Biological Activity Comparison of This Study and Literature

Wastewater	T (°C)	Membrane Configuration	OLR (kgCOD/m^3.d)	Biological Activity (gCOD$_r$/gMLVSS.d	Reference
Brewery wastewater	35	Cross-flow	12-20	0.39-0.53	Fakhru'l-Razi (1994)
Synthetic (VFA mixture)	55	Submerged gas lift	10-50	2	Jeison and van Lier (2008b)
Synthetic (Starch based)	55	External semi dead-end	6-12	0.24-0.30	This study

In addition, as discussed in Section 4.3.1 methane productivity increased with an increase in VFA concentration in hydrolytic effluent and loading conditions. Based on the result obtained that the maximum methane productivity was found to be 2.4 times higher than volume of the system. This performance can be considered as a good indicator of the overall system performance. Furthermore, MLVSS concentration also increased from 22.5 g/L to 25.1 g/L at loading rate 6 kgCOD/m^3.d with PVA-gel addition in hydrolytic reactor. Later as the OLR was changed to 8 and 12 kgCOD/m^3.d, the MLVSS also increased from 34.9 g/L and 40.0 g/L, respectively. An increasing in MLVSS concentration was due to an increase in VFA concentration from the hydrolytic effluent with PVA-gel addition and also increasing loading rate. Figure 4.15 demonstrated the average organic removal efficiency, biological activity, methane productivity and MLVSS concentration of the system. The detail data is given in Appendix D-5.

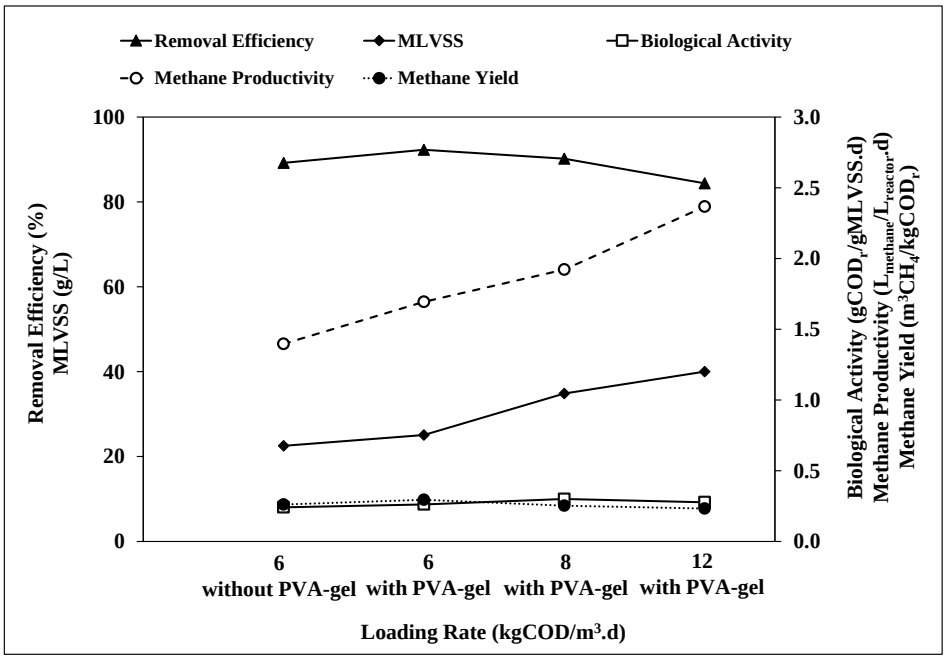


Figure 4.15 Average organic removal efficiency, biological activity, methane productivity, methane yield and MLVSS of the system

Moreover, methane yield is one of the crucial parameters with respect to the performance of anaerobic wastewater treatment. With a variation of only 0.23 to 0.29 $m^3CH_4/kgCOD_r$ (Figure 4.15), it can be safely said that during the course of reactor operation the system performance was stable and predictable. The stability was due to the system balance between methane production and organic removal rate. Furthermore, these observed values were in the range of reported studies done in AnMBR and high rate anaerobic wastewater treatment at 0.12-0.35 $m^3CH_4/kgCOD_r$. In addition, the observation, comparison of AnMBR and high rate anaerobic reactor presented the superiority of AnMBR to high rate anaerobic reactor as illustrated in Table 4.8.

Table 4.8 Methane Yield Comparison of This Study and Literature

Wastewater	T (°C)	Reactor Configuration	OLR ($kgCOD/m^3.d$)	Methane Yield ($m^3CH_4/kgCOD_r$)	Reference
Palm oil mill effluent	35	UASB	60	0.3	Borja et al. (1996)
Synthetic (Starch based)	55	Anaerobic upflow filters	1.2-17.2	0.19-0.27	Ahn and Forster (2000)
Slaughter house	37	Single Stage AnMBR Two Stage AnMBR	4.4-13.3 12.7	0.13-0.31 0.33	Saddoud and Sayadi (2007)
Dairy wastewater	50	Anaerobic SBR	1.6-12.8	0.12-0.26	Göblös et al. (2008)
Papermill wastewater	55	Anaerobic filter	1-12	0.24-0.32	Yilmaz et al. (2008)
Synthetic (Starch based)	55	Two Stage TAnMBR	6-12	0.23-0.29	This study

4.4.3 Pathway of organic carbon

The pathways of $COD_{influent}$ in two stage TAnMBR can be summarized as $COD_{VFA\&others}$, $COD_{methane}$, COD_{VSS} and COD_{acc}. The $COD_{VFA\&others}$ was inclusive of COD due to acetic acid, butyric acid and propionic acid in the effluent, and other COD's which converted to trace amount of CO_2, H_2, methane dissolved in the effluent and other types of VFA. $COD_{methane}$ was the part of organic matter that was measured as gaseous methane. COD_{vss} indicated the COD used for forming biomass. COD_{acc} was non-biodegradable organic matter but can be measured as a part of COD. The input and output organic mass (in form of COD) of two stage TAnMBR could be expressed as follow: $COD_{inf} = COD_{VFA\&others} + COD_{methane} + COD_{VSS} + COD_{acc}$.

In terms of COD balance at hydrolytic reactor, about 79.3%, 90.3%, 91.7% and 79.9% of COD_{inf} were converted to VFA under overall loading rate of 6 (without and with PVA-gel), 8 and 12 $kgCOD/m^3.d$, respectively. While the COD balance in methanogenic reactor, about 13.9%, 8.4%, 10.6% and 19.9% of COD_{inf} to methanogenic reactor remained in $COD_{VFA\&others}$ at overall loading rate of 6 (without and with PVA-gel), 8 and 12 $kgCOD/m^3.d$, respectively, and about 67.1%, 73.7%, 70.8% and 63.7% were converted to methane, respectively. Yet in the hydrolytic reactor, the major portion of the substrate was consumed while producing VFA which was utilized in the methanogenic reactor. The main portion of the substrate were converted while producing methane. Hence, at the optimum loading rate was 8 $kgCOD/m^3.d$ in order to achieve the high VFA concentration and organic removal as well as energy recovery. Nevertheless, methane had a potential to be dissolved in the liquid phase. Therefore, in order to improve the utilization of methane in practical

application, it is necessary to set a gas-water separator (gas strapping device) to recover dissolved methane before effluent discharge (Gao et al., 2014a; Gao et al., 2014b). Table 4.9 summarizes the results of the COD mass balance calculations obtained for each reactor. The detail of carbon balance calculation is given in Appendix H.

Table 4.9 COD Balance of Two Stage TAnMBR

Reactor	OLR (kgCOD/m^3.d)	COD_{inf} (g/d)	$COD_{VFA\&others}$ (g/d)	$COD_{methane}$ (g/d)	COD_{vss} (g/d)	COD_{acc} (g/d)
Hydrolytic Reactor	6 (without PVA-gel)	53.7	42.6	0.0	2.1	9.0
	6 (with PVA-gel)		48.5	0.0	2.0	3.2
	8 (with PVA-gel)	76.2	69.9	0.0	1.2	5.1
	12 (with PVA-gel)	107.6	86.0	0.0	1.0	20.6
Methanogenic Reactor	6 (without PVA-gel)	42.6	5.9	28.6	4.7	3.4
	6 (with PVA-gel)	48.5	4.1	35.7	5.3	3.4
	8 (with PVA-gel)	69.9	7.4	49.5	7.3	5.7
	12 (with PVA-gel)	86.0	17.1	54.8	10.2	3.9

4.4.4 VFA Distribution of two stage TAnMBR

Individual VFA is an important indicator of anaerobic wastewater treatment. Analysis of VFA distribution is valuable in order to optimize the performance of the system. As discussed in Section 2.1 and 2.2, acetic acid and *n*-butyric acid are more preferable VFA species for methane production while propionic acid is not encouraged because it is difficult to convert to acetic acid (low hydrogen partial pressure requirement). Therefore, the purpose of this study was to increase VFA concentration in hydrolytic effluent with a preferential predominance of acetic acid and *n*-butyric acid. Therefore, the intention of this section was to discuss the effect of PVA-gel addition in hydrolytic reactor of two stage TAnMBR on VFA distribution at three different loading conditions such as 6 (without and with PVA-gel), 8 (with PVA-gel) and 12 (with PVA-gel) kgCOD/m^3.d. Individual VFA was analyzed in synthetic wastewater as feed, at the effluent of hydrolytic reactor, at the effluent of methanogenic reactor and permeate at all OLR's. The results are presented in graphical format in Figure 4.16. Acetic acid, *n*-butyric acid and propionic acid were analyzed to represent the VFA distribution of two stage TAnMBR. A small amount of VFA concentration of 250-515 mg/L was observed in feed. This could be potentially due to the deterioration of synthetic wastewater during storage time. Therefore, to maintain the quality of feed, synthetic wastewater was prepared daily to minimize the feed deterioration.

In hydrolytic effluent, large amount of acetic acid and *n*-butyric was observed at all loading conditions. The hydrolytic effluent contained low propionic acid as compared with acetic acid and *n*-butyric acid. Acetic acid and *n*-butyric acid were; 1.3±0.1 g/L, 2.7±0.3 g/L (without PVA-gel) and 1.8±0.2 g/L, 2.6±0.4 g/L (with PVA-gel) at OLR 6 kgCOD/m^3.d, and were observed to increase to 2.1±0.1 g/L, 2.7±0.3 g/L and 3.1±0.1 g/L, 2.9±0.1 g/L at

loading rate 8 and 12 kgCOD/m^3.d, respectively. The significant increase in acetic acid in all conditions was due to an increment in biological activity as discussed in Section 4.2.3 ($p < 0.05$). Furthermore, biogas inhibitors such as propionic acid were observed below 550 mg/L in all conditions, which was lower than its toxic concentration at 1 g/L (Inanc et al., 1999). Typically, the accumulation of propionic acid could be observed to be more severe at thermophillic condition and/or with wastewater contain lactic acid, which subsequently converted to propionic acid (Khanal, 2008; Wang et al., 2009a). However, Speece et al. (2006) reported that the propionic acid accumulation could be minimized by suitable reactor configurations. In this regards, two stage AnMBR with PVA-gel addition in hydrolytic reactor has proved as an alternative reactor configuration which could potentially avoid the accumulation of propionic acid while simultaneously promoting the increase in acetic acid and *n*-butyric acid concentration in hydrolytic effluent.

As presented in Figure 4.16-4.19, the difference in concentration of VFA in hydrolytic effluent and methanogenic effluent shows the effectiveness of the system in terms of VFA removal efficiency. It was also observed that a small amount of VFA was being removed across the membrane. This was observed at 12-20% for all OLR's. Stuckey (2012) also observed that a substantial amount of VFA was found to decrease across membrane contributed which eventually contributed to membrane fouling. Furthermore, the VFA removal efficiency of methanogenic reactor was observed as 77.1% (without PVA-gel), 84.1% (with PVA-gel) at loading rate 6 kgCOD/m^3.d. Then, it decreased to 78.7% and 70.1% at loading rate 8 and 12 kgCOD/m^3.d with PVA-gel addition in hydrolytic reactor.

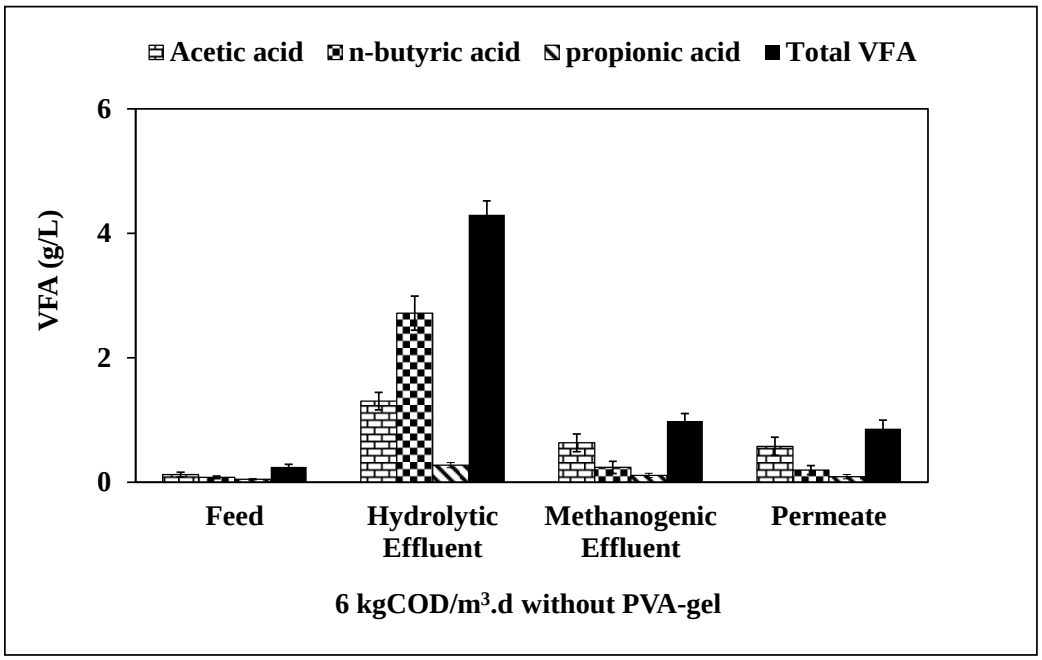


Figure 4.16 Contribution of VFA in two stage TAnMBR during loading rate 6 kgCOD/m^3.d without PVA-gel

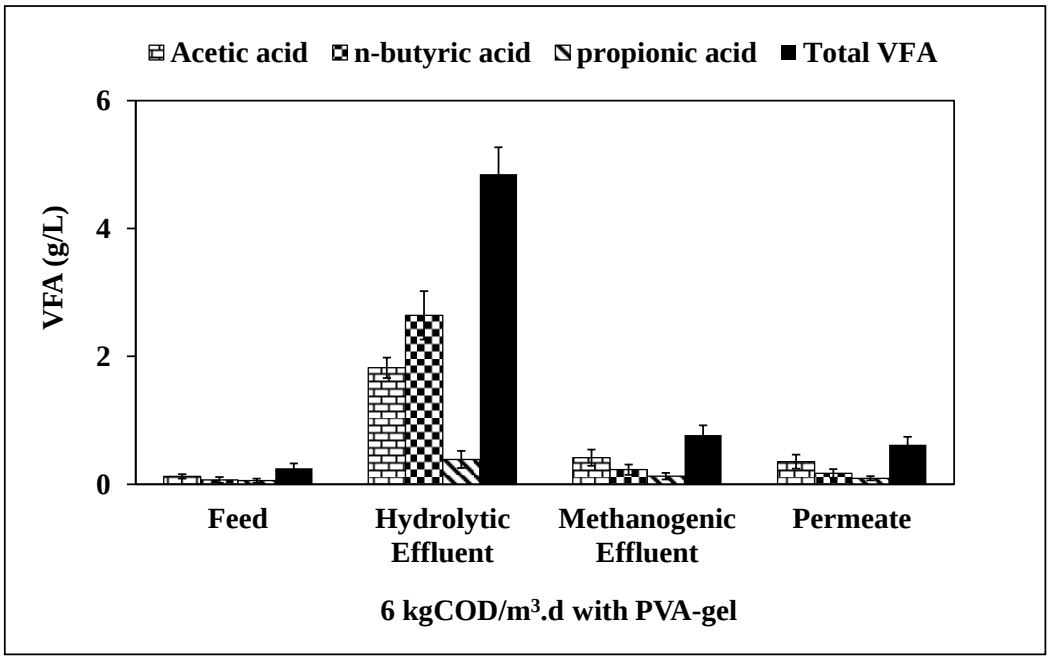


Figure 4.17 Contribution of VFA in two stage TAnMBR during loading rate 6 kgCOD/m^3.d with PVA-gel

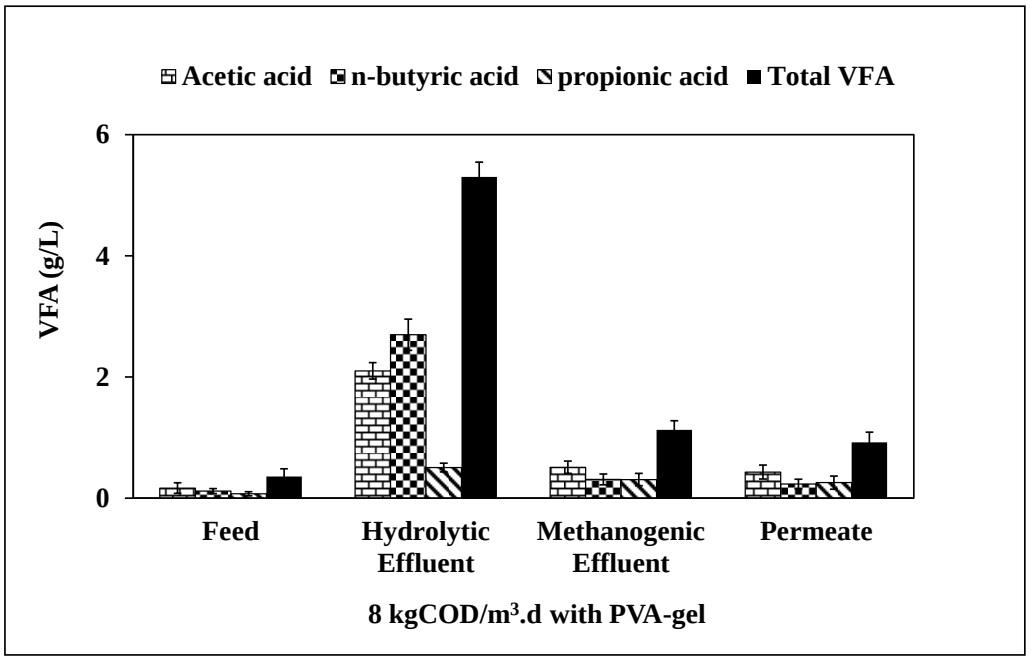


Figure 4.18 Contribution of VFA in two stage TAnMBR during loading rate 8 kgCOD/m^3.d with PVA-gel

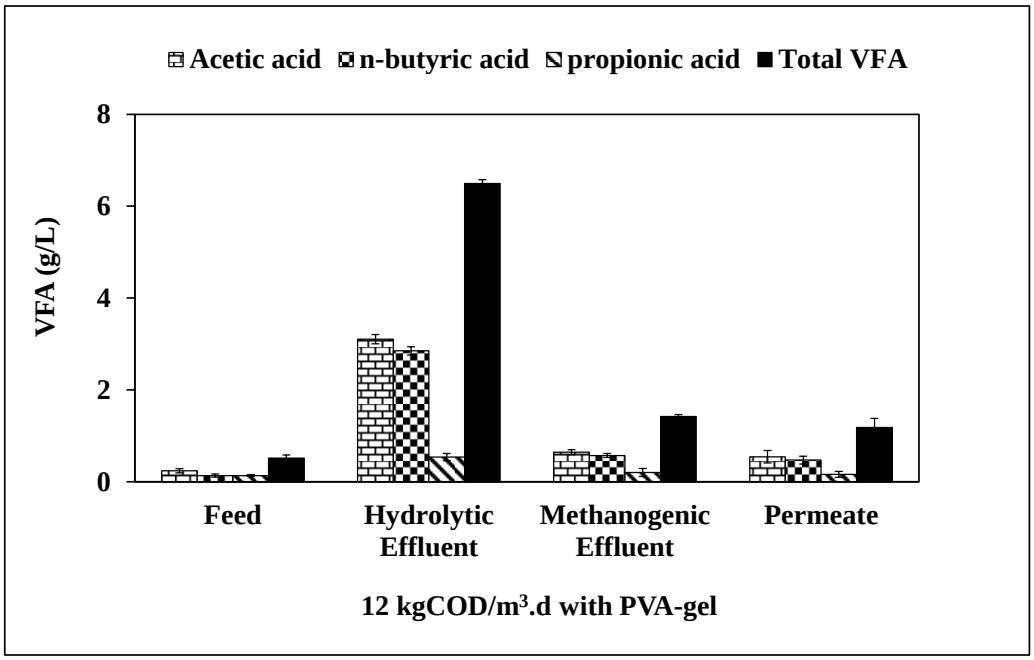


Figure 4.19 Contribution of VFA in two stage TAnMBR during loading rate 12 kgCOD/m^3.d with PVA-gel

4.5 Membrane Fouling Investigation

Once the membrane fouling was observed, suction pump and biomass recirculation pump were stopped. Then, after closing respective valves the membrane taken out from the housing prepared for fouling analysis in separate tank. Initially, DI water was used. Then, the base and acid washes were done in order to clean membrane. Membrane cleaning were done once the TMP reached 30-40 kPa which was observed to reoccur every 90-120 day during the reactor operation.

Membrane cleaning was carried out using alkaline and acid cleaning as discussed in Section 3.8. Furthermore, in between every cleaning step, membrane was rinsed with DI water until neutral solution was obtained. In addition, each step of membrane cleaning procedure, flux was measured to evaluate the hydrodynamic resistance. Furthermore, in AnMBR application, flux decreased due to increase in filtration resistance with time. Membrane fouling were identified as three group namely; removable fouling (remove by physical cleaning), reversible fouling (remove by chemical cleaning) and irreversible fouling (remain fouling after chemical cleaning). Membrane fouling could additionally take place on membrane surface or in the membrane pores due to the EPS deposition. Furthermore, EPS has also been identified as the key parameter in membrane fouling. The membrane fouling behavior in TAnMBR is discussed below.

4.5.1 Trans-membrane pressure (TMP) and flux

The TMP variation with time was monitored to investigate membrane fouling behavior at a constant flux. In this study, the system was operated in a constant flux and variable pressure mode. However, it was not possible to maintain a steady flux of 0.86 L/m^2.h (8 kgCOD/m^3.d) and 1.04 L/m^2.h (12 kgCOD/m^3.d) with constant TMP during whole reactor operation due to membrane fouling. The filtration resistance of membrane influenced the flux. To maintain a constant flux, flow rate was increased correspondingly by adjusting the suction rate of

pump. As a high TMP is a result of membrane fouling, it was used as a parameter-indicating requirement for membrane cleaning. The membrane cleaning procedure was conducted once the TMP increased to 30-40 kPa. The TMP and flux variation of the system are presented in Figure 4.20. The detail data is presented in Appendix E-1.

Membrane fouling occurred due to high bound EPS formation and deposition on the membrane surface or into membrane pore as discussed in following Section. Another potential for fouling could be associated to size of archaea dominating in the reactor sludge. Berube et al. (2006) reported that AnMBR operated at cross-flow mode contains a significantly smaller size of sludge (0.1-0.4 µm) compared to submerged AnMBR (50-500 µm). Furthermore, Vogelaar et al. (2002) pointed out that 16% of thermophillic sludge was smaller than 5 µm compared with only 4% in the mesophilic sludge volume. In addition, the precipitation of mineral salts on the membrane surface, under the influence of pH and temperature, could also cause membrane fouling in TAnMBR. It could be removed by chemical cleaning (acidic solution).

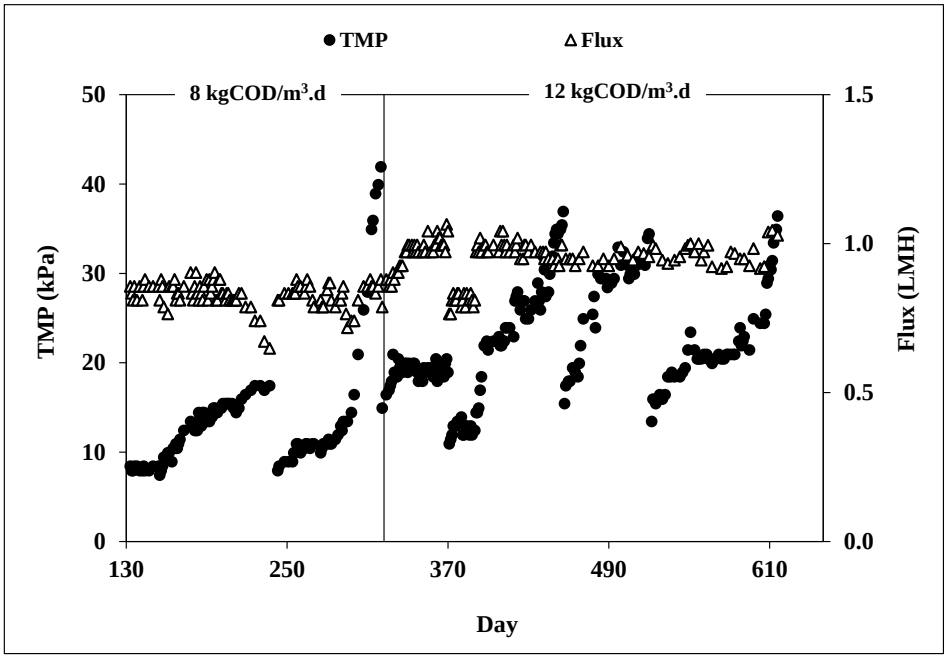


Figure 4.20 TMP and flux variation with time

4.5.2 Filtration resistance

The membrane filtration operations were terminated when TMP reached 30-40 kPa. The membrane module was removed and cake sludge was carefully scraped off the membrane using air and DI water. Then, as described in Section 3.8, the filtration resistance was measured.

Table 4.10 illustrates various resistances on the membrane at loading rates 8 and 12 kgCOD/m^3.d. It can be seen that the total filtration resistance ($R_t = R_m + R_{rm} + R_{re,\,or} + R_{re,\,ir} + R_{irr}$) at loading rate 12 kgCOD/m^3.d was higher than loading rate 8 kgCOD/m^3.d. The majority of the membrane fouling was caused by reversible organic fouling ($R_{re,\,or}$), followed by removable fouling (R_r). Removable fouling showed an increasing trend with an increase in loading rates and biomass concentration. This was due to higher possibility of microorganisms attaching on membrane surface or trapped in membrane pores. This result

was in agreement with Huang et al. (2013) that higher biomass concentration caused more particle deposition on membrane surface.

Membrane fouling is also caused by accumulation and adsorption of bound EPS on membrane surface and inside the membrane pore. EPS was found to have caused the reversible organic fouling, and could be removed by chemical cleaning (alkaline solution). It has been reported as a potential organic fouling agent in AnMBR (Aquino et al., 2006; Visvanathan et al., 2007; Lin et al., 2009; Lin et al., 2011). Based on the results obtained, high amount of reversible organic fouling elucidated that membrane fouling caused by bound EPS play an important role in the membrane filtration resistance.

Table 4.10 Filtration Resistance of the System

Item	Membrane Resistance (m^{-1})	
	OLR 8 kgCOD/m^3.d	**OLR 12 kgCOD/m^3.d**
Intrinsic membrane resistance (R_m)	3.81 x 10^8	3.81 x 10^8
Removable fouling resistance (R_{rm})	1.05 x 10^9	8.07 x 10^{10}
Reversible organic fouling resistance ($R_{re, or}$)	5.49 x 10^9	1.12 x 10^{11}
Reversible inorganic fouling resistance ($R_{re, ir}$)	4.32 x 10^8	3.71 x 10^8
Irreversible fouling resistance (R_{irr})	2.08 x 10^8	2.94 x 10^8
Total filtration resistance (R_t)	7.56 x 10^9	1.94 x 10^{11}

4.5.3 Bound EPS

In AnMBR, membrane fouling was largely affected the physiology of anaerobic sludge as well as the physico-chemical properties of the membrane material itself. EPS has been identified as the most significant biological factor contributing towards membrane fouling. It consists of a variety of polymeric materials such as protein, carbohydrate, lipid and nucleic acids (Jin et al., 2003). In this study, proteins and carbohydrates were considered as total EPS and it represents fouling on membrane surface. EPS could be classified into two forms namely bound and soluble EPS. Among the two forms, bound EPS had a negative effect on membrane filtration and significant effect on membrane fouling (Chang and Lee, 1998; Wang et al., 2009b). Furthermore, bound EPS was reported to be contributing to the organic reversible fouling (Visvanathan et al., 2007).

Bound EPS at loading rates 8 and 12 kgCOD/m^3.d were observed as 58.01±3.6 and 66.68±6.3 mg/gVSS, which corresponds to bound protein of 43.54±9.3 and 59.63±6.4 mg/gVSS and bound carbohydrate 14.47±4.2 and 7.05±0.7 mg/gVSS, respectively. The comparison of bound EPS, bound protein and bound carbohydrate at different loading rates are illustrated in Figure 4.21. Furthermore, protein substances were observed to be the predominant compounds contributing towards the fouling in AnMBR, accounting for 75.1 and 89.3% of bound EPS at loading rates 8 and 12 kgCOD/m^3.d, respectively. This was due to methanogenic archaea preferentially utilized carbohydrate as carbon source to produce methane and carbon dioxide. Similar observation were reported by Gao et al. (2010); Lin et al. (2011) and Mota et al. (2013). In this study, high protein concentration and low carbohydrate concentration at higher loading rates provided higher protein (PN) and carbohydrate (PS) ratio. The comparison of bound PN and PS ratio at different loading rates are presented in Figure 4.22. It was observed as 3.0±1.0 and 8.5±1.6 at loading rates 8 and

12 kgCOD/m^3.d, respectively. Furthermore, it has been reported that increasing PN/PS ratio could increase in sludge hydrophobicity (Liao et al., 2001). Furthermore, the high protein content led to hydrophobic sludge, consequently fouling the membrane by adsorption and by deposition during filtration. The higher bound PN/PS ratio potentially increased filtration resistance. This is agreement with the observation of Lin et al. (2009) that the sludge with higher PN/PS ratio in bound EPS would be more sticky (viscous) and thus favor to develop cake/gel formation on membrane surface or inside the pore. Based on these results, proteins were the majority contributors to membrane fouling under anaerobic condition, attributing to organic reversible fouling ($R_{re, or}$).

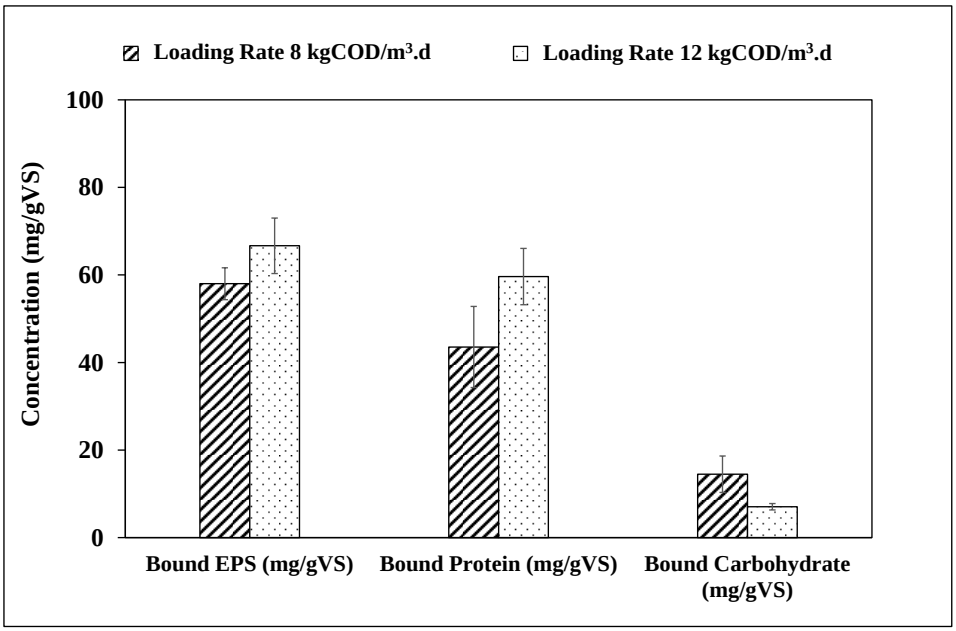


Figure 4.21 Comparison of bound EPS, bound protein and bound carbohydrate at different loading rate

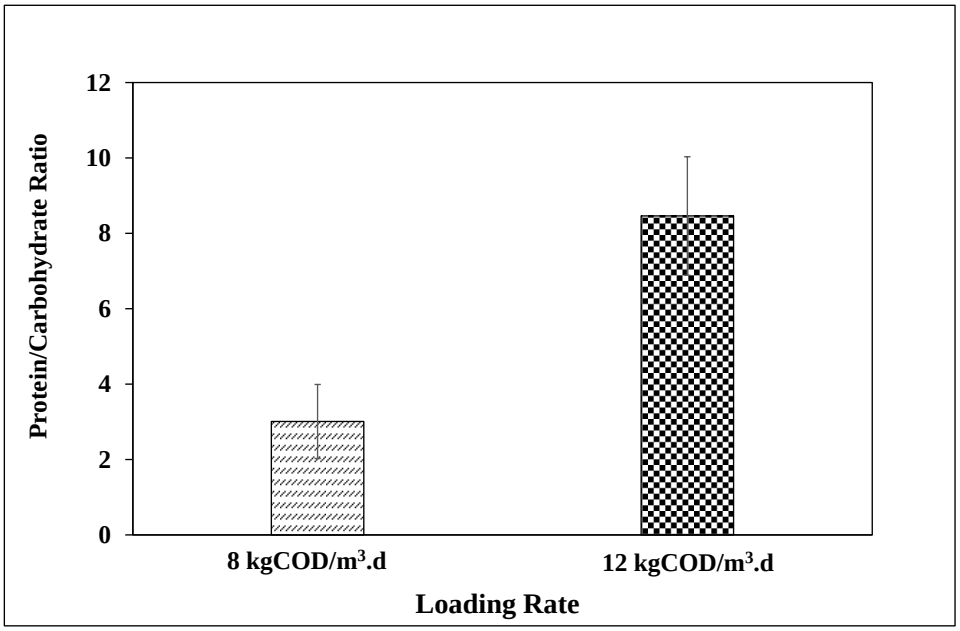


Figure 4.22 Comparison of bound protein and carbohydrate ratio at different loading rate

4.6 Energy Production and Greenhouse Gas Emission of Two Stage TAnMBR

Low energy requirement and energy production are the main advantage of anaerobic wastewater treatment. To evaluate this, the energy production and energy consumption were calculated and tabulated in Table 4.6. The net energy production gain from the system is determined by the quantity and quality of produced biogas. With respect to the experimental set up described in Section 3.7, hot water pump, heater, biomass recirculation pump, suction pump and mixing pump were the main energy consumption of this study. This was measured by watthour meter connected to two stage TAnMBR. The overall energy production from the reactor was calculated from methane yield. The energy calculation details are documented in Appendix F-1.

During the system operation, the energy consumption for all conditions were in the range of 12-15 kWh/m^3.d. Furthermore, the energy production at loading rates 6 (without PVA-gel), 6 (with PVA-gel), 8 (with PVA-gel) and 12 (with PVA-gel) kgCOD/m^3.d were 14.6, 16.8, 18.8 and 24.3 kWh/m^3, respectively. It could be inferred that when loading conditions was increased there was an increased in energy production due to higher organic removal rate and methane yield. The overall energy production and consumption in terms of kWh/m^3 are presented in Table 4.11.

Table 4.11 Energy Production

OLR (kgCOD/m^3.d)	Biogas Productivity ($L_{methane}/L_{reactor}$.d)	Methane Productivity ($L_{methane}/L_{reactor}$.d)	Energy Requirement (kWh/m^3)	Energy Production (kWh/m^3)
6 (without PVA-gel)	2.6	1.4	12-15	14.6
6 (with PVA-gel)	2.8	1.7	12-15	16.8
8 (with PVA-gel)	3.4	1.9	12-15	18.8
12 (with PVA-gel)	4.5	2.4	12-15	24.3

As per energy balance presented in Table 4.11, the energy production was higher than energy consumption. It indicated that this system is worthwhile to use as a two stage TAnMBR for high strength particulate wastewater treatment. Furthermore, the greenhouse gas (GHG) emissions were calculated to be 0.22-0.35 kgCO_2-eq/d during reactor operation. The calculation detail of GHG emission is given in Appendix F-2. Also, it should be noted that as the production capacity increased (methane productivity), GHG emission and energy production increase. Therefore, the methane production from the degradation process of anaerobic wastewater treatment should be considered for energy recovery and using in the wastewater treatment plant instead of releasing directly to the atmosphere. It is not only decreased the GHG emission but also gain the benefit of methane production for electricity consumption reduction as well. Figure 4.23 illustrates the energy balance and GHG emission of present study.

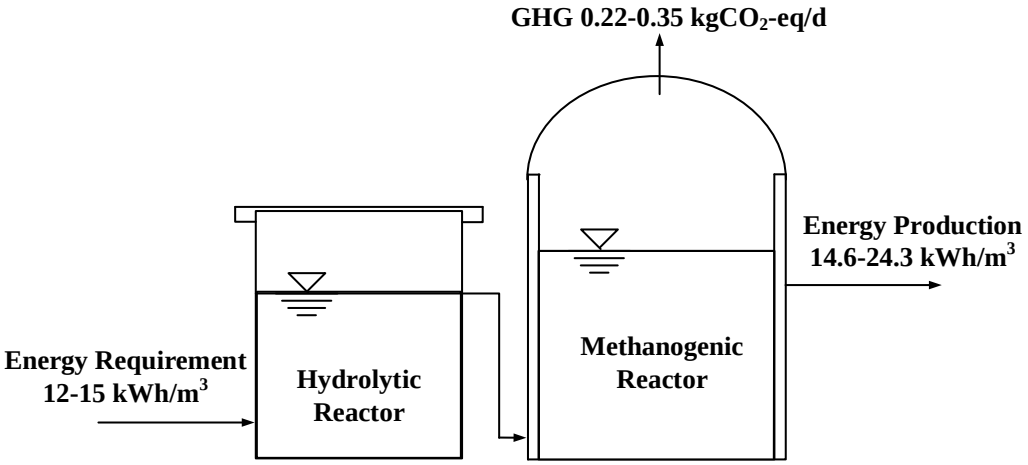


Figure 4.23 Energy balance and GHG emission of two stage TAnMBR

Chapter 5

Conclusions and Recommendations

The goals of this work was to assess a two stage thermophillic anaerobic membrane bioreactor (TAnMBR) for degradation of high strength particulate (tapioca starch based) synthetic wastewater. The influence of adding PVA-gel as biocarrier was also studied. The overall performance of a two stage TAnMBR treating high strength particulate wastewater at different loading rates was intensively studied to explore the sustainable loading rates in this research. Furthermore, membrane fouling was considered and investigated for the same. The energy recovery potential by treating tapioca starch based synthetic wastewater through two stage TAnMBR was also analyzed. Based on the results observed, following conclusions and recommendations have been drawn.

5.1 Conclusions

According to the first objective, the effect of PVA-gel as biocarrier on total VFA concentration and methane production of two stage TAnMBR were investigated.

The major conclusions of hydrolytic reactor's performance are summarized below:

1. An effective enrichment and acclimatization of two stage TAnMBR was done successfully. A gradual increase of temperature from 35°C to 55°C in steps of 2°C along with the loading rate was observed satisfactory.

2. Hydrolytic reactor pH remained unchanged and was observed to be within 5.4±0.5 for all loading conditions and pH adjustment in hydrolytic reactor was not required. This indicated the reactor stability. Furthermore, methanogenic reactor pH also observed to increase with increase in loading rate. This was due to the increasing concentration of NH_4HCO_3 in the synthetic wastewater.

3. Hydrolytic reactor produced acetic acid and *n*-butyric acid as the predominant VFA's, which are the preferable VFA species for methane production. Once the PVA-gel was added to hydrolytic reactor, VFA production significantly increased from 4.0±0.2 to 4.6±0.5 g/L ($p < 0.05$). Furthermore, VFA also significantly increased with increasing loading rate ($p < 0.05$). Average VFA production during loading rate 6, 8 and 12 kgCOD/m^3.d with PVA-gel addition was observed as 4.6±0.5, 4.9±0.1 and 6.0±0.1 g/L, respectively.

4. Soluble COD in hydrolytic effluent significantly increased from 11.5±0.6 g/L (without PVA-gel addition) to 13.1±2.5 g/L (with PVA-gel addition), while no significantly increased from 18.9±1.2 to 19.1±2.5 g/L when loading condition were increased to 8 and 12 kgCOD/m^3.d with PVA-gel addition ($p < 0.05$), respectively.

5. In addition, SS in hydrolytic reactor decreased from 10.8±1.4 to 5.8±1.0 and 4.3±0.4 g/L with significant increase in total VFA production from 4.6±0.5 to 4.9±0.2 and 6.0±0.1 g/L at OLR 6, 8 and 12 kgCOD/m^3.d with PVA-gel ($p < 0.05$). Indicating that SS in hydrolytic reactor was being utilized by acidogens to produce SCOD and VFA found in hydrolytic effluent.

6. The biological activity of hydrolytic reactor increased from 0.50 to 0.61 gVFA/gMLVSS.d when PVA-gel was added in hydrolytic reactor at loading rate 6 kgCOD/m^3.d with constant MLVSS concentration. These observations inferred the effectiveness of using PVA-gel as biocarrier in hydrolytic reactor to increase VFA production and biological activity. The increment in VFA production was due to an increase in biological activity of the hydrolytic reactor with 18.9, 20.3 and 29.9 g $VFA_{production}$/L of PVA-gel.d at loading rates 6, 8 and 12 kgCOD/m^3.d, respectively. Biomass attachment of 0.5 gVSS/gPVA-gel was also observed. Furthermore, the matured brown color PVA-gel had an average settling velocity 228 m/h, greater than that of unused PVA gel at 143 m/h.

The conclusions of methanogenic reactor's performance are summarized below:

1. Two stage thermophillic anaerobic membrane bioreactor was successfully applied for treating high strength particulate wastewater.

2. The organic removal rate was observed as 5.3, 5.5, 7.6 and 10.1 kgCOD/m^3.d at loading rate of 6 (without PVA-gel), 6 (with PVA-gel), 8 (with PVA-gel) and 12 (with PVA-gel) kgCOD/m^3.d with a COD removal efficiency of 84-92%. This indicates that the system was capable to remove almost all of the particulate matters in feed.

3. At steady stage condition, methane content in biogas produced from the system was observed to be in the range 53-60% for all loading conditions. Furthermore, it had shown an increment in methane productivity with PVA-gel addition. It could be further noted that methane productivity significantly increased from 1.4 to 1.7 $L_{methane}/L_{reactor}$.d once PVA-gel was added in hydrolytic reactor at loading rate 6 kgCOD/m^3.d ($p < 0.05$). The increase in methane productivity was due to increase in VFA concentration in the hydrolytic effluent. Furthermore, the two stage TAnMBR had shown a continuous increase in methane productivity with an increase in loading rate. Methane production was observed to be 1.7, 1.9 and 2.4 $L_{methane}/L_{reactor}$.d at loading rate 6, 8 and 12 kgCOD/m^3.d, respectively.

4. Biomass concentration of methanogenic reactor increased with each operating condition. This was one of the main advantage of membrane technology coupled with high rate anaerobic reactor to complete retains biomass within the system assuring no biomass washout from the system.

5. Methane yield from this study was in the range of 0.23 to 0.29 $m^3CH_4/kgCOD_r$.d. This indicated that the balance between methane production and COD removal rate without the accumulation of organic content in the system.

6. Biological activity of methanogenic reactor was in the range of 0.24 to 0.30 $kgCOD_r$/kgMLVSS.d. It is also showed the system balance in terms of COD removal rate and biomass concentration in the system. This indicated that there was a potential to go for higher loading condition and higher methane productivity.

7. The total membrane resistance (R_t) at loading rate 12 kgCOD/m^3.d was higher than loading rate 8 kgCOD/m^3.d. The majority of membrane fouling at higher loading condition was caused by accumulation of organic compounds which increased at higher loading rate as well as biomass concentration.

8. The bound EPS contents in the system increased at higher loading conditions. Furthermore, protein substances were observed to be the predominant compounds contributing towards the fouling in TAnMBR. High protein substances led to hydrophobic sludge, consequently fouling membrane by adsorption and deposition on membrane during membrane filtration.

9. The energy output from methane production based on energy recovery was observed as 14.6-24.3 kWh/m^3. It was identified as adequate to operate the system, which required energy input 12-15 kWh/m^3.

5.2 Recommendations for Future Research

1. The cultivated PVA-gel from hydrolytic reactor should be used as seed sludge to start up the hydrolytic reactor of two stage anaerobic reactor. Moreover, the effect of cultivated PVA-gel on degradation of organic matters to organic acids should be studied.

2. Biokinetics study should be done to prove the benefit of PVA-gel on biodegradation rate in terms of maximum specific growth rate (μ_m), specific yield (Y), saturation constant (K_s) should be evaluated to estimate the process efficiency.

3. Considering biokinetics, biological activity and biomechanisms in hydrolytic reactor with PVA-gel addition needs to be studied in more detail, and identifying microbial species, and quantifications through microbial techniques such as fluorescence *in situ* hybridization (FISH), polymerase chain reaction (PCR), and denaturing gradient gel electrophoresis (DGGE) at different loading conditions.

4. Sludge washout in hydrolytic reactor in higher loading rates should be eliminated. Considering the dispersed nature of hydrolytic bacteria, membrane filtration coupled with hydrolytic reactor can be studied and compared to the performance of hydrolytic reactor with PVA-gel addition as biocarrier in terms of VFA production.

5. The parameter governing the flux and membrane fouling in AnMBR should be varied and studied, for example, effect of HRT and F/M ratio on fouling behavior in TAnMBR. This should be studied in depth together with fouling analysis in TAnMBR.

6. In addition, anaerobic degradation model (Monod model, Contois model, Chen and Hashimoto model, Grau second order model, Stover-Kincannon model etc) must be developed in parallel with the HRT variation at a particular loading rate to find accurately the kinetics followed at a particular condition.

7. There is growing concern of recovering methane dissolved in the effluent. In this research much focus was not given to this issue by measurement but it was found by using carbon balance. Thus to ensure and increase the methane productivity of the system, striping of methane from the effluent needs to be addressed and effective primary data needs to be generated to address this issue.

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APPENDIX A
Two Stage Thermophilic Anaerobic Membrane Bioreactor System

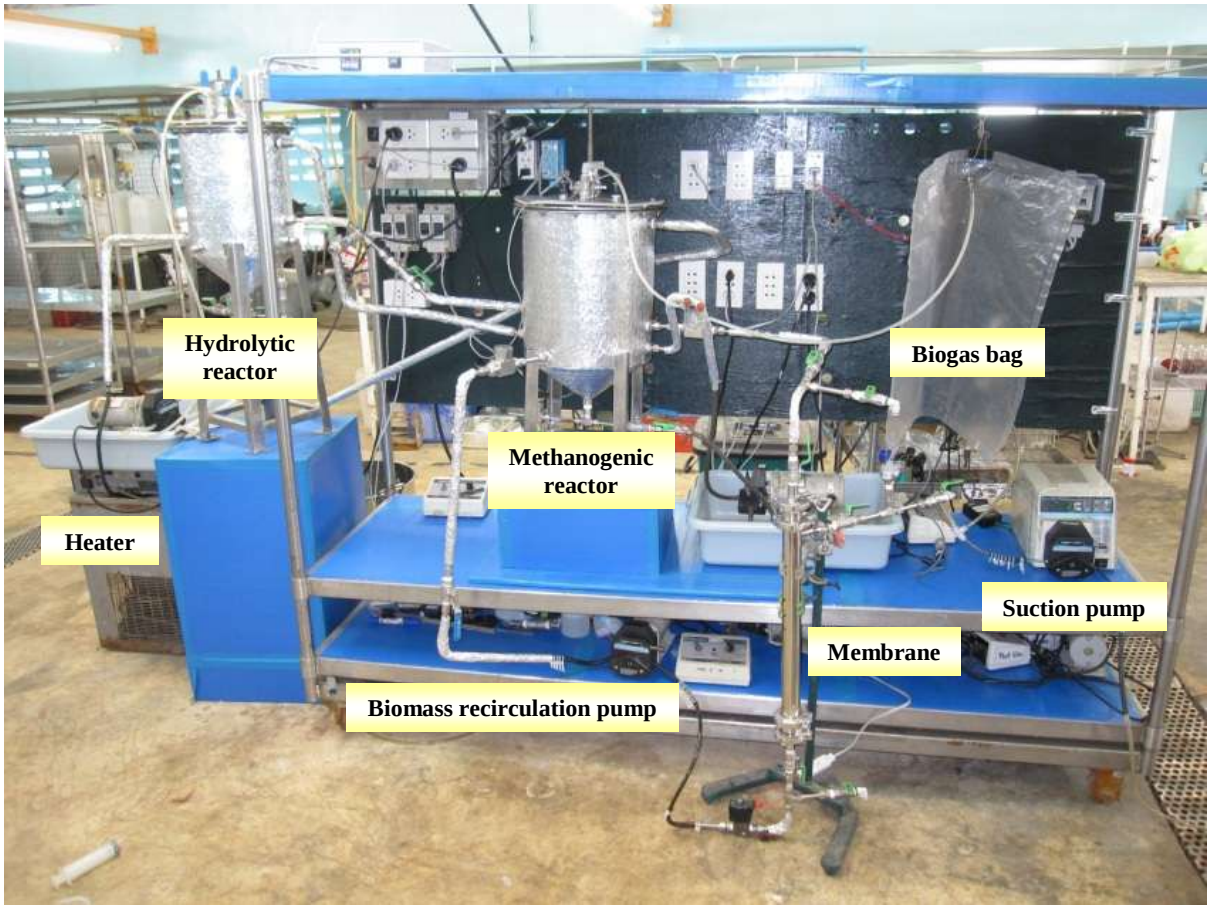

Figure A-1 Two stage thermophilic anaerobic membrane bioreactor

Figure A-2 PVA-gel

Figure A-3 Ceramic membrane

Figure A-4 Hydrolytic and methanogenic sludge

APPENDIX B
Standard Curves

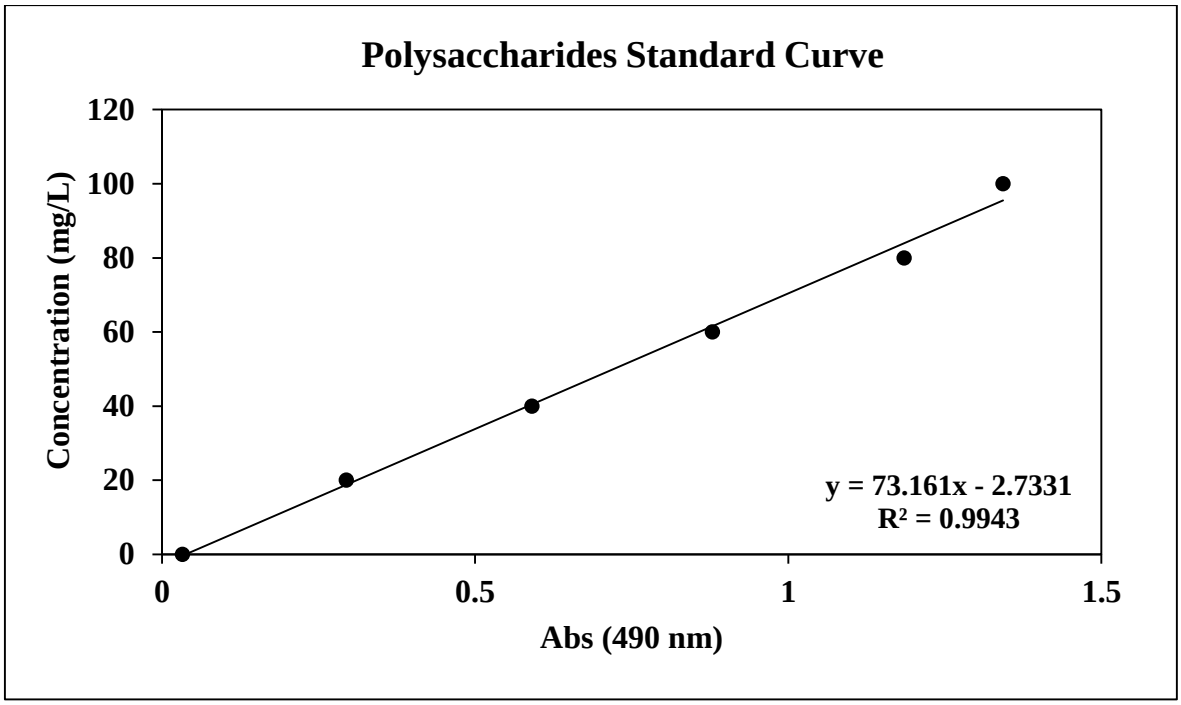

Figure B-1 Polysaccharides standard curve

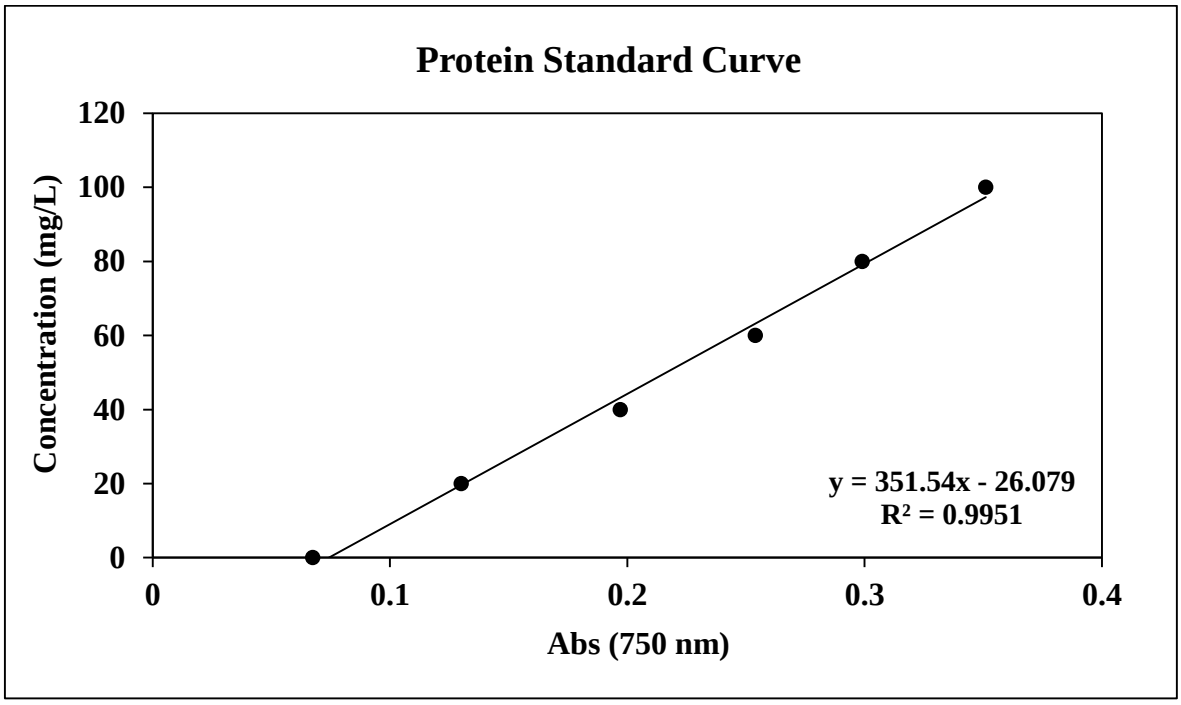

Figure B-2 Protein standard curve

APPENDIX C
Experimental Results during Isolation and Acclimatization

Table C-1 Temperature and pH Variation of Hydrolytic and Methanogenic Reactor during Isolation and Acclimatization Period

Days	Temperature and pH of the system			
	Hydrolytic Reactor		Methanogenic Reactor	
	Temperature (°C)	pH	Temperature (°C)	pH
1	35	5.52	35	7.14
7	37	5.56	37	7.03
14	39	5.73	39	7.32
17	39	5.52	39	7.14
18	39	6.05	39	6.91
19	39	5.42	39	6.63
20	39	5.30	39	7.15
21	39	5.10	39	7.03
22	39	5.45	39	7.10
23	39	5.37	39	7.08
24	39	5.40	39	7.10
25	39	5.54	39	6.80
26	39	5.30	39	7.10
27	39	6.12	39	7.14
28	39	6.20	39	6.80
29	39	6.30	39	6.70
30	39	5.73	39	7.10
31	39	5.10	39	6.84
32	39	4.82	39	6.81
33	39	4.78	39	6.61
36	39	4.87	39	7.30
37	39	4.63	39	7.05
39	39	4.67	39	7.18
40	39	4.59	39	6.80
41	41	4.69	41	7.07
42	41	5.23	41	7.08
43	41	5.00	41	7.30
44	41	5.65	41	7.18
45	41	5.45	41	7.30
46	41	5.18	41	7.50
47	41	5.46	41	7.30
48	41	5.38	41	7.45
49	43	5.34	43	7.58
50	43	5.12	43	7.46
51	43	5.54	43	7.30
55	43	4.95	43	7.85
57	43	5.60	43	7.67
58	43	5.29	43	7.43
71	45	5.47	45	7.83
62	45	5.30	45	7.49
63	45	5.28	45	7.30
64	45	5.01	45	6.94
65	45	4.92	45	6.82
66	45	5.24	45	6.93
67	45	5.27	45	6.88
68	45	5.07	45	7.07
69	45	5.13	45	6.94
70	45	5.56	45	6.87
71	45	5.40	45	7.13
72	45	5.31	45	6.93
73	45	5.13	45	7.03

Table C-1 Temperature and pH Variation of Hydrolytic and Methanogenic Reactor during Isolation and Acclimatization Period (Con't)

Days	Temperature and pH of the system			
	Hydrolytic Reactor		Methanogenic Reactor	
	Temperature (°C)	pH	Temperature (°C)	pH
75	45	5.79	45	6.97
76	45	5.78	45	7.10
78	47	5.67	47	7.19
79	47	5.55	47	7.28
80	47	5.41	47	7.43
81	47	5.22	47	7.55
82	47	5.10	47	7.59
83	47	5.33	47	7.62
84	47	5.31	47	7.64
85	47	5.29	47	7.34
86	47	5.47	47	7.63
87	47	5.44	47	7.59
88	47	5.24	47	7.20
90	47	5.45	47	7.45
91	47	5.29	47	7.39
92	47	5.17	47	7.31
93	49	5.78	49	7.30
94	49	5.27	49	7.26
95	49	5.06	49	7.47
96	49	5.61	49	7.11
97	49	5.35	49	7.03
100	51	4.80	51	6.80
101	51	5.40	51	7.40
103	51	4.85	51	7.38
105	51	5.45	51	7.32
107	53	5.30	53	7.55
111	53	5.10	53	6.70
116	53	5.25	53	6.67
121	53	5.43	53	6.50
125	55	5.36	55	7.31
127	55	5.10	55	7.12
128	55	5.12	55	6.92
129	55	5.52	55	7.44
130	55	5.25	55	7.77
132	55	4.91	55	7.75
135	55	4.86	55	7.70
137	55	4.83	55	7.82
139	55	4.81	55	7.63
142	55	5.13	55	7.36
143	55	4.86	55	7.38
144	55	5.55	55	7.47
145	55	5.20	55	7.12

Table C-2 Hydrolytic and Methanogenic Reactor OLR, MLVSS, VFA, COD Removal Efficiency and methane content during Isolation and Acclimatization Period

Days	Hydrolytic Reactor				Methanogenic Reactor			
	OLR ($kgCOD/m^3.d$)	MLVSS (g/L)	VFA (g/L)	CH_4 (%)	OLR ($kgCOD/m^3.d$)	MLVSS (g/L)	COD Removal (%)	CH_4 (%)
1	2	14.2	-	-	2	20.3	-	-
2	2	-	-	-	2	-	-	-
3	2	-	-	-	2	-	-	-
4	2	-	-	-	2	-	-	-
5	2	-	-	-	2	-	-	-
6	2	-	-	-	2	-	-	-
7	3	13.3	-	-	2.5	20.2	-	-
8	3	-	-	-	2.5	-	-	-
9	3	-	-	-	2.5	-	-	-
10	3	-	-	-	2.5	-	-	-
11	3	-	-	-	2.5	-	-	-
12	3	-	-	-	2.5	-	-	-
13	3	-	-	-	2.5	-	-	-
14	4	12.7	-	-	2.5	21.5	-	-
15	4	-	-	-	2.5	-	-	-
16	4	-	-	-	2.5	-	-	-
17	4	-	-	-	2.5	-	-	-
18	4	-	-	-	2.5	-	-	-
19	4	-	-	-	2.5	-	-	-
20	4	-	-	-	2.5	-	-	-
21	4	12.3	-	-	2.5	20.5	-	-
22	4	-	-	-	2.5	-	-	-
23	4	-	-	-	2.5	-	-	-
24	4	-	-	-	2.5	-	-	-
25	4	-	-	-	2.5	-	-	-
26	4	-	-	-	2.5	-	-	-
27	4	-	-	-	2.5	-	-	-
28	4	12.6	-	-	2.5	20.5	-	-
29	4	-	-	-	2.5	-	-	-
30	4	-	-	-	2.5	-	-	-
31	4	11.0	1.4	-	2.5	19.3	-	-
32	4	-	-	-	2.5	-	-	-
33	4	-	-	-	2.5	-	-	-
34	4	-	-	-	2.5	-	-	-
35	4	-	-	-	2.5	-	-	-
36	4	10.2	1.7	7.9	2.5	19.4	-	26.3
37	4	-	-	-	2.5	-	-	-
38	4	10.3	2.3	6.0	3	15.8	64.3	28.3
39	4	-	-	-	3	-	-	-
40	4	-	-	-	3	-	-	-
41	6	-	-	-	3	-	-	-
42	6	-	-	-	3	10.5	65.3	-
43	6	9.2	2.5	4.5	3	-	-	35.4
44	6	-	-	-	3	-	-	-
45	6	-	-	-	3	-	57.2	-
46	6	-	-	-	3	-	-	-
47	6	-	-	-	3	-	-	-
48	6	7.7	2.7	-	3	10.0	-	45
49	10	-	-	-	3.5	-	-	-
50	10	-	-	-	3.5	-	54.9	-
51	10	-	-	-	3.5	-	-	-
52	10	-	-	-	3.5	-	-	-
53	10	7.3	2.6	3.2	3.5	16.5	75.0	48.4
54	10	-	-	-	3.5	-	-	-
55	10	-	-	-	3.5	-	77.0	-
56	10	7.4	2.5	-	3.5	13.9	-	46.7
57	10	-	-	-	3.5	-	-	-

Table C-2 Hydrolytic and Methanogenic Reactor OLR, MLVSS, VFA, COD Removal Efficiency and methane content during Isolation and Acclimatization Period (Con't)

Days	Hydrolytic Reactor				Methanogenic Reactor			
	OLR ($kgCOD/m^3.d$)	MLVSS (g/L)	VFA (g/L)	CH_4 (%)	OLR ($kgCOD/m^3.d$)	MLVSS (g/L)	COD Removal (%)	CH_4 (%)
58	10	5.6	2.5	2.5	3.5	11.1	-	43.7
59	10	-	-	-	3.5	-	-	-
60	10	-	-	-	3.5	-	76.3	-
61	10	-	-	-	3.5	-	-	-
62	10	-	-	-	3.5	-	-	-
63	10	4.3	2.7	-	3.5	10.0	76.6	39.8
64	10	-	-	-	3.5	-	-	-
65	10	-	-	-	3.5	-	-	-
66	10	4.1	2.5	1.3	3.5	9.2	-	41.9
67	10	-	-	-	3.5	-	74.2	-
68	10	-	-	-	3.5	-	-	-
69	10	-	-	-	3.5	-	-	-
70	10	5.7	2.6	-	3.5	12.0	-	44.3
71	10	-	-	-	3.5	-	-	-
72	10	-	-	-	3.5	-	-	-
73	10	-	-	-	3.5	-	-	-
74	10	5.7	-	-	3.5	9.2	-	-
75	10	-	-	-	3.5	-	68.0	-
76	10	-	-	-	3.5	-	-	-
77	10	-	-	-	3.5	-	-	-
78	14.67	3.6	2.3	0.76	5.3	9.2	-	53.1
79	14.67	-	-	-	5.3	-	-	-
80	14.67	-	-	-	5.3	-	-	-
81	14.67	4.8	-	-	5.3	-	-	-
82	14.67	-	-	-	5.3	-	63.6	-
83	14.67	-	-	-	5.3	-	-	-
84	14.67	-	-	-	5.3	-	-	-
85	14.67	4.8	2.1	1.2	5.3	13.5	-	55.7
86	14.67	-	-	-	5.3	-	-	-
87	14.67	-	-	-	5.3	-	-	-
88	14.67	-	-	-	5.3	-	-	-
89	14.67	4.8	-	-	5.3	12.5	-	-
90	14.67	-	-	-	5.3	-	68.2	-
91	14.67	-	-	-	5.3	-	-	-
92	14.67	-	-	-	5.3	-	-	-
93	16	4.6	2.4	0.6	5.3	12.5	-	53.0
94	16	-	-	-	5.3	-	-	-
95	16	-	-	-	5.3	-	-	-
96	16	5.0	-	-	5.3	12.6	-	-
97	16	-	-	-	5.3	-	61.1	-
98	16	-	-	-	5.3	-	-	-
99	16	-	-	-	5.3	-	-	-
100	16	6.8	2.5	0.6	5.3	12.6	-	55.8
101	16	-	-	-	5.3	-	-	-
102	16	-	-	-	5.3	-	58.9	-
103	16	-	-	-	5.3	-	-	-
104	16	-	-	-	5.3	-	-	-
105	16	7.7	2.4	-	5.3	11.3	-	-
106	16	-	-	-	5.3	-	-	-
107	16	-	-	-	5.3	-	-	-
108	16	-	-	-	5.3	-	53.0	-
109	16	-	-	-	5.3	-	-	-
110	16	-	-	0.3	5.3	-	-	53.7
111	16	8.5	-	-	5.3	10.0	-	-
112	16	-	2.6	-	5.3	-	48.0	-
113	16	-	-	-	5.3	-	-	-
114	16	-	-	-	5.3		-	-

Table C-2 Hydrolytic and Methanogenic Reactor OLR, MLVSS, VFA, COD Removal Efficiency and methane content during Isolation and Acclimatization Period (Con't)

Days	Hydrolytic Reactor				Methanogenic Reactor			
	OLR ($kgCOD/m^3.d$)	MLVSS (g/L)	VFA (g/L)	CH_4 (%)	OLR ($kgCOD/m^3.d$)	MLVSS (g/L)	COD Removal (%)	CH_4 (%)
115	16	-	-	-	5.3	-	-	-
116	16	6.8	-	-	5.3	10.0	-	-
117	16	-	-	-	5.3	-	-	-
118	16	-	-	-	5.3	-	46.0	-
119	16	-	2.6	-	5.3	-	-	-
120	16	-	-	-	5.3	-	-	-
121	16	7.7	-	-	5.3	5.5	-	-
122	16	-	-	-	5.3	-	56.9	-
123	16	-	-	-	5.3	-	-	-
124	16	-	-	-	5.3	-	-	-
125	16	9.5	2.8	-	7	5.3	59.1	48.3
126	16	-	-	-	7	-	-	-
127	16	-	-	-	7	-	-	-
128	16	8.5	2.6	-	7	6.5	-	-
129	16	-	-	-	7	-	76.2	-
130	16	-	-	-	7	-	-	-
131	16	-	-	-	7	14.1	-	-
132	16	7.2	2.7	-	7	-	73.8	-
133	16	-	-	-	7	-	-	-
134	16	-	-	-	7	-	-	-
135	16	7.6	2.7	-	7	16.1	-	47.3
136	16	-	-	-	7	-	70.0	-
137	16	-	-	-	7	-	-	-
138	16	-	-	-	7	15.6	73.7	-
139	16	8.8	2.7	-	7	-	-	43.7
140	18.67	-	-	-	8	-	-	-
141	18.67	-	-	-	8	-	-	-
142	18.67	9.0	2.2	-	8	17.2	70.0	48.7
143	18.67	-	-	-	8	-	-	-
144	18.67	-	-	-	8	16.0	-	-
145	18.67	8.9	2.1	-	8	-	-	46.7

APPENDIX D
Experimental Results during Reactor Operation

Table D-1 VFA Production, MLSS, MLVSS, pH and Biological Activity of Hydrolytic Reactor during Reactor Operation

Days	pH	VFA Production (mg/L)	MLSS (g/L)	MLVSS (g/L)	Biological Activity ($gVFA_{production}$/gVSS.d)	Biological Activity ($gVFA_{production}$/L PVA-gel.d)
1	4.87	2100	9.49	9.00	-	-
5	4.80	2250	10.91	10.01	-	-
9	4.83	2800	10.27	9.68	-	-
12	4.90	2500	12.05	9.26	-	-
16	4.83	2950	11.62	9.16	-	-
19	4.71	3952	9.94	9.30	0.52	-
23	4.71	3707	12.92	11.30	0.40	-
26	4.65	3647	11.76	11.12	0.40	-
29	4.73	3579	10.16	9.40	0.47	-
33	4.75	3648	10.57	9.90	0.45	-
37	4.77	3325	11.02	10.77	0.38	-
40	4.90	3519	9.97	9.02	0.48	-
47	4.80	4012	10.37	9.84	0.50	-
54	4.90	4083	11.23	10.19	0.49	-
61	4.88	4053	10.48	9.87	0.51	-
68	4.85	4078	9.85	9.26	0.54	9.12
76	4.50	2218	11.71	9.64	0.28	11.41
79	4.51	2775	11.61	11.40	0.30	16.15
83	4.62	3927	11.28	10.88	0.45	14.22
86	4.53	3460	9.93	9.49	0.45	16.88
90	5.29	4105	10.70	9.63	0.53	18.04
93	5.61	4388	10.42	9.69	0.56	9.12
97	5.21	4641	9.05	8.44	0.68	19.08
100	5.12	5034	9.10	8.82	0.70	20.69
104	5.34	4963	9.88	8.90	0.69	20.40
107	5.23	5006	9.34	8.38	0.74	20.58
111	4.72	4987	11.56	9.36	0.66	20.50
114	4.78	4971	12.12	9.90	0.62	20.44
118	4.85	4782	10.88	8.81	0.67	19.66
121	4.95	4654	10.94	9.18	0.63	19.13
125	5.25	4710	12.77	10.12	0.57	19.36
128	5.05	4780	13.03	10.35	0.57	19.65
132	4.75	5350	11.73	9.85	0.67	22.00
135	4.88	5261	11.38	9.00	0.72	21.63
139	5.05	4627	9.53	8.86	0.64	19.02
142	4.75	4910	9.04	8.01	0.76	20.19
146	5.00	4439	8.27	7.69	0.71	18.25
149	5.10	4678	8.41	7.56	0.76	19.23
153	4.95	4629	8.57	7.88	0.72	19.03
156	5.25	4993	8.34	7.59	0.81	20.53
160	6.61	5110	7.92	7.05	0.89	21.01
163	5.87	4823	8.51	7.66	0.78	19.83
167	5.83	4897	5.73	5.20	1.16	20.13
170	5.50	4796	6.06	4.66	1.27	19.72
174	5.64	4925	5.06	4.80	1.27	20.25
177	5.75	4870	4.98	4.77	1.26	20.02
181	5.02	4484	5.63	4.89	1.13	18.43
184	6.15	4795	5.23	4.98	1.19	19.71
188	5.65	4858	5.12	4.97	1.21	19.97
191	5.20	4432	5.26	4.88	1.12	18.22
195	6.17	4597	5.01	4.71	1.20	18.90
198	5.89	4595	5.15	4.76	1.19	18.89
202	5.40	4415	5.23	5.11	1.07	18.15
205	6.19	4509	5.69	5.22	1.07	18.54
209	5.92	4361	5.42	5.11	1.05	17.93
212	6.12	4467	6.08	5.28	1.04	18.37
216	6.18	4369	6.65	5.18	1.04	17.96

Table D-1 VFA Production, MLSS, MLVSS, pH and Biological Activity of Hydrolytic Reactor during Reactor Operation (Con't)

Days	pH	VFA Production (mg/L)	MLSS (g/L)	MLVSS (g/L)	Biological Activity ($gVFA_{production}$/gVSS.d)	Biological Activity ($gVFA_{production}$/L PVA-gel.d)
219	6.13	4600	6.26	4.93	1.15	18.91
223	5.85	4613	6.64	3.89	1.46	18.97
226	6.29	4729	5.29	3.49	1.67	19.44
230	6.13	4913	7.02	5.21	1.16	20.20
233	6.18	4902	6.96	4.52	1.34	20.15
237	5.18	4937	6.46	4.55	1.34	20.29
243	5.43	5036	6.43	5.33	1.16	20.70
251	5.90	4918	4.70	4.33	1.40	20.22
258	5.82	5198	4.11	4.00	1.60	21.37
265	5.50	4993	3.98	3.52	1.75	20.53
272	5.32	4914	4.22	4.06	1.49	20.20
279	5.25	4940	4.92	4.13	1.48	20.31
286	5.10	4748	4.88	4.36	1.34	19.52
293	6.08	4694	3.79	3.64	1.59	19.30
300	5.38	5036	4.50	4.26	1.46	20.70
303	4.71	4888	5.64	5.36	1.12	20.10
310	4.67	5088	5.60	5.29	1.19	20.92
317	4.65	5016	5.80	5.68	1.09	20.62
324	5.52	4665	6.06	5.67	1.10	20.73
327	5.68	4791	5.98	5.39	1.19	21.30
331	5.38	5070	5.70	5.27	1.28	22.53
334	5.10	5082	4.84	4.60	1.47	22.59
338	6.80	4373	5.04	4.77	1.38	21.86
342	6.86	4833	5.16	4.59	1.58	24.16
345	7.11	4645	5.28	5.19	1.34	23.23
348	5.63	4777	4.88	4.61	1.55	23.88
352	5.15	5101	6.59	6.49	1.18	25.50
356	5.55	4942	6.34	6.25	1.19	24.71
359	5.01	5063	5.46	5.28	1.44	25.31
363	5.48	4948	5.99	5.61	1.32	24.74
366	5.05	5143	5.42	5.38	1.43	25.71
370	5.21	4912	5.16	4.82	1.53	24.56
374	5.12	5459	5.40	5.20	1.22	21.23
377	4.90	5729	5.50	5.19	1.29	22.28
381	4.90	5056	5.71	5.13	1.15	19.66
384	5.43	5325	5.74	5.41	1.15	20.71
388	4.86	4973	4.58	4.25	1.37	19.34
391	6.04	5363	5.87	5.31	1.52	26.82
395	5.55	5758	5.60	5.29	1.63	28.79
398	5.25	5691	4.06	3.93	2.18	28.46
401	6.43	5425	4.58	4.45	1.83	27.13
405	6.60	5356	3.91	3.78	2.13	26.78
408	6.07	5502	5.37	5.32	1.55	27.51
411	5.75	5741	4.98	4.82	1.79	28.71
415	6.78	5354	6.98	6.34	1.27	26.77
419	6.83	5411	7.26	6.96	1.17	27.06
422	6.89	5393	6.56	5.37	1.51	26.96
426	6.40	5541	6.44	5.83	1.43	27.70
429	6.33	5765	5.74	5.02	1.72	28.82
432	6.50	5949	5.10	4.90	1.82	29.75
436	5.35	6034	2.44	2.38	3.80	30.17
443	5.10	5994	2.76	2.58	3.48	29.97
450	5.35	6095	2.26	2.10	4.35	30.48
457	5.12	6164	2.24	2.00	4.62	30.82
464	5.20	6188	2.60	2.40	3.87	30.94
471	5.35	6094	2.64	2.54	3.60	30.47
478	4.70	5962	4.24	3.85	2.32	29.81

Table D-1 VFA Production, MLSS, MLVSS, pH and Biological Activity of Hydrolytic Reactor during Reactor Operation (Con't)

Days	pH	VFA Production (mg/L)	MLSS (g/L)	MLVSS (g/L)	Biological Activity ($gVFA_{production}$/gVSS.d)	Biological Activity ($gVFA_{production}$/L PVA-gel.d)
481	6.10	5914	4.20	3.75	2.37	29.57
485	6.20	5909	4.48	3.89	2.28	29.54
488	5.41	5994	4.26	3.81	2.36	29.97
492	5.43	6055	4.22	3.80	2.39	30.28
497	5.38	5949	4.28	3.90	2.29	29.75
504	5.63	5909	3.40	3.16	2.80	29.54
511	5.41	6095	4.00	3.73	2.45	30.48
518	5.12	6034	3.92	3.16	2.86	30.17
525	4.91	6033	3.78	3.65	2.48	30.16
532	5.38	6055	4.06	3.74	2.43	30.28
539	5.35	6031	3.74	3.50	2.58	30.16
546	5.48	6095	3.84	3.58	2.55	30.48
553	5.65	5949	4.22	3.82	2.34	29.75
560	5.33	6085	3.96	3.74	2.44	30.42
567	5.48	5807	4.52	4.24	2.05	29.03
574	5.15	5876	4.46	4.06	2.17	29.38
581	5.20	5947	4.76	4.62	1.93	29.74
588	5.33	5927	4.92	4.50	1.98	29.64
595	5.23	6028	4.46	4.27	2.12	30.14
602	5.68	5936	4.16	3.70	2.41	29.68
609	5.37	5943	4.43	4.07	2.19	29.71
616	5.61	5952	3.90	3.57	2.50	29.76

Table D-2 Volatile Fatty Acid (VFA) Composition of Two Stage TAnMBR

Days	Acetic Acid (mg/L)				Propionic Acid (mg/L)				*n*-Butyric Acid (mg/L)			
	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate
19	106.8	1423.6	290.8	251.4	46.0	202.2	117.3	49.2	36.9	2326.6	447.1	339.1
23	110.0	1277.3	390.4	363.1	60.0	224.5	112.3	55.2	42.0	2205.4	487.8	326.4
26	24.4	1194.8	645.3	547.8	63.9	258.1	161.1	137.0	53.0	2194.5	385.6	333.0
29	51.6	1575.3	316.2	229.8	40.6	123.9	74.0	57.0	68.5	1879.7	333.3	252.8
33	95.2	1222.6	563.3	492.0	54.8	252.4	112.6	99.5	39.5	2172.7	372.7	353.4
37	110.4	993.8	486.5	379.4	53.8	254.9	149.1	103.3	88.0	2076.4	326.5	252.6
40	137.5	1082.7	565.5	538.0	38.6	268.9	155.5	103.4	78.0	2167.3	385.6	304.7
47	137.5	1203.1	722.1	707.6	38.6	248.8	67.8	42.2	78.0	2559.8	236.4	190.4
52	161.6	1157.1	644.6	525.2	48.0	245.7	117.8	83.2	61.5	2638.4	343.2	267.5
54	130.3	1213.1	586.5	517.8	59.2	201.2	104.8	70.7	60.7	2668.6	328.8	236.4
56	115.0	1125.0	648.6	562.5	30.1	283.4	172.9	159.9	103.2	2617.7	133.4	124.7
59	133.3	1182.6	620.5	542.2	59.6	190.1	140.6	123.9	60.4	2671.7	177.9	159.7
61	123.5	1186.5	603.7	590.9	48.8	205.7	100.2	88.5	88.9	2660.3	224.9	196.6
68	59.8	1201.2	613.5	489.6	43.8	216.1	67.2	55.6	88.9	2660.3	224.9	196.6
76	141.6	742.8	540.2	465.1	109.8	184.2	176.4	100.6	87.1	1291.3	337.7	285.2
79	58.0	1102.5	410.6	291.8	54.1	192.3	94.2	75.3	133.9	1479.7	437.6	313.2
83	58.5	1450.6	722.0	600.4	26.6	220.4	130.6	102.7	168.4	2256.3	296.5	214.7
86	200.3	1409.1	390.3	319.9	156.2	128.9	120.1	86.3	131.2	1921.7	272.1	264.5
90	161.9	1746.5	274.9	223.3	55.3	291.4	183.7	127.0	64.3	2067.6	299.8	231.8
93	98.5	2041.5	441.9	371.9	80.2	192.1	185.7	143.6	106.3	2154.7	153.5	123.8
97	85.0	1548.2	461.3	395.9	67.3	254.3	162.7	138.2	61.4	2838.2	143.0	136.5
100	120.6	1799.8	317.7	277.3	77.3	255.0	133.3	126.0	74.5	2979.0	127.8	65.8
104	106.3	1684.6	244.0	222.0	40.9	342.3	79.5	74.0	81.0	2935.9	227.6	169.8
107	151.2	1692.1	342.3	287.8	52.2	251.3	101.2	71.3	49.4	3063.1	167.1	143.8
111	133.3	1666.5	362.2	346.4	29.8	274.1	183.4	103.6	78.1	3046.9	135.4	79.8
114	143.7	1734.7	423.4	346.4	44.3	574.0	183.4	103.6	19.7	2662.7	175.8	99.8
118	103.2	1864.5	295.8	234.2	61.1	535.9	88.2	81.4	19.7	2381.9	377.9	210.2
121	93.1	1730.9	547.3	504.1	44.6	437.2	44.8	41.6	38.1	2486.3	244.1	168.8
125	143.6	1646.7	497.1	462.7	74.9	392.0	74.5	31.4	38.1	2671.3	304.6	186.4
128	117.5	1744.2	374.3	305.4	54.0	484.8	106.3	68.5	28.9	2550.5	332.7	287.9
132	70.4	1812.9	404.6	334.8	42.7	457.5	126.3	77.1	50.9	3080.0	322.1	261.1
135	75.3	1970.6	443.5	397.2	62.6	470.2	101.1	70.8	61.4	2820.0	241.5	222.8
139	22.1	2162.2	522.7	446.9	81.2	555.2	120.0	106.0	59.9	1909.6	241.7	191.9
142	69.9	1734.3	560.5	494.6	73.7	474.7	117.4	93.1	73.2	2701.2	483.3	363.8

Table D-2 Volatile Fatty Acid (VFA) Composition of Two Stage TAnMBR (Con't)

Days	Acetic Acid (mg/L)				Propionic Acid (mg/L)				*n*-Butyric Acid (mg/L)			
	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate
146	79.1	1899.2	566.5	511.1	60.4	230.8	110.7	87.2	61.1	2308.9	257.1	203.6
149	79.1	1500.1	496.0	346.4	60.4	189.3	84.0	74.4	61.1	2988.4	485.0	393.8
153	70.4	1844.9	353.1	315.8	42.7	435.0	104.0	86.0	50.9	2348.9	435.4	360.0
156	75.7	1875.7	404.3	304.2	41.6	355.1	66.0	56.1	53.7	2762.2	412.7	383.8
160	70.4	1844.9	364.2	315.8	42.7	435.0	104.0	86.0	50.9	2829.8	435.4	360.0
163	70.4	1904.8	378.2	295.0	42.7	482.3	102.9	90.9	50.9	2435.7	299.1	236.9
167	75.7	1801.1	360.5	277.3	41.6	543.5	99.5	72.1	53.7	2552.2	351.2	330.2
170	75.7	1671.7	349.7	247.7	41.6	343.1	66.0	42.7	53.7	2781.2	382.6	293.8
174	79.1	1894.4	485.7	373.3	60.4	257.2	145.3	98.3	61.1	2773.8	430.6	355.0
177	75.7	1737.7	418.1	362.0	41.6	298.3	106.2	55.2	53.7	2834.2	477.9	410.9
181	98.3	1924.4	420.6	339.5	22.8	212.1	122.4	81.9	33.0	2347.4	363.7	325.2
184	117.5	2138.3	473.4	348.7	25.4	142.5	58.9	26.6	51.7	2514.5	413.4	334.7
188	117.5	1819.2	316.9	240.7	25.4	224.4	92.8	62.4	51.7	2814.2	529.2	425.2
191	117.5	1679.1	408.5	366.6	25.4	238.4	82.5	47.4	51.7	2514.7	479.5	329.7
195	131.6	1606.9	386.9	309.7	25.4	251.6	107.4	91.5	51.7	2738.1	352.2	293.0
198	138.6	1674.9	484.6	395.0	31.2	254.5	84.8	54.1	51.7	2665.3	500.9	410.8
202	135.5	1781.9	385.0	306.5	51.0	351.4	147.6	129.6	59.6	2282.1	438.7	379.6
205	135.5	1614.4	377.8	235.3	51.0	351.4	146.2	97.8	59.6	2543.6	415.1	379.6
209	35.7	1518.2	299.4	246.0	83.9	196.6	144.9	101.9	100.3	2646.2	426.9	367.7
212	57.1	1671.4	349.3	285.2	78.4	144.0	131.0	95.0	118.0	2652.1	334.6	296.7
216	107.0	1618.0	392.1	313.7	43.8	173.0	103.3	81.2	94.4	2577.5	341.7	248.2
219	75.7	1716.6	447.9	370.0	44.2	314.3	231.1	245.0	41.1	2569.5	343.6	205.4
223	171.1	2157.8	447.9	383.0	75.6	245.4	261.1	197.7	57.7	2210.0	438.4	376.9
226	99.3	2198.3	363.7	345.6	65.9	398.6	310.8	276.8	100.0	2132.4	379.6	217.4
230	82.1	2164.8	429.1	316.3	67.2	385.3	343.3	313.0	82.1	2362.5	375.5	355.1
233	116.7	2099.8	329.9	364.9	67.2	464.1	343.3	219.7	82.1	2338.0	542.8	472.7
237	99.3	1934.6	375.7	228.5	65.9	501.6	294.8	264.5	100.0	2500.3	506.1	389.5
243	103.4	2042.1	532.8	451.6	65.9	453.1	231.1	151.8	148.8	2540.6	391.8	306.1
251	107.0	1906.7	542.9	502.3	67.3	360.8	258.5	210.0	128.7	2650.5	302.0	240.8
258	250.6	1927.2	574.2	552.5	86.8	458.6	414.6	343.7	140.5	2812.2	230.7	214.1
265	242.9	1833.4	550.6	492.2	84.0	437.1	404.2	305.2	140.5	2722.4	236.3	159.5
272	332.1	1705.7	589.0	565.1	70.6	397.9	424.9	412.6	152.9	2810.5	341.9	276.6
279	72.3	2144.2	517.1	286.0	44.5	340.6	234.2	340.8	64.5	2455.6	346.9	281.6
286	51.7	1941.7	609.0	464.0	41.0	582.6	355.4	247.8	66.0	2223.5	350.6	326.5

Table D-2 Volatile Fatty Acid (VFA) Composition of Two Stage TAnMBR (Con't)

Days	Acetic Acid (mg/L)				Propionic Acid (mg/L)				*n*-Butyric Acid (mg/L)			
	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate
293	174.7	2163.5	461.8	309.6	39.6	507.7	446.4	385.7	54.7	2022.7	179.6	110.2
300	103.4	2042.1	532.8	451.6	65.9	453.1	231.1	151.8	148.8	2540.6	391.8	306.1
303	95.9	1857.1	588.8	542.9	130.6	388.1	176.5	113.6	85.4	2642.8	318.4	152.0
310	188.0	1937.2	300.6	245.9	129.1	461.0	203.5	97.7	122.5	2689.3	489.8	338.9
317	214.5	1830.5	351.8	334.2	41.1	360.9	231.0	226.9	183.3	2825.0	220.2	163.8
324	265.7	2289.1	337.2	297.8	209.6	280.0	226.4	221.6	183.8	2096.1	173.2	163.3
327	275.3	2186.5	421.6	371.6	215.8	346.4	231.2	215.8	186.9	2258.5	220.2	196.4
331	213.7	2370.9	508.0	352.1	134.2	440.2	181.9	205.4	111.6	2259.2	174.3	149.2
334	264.3	2150.0	482.7	360.3	64.3	470.0	193.3	170.3	130.3	2461.7	157.0	147.9
338	179.0	1961.2	640.2	497.0	96.6	318.7	158.4	138.3	171.5	2092.8	294.2	223.2
342	263.3	2204.1	685.2	625.8	108.0	322.7	124.2	88.7	104.8	2306.0	197.4	170.1
345	128.4	2011.5	532.1	464.7	106.1	463.9	164.5	70.1	152.4	2170.0	302.0	372.2
348	153.3	2123.7	506.0	464.7	52.2	663.7	160.1	132.4	65.5	1989.2	348.4	290.3
352	306.5	2117.9	466.8	410.1	115.2	430.5	124.1	104.7	40.8	2552.3	627.0	557.4
356	248.6	2010.7	637.5	604.5	64.1	349.3	211.3	186.4	176.0	2581.7	594.4	506.2
359	279.5	1979.8	534.8	450.4	71.6	244.9	125.5	97.8	122.6	2838.0	468.0	362.2
363	248.6	2040.0	639.5	497.2	64.1	291.1	175.3	147.3	176.0	2617.0	418.7	376.7
366	158.1	2291.9	688.9	632.2	61.6	408.1	207.9	177.1	107.4	2442.8	491.5	402.7
370	144.9	2206.1	821.0	780.6	84.7	393.8	216.8	186.4	125.3	2312.1	767.8	706.1
374	195.5	1934.6	605.9	530.4	56.1	358.5	235.5	209.2	63.0	3166.2	367.1	346.0
377	290.2	2126.5	1094.0	941.2	53.2	474.8	255.5	143.5	93.2	3127.4	810.5	742.5
381	239.0	1983.2	592.4	493.0	57.5	504.4	109.5	60.6	73.7	2568.4	525.3	451.5
384	254.1	2154.3	479.7	410.9	48.2	380.9	81.6	74.3	119.2	2790.2	520.0	377.6
388	181.5	2413.2	466.4	403.4	72.2	400.5	53.5	49.8	163.9	2159.2	398.7	288.0
391	249.3	2345.4	639.3	506.3	56.3	529.6	50.7	47.0	131.1	2488.4	467.3	414.6
395	198.8	2133.1	600.3	518.7	62.2	664.3	241.6	231.1	124.0	2960.8	563.4	464.8
398	250.6	2362.3	681.9	586.7	66.9	569.6	257.5	216.7	161.9	2759.3	563.4	521.4
401	239.1	2346.6	709.1	586.7	56.3	445.4	196.0	151.0	131.1	2633.0	406.2	364.2
405	206.4	2212.1	658.2	535.8	74.3	411.5	228.9	220.7	126.3	2732.0	458.6	427.1
408	180.4	2364.1	586.7	563.8	49.3	402.9	240.3	210.3	100.7	2735.4	479.5	458.6
411	292.6	2510.7	618.0	554.0	48.0	502.8	224.7	161.8	109.8	2727.7	528.4	453.3
415	160.6	2411.6	429.9	360.9	41.0	474.7	223.0	197.9	55.3	2467.7	322.3	294.9
419	278.3	2361.9	516.2	460.9	50.4	498.1	243.0	202.2	76.5	2551.3	374.7	322.3
422	214.1	2358.0	382.7	301.1	48.0	479.3	238.2	222.3	51.6	2555.3	584.3	479.5

Table D-2 Volatile Fatty Acid (VFA) Composition of Two Stage TAnMBR (Con't)

Days	Acetic Acid (mg/L)				Propionic Acid (mg/L)				*n*-Butyric Acid (mg/L)			
	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate	Feed	Hydrolytic Effluent	Methanogenic Effluent	Permeate
426	256.0	2520.2	369.1	301.1	64.6	536.0	324.6	239.6	101.5	2484.4	490.0	448.1
429	292.6	2796.2	314.7	260.3	48.0	502.8	283.1	209.1	109.8	2465.7	395.7	406.2
432	180.4	2935.7	382.7	355.5	46.7	402.9	214.0	167.6	100.7	2611.0	343.3	290.9
436	228.2	2915.1	464.3	382.7	54.9	407.4	243.0	211.2	105.0	2711.4	395.7	332.8
443	213.7	3009.5	421.2	380.6	48.0	495.6	222.1	191.8	109.8	2489.2	402.3	328.7
450	195.9	2974.0	502.3	471.9	67.3	415.1	331.2	288.8	118.0	2706.1	273.5	232.7
457	235.9	2903.6	395.8	366.8	61.0	439.6	146.2	137.9	126.3	2820.8	633.3	545.8
464	256.0	3065.6	483.2	421.2	64.6	463.0	361.5	331.2	101.5	2659.7	375.5	346.9
471	256.7	3136.1	809.0	812.6	70.7	355.0	101.5	93.0	125.5	2602.4	610.0	586.4
478	260.2	2754.8	791.2	727.1	69.5	292.3	83.7	62.4	124.1	2915.0	632.1	604.4
481	217.5	2886.7	698.6	677.2	74.2	252.0	109.8	79.0	194.8	2775.3	643.2	529.7
485	256.0	2756.6	702.1	543.5	64.6	311.5	129.6	108.9	101.5	2840.7	699.6	689.1
488	301.8	2795.2	755.6	680.7	46.6	328.1	102.7	90.8	124.0	2871.2	643.2	524.1
492	300.0	2857.6	769.8	698.6	49.3	346.1	106.2	87.3	116.9	2851.4	712.4	514.5
497	180.4	2935.7	782.1	681.9	43.3	402.9	243.0	202.2	100.7	2611.0	542.4	458.6
504	256.0	2756.6	755.6	639.7	64.6	311.5	118.5	99.2	101.5	2840.7	636.7	594.8
511	195.9	2974.0	613.9	573.3	67.3	415.4	331.2	288.8	118.0	2706.1	632.6	526.5
518	228.2	2915.1	668.3	573.1	54.9	407.4	243.0	211.2	105.0	2711.4	626.2	552.9
525	212.1	2938.0	654.7	654.7	61.1	431.6	275.5	252.0	111.5	2663.0	647.2	448.1
532	300.0	2857.6	766.3	662.9	39.3	346.1	106.2	87.3	116.9	2851.4	658.4	507.5
539	174.8	2997.0	791.2	727.1	56.3	339.1	119.2	79.0	137.0	2695.1	615.5	546.3
546	195.9	2974.0	674.8	644.3	67.3	415.4	331.2	288.8	118.0	2706.1	583.6	449.0
553	180.4	2935.7	659.7	586.7	49.3	402.9	243.0	202.2	100.7	2611.0	657.7	458.6
560	263.2	2934.5	727.7	600.3	67.3	413.6	243.0	183.5	111.2	2736.7	626.2	521.4
567	222.5	2813.3	536.1	462.0	86.4	404.3	205.3	150.9	162.4	2589.1	506.5	436.5
574	306.2	2777.7	472.6	409.2	100.8	499.2	170.3	144.4	179.0	2599.1	463.2	399.9
581	320.0	2745.8	504.3	347.8	106.3	526.7	248.0	196.2	142.4	2674.8	469.8	389.2
588	227.7	2755.6	514.9	409.2	99.0	462.8	242.8	166.4	171.0	2708.7	472.1	399.3
595	246.2	3029.0	529.7	361.6	74.9	342.6	224.0	163.9	193.4	2656.1	490.9	420.5
602	305.9	2904.3	542.4	365.8	83.8	429.5	239.6	152.2	142.1	2602.3	444.0	365.6
609	217.8	2640.7	557.2	372.1	91.7	573.7	236.3	178.7	174.4	2728.4	418.6	345.1
616	220.7	2780.6	510.7	337.2	101.1	486.0	237.6	165.8	179.4	2685.1	431.9	347.6

Table D-3 TCOD, SCOD, PCOD of Two Stage TAnMBR during Reactor Operation

Days	Feed			Hydrolytic Effluent			Permeate	Removal Efficiency (%)
	TCOD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	TCOD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	SCOD (mg/L)	
1	12032	3760	8272	20080	7520	12560	3008	75.0
5	15040	3384	11656	15792	6016	9776	1692	88.8
9	15040	3760	11280	27072	8272	18800	1692	88.8
12	11776	2944	8832	16928	8096	8832	1472	87.5
16	16016	6188	9828	24024	9464	14560	1820	88.6
19	15200	8400	6800	16800	10400	6400	1600	89.5
23	15608	6700	8908	18812	8932	9880	1710	89.0
26	16400	7200	9200	24000	13600	10400	1840	88.8
29	16816	6200	10616	24304	14112	10192	1700	89.9
33	14000	5820	8180	23280	10864	12416	1475	89.5
37	13968	6208	7760	24832	12416	12416	1552	88.9
40	15960	6460	9500	20520	11400	9120	1444	91.0
47	16544	4136	12408	15040	11280	3760	1579	90.5
54	14288	5264	9024	18048	10528	7520	1654	88.4
61	14616	5429	9187	15312	11832	3480	1601	89.0
68	15352	5322	10030	17230	11260	5970	1570	89.8
76	14896	2744	12152	18032	10192	7840	1646	88.9
79	12032	1880	10152	24064	9024	15040	1579	86.9
83	13536	3384	10152	20304	8272	12032	1654	87.8
86	11280	4512	6768	23312	7520	15792	1205	89.3
90	13104	2002	11102	12376	8736	3640	1100	91.6
93	13968	1552	12416	13192	9312	3880	1164	91.7
97	15520	3880	11640	13192	9312	3880	1242	92.0
100	16296	3492	12804	15520	9312	6208	1164	92.9
104	13248	4048	9200	18048	13248	4800	1000	92.5
107	14400	8800	5600	18400	15200	3200	1100	92.4
111	13192	12028	1164	18624	9312	9312	1009	92.4
114	13968	11640	2328	21728	10864	10864	1009	92.8
118	13680	5700	7980	15960	12160	3800	1140	91.7
121	14440	5100	9340	20520	16720	3800	1216	91.6
125	14720	9936	4784	18400	11776	6624	1120	92.4
128	14920	8800	6120	18400	15200	3200	1150	92.3
132	19400	7954	11446	18192	10854	7338	1320	93.2
135	18624	8536	10088	18968	13968	5000	1397	92.5
139	18800	8648	10152	18048	15792	2256	1580	91.6
142	22560	10152	12408	18792	17296	1496	1654	92.7
146	18400	3500	14900	17512	11040	6472	1480	92.0
149	19200	2800	16400	20000	14400	5600	1360	92.9
153	19200	3200	16000	19200	13600	5600	1200	93.8
156	19200	3600	15600	19200	14400	4800	1250	93.5
160	19464	7252	12212	17896	11760	6136	1254	93.6
163	18032	8232	9800	16464	14112	2352	1725	90.4
167	18624	8924	9700	17848	15520	2328	1979	89.4
170	19960	7980	11980	15960	15200	760	1748	91.2
174	19440	7740	11700	16560	14400	2160	2448	87.4
177	19200	8148	11052	16296	15200	1096	2406	87.5
181	19848	6596	13252	26384	13192	13192	1474	92.6
184	19000	8342	10658	18624	11640	6984	1707	91.0
188	19400	8600	10800	19000	16800	2200	1680	91.3
191	19400	8000	11400	21600	18400	3200	1680	91.3
195	18400	7360	11040	18400	16192	2208	1766	90.4
198	18400	6808	11592	19664	17664	2000	1619	91.2
202	19400	7360	12040	20000	16800	3200	1760	90.9

Table D-3 TCOD, SCOD, PCOD of Two Stage TAnMBR during Reactor Operation (Con't)

Days	Feed			Hydrolytic Effluent			Permeate	Removal Efficiency (%)
	TCOD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	TCOD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	SCOD (mg/L)	
205	19400	6080	13320	18400	16800	1600	1760	90.9
209	19600	6664	12936	18032	15680	2352	1725	91.2
212	19600	6212	13388	19600	16414	3186	1740	91.1
216	19400	3104	16296	17072	14744	2328	1630	91.6
219	19000	7600	11400	19760	16720	3040	1672	91.2
223	19200	8800	10400	20600	18400	2200	1440	92.5
226	20000	11200	8800	24800	17600	7200	1520	92.4
230	19600	8400	11200	17600	15200	2400	1520	92.2
233	18400	2400	16000	17600	15200	2400	1520	91.7
237	19200	3200	16000	19800	16800	3000	1600	91.7
243	20000	8000	12000	19200	17600	1600	1720	91.4
251	20000	5600	14400	19200	17600	1600	1840	90.8
258	19200	5600	13600	18400	17600	800	1520	92.1
265	23200	7200	16000	21600	20000	1600	2000	91.4
272	20800	5600	15200	19200	18400	800	1600	92.3
279	20250	6000	14250	22125	20250	1875	2250	88.9
286	21000	6750	14250	20250	18750	1500	2100	90.0
293	21750	6750	15000	21750	18750	3000	2225	89.8
300	20950	6275	14675	20831	19038	1794	2325	88.9
303	19200	4000	15200	24800	17600	7200	2060	89.3
310	20000	4800	15200	24000	19200	4800	2160	89.2
317	20253	2352	17901	24304	21168	3136	2117	89.5
324	20253	3717	16536	24368	19322	5046	2250	88.9
327	23520	3136	20384	23520	20384	3136	2950	87.5
331	24320	9360	14960	19440	18720	720	2522	89.6
334	25560	3960	21600	21960	21240	720	2400	90.6
338	24000	3600	20400	23200	20800	2400	2522	89.5
342	24000	4200	19800	21600	20800	800	2400	90.0
345	24304	6664	17640	18032	17248	784	2352	90.3
348	24304	8624	15680	17248	15680	1568	2587	89.4
352	24320	5684	18636	16072	14112	1960	3763	84.5
356	25560	6976	18584	15680	14896	784	3998	84.4
359	27440	8232	19208	23520	14800	8720	3763	86.3
363	28244	7056	21188	29792	19600	10192	3920	86.1
366	24444	3492	20952	25996	24444	1552	4423	81.9
370	25220	3492	21728	25996	23474	2522	8536	66.2
374	24056	5432	18624	19400	17848	1552	9467	60.6
377	20176	7760	12416	17848	14356	3492	9622	52.3
381	20176	11880	8296	14760	14040	720	4800	76.2
384	22680	10800	11880	14760	14040	720	2880	87.3
388	24832	12416	12416	25608	22504	3104	2328	90.6
391	26834	6208	20626	27160	23280	3880	3104	88.4
395	21952	7056	14896	25872	23520	2352	3920	82.1
398	23520	13328	10192	23520	18816	4704	4704	80.0
401	22504	9312	13192	19400	17848	1552	3725	83.4
405	24506	4656	19850	20952	16296	4656	3770	84.6
408	23560	4560	19000	28880	20520	8360	3800	83.9
411	24320	5320	19000	25840	22040	3800	3750	84.6
415	23260	4061	19198	26213	18091	8122	3809	83.6
419	23064	8184	14880	23808	19344	4464	3648	84.2
422	23040	8360	14680	28880	20520	8360	3800	83.5

Table D-3 TCOD, SCOD, PCOD of Two Stage TAnMBR during Reactor Operation (Con't)

Days	Feed			Hydrolytic Effluent			Permeate	Removal Efficiency (%)
	TCOD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	TCOD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	SCOD (mg/L)	
426	23560	7600	15960	22840	22040	800	3809	83.8
429	24800	8000	16800	24800	21600	3200	3520	85.8
432	25600	7200	18400	24000	21600	2400	3840	85.0
436	23064	12544	10520	21168	20384	784	3371	85.4
443	23520	10976	12544	19600	18816	784	3136	86.7
450	24304	8624	15680	21168	17248	3920	3200	86.8
457	24304	9408	14896	20384	18816	1568	3236	86.7
464	25088	10976	14112	21168	17248	3920	3293	86.9
471	24304	9408	14896	21168	19600	1568	3371	86.1
478	25088	7840	17248	21952	17248	4704	3648	85.5
481	24304	8624	15680	21168	17288	3880	3520	85.5
485	24304	9016	15288	19600	17248	2352	3450	85.8
488	24560	10528	14032	22560	17296	5264	3509	85.7
492	24816	12032	12784	21808	18048	3760	3610	85.5
497	23200	10400	12800	19200	16000	3200	3350	85.6
504	24560	10400	14160	19200	17600	1600	3840	84.4
511	24800	8000	16800	27200	21600	5600	4160	83.2
518	23520	12544	10976	20076	16856	3220	3685	84.3
525	25088	10192	14896	20384	17248	3136	3920	84.4
532	24400	10400	14000	19200	17671	1529	3784	84.5
539	24192	10986	13206	21189	17114	4075	3694	84.7
546	23520	10424	13096	21610	18098	3512	3609	84.7
553	23128	9608	13520	18424	15288	3136	3509	84.8
560	23560	9120	14440	22040	17480	4560	3488	85.2
567	25088	9408	15680	21952	18032	3920	3842	84.7
574	23520	9608	13912	18424	16464	1960	3763	84.0
581	25088	10192	14896	21168	19600	1568	3920	84.4
588	23560	10640	12920	22800	18620	4180	3842	83.7
595	25106	9599	15506	25844	22152	3692	3840	84.7
602	24000	11600	12400	26400	23200	3200	3840	84.0
609	22800	6840	15960	25080	20520	4560	3496	84.7
616	23560	8624	14936	24000	20000	4000	3750	84.1

Table D-4 Methane Production of Two Stage TAnMBR during Reactor Operation

Days	Biogas Production (L/d)	Methane Content (%)	Methane Production (L/d)	Methane Yield (m^3methane/kgCOD $_{removed}$)	Methane Production Rate ($L_{methane}/L_{reactor}$.d)
1	15.0	47.4	7.1	0.22	0.8
5	13.3	47.0	6.3	0.13	0.7
9	18.5	51.0	9.5	0.20	1.1
12	14.4	51.6	7.4	0.21	0.8
16	15.3	51.7	8.4	0.17	0.9
19	15.4	48.2	7.4	0.16	0.8
23	16.4	46.6	7.7	0.16	0.9
26	17.5	54.2	9.5	0.19	1.1
29	19.1	55.5	10.6	0.20	1.2
33	17.9	54.8	9.8	0.22	1.1
37	20.7	52.1	10.8	0.25	1.2
40	17.9	57.6	10.3	0.20	1.1
47	22.5	55.5	12.5	0.24	1.4
52	23.9	55.1	13.2	-	1.5
54	24.1	55.6	13.4	0.30	1.5
59	24.3	54.7	13.3	-	1.5
61	24.3	54.7	13.3	0.29	1.5
68	24.4	54.8	13.4	0.28	1.5
76	21.6	50.6	11.0	0.24	1.2
79	20.0	50.6	10.1	0.28	1.1
83	18.3	45.9	8.4	0.20	0.9
86	20.8	48.6	10.1	0.29	1.1
90	21.1	52.5	11.1	0.26	1.2
93	27.5	52.5	14.4	0.32	1.6
97	26.6	59.1	15.7	0.31	1.7
100	25.8	59.1	15.2	0.29	1.7
104	23.3	61.2	14.3	0.33	1.6
107	24.1	61.2	14.8	0.32	1.6
111	25.8	60.1	15.5	0.36	1.7
114	25.0	61.2	15.3	0.34	1.7
118	25.0	60.9	15.2	0.35	1.7
121	25.8	59.1	15.2	0.33	1.7
125	25.8	60.3	15.6	0.33	1.7
128	25.8	60.4	15.6	0.32	1.7
132	24.1	54.7	13.2	0.20	1.5
135	30.8	56.2	17.3	0.27	1.9
139	31.6	56.5	17.9	0.28	2.0
142	32.5	51.8	16.8	0.26	1.9
146	33.3	52.9	17.6	0.28	2.0
149	30.8	53.2	16.4	0.25	1.8
153	27.5	44.3	12.2	0.18	1.4
156	33.3	45.3	15.1	0.23	1.7
160	30.0	47.8	14.3	0.21	1.6
163	32.5	57.3	18.6	0.31	2.1
167	32.5	55.5	18.0	0.29	2.0
170	31.6	56.4	17.8	0.26	2.0
174	27.5	45.1	12.4	0.20	1.4
177	27.5	48.4	13.3	0.21	1.5
181	31.6	54.0	17.1	0.25	1.9
184	31.6	57.2	18.1	0.28	2.0
188	32.5	62.5	20.3	0.31	2.3
191	33.3	59.3	19.7	0.30	2.2
195	31.6	59.7	18.9	0.31	2.1
198	30.8	57.5	17.7	0.29	2.0
202	29.1	58.8	17.1	0.26	1.9
205	29.1	59.0	17.2	0.26	1.9
209	29.1	58.9	17.2	0.26	1.9
212	30.0	59.9	18.0	0.27	2.0
216	30.0	58.0	17.4	0.26	1.9

Table D-4 Methane Production of Two Stage TAnMBR during Reactor Operation (Con't)

Days	Biogas Production (L/d)	Methane Content (%)	Methane Production (L/d)	Methane Yield (m^3methane/kgCOD $_{removed}$)	Methane Production Rate ($L_{methane}/L_{reactor}$.d)
219	31.6	57.1	18.0	0.28	2.0
223	30.8	57.8	17.8	0.27	2.0
226	30.8	57.1	17.6	0.26	2.0
230	31.6	56.6	17.9	0.27	2.0
233	30.0	57.1	17.1	0.27	1.9
237	30.0	57.1	17.1	0.26	1.9
243	30.8	58.0	17.9	0.26	2.0
251	30.0	58.2	17.4	0.26	1.9
258	30.0	57.4	17.2	0.26	1.9
265	27.5	55.7	15.3	0.20	1.7
272	30.0	57.7	17.3	0.24	1.9
279	30.0	57.1	17.1	0.26	1.9
286	31.6	56.5	17.9	0.26	2.0
293	30.0	58.3	17.5	0.24	1.9
300	30.8	57.7	17.8	0.26	2.0
303	31.6	55.8	17.6	0.28	2.0
310	30.8	56.6	17.4	0.26	1.9
317	31.6	56.2	17.8	0.26	2.0
324	25.8	55.8	14.4	0.20	1.6
327	28.3	59.2	16.8	0.20	1.9
331	27.5	57.5	15.8	0.18	1.8
334	28.3	56.1	15.9	0.17	1.8
338	25.8	56.3	14.5	0.15	1.6
342	30.0	56.3	16.9	0.17	1.9
345	31.6	59.3	18.7	0.19	2.1
348	34.1	58.9	20.1	0.21	2.2
352	31.6	55.9	17.7	0.19	2.0
356	29.1	50.1	14.6	0.15	1.6
359	30.0	57.3	17.2	0.16	1.9
363	30.8	61.8	19.0	0.17	2.1
366	31.6	56.3	17.8	0.20	2.0
370	27.5	49.8	13.7	0.23	1.5
374	26.6	47.2	12.6	0.25	1.4
377	22.5	45.3	10.2	0.28	1.1
381	27.5	52.0	14.3	0.27	1.6
384	28.3	55.1	15.6	0.23	1.7
388	35.8	57.3	20.5	0.26	2.3
391	35.8	53.6	19.2	0.18	2.1
395	37.5	52.4	19.6	0.24	2.2
398	39.6	51.1	20.3	0.24	2.3
401	40.8	52.8	21.5	0.25	2.4
405	40.8	52.6	21.4	0.23	2.4
408	41.6	51.9	21.6	0.24	2.4
411	40.0	54.8	21.9	0.24	2.4
415	39.1	53.2	20.8	0.24	2.3
419	40.0	54.0	21.6	0.25	2.4
422	40.1	53.1	21.3	0.25	2.4
426	40.8	53.5	21.8	0.25	2.4
429	40.0	56.0	22.4	0.23	2.5
432	40.8	55.0	22.4	0.23	2.5
436	40.8	54.2	22.1	0.25	2.5
443	42.4	54.1	22.9	0.25	2.5
450	42.4	53.4	22.7	0.24	2.5
457	42.4	52.9	22.4	0.24	2.5
464	42.4	53.1	22.6	0.23	2.5
471	42.4	52.6	22.3	0.24	2.5
478	43.3	52.6	22.8	0.24	2.5

Table D-4 Methane Production of Two Stage TAnMBR during Reactor Operation (Con't)

Days	Biogas Production (L/d)	Methane Content (%)	Methane Production (L/d)	Methane Yield (m^3methane/kgCOD $_{removed}$)	Methane Production Rate ($L_{methane}/L_{reactor}$.d)
481	40.8	53.7	21.9	0.23	2.4
485	41.6	53.2	22.2	0.24	2.5
488	41.6	53.1	22.1	0.23	2.5
492	41.6	53.2	22.1	0.23	2.5
497	41.6	52.6	21.9	0.25	2.4
504	41.6	53.3	22.2	0.24	2.5
511	41.6	53.0	22.1	0.24	2.5
518	40.8	53.0	21.6	0.24	2.4
525	40.8	53.6	21.9	0.23	2.4
532	40.8	52.5	21.4	0.23	2.4
539	40.0	52.7	21.1	0.23	2.3
546	40.0	53.5	21.4	0.24	2.4
553	39.1	53.9	21.1	0.24	2.3
560	40.8	53.2	21.7	0.24	2.4
567	40.0	53.9	21.5	0.23	2.4
574	40.0	53.9	21.5	0.24	2.4
581	40.0	53.0	21.2	0.22	2.4
588	39.1	53.6	21.0	0.24	2.3
595	39.1	52.9	20.7	0.22	2.3
602	40.0	53.5	21.4	0.24	2.4
609	39.1	52.4	20.5	0.24	2.3
616	38.3	52.2	20.0	0.22	2.2

Table D-5 Removal Rates, COD, MLSS, MLVSS and Alkalinity of the Two Stage TAnMBR during Reactor Operation

Days	COD Inf (mg/L)	COD Eff (mg/L)	Removal Efficiency (%)	OLR ($kgCOD/m^3.d$)	ORR ($kgCOD/m^3.d$)	MLSS (g/L)	MLVSS (g/L)	Biological Activity (gCOD/gVSS.d)	pH	Ammonia (mg/L)	Alkalinity (mg/L)
1	12032	3008	75.0	4.7	3.5	22.6	17.3	0.30	7.3	364	1600
5	15040	1692	88.8	5.8	5.2	24.2	19.5	0.40	7.1	315	1900
9	15040	1692	88.8	5.8	5.2	22.5	16.8	0.46	7.3	308	2000
12	11776	1472	87.5	4.6	4.0	24.2	18.7	0.32	7.4	399	1950
16	16016	1820	88.6	6.2	5.5	21.0	16.9	0.49	7.2	357	1850
19	15200	1600	89.5	5.9	5.3	19.0	15.7	0.51	7.1	462	2350
24	15608	1710	89.0	6.1	5.4	20.0	16.4	0.49	7.3	399	2250
26	16400	1840	88.8	6.4	5.7	24.5	18.8	0.45	7.1	406	2400
30	16816	1700	89.9	6.5	5.9	25.4	22.2	0.40	7.0	350	2200
33	14000	1475	89.5	5.4	4.9	25.0	22.1	0.33	7.0	364	2300
37	13968	1552	88.9	5.4	4.8	24.3	21.0	0.34	7.0	406	2250
40	15960	1444	91.0	6.2	5.6	23.2	19.7	0.43	7.3	427	2700
47	16544	1579	90.5	6.4	5.8	24.9	22.5	0.39	7.2	658	2500
54	14288	1654	88.4	5.6	4.9	27.6	22.4	0.33	7.3	665	2600
61	14616	1601	89.0	5.7	5.1	24.1	21.8	0.35	7.1	679	2550
68	15352	1570	89.8	6.0	5.4	25.0	21.6	0.37	7.0	667	2600
76	14896	1646	88.9	5.8	5.2	27.3	24.2	0.32	7.0	679	2900
79	12032	1579	86.9	4.7	4.1	28.4	25.4	0.24	7.1	745	3200
83	13536	1654	87.8	5.3	4.6	30.3	24.7	0.28	7.4	616	3250
86	11280	1205	89.3	4.4	3.9	31.3	25.4	0.23	7.1	681	3450
90	13104	1100	91.6	5.1	4.7	27.5	23.1	0.30	7.2	658	3800
93	13968	1164	91.7	5.4	5.0	30.4	25.5	0.29	7.3	670	3850
97	15520	1242	92.0	6.0	5.6	30.8	26.4	0.32	7.3	711	3500
100	16296	1164	92.9	6.3	5.9	30.9	26.1	0.34	7.2	700	3500
104	13248	1000	92.5	5.2	4.8	27.9	24.4	0.29	7.2	651	3300
107	14400	1100	92.4	5.6	5.2	25.9	24.7	0.31	7.1	651	3450
111	13192	1009	92.4	5.1	4.7	31.7	25.8	0.28	7.3	632	3200
114	13968	1009	92.8	5.4	5.0	30.6	24.9	0.30	7.3	632	3200
118	13680	1140	91.7	5.3	4.9	29.4	24.4	0.30	7.4	637	3200
121	14440	1216	91.6	5.6	5.1	30.8	25.1	0.31	7.3	637	3250
125	14720	1120	92.4	5.7	5.3	34.6	24.3	0.33	7.3	658	4100
128	14920	1150	92.3	5.8	5.4	34.2	24.7	0.33	7.3	658	4100
132	19400	1320	93.2	8.0	7.4	34.8	27.5	0.41	7.3	553	3750
135	18624	1397	92.5	7.7	7.1	35.2	28.0	0.38	7.4	632	3750
139	18800	1580	91.6	7.7	7.1	30.7	27.0	0.39	7.5	777	4050

Table D-5 Removal Rates, COD, MLSS, MLVSS and Alkalinity of the Two Stage TAnMBR during Reactor Operation (Con't)

Days	COD Inf (mg/L)	COD Eff (mg/L)	Removal Efficiency (%)	OLR ($kgCOD/m^3.d$)	ORR ($kgCOD/m^3.d$)	MLSS (g/L)	MLVSS (g/L)	Biological Activity (gCOD/gVSS.d)	pH	Ammonia (mg/L)	Alkalinity (mg/L)
142	18800	1654	92.7	7.7	7.2	32.2	28.0	0.38	7.4	777	4050
146	18400	1480	92.0	7.6	7.0	31.7	26.8	0.39	7.3	749	4850
149	19200	1360	92.9	7.9	7.3	29.9	25.4	0.43	7.4	749	4850
153	19200	1200	93.8	7.9	7.4	28.3	23.3	0.48	7.4	700	4500
156	19200	1250	93.5	7.9	7.4	27.3	23.7	0.47	7.3	700	4500
160	19464	1254	93.6	8.0	7.5	20.6	18.9	0.59	7.0	693	2850
163	18032	1725	90.4	7.4	6.7	21.5	19.9	0.50	7.1	693	3350
167	18624	1979	89.4	7.7	6.8	20.8	18.1	0.57	7.0	728	3950
170	19960	1748	91.2	8.2	7.5	21.1	18.3	0.61	7.1	728	4000
174	19440	2448	87.4	8.0	7.0	17.5	15.6	0.67	7.0	742	3100
177	19200	2406	87.5	7.9	6.9	17.2	15.3	0.68	7.0	742	3200
181	19848	1474	92.6	8.2	7.6	18.0	15.9	0.71	7.2	665	3800
184	19000	1707	91.0	7.8	7.1	18.2	16.0	0.67	7.3	665	3800
188	19400	1680	91.3	8.0	7.3	18.5	16.0	0.68	7.4	721	4250
191	19400	1680	91.3	8.0	7.3	19.1	16.3	0.67	7.2	721	4250
195	18400	1766	90.4	7.6	6.8	18.2	16.1	0.64	7.3	693	4150
198	18400	1619	91.2	7.6	6.9	18.8	16.7	0.62	7.3	693	4150
202	19400	1760	90.9	8.0	7.3	20.1	17.8	0.61	7.2	742	3250
205	19400	1760	90.9	8.0	7.3	19.1	17.0	0.64	7.3	742	3350
209	19600	1725	91.2	8.1	7.3	19.8	17.5	0.63	7.2	749	3250
212	19600	1740	91.1	8.1	7.3	19.5	17.4	0.63	7.2	749	3400
216	19400	1630	91.6	8.0	7.3	19.6	17.4	0.63	7.2	714	3500
219	19000	1672	91.2	7.8	7.1	20.1	17.8	0.60	7.1	847	3350
223	19200	1440	92.5	7.9	7.3	41.6	37.5	0.29	7.2	749	3350
226	20000	1520	92.4	8.2	7.6	41.1	37.2	0.31	7.1	767	3350
230	19600	1520	92.2	8.1	7.4	27.5	33.5	0.33	7.2	784	3350
233	18400	1520	91.7	7.6	6.9	30.0	33.0	0.32	7.4	808	3350
237	19200	1600	91.7	7.9	7.2	24.0	35.5	0.31	7.3	749	3350
243	20000	1720	91.4	8.2	7.5	32.5	35.5	0.32	7.3	707	3750
251	20000	1840	90.8	8.2	7.5	27.0	34.0	0.33	7.3	784	3750
258	19200	1520	92.1	7.9	7.3	33.3	34.8	0.31	7.2	952	3550
265	23200	2000	91.4	9.5	8.7	24.3	34.0	0.38	7.3	784	3400
272	20800	1600	92.3	8.6	7.9	35.3	32.7	0.36	7.2	700	3200
279	20250	2250	88.9	8.3	7.4	35.5	36.3	0.31	7.2	840	3500
286	21000	2100	90.0	8.6	7.8	36.8	35.3	0.33	7.2	931	3550

Table D-5 Removal Rates, COD, MLSS, MLVSS and Alkalinity of the Two Stage TAnMBR during Reactor Operation (Con't)

Days	COD Inf (mg/L)	COD Eff (mg/L)	Removal Efficiency (%)	OLR ($kgCOD/m^3.d$)	ORR ($kgCOD/m^3.d$)	MLSS (g/L)	MLVSS (g/L)	Biological Activity (gCOD/gVSS.d)	pH	Ammonia (mg/L)	Alkalinity (mg/L)
293	21750	2225	89.8	8.9	8.0	37.3	37.3	0.32	7.2	798	4000
300	20950	2325	88.9	8.6	7.7	36.2	34.0	0.34	7.2	1025	3700
303	19200	2060	89.3	7.9	7.0	26.0	34.0	0.31	7.2	973	3850
310	20000	2160	89.2	8.2	7.3	30.0	34.5	0.32	7.1	1078	3950
317	20253	2117	89.5	8.3	7.5	36.0	36.8	0.30	7.2	1026	4200
324	20253	2250	88.9	9.0	8.0	33.3	37.8	0.32	7.2	1148	4100
327	23520	2950	87.5	10.5	9.1	31.3	37.7	0.36	7.2	1120	4200
331	24320	2522	89.6	10.8	9.7	31.5	36.3	0.40	7.1	931	4150
334	25560	2400	90.6	11.4	10.3	33.0	37.3	0.41	7.3	1022	4050
338	24000	2522	89.5	12.0	10.7	27.5	35.5	0.45	7.1	1008	3850
342	24000	2400	90.0	12.0	10.8	28.0	36.8	0.44	7.1	1057	4000
345	24304	2352	90.3	12.2	11.0	27.5	35.5	0.46	7.2	1274	4750
348	24304	2587	89.4	12.2	10.9	30.5	36.3	0.45	7.2	1218	4250
352	24320	3763	84.5	12.2	10.3	34.8	38.5	0.40	7.2	1155	3700
356	25560	3998	84.4	12.8	10.8	35.0	37.8	0.43	7.2	1176	3500
359	27440	3763	86.3	13.7	11.8	30.5	35.8	0.50	7.2	1050	4050
363	28244	3920	86.1	14.1	12.2	32.5	37.8	0.48	6.6	952	4050
366	24444	4423	81.9	12.2	10.0	33.2	38.8	0.39	7.1	819	3950
370	25220	8536	66.2	9.8	6.5	35.5	36.8	0.26	7.0	770	4750
374	24056	9467	60.6	9.4	5.7	37.0	37.8	0.23	7.0	833	4850
377	20176	9622	52.3	7.8	4.1	36.0	37.3	0.17	5.6	833	4550
381	20176	4800	76.2	7.8	6.0	31.5	38.5	0.23	7.0	756	4650
384	22680	2880	87.3	8.8	7.7	32.5	37.3	0.31	6.1	686	4850
388	24832	2328	90.6	9.7	8.8	32.5	37.8	0.35	7.3	665	5200
391	26834	3104	88.4	13.4	11.9	28.5	37.5	0.47	7.3	630	5050
395	21952	3920	82.1	11.0	9.0	34.3	38.0	0.36	7.4	1400	4000
398	23520	4704	80.0	11.8	9.4	33.0	37.3	0.38	7.3	1295	4150
401	22504	3725	83.4	11.3	9.4	37.0	38.5	0.37	7.1	721	4450
405	24506	3770	84.6	12.3	10.4	35.0	38.5	0.40	7.2	784	4550
408	23560	3800	83.9	11.8	9.9	33.0	36.5	0.41	7.1	812	4500
411	24320	3750	84.6	12.2	10.3	32.0	37.0	0.42	7.5	777	4600
415	23260	3809	83.6	11.6	9.7	36.3	38.8	0.38	7.2	805	4100
419	23064	3648	84.2	11.5	9.7	37.5	38.0	0.38	7.3	812	4200
422	23040	3800	83.5	11.5	9.6	40.5	37.8	0.38	7.1	903	3300
426	23560	3809	83.8	11.8	9.9	39.3	39.0	0.38	7.2	847	3600

Table D-5 Removal Rates, COD, MLSS, MLVSS and Alkalinity of the Two Stage TAnMBR during Reactor Operation (Con't)

Days	COD Inf (mg/L)	COD Eff (mg/L)	Removal Efficiency (%)	OLR ($kgCOD/m^3.d$)	ORR ($kgCOD/m^3.d$)	MLSS (g/L)	MLVSS (g/L)	Biological Activity (gCOD/gVSS.d)	pH	Ammonia (mg/L)	Alkalinity (mg/L)
429	24800	3520	85.8	12.4	10.6	38.3	37.3	0.43	7.2	1148	3050
432	25600	3840	85.0	12.8	10.9	37.8	38.3	0.43	7.2	1015	3150
436	23064	3371	85.4	11.5	9.8	45.0	40.0	0.37	7.1	1106	4650
443	23520	3136	86.7	11.8	10.2	44.5	39.5	0.39	7.2	1106	4050
450	24304	3200	86.8	12.2	10.6	45.5	39.5	0.40	7.1	994	3950
457	24304	3236	86.7	12.2	10.5	46.5	39.0	0.41	7.1	994	3800
464	25088	3293	86.9	12.5	10.9	43.5	39.3	0.42	7.1	1036	4000
471	24304	3371	86.1	12.2	10.5	41.8	39.8	0.39	7.2	896	3750
478	25088	3648	85.5	12.5	10.7	46.2	40.0	0.40	6.5	854	4600
481	24304	3520	85.5	12.2	10.4	47.6	39.0	0.40	6.8	952	5250
485	24304	3450	85.8	12.2	10.4	50.9	41.3	0.38	6.8	1036	4950
488	24560	3509	85.7	12.3	10.5	47.5	39.6	0.40	7.1	1078	4750
492	24816	3610	85.5	12.4	10.6	49.2	40.6	0.39	7.2	1092	4800
497	23200	3350	85.6	11.6	9.9	48.3	40.5	0.37	7.2	1036	4700
504	24560	3840	84.4	12.3	10.4	48.8	40.8	0.38	7.1	1050	4350
511	24800	4160	83.2	12.4	10.3	45.4	40.2	0.39	7.4	1162	4150
518	23520	3685	84.3	11.8	9.9	52.0	40.5	0.37	7.2	1120	4550
525	25088	3920	84.4	12.5	10.6	47.6	40.9	0.39	7.5	1106	4750
532	24400	3784	84.5	12.2	10.3	49.2	40.0	0.39	7.1	1064	4600
539	24192	3694	84.7	12.1	10.2	50.0	41.0	0.37	7.1	1036	4700
546	23520	3609	84.7	11.8	10.0	51.0	40.0	0.37	7.3	1106	4950
553	23128	3509	84.8	11.6	9.8	44.1	40.7	0.36	7.2	1302	4650
560	23560	3488	85.2	11.8	10.0	43.1	40.6	0.37	7.2	1260	4750
567	25088	3842	84.7	12.5	10.6	45.3	40.0	0.40	7.1	1148	4050
574	23520	3763	84.0	11.8	9.9	42.8	39.0	0.38	7.1	1015	4150
581	25088	3920	84.4	12.5	10.6	43.6	39.5	0.40	7.0	952	4780
588	23560	3842	83.7	11.8	9.9	40.8	39.4	0.38	7.1	1036	5500
595	25106	3840	84.7	12.6	10.6	45.5	39.5	0.40	7.1	1106	5750
602	24000	3840	84.0	12.0	10.1	41.0	39.5	0.38	7.2	1064	5250
609	22800	3496	84.7	11.4	9.7	41.3	39.3	0.37	7.2	1148	4875
616	23560	3750	84.1	11.8	9.9	46.0	39.5	0.38	7.2	1015	4900

APPENDIX E
Membrane Fouling Investigation

Table E-1 TMP and Flux Variation

Day	TMP (kPa)	Flux (LMH)	Day	TMP (kPa)	Flux (LMH)
133	8.4	0.86	199	14.9	0.88
134	7.9	0.83	200	14.9	0.88
135	7.9	0.81	201	14.9	0.83
136	8.4	0.86	202	15.4	0.83
138	8.4	0.81	204	15.4	0.81
140	7.9	0.86	205	15.4	0.83
142	7.9	0.81	206	15.4	0.83
143	8.4	0.86	207	15.4	0.81
144	7.9	0.88	209	15.4	0.81
147	7.9	0.86	210	14.9	0.81
150	8.4	0.86	211	14.9	0.81
154	8.4	0.86	212	14.4	0.81
155	7.4	0.81	214	14.9	0.83
156	7.9	0.88	216	15.9	0.83
157	8.4	0.86	219	16.4	0.79
158	9.4	0.79	223	16.9	0.79
161	9.9	0.76	226	17.4	0.74
162	9.9	0.86	230	17.4	0.74
164	8.9	0.86	233	16.9	0.67
165	10.4	0.86	237	17.4	0.65
166	10.4	0.88	243	7.9	0.81
167	10.9	0.81	244	8.4	0.81
168	10.4	0.83	248	8.9	0.83
169	10.9	0.83	251	8.9	0.83
170	11.4	0.81	254	8.9	0.83
173	12.4	0.86	255	9.9	0.83
178	13.4	0.90	256	9.9	0.83
179	12.9	0.83	257	10.9	0.88
180	12.9	0.81	258	10.9	0.86
181	12.4	0.86	260	9.9	0.86
182	12.4	0.90	263	10.4	0.83
183	12.4	0.83	264	10.9	0.86
184	14.4	0.81	265	10.9	0.88
185	13.9	0.86	267	10.4	0.86
186	12.9	0.83	269	10.9	0.81
187	14.4	0.81	270	10.9	0.79
188	14.4	0.86	275	9.9	0.81
190	13.4	0.88	276	10.4	0.79
191	13.9	0.81	277	10.9	0.79
192	13.4	0.88	280	10.9	0.84
193	13.9	0.83	281	11.4	0.87
194	13.9	0.86	282	10.9	0.87
195	14.9	0.88	283	10.9	0.81
196	14.9	0.90	286	11.4	0.79
197	14.4	0.81	288	11.9	0.81
198	14.4	0.81	290	12.9	0.81

Table E-1 TMP and Flux Variation (Con't)

Day	TMP (kPa)	Flux (LMH)	Day	TMP (kPa)	Flux (LMH)
291	12.4	0.83	363	18.4	1.02
292	13.4	0.86	364	18.4	1.02
294	13.4	0.76	365	18.9	1.00
295	13.4	0.72	366	19.4	1.00
298	14.4	0.74	367	18.4	1.00
300	16.4	0.74	368	19.9	0.97
303	20.9	0.81	369	20.4	1.06
307	25.9	0.86	370	18.9	1.04
310	27.9	0.86	371	10.9	0.76
312	27.9	0.88	372	11.4	0.76
313	34.9	0.86	373	11.9	0.81
314	35.9	0.86	374	12.9	0.83
316	38.9	0.83	375	12.9	0.81
318	39.9	0.86	376	12.9	0.81
320	41.9	0.88	377	13.4	0.83
321	14.9	0.79	378	13.4	0.83
324	16.4	0.88	380	13.9	0.79
326	16.9	0.86	381	11.9	0.81
327	17.4	0.88	382	11.9	0.83
328	17.9	0.86	383	12.9	0.79
329	20.9	0.90	384	12.9	0.83
330	18.9	0.88	386	11.9	0.81
332	18.4	0.90	387	12.9	0.83
333	20.4	0.90	388	11.9	0.81
334	19.4	0.93	389	12.4	0.79
335	19.9	0.93	390	12.4	0.81
336	18.9	0.93	391	14.4	0.97
338	19.9	0.97	392	14.4	1.00
339	19.9	0.97	393	14.9	0.97
340	18.9	1.00	394	16.9	1.02
341	19.9	1.00	395	18.4	0.97
343	19.4	1.00	397	21.9	1.00
344	19.9	0.97	398	21.9	1.00
345	19.9	1.00	399	22.4	0.97
346	19.4	0.97	400	21.4	0.97
347	18.9	1.00	404	22.4	1.00
348	17.9	0.97	408	22.9	0.97
351	17.9	0.97	409	21.9	1.04
352	19.4	1.00	410	21.9	1.00
353	18.9	0.97	411	22.4	1.04
355	19.4	1.04	412	22.4	1.00
357	19.4	0.97	413	23.9	0.97
359	18.4	0.97	414	23.9	1.00
360	19.4	1.00	416	23.9	0.97
361	20.4	1.00	419	22.9	1.00
362	17.9	1.04	420	26.9	0.97

Table E-1 TMP and Flux Variation (Con't)

Day	TMP (kPa)	Flux (LMH)	Day	TMP (kPa)	Flux (LMH)
421	27.4	1.00	490	28.9	0.93
422	27.9	1.02	492	28.9	-
424	25.9	0.95	494	29.4	0.95
426	26.9	0.95	497	32.9	-
427	26.9	1.00	499	30.9	0.99
428	24.9	1.00	501	32.4	-
430	24.9	0.97	504	30.9	0.96
432	25.9	1.00	505	29.4	-
435	26.9	0.97	506	29.9	-
437	28.9	-	508	30.9	0.94
438	26.9	-	509	29.9	-
439	25.9	0.97	511	30.9	-
440	27.9	-	512	31.9	0.97
441	31.9	0.97	513	31.9	-
442	30.4	0.95	516	31.9	0.97
443	27.4	0.97	517	30.9	-
445	27.9	0.95	518	31.9	-
446	29.9	-	519	33.9	-
447	31.4	-	520	34.4	0.96
448	31.9	0.95	522	13.4	-
449	33.4	0.93	523	15.9	0.99
450	34.4	-	525	15.4	0.98
451	34.9	-	527	15.9	-
452	34.4	0.95	528	16.4	-
453	34.9	0.93	530	15.9	0.94
454	34.9	1.00	532	16.4	-
455	35.4	1.00	534	18.4	0.93
456	36.9	-	536	18.4	-
457	15.4	-	537	18.9	-
458	17.4	0.95	539	18.4	0.94
460	17.9	-	543	18.4	0.96
461	17.9	0.95	545	18.9	-
463	19.4	0.95	547	19.4	0.98
465	18.9	0.93	549	21.4	0.99
467	18.4	-	551	23.4	1.00
468	19.9	0.95	554	21.4	0.97
469	21.9	-	556	20.4	-
480	23.9	-	557	20.4	1.00
471	24.9	0.97	559	20.4	0.94
478	25.4	0.93	561	20.9	0.97
479	27.4	-	563	20.9	-
482	29.9	0.93	564	20.4	0.99
484	29.4	-	565	20.4	-
486	29.4	0.95	567	19.9	0.92
488	29.4	-	569	20.4	-
489	28.4	-	570	20.4	-

Table E-1 TMP and Flux Variation (Con't)

Day	TMP (kPa)	Flux (LMH)	Day	TMP (kPa)	Flux (LMH)
572	20.9	-	598	24.9	0.98
574	20.4	0.92	603	24.4	0.92
576	20.4	-	606	24.4	0.92
578	20.9	0.92	607	25.4	-
579	20.9	-	608	28.9	-
581	20.9	0.97	609	29.4	1.04
583	20.9	-	610	30.9	-
584	20.9	0.97	611	30.4	-
587	22.4	-	612	31.4	1.04
588	23.9	0.95	613	33.4	-
589	21.9	-	614	34.4	-
590	22.4	-	615	34.9	-
591	22.9	0.95	616	36.4	1.03
595	21.4	0.93	-	-	-

Figure E-1 After clean with DI water

Figure E-2 After clean with alkaline solution

Figure E-3 After clean with acid solution

Hydrodynamic Resistance Investigation for Membrane Fouling Analysis

The effects of fouling on filtration performance can be expressed in terms of hydrodynamic resistance. The resistance-in-series model was applied to evaluate the characteristics of membrane fouling. According to this model, the permeate flux (J) can be expressed as below:

$$J=\frac{TMP}{\mu R_t}=\frac{TMP}{\mu(R_m+R_{rm}+R_{re}+R_{irr})}$$

Where TMP is transmembrane pressure, μ is the viscosity of the permeate, R_t is total resistance, R_m is the intrinsic membrane resistance, R_{rm} is the removable fouling, R_{re} is the reversible fouling and R_{irr} is irreversible fouling. The experimental procedure to determine each resistance value were as follows: (1) R_m was estimated by measuring the water flux of DI water; (2) R_t was evaluate by the final flux of biomass microfiltration and TMP; (3) the membrane was then flushed with DI water. After that, the DI water flux was measured to obtain the resistance of $R_m + R_{re} + R_{irr}$; (4) membrane was then clean with chemical solution. Then, DI water flux was measured again to get the resistance of $R_m + R_{irr}$. From the steps (1)-(4), R_t, R_m, R_{rm}, R_{re} and Ri_{rr} could be calculated.

Total Membrane Resistance (R_t)

Effect of membrane fouling on permeate flux decline can be explained by resistance-in-series model. In this model, the relationship between permeate flux and TMP is described by the following equation.

$$J=\frac{\Delta P}{\mu R_t}$$

Where J ($m^3/m^2.s$) is permeate flux, ΔP is transmembrane pressure (Pa), μ is permeate viscoscity (Pa.s), R_t is total membrane resistance (1/m); $R_t = R_m+R_{rm}+R_{re}+R_{ir,}$ R_m is initial membrane resistance of new membrane, R_{rm} is removable fouling resistance caused by biomass attachment (removed by rinsing with DI water, R_{re} is reversible fouling (removed by chemical cleaning) and R_{ir} is irreversible fouling.

Removable Membrane Fouling Investigation (R_{rm})

Fouled membrane was cleaned with DI water. Then, ceramic membrane was used to test the permeate flux with DI water for membrane resistance investigation. The membrane resistance caused by new membrane resistance, reversible and irreversible fouling can be calculated because removable fouling has already removed from ceramic membrane. The permeate flux from this experiment is used to calculate the removable membrane fouling resistance.

Removable fouling membrane resistance was calculated from equation below;

$$R_{rm} = R_t – (R_m + R_{re}+ R_{ir})$$

Reversible and Irreversible Membrane Fouling Investigation (R_{re} and R_{ir})

After cleaning the ceramic membrane with DI water, reversible and irreversible fouling are still remaining on membrane surfaces which need chemical cleaning to remove reversible fouling. The ceramic membrane was cleaned by NaOH at concentration 15 g/L and then clean with DI water until pH neutral. After that the ceramic membrane was cleaned with acid solution (H_3PO_4 + HNO_3) consequently clean with DI water until pH neutral.

Then, ceramic membrane was tested with DI water to measure permeate flux. The permeate flux is the new membrane resistance and irreversible fouling (R_m and R_{ir}) because both removable fouling and irreversible fouling have already removed by DI water and chemical cleaning respectively. The permeate flux from this experiment is used to calculate the irreversible fouling and reversible resistance.

Reversible fouling was calculated by following relationship;

$$R_{re} = R_t - (R_m + R_{rm} + R_{ir})$$

Irreversible fouling membrane resistance was calculated by relationship below;

$$R_{ir} = R_t - (R_m + R_{rm} + R_{re})$$

APPENDIX F
Energy Production and Greenhouse Gas Emission Calculation

F-1 Sample Calculation for Energy Consumption and Production

Consideration for calculation of energy production

- Energy input for the system consisted of hot water pump, heater, biomass recirculation pump, suction pump and mixing pump.
- Pump and heated operated 24 h/d.
- Power consumption of the system 12-15 kWh/m^3.d. (watthour meter)
- Calorific value of methane 9,000 kcal/m^3.
- Conversion unit from calorific value of methane to electricity 1 kcal = 1.16 x 10^{-3} kWh/m^3. Therefore, 9,000 kcal/m^3 = 10.44 kWh/m^3.

Example of calculation

Information

OLR 6 (with and without PVA-gel) kgCOD/m^3.d
- COD removal efficiency 89.6 and 92.3%
- Methane yield 0.26 and 0.29 m^3CH_4/$kgCOD_r$.d

OLR 8 and 12 (with PVA-gel) kgCOD/m^3.d
- COD removal efficiency 90.2 and 84.2%
- Methane yield 0.25 and 0.23 m^3CH_4/$kgCOD_r$.d

Energy production calculation

Methane production (without PVA-gel) = 6 kgCOD/m^3.d * 0.896 * 0.26 m^3CH_4/$kgCOD_r$
= 1.40 m^3CH_4/m^3.d
= 1.40 m^3CH_4/m^3.d * 10.44 kWh/m^3
= 14.62 kWh/m^3.d

Methane production (with PVA-gel) = 6 kgCOD/m^3.d * 0.923 * 0.29 m^3CH_4/$kgCOD_r$
= 1.61 m^3CH_4/m^3.d
= 1.61 m^3CH_4/m^3.d * 10.44 kWh/m^3
= 16.77 kWh/m^3.d

Methane production (with PVA-gel) = 8 kgCOD/m^3.d * 0.902 * 0.25 m^3CH_4/$kgCOD_r$
= 1.80 m^3CH_4/m^3.d
= 1.80 m^3CH_4/m^3.d * 10.44 kWh/m^3
= 18.83 kWh/m^3.d

Methane production (with PVA-gel) = 12 kgCOD/m^3.d * 0.842 * 0.23 m^3CH_4/$kgCOD_r$
= 2.32 m^3CH_4/m^3.d
= 2.32 m^3CH_4/m^3.d * 10.44 kWh/m^3
= 24.26 kWh/m^3.d

Energy consumption (Measuring by watthour meter)

Energy consumption
= 12-15 kWh/m^3.d

F-2 Greenhouse Gas Emission Calculation

The green house gas emission is measured in terms of carbon dioxide equivalent (CO_2-eq). Also, the effect of methane is 25 times higher than CO_2.

Example of calculation

Information

OLR 6 (with and without PVA-gel) kgCOD/m^3.d

- Pollution load = 15 g/L * 3.7 L/d = 55.5 g/d = 0.0555 kg/d

OLR 8 (with PVA-gel) kgCOD/m^3.d

- Pollution load = 20 g/L * 3.7 L/d = 74 g/d = 0.074 kg/d

OLR 12 (with PVA-gel) kgCOD/m^3.d

- Pollution load = 24 g/L * 4.5 L/d = 108 g/d = 0.108 kg/d

Greenhouse gas emission calculation

OLR 6 (without PVA-gel) kgCOD/m^3.d

= 0.0555 kg/d * 0.896* 0.26 $m^3CH_4/kgCOD_r$ *25 $kgCO_2$-eq/$kgCH_4$ * 0.66 $kgCH_4/m^3CH_4$

= 0.22 $kgCO_2$-eq/d

OLR 6 (with PVA-gel) kgCOD/m^3.d

= 0.0555 kg/d * 0.923* 0.29 $m^3CH_4/kgCOD_r$ *25 $kgCO_2$-eq/$kgCH_4$ * 0.66 $kgCH_4/m^3CH_4$

= 0.25 $kgCO_2$-eq/d

OLR 8 (with PVA-gel) kgCOD/m^3.d

= 0.074 kg/d * 0.902 * 0.25 $m^3CH_4/kgCOD_r$ *25 $kgCO_2$-eq/$kgCH_4$ * 0.66 $kgCH_4/m^3CH_4$

= 0.28 $kgCO_2$-eq/d

OLR 12 (with PVA-gel) kgCOD/m^3.d

= 0.108 kg/d * 0.842 * 0.23 $m^3CH_4/kgCOD_r$ *25 $kgCO_2$-eq/$kgCH_4$ * 0.66 $kgCH_4/m^3CH_4$

= 0.35 $kgCO_2$-eq/d

APPENDIX G
Statistical Analysis

Table G-1 Statistic Analysis using Analysis of Variance (one-way ANOVA)

ANOVA

Total VFA Concentration at loading rate 6 kgCOD/m^3.d with and without PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7218100.125	1	7218100.125	12.386	.001
Within Groups	17482657.375	30	582755.246		
Total	24700757.500	31			

ANOVA

Total VFA Concentration at loading rate 6 and 8 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2477990.669	1	2477990.669	10.924	.002
Within Groups	13383464.249	59	226838.377		
Total	15861454.918	60			

ANOVA

Total VFA Concentration at loading rate 8 and 12 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17453779.876	1	17453779.876	118.163	.000
Within Groups	15066390.345	102	147709.709		
Total	32520170.221	103			

ANOVA

ORR at loading rate 6 kgCOD/m^3.d with and without PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.245	1	.245	.738	.397
Within Groups	9.955	30	.332		
Total	10.200	31			

ANOVA

ORR at loading rate 6 and 8 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	66.247	1	66.247	393.606	.000
Within Groups	9.762	58	.168		
Total	76.009	59			

ANOVA

ORR at loading rate 8 and 12 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	168.494	1	168.494	144.739	.000
Within Groups	118.741	102	1.164		
Total	287.235	103			

ANOVA

Acetic acid at loading rate 6 kgCOD/m^3.d with and without PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1360560.134	1	1360560.134	21.834	.000
Within Groups	1869442.664	30	62314.755		
Total	3230002.799	31			

ANOVA

Acetic acid at loading rate 6 and 8 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	921270.432	1	921270.432	15.501	.000
Within Groups	3625434.794	61	59433.357		
Total	4546705.226	62			

ANOVA

Acetic acid at loading rate 8 and 12 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11630165.375	1	11630165.375	122.177	.000
Within Groups	9614272.489	101	95190.817		
Total	21244437.864	102			

ANOVA

Methane productivity at loading rate 6 kgCOD/m^3.d with and without PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.247	1	1.247	15.420	.000
Within Groups	2.749	34	.081		
Total	3.996	35			

ANOVA

Methane productivity at loading rate 6 and 8 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.898	1	1.898	39.537	.000
Within Groups	2.784	58	.048		
Total	4.682	59			

ANOVA

Methane productivity at loading rate 8 and 12 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.481	1	2.481	31.725	.000
Within Groups	7.821	100	.078		
Total	10.302	101			

ANOVA

SCOD at loading rate 6 kgCOD/m^3.d with and without PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	34303668.071	1	34303668.071	6.494	.018
Within Groups	121495316.889	23	5282405.082		
Total	155798984.960	24			

ANOVA

SCOD at loading rate 6 and 8 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	170172750.400	1	170172750.400	51.221	.000
Within Groups	53157523.600	16	3322345.225		
Total	223330274.000	17			

ANOVA

SCOD at loading rate 8 and 12 kgCOD/m^3.d with PVA-gel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.100	12	.258	1.033	.592
Within Groups	.500	2	.250		
Total	3.600	14			

APPENDIX H
Pathway of Organic Matter in Two Stage TAnMBR

H-1 Pathway of Organic Matter Calculation

The input and output organic mass (in form of COD) of two stage TAnMBR can be expresses as follows:

$COD_{influent} = COD_{VFA\&others} + COD_{methane} + COD_{vss} + COD_{accumulate}$	
$COD_{influent}$	COD concentration of tapioca starch based synthetic wastewater
$COD_{VFA\&others}$	COD concentrations of effluents VFA, including acetate, butyrate, propionate, other types of VFA, and the other patterns of COD which converted to trace amount of CO_2, H_2, methane dissolved in the effluent
$COD_{methane}$	$COD_{methane}$ was a part of organic matter that was measured in gaseous methane
COD_{vss}	COD_{vss} represents the organic matter contributing to biomass formation
$COD_{accumulate}$	COD concentration of complex organic matter that is non-biodegradable organic matter but can be measured as a part of COD

Carbon balance of hydrolytic reactor

Detailed calcuations:-

OLR 6 (with and without PVA-gel) kgCOD/m^3.d

- $COD_{influent}$
  = 14.5 g/L (53.7 g/d)

- $COD_{VFA\&others}$
  = 11.5 g/L (42.6 g/d) and 13.1 g/L (48.5 g/d)

- $COD_{methane}$
  = Methane gas was not observed in hydrolytic reactor

- COD_{vss} (OLR 6 kgCOD/m^3.d without PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(3.7 L/d)(9.8 g/L)
  = 2.1 g/d

- COD_{vss} (OLR 6 kgCOD/m^3.d with PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(3.7 L/d)(9.3 g/L)
  = 2.0 g/d

- COD_{acc} (OLR 6 kgCOD/m^3.d without PVA-gel)
  = 53.7 - (42.6 + 0.0 + 2.1) = 9.0 g/d

- COD_{acc} (OLR 6 kgCOD/m^3.d with PVA-gel)
  = 53.7 - (48.5 + 0.0 + 2.0) = 3.2 g/d

OLR 8 and 12 (with PVA-gel) kgCOD/m^3.d

- $COD_{influent}$
  = 20.6 g/L and 23.9 g/L (76.2 g/d and 107.6 g/d)

- $COD_{VFA\&others}$
  = 18.9 g/L and 19.1 g/L (69.9 g/d and 86.0 g/d)

- $COD_{methane}$
  = Methane gas was not observed in hydrolytic reactor

- COD_{vss} (OLR 8 kgCOD/m^3.d with PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(3.7 L/d)(5.5 g/L)
  = 1.2 g/d

- COD_{vss} (OLR 12 kgCOD/m^3.d with PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(4.5 L/d)(4.0 g/L)
  = 1.0 g/d

- COD_{acc} (OLR 6 kgCOD/m^3.d without PVA-gel)
  = 76.2 - (69.9 + 0.0 + 1.2) = 5.1 g/d

- COD_{acc} (OLR 6 kgCOD/m^3.d with PVA-gel)
  = 107.6 - (86.0 + 0.0 + 1.0) = 20.6 g/d

Carbon balance of methanogenic reactor

Detailed calcuations:-

OLR 6 (with and without PVA-gel) kgCOD/m^3.d

- $COD_{influent}$
  = 11.5 g/L and 13.1 g/L (42.6 g/d and 48.5 g/d)

- $COD_{VFA\&others}$
  = 1.6 g/L and 1.1 g/L (5.9 g/d and 4.1 g/d)

- $COD_{methane}$ (OLR 6 kgCOD/m^3.d without PVA-gel)
  = (12 L/d)/(0.42 L CH_4/ g COD) = 28.6 g/d

- $COD_{methane}$ (OLR 6 kgCOD/m^3.d with PVA-gel)
  = (15 L/d)/(0.42 L CH_4/ g COD) = 35.7 g/d

- COD_{vss} (OLR 6 kgCOD/m^3.d without PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(3.7 L/d)(22.5 g/L)
  = 4.7 g/d

- COD_{vss} (OLR 6 kgCOD/m^3.d with PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(3.7 L/d)(25.1 g/L)
  = 5.3 g/d

- COD_{acc} (OLR 6 kgCOD/m^3.d without PVA-gel)
  = 42.6 - (5.9 + 28.6 + 4.7) = 3.4 g/d

- COD_{acc} (OLR 6 kgCOD/m^3.d with PVA-gel)
  = 48.5 - (4.1 + 35.7 + 5.3) = 3.4 g/d

OLR 8 and 12 (with PVA-gel) kgCOD/m^3.d

- $COD_{influent}$
  = 18.9 g/L and 19.1 g/L (69.9 g/d and 86.0 g/d)

- $COD_{VFA\&others}$
  = 2.0 g/L and 3.8 g/L (7.4 g/d and 17.1 g/d)

- $COD_{methane}$ (OLR 8 kgCOD/m^3.d with PVA-gel)
  = (20.8 L/d)/(0.42 L CH_4/ g COD) = 49.5 g/d

- $COD_{methane}$ (OLR 12 kgCOD/m^3.d with PVA-gel)
  = (23 L/d)/(0.42 L CH_4/ g COD) = 54.8 g/d

- COD_{vss} (OLR 8 kgCOD/m^3.d with PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(3.7 L/d)(34.9 g/L)
  = 7.3 g/d

- COD_{vss} (OLR 12 kgCOD/m^3.d with PVA-gel)
  = (1.42 gCOD/gVSS)(0.04gVSS/gCOD)(4.5 L/d)(40.0 g/L)
  = 10.2 g/d

- COD_{acc} (OLR 8 kgCOD/m^3.d without PVA-gel)
  = 69.9 - (7.4 + 49.5 + 7.3) = 5.7 g/d

- COD_{acc} (OLR 12 kgCOD/m^3.d with PVA-gel)
  = 86.0 - (17.1 + 54.8 + 10.2) = 3.9 g/d

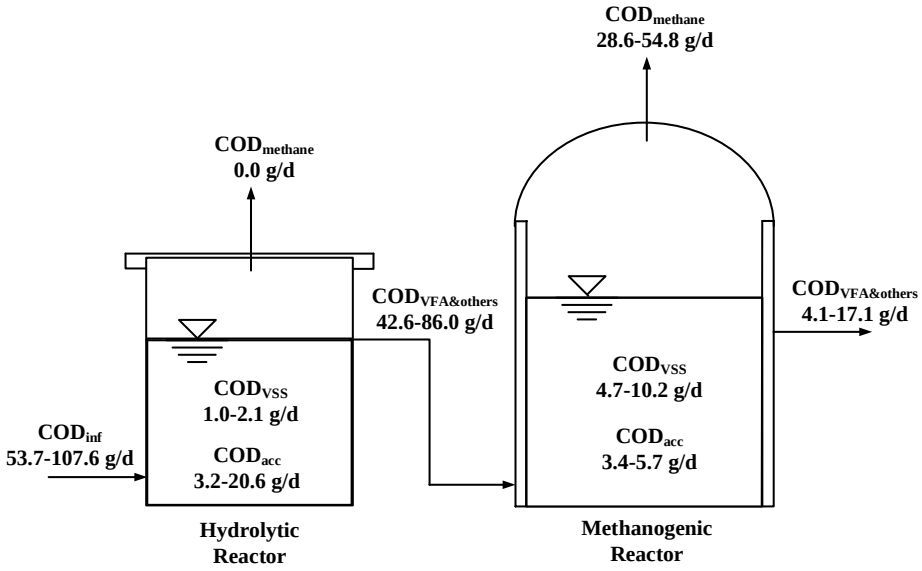

Figure H-1 Conversion of influent organic matter in two stage TAnMBR

List of Publications

International Conferences:

1. **Chaikasem, S.,** Abeynayaka, A. and Visvanathan, C. (2012). Effect of Biocarrier on the Performance of Two-Stage Thermophilic Anaerobic Membrane Bioreactor. Water and Environment Technology Conference, Tokyo, Japan, June 29-30, 2012.

2. **Chaikasem, S.,** Abeynayaka, A. and Visvanathan, C. (2013). Effect of Biocarrier on the Acidogenesis Process of Two-Stage Thermophilic Anaerobic Membrane Bioreactor. International Conference of Solid Waste 2013: Innovation in Technology and Management, Hong Kong Special Administrative Region, P.R. China, May 5-9, 2013.

3. **Chaikasem, S.,** Jacob, P. and Visvanathan, C. (2013). Improvement of Two-Stage Thermophilic Anaerobic Membrane Bioreactor Performance by Biocarrier Addition. 2013 International Environmental Engineering Conference and Annual Meeting of the Korean Society of Environmental Engineers: Convergence Technology, Seoul, Korea, June 11-13, 2013.

4. **Chaikasem, S.,** Jacob, P. and Visvanathan, C. (2014). Performance Evaluation of a Two-Stage Thermophilic Anaerobic Membrane Bioreactor for Treating High Strength Particulate Wastewater. The 4th IWA Regional Conference on Membrane Technology 2014, Ho Chi Minh City, Viet Nam, December 3-6, 2014.

Journal Publications:

1. **Chaikasem, S.,** Abeynayaka, A. and Visvanathan, C. (2014). Effect of PVA-gel as Biocarrier on Volatile Fatty Acids (VFA) Production of Two-Stage Thermophilic Anaerobic Membrane Bioreactor. *Bioresource Technology, 168,* 100-105.

2. **Chaikasem, S.**, Jacob, P. and Visvanathan, C. (2014). Improvement of Two-Stage Thermophilic Anaerobic Membrane Bioreactor Performance by Biocarrier Addition. *Desalination and Water Treatment,* 1-11.