

**ANAEROBIC CO-DIGESTION (ACD) OF GLYCERIN
PITCH (GP) WITH OLEOCHEMICAL EFFLUENT
(OCE)**

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ABSTRACT

Glycerin pitch (GP) with an average chemical oxygen demand (COD) of 973,750 mg/L produced from the oleo chemical industries have raised serious disposal problem. GP is the waste collected at the bottom of a distillation column that purifies crude glycerin. In some plants, this waste is mixed with 30% oleo chemical effluent (OCE) before treatment. Costly incineration or slow dosing of GP into conventional wastewater treatment plants are the only currently available treatment methods for GP. No study has been reported on using GP as co-substrate in anaerobic co-digestion (ACD) with OCE in addressing the disposal problem which formed the novelty of this study. Therefore, the aim of this research is to develop a cost effective ACD of GP with OCE. The research conducted including feasibility study, comparison study of ACD of GP with OCE at different digestion ratios, different reactor configurations and pre-treatment of substrate. The results revealed that continuous stirred tank reactor (CSTR) at an organic loading rate of (OLR) 4 g COD/L.day is chosen as the best option for co-digestion of GP with OCE treatment due to its great enhancement in the reduction of COD, oil and grease (O&G) and other parameters. COD removal of 70% is reported as the maximum degradation efficiency. The excellent performance is well beyond the target set >50% COD removal by the industry. It is concluded that GP as ACD substrate represents a cost-effective alternative to the current waste management technology.

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LIST OF ABBREVIATIONS

ACD	Anaerobic Co-digestion
ASBR	Anaerobic Sequencing Batch Reactor
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DGP	Diluted Glycerin Pitch
DO	Dissolved Oxygen
DOE	Department of Environment
EQA	Environment Quality Acts
EU	European Union
FiT	Feed in Tariff
FOG	Fat, Oil and Grease
GP	Glycerin Pitch
HRT	Hydraulic Retention Time
IAAB	Integrated Anerobic-Aerobic Bioreactor
ISCC	International Sustainability and Carbon Certification
LCFA	Long Chain Fatty Acid
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
MPB	Methane Producing Bacteria
OCE	Oleo Chemical Effluent
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
O&G	Oil and Grease
POME	Palm Oil Mill Effluent
RGWW	Refined Glycerin Wastewater
SLES	Sodium Laureth Sulfate
SRB	Sulfate Reducing Bacteria
TA	Total Alkalinity
TG	Total Glycerin
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus

TS	Total Solid
TSS	Total Suspended Solid
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VS	Volatile Solid
VSS	Volatile Suspended Solid
WWCS	Wastewater Characterisation Study
WWTP	Wastewater Treatment Plant

CHAPTER ONE

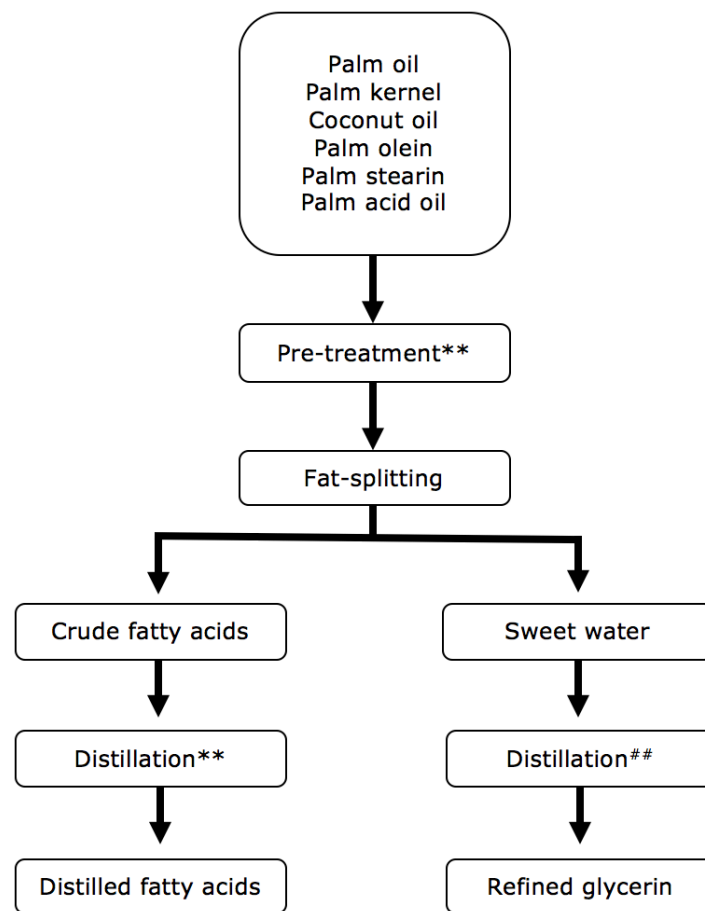
INTRODUCTION

1.1 Oleo chemical industry

Supply of fatty acids and glycerin in south east Asia has increased tremendously over the last five years which have accounted for approximately 80% of global production (Ciriminna et al. 2014; Wong 2016). From 2010-2015, global fatty acids and glycerin capacity exhibited two-fold growth (Ciriminna et al. 2014; Wong 2016). Malaysia is listed as one of the main oleo chemical production hubs due to the accessibility of raw materials and government incentives for value added palm industries (MIDA 2013; Wong 2016). The major oleo chemical exports are fatty acids (32%), methyl esters (20%), fatty alcohol (19%), soap noodles (15%) and glycerin (12%) (Foo 2015). These raw materials are used in manufacturing industries for various consumer applications such as detergents, soaps, personal care products, fragrance and flavouring sectors (Foo 2015). It is also widely demanded in industrial applications for lubricant, plastic, pharmaceutical, textile, rubber and paint sectors (Foo 2015). The country has a production capacity approximately 2,150,000 t/year and 240,000 t/year for fatty acids and glycerin respectively (Kifli et al. 1996; Wong 2016). Concurrent to the huge amount of production, contaminated wastes known as glycerin pitch (GP) and oleo chemical effluent (OCE) were generated.

A typical GP and OCE generation process is illustrated in Figure 1.1. GP is generated from the fat splitting process. Whereas, OCE is produced whenever vacuum distillation is performed to generate desired products of fatty acids and glycerin. OCE from the distillation process comprises the biggest portion of the effluent particularly in the production of fatty acids. Pre-treatment prior to fat splitting process is also likely to release OCE.

GP also known as saponification crude or hydrolyser crude glycerol or soap lye, a concentrate of the "sweet water" from fat hydrolysis or splitting (SDA 1990). This grade of glycerin is almost never consumed in any process except refining. GP is brownish viscous, alkaline and comprises of high organic contents (SDA 1990) whereas OCE is white cloudy, acidic and lower organic contents than GP. GP is originally produced in a stagnant form and it is often diluted with some portion of OCE by the industry to ensure it is flowable and ease of handling, hence termed as diluted GP (DGP). OCE contains glycerol, long chain fatty acids (LCFAs), grease and very small amount of phosphate, phosphorus, ammonia nitrogen and sulfate (SO_4^{4-}) (Zak & Rauckyte 2011). The properties of OCE can vary widely based on the types of manufacture products being conducted in the plant. If GP or OCE is discharged untreated with the properties as shown in Table 1.1, on reaching the river will undergo natural decomposition in which dissolved oxygen (DO) in the river water is rapidly depleted. This will bring destruction to aquatic life and natural ecosystem. In realising the pollution threat, the government had imposed parameter limits for the discharge of DGP and OCE into the environment through the enactment of Environmental Quality Acts (EQA) in 1978 as presented in the Table 1.1.



Sources of GP

** Sources of OCE

Figure 1.1: Source of generation of GP and OCE (Lee 1986)

Table 1.1: Characteristics of DGP and OCE and Department of Environment (DOE) standards for its discharge (EQA 1974).

General Parameter ^a	DGP		OCE		DOE standard
	Ave ^b	Range	Ave ^b	Range	
pH	12.1	11.7 – 12.9	4.3	3.6 – 5.5	5.5 - 9.0
Temperature	49.7	46.3 – 51.5	44.0	42.4 – 45.8	45.0
Biochemical oxygen demand (BOD)	202440	143250 – 267000	8223	4050 – 17970	50 ^c
Chemical oxygen demand (COD)	265806	143563 – 421500	10348	4000 – 38050	100
Total suspended solid (TSS)	1570	330 – 2360	259	24 – 818	100
Oil and grease (O&G)	27942	15614 – 47502	4065	3705 – 4297	10
Sulfate (SO ₄ ²⁻)	1645	145 – 3000	60	10 – 225	0.50

^aAll parameters in mg/L except pH and temperature (°C)

^b**Ave**, Average

^cSample incubated for 5 days at 20°C

1.2 GP and OCE treatment technologies

The enforcement of strict laws declared under the EQA has resulted in the establishment of technologies for the treatment of GP and OCE. Although there were various works performed on OCE treatment, aerobic digestion after chemical pre-treatment is the only widely employed method in the industry (Koo et al. 2011). On the other hand, GP has been categorised as a waste under Schedule S181 of the Environmental Regulations in Malaysia due to its high organic strength (Hidawati & Sakinah 2011). It is stored in drums and send for incineration by Kualiti Alam Waste Management Centre which is the primary body handling these scheduled wastes in Malaysia (Hidawati & Sakinah 2011). Since incineration can only be carried out strictly at prescribed premises, high charges are imposed for every tonne of waste disposed. Currently, industry is bearing the cost ranging USD 111,360 to USD 490,245 monthly for incineration at the average production of 435 tonnes of GP per month (Hidawati & Sakinah 2011). Due to the expensive treatment by incineration,

some industries also chose to dose GP into their OCE treatment plant with a constant small quantity as an alternative. The discharge from this method unfortunately could not comply to the standard regulations. Therefore, it is necessary to improvise the treatment system to produce greater quality of final treated effluent that could fulfil the disposal criteria enacted by the DOE at minimal cost.

1.3 Co-digestion technology

Anaerobic digestion (AD) is a series of biological processes performed in the absence of oxygen in which microorganism break down biodegradable materials and produce biogas (Monnet 2003). The end product is a mixture mainly of methane and carbon dioxide that is combusted to generate heat and electricity or can be processed into transportation fuels and renewable natural gas. The treatment of sewage sludge is one of the oldest and extensive applications. After the first energy generated in 1970s via AD, AD undergo intensive development particularly with the presence of immobilised biomass system to treat soluble effluents, and is considered established technology at present (Mata-Alvarez et al. 2000; Goulding & Power 2013). However, over the years AD of single substrates (mono-digestion) reports several downsides associate to substrate properties. For examples, (i) sewage sludge is portrayed by low organic loads (Murto et al. 2004; Kuglarz & Mrowiec 2007) (ii) organic fraction of municipal solid waste (OFMSW) has high amount of heavy metals (Kim et al. 2011; Cabbai et al. 2013) (iii) slaughterhouse wastes have methanogen inhibitors due to high concentration of nitrogen and/or LCFAs (Yuan 1995; Borja et al. 1998) (iv) animal manures have low organic loads and high nitrogen concentration (Astals et al. 2012; Astals et al. 2013) (v) crops and agro-industrial wastes have shortage of nitrogen and only produced seasonal (Fountoulakis & Manios 2009; Cavinato et al. 2010). Most of these challenges can be overcome by introducing co-substrate in what has been presently known as anaerobic co-digestion (ACD).

ACD which means AD of two or more substrates concurrently, is a viable alternative in lifting the downsides of mono-digestion. It also offers diversion opportunities to reduce landfill space and/or recycling goals for most municipalities. By incorporating substrate into ACD, not only the treatment facilities and energy costs can be reduced due to production of on-site power, greater methane generation eventually contribute to economic benefits (Mata-Alvarez et al. 2000). At the infant stage of research viewpoint, ACD emphasised on mixing substrates with the aim of improving the efficiency of AD. For instance, combining substrate of low nutrient with substrate of high nutrient could achieve ideal condition for macro and micronutrient stability (Mata-Alvarez et al. 2011). With this context, co-digestion is generating more methane than the summation of the methane produced in both single digestions, in other word, greater outcome may be attained.

Nevertheless, due to the current industrial perspective, the enhancement of methane production is primarily from the outcome of the increase in organic loading rate (OLR) rather than synergisms, all sort of substrate combinations is considered and used. The most common situation is when a major amount of a primary substrate; for example, manure or sewage sludge is mixed and digested together with minor amounts of a single, or a variety of additional substrate which is also known as co-substrate (Braun et al. 2002). High energy co-substrate such as fats, oils and grease are often used as it gives at least three times higher methane production (Mata-Alvarez et al. 2000).

Typically, the first selection benchmark is based on the transportation cost versus energy generation of co-substrate from the generation point to the AD plant. Despite the criteria, co-substrate selection and mixing ratio are vital factors to be accounted in order to meet the objectives of synergisms, dilute destructive constituent and maintain digestate quality. The current industry has emphasised the

goal in achieving >50% of COD removal of GP in order to ease further treatment processes and comply to discharge limits rather than to achieve biogas production. Hence, biogas production will not be the main investigation in this study as deeper exploration will be focused on COD reduction of GP through ACD to offer an innovation solution for GP treatment and its disposal. In addition, inhibition of ACD associate to SO_2^{4-} was found in substrate as they present in substantial concentration. Pre-treatment targeting the removal of inhibitor will be also conducted in this study.

1.4 Problem statement

In most cases, the oleo chemical refineries often dose GP in a small quantity into the existing OCE aerobic treatment plants to avoid costly disposal through incineration. This has led to more occasions of sudden or unexpected load to the treatment system which already subjected to high fluctuation to cope with the changes in the process streams. The GP generated is unable to be consumed totally by the treatment plant and incineration is ended as the only disposal way for the unconsumed GP. The final treated effluents from this practice not only fail in complying with the discharge standards but facing operational problems including challenges in handling the substrate.

GP is difficult to handle as it has low melting point and solidified at room temperature whilst making it difficult to flow. In industry practice, the GP generated is at high temperature (approximately 80°C) and transferred directly to the disposal drums before sending for incineration (Hidawati & Sakinah 2011). If GP is treated in OCE treatment plant, the hot GP is diluted instantly with OCE at the weight ratio of OCE to GP at 70: 30 to aid handling of this waste. This dispersed phase of GP in OCE is often precipitated as sludge during the chemical treatment due to its immiscibility

nature. Whereas the remaining GP is further aerobically digested in the existing OCE treatment plants, which often is difficult to be digested due to the high COD concentration of GP which is not suitable to undergo aerobic digestion which required lower strength of wastewater with COD concentration $<1,000$ mg/L (Chan et al. 2009). Biogas generation is not the primary objective in this study as the challenges currently faced by the industry focus on the difficulty of GP treatment and its disposal. This indicates that a more efficient treatment method is required for COD removal which represents the actual need and solution for the industry.

With the existing substrate availability in the factory, this study suggested that GP can be anaerobically co-digested with OCE as primary substrate. As GP is commonly paired with manure based substrate (e.g. pig manure) in ACD, no study in literature has been reported on the ACD of GP with OCE which formed the originality of this study. The treatment approach is projected to exhibit an improved performance as compared to the current technology with the least operational problems. This is due to positive synergism established in the digestion medium. ACD not only improves nutrient balance but also enhances pH buffer capacity. The high alkalinity and organic content of GP can be neutralised by OCE's acidity while providing the required organic strength for biogas production. ACD is anticipated to yield a lower strength discharge which can then go into existing treatment plant without creating shock loading. With this, all GP can be consumed while generating a final discharge that is satisfactory for further post treatments as needed by industry. It is clear that the economic advantages are cost saving and additional power supply as a result of shared equipment, easier handling of substrates and a more stable process.

To ensure an achievable treatment, prior to ACD, a characterisation and feasibility studies were conducted to investigate the suitability of GP and OCE for

ACD. This study allows to identify the important factors that will influence the synergism in ACD such as alkalinity, organic concentrations and SO_2^{4-} inhibition. Subsequently, biogas potential test (BPT) was used to prove both substrates can be anaerobically digested as well as to measure the potential biogas production. This preliminary study will demonstrate on how the data obtained can give an estimation of ACD performance before proceed to conduct the research in continuous systems. However, due to the immiscible characteristic of GP and OCE caused by high O&G content in GP, the combination of both became one of the biggest challenges in this research.

In order to overcome the mixing difficulty and high O&G content, the investigation of GP treatment with OCE was conducted at different OLR ratios using sequencing batch reactor (ASBR) and continuous stirred tank reactor (CSTR). The selected OLR ratio was later investigated at different reactor configurations under semi batch and continuous system (up-flow anaerobic sludge blanket, UASB reactor). ACD performance was evaluated based on the reactors' ability in coping substrate loading rate and the effectiveness of the mixing system involving manual mixing, agitator and recirculation pump. These mixing modes could allow a homogenous condition in reactor and increase surface area for contact between effluent and anaerobic digester sludge and therefore fitting the immiscible characteristic of substrates. The ACD performance evaluation at different OLR ratios and different reactor configurations not only to identify the influencing factors but to suggest the best treatment system and the optimum conditions to perform ACD. The chosen digestion ratio and reactor were then evaluated once again with and without pre-treated substrate. The pre-treatment was performed to reduce high TSS and inhibiting constituent, SO_2^{4-} found in substrate with the aim to increase overall COD removal.

1.5 Research objectives

The general objective of this study is to propose a novel treatment approach for ACD of GP with OCE. It is targeted to achieve high organic removal efficiencies with BOD and COD removal efficiencies of >50% as the requisite for subsequent chemical, physical and aerobic treatment.

The specific objectives of the present research are as follows:

1. To evaluate the feasibility of ACD of GP with OCE.
2. To evaluate and compare the performance of ACD of GP with OCE at different OLR ratios.
3. To evaluate the performance of chosen OLR ratios with different reactor configurations.
4. To determine the most feasible treatment system and the optimum conditions for the ACD of GP with OCE in achieving maximum COD removal.
5. To evaluate the performance of chosen reactor with and without pre-treated substrate targeting on inhibiting constituents.

1.6 Research scope

The present study focuses on the treatment system approach for an effective management of GP and OCE which is suitable for oleo chemical industries in Malaysia. High ACD treatment performance is not dictated by the type of substrates alone, but by various influencing factors including mixing, digestion ratio and nutrient balance, operating temperature, OLR and hydraulic retention time (HRT), digestion mode, type of reactors and inhibition of ACD. An optimum condition will lead to a high retention of biomass, maximum contact between the bacteria and substrate as well as development of good settling sludge (Chen et al. 2008; Kangle et al. 2012). An

effective ACD should also be determined based on well-founded scientific and engineering basis. Understanding the past research works is also equally important as a guideline for a better advancement in the field. Considering these, a detailed literature review on GP treatment studies and numerous ACD studies were evaluated with the aim of identifying the pros and cons of past and existing treatments, factors that affect the operation of ACD and suitable operating conditions range to be adopted in the current study.

To understand the characteristics of GP and OCE, and to ensure its suitability to be co-digested, characterisation and feasibility studies were conducted respectively. Several crucial parameters were measured in the characterisation study including COD, BOD, TSS, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), O&G, volatile fatty acid (VFA), total alkalinity (TA), SO_4^{2-} , sulfide (S^{2-}) and pH. Meanwhile, feasibility study was performed by computing the maximum potential methane and energy generation based on 85% COD removal efficiency.

Following this, BPT was utilised to verify the potential biogas production from the GP with OCE. Although this research is not targeting on biogas generation but focus on COD removal as emphasised by the need of industry, biogas generation will be considered as an added advantage. From BPT, possible occurrence of any inhibition could be identified and the ACD performance was analysed. Based on these and those obtained from literature, the three reactors namely ASBR, UASB and CSTR were developed to commission ACD in a complete system.

After the set-up of reactors, the operation of the reactors was first initiated by inoculating anaerobic sludge which was collected from the anaerobic pond treating palm oil mill effluent (POME). After start-up was completed successfully, ACD performance at different OLR ratios and reactor configurations were assessed based

on the overall organic removal efficiencies, reactor stability and biogas production rate. The factors which significantly affect the ACD performance were identified. Subsequently, the optimum OLR ratio and reactor system were selected based on the highest organic removal efficiencies with good stability.

Thereafter, a comprehensive pre-treatment study on the selected system was conducted with the aim to minimise inhibition issues and have a better control of the system. ACD performance was re-evaluated once again with and without pre-treated substrate. Finally, the optimum conditions for ACD of GP with OCE were attained and proposed.

1.7 Organisation of the thesis

This report consists of five chapters. A brief introduction on the status of oleo chemical industry, source and characteristics of GP and OCE, regulatory implementation towards the discharge of GP and OCE as well as its treatment approaches currently in practice were discussed in Chapter one (Introduction). This chapter also covers problem statements that deliver the origin and rationale to identify the research direction. This was then followed by the objectives exhibited with the research scope. The last section of this chapter was then sum up by the organisation of the thesis.

Chapter two (Literature Review) summarises the review of GP incorporates in ACD treatment from numerous laboratories, pilot, and full-scale studies. The influencing factors that affect the performance of ACD was explored and this includes mixing, digestion ratio and nutrient balance, operating temperature, OLR and HRT, digestion mode, type of reactors and inhibition of ACD. This section is important as it provides the understanding and how results from earlier research can be used to

address the feasibility of ACD. Lastly, the optimum operating conditions were also outlined along with a summary for this chapter.

Chapter three (Materials and Methods) includes lists of all materials, chemicals and analytical methods employed in the present study. This was followed by the experimental procedures which comprise sample analysis, operation of reactors and their processes, performance evaluation study of ACD of GP with OCE and pre-treatment of substrate. In order to display a well-defined picture on the experimental works, an experimental flowchart was illustrated as well.

Chapter four (Results and Discussion) is the essence of this thesis with four main studies. The first section of this chapter emphasises on the characterisation of substrates and feasibility of ACD by determining the potential methane production and energy potentials based on 85% COD removal efficiency. Following that, BPT was carried out. It was aimed to detect possible inhibition before proceeding to the continuous reactor. Meanwhile, the second and third sections discussed the effect of different OLR ratios and different reactor configurations respectively on the digestion efficiency, process stability and biogas production rate. The factors that influence the ACD performance were also deliberated. From the evaluation obtained, the optimum OLR ratio and the best reactor for the treatment of GP with OCE were determined and proposed. In the last section, pre-treatment was targeted to minimise detected constraints in order to further improve digestion efficiency. The pre-treated substrate was then embarked for ACD using the chosen reactor system.

Chapter five (Conclusions and Recommendations) concludes the work that has been commenced and main findings from the present study. The conclusions reveal the accomplishments of the recorded objectives throughout the study. Conclusively, recommendations for future study were proposed. These suggestions

were presented in view of their significance and importance related to the current research.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

In the degradation of GP, suitable technologies with the optimum operating conditions are essential to achieve required treatment efficiency. Due to the diminutive literature found on GP treatment, glycerin based substrate such as crude glycerin will be reviewed in this chapter as it is more commonly reported for its treatment performances. Crude glycerin is dark brown in colour, viscous, alkaline, possess approximately 20% glycerin, 6% fatty acids and 74% salt (Ooi et al. 2001; Yong et al. 2001), which has similarity to GP's characteristics but they are generated from different plant processes. GP also known as glycerin residue was resulted from the bottom of distillation column while crude glycerin is generated after a series of pre-treatment which involved extraction, filtration and distillation to recover glycerin from GP (Hazimah & Ooi 2000; Yong et al. 2001). The significant increase in glycerin production has created a surplus that has resulted in a dramatic decrease in crude glycerol prices (Yazdani & Gonzalez 2007). Hence, alternative to the use of crude glycerin is composting, in addition to other organic waste for biogas generation via ACD which explained the higher number of past studies conducted using crude glycerin as compared to GP (Yazdani & Gonzalez 2007).

A literature assessment on various types of approaches presently in use on glycerin based substrate is vital to gain understanding on how each method affects the treatment performance and to identify optimum operating settings that best suited for GP treatment. Therefore, this chapter delivers an overview ACD technology of glycerin based substrate with other substrates and factors influencing the

performance including the type of reactors and possible inhibition were discussed in order to suggest the optimum operating conditions for an effective ACD.

2.1 ACD studies

ACD has been recognised as economical and environmentally sustainable approach for glycerin based substrate an approach for increasing biogas generation in digester. Considering the widely reported effectiveness of ACD over mono-digestions, it is suggested to be a better solution for glycerin based effluent (co-substrate) to be co-digested with lower energy substrate like OCE (primary substrate). With ACD, it could form an ideal nutrient balance as well as pH level that complement both substrates deficiencies. Also, the establishment of positive synergisms in the digestion medium and the role as a supplier of missing nutrients, biogas yields is able to be improved (Mata-Alvarez et al. 2000). The capture of methane can be used as an energy source. As ACD can be conducted with current set-up and expertise, GP and OCE can be treated at the same time in the same plant without additional cost in transportation, extra process or facilities. Since high strength waste like crude glycerin often opted for landfill disposal, ACD also gives diversion opportunity to reduce landfill space and aiding the waste to achieve its recovery goals. The oleo chemical industry certainly can expect to see cost savings from incorporating wastes into their current plants that running mono-digestion mode. These include reduced energy costs due to production of on-site power resulted from biogas yields. Therefore, ACD is the key for this research.

Although ACD carries numerous benefits, many researchers were still facing difficulty in performing ACD and sometimes system upset mainly due to the inappropriate substrates ratio and operating conditions. For instance, this challenge was demonstrated by Wan et al. (2011) who reported a digestion failure when adding 75% or more of FOG feed as this immediately caused acidification of the digester. This is also supported by Astals et al. (2012) who reported system upset resulting in low methane yield when incorporating crude glycerin of >4% into the digester. Wan et al. (2011) further added that a relatively short HRT of 10 days employed could also contribute to the digestion failure due to washout of microorganisms during removal of treated wastewater. No study has been reported on reviewing the influencing factors of co-substrates on ACD and their optimum operating conditions. These areas were under explored which form the missing element in this research to answer the problems faced.

Despite of the possible disadvantages of ACD which likely contributed by the lack of knowledge in ACD practice, this review can provide research insights and serve as a guideline before conducting experiments related to ACD of effluent. This section discusses how results from numerous laboratories, pilot, and full-scale ACD studies can be used to address the feasibility and potential of glycerin based substrate used for ACD. The review also aims to provide recommendation for this research to achieve optimum operating conditions for ACD that can lead to minimum operational problems and maximum organics removal. The influencing factors that affect the operation of ACD treatment were also outlined.

2.2 Glycerin based substrate in ACD

Glycerin based effluent like crude glycerin is the by-product of glycerin production which is approximately 10% of the weight of the initial raw matter (EBB, 2010). Precisely, crude glycerin comprises the mixture of glycerin, alcohol, salts, water, free fatty acids, heavy metals, methyl esters, unreacted mono-, di- and tri-glycerides in diverging quantity depending on the quality of the raw matter and the chemical method employed to get glycerin (Pagliaro & Rossi 2008; Robra et al. 2010). Crude glycerin has to be disposed as waste in certain region due to the expensive treatment or refinement for small and medium plants (Pachauri & He 2006). Also, the current glycerin market cannot engage the large rise in by-product production (Johnson & Taconi 2007) and crude glycerin does not have a lot of direct consumptions due its impurities (Pagliaro & Rossi 2008). Johnson & Taconi (2007) explained that crude glycerin can be sold for USD 104 to 389 per tonne in other region depending on the glycerin purity and regional market availability. Hence, many researchers have been developing cost effective utilisations of crude glycerin in order to achieve a sustainable cost of glycerin production in the long run. One of the most promising solutions is using crude glycerin as a co-substrate in anaerobic digestion plants to produce renewable energy.

Crude glycerin has an average COD of 1,200,000mg/L and BOD of 97,080 mg/L while BOD/COD ratio was calculated to be 0.08, suggesting there is a high strength organic matter presents. It is considered not easily treatable by biological means as the ideal ratio for anaerobic digestion ranging from 0.3 to 0.8 (Metcalf & Eddy 2014). However, it is likely to be digested with the addition of lower strength waste such as cassava wastewater, potatoes processing wastewater, pig manure, sewage sludge, olive mill and slaughterhouse wastewater as listed in Table 2.1. Besides, high TSS >1,500 mg/L indicates a low settling capability because more suspended solids (non-degraded) are present and longer retention time is required

(Yong et al. 2001). Also, carbon/nitrogen (C/N) ratio of crude glycerin at 68 appears to be higher than optimum range from 20-30 for anaerobic digestion (Monnet 2003). This signifies that a rapid consumption of nitrogen (N) by the methanogens and could results in a lower gas production. Fortunately, the high nutrient (C/N ratio) in crude glycerin can act as a nutrient booster for waste with lower nutrient in order to achieve nutrient balance. Similarly, the high alkaline level of crude glycerin at pH averaging 10 can be counter-balanced if co-digest with acidic waste (Yong et al. 2001). For instance, OCE with lower nutrient at 24 (C/N ratio) and high acidity at pH 4.3 make a perfect match with crude glycerin to complement both characteristics. In addition, the digestion of crude glycerin may be difficult as high O&G concentration >28,000 mg/L tends to accumulate on the sludge surface and cause scum formation (Tchobanoglous et al. 2003). This downside can be rectified by adding crude glycerin as co-substrate into wastewater digester instead of digesting crude glycerin alone. As co-substrate added in a smaller amount, this could avoid the inhibition of degradation due to high O&G presents. Table 2.1 summarised various ACD studies with glycerin based substrate, each of the operating conditions will be further reviewed in the following section.

Table 2.1: Summary of various ACD studies with glycerin based substrate

No	Primary substrate	Co-substrate	Digestion ratio (V: V)	Reactor type ^a	Digestion mode ^b	Temp ^c	HRT ^d	OLR ^e	Methane production ^f			Reference
									Mono-digestion	Co-digestion	Improvement (%)	
1	Cassava wastewater	Crude glycerin	98: 2	ASBR	C	261	5	9.3 COD	R = 0.761	R = 1.287	69	Larsen et al. 2013
2	Potatoes processing wastewater	Pure glycerin Crude glycerin High conductivity glycerin	98: 2	UASB	C	35	20	8.2 COD	-	R = 0.48 R = 0.44 R = 0.30	-	Ma et al. 2008
3	Pig manure	Crude glycerin	96: 4 97: 3	UASB	SC SC	35 55	20 15	1.9 VS 2.6 VS	R = 0.754 R = 0.592	R = 3.630 R = 3.224	381 445	Astals et al. 2012 Astals et al. 2013
4	Sewage sludge	Crude glycerin	99: 1	CSTR	SC	35	27	7.8 COD	R = 0.247	R = 2.288	826	Rivero et al. 2014
5	Sewage sludge	Crude glycerin	99: 1	CSTR	B	35	35	-	R = 1.11	R = 2.35	112	Fountoulakis et al. 2010
6	Olive mill and slaughterhouse wastewater	Crude glycerin	99: 1	CSTR	C	35	46	-	R = 0.48	R = 1.21	152	Fountoulakis & Manios 2009
7	Orange peel waste	Residue glycerin	50: 50	CSTR CSTR	SC	35 55	30 30	1.52 VS 1.03 VS	$Y_{VS} = 0.327$ $Y_{VS} = 0.356$	$Y_{VS} = 0.385$ $Y_{VS} = 0.486$	18 37	Martín et al. 2013
8	Biodiesel manufacturing wastewater	Glycerin	-	ASBR	B	35	-	1.6 COD	$Y_{COD} = 0.292$	$Y_{COD} = 0.310$	6	Siles et al. 2010

^a**Reactor type:** ASBR, anaerobic sludge blanket reactor; UASB, up-flow anaerobic sludge blanket, CSTR, continuous sludge blanket reactor

^b**Digestion mode:** B, batch; C, continuous, SC, semi-continuous

^c**Temp,** Temperature: °C

^d**HRT,** Hydraulic retention time: days

^e**OLR,** Organic loading rate: COD, g COD/L.day; VS, g VS/L.day

^f**Methane production:** R, methane production rate (L/day); Y_{COD} , methane yield (L CH₄/g COD removed); Y_{VS} , methane yield (L CH₄/g VS)

2.3 Factors influencing ACD performance

Several factors affect the functioning of anaerobic digesters and feasibility of ACD where sufficient control is needed to prevent reactor failure. Taking into account of the characteristics of co-digesting substrates in this study, the few major influences that greatly affect ACD performance are mixing, digestion ratio and nutrient balance, operating temperature, OLR and HRT, digestion mode, type of reactor and inhibitors. These factors were greatly reviewed in order to gain deeper knowledge and consideration for future research. Also, summarised optimum operating conditions was presented.

2.3.1 Mixing

Mixing gives good contact between microbes and substrates, improves the ability of bacterial population to obtain nutrients and promote homogenous feed into the digesters (Leslie Grady et al. 1999). Mixing also reduces the forming of inhibitory intermediates such as scum and stabilises digestion conditions by preventing the development of temperature gradients (Leslie Grady et al. 1999). Overall treatment efficiency will be affected at different stages of pH and temperature when mixing is inadequate (Stafford 1982). Mixing can be done through mechanical mixing, biogas recirculation or through slurry recirculation (Stafford 1982). Mixing also improved gas production as compared to unmixed digesters (Karim et al. 2005). This has been proven in the study performed by Rizk et al. (2007) where ACD of wastewater treatment plant (WWTP) sludge with organic fraction of municipal solid waste (OFMSW) with no mixing system in a 70 L reactor under mesophilic condition, results showed that most biogas and methane generated only at the first month of operation. Therefore, it is suggested that mixing system is necessary to be installed in digester particularly in ACD to ensure homogeneity in digester.

In addition, the effects of different conditions of mechanical mixing on ACD operation studied by Stroot et al. (2001) has testified that low mixing speed (80 rpm) is more effective than high mixing speed (200 rpm). This is because excessive mixing can interrupt the microorganisms to perform their digestion. Hence, this indicates that setting optimum mixing speed is essential as it could substantially affect the digestion and biogas production rates. However, Karim et al. (2005) mentioned that mixing during start-up is not beneficial due to the fact that digester pH will be lowered, resulting in performance instability leads to delay start-up period. Under such circumstances, pH in digester should also be regulated from time to time in order to complement the mixing system for enhance digestibility.

2.3.2 Digestion ratio and nutrient balance

C/N ratio represents the correlation between the amount of carbon and nitrogen present in organic matter. This ratio is the balance of food a microbe needed to grow in order to perform digestion of organic matter. The optimum C/N ratio in anaerobic digesters is usually in between 20-30 (Monnet 2003). A rapid utilising of N by the methanogens results in a lower gas production and this is indicated with a high C/N ratio. Conversely, a lower C/N ratio causes ammonia deposition and pH surpass 8.5 which is considerably toxic to methanogenic bacteria. Therefore, in order to achieve an ideal ratio of C/N, it is suggested to have a mixture of waste with low and high C/N ratio.

Optimum digestion ratio for glycerin based substrate is relatively low at 1-4%. This is due to high C/N ratio of crude glycerin at 68 (Yong et al. 2001). If added at high concentration, a rapid consumption of N by the methanogens will result in low biogas production. A good illustration shown in Table 2.1 where both authors performed ACD of glycerin base effluent with different digestion ratios. Rivero et al.

(2014) showed to have a significant higher biogas improvement (826%) with lower digestion ratio (1%) as compared to Astals et al. (2013) who obtained lower biogas improvement even higher digestion ratio (4%) was applied. Studies also suggested that to ensure stable operation, addition of glycerin based substrate should not exceed 4% under mesophilic. This is likely due to the generation of high acetic acid and eventually causes a decrease in pH. When this happens, methane production will reduce because methanogens tend to consume a longer time to adjust and rectify the pH.

2.3.3 Operating temperature

ACD treatment at both mesophilic and thermophilic conditions are feasible depending on the type of substrates. From the studies gathered in Table 2.1, Astals et al. (2013) suggested that thermophilic ACD resulted in a higher methane improvement at 445% compared to mesophilic at 381% under similar operating conditions. Similar result was also portrayed by Martín et al. (2013) who showed that thermophilic resulted a higher methane improvement than mesophilic. However, the overall data also suggested that the improvement is not exclusively resulted by operating temperature but the ACD itself that increased the digestion and methane production. Mesophilic condition showed to have the highest methane improvement up to 826% (Rivero et al. 2014), likewise, other researchers also indicated the excellent methane improvement under mesophilic condition.

Mesophilic is a common operating temperature and has been widely in practice in majority cases for its better stabilisation. Although thermophilic is often desired for its high biochemical reaction and low retention time, it has high energy demand making the process costly and such system also sensitive to the sudden temperature fluctuation resulting in careful operation in regulating precise

temperature (Kardos et al. 2011; Ruffino et al. 2015). Considering the serious downsides of thermophilic, the fact that ACD is not solely dependent on operating temperature and effectiveness is mostly contributed by the active synergism created between substrates, mesophilic is preferred for the ACD of GP with OCE in this research.

2.3.4 OLR and HRT

OLR is a crucial parameter as it describes the amount of volatile solids to be fed into the digester each day. Volatile solids signify the amount of organic solids that can be digested. The optimum OLR and HRT are often depending on the type of substrates fed into the digester as the substrates determine the activity level of biochemical that will occur in the digester. Table 2.1 showed that low range OLR <2 g VS/L.day (in term of volatile solid) with long HRT of >30 days is favourable for glycerin based substrate and ideal to be adopted in ACD of GP with OCE. Rivero et al. (2014) have demonstrated that volatile solid (VS) removal up to 89% and methane yield of 1.5 LCH₄/g VS.day was achieved with OLR of 1 g VS/L.day. In terms of COD, OLR of 8 g COD/L.day with HRT >30 days is recommended. Under these operating conditions, COD removal and methane yield were able to achieve up to 93% and 0.3 L CH₄/g COD.day respectively (Rivero et al. 2014). ACD of glycerin based substrate would require a longer HRT. As methanogens have doubling time of 1-12 days (Jetten et al. 1992), HRT less than 30 days is insufficient for methanogens to work quick enough to convert acetic acid to methane. Subsequently, this will affect the performance of ACD as bacteria are unable to degrade glycerin based substrate. This is also reflected by Sulaiman et al. (2009) who observed a reduction in methane production when running under HRT of 10 days. Adding to the fact that glycerin based substrate has a high COD concentration averaging 1,200,000 mg/L; a longer HRT would be better in order to allow more contact time of substrates with digesting

bacteria to increase methane production. It is also important to add glycerin based substrate gradually to allow bacteria to acclimatise in order to gain better results at increasing OLR.

2.3.5 Digestion mode

Digesting glycerin based substrate in semi-continuous mode appeared to be a desirable option as validated in Table 2.1. This mode could achieve a higher methane production up to 826% as compared to batch (up to 112%) and continuous modes (up to 152%). Continuous mode is commonly applied for liquid waste while batch mode for settled sludge. However, feeding strategy with a small quantity of substrate or continuous mode appears to be more preferable than feeding large quantity of substrate at once (Piao et al. 2013). This is supported by Golkowska et al. (2012) who demonstrated that an increase in feeding frequency from semi-continuous to continuous mode resulted in proportional acceleration of substrate degradation for comparable OLR. According to Jard et al. (2012), AD usually produces higher methane yield when run in a continuous mode than in batch mode due to the progressive adaptation of microbial population. Batch mode may provide a good estimate of the substrate capacity to be degraded as it allows longer contact time between substrates and microbes. However, continuous mode is more assuring on its reliable outcomes as the progressive adaptation of microbial population could provide a healthy condition in the digester without overloading.

2.3.6 Type of reactors

Generally, there are three types of reactors commonly used in the industry associates with ACD of crude glycerin. These reactors are namely ASBR, CSTR and UASB. A schematic of ASBR is shown in Figure 2.1. ASBR operates in four steps cycle: feed, react, settle and decant (Akil & Jayanthi 2012). The first step involves the addition of substrate to the reactor where content will be mixed manually/mechanically to allow contact between organics and anaerobes. The conversion of biodegradable organic matters to biogas was achieved during reaction step. Food to microorganism (F/M) ratio is high at the beginning of this step. At the end of reaction period, the low F/M favours flocculation and settling of biomass. Anaerobes with poor settling characteristic were removed from the reactor at sludge discharge point while biogas was collected at biogas outlet port. The clarified supernatant (treated effluent) was also discharged from the reactor.

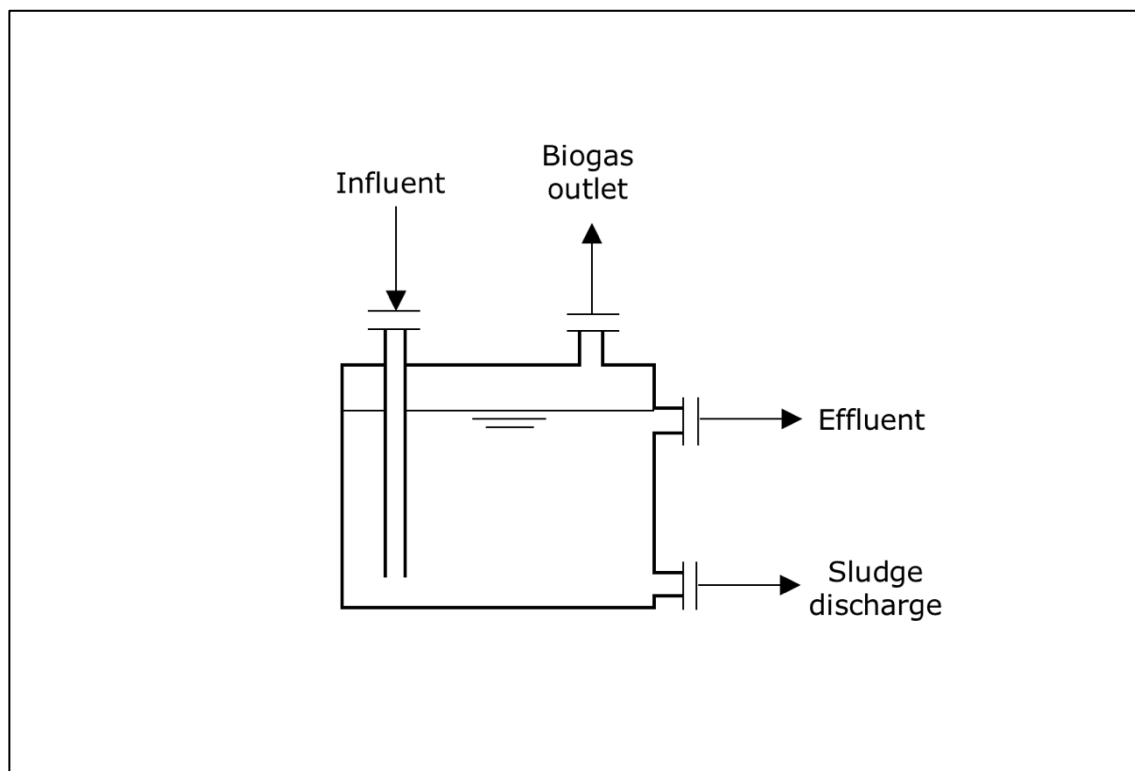


Figure 2.1: Schematic representation of ASBR (Tansengco et al. 2016)

Meanwhile in CSTR, it operates on a steady state basis, normally used for a homogenous liquid phase reaction. A schematic of CSTR is illustrated in Figure 2.2 where substrate was fed into the reactor through inlet port at the top of reactor. The content was mixed thoroughly by stirring device (agitator) and pH was adjusted regularly. The content in the output product has the same properties as to the content inside the reactor due to uniform stirring. The treated effluent was then drawn out from the reactor.

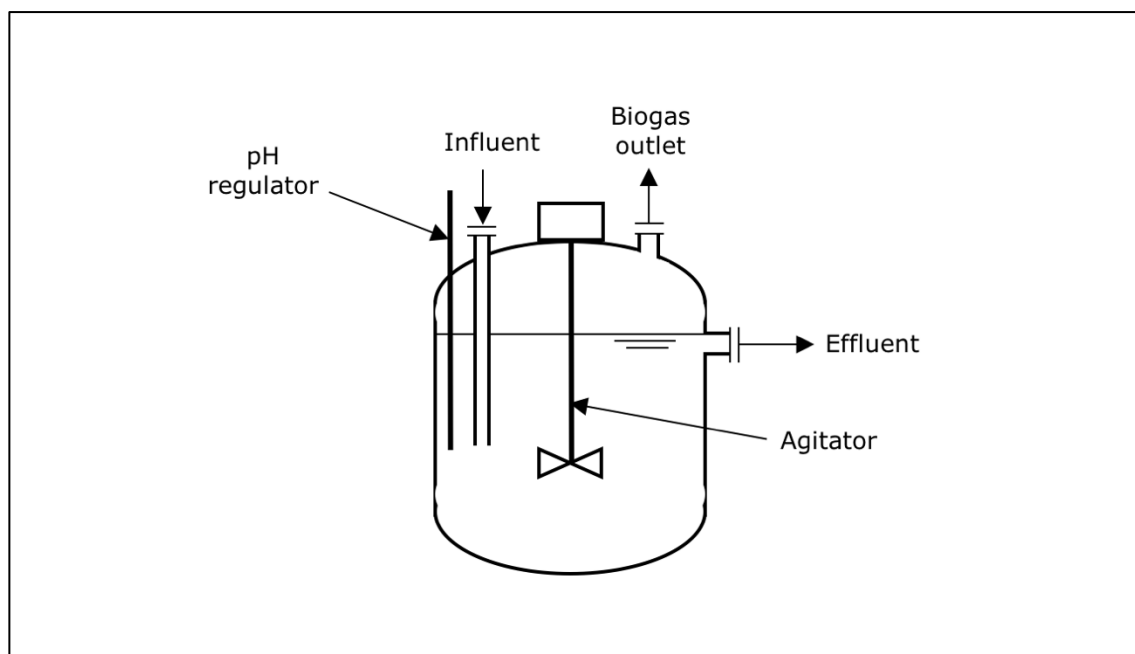


Figure 2.2: Schematic representation of CSTR (Usack et al. 2012)

On the other hand, substrate enters UASB from the bottom and flows upwards as schematised in Figure 2.3. A dense sludge bed with high anaerobes activity and sludge blanket which suspends in the reactor filter and treat the effluent as the effluent flows through it (von Sperling & de Lemos Chernicharo 2005; Vlyssides et al. 2009). The process was based on the development of dense granules (1-4 mm in diameter) formed by anaerobes, because of their weight, resist being washed out in the up-flow (von Sperling & de Lemos Chernicharo 2005). The anaerobes in the sludge degrade organic compound and as a result, biogas is released. Larger granules of sludge formed over time will act as filter for smaller particles as the

effluent rises through the sludge. Due to the up-flow routine, granule forming organisms are favourably accumulated while others were washed out. Sloped walls deflect material that reaches the top of the reactor downwards while the top part of the reactor serves as a gas-liquid-solid separator (GLSS) to allow separation of biogas and washed out of solids from the liquid phase. The treated effluent was collected at the discharge point.

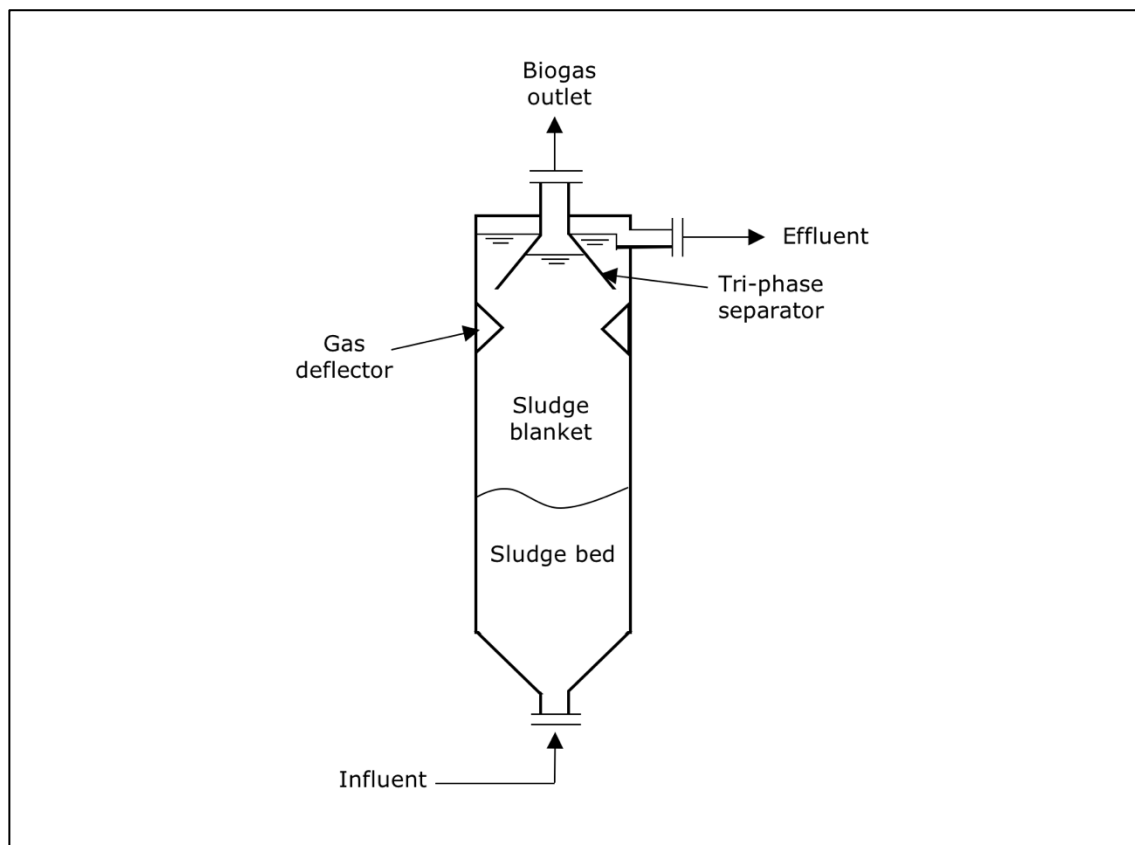


Figure 2.3: Schematic representation of UASB (von Sperling & de Lemos Chernicharo 2005).

ACD of glycerin based substrate using UASB and CSTR seemed to be a pleasing option. This is reflected by effectiveness in co-digestion and advantages of the reactors through the concerted research and development efforts (Frostell 1983; Cervantes et al. 2006) which are documented in Table 2.1 and 2.2 respectively. Despite the disadvantages, it can be seen that UASB and CSTR are operationally and

economically advantageous to adopt in the ACD of GP with OCE in terms of high digestion efficiency and homogenous mixing respectively. Homogenous mixing and continuous mode are equally crucial in ACD to ensure two different co-digesting substrates are well mixed and to achieve progressive adaptation of microbial population in the reactor.

Besides, it is important to be reminded that substrate with high fat content could have adverse impact on the performance. In this case, GP with high fat content may prevent system from operating at high OLRs and can cause depreciation of microbial activities and wash out of active biomass (Torkian et al. 2003). Fortunately, in majority cases, UASB technology are reported to remove >60% of COD from most types of effluent hence making it a promising option. On the other hand, ASBR is less preferred for ACD of crude glycerin because mixing may be not thorough in the system. Since the substrate is highly vicious and the importance of ACD highlights on homogenous condition, uniform mixing is vital. Nevertheless, ASBR portrayed the benefits of achieving high degradation of organics, manual mixing (no energy consumption) and simplicity of reactor making it worthwhile to be explored to learn ACD performance. Despite that UASB and CSTR are viewed to be more ideal to be implemented in this research, ASBR should also be employed in this research to prove its benefits and compare the performances between reactors.

Table 2.2: The advantages and disadvantages of ASBR, CSTR and UASB

Type of reactor ^a	Advantages	Disadvantages
ASBR	• High conversion per unit volume can be obtained by leaving the reactant in the reactor for long periods of time • Flexibility of operation-same reactor can produce one product one time and a different product the next	• High operating cost • Product quality more variable than with continuous operation • High labour costs per batch, the variability of products from batch to batch, and the difficulty of large-scale production
CSTR	• Continuous operation • Homogenous mixing of substrate • Good temperature control is easily maintained • Reactor has large heat capacity • Low operating (labour) cost	• Conversion of reactant to product per volume of reactor is small compared to other flow reactors • By-passing and channelling possible with poor agitation • Poor settling of sludge
UASB	• High reduction in organic pollutant and conversion into biogas • Can withstand high organic and hydraulic loading rates • Less disposal of sludge (and, thus, infrequent desludging required) • Biogas can be used for energy (but usually first requires scrubbing) • No aeration system required (thus little energy consumption) • Effluent is rich in nutrients and can be used for agricultural irrigation	• Difficult to maintain proper hydraulic conditions (up-flow and settling rate must be balanced) • Long start up time • Treatment may be unstable with variable hydraulic and organic loads. • Constant source of electricity is required • Effluent and sludge require further treatment and/or appropriate discharge

^a**Type of reactor:** UASB, up-flow anaerobic sludge blanket; CSTR, continuous stirred tank reactor; ASBR, anaerobic sludge blanket reactor

2.3.7 Inhibition of ACD

There are various type of inhibitory substance and are often the main cause of ACD failure as they may be present in substantial concentration in effluent. The inhibitors commonly present in digesters with ACD of glycerin based substrate include SO_4^{2-} , S^{2-} and organic such as LCFAs.

SO_4^{2-} is usually reduced to S^{2-} by sulfate reducing bacteria (SRB) and this reduction is carried out by two groups of SBR namely incomplete (reduces lactate to acetate and carbon dioxide) and complete oxidisers (reduces acetate to carbon dioxide and bicarbonate) (Koster et al. 1986; Hilton & Oleszkiewicz 1988). Two stages of inhibitions may occur during the reduction process: (1) Primary inhibition results from competition for common organic and inorganic substrates from SRB which restrain methane production (Harada et al. 1994). As reported by Oude Elferink et al. (1994), SRB could degrade LCFAs, organic acids, aromatic compounds, ethanol and other alcohols as well as have high affinity for reduced substrates. Due to the various substrate utilisation shown by SRB, they compete with methanogens or acetogens for available acetate, hydrogen, propionate and butyrate in AD (Colleran et al. 1995). (2) Secondary inhibition is due to toxicity of S^{2-} to various bacteria groups (Anderson et al. 1982; Oude Elferink et al. 1994; Colleran et al. 1998). S^{2-} in the form of hydrogen sulfide is toxic to methanogens as well as to SRB themselves which supported by the studies of Conn et al. (1987), Tursman & Cork (1988), Vogels et al. (1988) and O'Flaherty et al. (1999). The researchers suggested that this is because it can diffuse into cell membrane and then denature the native proteins in it which disrupt various co-enzyme sulfide linkage and assimilatory metabolism of sulfur, which is a required nutrient for methanogens. Parkin et al. (1990) further added that inhibitory S^{2-} concentration is >100 mg/L. Although such inhibition could be overcome by dilution of effluent, it is not often and not desirable as this also increases reactor volume and size. Alternatively, chemical reactions with

addition of calcium chloride to initiate coagulation followed by polymer for flocculation process is recommended for SO_4^{2-} removal (Oude Elferink et al. 1994; Song et al. 2001).

Besides SO_4^{2-} and S^{2-} , organic substances which are poorly soluble in waste or adsorbed to the sludge surface causes membrane to swell and leak, inhibit ion gradients and subsequently causing cell lysis. LCFAs is identified as inhibitor in effluent rich in fatty materials. It has been reported to be inhibitory at a low concentration of gram-positive bacteria (Kabara & Vrable 1977). Methanogens is constrained by LCFAs due to their cell wall which mimics the gram-positive bacteria (Zeikus 1977). Through adsorption onto the cell wall or membrane, LCFAs exhibit acute toxicity towards anaerobes which interfere the protective and transport functions (Rinzema et al. 1994). In addition, sorption of light layer of LCFAs to biomass results in sludge floatation and eventually sludge wash out (Rinzema et al. 1989). This has been observed in Hwu et al. (1998)'s studies where granular sludge floatation occurred in UASB reactors. Type of sludge could also influence the LCFAs toxicity as it is more correlated to the sludge physical characteristics rather than biological characteristics. This is explained by Hwu et al. (1996) that suspended and flocculent sludge which have higher specific surface area showed a greater inhibition than granular sludge. Other researchers also suggested that LCFAs exerted a bactericidal effect and no adaptation of methanogens occurred (Hanaki et al. 1981; Koster & Cramer 1987; Angelidaki & Ahring 1992). Addition of calcium has been reported to reduce LCFAs inhibition through the formation of insoluble salts (Hanaki et al. 1981). Nevertheless, this method could not solve the problem of sludge floatation. Hence, pre-treatment such as induction of specific enzyme for degradation or chemical reaction (coagulation and flocculation) may need to be further explored.

2.4 Operating conditions for ACD of crude glycerin

Table 2.3 presented the summary of optimum operating conditions for glycerin based substrate to achieve stability in ACD performance. These optimum operating conditions will be adopted in the ACD of GP with OCE.

Table 2.3: Optimum operating conditions for crude glycerin as co-substrate

Operating condition	Crude glycerin
Type of reactor^a	ASBR, CSTR or UASB
Digestion volume ratio	1-4%
Digestion mode	Semi-continuous
Temperature	Mesophilic (28-38°C)
HRT	>30 days
OLR	In terms of VS: <2 g VS/L.day In terms of COD: $\approx$ 8 g COD/L.day

^a**Type of reactor:** ASBR, anaerobic sludge blanket reactor; CSTR, continuous stirred tank reactor; UASB, up-flow anaerobic sludge blanket

2.5 Summary

Glycerin based effluent which often is used as co-substrate in treatment plant was proven to gain higher methane yield compared to the mono digestions. Hence, it can be concluded that glycerin based substrate is potentially feasible in boosting up biogas production in ACD. It is also economically feasible to co-digest the mentioned co-substrate in other industries such as oleo chemical, pesticides, palm oil mill, mining and agriculture industries as it can reduce cost from shared equipment, easier handling of feedstock, and a more stable process in general. ASBR, UASB or CSTR are suggested for GP treatment to compare the ACD performances in this research. Whereas, optimum operating conditions include slow mixing system, mixture of wastes with low and high C/N ratio and semi-continuous

mode are recommended. Mesophilic condition and low range OLR <2 g VS/L.day with HRT >20 days are preferred. Pre-treatment also is advised to be conducted to reduce inhibitors effect in ACD. Further research is required to develop deeper understanding for ACD improvement on bio-waste as a main substrate. This is because bio-waste as a main substrate is still lacking in research as compared to manures or sewage sludge. In addition, despite the fact that mixed liquor suspended solid (MLSS) concentration plays a big role in ACD, the effect in terms of its optimum concentration on the ACD performance was found to be deficient in literature. Hence, this area should be further studied.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental flow chart

The experimental work carried out in the present research which involved feasibility studies, ACD performance evaluation with different reactors and pre-treatment is presented in a flow chart in Figure 3.1.

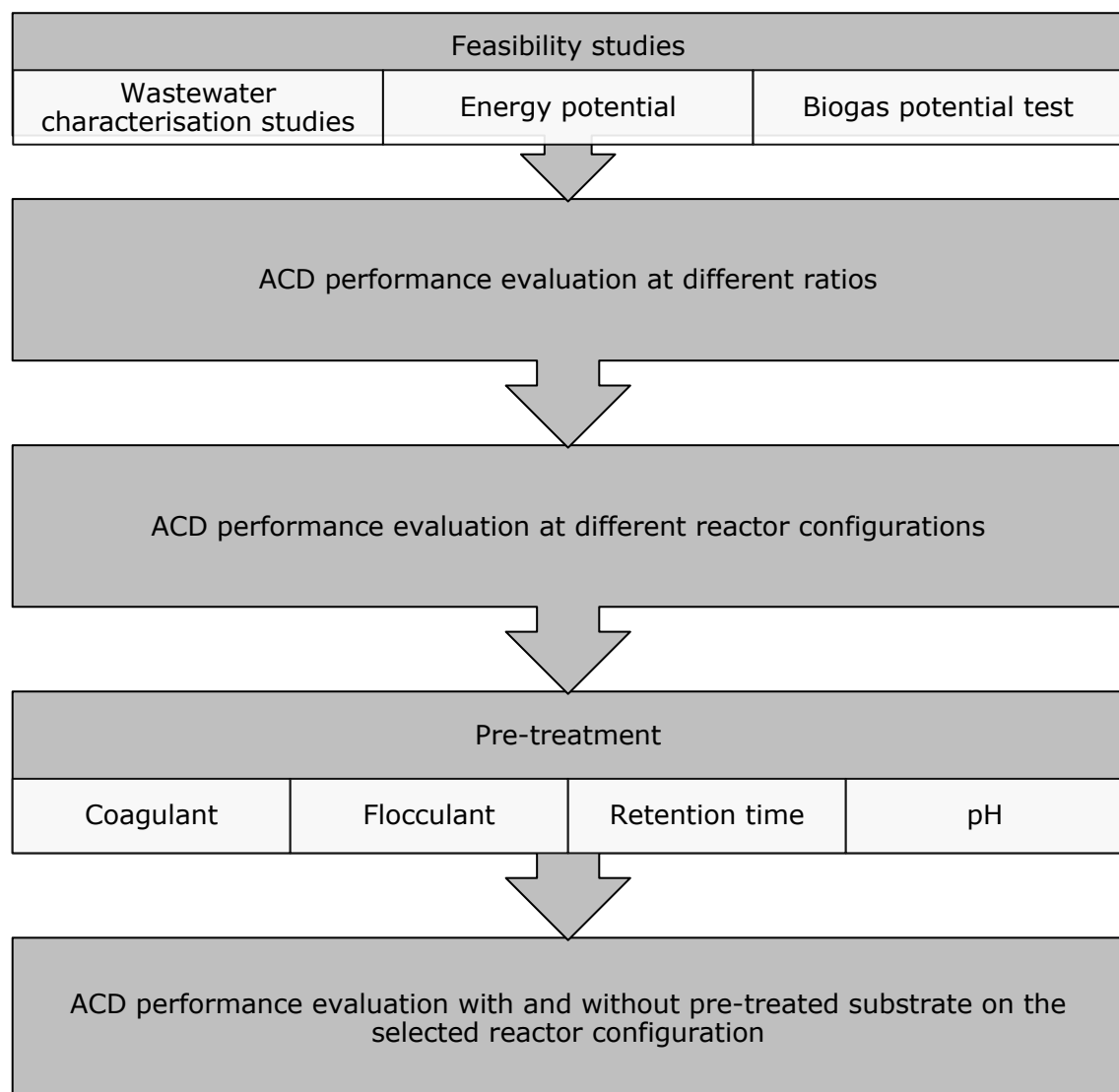


Figure 3.1: Flow chart of overall experimental activities

3.2 Materials

The chemicals and reagents in analytical and industrial grades that have been used throughout the study are tabulated in Table 3.1.

Table 3.1: List of chemicals and reagents used

Chemical / Reagent	Purpose	Brand
COD digestion reagent vial	Chemical oxygen demand (COD) analysis	HACH, Loveland, CO
Nitrification inhibitor formula 2533	Biochemical oxygen demand (BOD) analysis	HACH, Loveland, CO
Total organic carbon direct method high range test 'N tube™ reagent set	Total Organic Carbon (TOC) analysis	HACH, Loveland, CO
Test 'N tube total™ nitrogen reagent set	Total nitrogen (TN) analysis	HACH, Loveland, CO
Nitrogen reagent set	Total kjedahl nitrogen (TKN) analysis	HACH, Loveland, CO
n-hexane 99.6%, GR for analysis, ACS	Oil & grease (O&G) analysis	Merck, Germany
Total high range phosphorus test 'N tube reagent set	Total phosphorus (TP) analysis	HACH, Loveland, CO
Volatile acid reagent set	Volatile fatty acid (VFA) analysis	HACH, Loveland, CO
Sulfuric acid	Total alkalinity (TA) analysis	Merck, Germany
Sodium bicarbonate (NaHCO ₃)	pH adjustment	Bendosen, UK
Calcium chloride (CaCl ₂)	Pre-treatment: Coagulation	Merck, Germany
Flocculant FO 4650 SH	Pre-treatment: Flocculation	SNF Floerger, France
Hydrochloric acid (HCl)	Pre-treatment: pH adjustment	Merck, Germany
Glycerol 99.9%	Total glycerin (TG) analysis	Dr Ehrenstorfer
Acetonitrile (ACN)	Flushing high performance liquid chromatography (HPLC)	Merck, Germany
SulfaVer® reagent powder pillows	Sulfate (SO ₄ ²⁻) analysis	HACH, Loveland, CO
Sulfide reagent set	Sulfide (S ²⁻) analysis	HACH, Loveland, CO

3.3 Analytical methods

Table 3.2 presented the summary list of analytical methods exercised in determining the characteristics of all the samples involved throughout the study. These included samples of anaerobic sludge, raw substrates - diluted GP (DGP) and OCE, influent, treated effluent and pre-treated substrates. Also, all equipment used in performing the sample analyses are listed in Table 3.2. All analyses were conducted in triplicate to obtain the average data.

Table 3.2: List of parameters and analyses methods employed

Parameter	Reference No.	Analytical method	Equipment used
pH	-	-	pH meter (HACH SensION+, Loveland, CO)
Temperature	-	-	pH meter (HACH SensION+, Loveland, CO)
Dissolved oxygen (DO)	-	-	DO meter (YSI 5100, USA)
Biochemical oxygen demand (BOD)	HACH 8043	3-day BOD test	BOD incubator (HACH, Loveland, CO)
Chemical oxygen demand (COD)	HACH 8000	Reactor digestion method	HACH DRB200 reactor, DR2800 spectrophotometer
Total solids (TS)	APHA 2540B	Total solids, dried at 103-105°C	Oven (Mettler, Germany)
Total suspended solids (TSS)	HACH 8006	Photometric method	HACH DRB2800 spectrophotometer
Volatile suspended solids (VSS)	APHA 2540E	Volatile solids, dried at 550°C	Muffled furnace (Carbolite, UK)
Total Organic Carbon (TOC)	HACH 10128	Direct method high range text 'N tube™ reagent	HACH DRB200 reactor DRB2800 spectrophotometer
Total nitrogen (TN)	HACH 10072	Persulfate digestion method	HACH DRB200 reactor DRB2800 spectrophotometer
Total kjedahl nitrogen (TKN)	HACH 8075	Nessler's method	HACH DRB200 reactor DRB2800 spectrophotometer

Total phosphorus (TP)	HACH 10127	Molybdovanadate method with acid persulfate digestion	HACH DRB200 reactor DRB2800 spectrophotometer
Oil & grease (O&G)	USEDPA Method 1664	n-hexane gravimetric method	Rotary evaporator (Laborota 4001, Heidolph, UK) Centrifuge (Eppendorf 5430, Germany)
Volatile fatty acid (VFA)	HACH 8196	Esterification for volatile acids	HACH DRB2800 spectrophotometer
Total alkalinity (TA)	HACH 8193	Titration method with sulphuric acid	pH meter (HACH SensION+, Loveland, CO)
Total glycerin (TG)	ASTM D 6584-00	Gas chromatography	HPLC Agilent Afinity 2600 (Agilent, USA) Zorbax carbohydrate analysis column 4.6x250mm, 5 μ m (Agilent, USA)
Sulfate (SO ₄ ²⁻)	HACH 8051	USEPA sulfaVer 4 method	HACH DRB200 reactor DRB2800 spectrophotometer
Sulfide (S ²⁻)	HACH 8131	USEPA methylene blue method	HACH DRB200 reactor DRB2800 spectrophotometer
Biogas composition	-	-	Biogas 5000 Standard (Geotech, UK)

3.4 Sampling methods

Anaerobic sludge obtained from anaerobic tank treating POME in Sime Darby West Oil Mill, Carey Island, Selangor state of Malaysia was used in the entire study. The characteristics of anaerobic sludge are tabulated in Table 3.3, VSS/TSS ratio is the volatile fraction which signifies the biologically active quantity of solids in the sludge.

Table 3.3: Characteristics of anaerobic sludge

Parameter ^a	Unit	Anaerobic sludge	
		Average	Range
TSS	g/L	18.9	18.4 - 19.2
VSS	g/L	10.3	10.0 - 10.3
VSS/TSS	-	0.54	0.54 - 0.54

^a**Parameter:** TSS, total suspended solid; VSS, volatile suspended solid

Whereas for DGP and OCE, a series of ten days sampling collection was conducted at a refinery factory, Selangor state of Malaysia. There were three sampling points for DGP termed as DGP 1, DGP 2 and DGP 3; and three sampling points for OCE named as OCE 1, OCE 2 and OCE 3. However, the three sampling points for both DGP and OCE were denoted as DGP and OCE only in this study as the data presented in the form of average of all three sampling points.

Also, it is important to note that GP is originally produced in a stagnant form and it was mixed or diluted with some portion of OCE with OCE: GP volume ratio of 70:30 by the industry to ensure it is flowable and ease the sample collection. Hence, DGP as per mentioned ratio was used throughout the experiments. Whereas, the results will be presented in OLR basis (OLR of OCE: OLR of GP) to serve as a functional reference for other researchers and hence will be reported as OLR_{GP} only in the entire study.

3.5 Feasibility studies of ACD of GP with OCE

3.5.1 Wastewater Characterisation Studies (WWCS)

The WWCS is divided into two parts; on-site and off-site analyses. The on-site samples of the effluent were collected at two hours interval from 8.00 am to 6.00 pm daily. On the other hand, the off-site measurements (BOD, COD, TSS, TOC, TN, TKN, TP, O&G, TG, VFA, TA, SO_4^{2-} and S^{2-}) were performed at composite samples in the Engineering Research Laboratory of the University of Nottingham, Malaysia Campus in accordance to analysis methods listed in Table 3.2.

3.5.2 Energy potential

Evaluation of the suitability and potential of ACD for DGP with OCE is conducted based on the results obtained from the WWCS. This evaluation is reviewed from the estimation of potential methane production rate (F_{CH_4}) and maximum energy potential (F_{kWh}).

Table 3.4: Main parameters employed in estimating the methane and energy potentials

No.	Symbol	Unit	Description	Equation (Eq)	Eq. No.
1	F_{CH_4}	m ³ /day	Methane production	$\frac{COD_{in} \cdot \%COD \cdot Q_{in} \cdot Y_{CH_4}}{1000}$	3.1
2	V_{gas}	m ³ /mole	Volume occupied by 1 mole of gas	$\frac{nRT}{P}$	3.2
3	Q_{CH_4}	mole/day	Conversion of methane in m ³ /day to mole/day	$\frac{F_{CH_4}}{V_{gas} \cdot 1000}$	3.3
4	M_{CH_4}	g/day	Mass of methane	$Q_{CH_4} \cdot MW_{CH_4}$	3.4
5	F_{kWh}	kJ/day	Energy content of gas	$M_{CH_4} \cdot 50.1 \text{ kJ/g}$	3.5

where %COD is the COD removal efficiency (%), Y_{CH_4} is the CH_4 obtained per COD converted under anaerobic condition (L CH_4 /g COD), n is the number of mole (mole), R is gas constant (atm.L/mole.K), T is the temperature of influent (K), P is the atmospheric pressure (atm), and MW_{CH_4} is the molecular weight of methane (g CH_4 /mole).

F_{CH_4} can be accessed by using Eq. 3.1 and assuming Y_{CH_4} is 0.24 L CH_4 /g COD and %COD equivalent to 85%. On the other hand, V_{gas} can be calculated, first by obtaining the volume occupied by 1 mole of gas assuming R is 0.082057 atm.L/mole.K, P is 1 atm and n is 1 mole (Eq. 3.2). The volume occupied by 1 mole of gas is then incorporated into Eq. 3.3 in order to convert methane production from m^3 /day to mole/day. Methane production in mole/day (Q_{CH_4}) will be further multiplied by methane molecular weight to achieve mass of methane (M_{CH_4}) in Eq. 3.4. M_{CH_4} will then be used in Eq. 3.5 to find the energy content (F_{kWh}) which can be calculated by using the factor 50.1 kJ/g methane as given by Metcalf & Eddy (2014). Since $1 W = 1 J/s$, energy content in gas (kJ/day) can be converted into KW by dividing 86400 s which subject to gas engine at 40% efficiency.

3.5.3 Biogas potential test (BPT)

A 1 L conical flask (T 101) was used as a "reactor" for the BPT with an effective working volume of 850 ml to determine the biogas potential of DGP and OCE individually along with POME as a control. A schematic diagram of BPT is depicted in Figure 3.2. POME was chosen as a control due to its known biogas potential that has been widely researched, hence suitable as a point of comparison. The individual set up of DGP, OCE and POME as substrate, each have been replicated thrice. Substrate with a volume of 50 ml and anaerobic sludge of 800 ml were fed into the reactor that operated mesophically. Prior to feeding, substrate was analysed for its initial COD

concentration. It was air sealed with only a gas stream connected to the water displacement system (T 102) to measure the volume of biogas generated. Each substrate was incubated for 30 days and the digested effluent was collected to analyse for the final COD concentration. The volume of biogas generated was also recorded daily.

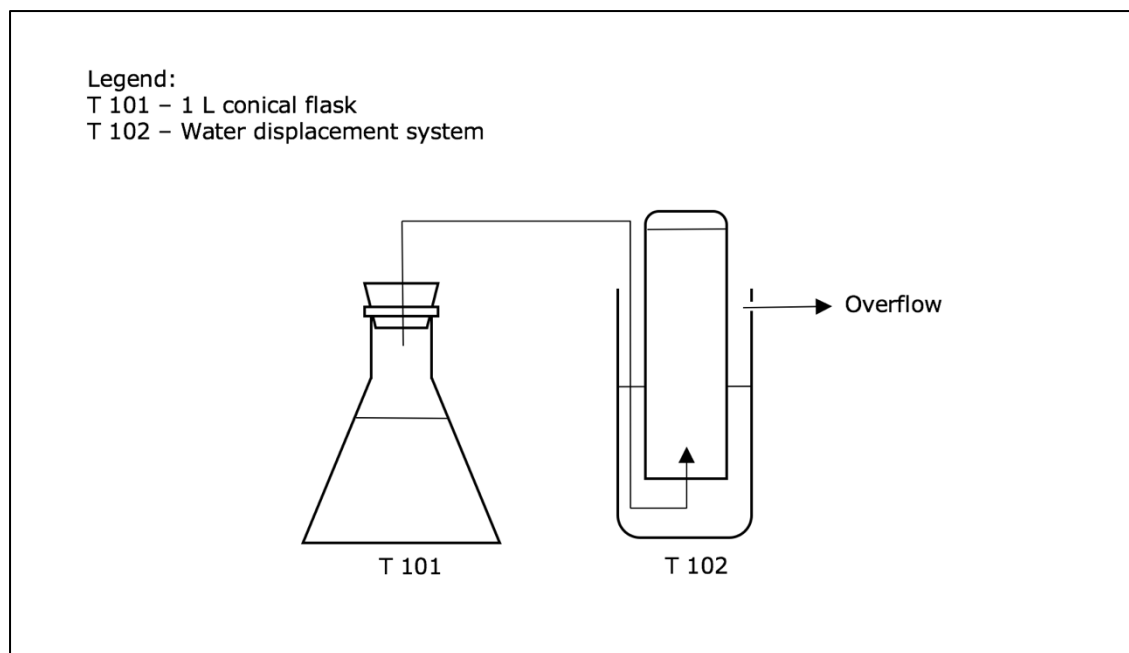


Figure 3.2: Schematic diagram of BPT

3.6 Bioreactors performance and stability parameters

This research was designed with a series of parameters to be tested along the experiment. The ACD performance of GP with OCE at different ratios and reactor configurations and pre-treated substrate was regulated and accessed in terms of overall organic removals of BOD, COD, TSS, TG, O&G, SO_4^{2-} and S^{2-} as well as biogas production rate and its composition. The process stability was analysed in the aspects of pH, VFA/TA, mixed liquor volatile suspended solid (MLVSS) concentration and F/M ratio. The main parameters used and their calculation methods are tabulated in Table 3.5.

Table 3.5: Main parameters employed in the present study

No.	Symbol	Unit	Description	Equation	Eq. No.
1	OLR	g COD/L.day	OLR	$\frac{Q_{in} \cdot COD_{in}}{V_r}$	3.6
2	%V	%	Percentage volume of substrate	$\frac{Q_{in}}{\Sigma Q_{in}} \times 100$	3.7
3	V _{in}	L	Total volume of influent	$\frac{V_i \cdot \%V}{100}$	3.8
4	%COD	%	COD removal efficiency	$\frac{COD_{in} - COD_{out}}{COD_{in}} \times 100$	3.9
5	%BOD	%	BOD removal efficiency	$\frac{BOD_{in} - BOD_{out}}{BOD_{in}} \times 100$	3.10
6	HRT	day	Hydraulic retention time	$\frac{V_r}{Q_{in}}$	3.11
7	R	-	Recirculation ratio	$\frac{Q_r}{Q_{in}}$	3.12
8	Q _{in}	L/day	Flow rate of influent	$\frac{OLR \cdot V_r}{COD_{in}}$	3.13
9	Y _M	L CH ₄ /g COD _{removed}	Methane yield	$\frac{Q_{CH_4}}{Q_{in} (COD_{in} - COD_{out})}$	3.14

where Q_{in} is the flow rate of influent (L/day), V_r is the volume of the bioreactor (L), V_i is the volume of substrate in bioreactor (L), COD_{in} is the influent COD concentration (mg/L), COD_{out} is the COD concentration of digested effluent (mg/L), BOD_{in} is the influent BOD concentration (mg/L), BOD_{out} is the BOD concentration digested effluent (mg/L), MLVSS is the biomass concentration (mg/L), Q_{CH₄} is the methane production rate (L/day) and Q_r is the recirculation flow rate (L/day).

The COD_{in} was measured prior to calculation of the correct Q_{in} by using Eq. 3.13. Biogas production was measured by using water displacement method with a 2 L inverted water-filled graduated cylinder for a collection period of 24 hours, once a day and average value for the particular day was recorded (Kumar et al. 2008).

3.7 Experimental strategy of bioreactors

There are two parts in the evaluation of ACD performance. Firstly, it was accessed at different ratios under ASBR and CSTR. A series of different OLR ratio combinations designated under 4 g COD/L.day (4: 0, 3: 1, 2: 2, 1: 3 and 0: 4 of OCE: GP) was evaluated. The OLR was fixed at 4 g COD/L.day throughout the experiment which is also corresponding to the normal loading rate applied to the anaerobic inoculums collected from palm oil mill (Chan et al. 2012). The OLR of GP was increased by reducing OLR of OCE from 4 to 3, 2, 1 and finally 0. This series aim to reduce the organic load impact of GP on anaerobic microorganism. Although the actual OLR of OCE: GP obtained throughout the experiment were varies, it was able to be regulated and close to the designated OLR as shown in Table 3.6. Besides, note that DGP comprises 70: 30 of OCE: GP (v: v) which means OCE portion is present in the last OLR ratio but the contribution of OCE to OLR is negligible. Concurrently, the Q_{in} was adjusted accordingly on different ratios. The OLR ratio with the highest organic removals was chosen for the second evaluation. In the second part, ACD performance was studied at different reactor configurations by using the chosen OLR ratio obtained.

Prior to the experiment, 1.0 L, 0.3 and 5.0 L of anaerobic sludge were placed into the ASBR, CSTR and UASB respectively. These volumes of sludge also represent 25%, 30% and 22% of the effective volume of the respective reactor which regarded as common percentage in anaerobic reactor (Chan et al. 2012). Oxygen-free nitrogen gas was charged into the reactor from its bottom / purging outlet to replace the air inside. No feed was added to the anaerobic sludge at this stage. The sludge was then left at rest for 24 hours to allow gradual adaptation to ambient temperature. At the same time, for UASB, the recirculation pump as shown in Figure 3.8 was initiated at a recirculation ratio, R (Eq. 3.12) of 15 to ensure mixing in the reactor. After the completion of the rest period, ASBR and CSTR were fed with OLR_{GP}

0 (OLR ratio of OCE: GP at 4: 0) with influent flow rate (Q_{in}) of 1.160 L/day and 0.230 L/day respectively in a series of five ratios as presented in Table 3.6. The second ratio was proceeded when the COD removal efficiency remains relatively constant within 5% for three consecutive days. Note that HRT will be the same for both reactors. This is because Q_{in} which obtained from the same OLR and COD_{in} will give the same HRT despite the varies in V_r .

Table 3.6: Operational flow rates at different ratios

Design ratio ^a	ASBR						Actual ratio ^b	CSTR						Actual ratio ^b	HRT ^e
	OCE		DGP					OCE		DGP					
			OCE		GP					OCE		GP			
	Q _{in} ^c	V _{in} ^d	Q _{in} ^c	V _{in} ^d	Q _{in} ^c	V _{in} ^d		Q _{in} ^c	V _{in} ^d	Q _{in} ^c	V _{in} ^d	Q _{in} ^c	V _{in} ^d		
4: 0	1.160	3.000	0.000	0.000	0.000	0.000	4.105: 0.000	0.230	0.595	0.000	0.000	0.000	0.000	4.109: 0.000	2.6
3: 1	0.870	2.960	0.008	0.028	0.004	0.012	3.007: 1.129	0.172	0.587	0.002	0.006	0.001	0.002	2.892: 1.086	3.4
2: 2	0.580	2.884	0.016	0.081	0.007	0.035	1.955: 2.163	0.115	0.572	0.003	0.016	0.001	0.007	1.955: 2.163	5.0
1: 3	0.290	2.676	0.025	0.226	0.011	0.097	0.994: 3.128	0.057	0.531	0.005	0.045	0.002	0.019	0.994: 3.128	9.2
0: 4	0.000	0.000	0.033	2.100	0.014	0.900	0.100: 4.030	0.000	0.000	0.006	0.417	0.003	0.179	0.100: 4.033	64.2

^a**Design ratio**, OLR ratio of OCE: GP designated: g COD/L.day (Calculated based on Eq 3.6)

^b**Actual ratio**, OLR ratio of OCE: GP obtained: g COD/L.day (Calculated based on Eq 3.6)

^c**Q_{in}**, Flow rate of influent: L/day (Calculated based on Eq 3.13)

^d**V_{in}**, Total volume of influent: L (Calculated based on Eq 3.8)

^e**HRT**, Hydraulic retention time: days (Calculated based on Eq 3.11)

3.7.1 Anaerobic Sequential Batch Reactor (ASBR)

Figure 3.3 illustrated the actual view of ASBR with an effective working volume of 4 L, operated mesophically in semi-continuous mode. All ports were sealed during the experiment in order to maintain anaerobic conditions and to prevent biogas from escaping. Substrates mixture were fed daily to the reactor through the opening inlet (F1) as schematised in Figure 3.4. The ASBR (T 101) was shaken by hand twice a day to enhance mixing. The biogas generated will be then channelled to the gas holder which uses a water displacement system (T 102) to determine the biogas volume while biogas composition was recorded through gas sampling point (SP 3) using a biogas analyser (Geotech 5000 Standard, UK). The treated effluent and sludge were sampled at the upper (SP 2) and lower (SP 1) outlet points of ASBR respectively. Samples collected were analysed as described in Section 3.4 whereas the remaining samples were returned to the reactor to reduce the loss of biomass in the system.

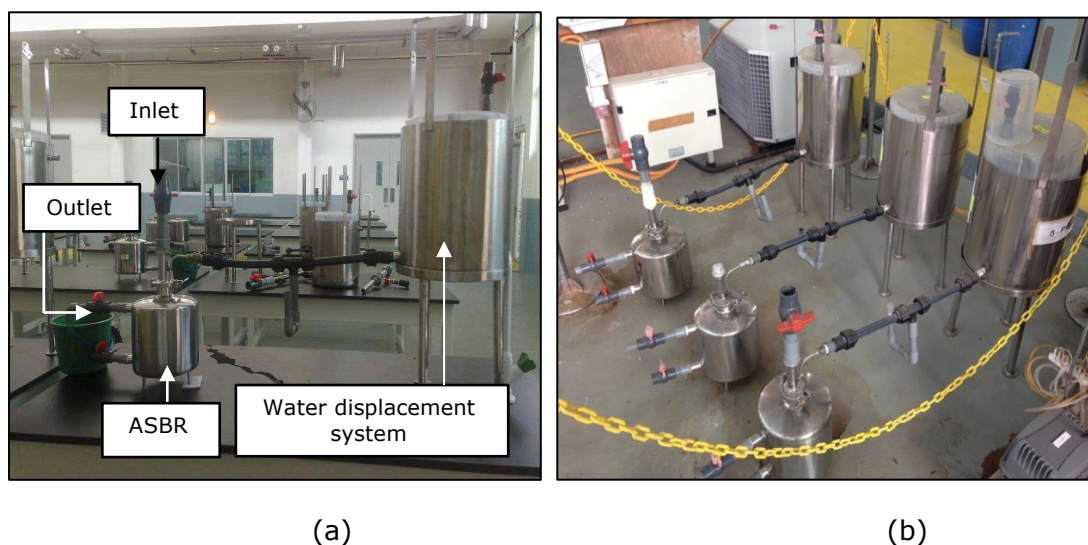


Figure 3.3: Actual view of ASBR system, (a) front view and (b) top view

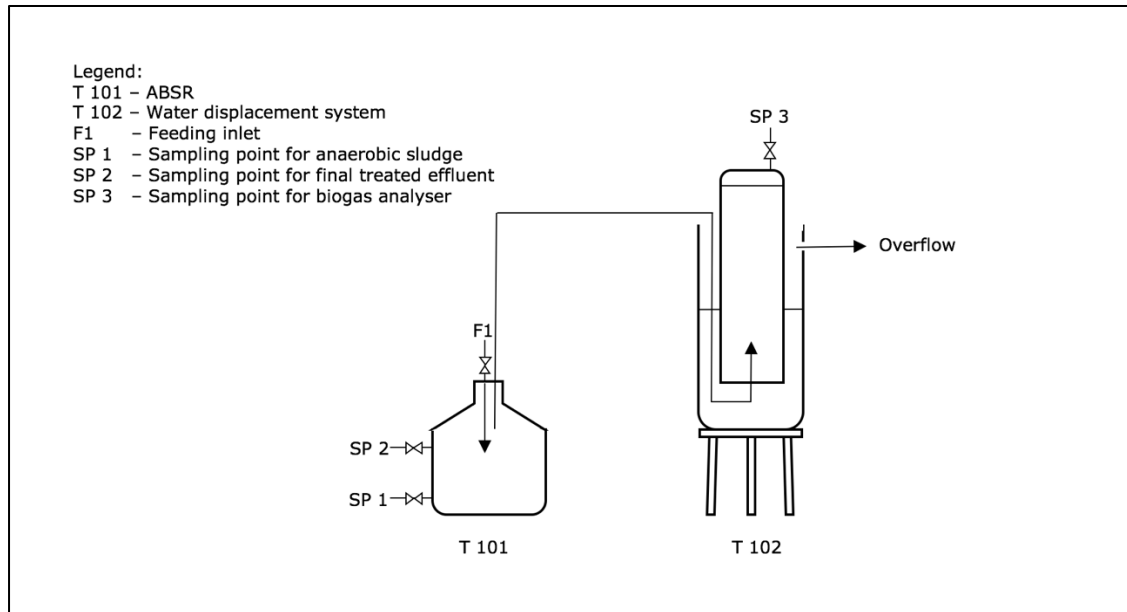


Figure 3.4: Schematic diagram of ASBR

3.7.2 Continuous Stirred Tank Reactor (CSTR)

Figure 3.5 displayed the actual view of CSTR which is also known as minifors (Infors HT, Switzerland). Substrates mixture were fed daily through inlet (F1) to the reactor as schematised in Figure 3.6. The effluent in reactor (T 101) was mixed by a stirrer of two impellers stirring at 150 rpm in a continuous mode to provide homogenous mixing of substrate with sludge. The pH control in the system were regulated with two dosing pumps connected to acidic and alkaline buffers while oxygen concentration and temperature were regulated online. Besides, a gas stream outlet (SP 3) was designed to determine biogas composition using biogas analyser (Geotech 5000 Standard, UK). This outlet also connected to the water displacement system (T 102) to measure the volume of biogas produced. Biogas produced in CSTR was diverted through the outlet to an overturned measuring cylinder and displaced the water from the cylinder into the container. Both treated effluent and sludge were collected via an adjustable sampling point at SP 2 and SP 1 respectively.

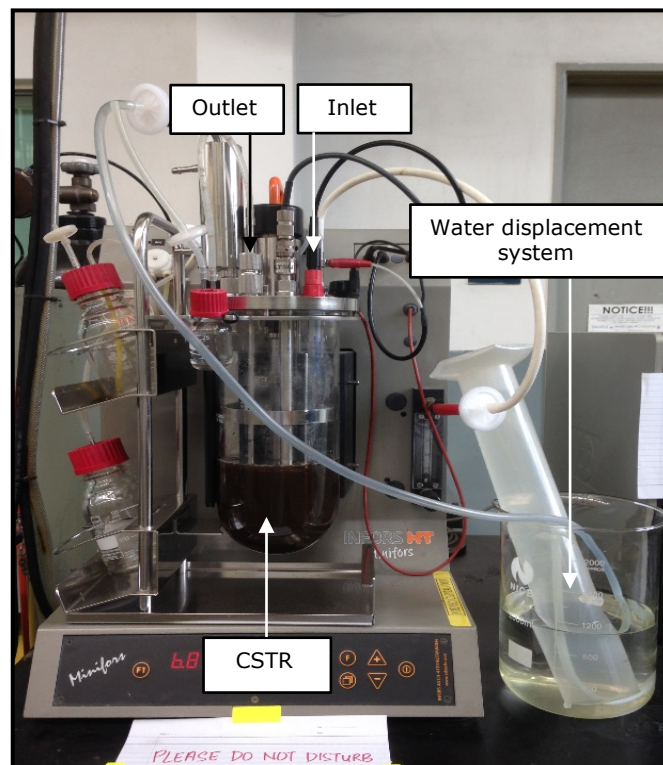


Figure 3.5: Actual view of CSTR

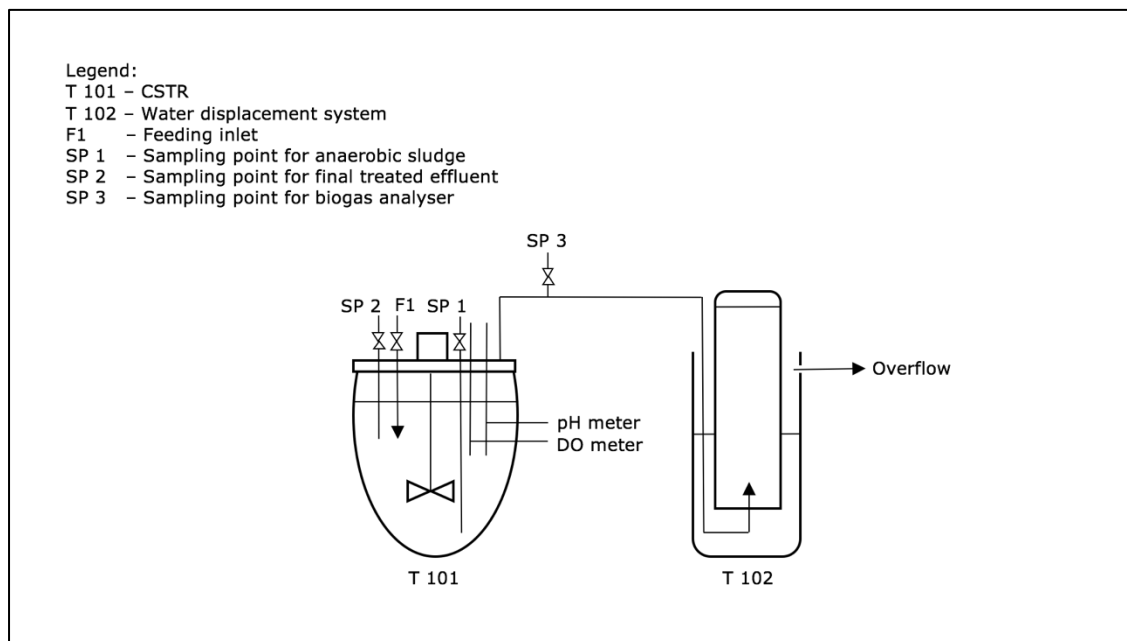


Figure 3.6: Schematic diagram of CSTR

3.7.3 Up-flow Sludge Blanket (UASB) reactor

Figure 3.7 showed the actual view of UASB. From the feed tank (T 101), the mixture of substrates was continuously fed to the reactor compartment (T 102) with the aid of a peristaltic feed pump (P 101) (Longer pump, China) as schematised in Figure 3.8. The effluent in the sludge blanket of the reactor from recirculation outlet point (W1) was recirculated to the recirculation inlet point (R1) at the bottom compartment by using peristaltic pump (P 102) (IBS, Switzerland) in order to achieve uniform and homogenous distribution of substrates along the sludge bed. The effluent streamed upward and overflowed as final treated effluent which can be collected at sampling point (SP 2) located at the top of the reactor. Whereas, sludge can be sampled at sampling point (SP 1) designated at the sludge bed of the reactor. The GLSS was also connected to a water displacement system (T 103) to measure the volume of biogas produced whereas a gas sampling point (SP 3) was designed to determine biogas composition by using a biogas analyser (Geotech 5000 Standard, UK).

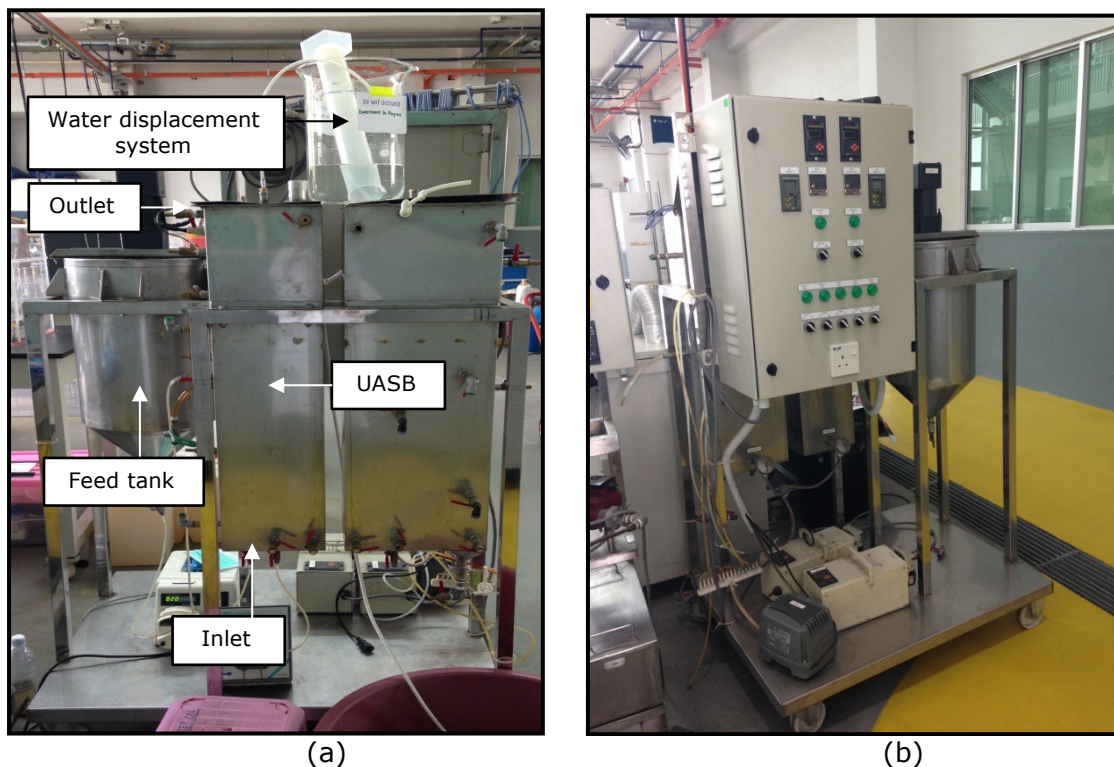


Figure 0.7: Actual view of UASB system, (a) front view and (b) rear view

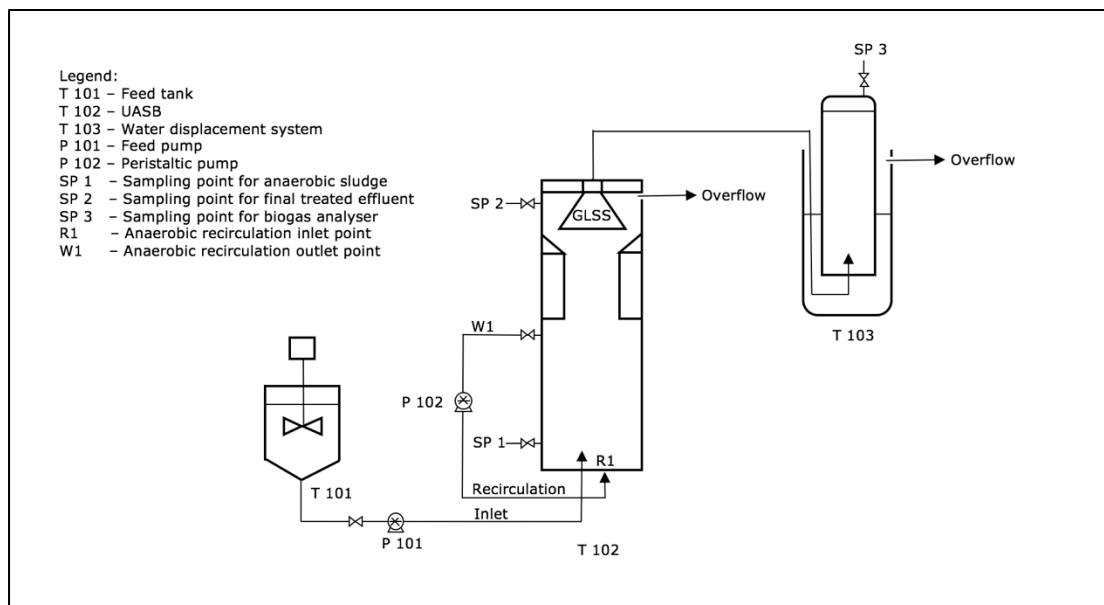


Figure 3.8: Schematic diagram of ACD

3.8 ACD performance with pre-treatment

3.8.1 Pre-treatment of GP for SO_4^{2-} removal

In order to assess SO_4^{2-} inhibition in ACD treatment and to improve performance of the chosen reactor, pre-treatment was conducted on the chosen OLR of OCE: GP. Jar test was used to identify the suitable chemical physical pre-treatment conditions. There are four phases involved in jar test. First phase termed as coagulation where calcium chloride (CaCl_2) (Merck, Germany) was used as a coagulant to destabilise colloidal suspensions and form precipitation. Whereas, commercial cationic polymer (SNF Floerger, France) was applied in the second phase as flocculant to promote aggregation of colloids, combining small floc to a bigger floc which eventually form two separate layers in the effluent. Following that, retention time of CaCl_2 and polymer (third phase) as well as pH (last phase) were explored to achieve an optimum point for maximum reduction of SO_4^{2-} concentration.

In this study, DGP placed into five units of beakers with effective volume of 200 ml along with one control. This set is elucidated in Figure 3.9 and each beaker

is labelled as “jar” in this study. At phase (1), the addition of increasing CaCl_2 dosages were added into respective jar according to Table 3.7. The dosages range have been arrived based on trial and error. During the optimisation of coagulant dosages, other variables (flocculant dosage, retention time of coagulant and flocculant, and pH) were unregulated and fixed at 0.005 mg/L, 1 ml, 5 ml and 11.80 respectively. The pre-treated substrate in supernatant form was separated from the precipitate by filtration using filter paper as shown in Figure 3.10. The pre-treated substrate was then analysed for SO_4^{2-} , COD and pH. The dosage with highest reduction of SO_4^{2-} was chosen to proceed to the next phase: (2) polymer dosages followed by (3) retention time of CaCl_2 and polymer, and (4) pH adjustment using HCl. Similar procedures applied to each performing phase, where other variables will be kept constant when optimising one parameter.

Table 3.7: Operating dosages, retention time and pH of pre-treatment

Run	Optimisation variables	Unit	Jar 1	Jar 2	Jar 3	Jar 4	Jar 5
1	CaCl_2	mg/L	12.5	25	37.5	50	62.5
2	Polymer	mg/L	0.005	0.010	0.015	0.020	0.025
3(a)	Retention time CaCl_2	min	1	2	3	4	5
(b)	Retention time polymer	min	5	10	15	20	25
4	pH	-	7	8	9	10	11

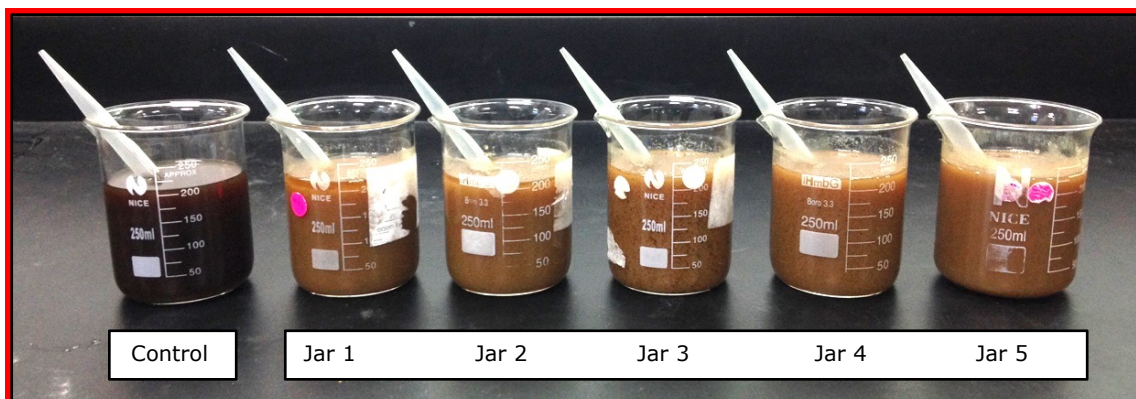


Figure 3.9: Optimisation set of substrate with coagulant-flocculant dosages

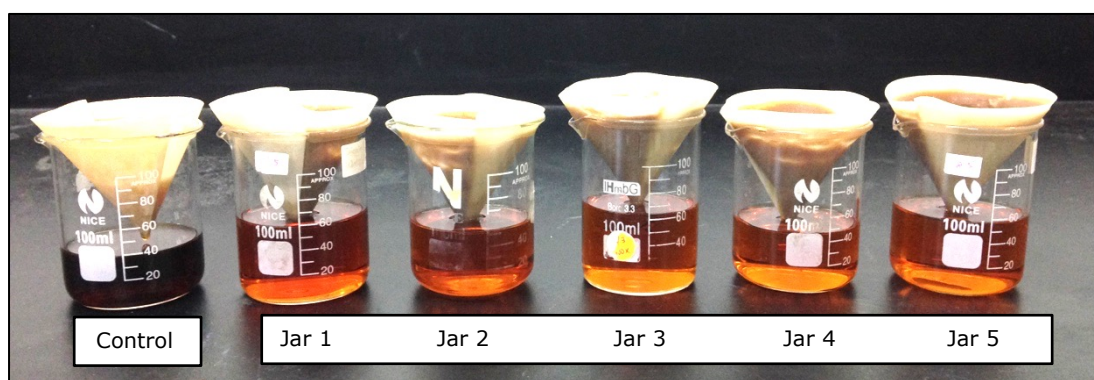


Figure 3.10: Filtration of pre-treated substrate

3.8.2 ACD of pre-treated GP using the chosen reactor

Based on the results obtained from ACD of GP with OCE under Section 3.7, the reactor with the highest organic removals was chosen for subsequent ACD with and without pre-treated DGP. Two sets of substrate at OLR_{GP} 4 were prepared. One using pre-treated substrate and another without pre-treatment as control. Both substrates are subjected to ACD using the chosen reactor with similar operation conditions, performance and stability analyses as listed in Section 3.7 and 3.6 respectively.

CHAPTER FOUR

RESULTS AND DISCUSSION

The findings and results obtained throughout the current research are presented and discussed as follow:

1. Wastewater characterisation and feasibility studies

The results of the characterisation of DGP and OCE were presented. Following this, the F_{CH_4} and $F_{k_{Wh}}$ based on 85% COD removal efficiency were discussed. Also, the suitability of DGP and OCE for ACD treatment was explained to point out several critical drawbacks to be taken into consideration if GP co-digests with OCE. In order to prove both substrates can be anaerobically digested, BPT was presented to measure the potential biogas production.

2. Performance evaluation of ACD of GP with OCE at different OLR ratios

In this section, the effect of different OLR ratios on the ability to handle OLR of GP, treatment efficiencies and reactor stability were presented. The factors which significantly affect the ACD performance of GP with OCE at different OLR ratios were identified and discussed. The performance of different OLR ratios was compared in order to identify the best OLR ratio for the treatment of GP with OCE.

3. Performance evaluation of ACD of GP with OCE at different reactor configurations

This section delivered the comparison of different reactor configurations under the chosen OLR ratio in order to select the best reactor for ACD of GP with OCE. The start-up of the reactors, the effect of different reactor configurations which involves different mixing mechanism on the digestion efficiency and reactor stability were reported. Aspects that influence the ACD performance at different reactors were also highlighted.

4. Performance evaluation of ACD with pre-treated GP using the chosen reactor

This segment discussed on the pre-treatment for GP by identifying the ideal dosages of calcium chloride (CaCl_2) and polymer along with retention time and pH. These variables were to stimulate coagulation and flocculation processes for the removal of SO_4^{2-} which is found to be the inhibitor in ACD. The pre-treated substrate was then placed into chosen reactor for ACD process. The performance on digestion efficiency, biogas production rate and process stability were discussed and compared.

4.1 WWCS

GP and OCE averaging 99 t/month and 12,927 t/month were generated respectively. The data was recorded based on a typical processing plant in Malaysia as presented in Table 4.1. The generation of GP is originally produced in a stagnant form and later it was mixed with large portion of OCE (OCE: GP volume ratio of 70: 30) to ensure it is flowable and ease handling process. Hence, DGP as per mentioned volume ratio was used throughout the experiments. The results were later presented in OLR ratio basis to serve as a functional reference for other researchers as emphasised in Chapter 3 – Section 3.4.

Table 4.1: Product and wastewater generations on monthly basis based on a typical processing plant in Malaysia.

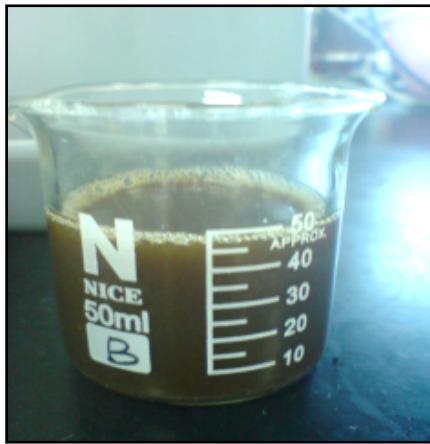
	t/month		Standard Deviation
	Average	Range	
Raw materials	-	-	-
a) Palm kernel oil	21,841	11,688 - 25,884	3,983
b) Water	18,484	18,896 - 18,950	-
Production	25,216	17,520 - 28,602	3,573
a) Refined glycerin	2,645	1,794 - 3,368	450
b) Fatty acids	22,571	15,726 - 25,234	-
Wastewaters generation	15,109	13,064 - 16,232	1,073
a) GP	99	-	314
b) OCE	12,927	-	462
c) Wash water	2,083	-	-

WWCS conducted consists of on-site and off-site analyses as summarised in Table 4.2. Characteristics of DGP and OCE under each analysis parameter will be further discussed in the following sub-sections.

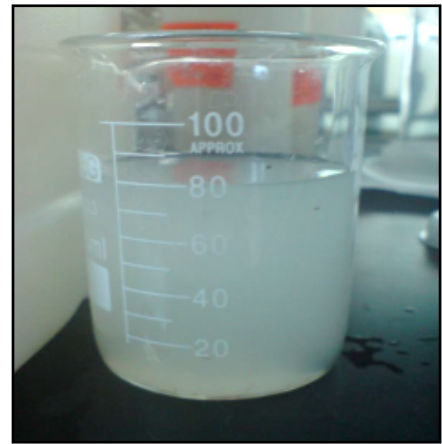
Table 4.2: Summary of various analysis parameters with DGP and OCE

Analysis parameter	Unit	DGP			OCE		
		Average	Range	Standard deviation	Average	Range	Standard deviation
Off-site							
BOD	mg/L	202440	143250 – 267000	41096	8223	4050 – 17970	3316
COD	mg/L	265806	143563 – 421500	91140	10348	4000 – 38050	7534
TSS	mg/L	1570	330 – 2360	721	259	24 – 818	177
TOC	mg/L	62310	34800 – 97000	27589	2263	1800 – 3200	710
TN	mg/L	461	40 – 880	328	94	4 – 1000	213
TKN	mg/L	239	28 – 652.81	198	2	2 – 58	10
TP	mg/L	4389	453 – 8270	2395	228	72 – 408	106
TA	mg/L	19528	12070 – 26360	5152	70	-55 – 1670	313
VFA	mg/L	7728	3290 – 12120	3521	637	92 - 1908	484
TG	mg/L	16.94	14.45 – 17.34	0.83	0	0	0
O&G	mg/L	27942	15614 – 47502	11991	4065	3705 – 4297	168
S ²⁻	µg/L	15424	460 – 34000	10772	506	100 – 1340	321
SO ₄ ²⁻	mg/L	1645	145 – 3000	831	60	10 – 225	51
On-site							
Weather	-		Sunny			Sunny	
Appearance	-		Cloudy, brown			Cloudy, white	
pH	-	12.10	11.65 – 12.87	0.40	4.30	3.59 – 5.46	0.40
Temperature	°C	49.7	46.3 – 51.5	2.0	44.0	42.4 – 45.8	1.1
Flow rate	m ³ /day	23.4	11.1 – 45.7	15.3	367.2	180.0 – 554.4	108.0

4.1.1 On-site parameters



(a)



(b)

Figure 4.1: (a) DGP and (b) OCE

Figure 4.1 showed that DGP is cloudy brown in appearance and OCE is cloudy white throughout the sampling period. Besides, pH was observed to be very alkaline at 12.1 in DGP and acidic at 4.3 in OCE. This indicates that both DGP and OCE could be counter-balanced by each other if co-digested in the digester to achieve a neutral pH which is suitable for microorganisms' growth. The weather remained sunny throughout the sampling period and the temperature of DGP and OCE were noticed to be relatively close averaging 49.7°C and 44.0°C respectively. These high temperatures were due to the heat from production activities. It should be noted that a sudden change or fluctuating temperature could result in a high rate of mortality of microorganism (Wu et al. 2006). The optimum temperature for bacterial activity is in the range from 25°C to 35°C (Kardos et al. 2011) and the high temperature of DGP and OCE can be cooled down to the desired optimum temperature mentioned prior to ACD. Lastly, the average flow rate of 367.2 m³/d was obtained for OCE while GP have relatively lower flow rate averaging at 23.4 m³/d. The difference was due to the different processes used to produce these wastes. It was also noticed that the physical form OCE presents in a flowable liquid

while DGP is in viscous liquid state which could not flow vigorously. Therefore, aspects such as the mixture ratio, mode of mixing and recirculation ratio are needed to be taken into account when designing reactor for ACD to ensure well mixing and no clogging in the system.

4.1.2 Off-site parameters

4.1.2.1 BOD, COD and TSS

Results revealed that the BOD concentration for OCE samples appeared to be lower than DGP by almost 25 times. This indicates the amounts of organic matter that can be oxidised biochemically by microorganisms are relatively high in DGP compared to OCE. This analysis also implies the high carbon content in the DGP is accessible for microbial growth.

Similar to BOD, COD concentration in OCE showed to be lower than DGP by 25 times. This reflects the high amount of oxygen needed to fully oxidise both organic and inorganic compounds present in DGP. This analysis also suggests that the higher COD in DGP would require a longer retention time for AD compared to OCE.

According to Metcalf & Eddy (2014), BOD/COD ratio for untreated effluent is usually in the range from 0.3 to 0.8. If BOD/COD ratio is >0.5 , the effluent is considered to be easily treatable by biological means. If the ratio is below about 0.3, either the effluent may have some toxic components or acclimated microorganisms may be required in its stabilisation. BOD/COD ratio in this study was calculated to be 0.8 for both DGP and OCE, this suggests that the substrates are readily to be treated biologically. It is also vital to note that the current high BOD/COD ratio of OCE and DGP is more agreeable to be treated anaerobically because the suggested

range for anaerobic digestion is >0.7 and 0.8 as recommended by Metcalf & Eddy (2014). Presently the industry treating OCE aerobically should be rectified in the future to have a more effective treatment.

TSS is the amount of total solids insoluble in wastewater and retained by a filter. DGP was observed to be six times higher TSS than that in OCE. This shows the low settling capability of DGP and longer retention time is required for digestion as it contained of more insoluble substance. Pre-treatment of effluent may also be needed to allow suspended solids to settle before adding it as feed into anaerobic digestion system.

4.1.2.2 TOC, TN, TKN and TP

Organic carbon in effluent denotes to many diverse carbon-containing organic compounds in several oxidation stages. Therefore, TOC means the amount of carbon bound in an organic compound in an effluent. Contrary to BOD and COD, TOC is independent of the oxidation state of the organic compounds. It does not include other organic elements such as hydrogen or nitrogen, or inorganic elements that might contribute to BOD or COD. Results revealed that TOC content in DGP is higher than OCE by 32 times. The high TOC in DGP reflects on the abundant amount of organic carbon available for microorganisms' consumption to produce biogas compared to that in OCE. Hence, DGP is suggested to be able to provide carbon source to compensate the lower carbon content in OCE; making them as suitable co-substrates in ACD.

TN is the sum of all the various forms of nitrogen (N) being oxidised into nitrate while TKN is the sum of organic N and ammonia N. Concentration of TN in DGP was about 300 mg/L higher than OCE. High N content in GP implies the greater accessibility of nutrient content to the microorganisms' growth. This also suggests

the high treatability of DGP by biological means and could counter-balance the lower nutrient content of OCE if co-digestion takes place. Likewise, TKN content in DGP was similarly high, approximately 200 mg/L higher than OCE. This shows the high amount of organic material such as proteins and urea present in DGP will also contribute to the growth of microorganisms.

In order to evaluate the sufficiency of nutrient content of samples, relationship between the amount of carbon (C) and N present in organic materials can be represented by C/N ratio. C/N ratios of 20-30 is considered optimum in anaerobic digesters (Monnet 2003). The calculated C/N ratios in this study are 135 and 24 in DGP and OCE respectively. The high ratio in DGP signifies nutrient limitation (N composition is limited) in providing essential element for the synthesis of amino acids, nucleic acids and proteins which will be converted to ammonia. Ammonia often help in the neutralisation of volatile acids to achieve neutral pH conditions for essential cell growth. Thus, N deficiency could affect the growth rate of anaerobes and results in lower biogas production. On the other hand, C/N ratio in OCE fall within the optimum. As suggested by Monnet (2003), an ideal C/N ratio of the feedstock materials can be achieved by mixing effluent of low and high C/N ratios, in this case OCE and DGP.

Phosphorus (P) naturally present in effluent and it is fundamental to the growth of algae and other biological organisms. Insufficient P content in effluent could cause limiting nutrient for the growth of microorganisms in a body of effluent. TP concentration in OCE was noticed to be significantly lower compared to that in DGP. This could be due to a lower percentage of phospholipids present in the overall oil and grease (O&G) content of OCE (Poh et al. 2010). The optimum P or nutrient content can be determined by the ratio of COD: N: P. This ratio should be approximately 250: 5: 1 for effluent to be treated anaerobically (Metcalf & Eddy 2014). The calculated ratio for DGP is 250: 0.4: 4.1 while OCE is 250: 2.3: 5.5.

These ratios suggest that N is still lacking while P is more than sufficient for anaerobic treatment for both substrates. Although the N ratios obtained were lower than the recommended value, the inadequacy of N can be resolved by adding N source such as manure, crop residues or chemical such as urea (Seghezzo et al. 1998; Lehtomäki et al. 2007; Yossan et al. 2012).

4.1.2.3 TA and VFA

VFA are classified as water-soluble fatty acids that can be distilled at atmosphere pressure and have low boiling point. VFA are degraded during acidogenesis phase in anaerobic digestion into hydrogen and acetic acid which act as substrate by methane-forming bacteria. VFA content in DGP measured at 7,728 mg/L of acetic acid was testified higher than that in OCE by 12 times. This denotes that more VFA will be degraded during digestion and available for methanogens consumption leading to higher biogas production. However, VFA is recommended to be <2000 mg/L for effective anaerobic treatment and concentration above that can trigger toxicity and constrain the activity of bacteria in digestion system which leads to system failure (Shivayogimath & Ramanujam 1999; Chan et al. 2012). Nonetheless, the significantly high VFA detected in DGP can be managed by reducing the OLR for digestion.

Alkalinity is determined by titrating against a standard acid, the results are expressed in terms of calcium carbonate, mg/L as CaCO₃. Alkalinity in effluent is often caused by the presence of carbonates, bicarbonates and hydroxides. For instance, magnesium, potassium, calcium and ammonia are the most common elements. The alkalinity in effluent aids to resist changes in pH caused by acids addition. It is important to make sure the concentration of alkalinity in effluent to be optimum (>2000 mg/L) for chemical and biological treatment processes (Chan et al.

2012). This study displayed a high TA concentration for DGP compared to that in OCE. Fortunately, the high alkalinity of DGP could be counter-balanced by co-digesting with OCE.

The ratio of VFA/TA can be used to evaluate the stability of ACD and the process is considered to operate desirably when the ratio is $<0.3-0.4$ (Fannin 1987). The ratio in DGP is estimated suitable for ACD at 0.4 whereas OCE has a higher ratio at 9.1. The high VFA content in OCE implies that if placed under anaerobic digestion, the amount is more than it can be consumed by acetogenic and methanogenic bacteria. Fortunately, the high TA concentration in GP makes a good match to counter-balance the downside of OCE. Hence under ACD of DGP with OCE, some amount of alkalinity will be used to neutralise the high VFA concentration for maintaining the satisfactory pH level.

In this study, pH was observed to be very alkaline in DGP and acidic in OCE. Moreover, Buyukkamaci & Filibeli (2004) reported that the source of effluent with high COD concentration is the cause of high VFA concentration. As evidence, high concentration of COD observed in GP also corresponding to the high concentration of VFA. This is supported by Chan et al. (2012) who testified the COD and VFA concentrations in POME during digestion. This result also complements with the outcome achieved by Zhang et al. (2008) and Borja & Banks (1994) which disclosed that about two third of the anaerobically digested COD was caused by VFA. Therefore, this verifies the optimistic correlation present between COD and VFA concentrations.

4.1.2.4 TG and O&G

TG represents traces of glycerin that is commonly present in DGP. This is because during the production process of refined glycerin via distillation, some of the

glycerin traces will also be transferred into the waste portion. TG could be recovered in further purification process to increase revenue, however the low TG averaging 16.94 mg/L detected is considered not necessary. Although there is no guideline of the anaerobic safe limit for TG up to date, TG is viewed as favourable to ACD as it acts as a good carbon source. In contrast, O&G has slow natural degradation and insolubility in water, often undesirable in ACD. High O&G at 27,942 mg/L may lead to incomplete or low conversion of oil or fat into the desired mono-alkyl esters.

O&G was found to be present in DGP in a high amount due to the effluent itself as a by-product of oil splitting process (Hidawati & Sakinah 2011). This points out that the digestion of DGP could be challenging as high O&G concentration tends to accumulate on the sludge surface and cause scum formation (Tchobanoglous et al. 2003). This will eventually cause the generation of foul odours and reduce the rate of microbial degradation resulting in lower digestion efficiency. However, referring to Chan et al. (2012), anaerobic microorganism are robust as they are highly acclimatised to intense condition such as high O&G presents in POME during digestion. They are able to biodegrade the O&G up to 99.7% in their study through the process of hydrolysis (Lee 2006). Further step that can be taken also includes physical removal and increase surface area for contact between substrate and microbes.

4.1.2.5 SO_4^{2-} and S^{2-}

SO_4^{2-} is an usual constituent of many industrial effluent (O'Flaherty et al. 1998). SO_4^{2-} is reduced to S^{2-} by sulfate reducing bacteria (SRB) in anaerobic reactors (Koster et al. 1986; Hilton & Oleszkiewicz 1988). SO_4^{2-} and S^{2-} concentrations are also an indication of odour-forming potential and the treatability of an effluent. SRB consumes SO_4^{2-} as one of the main substrates to produce hydrogen sulfide (H_2S) which gives the foul odours in the effluent. Both SO_4^{2-} and

S^{2-} concentrations in DGP were observed to be substantially high at 1,645 mg/L and 15,424 mg/L respectively which have surpassed the anaerobic safe limit of <750 mg/L for SO_4^{2-} and <100 mg/L for S^{2-} (Celis-García et al. 2004). Although SO_4^{2-} concentration in OCE (60 mg/L) is well below the suggested threshold, S^{2-} concentration of 506 mg/L has exceeded the advised concentration. These suggest the risk of increase growth of SRB resulting in competition and reduce methane producing bacteria (MPB) in return. This is certainly not desirable for biogas generation. Alternatively, S^{2-} concentration can be reduced by introducing S^{2-} removal step such as physio-chemical techniques (stripping), chemical reactions (coagulation, oxidation, precipitation) or biological conversions (partial oxidation to elemental sulphur) (Oude Elferink et al. 1994; Song et al. 2001).

4.2 Feasibility study

Feasibility study was conducted in order to evaluate the potential methane production rate (F_{CH_4}) and maximum energy potential (F_{kWh}) for individual DGP and OCE. This study could also serve as an indication on energy consumption expenditure via ACD over conventional mono-digestion. With this measure, the suitability and potential of ACD of DGP with OCE can be accessed. Table 4.3 presented the F_{CH_4} and F_{kWh} estimated based flow rates recorded in Table 4.1. and assumptions that COD removal efficiency is 85%, CH_4 obtained per COD converted under anaerobic condition is 0.24 L CH_4 /g COD and gas engine efficiency is 40% (Metcalf & Eddy 2014).

Table 4.3: The potential methane production rate and energy content in DGP and OCE

Effluent	F_{CH_4} (m ³ /d)		F_{kWh} (kWh)	
	Average	Range	Average	Range
DGP	1.269	0.324 – 3.931	4.48	1.14 – 13.88
OCE	0.775	0.147 – 4.303	2.74	0.52 – 15.19

Results revealed that DGP is expected to produce higher F_{CH_4} and F_{kWh} as compared to that in OCE. DGP and OCE could generate a maximum of 4.48 and 2.74 kWh respectively. Since the potential F_{kWh} achieved is relatively low in both DGP and OCE, the F_{CH_4} is recommended to be channelled to the existing boilers or within factory use.

4.2.1 The suitability of DGP and OCE for ACD treatment

This study has pointed out several critical drawbacks to be taken into consideration if DGP co-digests with OCE. Firstly, the high O&G content in DGP might affect the ACD performance by constraining granular sludge formation, promote scum formation, clog pipe lines and make the digestion difficult (Lettinga & Hulshoff Pol 1991; Gerardi 2003). However, three suggested methods can be applied to solve the high O&G content: 1) Physical removal of O&G content before treatment as currently being done in most effluent treatment plant, 2) increase the surface area for contact between effluent and anaerobic digester sludge and 3) the use of commercial effluent treatment bacteria such as Novozymes Bi-Chem SM700 or enzyme lipase to seed bioreactor system (Poh et al. 2010). The bacteria are customised to deal with high O&G loads in order to assist in the digestion of O&G as well as to allow the bioreactor to handle sudden shock loads.

The high SO_4^{2-} and S^{2-} concentrations found in DGP may cause toxicity in ACD (Gerardi 2003). Although SRB will reduce SO_4^{2-} in the feed to produce H_2S in anaerobic system, which in turn inhibits the metabolic activity of anaerobic bacteria, there are several approaches of handling high SO_4^{2-} and S^{2-} loads in anaerobic systems as proposed by Gerardi (2003). This includes dilution, separation and treatment of SO_4^{2-} and S^{2-} effluent stream, precipitation of the S^{2-} by adding heavy metals such as iron as well as scrubbing and recirculating digester biogas.

Furthermore, the high TSS appeared in DGP might have result in a slight challenge for ACD treatment. This means a longer HRT is needed in order to effectively eliminate and decompose solid particulate matter in the effluent. The high TSS could also cause digester upsets by excess scum and foam formation resulting in washout of bacteria from a biological treatment system (Lettinga & Hulshoff Pol 1991). According to Zinatizadeh et al. (2007), this situation can be fixed by pre-treatment of effluent allowing suspended solids to settle before adding it as feed into anaerobic digestion system. Fortunately, pre-treatment may not be needed when the installation of integrated sludge clarifier/liquid flow management is made available in bioreactor (Borja et al. 1996; Zinatizadeh et al. 2006).

Despite the possible downsides caused by the characteristics of DGP and OCE, there are two major advantages of ACD include balancing nutrient and alkalinity. From the results obtained, the high TOC content in DGP signifies abundance carbon-containing organic compounds available for the bacterial fixation and subsequent biogas generation. Hence, DGP could serve as a carbon provider in order to counterweigh the lower TOC content in OCE. In addition, the large quantity of biochemically degradable matter present in DGP indicated by the high BOD and COD which in turn convert into a high organic content. It facilitates ACD process as the high organic matter would provide a rich food source and promote the growth of microorganism accountable for degradation roles. Although BOD/COD ratios of DGP

at 0.8 is considered optimum ratio (>0.5) for high treatability by biological means, it is crucial to adjust to a suitable ACD mixing volume of DGP as suggested earlier to avoid shock loading. On a side note, although concentration of nitrogenous content (TN and TKN) in DGP is observed to be insufficient for an ideal ACD environment, this can be easily rectified by adding N source.

Apart from that, the exceeded VFA concentration in DGP and OCE could interrupt TA concentration in system. As suggested by Shivayogimath & Ramanujam (1999) and Chan et al. (2012), extensive care need to be taken to ensure VFA <500 mg/L for effective ACD treatment. This can be compromised by adding more alkaline or reducing OLR in digestion system. Providently, the high TA in DGP with concentration of $>2,000$ mg/L is another advantage in ACD as a result of its contribution in effective anaerobic treatment (Metcalf & Eddy 2014). Due to the high alkalinity consumption of OCE if it were to anaerobically digest alone, DGP makes a good candidate as alkalinity supplier and subsequently cost saving on chemical needed to equalise the balance of pH in the system.

To sum up, the WWCS data shows DGP and OCE are highly suitable for ACD treatment. Although some measures of pre-treatment were proposed in order to optimise the digestion condition in DGP, these measures can be avoided using ACD by co-digesting DGP with OCE as a counter-balance substrate. Although results from the feasibility study shows that DGP could not contribute to high energy pay back, it is not the main concern in this study. Instead, the compatibility of the two substrates were promising in solving the disposal issue of DGP besides minimising expenditure on chemical usage making ACD cost effective. Following energy potential, BPT to measure the potential biogas production in order to prove both substrates can be anaerobically digested will be discussed in the following section.

4.2.2 BPT

BPT was carried out to confirm the feasibility of DGP digestion and biogas collection before proceed to commence the laboratory scale treatment using designated reactors with digestion ratios. Performance in terms of cumulative biogas production and COD removal of each single substrate (POME, DGP and OCE) are evaluated and presented in Table 4.4. POME is used as the control under anaerobic treatment (Chan et al. 2012) and chosen as an indicator of comparison. It was carried out under mesophilic condition in batch mode with HRT of 30 days. Based on Table 4.4, POME has the highest cumulative biogas production rate as compared to DGP and OCE by 45% and 67% respectively. In contrast, DGP portrayed highest COD removal than POME and OCE by 33% and 93% respectively. Even though COD removal in DGP does not correspond to the biogas production obtained, it implies the high substrate potential in ACD but not in biogas generation. Furthermore, BPT has verified the findings summarised in Section 4.2 in regards to the low F_{CH_4} achieved in DGP and OCE.

The results also confirmed that there is no inhibition occurrence in the biogas production and the potential of both substrates (DGP and OCE) to be digested together in a reactor. While this study concluded the high degradability of DGP in ACD, it is important to emphasise that BPT analysis indicates the potential of substrates under perfect laboratory conditions. The analysis does not indicate how the substrate will behave in actual digester and uncertainty whether ACD mixture will be toxic in higher doses than the small amount used in BPT. For this, ACD performance evaluation conducted under different OLR ratios followed by different reactor configurations in laboratory scale were discussed in the subsequent sections.

Table 4.4: Biogas potential test of POME, DGP and OCE

Parameter	Unit	POME	DGP	OCE
COD removal	%	47	70	5
Cumulative biogas production	L	0.760	0.418	0.261

Although feasibility study including BPT has illustrated a poor potential of biogas production of substrates that is not commercially profitable to be converted into energy usage, it is not part of the focus in this study. In this case, COD removal is the core study in meeting the industry needs to first solve the GP treatment and disposal difficulties. Hence, a good potential of biogas production will only be an added advantage to the overall results but not essential.

4.3 Performance evaluation of ACD of GP with OCE at different OLR ratios

Various exploration have been done on ACD particularly on a close family of GP, crude glycerin which is the primary by-product of oleo chemical and biodiesel production but not on GP itself (Sulaiman et al. 2009; Fountoulakis et al. 2010; Yang et al. 2012; Astals et al. 2013; Athanasoulia et al. 2014). Reliable information pertaining to the research area is not abundant in the literature. Hence, it is essential to conduct a preliminary study for the ACD of GP with OCE before a full system being established. In recent years, ASBR has been utilised as a competent technology for industrial and municipal wastewater treatment. This is due to the reason that it is single tank configuration and high efficiency in BOD and TSS removals (89-98% and 85-95% respectively) (Mahvi 2008). It is minimal design and yet produces high quality effluent making it cost saving and manageable in the operation. Besides, CSTR also been widely investigated as a suitable type of reactor for ACD due to its benefits including homogenous mixing of substrate and continuous operation with

an easy access to control pH, mixing speed, oxygen concentration and temperature online (Frostell 1983; Cervantes et al. 2006). Therefore, ASBR and CSTR were used to verify degradability level in order to attain the OLR_{GP} that is well performed. Both reactors were expected to show COD removal that is able to meet the industry target at >50%. Factors affecting the performances of organic removal efficiencies, reactor stability, nutrient balance and biogas production rate were studied.

The reactors were operated in a series of five different ratios on OLR basis (4: 0, 3: 1, 2: 2, 1: 3 and 0: 4 of OCE: GP). The OLR ratios combination remained under 4 g COD/L.day. The fixed OLR at 4 g COD/L.day was chosen in designing the ratios as it is corresponding to the normal loading rate applied to the anaerobic inoculums collected from palm oil mill (Chan et al. 2012). All ratios were run in parallel in its respective ASBR whereas in CSTR, all ratios run after another in the same reactor. The maximum applicable OLR of GP (OLR_{GP}) is defined at the stage prior to the reactor failure. Under the failure stage, the ASBR will fail to self-sustain to the ideal reactor condition (pH <6.0), lose stability (VFA/TA >0.4) and experience low organic removal efficiency (COD <50%). A compilation of specific operating and feed conditions applied to ASBR and CSTR along with operating mode of ASBR are presented in Table 4.5 and Table 4.6 respectively.

Table 4.5: Specific operating and feed conditions of ASBR and CSTR

Parameter	Unit	OLR _{GP}									
		0		1		2		3		4	
		Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
ASBR											
Influent BOD	mg/L	8422	7050 - 8670	10293	10030 - 11450	15613	15100 - 16730	29448	28177 - 30200	202482	198000 - 208710
Influent COD	mg/L	10620	9700 - 11620	14078	12340 - 14930	20479	20100 - 23450	38058	36800 - 38960	265153	260500 - 270900
Influent TSS	mg/L	248	50 - 334	254	155 - 500	352	209 - 420	430	334 - 478	1546	1344 - 1734
Influent TG	mg/L	0.00	0.00	0.51	0.44 – 0.55	0.67	0.60 – 0.73	1.88	0.24 – 3.15	16.46	15.00 – 17.37
Influent O&G	mg/L	4288	3882 - 4500	4414	3890 - 4670	4982	4870 - 5230	6655	6505 - 6810	27790	25600 - 28450
Influent SO ₄ ²⁻	mg/L	64	55 -71	95	85 - 120	130	114 - 198	234	220 - 256	1644	1560 - 1708
Influent S ²⁻	mg/L	533	410 - 560	723	550 - 770	1121	1011 - 1237	2146	2080 - 2256	15376	14550 - 15677
Influent TA	mg/L	78	68 - 101	350	303 - 398	848	811 - 901	2176	2080 - 2260	19492	19220 - 20300
Influent VFA	mg/L	644	613 - 670	727	710 - 777	923	909 - 1345	1451	1344 - 1530	7746	6890 - 8000
pH	-	7.19	4.32 – 7.54	7.20	4.43 – 7.69	7.13	5.96 – 7.69	7.04	5.62 – 7.66	7.11	6.45 – 10.65
MLVSS	g/L	18.82	15.50 – 19.90	19.28	8.40 – 25.00	25.10	18.50 – 25.90	22.30	18.70 – 27.60	21.43	19.80 – 25.60
F/M ratio	g BOD/g MLVSS.d	0.13	0.11 – 0.15	0.16	0.12 – 0.39	0.09	0.09 – 0.13	0.11	0.08 – 0.13	0.11	0.09 – 0.12
CSTR											
Influent BOD	mg/L	8390	6780 - 8830	10312	9770 - 10910	15613	14300 - 18520	29448	28700 - 31800	202597	200750 - 210700
Influent COD	mg/L	10630	10030 -11800	13540	12050 - 14010	20479	19100 - 21600	35058	33400 - 38970	265357	254500 - 274500
Influent TSS	mg/L	298	218 - 380	283	211 - 360	352	209 - 488	430	334 - 511	1528	980 - 1760
Influent TG	mg/L	0.00	0.00	0.49	0.39 – 0.55	0.66	0.59 – 0.78	2.51	2.05 – 4.44	17.32	14.40 – 18.10
Influent O&G	mg/L	3963	3765 - 4270	4342	4310 - 4720	4982	4870 - 5420	6655	6620 -7550	27972	27050 - 28900

Influent SO₄²⁻	mg/L	62	53 - 70	83	75 - 109	130	120 - 198	234	211 - 256	1635	1600 - 1760
Influent S²⁻	mg/L	527	504 - 567	689	620 - 704	1121	1045 - 1170	2146	2070 - 2210	15375	14890 - 15800
Influent TA	mg/L	79	73 - 101	323	316 - 394	848	811 - 901	2176	2090 - 2278	19390	18450 - 20544
Influent VFA	mg/L	634	611 - 678	759	723 - 871	923	909 - 1090	1451	1400 - 1760	7688	7080 - 8100
pH	-	6.78	4.50 - 7.50	6.74	5.02 - 7.12	7.07	6.07 - 7.89	7.37	5.06 - 7.80	8.57	6.50 - 11.80
MLVSS	g/L	19.33	8.90 - 24.00	20.62	9.80 - 23.40	21.50	20.00 - 25.00	21.97	20.60 - 26.50	22.15	17.20 - 26.30
F/M ratio	g BOD/g MLVSS.d	0.12	0.08 - 0.25	0.10	0.09 - 0.22	0.10	0.09 - 0.11	0.10	0.09 - 0.11	0.10	0.08 - 0.17

Table 4.6: Operating mode of ASBR

Parameter	Value
Digestion mode	Semi-batch
Cycle length	24 hrs
Filling period	1 min
Anaerobic period	22 hrs
Settling period	2 hrs
Withdrawing period	5 min

4.3.1 BOD, COD and TSS removal efficiencies

The BOD and COD performance profiles at various OLR_{GP} are shown in Figure 4.2. and Figure 4.3. In ASBR, the highest BOD and COD removal efficiencies of 83% and 92% respectively were achieved at OLR_{GP} 1 whereas in CSTR, highest removals of 65% for both BOD and COD were obtained at OLR_{GP} 4. The current removal efficiencies are considered satisfactory as the ideal COD removal efficiency is targeted to be at least 50% and above. This will greatly reduce the amount of COD load into the subsequent treatment of chemical physical followed by aeration.

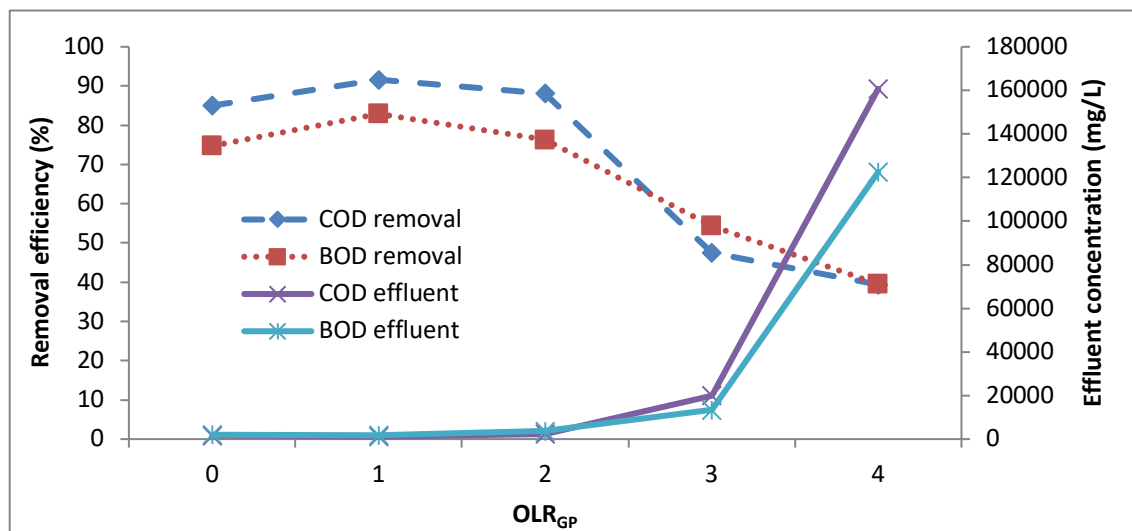


Figure 4.2: BOD and COD removal efficiencies at different OLR_{GP} in ASBR

Note: Total $OLR = 4 \text{ g COD/L.day}$ (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

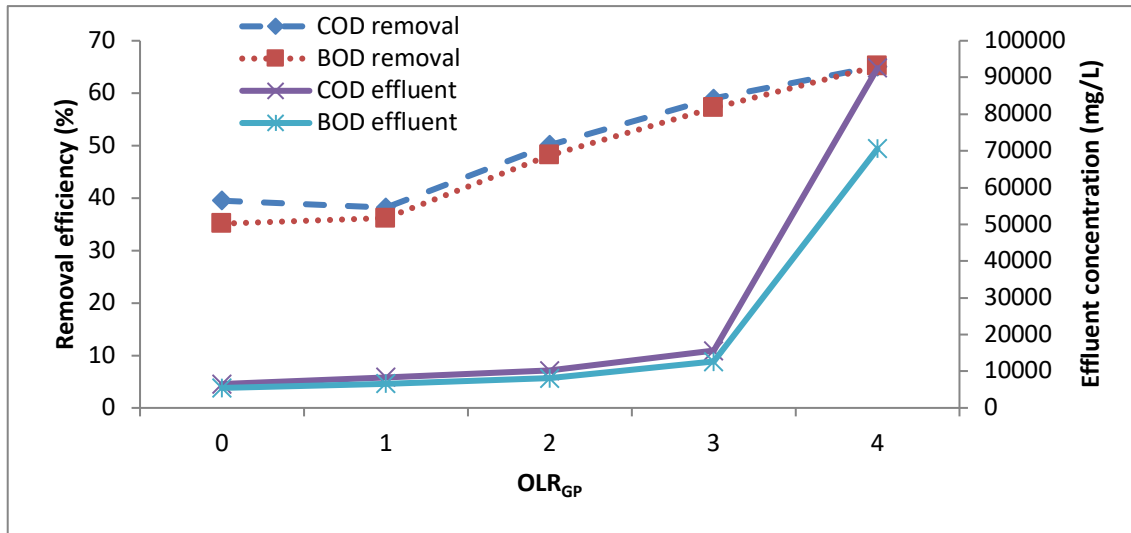


Figure 4.3: BOD and COD removal efficiencies at different OLR_{GP} in CSTR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis –
4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

In ASBR, COD removals were decreased with the increased of OLR_{GP}, this indicates the addition of GP stresses the ACD and decreased the digestion efficiencies. The lower efficiencies also imply that the anaerobes were not acclimatised appropriately to the substrates. In contrast, CSTR showed an increasing COD removal trend from OLR_{GP} 1 up to OLR_{GP} 4 (38% up to 65%). This indicates that CSTR could tolerate the addition of GP into the system and could break down GP. The excellent performance is relatable to the active portion of inoculum, MLVSS: MLSS which was maintained at ≥ 0.80 for an optimum condition in the reactor. The increased of GP concentration does not adversely affect MLVSS but providing more organic substance for the growth of anaerobes. This is also supported by Leslie Grady et al. (1999) who suggested that the specific growth rate of the microorganisms increase when substrate concentration increases. The increasing trend in CSTR from 38% to 65% during the increment ratio from OLR_{GP} 1 to OLR_{GP} 4 also corresponds to the previous studies reported for increasing loading of crude glycerin tested using CSTR (Fountoulakis & Manios 2009; Fountoulakis et al. 2010; Martín et al. 2013; Rivero et al. 2014). Besides, BOD removal at OLR_{GP} 1 to OLR_{GP} 4 followed a similar pattern to that of COD removal which subsequently confirmed the results obtained

for COD removal.

Although OLR_{GP} 1 in ASBR appeared to have superior performance as compared to the rest, it may not be an ideal ratio considering the mixture of higher volume of OCE. The high dilution of GP with OCE will contribute to the cost of pumping, energy and up-size the reactor making it less economical. Also, this may not be in lined with the current research objective that is to develop a cost effective ACD approach. Likewise, digesting the highest OLR_{GP} 4 in ASBR may not be the best approach as current results showed a poor performance not meeting the desire removal efficiency. Although OLR_{GP} 4 tends to stay longer in the reactor, it displayed the lowest BOD removal of 39% and COD of 39%. As described earlier, the removal efficiency is affected by MLVSS and referring to that in Figure 4.4, it is detected slightly declined from 25,100 mg/L (OLR_{GP} 2) to 22,300 mg/L (OLR_{GP} 3) and 21,433 mg/L (OLR_{GP} 4) despite maintaining within the optimum range mentioned. This indicates the substrates may not be in full contact with anaerobes as there is no mixing or recirculation in the system. As stated by Peter et al. (2001) and Gómez et al. (2006), hydraulic recirculation greatly enhanced the mass transfer between substrates and anaerobes. This corresponds to CSTR where the aid of agitation has greatly enhanced activity of anaerobes and the system is able to cope with the highest OLR of GP (OLR_{GP} 4).

On the other hand, the highest TSS removal was observed at OLR_{GP} 4 in CSTR from 1,528 mg/L reduced to 592 mg/ (61% removal efficiency). Whereas, no removal of TSS was detected at all OLR_{GP} in ASBR hence not illustrated in Figure 4.2. The TSS concentration of treated effluent in ASBR is ranging 567 mg/L to 2,571 mg/L. The turbidity in TSS treated effluent is contributed by excess biomass as portrayed in F/M ratio (Figure 4.4). Similarly, although same performance (no removal of TSS) was recorded across ratio OLR_{GP} 0 to OLR_{GP} 3 in CSTR and therefore not shown in Figure 4.3, the addition of TSS in treated effluent (ranging 484 mg/L

to 513 mg/L) is contributed by biomass concentration that attached or carried together with suspended solids of substrate in treated effluent. Nevertheless, the increased was remained <513 mg/L and not causing a severe problem in the system such as scum formation.

Unlike ASBR, CSTR showed a clear separation of inoculums and substrates when the system let to rest or during settling which reflects a healthy system without any floating substances such as O&G. Therefore, the observation confirmed that the immiscible phenomena were not present in CSTR. Clearly, this suggests the stirring plays a crucial role in digesting GP as this allows homogenous mixing and permits contact between microbes with substrate. This study reveals that CSTR may be a better choice for the immiscible substrates (GP with OCE) due to the effective agitation mixing in achieving the desired COD removal.

4.3.2 MLVSS concentration and F/M

The MLVSS and F/M values were monitored systematically in order to study the ongoing biochemical activity. Their averages at various OLR_{GP} are shown in Figure 4.4 and Figure 4.5. In ASBR, it was observed that MLVSS increased from initial OLR_{GP} 0 (18,817 mg/L) progressing to OLR_{GP} 1 and subsequently OLR_{GP} 2 at its maximum average of 25,100 mg/L. This suggests a healthy microbes' growth in the process despite the increase of ratio. However, as soon as it reaches ratio OLR_{GP} 3, MLVSS started to show reduction and such decline continue gradually until ratio OLR_{GP} 4 where MLVSS of 21,433 mg/L was detected at this point. MLVSS was affected at OLR_{GP} 3 and OLR_{GP} 4. This is also reflected in higher TSS concentration effluent produced, 70% increased from OLR_{GP} 2 to OLR_{GP} 3 and 180% increased from OLR_{GP} 3 to OLR_{GP} 4. Similarly, in CSTR, increasing trend was seen in MLVSS from OLR_{GP} 0 to OLR_{GP} 3 and later slight decreased from OLR_{GP} 3 to OLR_{GP} 4.

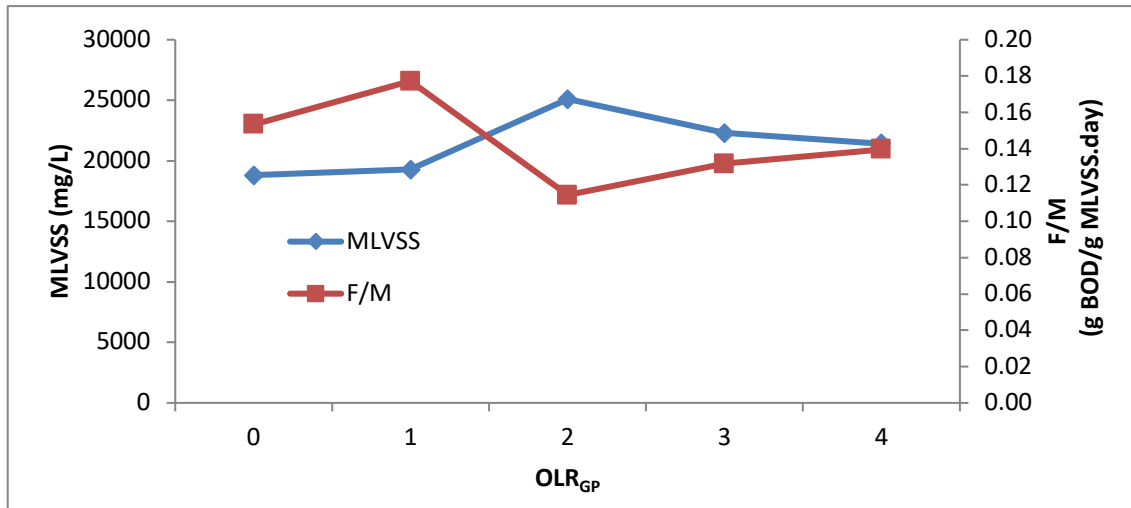


Figure 4.4: MLVSS concentration and F/M ratio at different OLR_{GP} under ASBR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis –
4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

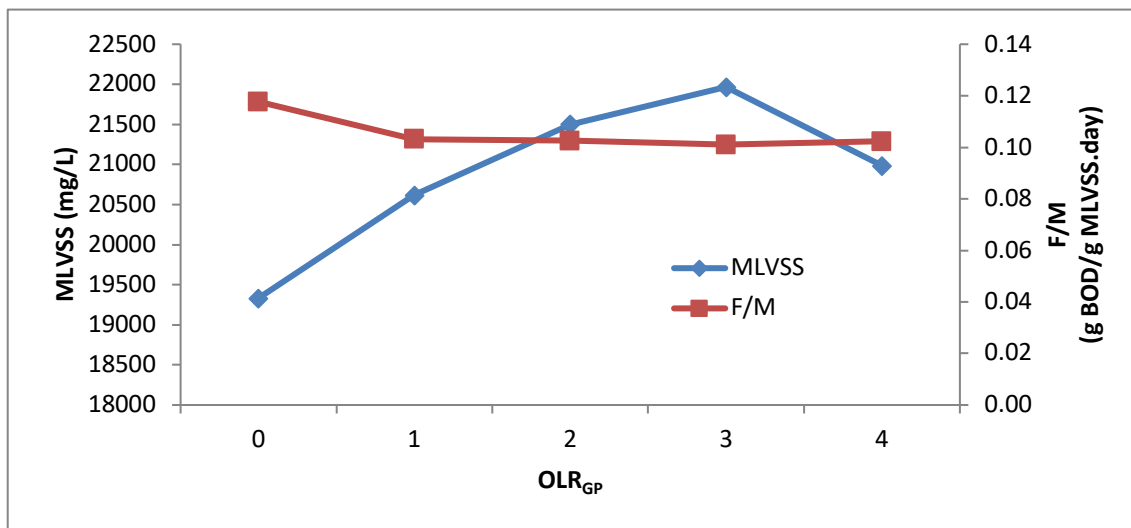


Figure 4.5: MLVSS concentration and F/M ratio at different OLR_{GP} under CSTR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis –
4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

Despite the decline in MLVSS where sludge washout was observed, F/M recorded at all OLR_{GP} ranging 0.11-0.16 g COD/g MLVSS.d (ASBR) and 0.10–0.12 g BOD/g MLVSS.day (CSTR) is lower than the typical value in conventional anaerobic system ranging 0.25-0.39 g COD/g MLVSS.d and complex mix anaerobic system ranging 0.20-0.60 g BOD/g MLVSS.day respectively (Tchobanoglous & Burton 1991; Chan et al. 2012). This denotes that anaerobes population was adequate with the

applied loading rate to biodegrade the organic matter effectively. In fact, present in excessive MLVSS has caused the turbidity in treated effluent. This is contributed by the regular top-up of anaerobes to ensure the system stability. The excess biomass concentration reflected by F/M ratio also concurs with the volatile fraction of MLVSS: MLSS observed at all ratios (≥ 0.80). As the value is what suggested by Metcalf & Eddy (2014) at 0.85 and Woodside & Kocurek (1997) at 0.80, it indicates sufficient biologically active portion of the solids in the mixed liquor. As MLVSS concentration detected gradually decreased due to sludge washed out and COD removal was well above the target, the excess MLVSS was not considered as an issue in this study.

4.3.3 pH, alkalinity and VFA concentration

Alkalinity was added to keep pH above 7.0 at all OLR_{GP} throughout the experimental stages. This is because the growth rate of methanogens is significantly affected at pH below 6.6 while disintegration of microbial granules occurred at pH with excessive alkalinity (Mosey & Fernandes, 1989; Sandberg & Ahring, 1992). In order to provide favourable condition for the functioning of anaerobes to give maximum performance, buffer was added and may contribute to chemical cost.

Figure 4.6 and Figure 4.7 displayed the pH, TA and VFA concentrations at different OLR_{GP} under ASBR and CSTR respectively. VFA has depicted concentration exceeded optimum < 500 mg/L (Shivayogimath & Ramanujam 1999) at OLR_{GP} 1 in ASBR. It continues to increase gradually until OLR_{GP} 3 where a steep increase of 49% was observed before it reaches highest peak at 7,040 mg/L at the end of OLR_{GP} 4. Same trend was observed in CSTR. The high VFA is contributed by substrate itself. Since the existing TA of substrate did not provide sufficient buffering capacity in neutralising VFA concentration in the reactor, sodium bicarbonate was added as additional alkalinity in ACD.

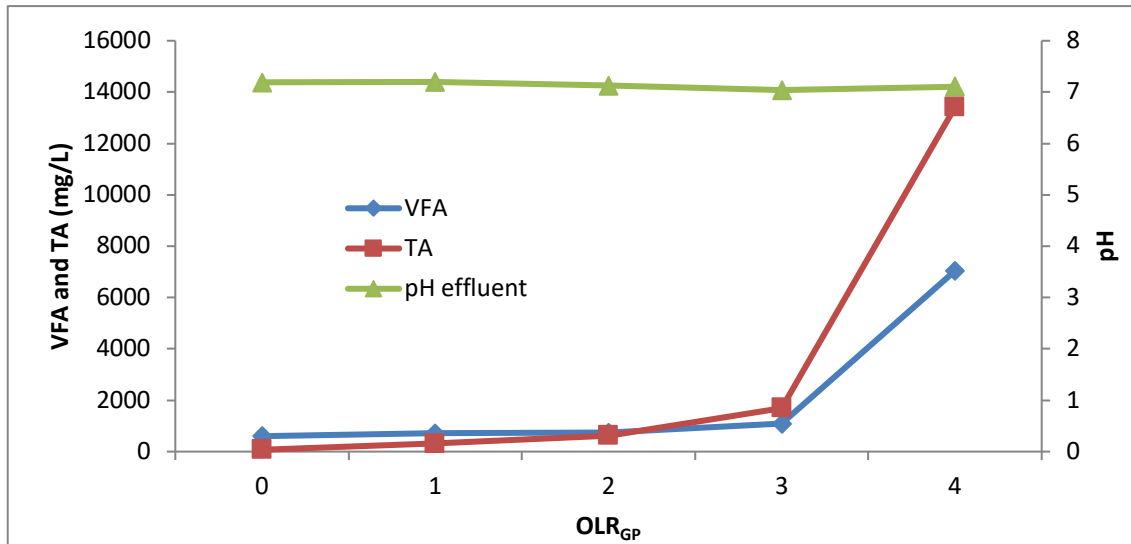


Figure 4.6: pH, alkalinity and VFA concentration at different OLR_{GP} under ASBR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

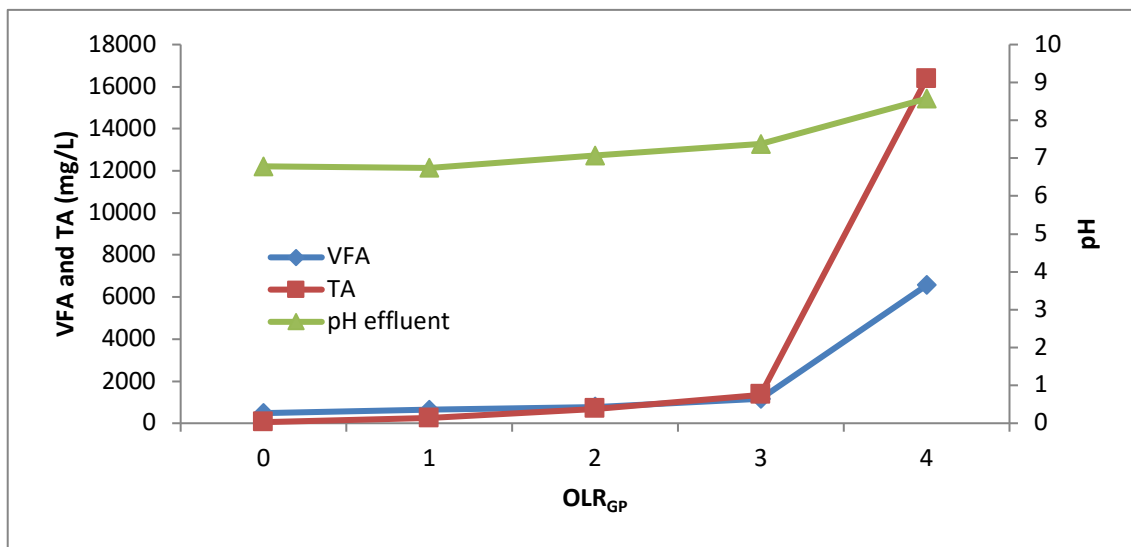


Figure 4.7: pH, alkalinity and VFA concentration at different OLR_{GP} under CSTR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

The effluent pH under ASBR remained stable ranging 7.04 to 7.20 at all OLR_{GP} and it is attributable to the alkalinity enhancement added. Likewise, CSTR exhibited similar pH range of 6.74 to 7.37 at OLR_{GP} 0 to OLR_{GP} 3, except for OLR_{GP} 4 which remained at pH averaging 8.57. Although pH 6.5-7.5 is regarded as ideal for ACD

treatment (Metcalf & Eddy 2014), buffer was only provided at the start and supplementation has been stopped at OLR_{GP} 4. This is because the digesting effluent in the reactor constantly retained its pH back to ≈ 8.0 regardless of the applied supplement. Hence, the pH was left unregulated to evaluate the performance with the self-adjusting anaerobes.

Direct measurement of pH is however not as reliable as buffer capacity as the accumulation of VFA will reduce buffering capacity rapidly before the pH decreases. Hence, the system stabilisation can be indicated by the ratio of VFA/TA and referring to that, the high VFA/TA observed at OLR_{GP} 0 to OLR_{GP} 3 in both reactors has exceeded recommended ratio by Fannin (1987) at $<0.3-0.4$ from digester failure. The high VFA concentration signifies methanogenic inhibition towards toxicity of substrates. At these OLR_{GP} , acid forming bacteria displace methanogens resulting in increasing VFA concentration. The ideal pH regulated by additional buffer into the reactors also did not contribute to the TA level. Lack of buffering capacity from substrate GP (TA $<2,000$ mg/L) at these OLR_{GP} regardless of the presence of the mixing aid is caused by the diffusion of VFA into the cell of anaerobes (Poh & Chong 2010; Chan et al. 2012). This means more buffers is needed in the treatment process to achieve VFA/TA stability and the process became dependent to chemical aids.

Nevertheless, the increase of GP along the ratios also results in a lower VFA/TA which is closer to the favourable operating condition. VFA/TA was noticed to be improving gradually from ratio OLR_{GP} 1 up to OLR_{GP} 4 at 8.47 to 0.40 (ASBR) and 9.28 to 0.40 (CSTR). At OLR_{GP} 4, VFA/TA at 0.40 is considered to achieve stability in both reactors.

4.3.4 TG and O&G removal efficiencies

TG also known as total glycerin content and it reflects the quality of glycerin itself. Generally, it is favourable in ACD as it represents carbon source. Whereas, O&G is not favourable in ACD as it reflects the organic compounds present which will cause fouling, deposits formation and clogging at nozzles and valves.

The TG and O&G performance profiles at different OLR_{GP} are illustrated in Figure 4.8 and Figure 4.9 respectively. The highest TG and O&G removals were achieved at OLR_{GP} 1 (42% and 60% respectively) in ASBR and OLR_{GP} 4 in CSTR (72% and 74% respectively). It can be evidently seen that the increased of OLR_{GP} substantially influenced the TG and O&G removal efficiencies. The trend of TG and O&G removals demonstrates similarity with BOD and COD removals. In ASBR, the results denote that higher GP loading rate discourages the activity of anaerobes to give an effective degradation as seen in the decline of TG removal in the following OLR_{GP} 2, OLR_{GP} 3 and OLR_{GP} 4 at 25%, 16% and 10% whereas O&G removal at 36%, 31% and 29% respectively. Unlike CSTR, anaerobes activity has improved along with the increased of GP loading rate. This implies that the agitation mixing could form a homogenous condition in CSTR and consequently increased the contact between anaerobes and substrates which promoted the degradation activity. This is also reflected in the increased of TG removal (19%, 32%, 69% and 72%) and O&G removal (62%, 65%, 70% and 74%) with increasing OLR_{GP} from OLR_{GP} 1 to OLR_{GP} 4 respectively. These removals have apparently improved as compared to those achieved in ASBR.

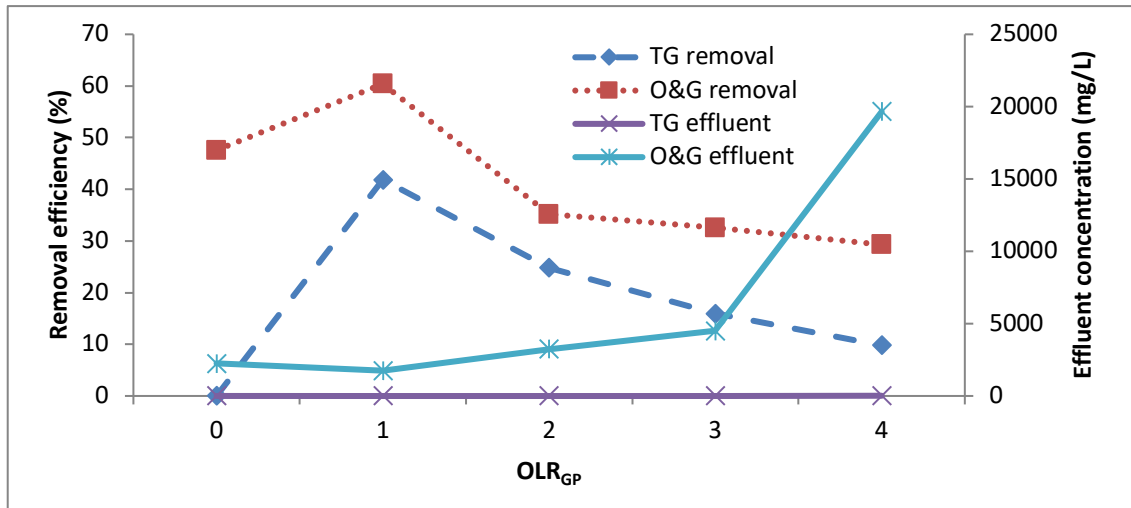


Figure 4.8: Effluent TG and O&G⁻ and its removal efficiencies at different OLR_{GP} in ASBR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

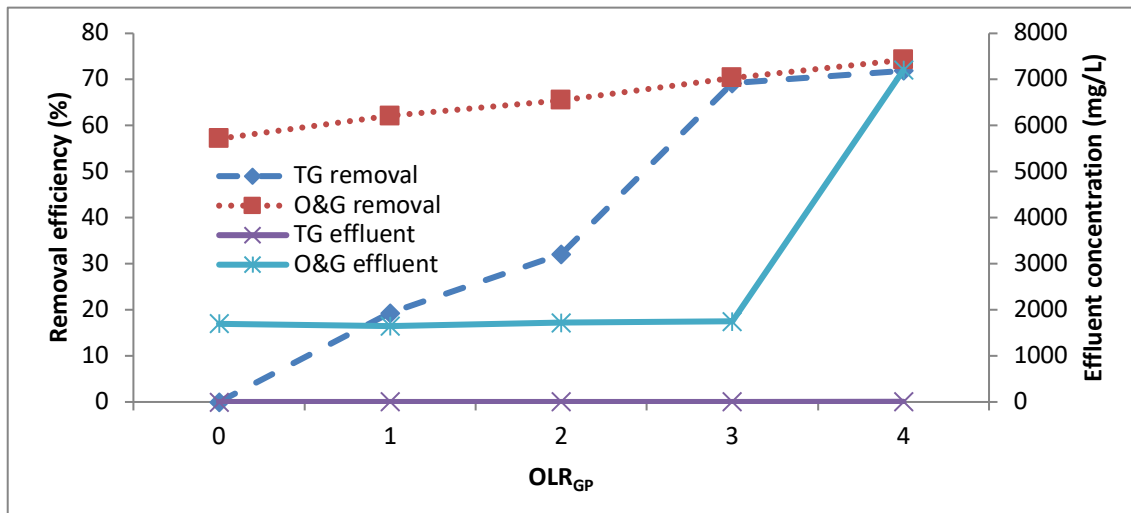


Figure 4.9: Effluent TG and O&G⁻ and its removal efficiencies at different OLR_{GP} in CSTR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

Despite the high removal efficiencies achieved in CSTR meeting the industry requirement as per COD removal, the effluent O&G concentration at all OLR_{GP} were >1,600 mg/L. This indicates that O&G was not completely degraded in CSTR. The trend of effluent concentration also corresponds to effluent TG observed. This is due

the presence of inhibitor, LCFAs in substrate which is the major contributor to O&G that is difficult to be digested. The accumulation of LCFAs could increase the lag phase of methanogenic activity and rigorously hinder ACD process by shock loading of LCFAs (Long et al 2012, Alves et al 2009). Primary treatment often employed in industry to reduce fat levels along with coarse suspended solids in untreated effluent which eventually ease the operation of ACD and produce a quality of discharge effluent (Husband 1992). Hence, it is not surprising the amount O&G present in effluent without undergo primary treatment has formed an intense condition in CSTR.

In addition, it is worth to mention that non-degraded O&G was not suspended or formed immiscible layers in the substrates which is attributable to the homogenous form in CSTR. It does not cause the common problems such as fouling, clogging at nozzles and valves, and scum formation except for sludge washed out. The sludge washed out in CSTR may be contributed by light layer formed by LCFAs to biomass which promotes sludge floatation (Rinzema et al. 1989). This has been agreed by Alves et al (2009) who reported two main problems associate with high O&G: (1) sludge floatation and biomass washout caused by the adsorption of lipids/LCFAs on the biomass and (2) inhibition of acetogens and methanogens by LCFAs. This is also in-line with Long et al (2012) and other researchers who encountered the same issues but most of these studies relate to high rate digestion system, such as CSTR.

Overall, the experimental results validate that ASBR has reached its threshold degradation point of TG and O&G at OLR_{GP} 1. Although HRT increased along with the increasing ratios providing sufficient degradation time, the microbial activity could not cope with the intense condition in ASBR. As ASBR system only allows substrate to pass through the sludge bed once without any aid of recirculation, CSTR with OLR_{GP} 4 is able to attain the highest removal of TG and O&G as shown in Figure 4.9.

4.3.5 SO_4^{2-} and S^{2-} removal efficiencies

The resulting effluent and removal efficiencies of SO_4^{2-} and S^{2-} at different OLR_{GP} are shown in Figure 4.10 and Figure 4.11. The highest removals of SO_4^{2-} and S^{2-} were observed at OLR_{GP} 1 in ASBR (75% and 86% respectively) and OLR_{GP} 4 in CSTR (66% and 68% respectively). The high removals of SO_4^{2-} are unfortunately regarded as undesirable performance in ACD as it indicates high conversion into S^{2-} by SRB. This may be dangerous in the system because when more SO_4^{2-} was reduced, not only the resulting S^{2-} is harmful to various microbes (Anderson et al. 1982; Oude Elferink et al. 1994; Colleran et al. 1995; Colleran et al. 1998), the active SRB would compete with MPB for common organic and inorganic substrates and disrupt methanogenesis (Oude Elferink et al. 1994).

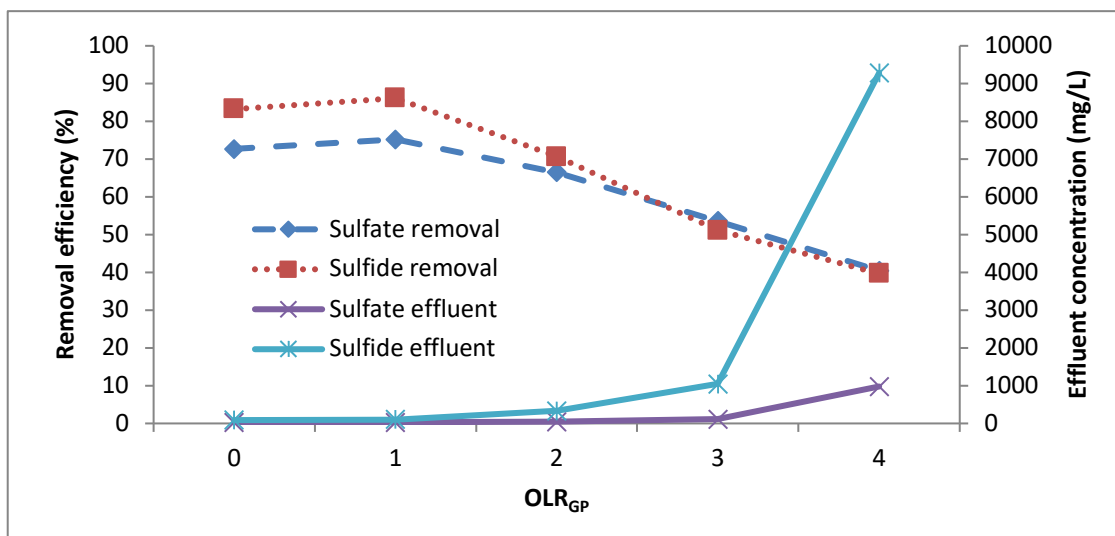


Figure 4.10: Effluent SO_4^{2-} and S^{2-} and its removal efficiencies at different OLR_{GP} in ASBR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

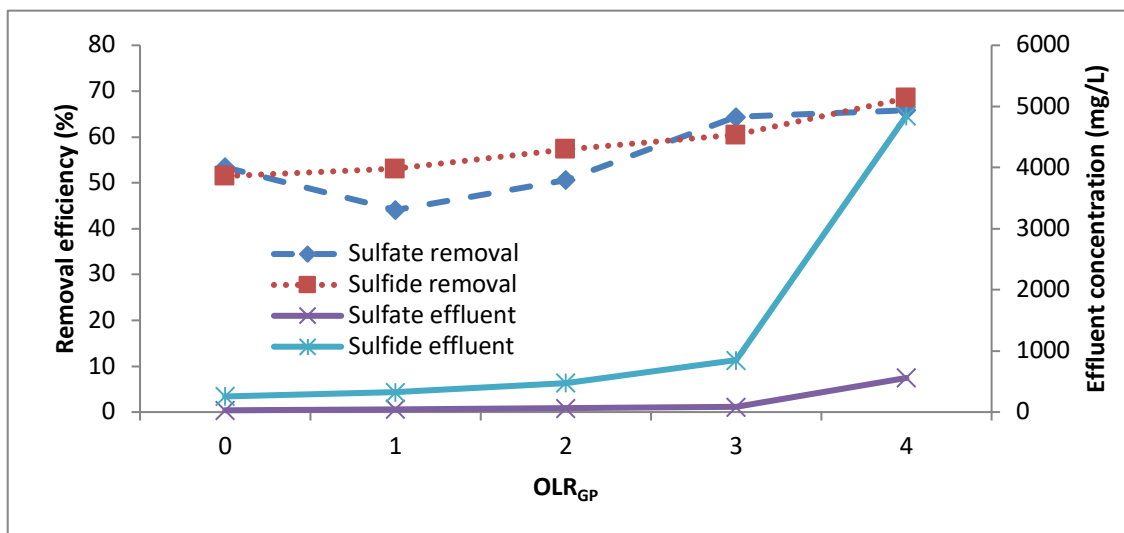


Figure 4.11: Effluent SO_4^{2-} and S^{2-} and its removal efficiencies at different OLR_{GP} in CSTR

Note: Total OLR = 4 g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

In CSTR, the increasing GP loading has resulted in an increased in SO_4^{2-} and S^{2-} removals. Increased substrate loading indicates higher organic compounds made available for SO_4^{2-} reducers for oxidation to obtain energy. Generally, SO_4^{2-} reducers have better growth kinetic properties than methanogens and tend to compete for the common substrates hydrogen, formate and acetate as well as involved in the degradation of substrates like propionate and butyrate. With the presence of SO_4^{2-} reducers, the substrates degradation favour the SO_4^{2-} reduction pathway more than methanogenesis (Oude Elferink Stefanie et al. 1994). This explains the interference of methanogenesis by the high SO_4^{2-} reduction which also resulted in high S^{2-} in this study.

In spite of the effluent SO_4^{2-} kept at <750 mg/L as advised (Celis-García et al. 2004), the high effluent S^{2-} averaging 100 mg/L and 4,848 mg/L at OLR_{GP} 1 (ASBR) and OLR_{GP} 4 (CSTR) has surpassed the recommended concentration of <100 mg/L (Celis-García et al. 2004). When the limit is equal / has been exceeded, MPB was constrained by SRB because the reduced amount of SO_4^{2-} has imposed toxicity

to MPB. This is caused by H_2S which is a toxic form of S^{2-} (Tursman & Cork 1988). It can diffuse into cell membrane and denature protein in cytoplasm through the formation of S^{2-} and disulfide linkage between polypeptide chains (Conn et al. 1987). The formation not only interferes with various coenzyme sulfide linkage, but disrupts the assimilatory metabolism of sulfur, which is a nutrient required for methanogens (Vogels et al. 1988; O'Flaherty et al. 1999). As reported by Parkin et al. (1990) and Celis-García et al. (2004), S^{2-} concentration of >100 mg/L is inhibitory and would be considered lethal to the reactor. The aftermath of the competition has also exhibited by the gradual decline and negligible biogas production rate in Figure 4.12 and Figure 4.13.

This investigation revealed that S^{2-} could be one of the key downsides especially in the generation of biogas. Although in term of COD removal, it was meeting the target but the high effluent S^{2-} is undoubtedly a challenge in the production of biogas. To improve the situation, pre-treatment to reduce S^{2-} prior to ACD could be the alternative in order to achieve better overall results overall.

4.3.6 Biogas production rate, biogas yield and methane gas composition

The profiles of biogas production rate, biogas yield and methane composition are exhibited in Figure 4.12 and 4.13. The highest biogas production of 1.493 L/day with calculated biogas yield of 0.116 L biogas/g COD removed.day was achieved at OLR_{GP} 1 (ASBR). Similarly, CSTR at OLR_{GP} 1 achieved maximum biogas production of 0.003 L/day, which corresponds to biogas yield of 0.001 L biogas/g COD removed.day. This signifies the maximum activity of methanogens accomplished at OLR_{GP} 1. Following OLR_{GP} 1, biogas production rate did not portray a well performance averaging 0.519 L/day and 0.118 L/day (ASBR) and 0.001 L/day and 0.002 L/day (CSTR) at OLR_{GP} 2 and OLR_{GP} 3 respectively. These indicate very

negligible conversion of biogas occurred. The poor performance was detected at OLR_{GP} 4 with no biogas collected in both reactors.

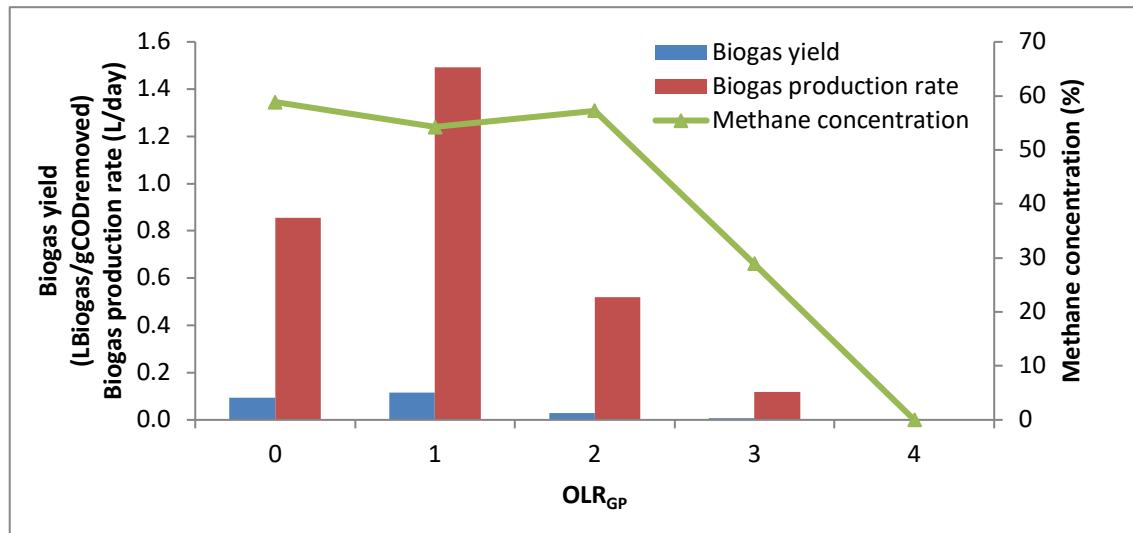


Figure 4.12: Biogas production rate, yield and methane gas composition at different OLR_{GP} in ASBR

Note: Total $OLR = 4$ g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

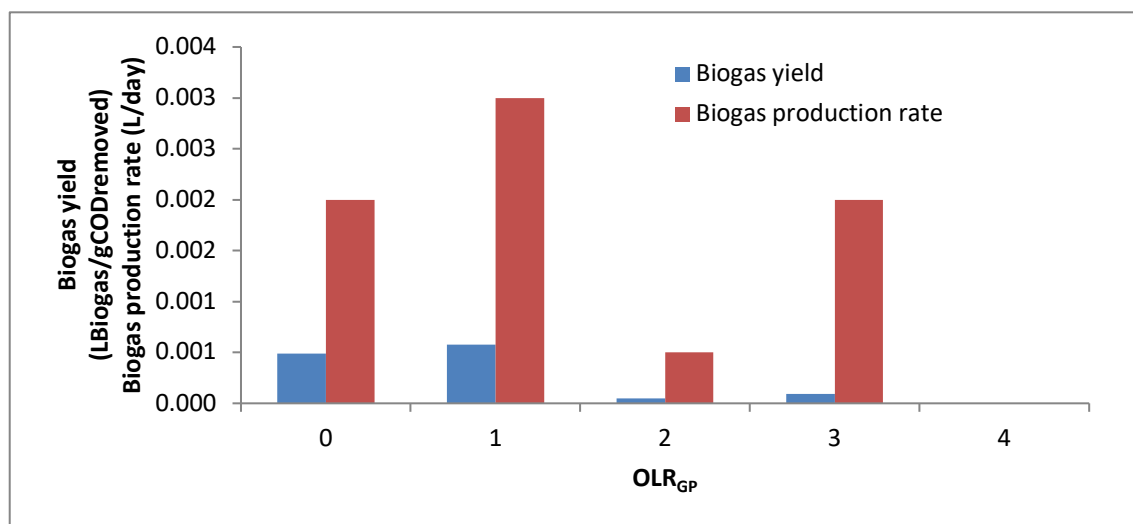


Figure 4.13: Biogas production rate and yield at different ratios in CSTR

Note: Total $OLR = 4$ g COD/L.day (OCE: GP ratio in OLR basis – 4: 0, 3: 1, 2: 2, 1: 3, 0: 4)

In ASBR, the highest methane concentration recorded was at ratio OLR_{GP} 0 (59%) followed by OLR_{GP} 1 (54%), OLR_{GP} 2 (57%) and ratio OLR_{GP} 3 (29%). The composition of methane at OLR_{GP} 0, OLR_{GP} 1 and OLR_{GP} 2 are considered satisfactory as the desire concentration often fall within 50-60% (Metcalf & Eddy 2014). Despite being observed that the highest methanogenic activity was at OLR_{GP} 1 (ASBR) as evidenced by the highest COD removal of 92% and satisfactory methane content of 54%, the biogas production rate (1.493 L/day) was at its maximum level and is not comparable to the estimated biogas production of 5.024 L/day using Eq. 3.1. Similar to CSTR, it was noticeably very negligible and could not represent the true rate of biogas production in this study. Since biogas produced was too little to be measured for its composition, no methane composition is detected and biogas yield was presented in this study instead of methane yield.

In ASBR, the poor performance in biogas production rate and its yield may be caused by three influences: (1) Less contact between substrate and anaerobes as there is no recirculation. (2) Tension caused to methanogens by high effluent S^{2-} . When loading rate of GP was increased, unbalance process occurred as S^{2-} in effluent also increased from 329 mg/L to 9,279 mg/L (OLR_{GP} 2 to OLR_{GP} 4). The high production is toxic and the rate of acid formation exceeds the production rate of methane. The finding suggests that methanogenesis inhibition is in line with the studies quoted by Celis-García et al. (2004). The inhibition is influenced by the destructive form of S^{2-} that could denature existing proteins in the cell by membrane diffusion. (3) Inhibition of methanogens caused by high VFA and the decrease of pH. This is due to the high effluent VFA at 708 mg/L exceeded the optimum of >500 mg/L and is considered not an ideal condition for methanogens (Shivayogimath & Ramanujam 1999; Chan et al. 2012). Such condition could disrupt cell from its metabolism (Shivayogimath and Ramanujam, 1999; Ferreira 2013). It is supported

by Wang et al. (2009) who observed a significant methane reduction when VFA was detected high which rationalises the low biogas achieved.

On the other hand, biogas was failed to be generated in CSTR may be associated with two factors: (1) Inhibition of methanogens by high O&G. This is because the converted COD is not utilised quick enough in further degradation (acidogenesis and acetogenesis) and caused inhibitory onto methanogens. Generally, lipids of O&G are hydrolysed to glycerol and free LCFAs which will then be degraded anaerobically via the β -oxidation pathway to acetate and hydrogen (Cirne et al. 2007; Pittaway 2011). The degraded products will be metabolised by methanogens into biogas. The high accumulation of LCFAs has eventually disrupt the metabolism of methanogens that include food transportation across the membrane (Kabara & Vrable 1977; Zeikus 1977; Ferreira 2013). This inhibitory potential also has been reported by various researchers as described in Section 4.3.4. (2) Inhibition of methanogens by high S^{2-} , which also faced in ASBR.

In a nutshell, CSTR at OLR_{GP} 4 would be highly preferred for further investigation and is regarded as the maximum applicable loading rate of GP as compared to ASBR at OLR_{GP} 1. Although ACD in CSTR failed to generate biogas due to the main setbacks discussed above, it is not the main focus in this study. With agitation mixing in CSTR, it solves immiscibility difficulties which lead to the excellent ACD performance that is now meeting the criterion of industry and objective of this study. This was shown with the high BOD and COD removal efficiencies at 65% and 65% respectively. Same goes to O&G and TG removal efficiencies at 74% and 72% respectively. Nevertheless, it is also important to note that ASBR gives a bench mark of how ACD would behave. Whereas the problems encountered in ASBR could be improved and avoided in the future investigation by: (1) having recirculation in the reactor to improve contact between substrate and anaerobes, (2) the increase interaction could ultimately enhance the population MPB to compete SRB for the

reduction S^{2-} toxicity and (3) regulate alkaline level to counter balance the acid formation in ASBR.

4.3.7 Optimum OLR_{GP}

Table 4.7 presented the summary performance at the optimum different OLR_{GP} identified for ASBR and CSTR. Based on the performance evaluation, the optimum OLR_{GP} in both ASBR and CSTR achieved remarkable overall COD removal meeting the industry needs >50%. In order to justify the most suitable OLR_{GP} for the ACD of GP with OCE, four main criteria were identified: (1) the mixing mechanisms (2) the ability to handle OLR of GP (3) treatment efficiencies in terms of BOD, COD, TSS, TG and O&G removals (4) reactor stability in terms of pH, TA, VFA, SO_4^{2-} and S^{2-} .

Table 4.7: Overall ACD performance at different OLR_{GP}

Parameter	Unit	ASBR	CSTR
Optimum OLR_{GP}	-	OLR_{GP} 1	OLR_{GP} 4
BOD removal	%	83	65
COD removal	%	92	65
TSS removal	%	0	61
TG removal	%	42	72
O&G removal	%	60	74
Biogas production rate	L/day	1.493	0
Biogas yield	L biogas/g COD removed.day	0.116	0
Methane composition	%	54	0
Effluent BOD	mg/L	1,760	70,580
Effluent COD	mg/L	1,177	92,537
Effluent TSS	mg/L	597	592
Effluent TG	mg/L	0.26	4.88
Effluent O&G	mg/L	1,748	7,205
Effluent SO_4^{2-}	mg/L	23	559
Effluent S^{2-}	mg/L	100	4,848
Effluent pH	mg/L	7.20	8.57
Effluent TA	mg/L	312	16,378

Effluent VFA	mg/L	708	6,558
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The substrates in CSTR are observed to be well-mixed despite the density differences in GP and OCE. The fairly good performance at the highest OLR_{GP} 4 was credited to the excellent contact between biomass and substrates with the aid of agitation mixing. The agitation mixing in CSTR also reduces substrate inhibition such as concentration gradient and accumulation of intermediates (Mohan et al. 2007). Unlike CSTR, ASBR has no mixing mechanism in the reactor and hence does not homogenise the two substrates mixture at a higher OLR_{GP} . It is undeniably true that the low density of oil and grease in GP making the miscibility factor a challenge, this study however revealed that mixing in CSTR is a necessity in treating immiscible substrates like GP and OCE.

Besides, it is undoubtedly seen that the ACD in CSTR are more proficient in coping with higher OLR of GP (4 g COD/L.day) as compared to ASBR (1 g COD/L.day). This is because applying a higher OLR will result in a smaller reactor volume which will considerably reduce the capital cost of the reactor. Moreover, higher OLR of substrates may be applicable as CSTR permit the systems to be more tolerant to the high organic shock. On the other hand, opposite trend was observed in ASBR. Lower OLR of GP (ASBR) also indicates that higher OCE volume is needed to be dosed into the system which eventually not only increases the sizing of reactor but slow degradation of GP quantity. Hence, the OLR_{GP} 4 (CSTR) is superior than OLR_{GP} 1 (ASBR) in terms of higher loading rate of GP that can be employed in the ACD treatment of GP.

Referring to the organic removal efficiencies, despite that $OLR_{GP\ 1}$ (ASBR) demonstrated the highest BOD and COD removals of 83% and 92% respectively, the highest TSS, TG and O&G was achieved in $OLR_{GP\ 4}$ (CSTR) (61%, 72% and 74% respectively). Although $OLR_{GP\ 4}$ (CSTR) may not be superior as compared to $OLR_{GP\ 1}$ (ASBR) in terms of BOD and COD removals, the objective to reduce at least 50% of the pollutant is met in this research. With this reduction, post treatment is made possible at the later stage for the industry. At $OLR_{GP\ 1}$ (ASBR), the high dilution of GP with OCE is no surprise has reduced an amount of influent colloidal COD and eases the ACD treatment. High dilution of OCE also denotes higher capital for reactor up-sizing, it's pumping and energy needed making it less economical. It is also clear that the high treatment efficiency in CSTR with higher OLR of GP (4 g COD/L.day) has outweighed the treatment efficiencies accomplished in ASBR. Thus in overall, $OLR_{GP\ 4}$ (CSTR) scores better feasibility and higher effectiveness than $OLR_{GP\ 1}$ (ASBR).

In terms of stability, it is considered most stable based at $OLR_{GP\ 4}$ (CSTR) with optimum VFA/TA ratio of 0.40 observed with no buffer aid. Meanwhile, sodium bicarbonate which acts as a buffer was constantly added in $OLR_{GP\ 1}$ (ASBR) in adjusting the pH leading to higher operational cost. The steady profile in $OLR_{GP\ 4}$ (CSTR) is also unquestionably better than $OLR_{GP\ 1}$ (ASBR) which can only sustain its performance without any operational problems at the lowest OLR of GP (1 g COD/L.day).

In conclusion, $OLR_{GP\ 4}$ in CSTR is suggested for the operation of ACD in treating GP with OCE due to its competency in forming homogenous mixture for immiscible substrates, high ability to cope with high loading rate, high overall treatment efficiencies and good system stability which ease operational drawbacks. Therefore, $OLR_{GP\ 4}$ is ideal to be the chosen OLR_{GP} for further investigation in comparing the reactor configurations.

4.4 Performance evaluation of ACD of GP with OCE at different reactor configurations

With the chosen OLR_{GP} suggested from the previous findings in Section 4.3 (OLR_{GP} 4 in CSTR), it is worthwhile to confirm the feasibility of ACD of GP with OCE at different reactor configurations and to determine the best configuration that fits the industrial needs. Since mixing plays a strong role in this study and was discovered ineffective for ACD in ASBR (without mixing mechanism) with low OLR_{GP} and stability achieved, it is crucial to conduct ACD in different reactor configurations in order to compare both semi batch and continuous systems in solving miscibility of substrates and improving the overall performances. UASB has been widely reviewed as a suitable type of reactor for ACD due to its advantages includes higher removals of organic pollutant and conversion into biogas as well as less disposal of sludge (Chapter 2 - Section 2.4.6). For these reasons, UASB was used to confirm the degradability of the chosen OLR_{GP} . Both CSTR (semi batch system) and UASB (continuous system) was compared and the effect of mixing mechanisms (agitation and recirculation respectively) on ACD performances including organic removal efficiencies, reactor stability, nutrient balance and biogas production rate were studied.

Each reactor was operated based on the chosen OLR_{GP} 4. As suggested by Chan et al. (2012), at least 3 cycles of HRT were required in order to obtain a stable and actual digestion efficiencies in both reactor systems. In this study, 4 cycles of HRT (64.2 days) were conducted to further validate the results. The optimum reactor configuration is selected based on the highest organic removal efficiency (COD >50%) along with good stability in reactor (pH >6.0 and VFA/TA <0.4). Operating flow rates along with a summation of specific operating and feed conditions applied to the different reactors are presented in Table 4.8 and Table 4.9 respectively.

Table 4.8: Operational flow rates at different reactor configurations

		CSTR	UASB
Design ratio^a		0: 4	0: 4
Actual ratio^b		0.100 : 4.033	0.089: 3.584
HRT		64.2	64.2
1) OCE	Q_{in}^c	0.000	0.000
	V_{in}^d	0.000	0.000
2) DGP			
a) OCE	Q_{in}^c	0.006	0.196
	V_{in}^d	0.417	5.400
b) GP	Q_{in}^c	0.003	0.084
	V_{in}^d	0.179	12.600

^a**Design ratio**, OLR ratio of OCE: GP designated: g COD/L.day (Calculated based on Eq 3.6)

^b**Actual ratio**, OLR ratio of OCE: GP obtained: g COD/L.day (Calculated based on Eq 3.6)

^c**Q_{in}**, Flow rate of influent: L/day (Calculated based on Eq 3.13)

^d**V_{in}**, Total volume of influent: L (Calculated based on Eq 3.8)

^e**HRT**, Hydraulic retention time: days (Calculated based on Eq 3.11)

Table 4.9: Specific operating and feed conditions of CSTR and UASB

Paramater	Unit	OLR _{GP} 4			
		CSTR		UASB	
		Average	Range	Average	Range
Influent BOD	mg/L	202597	200750 - 210700	202510	187000 - 210700
Influent COD	mg/L	265357	254500 - 274500	233060	204449 - 258000
Influent TSS	mg/L	1528	980 - 1760	1578	1450 - 1890
Influent TG	mg/L	17.32	14.40 - 18.10	16.94	14.45 - 17.65
Influent O&G	mg/L	27972	27050 - 28900	27318	21500 - 29100
Influent SO₄²⁻	mg/L	1635	1600 - 1760	1862	1605 - 1890
Influent S²⁻	mg/L	15375	14890 - 15800	15828	14500 - 17040
Influent TA	mg/L	19390	18450 - 20544	18733	17300 - 19990
Influent VFA	mg/L	7688	7080 - 8100	7660	5980 - 8000
pH	-	8.57	6.50 - 11.80	6.74	5.40 - 6.84
MLVSS	g/L	22.15	17.20 - 26.30	34.28	20.60 - 42.00
F/M ratio	g BOD/g MLVSS.d	0.10	0.08 - 0.17	0.10	0.08 - 0.17

4.4.1 BOD, COD and TSS removal efficiencies

The performance of BOD and COD removal efficiencies and effluent concentrations at different reactors are presented in Figure 4.14 and Figure 4.15 respectively. In the ACD performance, the highest BOD, COD and TSS removal efficiencies were achieved in CSTR with 65%, 65% and 61% removals respectively. Whereas, the removals were reported at 48%, 36% and 0% respectively in UASB. During the start-up, both reactors were at 40% COD removal. COD removals in UASB were then drastically reduced until it reached the lowest at -28% (meaning no removal but addition of COD concentration in effluent), unlike CSTR which experienced a gradual increased in COD removal. This implies that the UASB could not tolerate GP in the system. The intense condition formed in the UASB leading to deterioration of performance as system could not break down GP. Nevertheless, the system was able to adapt to the condition after the fall. As a result, the unsteady performance in UASB took a longer acclimatisation period (27 weeks) as compared to CSTR (19 weeks) before both reached steady state. BOD removal followed a similar pattern to that of COD removal in both reactors which subsequently confirmed the results obtained for COD removal. Unfortunately, UASB could not meet the ideal COD removal efficiency which targeted to be at least 50% and above according to industry desires, the current removal efficiency at 36% was considered not satisfactory.

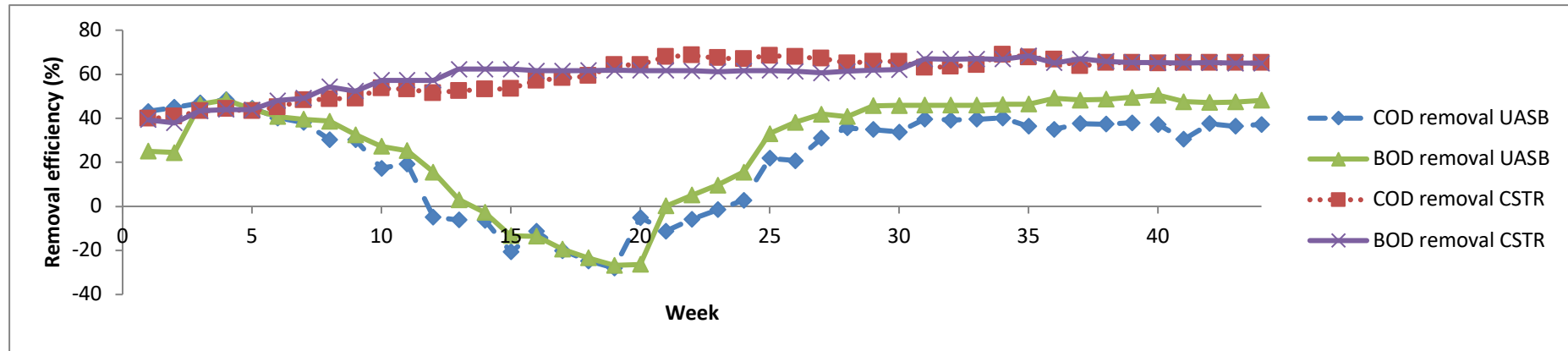


Figure 4.14: BOD and COD removal efficiencies at different reactors

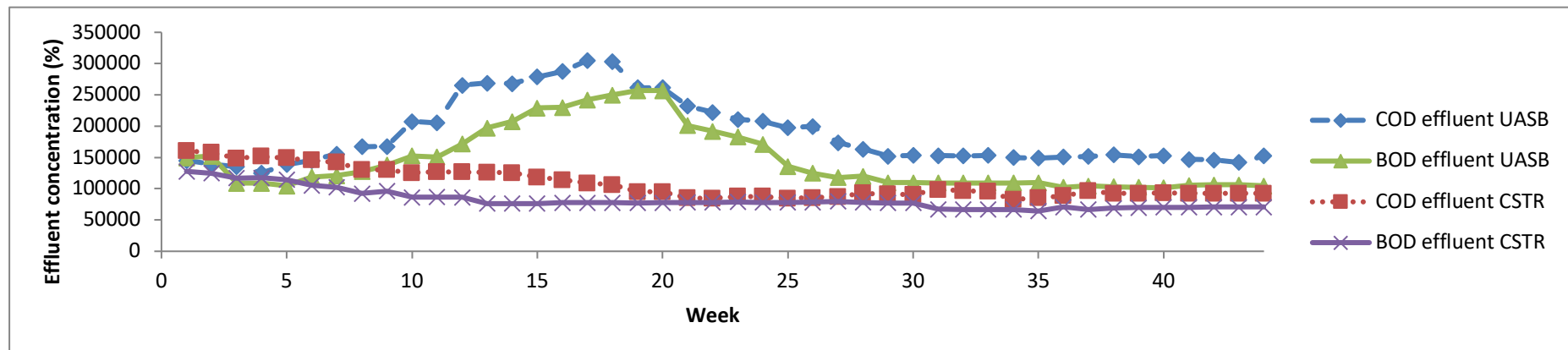


Figure 4.15: BOD and COD effluent concentrations at different reactors

UASB was adopted by Ma et al. (2008) and Astals et al. (2012) in co-digesting crude glycerin. Both have reported >85% COD removal efficiency at an OLR of 8.2 g COD/L.day and 1.9 g VS/L.day respectively. However, such performance did not correspond to the current study whereby COD removal efficiency of <50% was observed at OLR of 4 g COD/L.day. Although both mixing (feed tank) and recirculation (inside the reactor) were applied to ensure a homogenous blend between GP and OCE, the substrates in the system were spotted to form two immiscible layers. This was apparent as undigested GP was observed floating at the effluent point of UASB during acclimatisation. This suggests that GP has quickly escaped from sludge bed and floated up to the effluent point before it has enough contact with the anaerobes. This also explains the low COD removal as high concentration in final effluent was contributed by GP which has escaped from the sludge bed.

On the other hand, TSS removal was not detected in UASB but an increase trend in effluent concentration was observed from 1,700 mg/L (Week 1) up to 3,760 mg/L (Week 44) and hence not presented in Figure 4.14 and Figure 4.15. This means that there is no removal but an addition of TSS in the effluent. This is caused by the excess sludge and formation of scum along with escaped GP seen at effluent point during the operation of UASB. The results also reflect the incomplete of ACD. Although the present study employed hydraulic recirculation, it is proven not effective. This study reveals that UASB may be not suitable system for ACD due to the immiscible characteristics of digesting substrates (GP with OCE) as compared to CSTR. Likewise, although CSTR experienced the same trend (no removal of TSS) therefore not exhibited in Figure 4.14 and Figure 4.15, it only occurred during acclimatisation stage until Week 31 and subsequently showed gradual improvement up to 61% removal during steady state.

4.4.2 MLVSS concentration and F/M

MLVSS and F/M values were monitored regularly in order to understand the ongoing biochemical activity and behaviour at different reactors are shown in Figure 4.16 and Figure 4.17 respectively. At the acclimatisation stage, MLVSS concentration in UASB was declined to 20,600 mg/L (Week 7) with corresponding F/M averaging 0.17 g BOD/g MLVSS.day due to the washed out of aged sludge which often difficult to settle. This has caused poor performance in TSS removal whereby TSS concentration in effluent increased 137% from Week 1 to Week 44. Another factor contributed to the turbidity in final effluent is the scum along with undigested GP that have been escaped from the sludge bed. Scum deposition seen at the discharge point is attributable to the presence of O&G from GP which was not fully digested.

It was then gradually increased back to its normal concentration towards steady state (33,200 mg/L with corresponding F/M averaging 0.10 g BOD/g MLVSS.day. This indicates adaptation of anaerobes towards the loading rate of GP, they grow and develop into new cells. Nevertheless, the system still has excess biomass concentration for ACD to take place. This is reflected by the low F/M ratio ranging 0.08-0.17 g BOD/g MLVSS.day compared to the optimum ranging 0.25-0.39 g BOD/g MLVSS.day (Tchobanoglous & Burton, 1991; Chan et al., 2012). Although MLVSS showed in excess in UASB, sludge washed out is apparent and additional sludge has been constantly added throughout the experiment. The scum formation was also the worst observed at UASB during acclimatisation. These are reflected in the low COD, BOD and TSS removal efficiencies achieved. Therefore, the GLSS in UASB is clearly not effective for ACD of GP with OCE in retaining the biomass, a separate or enhanced settling compartment is recommended to improve the situation in the future investigation.

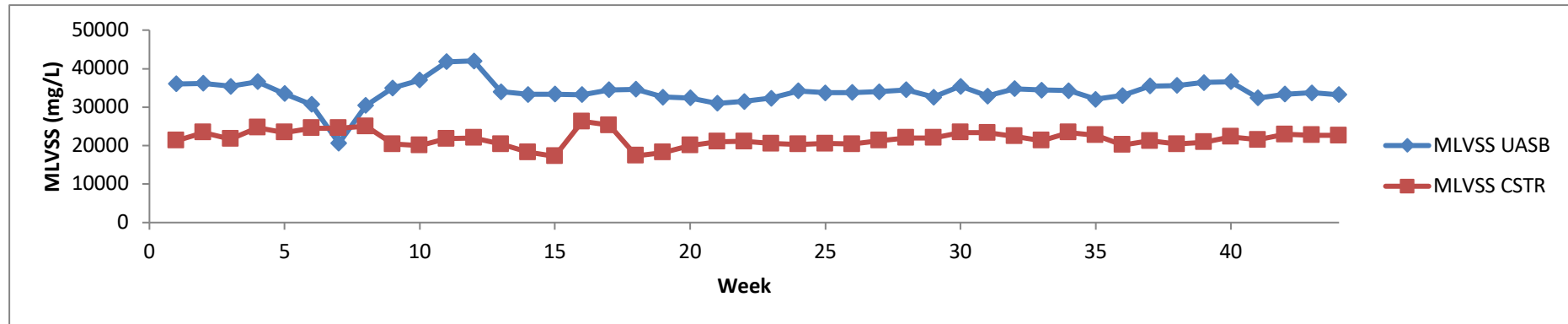


Figure 4.16: MLVSS concentration at different reactors

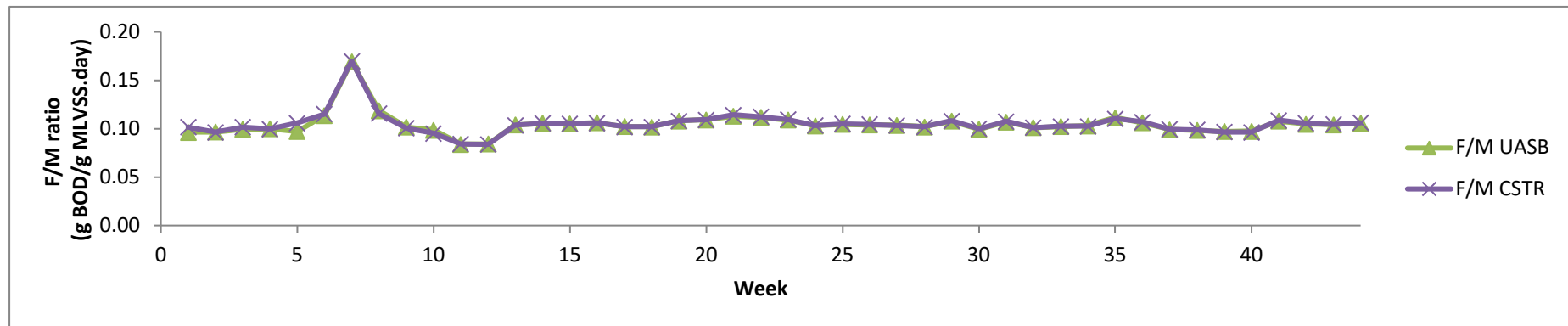


Figure 4.17: F/M ratio at different reactors

Likewise, CSTR has the same trend of steady state with MLVSS of 22,150 mg/L and its corresponding F/M averaging 0.10 g BOD/g MLVSS.day. However, CSTR appeared to have more stable MLVSS concentration during acclimatisation stage with no sign of nutrient imbalance or operational issue such as scum formation. This also reflects the reactor capability in utilising and retaining the biomass.

4.4.3 pH, alkalinity and VFA concentration

Due to instability of VFA/TA in both reactors, alkalinity was supplemented to the system for pH adjustment throughout the experiment. Reason for this is to ensure the methanogenic activity would not be affected severely which attentively agrees with those reported in the literatures (Ma et al. 2008; Yi Jing Chan et al. 2012; Li 2012). As such, the operational costs especially the chemical costs need to be considered.

Figure 4.18 and Figure 4.19 depicted the profile of VFA, TA and pH at different reactors. The VFA concentration at UASB and CSTR were constant averaging 2,548 mg/L and 6,558 mg/L respectively throughout the experiment. The concentration have exceeded the optimum of 500 mg/L for ACD (Shivayogimath & Ramanujam 1999). This indicates that there may be loss of methanogenic potential and unsatisfactory balance between acidogens, acetogens and methanogens in the biomass. The high VFA is contributed by the substrate characteristic itself. On the other hand, TA concentration in UASB and CSTR were also at constant averaging 1,647 mg/L and 16,378 mg/L. It is expected that TA supplement will not be needed as the loading rate of GP shall contribute its high alkalinity in the ACD. Unfortunately, that is not the case. The lower VFA and TA in UASB are caused by the escaped of GP (main VFA and TA contributors) from the sludge blanket and hence not providing the alkalinity needed. Although VFA at UASB shown to have 67% reduction as compared

to CSTR with 15%, it is contributed by the constant dosing of TA supplement into the system to provide adequate buffering capacity. This is through the neutralisation of hydrogen ions (discharged from the VFA) together with carbonates (dissociated from the carbonic acid) and the bicarbonate alkalinity inside the UASB.

Despite the addition of TA, UASB fail to achieve stability as evidenced by the VFA/TA ratio of 1.59 which surpassed the recommended range of <0.30-0.40 (Fannin, 1987). This denotes methanogenic inhibition towards toxicity of substrates. At this stage, acid forming bacteria displace methanogens and free VFAs diffused into the cells of anaerobes and ionised to decrease the pH (pH however does not show a great decrease as TA is constantly supplemented). The high VFA/TA ratio and the loss of buffering capacity (TA <2,000 mg/L) has led to souring (pH $\approx$ 6) and failure of the reactor. This means deterioration of the system.

In the contrary, CSTR with homogenous mixing has promoted reactor stability where TA was reduced 16% for the neutralisation of VFA content. TA reduction was rather little hence reflected in pH 8.57 more to the high side. Despite pH and VFA exceeded optimum >500 mg/L, the system is considered stable with VFA/TA of 0.40. Nevertheless, the high VFA detected may be threatening to methanogenic pathway.

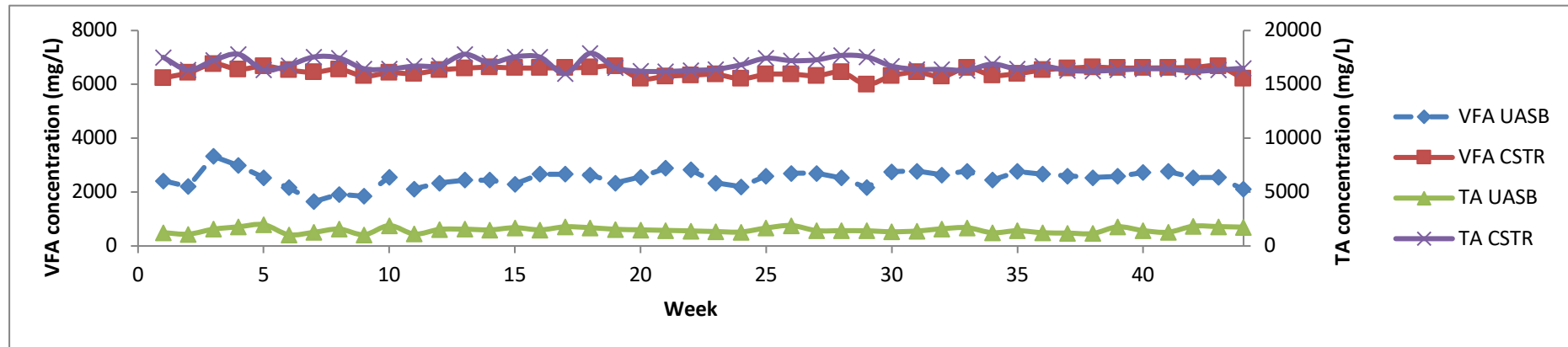


Figure 4.18: TA and VFA concentration at different reactors

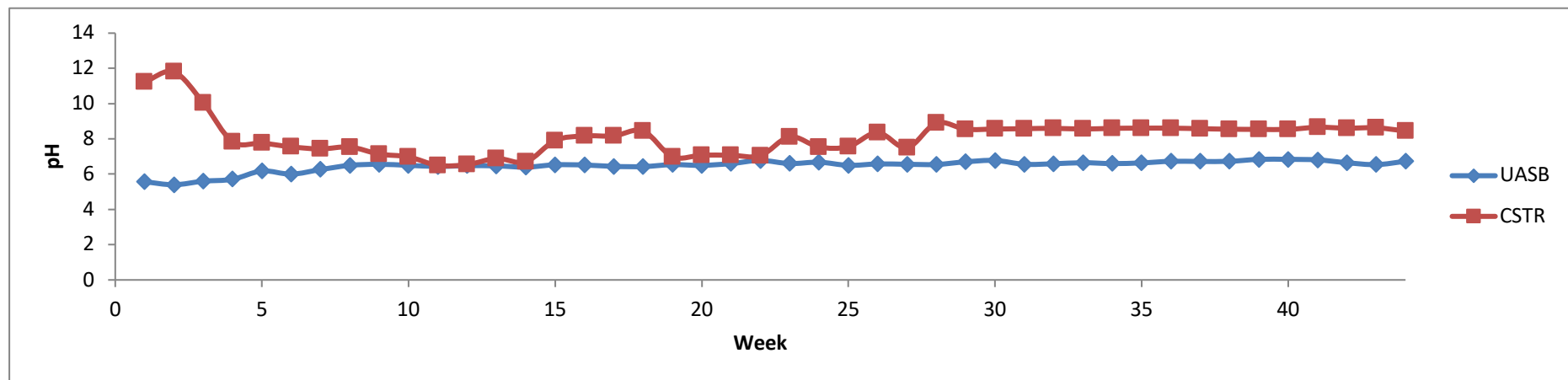


Figure 4.19: pH at different reactors

4.4.4 TG and O&G removal efficiencies

The TG and O&G performance profiles in term of removal efficiency and final effluent concentration are shown in Figure 4.20 and Figure 4.21 respectively. The highest TG removal efficiency of 72% and O&G removal efficiency of 74% were achieved in CSTR. Meanwhile, UASB obtained 38% and 57% of TG and O&G respectively. In CSTR, acclimatisation period was stable along with gradual incline of removal efficiencies up to its highest points at steady state. In contrast, it can be evidently seen that in UASB, removal efficiency dropped to its lowest 0% (TG) and -22% (O&G) prior to steady state. The trend of removals demonstrates similarity with BOD and COD removals.

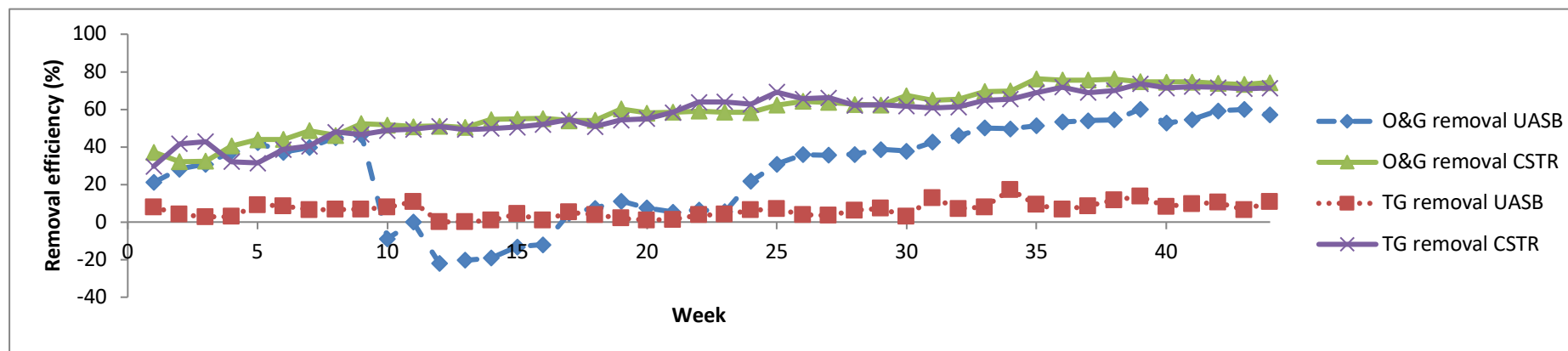


Figure 4.20: TG and O&G removal efficiencies at different reactors

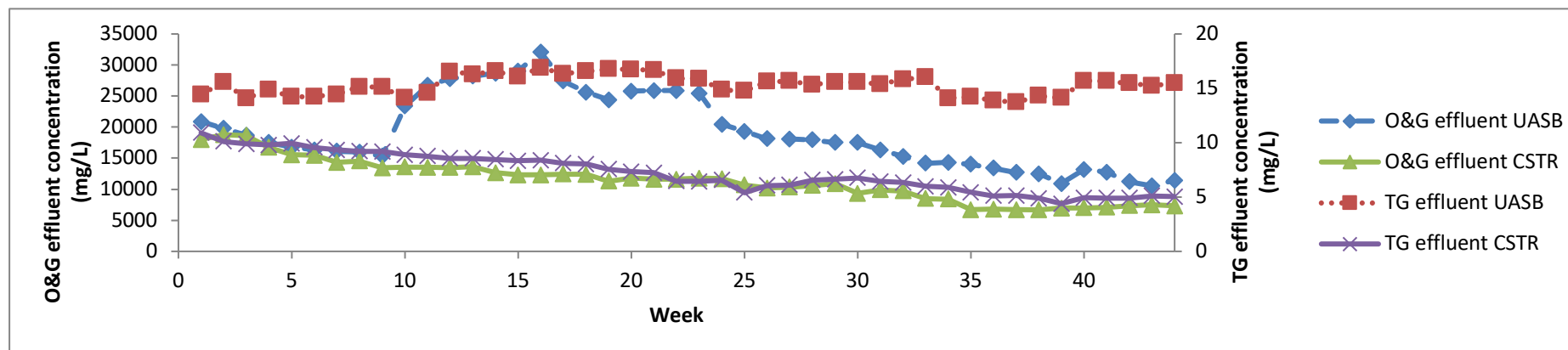


Figure 4.21: TG and O&G final effluent concentrations at different reactors

Despite the positive removals trend observed at the steady state, the effluent O&G concentrations in UASB (11,680 mg/L) is 62% higher than that in CSTR (7,205 mg/L). The high O&G in final effluent indicates that it did not degrade in the system. The non-degraded O&G floating at the top of UASB (discharge point) also caused scum deposition, fouling and as a result, frequent clogging at nozzles and valves faced throughout the experiment. This is also reflected in the negative removal observed in Week 10 to Week 16 and the longer acclimatisation period took up to Week 33. Fortunately, these were not observed in CSTR.

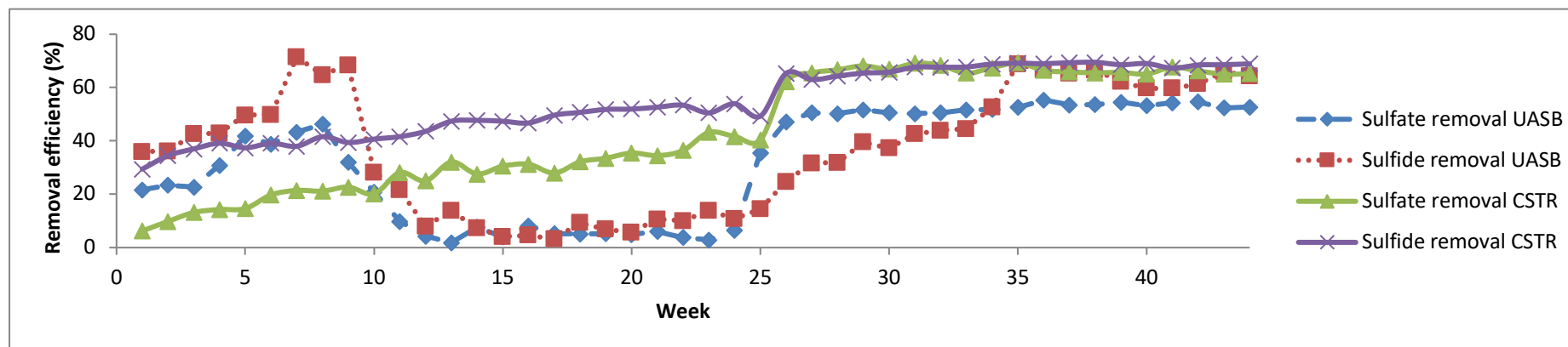
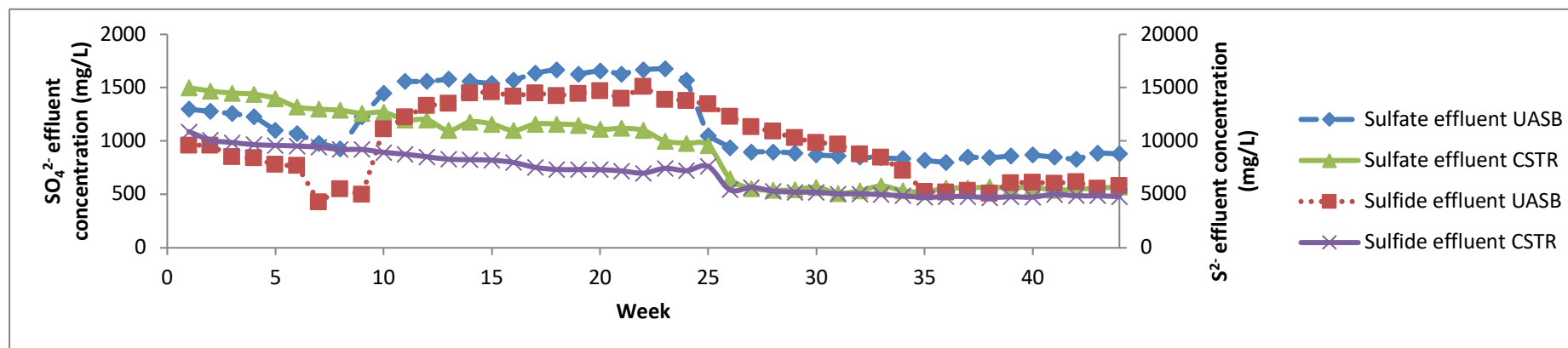
Although uncertainty remains as to the threshold concentration of O&G that can be managed within anaerobic digester, there are some indications from the past research works. Hwu et al (1998) reported that sludge floatation occurred when LCFAs concentration reached 263 mg/L whereas Yuan (1995) noticed methanogenesis inhibition happened when LCFAs is greater than 400 mg/L. Thus, this corresponds to the occurrence of sludge washed out in UASB reported as described in Section 4.4.2. Such instability in UASB also encountered by Tchobanoglous et al. (2003) who explained that the high O&G tends to accumulate on the sludge surface and resulting in foaming and scum formation. The O&G concentration that surpassed the suggested safe limit by Hwu et al (1998) and Yuan (1995) also indicates potential methanogenic disruption leading to poor biogas collection.

The high concentration of O&G in effluent is likely affected by the recirculation in UASB. With such mixing, ACD did not attain higher removal efficiencies and this is attributed to the escape of GP from sludge bed straight to the effluent point. Two immiscible layers were also observed in the final effluent showing inhomogeneous ACD in the reactor. As influent GP comprises of high O&G averaging 27,942 mg/L, the low density substrate is unable to merge easily with OCE without a thorough mixing. This experimental result has proven that UASB is not a suitable system for

immiscible substrates like GP and OCE. As UASB system could not blend the two substrates and recirculation alone was not effective, CSTR would be a better choice to unleash the full potential of ACD in attaining a higher removal of TG and O&G. Therefore, pre-treatment such as chemical processing (pH adjustment, coagulation-flocculation or adding chemical de-emulsifier) or physical/filtration approaches (membrane filtration, activated carbon, clay based materials or evaporation/distillation) should be explored in order to further boost the effluent quality.

4.4.5 SO_4^{2-} and S^{2-} removal efficiencies

The removal efficiencies and final effluent concentrations of SO_4^{2-} and S^{2-} are illustrated in Figure 4.22 and Figure 4.23 respectively. The highest removals of SO_4^{2-} and S^{2-} were detected in CSTR with 66% and 68% respectively. Whereas, the maximum SO_4^{2-} and S^{2-} removals in UASB were at 54% and 62% respectively. In CSTR, removal efficiencies were increased gradually from Week 1 up to steady state, unlike UASB which experienced a downfall on Week 10 during acclimatisation. UASB clearly struggled for a long acclimatisation period (18 weeks) before it could reach its steady state on Week 29 onwards. The trend is also similar to TG and O&G reported which suggests that UASB could not break down the targeted substances as compared to CSTR. This is due to the poor homogenous condition in the UASB where substrates only get to be in contact with anaerobes under long operation days via recirculation mixing.

Figure 4.22: SO_4^{2-} and S^{2-} removal efficiencies at different reactorsFigure 4.23: SO_4^{2-} and S^{2-} final effluent concentrations at different reactors

However, the high removal efficiency of SO_4^{2-} is not equivalent to excellent ACD. In fact, SO_4^{2-} reduction is only optimal at a relatively low level of SO_4^{2-} concentration (<750 mg/L). This study revealed that more SO_4^{2-} was converted into S^{2-} by SRB. SRB (1) generates S^{2-} which is inhibitory to SRB and MPB. (2) reduces rate of methanogenesis (3) decreases the quantity of methane produced by competing for available carbon and hydrogen. As a result, the final concentrations of S^{2-} captured at 5,830 mg/L in UASB and 4,800 mg/L in CSTR greatly surpassed the safe limit recommended at <100 mg/L (Celis-García et al. 2004). S^{2-} was reported to be toxic to unacclimated methanogens at concentration of 50 mg/L (Parkin et al. 1983) while (Maillacheruvu & Parkin 1996) discovered that H_2S inhibited acetotrophic methanogens much more than hydrogenotrophic methanogens. The inhibitory concentration for acetrophic and hydrogenotrophic methanogens was 160 mg/L and 220 mg/L respectively (Yamaguchi et al. 1999). Not only S^{2-} can denature proteins and interfere with key metabolic enzymes in the cells (Madigan et al. 2003), it also disrupt assimilation of sulfur and affect intercellular pH (Visser 1995). Therefore, uncoupling growth from energy production and cell maintenance required more energy (Okabe et al. 1995). The outcome of inhibition is also reflected by the poor biogas production rate and its yield at UASB and CSTR in Figure 4.24.

This study revealed that S^{2-} could be one of the major drawbacks in ACD for both reactors. In most cases, biological treatment of sulfate-rich wastewater was not widely practiced due to the production of H_2S that leads to corrosion, increased effluent COD, odour and toxicity. Hence, to minimise process failure, pre-treatment in reducing the concentrations of SO_4^{2-} and S^{2-} in influent should be taken into the next step of investigation.

4.4.6 Biogas production rate

The biogas production rate at different reactors is exhibited in Figure 4.24. The highest biogas production of 0.300 L/day was achieved in UASB (Day 84) as compared to CSTR with 0.009 L/day (Week 12). The result however does not make an improvement and continue to reduce following the highest peak. This indicates very negligible conversion of biogas occurred. The worse performance was recorded at the end of steady state with no biogas production and hence methane was not detected. Although UASB appeared to have higher biogas production, both reactors have a poor performance and the negligible amount of biogas produced was too little to be measured neither for its composition nor biogas yield.

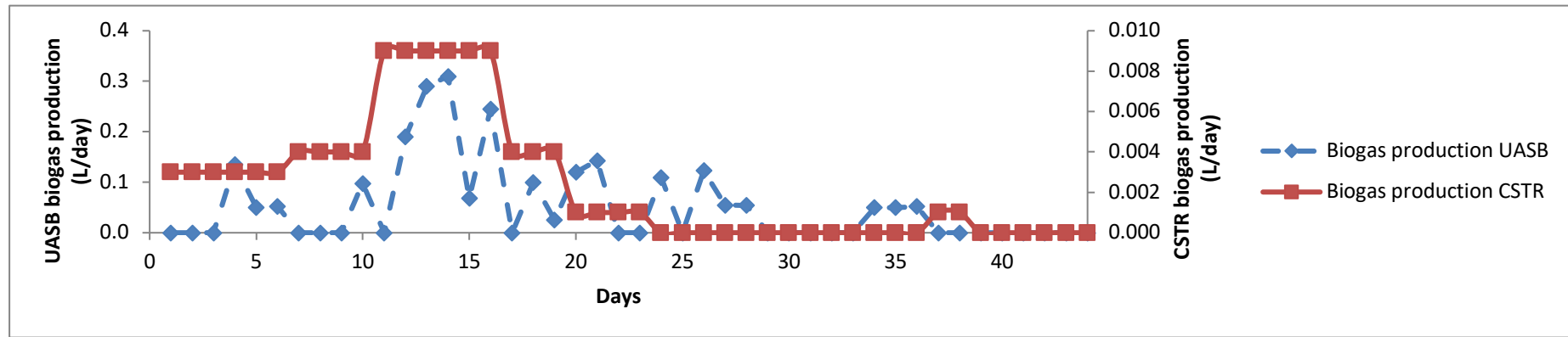


Figure 4.24: Biogas production rate at different reactors

Even though CSTR obtained highest COD removal (65%), there is no sign of methanogenic activity as reported by the undetectable biogas. Also, drastic drop was observed in biogas production rate. As reviewed in Section 2.2, ACD with glycerin based substrate generally could not achieve a high biogas production as compared to the established high energy substrate like POME treatment. The methane production rate obtained with glycerin based substrate is often <4 L/day which result in the low conversion into electricity (Ma et al. 2008; Fountoulakis & Manios 2009; Fountoulakis et al. 2010; Siles et al. 2010; Astals et al. 2012; Astals et al. 2013; Larsen et al. 2013; Rivero et al. 2014). This also explained the reason Malaysian government incentives has been focusing on palm industries (MIDA 2013; Wong 2016). Same goes to the feed in tariff (FiT) scheme or Clean-Energy-Cash-Back scheme introduced where major renewable energy producers were handling palm based substrate like POME (SEDA 2018). It is parallels to the low potential of F_{CH_4} and F_{kWh} , in DGP as discussed in Section 4.2. The research in ACD using glycerin based substrate is certainly at infancy and in-depth explorations worth to be conducted to comprehend the overall synergism of this substrate in ACD process. Biogas production is not the key focus as COD reduction represents the main objective in fulfilling industry's need; nevertheless, this study disclosed the difficulties encountered and it is values added for the next investigations.

This study revealed that there are four major drawbacks in UASB leading to the poor biogas production: (1) The unsuitable mixing system in UASB for immiscible substrates. The recirculation system applied in the UASB is inadequate to form a homogenous mixture. As a result, the poor biomass contact has lessened the ability of microbes to capture organic matter and obtain nutrients from GP. (2) Inhibition of methanogens caused by high VFA. Clearly, the high VFA concentration in both reactors has imposed impact on methanogenic pathways. It is supported by Wang et al. (2009) who observed a significant methane reduction when VFA was high in concentration. Although both reactors have exceeded the safe limit of <500 mg/L,

UASB has performed poorly caused by the escaped GP resulting in instability of reactor. (3) Inhibition of methanogens caused by high O&G. The floating residues of GP in UASB observed with high O&G not only contributed to the high TSS in treated effluent and scum formation in the system, it also resulted in sludge washout and ultimately disrupted methanogens activity. This is caused by the inhibitory effect of LCFAs by adsorption onto methanogens where its metabolism including food transportation across the membrane was inhibited (Kabara & Vrable 1977; Zeikus 1977; Ferreira 2013). This inhibitory effect also has been reported by various researchers on acetogens (Rinzema et al. 1989), acetoclastic methanogenic archaea (Koster & Cramer 1987; Lalman & Bagley 2000) and hydrogenotrophic methanogenic archaea (Hanaki et al. 1981; Lalman & Bagley 2000). (4) Inhibition of methanogens caused by lethal SO_4^{2-} and S^{2-} . They caused two stages inhibition in ACD. First stage, MPB activity is constrained by SRB due to the active degradation of SRB on various organic compounds in the metabolic pathways. The growth of SRB was contributed by the high concentration of SO_4^{2-} of substrate. Second stage, the conversion SO_4^{2-} has also occasioned in high S^{2-} concentration lead to toxicity to MPB. This is in-line with Celis-García et al. (2004)'s study who stated at least 50% methanogenesis inhibition as the destructive form S^{2-} could denature existing proteins in the cell by membrane diffusion. Similarly, CSTR faced the same challenges: (2) and (4).

Despite the drawbacks faced by both reactors, CSTR is preferred as compared to UASB due to its agitation mixing which solve the major difficulty in ACD - immiscibility of substrates characteristic. Ideal reactor, CSTR is selected for further investigation in order to achieve the objectives of this study. Although UASB failed to perform to its fullest potential, it serves as a good indication on substrates miscibility, the flaws in the system and parameters to be accounted for improvement.

4.4.7 Optimum reactor configuration

Table 4.10 presented the summary performance at different reactor configurations. The ideal reactor was defined based on three factors: (1) Mixing mechanisms. (2) Treatment efficiencies in terms of BOD, COD, TSS, TG and O&G removals. (3) Reactor stability in terms of pH, TA, VFA, SO_4^{2-} and S^{2-} .

Table 4.10: Overall ACD performance at different reactor configurations

Parameter	Unit	UASB	CSTR
Optimum OLR_{GP}	-	OLR _{GP} 4	OLR _{GP} 4
BOD removal	%	48	65
COD removal	%	36	65
TSS removal	%	0	61
TG removal	%	38	72
O&G removal	%	57	74
Biogas production rate	L/day	0	0
Biogas yield	L biogas/g COD removed.day	0	0
Methane composition	%	0	0
Effluent BOD	mg/L	104,435	70,580
Effluent COD	mg/L	148,610	92,537
Effluent TSS	mg/L	3,740	592
Effluent TG	mg/L	10.42	4.88
Effluent O&G	mg/L	11,680	7,205
Effluent SO_4^{2-}	mg/L	1,788	559
Effluent S^{2-}	mg/L	5,970	4,848
Effluent pH	mg/L	6.74	8.57
Effluent TA	mg/L	1,647	16,378
Effluent VFA	mg/L	2,548	6,558

The substrates in CSTR are observed to be well-mixed despite the density differences in GP and OCE. The substantial improvement in organic removal efficiency up to 65% using agitation mixing in the system is a sign of enhancement in mass transfer between substrates and the sludge. The agitation mixing in CSTR which formed hydrodynamic behaviour also reduces substrate inhibition such as concentration gradient and accumulation of intermediates (Mohan et al. 2007). It is

undeniably true that the low density of GP making the miscibility factor a challenge, this study however revealed that agitation mixing (CSTR) is more competent in handling for immiscible substrates in ACD as compared to recirculation mixing (UASB).

Besides, CSTR achieved overall higher removal efficiencies (65% BOD, 65% COD, 61% TSS, 72% TG and 74% O&G) as compared to UASB which fail to meet the target COD >50%. UASB did not portray a good performance due to its system not being able to handle immiscible substrates. Although UASB was operated under the optimum recirculation ratio for ACD as adopted from literature, it does not homogenise the two substrates mixture and escaped of untreated GP from the treatment was observed.

In addition, CSTR is considered to have a steadier profile in reactor stability due to its optimum VFA/TA ratio of 4.0 and non-dependent on buffer aid. Not only that UASB constantly dependent on buffer leading to higher operational cost, it suffered from clogging issue and scum formation throughout the operation. Overall, the CSTR scores better feasibility and higher effectiveness than UASB for ACD of GP with OCE. The excellent performance of CSTR in the three factors discussed, this study concluded that CSTR is the ideal reactor to be used to further investigate the challenges faced and to boost organic removal efficiency.

4.5 Jar test for substrate pre-treatment

There are many factors govern the performance of CSTR where pre-treatment is required to maximise its performance at a lower operating and capital costs. From the realistic and economic points of view, pre-treatment of GP to ease ACD treatment and yield a lower capital and operating costs will be advantageous. This pre-treatment study primarily focused on removing SO_4^{2-} and soap contents. The high

SO_4^{2-} present has caused inhibition in the CSTR system during the treatment. The inhibition was due to competition for substrates from SRB which suppresses methane production and results from toxicity of S^{2-} to various bacteria groups. The overall optimum of variables was determined based on the highest SO_4^{2-} removal with minimum dosage. The selected variables of (A) dosage of coagulant (B) dosage of flocculant (C) retention time of both coagulant and flocculant and (D) pH were optimised against the critical responses of SO_4^{2-} and soap as shown in Table 4.11 to Table 4.14.

SO_4^{2-} content resembles the amount of soap. Precisely, SO_4^{2-} is present in soap and it behaves similarly as sodium laureth sulfate (SLES) which is an anionic detergent and surfactant that is usually found in various personal care products. Effluent rich in SO_4^{2-} is undesirable in industrial operation due to the production of H_2S during biological treatment. As high soap content also indicates the incomplete degradation of SO_4^{2-} , it is presumed that soap is destructive compound for methanogens equivalent to SO_4^{2-} . Not only the competitive interactions between SRB and MPB, the H_2S generated also caused physical chemical corrosion, odour and biological constraint which lead to process break down (Hulshoff Pol et al. 1998). Therefore, soap is interconnects with SO_4^{2-} in this research. Despite the shortage of research on soap threshold concentration for ACD, the concentration of SO_4^{2-} is able to provide an indication on the tolerance limit as mirrored by its removal efficiency.

4.5.1 Effects of coagulant and flocculant dosages on SO_4^{2-} and soap removals

It is important to note that CaCl_2 which acts as a coagulant is preferred in this study over the commonly used sodium hydroxide and calcium hydroxide because CaCl_2 is neutral in pH. Whereas, sodium hydroxide and calcium hydroxide are alkaline. Since effluent and coagulant are not supposed to be the same pH, the usage of such coagulants could defeat the purpose of creating pH difference knowing that GP itself is in alkaline state. The influence of different dosages of CaCl_2 and polymer on the removal of SO_4^{2-} and soap are shown in Table 4.11 and Table 4.12 respectively.

Table 4.11: SO_4^{2-} and soap removals at various CaCl_2 dosages

Response	Unit	Stage 1: CaCl_2 dosage				
	mg/L	17.5	20.0	22.5	25.0	27.5
SO_4^{2-} removal	%	94	95	96	96	94
Soap removal	%	26	27	29	31	28

Table 4.12: SO_4^{2-} and soap removals at various polymer dosages

	Unit	Stage 2: Polymer dosage				
	mg/L	0.005	0.010	0.015	0.020	0.025
SO_4^{2-} removal	%	89	93	94	96	95
Soap removal	%	15	21	26	31	29

Results showed that the optimum CaCl_2 and polymer dosages fall at 25 mg/L and 0.02 mg/L respectively achieved highest SO_4^{2-} and soap removal efficiencies.

The increased in CaCl_2 dosage also increases SO_4^{2-} and soap removals up to 96% and 31% respectively. The removals did not show further increment with higher dosage of CaCl_2 , in fact the surplus after threshold point results in overdosing as reflected in the dropped of SO_4^{2-} and soap removals. This reduction is likely to be caused by the charge reversal and destabilisation of colloidal particles which agrees to what reported by Yukselen & Gregory (2004).

The results also revealed that CaCl_2 could be a viable coagulant in managing high SO_4^{2-} problems in wastewater. The superior performance SO_4^{2-} removal efficiencies could be contributed by the active chemical reaction. This is because calcium ions hydrolysed quickly in the effluent and initiates the occurrence of destabilisation of particles. Destabilisation was formed between the charges opposite to those of suspended solids are added to the effluent to neutralise the negative charges on dispersed non-settable solids such as organic substances (MRWA 2003). When the charge is neutralised, the small suspended particles are capable of sticking together hence better removal efficiencies.

Although the performance of biogas production is not evaluated during pre-treatment as the primary aim is to reduce SO_4^{2-} and soap contents prior to ACD, the addition of CaCl_2 will not inhibit methanogenesis. Many have reported no inhibitory effect and effluent characteristic showed improvement in overall AD performance with the addition of CaCl_2 (van Langerak et al. 1998; Sharma & Singh 2001; Ahn et al. 2006; Kumar et al. 2016). On top of the precipitation mechanism, the protective adsorption of calcium ions could also take positive effects on the reduction of methanogenesis inhibition (Fukushi et al. 2010). However, it is important to note that CaCl_2 was added during pre-treatment and not during the ACD treatment itself. It precipitated as calcium sulfate (CaSO_4) and was removed from effluent. This means coagulant has been removed along with the targeted compounds prior to ACD hence not affecting the treatment process.

CaCl₂ also unveiled similar good performance in the responses with low dosage of flocculant (<0.025 mg/L of polymer) needed in this study as shown in Table 4.12. Small dosage of polymer is sufficient in increasing the particle size from submicroscopic micro-floc to visible suspended particles. This happened when the collision of the micro-floc particles causes them to bond to produce larger visible flocs which is now termed as macro-floc which ease the filtration process at the later stage in producing an improved effluent quality.

4.5.2 Effects of retention time and pH on SO₄²⁻ and soap removals

It is worth to note that rapid mixing with high energy was applied to fully disperse coagulant and promote particle collisions in order to obtain proficient coagulation and formation of micro-flocs. Consequently, slow mixing was employed to bring micro-flocs into contact with each other during flocculation. This is to maximise the number of collision between particles and flocs without breaking the flocs up through rapid mixing. The effect of retention time of CaCl₂ and polymer on the removal of SO₄²⁻ and soap is shown in Table 4.13.

Table 4.13: SO₄²⁻ and soap removals at various retention time of CaCl₂ and polymer

	Unit	Retention time of CaCl ₂				
	min	1	2	3	4	5
SO ₄ ²⁻ removal	%	97	96	96	96	96
Soap removal	%	31	31	31	31	31

	Unit	Retention time of polymer				
	min	5	10	15	20	25
SO ₄ ²⁻ removal	%	97	97	96	96	96
Soap removal	%	31	31	31	31	31

Referring to Table 4.13, SO_4^{2-} removal was observed highest at 1 min and 5 min retention time for CaCl_2 and polymer respectively and no sign of changes in soap removal. Soap removal at 31% is considered the maximum efficiency regardless the retention time applied. Retention time >1 min (CaCl_2) and >5 min (polymer) showed no further improvement in SO_4^{2-} removal. This infers that the retention time was not a significant influence on the critical responses. These responses are not sensitive to the mixing time and the lowest retention time applied is considered adequate in dispersing coagulant and allowing the formation of macro-flocs. This study agreed to the suggested effective contact time of 1 to 3 min for coagulation with rapid mix chamber whereas 15 or 20 min to an hour or more for flocculation with slow mix chamber (MRWA 2003). Although the retention time for polymer is observed shorter than recommended, the adequacy has permitted SO_4^{2-} and soap removals in sedimentation process. This is likely to be due to the rapid reduction of SO_4^{2-} and soap that reached equilibrium at a minimum retention time. Similar behaviour were observed by Szabo et al. (2008) and Georgantas & Grigoropoulou (2003).

On the other hand, the influence of pH on the removals of SO_4^{2-} and soap is shown in Table 4.14. Usually pH will not only affect the surface charge of coagulants, it also impacts the stabilisation of suspension. The effectiveness of solubility of CaCl_2 is affected by low or high pH. In optimum form, the flocs were rigid and settled well in less than 15 min. In this study, the reduction of SO_4^{2-} and soap were observed to be good at pH 8. The results were also parallel with the research performed by Ebeling et al. (2003) and Bina et al. (2009). The best performance of responses was concluded at pH 8 as it showed no further improvement in SO_4^{2-} but slight decreased in soap removal with further increased in pH.

Table 4.14: SO_4^{2-} and soap removals at various pH

	Unit	pH				
	-	7	8	9	10	11
SO_4^{2-} removal	%	96	97	97	97	97
Soap removal	%	32	33	33	32	32

In neutral state, polymer is able to produce larger and denser floc due to more coiled structure. Whereas in alkaline state, the structure becomes extended chain with more charged, hence produced smaller and looser flocs. Despite that the optimum pH shown here favour alkaline condition, the effect of pH on coagulant efficiency and the removals of SO_4^{2-} and soap were insignificant. The evidence also indicates that the neutralisation charge neither a primary mechanism in regulating the formation of floc in the coagulant (Huang & Chen 1996) nor the removal efficiencies of SO_4^{2-} and soap. As GP itself is alkaline in nature, pH may not necessary to be regulated in this case hence saving chemical cost.

4.5.3 Optimum conditions for substrate pre-treatment

Table 4.15 presented the summary of optimum conditions for substrate pre-treatment. This could aid as a guide to obtain highest removal of SO_4^{2-} and soap in effluent like GP.

Table 4.15: Optimum conditions for substrate pre-treatment

Parameter	Unit	Optimum condition
CaCl₂ dosage	mg/L	25
Polymer dosage	mg/L	0.02
Retention time a) CaCl₂	min	1
b) Polymer	min	5
pH	-	8

4.6 Performance evaluation of ACD of pre-treated substrate using the chosen reactor

As the previous results using CSTR revealed that SO_4^{2-} and S^{2-} are one of the major drawbacks in the system, substrate GP was pre-treated via coagulation-flocculation process in order to rectify the problems. Hence, it is vital to place pre-treated GP on test to verify if the reduced SO_4^{2-} and soap contents could improve the overall performance of the system. By using the same operational flow rate and conditions CSTR (Table 4.8 and Table 4.9), the pre-treated GP is presumed to show enhancement in COD removal. This is because coagulation-flocculation process has been widely researched as a suitable pre-treatment due to its ability in removing SO_4^{2-} and soap content in substrate with >50% efficiency using calcium coagulant (van Langerak et al. 1998; Sharma & Singh 2001; Ahn et al. 2006; Kumar et al. 2016). The pre-treated GP with lesser colloidal particles not only ease the treatment process but enhance methanogenesis and overall organic degradation. Therefore, CSTR with pre-treated GP was performed to verify the hypotheses. Aspects influencing the performances of organic removal efficiencies, reactor stability, nutrient balance and biogas production rate were analysed.

Unlike previous ACD with various OLR_{GP} , this experiment was investigated at only one OLR_{GP} (OLR_{GP} 4) which has been concluded ideal in order to evaluate the maximum COD removal. There were two sets of the same OLR_{GP} 4 where one acts as a control and the other serves as variable (pre-treated substrate). Both sets will be termed as "control" and "pre-treated substrate" only in the entire discussion. The highest response achieved is regarded as threshold point prior to reactor breakdown.

4.6.1 BOD, COD and TSS removal efficiencies

The performance of control and pre-treated substrate using CSTR in terms of BOD, COD and TSS removal efficiencies are consolidated and presented in Table 4.16. The COD removal efficiency of pre-treated substrate increased to a maximum of 70%. The 5% increment as compared to control indicates the improvement of microbial activity and the highest microbial activity was achieved with pre-treatment. The improvement was contributed by the high biodegradation efficiency of CSTR where most recalcitrant fraction and colloidal matter were eliminated by pre-treatment prior to the ACD. This has resulted in a more easily biodegradable influent to the reactor. This shows that pre-treatment served an important polishing step in the system and such improvement also resembles the findings in various research that has been testified using pre-treated substrate (van Langerak et al. 1998; Sharma & Singh 2001; Ahn et al. 2006; Kumar et al. 2016). Although scum was observed at the top of CSTR at the beginning of operation (first 10 days) indicates the instability of CSTR during the start-up, there is no severe system inhibition to the ACD as improved performance was observed.

Table 4.16: BOD, COD and TSS removal efficiencies and its effluent concentrations of control and pre-treated substrate in CSTR

Parameter		Control	Pre-treated substrate
BOD	Removal efficiency (%)	65	69
	Effluent concentration (mg/L)	70,770	63,542
COD	Removal efficiency (%)	65	70
	Effluent concentration (mg/L)	92,415	76,945
TSS	Removal efficiency (%)	70	0
	Effluent concentration (mg/L)	499	813

Besides, BOD removal efficiency followed a similar pattern to that of COD removal which subsequently validated the outcomes obtained for COD removal. Although the COD removal is well above 50% as desired in this study and pre-treatment is able to further boost up the efficiency, the final effluent produced with pre-treated substrate has a high BOD concentration of 63,542 mg/L. Further treatment steps such as membrane filtration or aerobic treatment may need to be taken into consideration if necessary. Based on literatures, coagulation-flocculation was employed in various effluent including sanitary landfill leachates, slaughterhouse effluent, beverage industrial effluent, pulp and paper mill effluent in reducing organic matter (Tatsi et al. 2003; Amuda & Alade 2006; Amuda & Amoo 2007; Ahmad et al. 2008). All of them have reported >80% COD removal efficiency whereas results in this study showed lower performance where maximum BOD and COD removals were insignificantly increased by 7% and 5%. This is because the main objective of the pre-treatment is to reduce SO_4^{2-} and soap contents instead of COD. Hence, in term of SO_4^{2-} and soap reductions, the performance of pre-treatment coupled with ACD in CSTR was considered excellent. As the objective has been defined clear, COD reduction is not expected during pre-treatment but in ACD instead as CSTR has high organic removal efficiency.

On the other hand, highest TSS removal was observed at control with 70% efficiency whereas no removal with pre-treated substrate but a higher effluent TSS of 813 mg/L as compared to control at 499 mg/L was detected. Although there is no removal with pre-treated substrate, it is important to note that coagulation-flocculation followed by sedimentation processes itself has significantly removed TSS from 1,643 mg/L to 723 mg/L (56% reduction) prior to ACD. Even so, it is worth to highlight that final effluent TSS after ACD was observed lower at control than that with pre-treated substrate. Pre-treated substrate also did not aid TSS reduction during ACD in CSTR. This study revealed that pre-treatment is advantageous in

polishing BOD and COD removals but may not be effective in reducing the final effluent TSS.

4.6.2 MLVSS concentration and F/M

The average MLVSS and F/M values of control and pre-treated substrate are shown in the Table 4.17. The system experienced depletion of sludge hence additional sludge was refilled twice on Week 2 and Week 9. After one cycle of HRT was completed (64.2 days), the depletion was observed to decline gradually. MLVSS concentration at control was recorded averaging 11,615 mg/L with corresponding F/M at 0.11 g BOD/g MLVSS.d at the end of steady state where COD reached its maximum at 65%. COD removal however does not decline despite the gradually declined of MLVSS. This indicates the microbial activities of anaerobes were high, with numerous of active sludge as evidenced by high TSS/VSS of the sludge (1.21) in the system. Likewise, pre-treated substrate portrayed the same trend in MLVSS averaging 20,300 mg/L that resembling F/M at 0.11 g BOD/g MLVSS.d. Although the recalcitrant compounds including SO_4^{2-} were removed during pre-treatment prior to ACD, the amount of active biomass does not significantly increase as evidenced by the TSS/VSS ratio (1.20).

Table 4.17: MLVSS concentration and F/M ratio of control and pre-treated substrate in CSTR

Parameter	Control	Pre-treated substrate
MLVSS (mg/L)	19,950	20,300
F/M	0.11	0.11

F/M ratios of both control and pre-treated substrate were below the recommended effective F/M ratio of 0.2-0.6 g BOD/g MLVSS.day for CSTR (Tchobanoglous & Burton, 1991). This shows that food is inadequate while anaerobes population is sufficient in the system. Nonetheless, excess anaerobes could also cause long sludge settling period and high effluent TSS (Hua et al. 2007). This is relatable to the current findings where turbid final effluent TSS of 499 mg/L and 813 mg/L at control and with pre-treated substrate respectively was observed. This is because the outflow from CSTR does not only consist of treated effluent but along with a portion of washout sludge. Fortunately, the sludge washout and excess MLVSS are not deliberated as a concern in this study due the fact that the COD removal was well above the goal and not affected by the changes in MLVSS.

4.6.3 pH, alkalinity and VFA concentration

In order to keep an optimum pH throughout the experiment, alkalinity was provided to the system. Such supplement was offered to evaluate the maximum performance under encouraging condition for the performing anaerobes without the influence of the instability of VFA/TA ratio observed in CSTR. Therefore, chemical cost should be taken into account. Table 4.18 depicted the average effluent pH, TA and VFA concentration of control and pre-treated substrate.

Table 4.18: pH, TA and VFA effluent concentrations of control and pre-treated substrate in CSTR

Parameter	Control	Pre-treated substrate
VFA effluent concentration (mg/L)	6,390	6,723
TA effluent concentration (mg/L)	16,107	11,205
pH effluent	6.45	7.25

The VFA concentration with pre-treated substrate was noticed to be 5% higher than control. Nevertheless, both concentrations were distinctively surpassed the optimum of <500 mg/L for ACD (Shivayogimath & Ramanujam 1999) which could lead to the loss of methanogenic potential. With the aids of buffer added, the effluent pH remained constant averaging 6.45 and 7.25 at control and with pre-treated substrate respectively. Although pre-treatment revealed that pH 8-9 has the tendency to give highest SO_4^{2-} and soap removals and the influent was regulated at pH 8 prior to ACD, pre-treated substrate in CSTR has been constantly retained its pH ≈ 7 regardless of the pH adjustment. This is because the ideal pH for anaerobic process is generally lies within 6.8-7.2 while pH 7.5 is reported to be optimal for ACD (Zhai et al. 2015). Hence, anaerobes tend to have self-regulation on preference pH to suit its growth and development. Buffer was only added at the start and discontinued to leave pH unregulated. This is to evaluate the maximum performance with the self-adjusting anaerobes which favour pH 7 in degradation.

TA concentration with both control and pre-treated substrate was observed well above the optimal >2,000 mg/L for a stable condition for anaerobes to perform in CSTR. Despite the positive TA levels observed, pre-treated substrate showed insufficient TA to provide buffering capacity in neutralising the high VFA content in CSTR. Evidently, it can be seen that CSTR experienced instability as reflected by the

VFA/TA of 0.60 which exceeded the suggested limit of <0.3-0.4 from digester failure (Fannin 1987). Unlike pre-treated substrate, VFA/TA of 0.40 at control is relatively satisfactory. The instability with pre-treated substrate is due to the inadequacy of TA in neutralising the high VFA present in the system. TA was reduced during pre-treatment (prior to ACD) in achieving pH 8 mentioned earlier. Hence, influent TA was lower and it leads to the poor neutralisation of VFA. This also signifies methanogenic inhibition due to the high VFA concentration of substrate itself. Control on the other hand could performed better as influent pH was not regulated, the high TA in substrate itself could provide sufficient buffering capacity in CSTR. Thus, influent pH should not be regulated during pre-treatment in order to preserve the maximum TA during ACD in CSTR.

4.6.4 O&G and TG removal efficiencies

The TG and O&G performances are elucidated in Table 4.19. TG was observed 8% higher with pre-treated substrate as compared to control. Meanwhile, a significant reduction of O&G content was observed with pre-treated substrate, which is 32% higher than control. The optimistic reduction with pre-treated substrate was highly attributed to the high performance during pre-treatment process (prior to ACD) where it has significantly reduced TG and O&G to 60% and 93% respectively. The impressive reduction of TG and O&G with pre-treated substrate under ACD has resulted in an exceptionally satisfactory final effluent after ACD. It is meeting the local discharge limit of <50 mg/L for O&G without the need to go through further treatment steps. This indicates pre-treatment played a big role in the process as it reduced fat levels along with coarse suspended solids making the influent more degradable as evident in the increased of BOD and COD removals.

Table 4.19: TG and O&G removal efficiencies and its effluent concentrations of control and pre-treated substrate in CSTR

	Parameter	Control	Pre-treated substrate
TG	Removal efficiency (%)	72	79
	Effluent concentration (mg/L)	4.53	1.37
O&G	Removal efficiency (%)	74	98
	Effluent concentration (mg/L)	7,388	40

The two steps degradation in this study (pre-treatment followed by ACD) has extensively break down the LCFAs, the major contributor to O&G (Husband 1992). This is because wastes containing emulsified oils can be clarified by coagulation and the emulsion is broken through the addition CaCl_2 , which is often the coagulant choice for soap and detergent manufacture wastewater (Wang et al. 2005). CaCl_2 has been used in the treatment where soapy constituents are precipitated as insoluble calcium soaps of fairly satisfactory flocculating and settling properties. Treatment with CaCl_2 also can be used to remove practically all grease and suspended solids and a major part of the suspended BOD (Wang et al. 2005). Therefore, the CaCl_2 coagulant added during pre-treatment in this study acts as an emulsion breaking agent for oily wastes besides reducing SO_4^{2-} and soap contents. The high O&G reduction at 93% during pre-treatment itself has indicates a superior performance. The outstanding results also corresponds to Wang et al. (2005) and Tong et al. (2013) who performed similar pre-treatment in demulsifying heavy oil wastewater. As a result, the pre-treated substrate increased degradation activity in CSTR as observed in this study.

As discussed in Section 4.4.4, the high O&G concentration in final effluent recorded at 7,205 mg/L in CSTR (without pre-treatment of GP) may be one of the factors obstructing methanogenesis due to the presence of high LCFAs that often

hinder the cell wall/membrane of anaerobes. However, with pre-treated GP, even the effluent concentration of O&G produced as low as 40 mg/L, the biogas production profile still does not correspond to the outcome of O&G. This proves that LCFAs may not be the inhibition factor for methanogenesis.

4.6.5 SO_4^{2-} and S^{2-} concentrations

The average effluent concentrations and removal efficiencies of SO_4^{2-} and S^{2-} are reflected in Table 4.20. It was observed that despite the lower removal efficiencies recorded, pre-treated substrate achieved lower SO_4^{2-} and S^{2-} final concentrations as compared to that in control. The great reduction was contributed by pre-treatment (prior to ACD), the process has significantly brought down SO_4^{2-} concentration to 114 mg/L (97% reduction) in average before ACD is commence. NOT TALLY with table 4.20 The results corresponds to the studies done by Benatti et al. (2009) where authors suggested the precipitation of CaCl_2 could reduce SO_4^{2-} content up to 99% in raw or oxidised wastewater. This happened because precipitation involves charge neutralisation in which a cationic species (Ca^{++}) combine irreversibly with negatively charged contaminants. In this study, CaCl_2 reacts with SO_4^{2-} to form insoluble calcium sulfate which later formed a floc and removed through sedimentation. Hence, the findings suggest that the optimistic removal of targeted compounds was resulted by the effectiveness of pre-treatment.

Table 4.20: SO_4^{2-} and S^{2-} removal efficiencies and its effluent concentrations of control and pre-treated substrate in CSTR

Parameter		Control	Pre-treated substrate
SO_4^{2-}	Removal efficiency (%)	66	55
	Effluent concentration (mg/L)	580	51
S^{2-}	Removal efficiency (%)	71	26
	Effluent concentration (mg/L)	4,565	370

During ACD, SO_4^{2-} and S^{2-} concentrations in pre-treated substrate was further reduced to an impressive final effluent. Unlike control which showed higher final effluent SO_4^{2-} and S^{2-} at 580 mg/L and 4,565 mg/L respectively, pre-treatment clearly an effective contributor in the reduction of both removals. With pre-treated substrate, it can be perceived that the final effluent SO_4^{2-} at 51 mg/L is well below <750 mg/L as advised. This denotes higher conversion of SO_4^{2-} into S^{2-} by SRB and could inhibit methanogenesis. It is also reflected by the high S^{2-} effluent concentration averaging 370 mg/L which unfortunately could not complied to the recommended concentration of <100 mg/L (Celis-García et al. 2004).

Production of S^{2-} has always been a major problem associated with ACD treatment of SO_4^{2-} rich effluent. Although SO_4^{2-} and S^{2-} have been significantly reduced during pre-treatment followed by ACD, the reduction is not sufficient for S^{2-} . As a result, MPB activity was restrained by SRB in this study. The toxicity compelled on MPB by S^{2-} is up to 4 times and 46 times more than the limit with pre-treated substrate and control respectively is considered prone to methanogenesis inhibition (Parkin et al. 1990; Celis-García et al. 2004). The inhibition is caused by the growth kinetics properties SO_4^{2-} reducers which cause substrates degradation favour the SO_4^{2-} reduction pathway more than methanogenesis (Oude Elferink Stefanie et al. 1994). The inhibitory effects in the system by the outcome of the competition are

also mirrored by the poor biogas production rate (0.109 L/day) and its yield (0.081 L/day). This has also been experienced by various studies (Anderson et al. 1982; Parkin et al. 1990; Harada et al. 1994). This study suggested that pre-treatment step was not adequate in reducing S^{2-} in ACD. S^{2-} remained as the major drawback in the generation of biogas despite the high COD removal meeting the target.

4.6.6 Biogas yield, biogas production rate and methane composition

The biogas production was recorded averaging 0.109 L/day and 0.081 L/day at control and with pre-treated substrate respectively at the first 3 weeks. The trend was observed to experience decline in the production from Week 1 onwards and unfortunately no biogas production was generated throughout the experiment after Week 3. Hence no data is able to be captured and presented. The declined biogas generation at the early stage indicates the system was undergoing methanogenesis inhibition, the biogas generated was also very negligible and could not offer meaningful information.

Although pre-treatment has been a great player in the reduction of SO_4^{2-} and soap contents, it does not restore the biogas production problems faced by ACD under CSTR. Under optimum conditions, biogas was failed to be produced in CSTR is likely to be correlated to two aspects: (1) Inhibition of methanogens by the high VFA. The limiting step is either acetogenic or methanogenic. During ACD, the high organic acids present in substrate could not be utilised by acetogens and methanogens. The accumulations of VFA have completely constrained the methanogenic activity resulting in no biogas production in the system, regardless of the pH value. (2) Inhibition of methanogens caused by lethal S^{2-} . S^{2-} is reduced by SRB, the concentration exceeded the safe limit of >100 mg/L is considered corrosive and caused problem of methanogenesis deterioration during ACD (Chen et al. 2008).

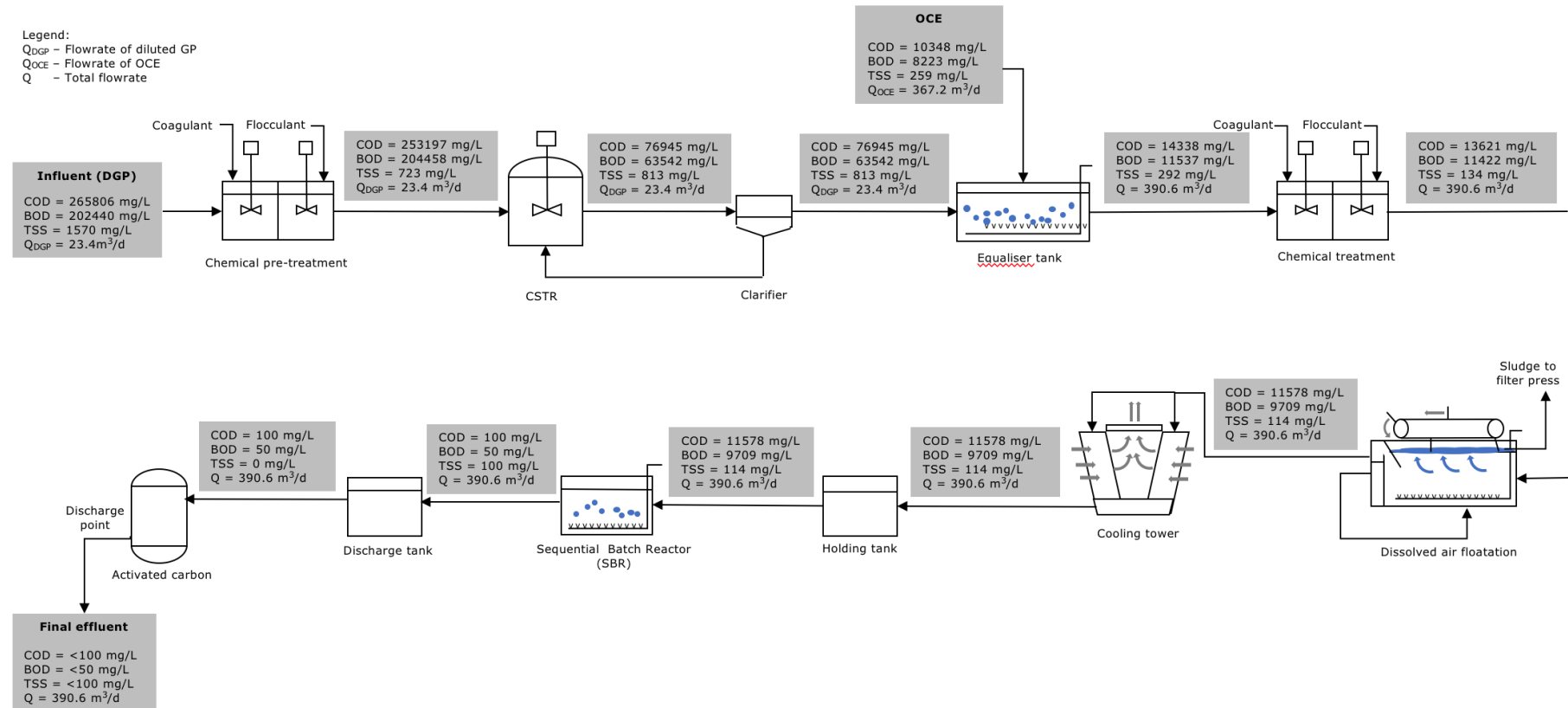
Although pre-treatment have significantly reduced the S^{2-} concentration, it is not sufficient at this stage.

Overall, this study revealed that pre-treatment prior to ACD in CSTR has improved the removal efficiencies of BOD, COD, TG, O&G, SO_4^{2-} and S^{2-} . Specifically, 70% COD removal is regarded as the maximum efficiency of the degradation. The excellent performance is meeting the objective of this research. ACD in CSTR however failed to generate biogas due to the main setbacks discussed. Further exploration in additional treatment step or two stage system (acidogenic reactor followed by methanogenic reactor) in ACD could be an alternative. Such system not only able to facilitate the control of the operational parameters of both reactors separately making ACD pathways more balance but also proficient in increasing the efficiency of conversion of biogas (Luo et al. 2011). However, it may not be necessary because it is clear that the potential of biogas generation is generally low for substrate like GP and OCE as verified in feasibility study. Nevertheless, pre-treatment serves as a great polishing step for substrate with high SO_4^{2-} and soap contents.

4.7 Process solution for GP in oleo chemical industry

Figure 4.25 illustrated a process flow in current oleo chemical industry associates with GP solution. It was shown that ACD of pre-treated GP under CSTR has effectively reduced 70% of COD content. This not only eases the following treatment steps but also minimise the operational problems faced due to shock loading. As a results, final effluent could achieve COD and BOD of <100 mg/L and <50 mg/L respectively and meeting the discharge limit.

Figure 4.25: Process solution for GP in oleo chemical industry



CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

It is proposed that a novel ACD of GP with OCE is effective using CSTR in the present research. Through the investigation of the feasibility performance of ACD, it was found that CSTR system produced better treatment efficiencies in terms of COD, BOD, TSS, TG and O&G (65%, 65%, 61%, 72% and 74% respectively) with the highest GP loading rate of 4 g COD/L.day as compared to UASB (36%, 48%, 0%, 38% and 57% respectively) at OLR 4 g COD/L.day and ASBR (92%, 83%, 0%, 42% and 60% respectively) at OLR 1 g COD/L.day. This is attributed to the CSTR's competency in forming homogenous mixture for immiscible substrates, hence higher ability to cope with high loading rate and overall treatment efficiencies. CSTR system is able to operate in higher stability with highest GP loading rate. The low VFA/TA ratio of 0.40 and high effluent pH of 8.57 observed in CSTR without buffer aid is also cost saving. Effective and optimised operating conditions of ACD treatment system using CSTR with pre-treated GP were proposed conclusively in this research. This is due to its great enhancement in the reduction of overall treatment efficiencies, further boosted COD removal up to 70%, which is regarded as the maximum efficiency of the degradation. The excellent performance has met the objective of this research of >50% COD removal as targeted by the industry. Although 96% of SO_4^{2-} has been removed from substrate, the remaining SO_4^{2-} concentration has surpassed the safe limit for ACD. This could be the major drawback in this research which required further investigation. Nevertheless, the findings in this research have solved the current GP treatment and disposal problems.

5.2 Recommendations

- 1) A scale up analysis of the CSTR is recommended. A pilot plant can be installed in refinery industry for long term assessment on the performance of the system. This pilot plant will be an undertaking that helps in progressing towards commercialising the CSTR which offers effective ACD of immiscible substrate from the refinery and solving the wastewater discharge challenge.
- 2) In order to comprehend the feasibility of CSTR in economical aspect, a full cost and economy evaluations on the industry scale of CSTR can be performed. Comparison can be made with the current GP treatment management adopted by refinery factories. A cost benefit analysis utilising the GP as potential ACD substrate should be included to compute on its potential on a standard shift from sending for incineration/dosing back into existing plants into beneficial utilisation in digesting more than one substrate.
- 3) Due to the biogas challenges faced by GP and in order to have further breakthrough in this research, two stage system model (acidogenic reactor followed by methanogenic reactor) can be built. It is purposed to facilitate the control of the operational parameters of both reactors separately making ACD pathways more balance but also proficient in increasing the efficiency of conversion of biogas.

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LIST OF PUBLICATIONS**INTERNATIONAL JOURNALS**

1. Wei Ling Chow, Yi Jing Chan and Mei Fong Chong. A New Energy Source from the Anaerobic Co-digestion (ACD) Treatment of Oleo Chemical Effluent with Glycerin Pitch. DOI: 10.1002/apj.1902 (Published in Asia-Pacific Journal of Chemical Engineering).
2. Wei Ling Chow, Yi Jing Chan, Mei Fong Chong, Phaik Eong Poh. Co-digestion Studies of Saleable Glycerin with POME and Poultry Manure. (Published in Journal of Engineering Science and Technology).

INTERNATIONAL CONFERENCE AND PROCEEDING

1. Wei Ling Chow, Yi Jing Chan and Mei Fong Chong. A Comparative Study on Co-digestion of Glycerin Pitch with Oleo Chemical Effluent by using Different Reactors. World Sustainable Energy Day (WSED) 2016, Stadthalle Wels Pollheimerstrasse, Wels, Austria (24th – 26th February 2016) (Poster presentation).
2. Wei Ling Chow, Yi Jing Chan and Mei Fong Chong. Energy Potential of Co-digestion Treatment of Wastes from Oleo Chemical Industry. CHEMECA 2014, Perth Convention and Exhibition Centre, Perth, Australia (28th - 1st October 2014) (Oral presentation).

LOCAL CONFERENCE AND PROCEEDING

1. Wei Ling Chow, Yi Jing Chan, Mei Fong Chong and Phaik Eong Poh. Co-digestion Studies of Glycerin with Palm Oil Mill Effluent (POME) and Poultry Manure. 27th SOMChE & 21st RSCE 2014, Taylor's University Lakeside Campus, Selangor, Malaysia (29th - 30th October 2014) (Oral presentation).
2. Wei Ling Chow, Yi Jing Chan, Mei Fong Chong, Balinderjit Singh, Shin Zhi Hii, Sin Yee Ler and Zhi Yao Lim. Performance and Kinetic Evaluation of Sequencing Batch Reactor (SBR) for the Treatment of Anaerobically Digested Palm Oil Mill Effluent (POME). 5th ICENV 2015, Eastin Hotel, Penang, Malaysia (18th - 19th August 2015) (Oral presentation).

LIST OF AWARDS

1. Wei Ling Chow, Yi Jing Chan and Mei Fong Chong. Images of Postgraduate Experience Photography Competition 2015, the University of Nottingham Malaysia Campus, Selangor, Malaysia (20th November 2015) (Image – Overall winner and people’s choice awards).
2. Wei Ling Chow, Yi Jing Chan and Mei Fong Chong. Wastewater Treatment Generates Energy. Research Showcase 2015, the University of Nottingham Malaysia Campus, Selangor, Malaysia (8th May 2015) (Poster presentation – Best poster award).
3. Wei Ling Chow, Yi Jing Chan and Mei Fong Chong. A New Energy Source from Oleo Chemical. Faculty of Engineering Postgraduate Research Showcase, the University of Nottingham Malaysia Campus, Selangor, Malaysia (23rd September 2014) (Poster presentation – First runner-up).
4. Wei Ling Chow, Yi Jing Chan and Mei Fong Chong. A New Energy Source for Oleo Chemical Industry. Malaysia World Water Day 2014 Roundtable Discussion, Concorde Hotel Shah Alam, Selangor, Malaysia (5th June 2014) (Poster presentation – First prize winner).

