BIOGAS PRODUCTION FROM PALM OIL MILL EFFLUENT (POME) USING MONO- AND CO-DIGESTION WITH PILOT-SCALE INTEGRATED ANAEROBIC-AEROBIC BIOREACTOR (IAAB)

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ABSTRACT

In the preliminary stage, start-up, long-term performance evaluation [organic loading rate (OLR) 10.5 gCOD/L.day to 32.5 gCOD/L.day] and kinetic analysis of the pilot-scale IAAB were conducted at mesophilic condition (28°C). Based on the performance studies of the pilot-scale IAAB at different OLRs, the maximum applicable OLR_{an} at which mesophilic system could maintain steady state operation was at OLR_{an} 30.0 gCOD/L.day. The quality of the final treated effluent remained stable with high percent of compliance with the discharge limit of 100 mg/L. Modified Stover Kincannon, Grau second-order and Monod kinetic models were applied to the pilot-scale IAAB at OLR_{an} 10.5 - 32.0 gCOD/L.day. It was concluded that Stover Kincannon model is the most appropriate model to estimate the performance of the anaerobic (R^2 of 0.9982) and aerobic systems of the pilot-scale IAAB (R^2 of 0.9998).

The pilot-scale IAAB was then operated at OLR_{an} 30.0 gCOD/L.day for a period of 118 days to produce a set of reliable data that can be used to convince the palm oil millers to accept the new approach of POME treatment technology. The average overall $BOD_{removal}$, $COD_{removal}$, and $TSS_{removal}$ achieved by the pilot-scale IAAB at OLR_{an} 30.0 gCOD/L.day (corresponding to average OLR_a 2.86 ± 0.37 gCOD/L.day) were more than 99%. This had also resulted in the achievement of BOD <20 mg/L and TSS <400 mg/L throughout the study period with 70% and 100% of compliance, respectively. Overall, operating the pilot-scale IAAB at OLR_{an} 30.0 gCOD/L.day was selected as the most suitable prototype to treat large volume of POME due to its ability to operate at lower HRT, higher overall treatment removal efficiencies (more than 99%), methane yield (0.0980 $LCH_4/gCOD_{removed}$) and purity of methane (60.65%). In order to boost up the biogas generation and achieve high quality of final treated effluent with BOD <20 mg/L, anaerobic co-digestion of *Moringa Oleifera*, a natural coagulant with POME was first conducted at laboratory-scale. Co-digestion of POME with the one-time fed *M. oleifera* filtrate showed an improved 92% $TSS_{removal}$, 94% $BOD_{removal}$ and methane yield of 24.3 mL $CH_4/gCOD_{removed}$. Methanogen bacterial DNAs isolated from POME, granular sludge and anaerobically-treated POME was identified as *Methanoculleus* spp., *Methanolinea* spp. and *Methanoculleus* spp., respectively.

Quantitative polymerase chain reaction (qPCR) conducted confirmed the highest concentration of methanogen DNA in the POME-*M. oleifera* co-digestion (one-time feed). Therefore, this co-digestion system was feasible and efficient in enhancing methane yield (69%) and $TSS_{removal}$ (59.5%), respectively compared to those of mono-digestion. The pilot-scale IAAB was then operated in co-digestion mode for a period of 35 days at OLR_{an} 30.0 gCOD/L.day (corresponding to average OLR_a 3.25 ± 0.30 gCOD/L.day). Throughout the study period, the BOD of the final treated effluent produced during the operation of the pilot-scale IAAB in co-digestion mode exhibited high percentage (74%) of compliance towards the discharge limit of BOD <20 mg/L.

Significant findings were obtained by comparing between mono- and co-digestion studies conducted using pilot-scale IAAB under the same operating condition of OLR_{an} 30.0 gCOD/L.day. *M. oleifera* filtrate provided an improved $BOD_{removal}$ (94-95%), $COD_{removal}$ (93-94%) and $TSS_{removal}$ (89-97%) as compared to mono-digestion ($BOD_{removal}$: 93-94%; $COD_{removal}$: 92-93%; $TSS_{removal}$: 86-90%). The average methane yield and methane purity obtained during co-digestion (0.1353 ± 0.01 $LCH_4/gCOD_{removed}$ and 64 ± 0.44%, respectively) were higher than the mono-digestion (0.0980 ± 0.01 $LCH_4/gCOD_{removed}$ and 61 ± 2.63%, respectively). The incorporation of *M. oleifera* filtrate had increased the biogas production by 72%. Such significant result was due to the protein content in the mixture of the POME and *M. oleifera* filtrate and the coagulant effect exhibited by the shelled of *M. oleifera* filtrate.

Besides, this was also contributed by the balanced carbon-to-nitrogen ratio (C/N) composition exhibited by the mixture of POME and *M. oleifera* filtrate. Most importantly, the addition of *M. oleifera* filtrate into the digestion of POME resulted in the production of final treated effluent with BOD of less than 20 mg/L at a much lower bacteria concentration and higher F/M_a of 18,012 - 20,000 mg/L and 0.185 - 0.187 gCOD/gMLVSS.day, respectively as compared to the mono-digestion. These advantages ascertain the superiority of the pilot-scale IAAB than the laboratory-scale IAAB in terms of POME treatment or other high strength wastewater at a higher OLR_{an} and shorter HRT for the production of final treated effluent with BOD of less than 20 mg/L. Future work will focus on up-scaling the pilot-plant IAAB into commercial plant. This is aimed to evaluate the performance of the commercial plant on the constant fluctuation of the characteristics of POME and at high loading rate. Hence, the data produced can be compared with the existing POME treatment, which will act an alternative for palm oil millers to adopt such technology for POME treatment.

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LIST OF ABBREVIATION

BOD	Biochemical Oxygen Demand
$BOD_{removal}$	Biochemical Oxygen Demand Removal Efficiency
CDM	Clean Development Mechanism
CER	Carbon Emissions Reduction
COD	Chemical Oxygen Demand
$COD_{removal}$	Chemical Oxygen Demand Removal Efficiency
CPKO	Crude Palm Kernel Oil
CPO	Crude Palm Oil
CSTR	Continuous Stirred Tank Reactor
C/N	Carbon to Nitrogen
DGGE	Denaturing Gradient Gel Electrophoresis
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOE	Department of Environment
EFB	Empty Fruit Bunch
EGSB	Expanded Granular Sludge Bed
FFB	Fresh Fruit Bunch
FiT	Feed-in Tariff
F/M	Food-to-Microorganisms
GDP	Gross Domestic Product

GHG	Greenhouse Gas
GLSS	Gas-liquid-solid separator
HRT	Hydraulic Retention Time
IAAB	Integrated Anaerobic-Aerobic Bioreactor
KP	Kyoto Protocol
$Lignin_{removal}$	Lignin Removal Efficiency
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
NKEA	National Key Economic Area
NREPAP	National Renewable Energy Policy and Action Plan
OLR	Organic Loading Rate
OPF	Oil Palm Frond
OPT	Oil Palm Trunk
O&G	Oil & Grease
PCR	Polymerase Chain Reaction
PK	Palm Kernel
PKC	Palm Kernel Cake
PKN	Palm Kernel Nut
PKS	Palm Kernel Shell
PMF	Palm Mesocarp Fibre
POME	Palm Oil Mill Effluent
qPCR	Quantitative PCR
RAS	Returned Activated Sludge
REGEN	Waste Recovery and Regeneration
RFLP	Restriction Fragment Length Polymorphisms

R&D	Research and Development
R_{RAS}	Returned Activated Sludge Ratio
SRT	Solid Retention Time
SS	Suspended Solid
SVI	Sludge Volume Index
TA	Total Alkalinity
TOC	Total Organic Carbon
TN	Total Nitrogen
$TN_{removal}$	Total Nitrogen Removal Efficiency
TP	Total Phosphorus
$TP_{removal}$	Total Phosphorus Removal Efficiency
TS	Total Solid
TSS	Total Suspended Solid
$TSS_{removal}$	Total Suspended Solid Removal Efficiency
TVS	Total Volatile Solid
$TVS_{removal}$	Total Volatile Solid Removal Efficiency
UASB	Upflow anaerobic sludge bed
UASB-HCPB	Up-flow anaerobic sludge blanket-hollow centred packed bed
UASFF	Upflow anaerobic sludge fixed film
UNFCCC	United Nations Framework Convention on Climate Change
VFA	Volatile Fatty Acid
WAS	Waste Activated Sludge

CHAPTER ONE

INTRODUCTION

1.1 Overview of Palm Oil Industry

Biomass energy has emerged as a credible fuel resource. Oil palm processing feed stock is among notable feed stocks that can be used in the production of biofuels, viz. biogas, (Ohimain and Izah, 2014) bioethanol, biodiesel, bio-methanol, bio-butanol, bio-oil, briquette, bio-hydrogen, and bio-electricity. These sources of renewable energy are produced using different approaches of conversion technologies. Global biogas market revenue is poised to exceed USD 110 billion by 2025, as reported in the latest study by Global Market Insights, Inc (Global Market Insights, 2019). Ongoing initiatives toward the utilisation of industrial, municipal and energy crops coupled with growing penetration of biofuels across the transportation sector has created a favourable business scenario. Increasing efforts toward the deployment of sustainable energy technologies along with the introduction of innovative financial instruments, incentives and subsidy schemes will proliferate the business landscape. In addition, ongoing efforts towards reducing out dependence on fossil fuel sources for power generation will enhance the industry outlook (Global Market Insight, 2019).

Biogas is currently the top-notch interest in Malaysia due to its high-quality fuel properties and is able to be utilised in various energy services, viz. heat, electricity and transportation fuel (Mshandete and Parawira, 2009; Ohimain and Izah, 2017). The use of biogas will reduce the demand of fossil fuel, which is the current goals of many countries. This will aid in reducing the environmental impacts, e.g. global warming and pollution, improve sanitation, reduce demand for wood and charcoal for cooking, etc. (Mshandete and Parawira, 2009; Paepatung et al., 2009). Bioconversion signifies biological transformation of waste and/or the reformation of complex organic waste into valuable metabolite using the approach of biological processes or microorganisms (bacteria, yeast, and fungi), which is carried out using anaerobic digesters (Salihu and Alam, 2012; Ohimain and Izah, 2017). It can be used to produce biogas in a large scale with the condition of sufficient volumes of raw materials by utilising anoxic microbial cells to stabilise the organic matters resulting in the production of methane (CH_4) and carbon dioxide (CO_2) as major components in biogas (Irvan et al., 2012; Ohimain and Izah, 2017).

At present, the palm oil industry is being dominated by the Southeast Asia region. Due to its suitability to regional climatic conditions and high yield rates, palm oil is the main biodiesel feedstock in Southeast Asia (Mukherjee and Sovacool, 2014; Iskandar et al., 2018). To date, Malaysia and Indonesia are the world's biggest palm oil producers, where the production by both countries made up 85% of the world palm oil production. In the year 2011, palm oil plantations in both the countries produced over 53 million metric tonnes of palm oil on 16 million hectares of land (Iskandar et al., 2018).

This allows us to infer that palm oil is the key economic driver and a crucial component of gross domestic product (GDP) in both the countries (Nambiappan et al., 2018). It is interesting to note that Malaysia has issued almost ~39% and ~44% of global palm oil production and global export for palm oil, respectively (Iskandar et al., 2018). As reported by Teoh, (2000), the yearly production of palm oil industry in Malaysia will reach ~15.4 tonnes in 2016-2020. As a result, Malaysia will be able to produce 21,000 metric tonnes of palm oil from 453 operating oil mills in Malaysia (245 mills in Peninsular Malaysia and 208 mills in Sabah and Sarawak) (Iskandar et al., 2018). As reported by Foo and Hameed, (2010), every tonne of crude palm oil (CPO) produced from the fresh fruit bunch (FFBs) resulted in approximately 6 tonnes of waste palm fronds, 5 tonnes of empty fruit bunches (EFBs), 1 tonne of palm trunks, 1 tonne of press fibre, 500 kg of palm kernel endocarp, 250 kg of palm kernel press cake, and 100 tonnes of palm oil mill effluent (POME).

In this scenario, POME could be a great source of raw material for biogas production in Malaysia. This will be a great potential for Malaysia to transform the image of oil palm industry to a "green image industry". The continuous utilisation of biomass waste of POME will reduce the dependency on refined petroleum fuel products, boost electricity generation and lower environmental impact (Chotwattanasak and Puetpaiboon, 2011). Chungsiriporn et al. (2006); Tabassum et al. (2015) reported that 1.5 m^3 of water is used to process 1 tonne of FFB and 50% of the water used ends up as POME. The volume of POME generated from the 30-tonne of FFB per hour is 21 m^3/hour of POME (equivalent to 400 m^3/day). It is assumed that the amount of electricity generated from the 30 tonnes of FFB/hour is 1.1 MWe. If it is not managed properly, it could lead to a disastrous impact on the environment (Tabassum et al., 2015). This is because the oxygen depletion of raw POME is 100 times more toxic than that of raw sewage (Madaki and Lau, 2013). Hence, it is not permissible to directly discharge the POME into waterways.

Such amount will be able to generate methane with yearly burning rate of 12.0 million litres of fuel oil (Ohimain and Izah, 2017). Additionally, biogas produced from the covered lagoon system can be a source of fuel for electricity generation. This is supported by the evidence that biogas produced from POME has a higher energy content (34.5 MJ/m^3) as compared to the conventional biogas stove (20 MJ/m^3) (Foo and Hameed, 2010; Ohimain and Izah, 2017). The Malaysian biogas industry has picked up since 2016 driven primarily by the palm oil industry. The government has proposed that all the POME produced has to be digested by means of anaerobic digestion as part of the National Transformation Programme, Key Economic Area and introduction of feed-in tariffs for the electricity produced. As reported by SEDA (2018), the biogas industry has grown by 400% since 2014 as supported by the increased in electricity produced annually. However, there is much still to be done for utilisation of agricultural, municipal and industrial organic waste. With the palm oil industry growing year on year, capturing methane from POME presents a very unique opportunity for country like us. The approach of anaerobic digestion has been successfully carried out in Serting Hilir Palm Oil Mill (1.2 MW of electricity per hour is produced from 770 m^3/day of POME) and Tanah Makmur Biogas Power Plant at Sri Jelutung Palm Oil Mill (total electricity generated since October 2017 is ~13.4 million kilowatt-hours, which is sufficient to supply electricity to 3,400 homes) (Jain, 2019). It is estimated that ~RM 25 million is required for a palm oil mill to treat the POME produced. Malaysia possesses a huge potential on renewable energy resources. If all the POME produced is being utilised in a proper way, Malaysia can fulfil its total energy need in a sustainable manner.

POME is generated from three different sources, namely steriliser condensate, decanter or separator sludge and hydrocyclone waste (Table 1.1). It is a type of high strength wastewater which comprises of 95%-96% water, 0.6%-0.7% oil, 4%-5% total solids (TS) (including 2%-4% suspended solids (SS)), and high concentration of organic nitrogen (Khalid and Mustafa, 1992; Iskandar et al., 2018). Such characteristics of POME may not be similar during the high and low crop seasons, which will affect the operations and quality control of a processing plant, resulting in different biogas production rates. The typical texture of POME is thick, brownish, colloidal slurry of water from the crushing of the palm fruit mesocarp.

It has a foul smell with organic materials, viz. lignin, carotene, phenolic and pectin with concentrations of 4,700 ppm, 8 ppm, 5,800 ppm, and 3,400 ppm, respectively (Ho et al., 1984; Sundram et al., 2003). These will be converted into biogas through the interactions of microbes and other factors that may influence the production under anoxic condition – anaerobic digestion. Furthermore, if the POME is discharged without prior treatment, the biochemical oxygen demand (BOD) produced in year 2009 was estimated to be 1,095 million kg. In another words, the BOD value produced is equivalent to the waste generated by 75 million people, which is nearly thrice the current population in Malaysia (Ahmad and Chan, 2009).

Table 1.1: The characteristics of raw POME (Loh et al., 2017)

Parameter	Unit	Average	Range
pH	-	4.5	3.3-5.7
Biochemical Oxygen Demand	mg/L	21,060	18,225-23,900
Chemical Oxygen Demand (COD)	mg/L	50,340	45,818-54,861
Suspended Solid	mg/L	27,883	24,846-30,920
Oil & Grease (O&G)	mg/L	7,213	5,614-8,812
Volatile Fatty Acid (VFA)	mg/L	1,060	1,057-1,073

1.2 Journey of Palm Oil Mill Effluent (POME) Treatment

It is essential to come up with a solution to treat POME effectively to minimise any environmental pollution associated with POME. Biogas production technology has been utilised in the past decades for the purpose of converting different types of biomass wastes into valuable added products without the presence of oxygen. This is due to the higher cell retention times of 4-10 folds greater than those technologies that operated under aerobic treatment manner. Additionally, the microbial cells used for biogas production, i.e. methanogenesis is mostly present under anaerobic environment. As a result, anaerobic digestion technologies have outweighed the aerobic digestion technologies in importance. When POME is used as a source of substrate for biogas production, it will typically produce biogas consisting of CH_4 and CO_2 in 65% and 35% proportions, respectively (Sridhar and AdeOluwa, 2009; Ohimain and Izah, 2014). It is worth noting that the methane produced from the anaerobic digestion of POME has a great potential for power generation using gas engine. Sridhar and AdeOluwa, (2009) reported that typical production of biogas during the high crop season of FFB ranges from 20 m^3CH_4/m^3 biogas to 28 m^3CH_4/m^3 biogas. Such amount of biogas produced is able to generate approximately 36kWh to 50.4kWh of electricity, which is equivalent to 18% to 25% power generation efficiency. Ugoji (1997) stated that the use of anaerobic digester in treating POME is able to produce 2.4cm^3 of biogas/m^3 of digester vol/day. The power generated from POME can be sold to local national grid for domestic, industrial or commercial purposes.

The Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations in 1977 was enacted under the Environment Quality Act of 1974, Department of Environment (DOE) Malaysia (DOE, 1977). This enactment describes the characteristics and standards of POME to be discharged into the watercourses after the treatment. As a result, all the industries involved in CPO production were required to develop methods to treat POME prior to discharge into streams. Additionally, the stringent discharge limit set by the DOE especially in terms of BOD has further emphasised the importance of POME treatment, which has drawn attention from many researches to lower the BOD of mill discharges. To date, the BOD discharge limit has been reduced from 100 to 20 mg/L, especially in very sensitive areas such as Sabah and Sarawak. Thus, this has drawn the attention of many palm oil millers to look for an effective POME treatment system that will allow them to reach the BOD of 20 mg/L constantly.

Sporadic researches have been conducted on the methods of POME treatment. Open lagoon ponding system is the most conventional way for treating POME and more than 85% of palm oil mills in Malaysia have adopted this method in treating POME (Madaki and Lau, 2013). Open lagoon ponding system is normally inclusive of sand and oil traps, cooling ponds, acidification ponds, anaerobic ponds, facultative ponds and aerobic ponds. Anaerobic ponds are able to digest high amounts of solids and are inexpensive as no aeration is involved during the process. Additionally, the utilisation of the microbial cells in the anaerobic pond will result in production of pre-treated water that has high percentage of nitrogen and phosphorus that can be used as a source of fertiliser. The anaerobic digestion of the POME is able to produce effluent with low surpluses of sludge (5-10%) (Rahayu et al., 2015). Yacob et al. (2006) concluded that open lagoon ponding system recorded higher organic conversion efficiency (97.80%) than open digesting tank whereby every kilogram of COD removed, 237g of methane will be produced or 12.36kg of methane/tonne of POME at a hydraulic retention time (HRT) of 40 days. This treatment also results in the anthropogenic release of greenhouse gases (GHG), such as CO_2 and CH_4 gases, which are recognised as one of the main causes of global warming that results in climate changes around the world (Madaki and Lau, 2013). Even though high COD removal efficiency ($COD_{removal}$) is achieved using this method, some drawbacks such as long HRT and huge land area required for the pond (1-5 ha), frequent solid accumulation at the bottom of the pond, and the inability to capture the release of biogas have resulted in continuous studies being carried out to improve the present system.

In recent years, covered lagoon technology is improvised and showcased some successful track record in biogas capturing. This improvement has resulted in effluent produced with less than 5,000 mg/L COD (at least 90% $COD_{removal}$). Additionally, the improved system has built-in gas-tight cover that carries a function of capturing the released biogas during the anaerobic digestion process. Most importantly, the investment cost of covered lagoon is lower than the aforementioned technologies (Loh et al., 2017). Therefore, there are currently more than 19% of the palm oil mills adopting covered lagoon system. (Loh et al., 2017; Choong et al., 2018 and Yap et al., 2018).

However, the effluent produced after anaerobic digestion still contained high concentrations of solids that require further treatment to reduce the solid concentration. As reported by Shirai et al. (2003), the use of anaerobic covered lagoon enables the system to capture at least 45% of the CH_4 gas. Despite the numerous set ups of covered lagoon in Malaysia, there is scarcity of published data on their actual treatment performances and biogas yield. The limitation of lands available for POME treatment has pushed the industry to look for other alternative treatment aiming to reduce the carbon footprint. Efforts have been made for better environmental management of the palm oil industry in our country since 1980's. Based on the increase in annual production of POME produced by all the palm oil mills in Malaysia, coupled with the more stringent BOD discharge limit, it is no doubt that the conventional ponding system or covered lagoon technology is not sufficient to treat the POME. In order to comply with the new BOD discharge limit (20 mg/L), improved high-rate anaerobic bioreactor equipped with biogas capturing system such as hybrid plug-flow system of up flow anaerobic sludge beds (UASB) and expanded granular sludge bed (EGSB), and closed anaerobic reactor equipped with continuous stirring device have been adopted in the treatment of POME (Loh et al., 2017; Yap et al., 2018). These systems are able to achieve higher treatment efficiency with BOD/COD removal efficiencies of 80%–90% at low HRT (Loh et al., 2017). However, these systems require high capital investment, operating and maintenance costs and also suffers from some operational issues such as scum formation, sludge washout and low $TSS_{removal}$.

1.3 Challenges in POME Treatment

There are many challenges faced in the operation of POME biogas plants. The main challenge lies within the characteristics of POME itself which contains high concentration of TSS and O&G (Loh et al., 2017). The high TSS concentration in POME causes the liquid to be viscous (Poh et al., 2010) and intense mixing is required to ensure the maximum contact between the TSS with those bacteria suspended in the anaerobic sludge. The high content of O&G will solidify once the temperature of POME is being decreased to less than 40 °C and in contact with the mixture in the anaerobic digester. These solids will form a scum layer floating on top of the liquid surface of the reactor whilst making it difficult to be digested by the bacteria which mostly suspended at the bottom of the reactor. It is postulated that if the TSS and O&G can be coagulated and settled, efficient contact with the anaerobic sludge can be established to enhance the digestion efficiency of these components. This will prolong the retention time of TSS and O&G in the reactor for efficient conversion from complex matters into soluble matters in a simpler form. The scum formation can be minimised or eliminated too.

Though POME can be used as a sole substrate for anaerobic digestion to produce methane, the drastic fluctuation of POME, the quantity of feed-stock, inconsistent carbon to nitrogen (C/N) ratio, poor buffering capacity, and low pH of the substrate may be the limiting factors for low yield of biogas production from single digestion of the substrates (Demirel and Scherer, 2008 ; Sidik et al., 2013; Nurliyana et al., 2015).

In order to improve the biogas yield rate for anaerobic digestion of organic matter or biomass, co-digesting more than one type of organic matter would be able to complement the missing nutrients in the anaerobic digesters (Mata-Alvarez et al., 2000; Sidik et al., 2013).

1.4 Anaerobic Co-Digestion

Co-digestion, as suggested by various researchers viz. Demirel and Scherer, (2008); Sidik et al. (2013); Nurliyana et al. (2015), may provide an intermediate breakdown of the homogenous mixture to overcome the inhibition conditions, e.g. high nitrogen content, lack of trace elements or overload of light metal ions. To date, anaerobic co-digestion involving variety of substrate can be used as a recipe to improve the biogas yield as well as stability of the anaerobic process. Additionally, the use of more than one substrate for anaerobic digestion is able to balance up the C/N ratio, which is vitally important during the start-up of the digestion. For instance, study conducted by Nurliyana et al. (2015) showed that the co-digestion of POME and EFB with C/N of 45 was able to increase methane production from 0.17 to 2.03 L CH_4. Nonetheless, co-digestion process of POME with substrate of high carbon contents and buffering capacity is yet to be an attractive option to provide an optimum solution that will improve the methane production efficiency.

Moringa oleifera is commonly known as the 'horse-radish' tree or 'drumstick' tree with most of its parts useful for various applications. In Sudan, it has been traditionally used in water purification (Ndabigengesere and Subba Narasiah, 1998). Economic viability and environmental side effects of aluminium and iron salts have sparked the interest in the use of natural and organic coagulants from plant materials, especially *Moringa oleifera* seed (Sutherland et al., 1994; Muyibi and Okuofu, 1995; Ndabigengesere et al., 1995; Ghebremichael et al., 2005; Bhatia et al., 2007; Sarpong and Richardson, 2010; Sánchez M et al., 2012). *M. oleifera* extract has shown to have large coagulating effects on total suspended solid removal efficiency ($TSS_{removal}$) and $COD_{removal}$ with least 95% and 50% respectively (Bhatia et al., 2007a; Othman et al., 2008). Additionally, effluent treated with *M. oleifera* extract produced smaller volume of sludge as compared to conventional coagulant (Bhatia et al., 2007a), which can be used as a source of fertiliser or animal feed. *M. oleifera*, as a carbon source of 54.8%, will be able to complement the high nitrogen content in POME of 2.71% in the digestion process (Ndabigengesere et al., 1995; Baharuddin et al., 2010). Hence, co-digestion of substrates with high carbon content is able to support the growth of microbes that responsible for the digestion process as well as increasing the biogas yield.

1.5 Methanogen

Methanogens or methanogenic archaea are vital in the anaerobic microbial degradation of organic waste with the resultant production of methane. However, despite their key role as the terminal oxidisers in a complex microbial community, controlling the flux of carbon through the anaerobic degradative pathway still remained an unresolved mystery for the treatment of all kinds of wastewaters.

Very little is known about the methanogen community structure due to the difficulty in cultivating their growth or absence of rapid detection and identification tools to study their community in depth. They are the members of the domain Archaea placed together with halophilic and thermophilic archaea are known to produce large amount of atmospheric methane. To date, there are only five orders of methanogens that were described (*Methanosarcinales*, *Methanopyrales*, *Methanococcales*, *Methanomicrobiales*, and *Methanobacteriales*) (Hales et al., 1996). Methanogens are non-culturable and require specific laboratory settings (Widdel, 1986; Wolfe and Metcalf, 2010; Kenters et al., 2011; Wolfe, 2011; Khelaifia et al., 2013). Since they are the major producer of methane gas, it is essential to understand the mechanism of methanogenesis especially the surviving nature of methanogen during the anaerobic digestion. This will be carried out by adopting molecular techniques, viz. restriction fragment length polymorphisms (RFLP), denaturing gradient gel electrophoresis (DGGE), polymerase chain reaction (PCR)-based techniques that mainly target the 16S rDNA for identification purposes coupled with quantitative PCR (qPCR). These methods as compared to the conventional ones (cultivation manner) will aid in reducing the overall time as well as any external factors that may inhibit the growth of methanogen whereby DNA of the methanogen will be subjected for further treatments.

1.6 Problem Statements

As mentioned in Section 1.3, the main challenge lies within the characteristics of POME itself that contains high concentration of TSS, as well as O&G (Loh et al., 2017). POME has a typical range of 28,846 mg/L to 30,920 mg/L and 5,614 mg/L to 8,812 mg/L for TSS and O&G respectively (Loh et al., 2017). The treatment of POME in all palm oil mills in Malaysia are the major contributor of GHG due to the released of methane gas into the environment from anaerobic ponds or open digester tanks (Yacob et al., 2005; Nurliyana et al., 2015). It is dubbed as one of the most threatening GHG as it is estimated that methane has the ability to capture heat 25 to 30 times more effective than carbon dioxide. However, it is an important energy source if it is captured and utilised. The issue of green environment and depletion of non-renewable fossil fuels have attracted the use of methane produced during POME treatment as a new source of energy. Despite the attractive incentive schemes offered by the Malaysian government, small holders or private millers still have reservations installing biogas trapping or methane avoidance facilities in their palm oil mills due to: (a) slow rate of installation of biogas plants; (b) limited use of the surplus energy generated; (c) tedious procedural requirement related to grid interconnectivity matters, and; (d) limited biogas quota (Loh et al., 2017). The issue of stringent discharge limit of BOD 20 mg/L and limited land has encouraged the research and development (R&D) in POME treatment in Malaysia towards the direction of anaerobic-aerobic treatment processes using closed high-rate bioreactor system.

Integrated Anaerobic-Aerobic Bioreactor (IAAB) is a type of high-rate bioreactor that combines both the anaerobic and aerobic processes. The IAAB was designed by Chan et al. (2012), as shown in Figure 2.17 (Chapter 2, Section 2.7.1). The design of the anaerobic compartment of the IAAB incorporates the technology of up-flow anaerobic sludge fixed film (UASFF), which is the combination of up-flow anaerobic filter and up-flow anaerobic sludge blanket (UASB) (Chan et al., 2012). Besides that, the technology deployed in the aerobic compartment of the IAAB is the activated sludge technology. The IAAB system designed by Chan et al. (2012) has its own uniqueness as compared to the other high-rate bioreactor system (Chan et al., 2009). The uniqueness in the anaerobic compartment of the IAAB are (a) sludge recycling (b) development of sludge bed (c) Middle portion of the IAAB: able to retain biomass (d) Top portion of the IAAB: separation of biogas and washed-out solids from the liquid phase (Chan et al., 2012). The uniqueness of the aerobic and sedimentation system of the IAAB are (a) sludge recycling (b) formation of flocculent settleability solid that can be removed by gravity settling in the settling tank. The remarkable performance of the IAAB obtained during the treatment of POME (high strength wastewater) with organic removal efficiencies of at least 90% (Chan et al., 2012a) mainly contributed by the aforementioned uniqueness present in both the compartments, i.e. anaerobic and aerobic. This allow us to infer that the configuration of the IAAB, i.e. anaerobic process followed by aerobic process is one of the most effective way in handling high strength wastewater (POME).

IAAB has a great potential in treating industrial type of high strength wastewater to achieve high removal efficiencies of at least 90%. It may be an alternative for the conventional anaerobic-aerobic system, viz. ponding system as it does not require large land area, reduction in carbon footprint, able to trap methane gas produced during anaerobic digestion, able to retain high biomass concentration in the anaerobic compartment, reduction of organic and inorganic matter in the wastewater (with removal efficiencies of at least 90%), and able to produce final treated effluent that abides the discharge limit. Despite the positive results obtained during the study conducted by Chan et al. (2012), it is never easy to commercialise this high-end prototype in the industry. This is because (a) large investment cost is required; (b) lack of data to convince the palm oil millers to accept the new technology as the developed prototype was mainly tested in the laboratory stage.

The optimum operating conditions and reaction kinetics of the laboratory scale IAAB have been comprehesively investigated by Chan et al., (2012). However, there are other factors that may change during the transformation to full scale plant such as fluid dynamics and homogeneity in the IAAB. It is difficult to predict the behavior of the anaerobic digestion process and the effects of different operating parameters on biogas production in full scale operation. Therefore, pilot scale investigation is necessitated to understand the anaerobic digestion process performance under dynamic organic loading conditions, and thus the limits for acceptable industrial operative settings can be identified (Cabbai et al., 2016). Most importantly, it can demonstrate the commercial feasibility of the IAAB system. Therefore, The IAAB used in this study is in the pilot-scale configuration, which was placed at one of the palm oil mills in Malaysia. This is to identify the performance of the pilot-scale IAAB under the condition of constant fluctuation of the characteristics of the POME produced. The data produced in this study will become the initial move for the palm oil millers to adapt such approach in replacing the conventional treatment system.

Although POME can be used as a sole substrate for anaerobic digestion to produce methane, the drastic fluctuation characteristics of POME, the quantity of feed-stock, inconsistent C/N ratio, poor buffering capacity, and low pH of the substrate may be the limiting factors for low biogas yields from single digestion of substrates (Demirel and Scherer, 2008; Sidik et al., 2013; Nurliyana et al., 2015). In order to improve the biogas yield for anaerobic digestion of organic matter or biomass, co-digesting more than one type of organic matter will complement the missing nutrients in the anaerobic digesters (Mata-Alvarez et al., 2000; Sidik et al., 2013). This is performed using a laboratory-scale anaerobic digestion to determine the effects of *Moringa Oleifera* towards the treatment of POME to determine the optimised anaerobic operating condition. This will be implemented in the pilot-scale IAAB, which is expected to reduce the solid concentration in the POME, as well as increasing the biogas yield.

1.7 Research Objectives

The general objective of the entire study is to determine the optimum operating parameters to produce final treated effluent with BOD 20 mg/L by using the integrated anaerobic-aerobic bioreactor (IAAB) in the pilot scale. The pilot-scale IAAB is aimed to achieve high COD, BOD and TSS removal efficiencies, high methane yield with at least 60% methane purity, and generate the final treated effluent to the EQA 1974 requirement. Two conditions were proposed in this study, mono-digestion and co-digestion.

The specific objectives of this research are as follows:

1. To compare two different anaerobic systems for POME treatment by obtaining a complete, up-to-date and representative characteristics of POME, anaerobically-treated effluent, aerobically-treated effluent, and final discharge effluent.
2. To determine the optimum concentration of *M. oleifera* extract for co-digestion by conducting jar tests followed by identifying the most suitable co-digestion condition by performing a laboratory-scale study.
3. To identify and quantify the methanogen in each treatment using Sanger Sequencing and real-time/quantitative polymerase chain reaction (qPCR).
4. To optimise the performance of the pilot-scale IAAB at OLR 30.0 gCOD/L.day for the achievement of BOD 20 mg/L.
5. To evaluate the performance of the pilot-scale IAAB by co-digesting *M. oleifera* extract with POME at OLR 30.0 gCOD/L.day.

1.8 Research Scope

The following research scopes are carried out to ensure that the research objectives (Section 1.7) are achieved. In order to compare the performance of different POME treatment systems, characterisation of raw POME, anaerobically-treated POME, aerobically-treated POME and final treated effluent were conducted at three different palm oil mills. Several important parameters, viz. COD, BOD, TSS, total volatile solid (TVS), total nitrogen (TN), total phosphorus (TP), lignin, O&G, and pH were measured in the characterisation study. This is aimed to provide an up-to-date and representative POME characteristics and contaminants dedegradation performance of the three commonly used POME treatment methods in Malaysia. It will also aid in method selection and equipment sizing for POME as well as other industrial-scale wastewater treatment and management. Following this, the operation of the pilot-scale IAAB was initiated by inoculating anaerobic sludge collected from the anaerobic pond that treats POME. The aerobic compartment was inoculated with the acclimatised activated sludge obtained from the aerobic pond that treats anaerobically-treated POME. After the completion of the start-up process, a long-term performance evaluation on the pilot-scale IAAB towards the approach of discharge effluent of BOD 20 mg/L was carried out at various organic loading rates (OLR) to identify the most significant dependant factors that will affect the performance of anaerobic and aerobic systems. OLRs, mixed liquor suspended solid (MLSS) concentration, food-to-microorganism (F/M) ratio, pH, and dissolved oxygen (DO) concentration will be investigated and defined from the long-term performance study of the pilot-scale IAAB. Additionally, the approach of kinetic modelling will be used in this present study to describe the specific parameters that govern the performance of the system and to estimate the treatment efficiencies of full- scale reactors using the same operating conditions. This study will demonstrate how the significant parameters affect the performance of the pilot-scale IAAB towards BOD 20 mg/L.

The performance of the pilot-scale IAAB was assessed based on the overall BOD, COD, TSS, TP, TN, and lignin removal efficiencies, methane production rate, methane composition, methane yield, effluent pH, VFA, and total alkalinity (TA) concentrations. The kinetic constants obtained from the anaerobic and aerobic processes may act as a yardstick to describe and predict the performance of the pilot-scale IAAB system. Additionally, the obtained kinetic constants from both the processes can be used as a comparison tool with the laboratory IAAB. The experimental results obtained from these performance studies were able to fit into several established mathematical models, viz. Monod, Stover-Kincannon, Grau second-order, and Contois kinetic models, to obtain suitable substrate removal kinetic models and relevant kinetic constants under mesophilic condition. Secondly, a comprehensive optimisation study on the operating conditions was carried out aiming to achieve stable performance of the pilot-scale IAAB in terms of overall COD, TSS, and BOD removal efficiencies of more than 98%, high purity of methane gas of more than 55% and final discharge effluent that has BOD of 20 mg/L.

In order to increase biogas yield, as well as producing final discharge effluent with on-spec BOD (< 20 mg/L) and TSS (< 50 mg/L), the concept of coagulation was taken into consideration. In this study, *M. oleifera* was chosen to co-digest with POME. A jar test was conducted to determine the most optimum concentration required to coagulate the POME. The performance of the jar test was determined based on the COD, TSS, BOD, and O&G removal efficiencies, as well as its pH. Following this, a laboratory-scale anaerobic digestion was utilised to evaluate the performance of the anaerobic system in terms of BOD, COD and TSS removal efficiencies, which is aimed to determine the optimum co-digestion conditions. The optimised operating parameters obtained was carried out using the pilot-scale IAAB, where its performance was evaluated in terms of BOD, COD and TSS removal efficiencies, methane production rate, methane composition, methane yield, effluent pH, VFA and TA concentrations. The performance of the mono- and co-digestion of the pilot-scale IAAB were compared.

1.9 Thesis Organisation

The summary of the entire research aiming to promote the usage of green energy and produced final treated effluent with BOD < 20 mg/L and TSS < 100 mg/L using the approaches of pilot-scale IAAB is illustrated in the flowchart as shown in Figure 1.1.

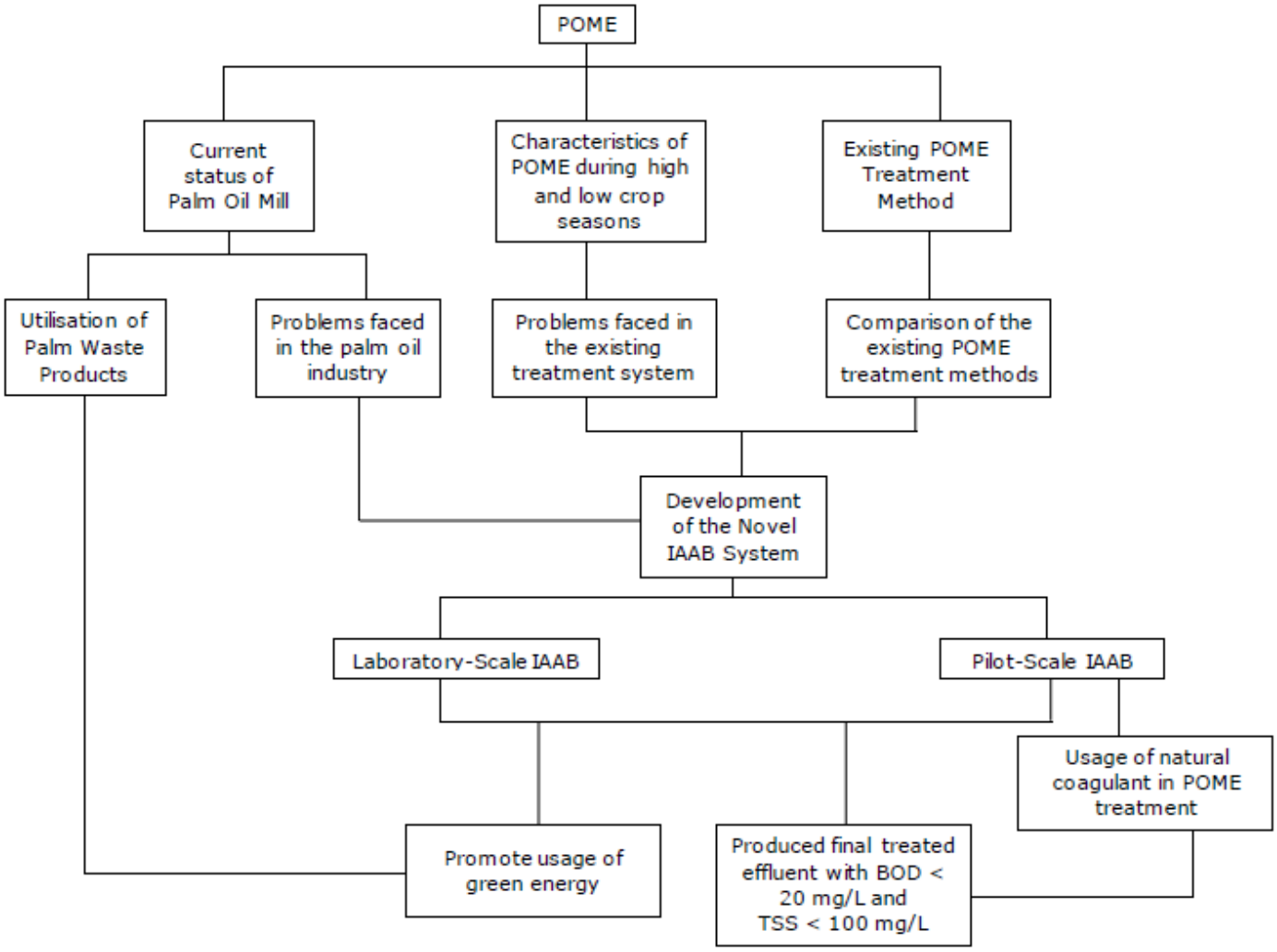


Figure 1.1: Summary of the entire research study using the pilot-scale IAAB

This report consists of six chapters. Chapter One (Introduction) briefly explained the current status of palm oil industry in Malaysia, source and characteristics of palm oil mill effluent (POME) at low and high crop seasons, obstacles faced in the treatment of POME, way to improve the existing anaerobic digestion, i.e., anaerobic co-digestion, and, methanogen identification in the anaerobic digestion process. This chapter also includes problem statements consisting of the basis and rationale during the identification of the research direction. Additionally, the background of the high-rate bioreactor, i.e., integrated anaerobic-aerobic bioreactor (IAAB), potential and challenges in commercialising the IAAB in the palm oil industry was also briefly explained in this chapter as well. This was then followed by the objectives presented with the research scope. An organisation of this thesis was also summarised in the final section of this chapter.

Chapter Two (Literature Review) covers the review of the palm oil industry in Malaysia mainly consisting of utilisation of the waste product, viz. empty fruit bunch (EFB) and POME by converting them into valuable added product. The usages of these products in terms of power generation were highlighted in this section as well. The obstacles faced by the palm oil industry in developing the palm biomass sector for sustainable development in Malaysia was also discussed in the categories of biogas (bio-energy) and value-added product. The design concept of the physical configuration of the laboratory-scale IAAB and the results obtained were discussed thoroughly. The design feature of the pilot-scale IAAB was deliberated thoroughly together with the process flow diagram (PFD). The development of kinetic model for POME treatment which would be used to determine the efficiency of the respective POME treatment used, i.e., conventional anaerobic-aerobic treatment and IAAB systems. Parameters and ways to overcome the methanogen inhibition that occurred frequently during the anaerobic digestion were also presented. Lastly, the concept of anaerobic co-digestion which would act as an alternative for the existing anaerobic digestion together with the choices of co-substrates available were also presented.

Chapter Three (Materials and Methods) provides the list of all the materials, chemicals and analytical methods used in to conduct the present study. This was followed by experimental procedure inclusive of sample analysis, performance evaluation study and kinetic analysis. An experimental flowchart that illustrates a clearer picture on the experimental works involved was also presented.

Chapter Four (Results and Discussion) is the core of this thesis with three main studies. The first section of this chapter thoroughly compares the existing industrial scale anaerobic systems used for the treatment of POME. This was aimed to identify the most suitable type of industrial scale anaerobic system for the treatment of POME with constant fluctuation of its characteristics and optimum parameters that allow the production of final discharge effluent that abides the discharge limit set by the DOE, Malaysia. The results of the characterisation of raw POME, anaerobically-treated POME and aerobically-treated POME based on the analysis methods were presented.

The second section discussed the anaerobic co-digestion of POME with *M. oleifera* extract performed at laboratory-scale. The optimum concentration of *M. oleifera* extract to coagulate the solids in POME was carried out using the concept of jar test. The determined optimum concentration of *M. oleifera* extract was then applied to the anaerobic laboratory-scale study aiming to compare the performance of mono- and co-digestion of POME with or without *M. oleifera* extract. The third section of this chapter presented the start-up, steady state performance, kinetic evaluation, mono-digestion, and, co-digestion carried out using the pilot-scale IAAB under mesophilic condition. The detailed start-up procedure for each process, i.e. anaerobic, aerobic and sedimentation were presented and discussed. The maximum loading capacity and kinetic parameters of the pilot-scale IAAB using several established mathematical models, viz. Modified Stover-Kincannon, Monod, Contois, and Grau Second-Order were determined and discussed. The performance of the pilot-scale IAAB were carried out for the mono- and co-digestion assay at the optimum anaerobic OLR and concentration of *M. oleifera* extract. In the last section, the performance of the pilot-scale IAAB at mono- and co-digestion assay were compared to determine the most suitable POME treatment method.

Chapter Five (Conclusions and Recommendations) concludes the work that has been carried out and the important outcomes obtained from this research study. The conclusions reflect the achievements of the listed objectives that were obtained throughout the study. Finally, the recommendations for future study were listed as well. These recommendations were presented in view of their significance and importance related to the current research.

Chapter Six (References) contained the list of references used for this study.

Chapter Seven (Appendices) contained the list of tables and figures that supports this study.

CHAPTER TWO

LITERATURE REVIEW

Oil palm, scientifically known as *Elaeis guineensis Jacg*., produces two distinct types of oils: CPO from the mesocarp, and crude palm kernel oil (CPKO) from the kernel. Such oils possess excellent cooking properties, and they are widely used for both edible (e.g. cooking oil, margarine, shortening, butter, etc.) and non-edible applications (e.g. soap, cosmetics, detergents, etc.) (Yap et al., 2018). Palm oil was the most consumed vegetable oil in 2015/16, accounting for 60.96 million tonnes (Mt) out of 178.31 Mt total vegetable oil produced globally. In addition, the consumption of palm oil is projected to increase and it is critical to have sustainable production to support the world demand. Malaysia is the second largest palm oil producer, after Indonesia, in the world with 5.74 million hectares of land planted with oil palm in 2016 (MPOB, 2017). Currently, it forms more than half of the agricultural land use in Malaysia, covering 24% of land mass (MPOB, 2015). The palm oil industry in Malaysia accounts for approximately 7% of total Malaysian GDP in 2014. It has faced many challenges that cause the reduction of overall productivity, which include climate change, pests, and diseases. Monoculture of oil palm, high usage of chemical fertilizers, underutilizing biomass generated, and high concentrations of pollutants in the milling wastewater are among the sustainability issues faced by the palm oil industry.

The primary palm products are CPO and CPKO that are extracted from the palm FFB and palm kernel (PK), respectively. Malaysia produces approximately 17.3 Mt of CPO in 2016 (MPOB, 2017). Due to the high production of CPO, a large quantity of biomass and POME are generated. For every unit of FFB processed in the palm oil mill, approximately 22% of the weight of FFB is generated as CPO and approximately 70% of the weight of FFB is generated as by-product/waste. This "by-product" or "waste" from the palm oil mill has been identified as one of the main biomass sources in the country. The palm oil waste, which is generally known as oil palm biomass (hereinafter to be known as palm-based biomass), includes palm EFB, palm mesocarp fiber (PMF), palm kernel shell (PKS), palm kernel cake (PKC) and POME. Malaysia generated approximately 80 Mt (dry weight basis, dwb) in 2014 of oil palm biomass (Ng et al., 2012; Loh, 2017). This value indicates the enormous opportunities and green potentials for the palm oil industry in the country.

2.1 Palm Oil Industry

In general, palm oil industry can be divided into plantation (agronomy and estate management), processing (milling and refinery) and supply chain management (consumer and transportation). FFB is harvested from plantations and processed in palm oil mills to extract the CPO. The oil is sent to refineries to convert into other palm-based products (shortening, soap, cosmetics, etc.). A huge amount of biomass and wastewater are generated in the process.

Figure 2.1 shows the typical CPO milling process. Palm fruit arrives at palm oil mills in bunches, which are known as FFB. FFB consists of fruits embedded in spikelets that grow on the main stems. FFB is first sent to a steriliser for cooking. They are heated under pressurised steam (approximately 3 bars) to weaken the fruit stems and to denature the oil-splitting enzymes in fruits that promote hydrolysis and autoxidation. After sterilisation, FFB is sent to the threshing unit. In this unit, fruitlets are detached from the main stems, leaving behind spikelets on the stems which are generally called EFB (Liew et al., 2015).

The loose fruitlets are then sent to the extraction unit. These fruitlets are first mechanically pressed to extract the oil. This oil contains impurities, e.g. water, fibrous material, cell debris, etc., which must be removed prior to storage. PMF and nuts, or palm kernel nuts, are left behind after oil extraction. The impurities in the CPO are removed via purification units, which then form sludge. The CPO is retained and sent to storage tanks. On the other hand, palm kernel nuts (PKN) are sent to crushers and oil extraction units for palm kernel oil extraction. Both CPO and CPKO then appear as the main products of a palm oil mill (Ng et al., 2015).

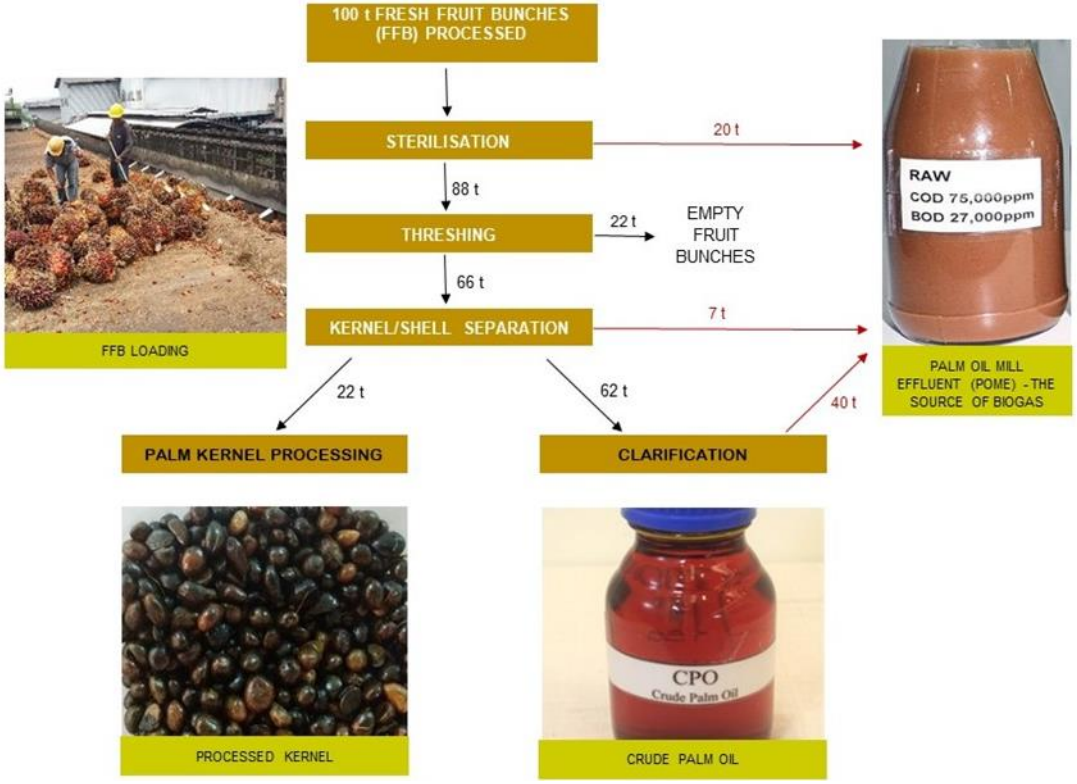


Figure 2.1: Typical palm oil milling process (red arrows indicate the flow rate of POME) (Loh et al., 2013; Yap et al., 2017; Yap et al., 2018)

The by-products and wastes generated at each stage of milling process are indicated in Figure 2.2. POME is the collective wastewater generated from palm oil mills. While about 20 t of CPO are produced from every 100 t of FFB processed, about 67 t of POME are generated (Figure 2.2) (Loh et al., 2013). POME is generated from 3 different sources: (a) separator sludge from the clarification of CPO (0.4 t/t FFB); (b) steriliser condensate from FFB sterilisation (0.2 t/t FFB); (c) effluent from wet separation of kernel and shell (0.07 t/t FFB) (Liew et al., 2015; Yap et al., 2017). The large amount of POME ~58 Mt (wet basis) deriving from 85.84 Mt of FFB processed in 2016 (MPOB, 2017) or 64 Mt in 2014 (Loh et al., 2017) is of high concern. This is because this poses a negative impact to the environment coupled with the hidden potential to maximise the "waste to wealth" monetary value. It is estimated that Malaysia produces about 80 Mt (dry weight basis, dwb) of oil palm biomass yearly (SEDA, 2012; Loh et al., 2017). The oil plam biomass produced from palm oil mills and oil palm plantations, which will be discussed in details in Section 2.2.

2.2 Solid Biomass from Palm Oil Mill

In Malaysia, more than 85% of the total biomass in the country is derived from the palm oil industry. Inefficient management and disposal of such biomass cause sustainability issues. On the contrary, efficient utilisation of the biomass by applying "waste-to-wealth" concept can strengthen the economy and sustainable development in the country. In palm oil mills, major solid biomasses are EFB, PMF and PKS.

These biomasses are produced at a rate of 22%, 13% and 5%, respectively, per unit weight of FFB processed (Yacob, 2006; Loh et al., 2017). Solid oil palm biomass is lignocellulosic material. Due to steam sterilisation of FFB during the milling stage, EFB absorbs part of the moisture, which leads to its high moisture content (>50 wt.%). Pre-treatment of EFB is typically required prior to further processing into value-added products. On the other hand, the lignocellulosic biomass contains residue oil, which leads to its relatively high calorific value, where the energy derived can be recovered for efficient use. Table 2.1 shows some of the fuel properties of PMF, PKS and EFB. It shall be noted that these values vary with the sources of palm-based biomass, depending on the geographical locations.

Nevertheless, it is worth noting that the above mentioned are palm-based biomasses from palm oil mill. There is a significant amount of biomass generated from oil palm plantations. These include pruned oil palm fronds (OPF) collected during the harvesting period and oil palm trunks (OPT) felled during the replanting period. As of 2014, approximately 42.1 Mt of OPF and 7.2 Mt OPT (25 years cycle replanting) were returned to the field as soil mulching and soil moisture conservation practices (Yacob, 2006; Loh, 2017).

Table 2.1: Moisture content and calorific values of typical palm biomasses from palm oil mill

	Moisture content (wt.%)	High heating value (kcal/kg)	Low heating value (kcal/kg)
PMF	30	2,604	2,213
PKS	23	4,147	3,756
EFB	43	2,416	2,025

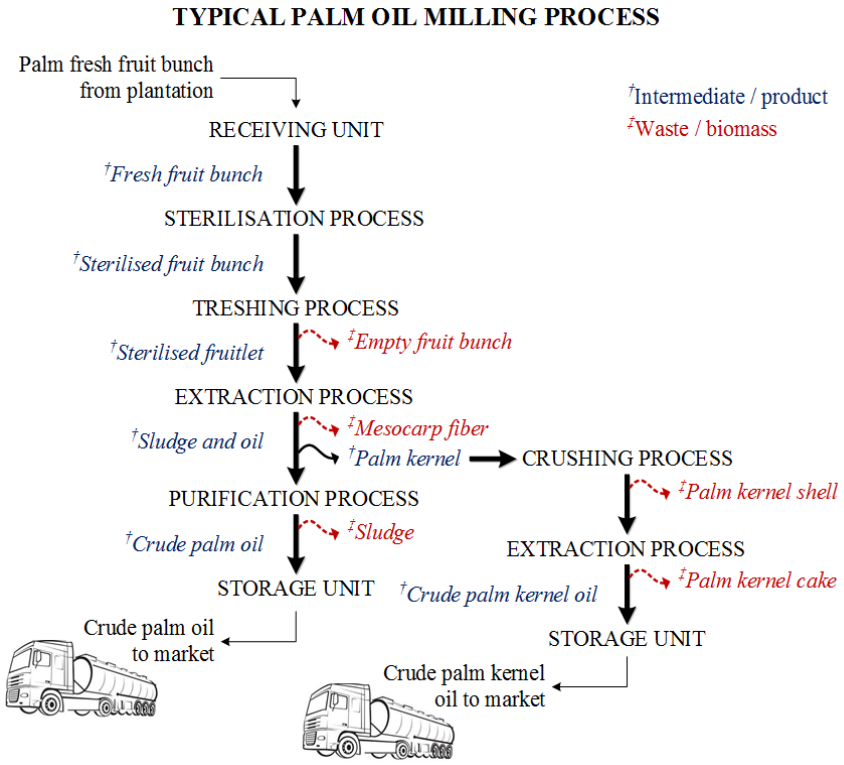


Figure 2.2: Oil palm biomass generated from palm oil mills (after Ng et al., 2015; Yap et al., 2018)

2.2.1 Oil Palm Biomass for Value-Added Products Generation

Solid oil palm biomass is increasingly being converted into value-added products, e.g. dried long fibre (Abdul Khalil et al., 2010; Nasrin et al., 2011), briquettes and pellets (Nasrin et al., 2011; Ng and Ng, 2013), bio-fertiliser (Sulong et al., 2008; Loh et al., 2013), etc. However, there are still many palm oil mill owners who retain the biomass for mulching. Over the decades, technologies were established to convert such biomass into value-added products. These include pelletising and torrefaction processes to produce pellets from EFB (Ng and Ng, 2013, Sukiran et al., 2017), fiberising process to produce dried long fibres (Eureka Synergy, 2015), screw extrusion and piston press briquetting process to produce briquettes (GGS, 2015), as well as composting process to convert EFB, spent bleaching earth and sludge into bio-fertilisers (Sulong et al., 2008; Loh et al., 2013), etc. Recently, more technologies are developed to convert these semi-finished products into value-added products. For example, dried long fibre is pressed and knitted into fibre mats (Eureka Synergy, 2015), the pellet is processed into edible pellets (animal feed) (Abdul Rahman, 2010; Loh et al., 2013), etc. The biodegradable fibre mat has shown potential in combatting soil corrosion, accelerating seed germination and plant growth, suppressing weed and so on. The development of pelletising technology to produce fuel pellet as edible pellet has further revealed the potential of oil palm biomass from the palm oil industry. Currently, the edible pellet/animal feed produced is used for fish cultivation. Figure 2.3 shows samples of palm-based value-added products that are currently being commercialised.



(a) dried long fiber



(b) bio-fertiliser



(c) pellet (biofuel)



(d) fiber mat

Figure 2.3: Value-added products from oil palm biomass (Loh et al., 2013; Eureka Synergy, 2017; Yap et al., 2018)

2.2.2 Oil Palm Biomass for Power Generation

Apart from producing value-added products (as discussed thoroughly in Section 2.2.1), oil palm biomass has been typically recovered for its energy content. These biomasses contain a high calorific value, which can be recovered to generate heat and electricity. In Malaysia, PMF and PKS are commonly fed to the palm oil mill biomass boilers to generate steam for heating and power through a steam turbine. EFB is less commonly used due to its high moisture content, slagging and low ash fusion temperature problems (Azeus, 2017). Nevertheless, a combination of PMS, PKS and EFB constitutes about 42% of boiler fuels for palm oil production (Umar et al., 2014).

Malaysia targets 11% of renewable energy source to its power generation by 2020 (Shumkov, 2017). Oil palm biomass as one of the renewable energy sources could contribute significantly to achieve the target due to its abundance. Considering that most of the palm oil mills are typically located outskirt or near to oil palm plantation, these mills may not have easy access to electricity from the national grid and so they generate power to primarily operate their mills and support daily activities using boilers and turbines. In recent years, some millers export the excess power from the biogas power plant to the grid through Feed-in Tariff scheme (SEDA, 2017). The survey by Umar et al. (2014), which received 20% responses from the operating palm oil mills in Malaysia, reported that 86% of the respondents utilised two-thirds of their biomass energy generated for self-consumption.

2.3 Liquid Effluent from Palm Oil Mill

POME – the liquid waste from palm oil milling process – is usually hot (~70°-80°C) and discharges into a holding pond as shown in Figure 2.4. It is a viscous, brownish liquid with high organic and solids contents. Table 2.2 shows typical POME characteristics from palm oil mills in Malaysia (Poh and Chong, 2010; Loh et al., 2013; Loh et al., 2017). The direct discharge of POME into a watercourse without proper treatment is undesirable due to its high polluting potential. In conjunction with the development of oil palm plantations in an exponential manner (Madaki and Lau, 2013) since 1970's, the palm oil milling activities in Malaysia have bloomed in the same trend. The number of palm oil mills has increased alongside the increased amount of FFB being processed, as shown in Figure 2.5 (MPOB, 2015). Therefore, the generation of POME is expected to increase and it requires proper treatment before being released as final discharge. Otherwise, it can be a major source of organic pollutant due to its high BOD and COD content.



Figure 2.4: Raw palm oil mill effluent being discharged into a holding pond (Yap et al., 2018)

Table 2.2: Typical palm oil mill effluent, POME, characteristics (Poh and Chong, 2010; Loh et al., 2013; Loh et al., 2017)

Parameter	Mean	Range
pH	4.5	3.3-5.7
Temperature[b]	80	70-90
BOD[a]	21,060	18,225-23,900
COD[a]	50,340	45,818-54,861
TS[a]	54,748	46,304-63,192
Suspended solids[a]	18,000	7,100-24,500
O&G[a]	7,213	5,614-8,812
TN[a]	725	670-780
Ammoniacal nitrogen[a]	89	77-101
VFA[a]	1,060	1,057-1,073
N[a]	651	554-747
P[a]	122	109-136
K[a]	1,869	1,696-2,043
Mg[a]	288	279-296
Ca[a]	286	282-290
Fe[a]	66	65-67
Zn[a]	1.98	1.24-2.72
Cu[a]	0.85	0.80-0.90
Mn[a]	2.80	2.67-2.93
Ni[a]	1.27	0.10-2.70

[a] All parameters are measured in mg/L, except pH. [b] Temperature is measured in °C.

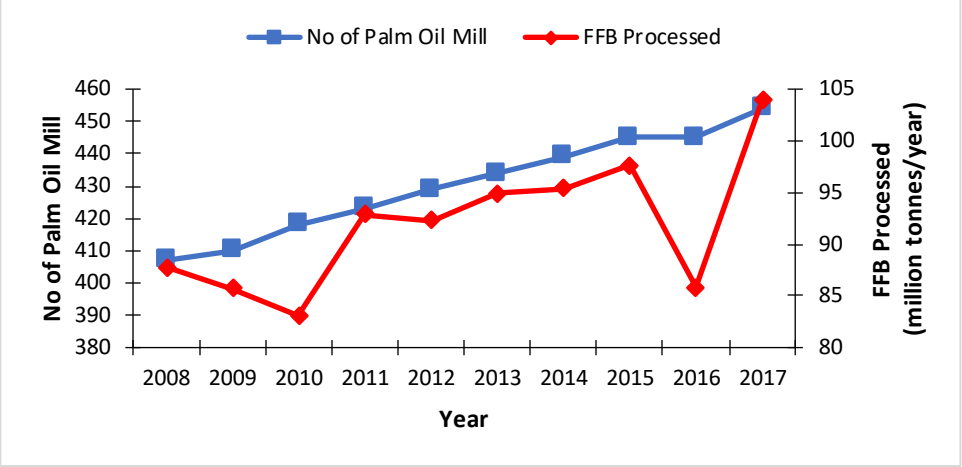


Figure 2.5: Palm milling activities in Malaysia (MPOB, 2017)

2.3.1 POME for Power Generation

Biological treatment consisting of both anaerobic and aerobic/facultative processes (Figure 2.6) is commonly used to treat POME to produce treated effluent with BOD of 100 mg/L, as required by the Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations, 1977 (Legal Research Board, 2008; Liew et al., 2015; Yap et al., 2017). However, ponding system is unable to produce final treated effluent consistently with BOD of 20 mg/L as enforced by the DOE, especially in environmentally-sensitive locations of the country. The ponding system requires a large footprint as a long retention time is required to break down the organic matters, which in turn produces biogas without being captured and utilised. For a typical palm oil mill having an FFB processing capacity of 45 t per hour, 6 ponds with a total volume of 200,000 m^3 are required. This takes up about 70% of the total CPO milling area. The treatment ponds require frequent desludging of the settled POME to avoid short circuit of anaerobic process. Scum layer formed on the pond surface needs to be removed by mechanical means, which is tedious due to the resulting thickened and hardened greasy solids covering a huge surface area.



(a)



(b)

Figure 2.6: Typical (a) anaerobic and (b) aerobic ponds of a palm oil mill

As POME is easily broken down by the naturally occurring microorganisms during the anaerobic process in the ponding system, it can be a potential renewable substrate for biogas production (Yap et al., 2017). Undeniably, POME is one of the major substrates for biogas generation in Malaysia along with many other wastewater sources including rubber, animal manure, sewage and other industrial wastewaters. The total power output (electricity) will be around 480 MW (Loh, 2017; Loh et al., 2017) from 445 palm oil mills provided the following criteria are met: (a) Biogas capturing systems with a $COD_{removal}$ of 95%; (b) methane yield of 0.27 LCH_4 /$gCOD_{removed}$ at STP (Chan, et al., 2013); (c) the gas engine has an electrical conversion efficiency of 35%, and; (d) the biogas power plant operates for 7,200 hours annually (Loh, 2017; Loh et al., 2017). The average power output of a palm oil mill with biogas trapping is approximately 1 MW as shown in Figure 2.6.

The huge power potential of POME for climate change mitigation forms the key driver for biogas development in Malaysia (Yap et al., 2017). Covered lagoon and continuous stirred tank reactor (CSTR) are the two most-commonly-used biogas capturing technologies, coupled with other newer options such as the UASB and the EGSB (Chan et al., 2009; Loh et al., 2013; Loh et al., 2017; Yap et al., 2017).

Nevertheless, most of these technologies are still unable to completely break down the organic matters to meet the more stringent POME discharge requirement set forth by DOE for a cleaner environment in the future (Liew et al., 2015). The conventional ponds employing tertiary polishing system are still needed to further treat POME to meet this requirement. The installation of biodigester will minimise the formation of scum in the ponds and improve the quality of the treated effluent. Biogas system alone may be retrofitted and built upon the existing ponds, but in some cases require additional footprint to the already large treatment area, and thus may not be a solution for a holistic effluent treatment. As oil palm is a seasonal crop, POME is usually produced at its highest volume in the middle of the year (high crop season) and drops to a minimum towards the end of the year (low crop season). This is one of the major challenges faced by the biogas operators as they need to accommodate such fluctuating situation: (a) flaring the excess biogas during high crop season; (b) facing insufficient POME for biogas generation, which affects its intended use during the low crop season. The common obstacles faced by palm oil millers and operators are the complexity of biogas plant operation, potential explosion and corrosion hazards, although the biogas system promotes the utilisation of renewable energy and reduces GHG emissions into the environment (Yap et al., 2017). Hence, most of the biogas plants in palm oil mills only have an average total power output of around 1 MW instead of 2 MW, as shown in Figure 2.7.

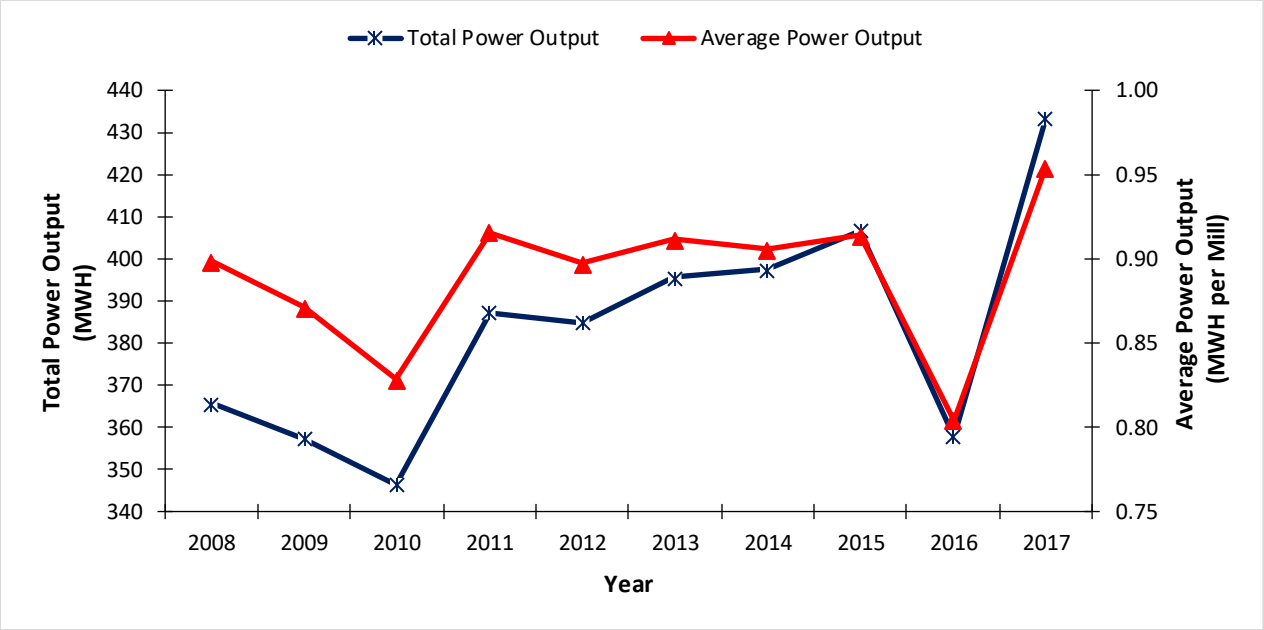


Figure 2.7: The potential total power output via biogas capturing from the available palm oil mill effluents in Malaysia (based on yearly palm oil mills; source: www.mpob.gov.my)

2.3.2 Development of Biogas Facility in Malaysia

The biogas business in Malaysia especially from the oil palm sector, like many other businesses, is profit-driven and supported by the government in the forms of several regulations and policies, environmental incentives and financial assistance.

Currently, new and existing palm oil mills requiring throughput expansion are mandated to be equipped with biogas-trapping facilities (MPOB, 2014; Loh et al., 2017). With more active promotion and awareness creation under the National Key Economic Area (NKEA) Entry Point Project 5 (PEMANDU, 2010; Loh et al., 2017), the millers have taken a more proactive role now in biogas capture. Since the ratification of the Clean Development Mechanism (CDM) in 2002 by Malaysia, several palm-based biogas/biomass projects were on board. The CDM under the Kyoto Protocol (KP) to the United Nations Framework Convention on Climate Change (UNFCCC) (enforced in 2005) is a scheme to promote sustainable development via GHG emissions reduction by introducing carbon emissions reduction (CER) units/credits (UNFCCC 1998). This was implemented by the Malaysian Green Technology Corporation (the then Malaysia Energy Centre, PTM) as the technical secretariat in overseeing and supporting the CDM in Malaysia (MGTC, 2014). The biogas produced from the treatment of POME in the palm oil industry was qualified to earn CER under CDM (Figure 2.8), as these activities have shown additional values by turning the previously unutilised and released biogas (methane) into valuable renewable energy that helps to reduce GHG emissions into the atmosphere.

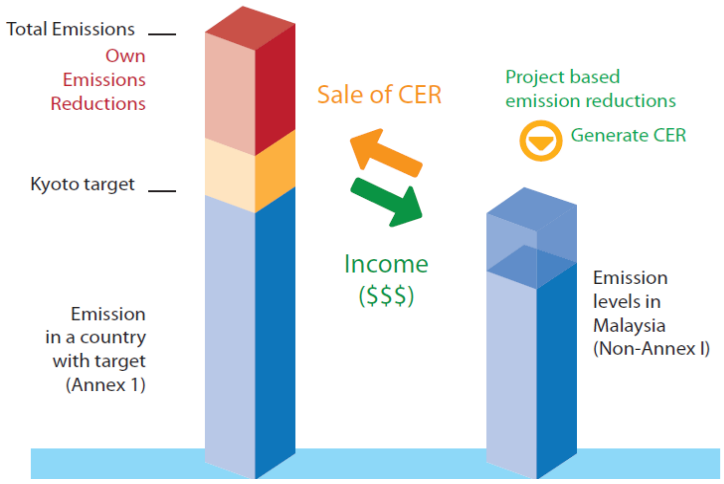


Figure 2.8: Mechanism to earn carbon credits through the sale of CER (MGTC, 2014)

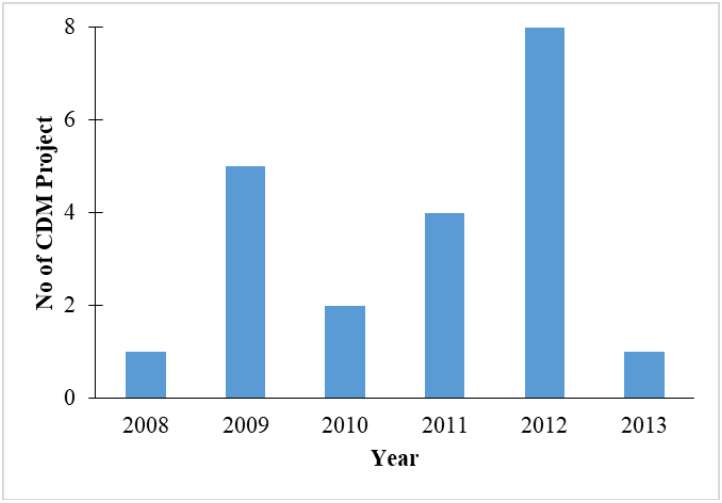


Figure 2.9: Number of biogas projects in Malaysia under CDM (CDM, 2013)

With the successful implementation of the first CDM project in the year 2008, the number of CDM biogas projects in Malaysia showed an increasing trend with most installation performed in 2012, before the CDM drew to its end in its first KP commitment period as shown in Figure 2.8. Towards the end of CDM, there were 21 projects (out of 205 registered projects from Malaysia) and 90% of these projects were using POME to generate biogas (CDM, 2013; Yap et al., 2017). As shown in Figure 2.9, a drastic drop in CDM projects was observed in 2013 due to the uncertainties of CDM's future, resulting in a severe drop of CER price from an average of €12.52/t in the year 2010 to €3.93/t in the year 2012 (CDM, 2012).

The biogas development in Malaysia did not stop with the end of first commitment period of KP when the National Renewable Energy Policy and Action Plan (NREPAP) was approved by the Cabinet in April 2011 (SEDA, 2014). Following the first strategic thrust under NREPAP to encourage the use of indigenous resources such as POME for biogas production, the Feed-in Tariff (FiT) of RM0.32 per kWh for renewable electricity production from biogas was introduced under the Renewable Energy (RE) Act 2011 [Act 725] (SEDA, 2016). Under this Act, a statutory body - the Sustainable Energy Development Authority of Malaysia (SEDA Malaysia) - was formed to administer and manage the implementation of the FiT. Starting 1st January 2014, biogas produced from POME as an agricultural waste is eligible to enjoy an additional bonus, making the FiT an approximate RM 0.40 (basic rate plus additional bonus using POME) (KeTTHA, 2011; SEDA, 2014) per kWh through the approved Distribution Licensees (DL) (SEDA, 2017) under the revised FiT tariff structure (Table 2.2). In Malaysia, the approved DL's are Tenaga National Berhad (TNB), Sabah Electricity Sdn Bhd (SESB) and Northern Utility Resources Bhd (NUR), which are responsible for the payment to the Feed-In-Approval Holder (FiAH) on the continuous supply of renewable energy for a long-term basis of 16 years. The FiT rates throughout the five-year period are shown in Table 2.3.

As shown in Table 2.3, FiT rates were adjusted in 2014. The FiT quotas for biogas from POME are supported by the Renewable Energy Fund imposed on consumers' electricity bills at a rate of 1.6%, which is very much dependent on the availability of the fund (Loh et al., 2017; Yap et al., 2017). As reported by the Daily Express, (2015), there are already 12 biogas plants from POME connected to the national grid through the FiT scheme. Additionally, at least another 79 palm oil mills are in the process of connecting to the grid, showing at least 91 palm oil mills will be connected to the grid in the near future. The government has also identified 113 potential palm oil mills for the FiT programme (Daily Express, 2015) as these palm oil mills are situated near electricity power sub-stations, which can absorb the electricity generated. Therefore, the current FiT rate is promising in supporting the setup of biogas capturing infrastructure with the feasible return of investment. With the revised FiT rate in 2014 as shown in Table 2.3, the number of applicants for grid connection has increased. In fact, all quota offered till 2019 as shown in Figure 2.10 was already taken up and there is an increased number of biogas plants since 2012 as shown in Figure 2.11, in which the total installed capacity for 2018 and 2019 are expected to further increase (SEDA, 2017).

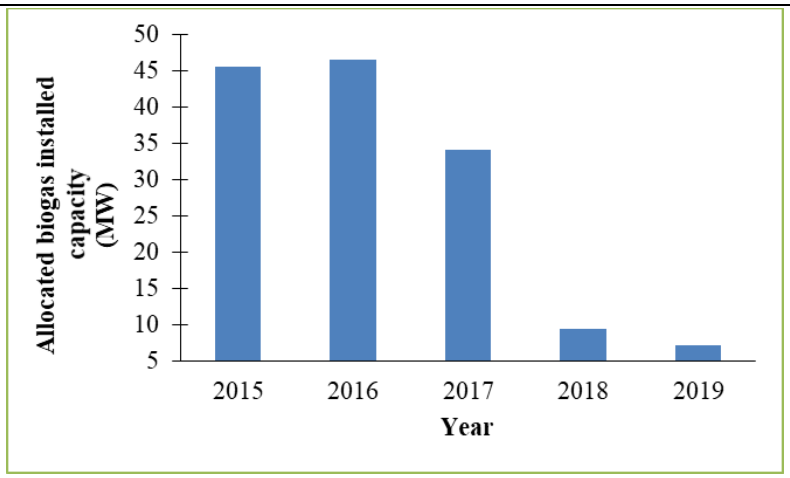


Figure 2.10: Feed-in-tariff (FiT) quota for biogas on an annual basis (SEDA, 2017)

Table 2.3: Comparison of FiT rates for biogas starting from the year 2012 to 2017 (SEDA, 2017)

Description	FiT Rates (RM per kWh)					
	2012	2013	2014*	2015	2016	2017
Basic FiT rates having an installed capacity of:						
Up to and including 4MW	0.3200	0.3184	0.3184	0.3184	0.3184	0.3184
4MW until 10MW	0.3000	0.2985	0.2985	0.2985	0.2985	0.2985
10MW until 30MW	0.2800	0.2786	0.2786	0.2786	0.2786	0.2786
Bonus FiT rates having the following criteria (one or more):						
Use of gas engine technology with an electrical efficiency of above 40%	+0.0200	+0.0199	+0.0199	+0.0199	+0.0199	+0.0199
Use of locally-manufactured or -assembled gas engine technology	+0.0100	+0.0100	+0.0500	+0.0500	+0.0500	+0.0500
use of landfill, sewage gas or agricultural waste* including animal waste as fuel source	+ 0.080	+0.0786	+0.0786	+0.0786	+0.0786	+0.0786

*POME as an agricultural waste is eligible for an additional bonus.

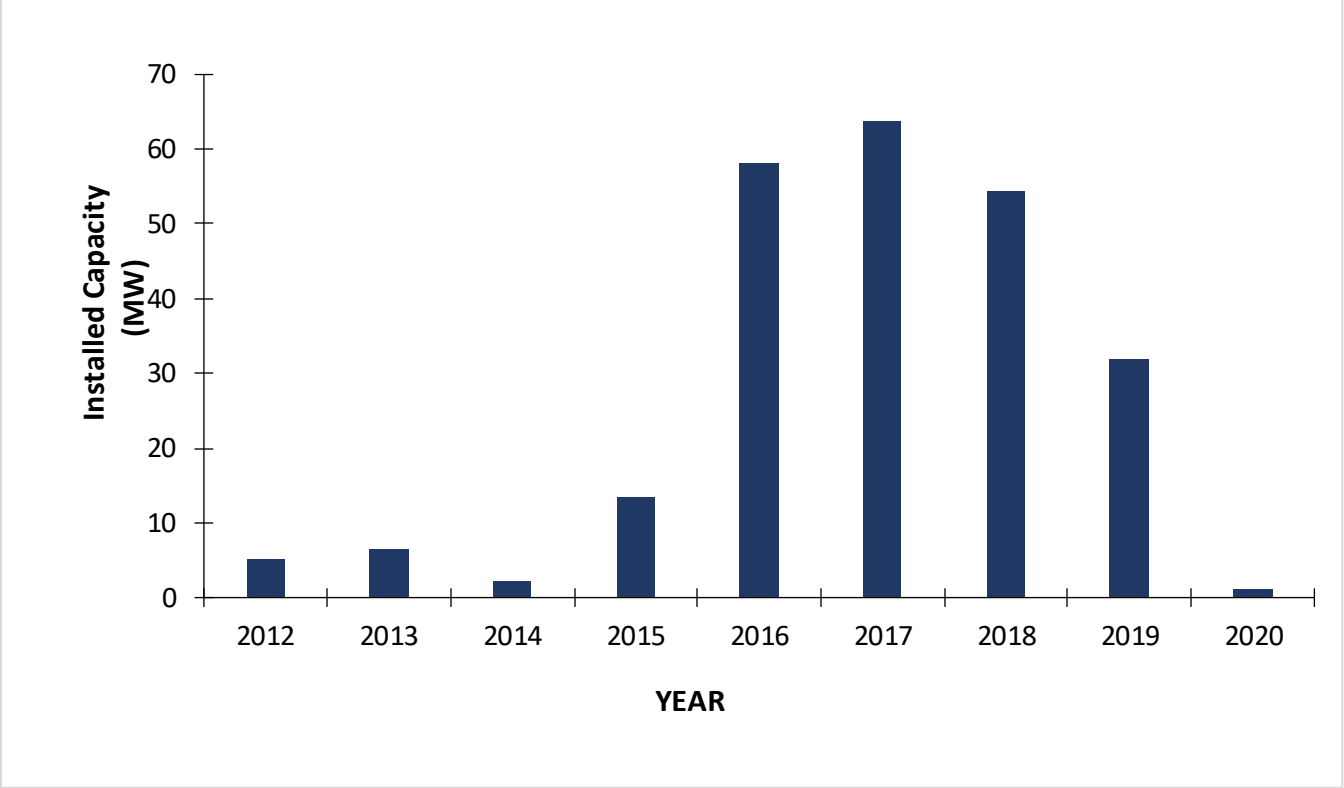


Figure 2.11: Total installed capacity of the biogas plants (both commissioned and in-progress) in Malaysia (SEDA, 2017)

Table 2.4: FiAH applications approved as of 2015 for quota offered up to 2018, excluding solar (SEDA, 2015; SEDA, 2017; SEDA, 2018)

RE Resources	Capacity (MW)	Total Capacity (%)
Biogas	164.02	14.21
Biomass	348.79	30.22
Small Hydro	279.64	24.23
Solar (Individual)	60.54	5.24
Solar (Community)	6.26	0.54
Solar (Non-Individual)	258.01	22.35
Total	**1,154.26**	**100**

Table 2.4 shows the FiAH applications approved as of 2015 for quota offered up to 2018, excluding solar (SEDA, 2015; SEDA, 2017; SEDA, 2018). It can be seen that biogas forms one of the major sources of renewable energy besides biomass, solar and hydro powers. If all the approved FiAHs with a capacity of 164.02 MW (SEDA, 2015) is commissioned and connected to the grid by 2017, it will contribute to about 23% of the total potential energy of 480 MW (Loh et al., 2017) from biogas of POME. If all palm oil mills capture biogas from POME for electricity generation, it will contribute to about 3% of the electricity consumption in 2012 and 12% of the envisaged renewable energy generation (4,000 MW) by 2030 in Malaysia (Loh et al., 2017). Therefore, the FiT under the Renewable Energy Act seems appropriate to incentivise and encourage the supply of biogas from POME to the national grid by the Malaysian palm oil industry.

While the Malaysian government was offering attractive incentives, a new legislation was also introduced. Effective 1st January 2014, all new palm oil mills and the existing ones applying for throughput expansion in the country were required to install full biogas capture or methane avoidance facilities (MPOB,2014; Loh et al., 2014; Loh et al., 2017). This mandate was to encourage further development of biogas plants in Malaysia and open more options for biogas utilisation.

The POME treatment for capturing biogas in Malaysia has come a long way (Figure 2.12). From being environmentally-unfriendly and smelly, the released biogas from POME treatment has been monetised during the CDM active period: 2008-2012. With the expiry of the first KP commitment period, palm oil millers then saw its value as boiler fuel. The enforced Renewable Energy Act, as well as the rewarding FiT, have pushed the direction of biogas from POME towards producing electricity for the national grid. The biogas produced can be utilised in various manners, e.g. combined heat and power (CHP), co-firing in palm oil mill biomass boilers, electricity generation (on- and off-grid), etc. Up to December 2016, there were 92 completed biogas plants, 9 under construction and 145 under planning (Figure 2.13) (Loh et al., 2017). Of these, 20 biogas plants are connected to the national grid, 6 under constructions and 23 at various stages of planning, totalling 29 biogas plants in the pipeline to come on board for grid connection in the next few years, based on the number of approved FiAHs (Loh et al., 2017). Compared to Thailand, which has started more than 10 years earlier in biogas (Suwanasri et al., 2015), the recent biogas development in Malaysia has been quite encouraging as was evidenced by the increasing number of biogas plants connected to the grid. However, there is still a big gap to achieve the NKEA target of having all palm oil mills to be equipped with biogas plants by 2020 due to: (a) low rates of FiT before revision; (b) current setting of biogas quotas (Figure 2.10); (c) slow installation rates of biogas plants; (d) limited use of the surplus energy generated, and; (e) tedious procedural requirement relating to grid interconnectivity matters (Loh et al., 2017). There is still much effort needed to encourage more biogas plant uptake. The development of POME treatment to meet the newly enforced stringent discharge limit of BOD 20 mg/L has always been prioritised rather than producing renewable energy. As such, methane avoidance technology may be an alternative if it is proven to be feasible and economical as no discharge is anticipated (Yap et al., 2017).

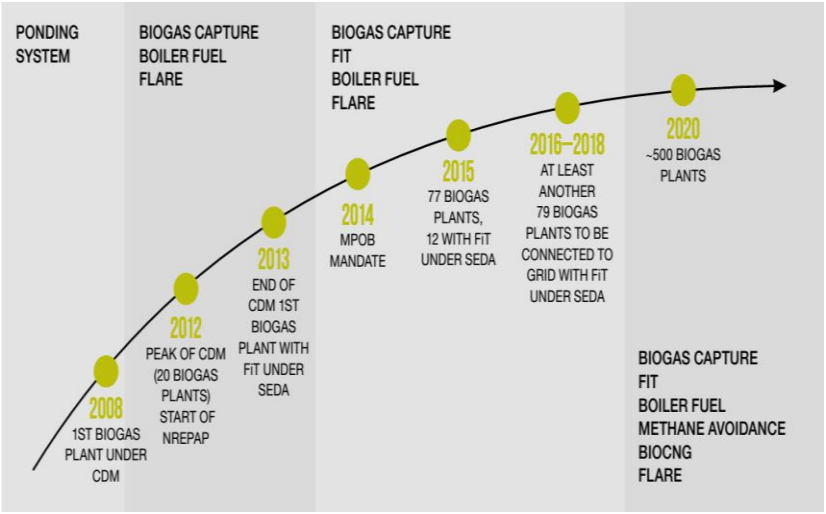


Figure 2.12: Timeline of biogas development in Malaysia (Yap et al., 2017)

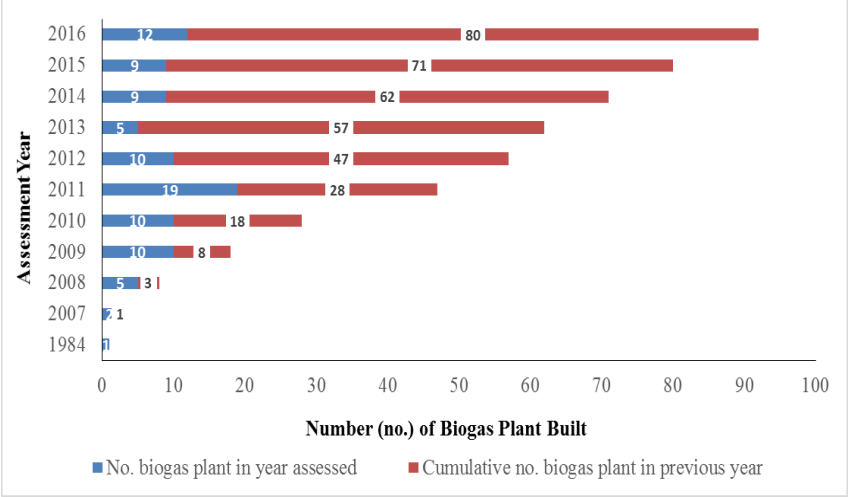


Figure 2.13: Number of biogas plants in Malaysia from year 2007 to 2016 (Loh et al., 2017)

The biogas generated from POME can also be used for rural electrification, i.e. supplying electricity to nearby housing areas. As reported by ST, (2014), this has been practiced widely in Sabah. Since the 1920s, a total installed capacity of 705 MW from 1,005 private licenses was connected off-grid, mostly fueled by palm oil and food wastes, while diesel only contributed to a small fraction.

Along the learning cycle in biogas capturing and utilisation in the industry, the R&D in POME treatment in Malaysia has moved towards using closed, high-rate bioreactor system due to limited lands for treatment ponds and cost-effectiveness of such system. The use of highly efficient bioreactors coupled with maximum recovery of useful energy from POME has resulted in decent technological efficiency and stability. High-rate bioreactors such as UASB (Borja and Banks, 1994; Loh et al., 2013), upflow anaerobic sludge fixed film (UASFF) (Najafpour et al., 2006), CSTR (Tong and Jaafar, 2006), and IAAB (Chan et al., 2013) have been used to treat POME either in laboratory or pilot scales with $COD_{removal}$ of at least 90% (Yap et al., 2017).

Even though the obtained result is quite promising, the palm oil industry has a reservation on using them due to the limited numbers of commercial data or track records. To promote such technology to the palm oil industry, more works are required to show the efficiency of a workable biogas technology. This can be done by collaborating with the industry to produce commercial data.

2.4 Performance Study of Various Biological Treatment Systems

It is important to compare the performance of different biological treatment systems with the IAAB system used in this study, as this allows us to justify the effectiveness of the method proposed in this study compared to the other systems available, especially on the high-rate bioreactors systems with the ability to capture the produced methane gas. The biogas captured is essential to the palm oil millers due to the enactment of the rule by the local authority, of which the allocation of at least one biogas plant in each palm oil mill by year 2020 has become mandatory.

Since 1980s, the treatment of POME has relied on the biological treatment of POME, mainly by anaerobic means. The most common method used for the treatment of POME is the conventional ponding system. It is a system that uses many anaerobic and facultative ponds, which is highly dependent on the microbial cells to degrade the compounds in POME. This system cannot be used as a long-term solution due to the inability to capture the released methane gas.

To solve these issues, modification has been carried out on the conventional ponding system by applying a cover on top of the ponds. The covered system allows the capture of the released methane gas during the AD process, but large areas of land required is still one of the disadvantages. Additionally, an HRT of 45 days is required for an effective AD process. The vast amounts of lands needed, as well as a long HRT, has resulted in large numbers of research being carried out, especially on the high rate bioreactor system.

Due to the advancement of technology, improvement has been done in the use of smaller-sized equipment, which will result in a reduction in HRT. The modified anaerobic treatment methods include anaerobic filtration, fluidised bed reactor, UASB reactor, UASFF reactor, continuous stirred tank reactor (CSTR), anaerobic contact digestion, up-flow anaerobic sludge blanket-hollow centred packed bed (UASB-HCPB) reactor, and IAAB. Each of these methods has been used to treat POME but has only been done so in the research level. The comparison of performance of various anaerobic treatment methods on POME is shown in Table 2.5.

Table 2.5: The performance of various anaerobic treatment methods on POME

Anaerobic Treatment Method	OLR_{an} $\left(\frac{kg_{COD}}{m^3 day}\right)$	HRT_{an} (days)	Methane Composition (%)	$COD_{removal}$	References
Anaerobic Pond	1.40	40.00	54.40	97.80	(Yacob et al., 2006)
Anaerobic Digester	2.16	20.00	36.00	80.70	(Yacob et al., 2005)
Anaerobic Filtration	4.50	15.00	63.00	94.00	(Borja and Banks, 1994)
Fluidised Bed Reactor	40.00	0.25	N/A	78.00	(Borja and Banks, 1995)
UASB reactor	10.63	4.00	54.20	98.40	(Borja and Banks, 1994)
UASFF reactor	11.58	3.00	80.10	97.00	(Najafpour et al., 2006)
CSTR	3.33	18.00	62.50	80.00	(Tong and Jaafar, 2006)
Anaerobic contact process	3.44	4.70	63.00	93.30	(Ibrahim et al., 1985)
UASB-HCPB reactor	6.88	4.00	66.90	88.80	(Poh and Chong, 2014)
Laboratory-scale IAAB	10.50	28.00	64.00	91.80*	(Chan et al., 2012)

N/A: Data Not Available; *: $COD_{removal}$ of anaerobic compartment only

Even though fluidised bed reactor system is able to treat POME at a higher OLR_{an} (40.0 kgCOD/m^3.day) with a short HRT_{an} (0.25 days), the inability to capture the released methane gas has become one of its major disadvantages. By comparing the anaerobic compartment of the laboratory-scale IAAB (Chan et al.,2012) with the UASFF reactor (Najafpour et al.,2006), it shows that OLR_{an} and HRT_{an} affected the $COD_{removal}$ and the obtained methane composition. A better performance was demonstrated by the UASFF reactor with the highest methane composition (80.10%). This is because the UASFF technology is a combination of UASB and upflow anaerobic filter, whereby it allowed the occurrence of rapid acclimatisation period for the microbial cells in the sludge to adapt to the new condition. Additionally, the middle portion of the UASFF functioning as biomass mobiliser is able to reduce the loss of sludge. As a result, it is able to maintain a large population of anaerobic microbial cells in the bottom of the UASFF to ensure efficient breakdown of incoming wastewater rapidly, as reflected on the high $COD_{removal}$. This concludes that both the UASB and UASFF bioreactors have great potential in POME treatment. Despite the positive results obtained in each of the laboratory-scale study of the POME treatment, these bioreactors only allow the occurrence of single treatment. The effluent treated from these bioreactors has to be subjected to post-treatment methods (HRT_{an} of at least 20 days) before releasing into the waterways. Hence, it is advisable to treat wastewater using a new prototype that is able to shorten the HRT_{an}, with minimal amount of space needed, a high loading rate and is able to capture the released methane gas, which will change the perspective of people towards the palm oil industry. This will be discussed in details in the subsequents sections 2.5.

2.5 Waste Recovery and Regeneration (REGEN) System for Palm Oil Industry

Various treatment systems/technologies have been developed and implemented in the palm oil industry. However, there is no single technology that tackles all the solid and liquid wastes in the palm oil mill. Most palm oil mills implement one or more biomass conversion technologies, yet none has successfully implemented zero waste management, which eliminates all wastes, while securing attractive net earnings. Recognising this gap in technology, an integrated system - Waste Recovery and Regeneration (REGEN) system - for palm oil mill is introduced. Figures 2.14 and 2.15 show the concept and process block diagram of REGEN system respectively.

As shown in Figures 2.14 and 2.15, REGEN system integrates all possible technologies to convert both the solid and liquid biomasses generated from the palm oil milling process into value-added products. Solid waste is processed via technologies such as pelletising system, fiberising system, composting system and other conversion processes based on the owner's interest. The system is flexible to mix-and-match different technologies to produce a variety of products and, therefore, is able to produce multiple products based on real-time market demand. The allocation of biomass in the system based on different market demand and biomass availability allows for full utilisation of biomass.

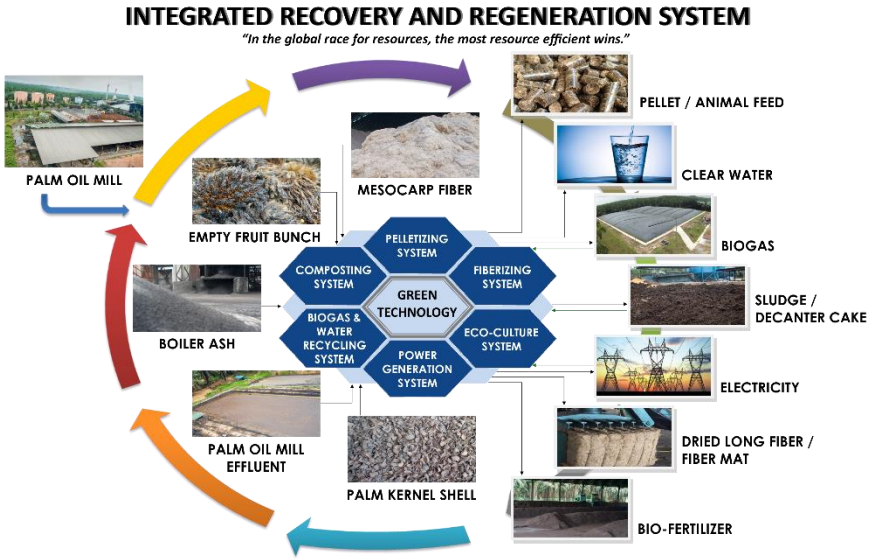


Figure 2.14: Concept of REGEN System (Ng et al., 2015)

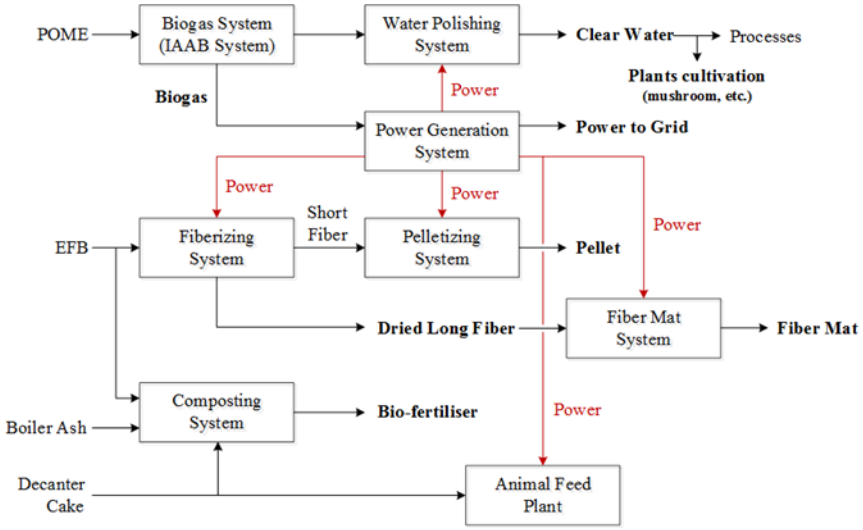
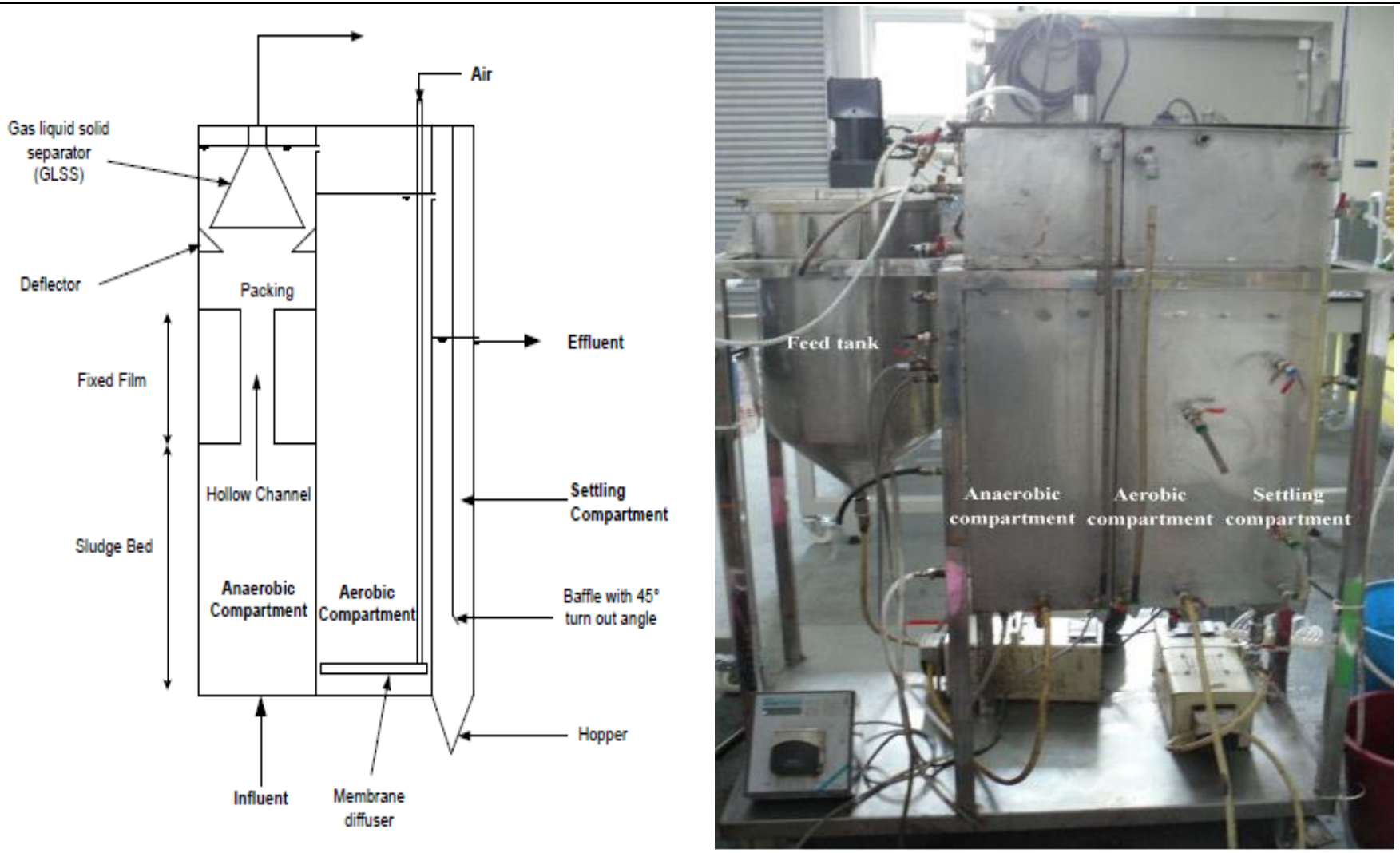


Figure 2.15: Process block diagram of REGEN System (Ng et al., 2015)

In the system, the liquid waste - POME - is treated in an efficient biogas system known as the Integrated Anaerobic and Aerobic Bioreactor (IAAB) system, which will be discussed in detail in Section 2.5.1 (Chan et al., 2012). The IAAB system consists of three compartments: anaerobic, aerobic and settling compartments, with an optional polishing system. The IAAB system is able to treat POME to meet the country's environment standard consistently. The treated water after settling compartment has BOD < 30 mg/L, COD < 500 mg/L, TSS < 45 mg/L and pH ~ 7.4. In the IAAB system, sufficient power can be generated from biogas for the entire REGEN system. Biogas is generated from POME in the anaerobic compartment. The collected biogas is then treated and used to generate power via gas engine to support the energy requirement of the REGEN system. The IAAB system developed is a high-rate reactor and it is able to control and stabilise the quality of treated POME. Once the POME is treated through the anaerobic, aerobic and settling compartments, it can be further polished to reduce the COD and TSS content in POME. After the polishing system, high-quality water is generated and can be reused and recycled to the palm oil mill. Note that the IAAB system produces sufficient power for the entire system, thus no external power is required. In addition, IAAB system implicates small footprint and requires shorter retention time compared to the conventional biogas technologies (covered lagoon and tank system) that produce the same amount of energy. In this system, POME is fully treated and recovered. The treated POME meets the environmental discharge limit steadily and the water is further polished for recycling and reuses in the palm oil mill. It is noteworthy that clear water quality can be achieved. Nevertheless, the IAAB system can be replaced by common POME treatment systems which generate biogas and electricity, depending on the owner's choice of technology. The REGEN system is energy self-sustained and is able to generate excess power for energy export.

2.5.1 Laboratory-Scale IAAB

Studies have shown that combining and optimising the anaerobic and aerobic processes will enhance the treatment process of POME (Chan et al., 2012; Loh et al., 2013; Yap et al., 2017). This may be the future of treating POME due to land limitations. The basic configuration of the laboratory-scale IAAB is illustrated in Figure 2.16. It is a single bioreactor with three different compartments, which are designed for different processes (Chan et al., 2012). The first, second and third compartments are designated for anaerobic process, aerobic process and sedimentation, respectively.



(a) (b)

Figure 2.16: (a) Basic Configuration of the Laboratory-Scale IAAB (b) Actual View of the Laboratory-Scale IAAB (Chan et al., 2012)

The anaerobic compartment is a combination of the UASB and UASFF reactors. It is divided into three parts that have their respective function. The lower part of the compartment is responsible for the development of sludge bed, where the influent will enter from the bottom. It is similar to the concept of the UASB reactor. The middle part of the compartment functions as a biomass immobiliser, which will reduce the loss of sludge during the conversion of organic matter into methane gas. Lastly, the top part of the anaerobic compartment functions as a gas-liquid-solid separator (GLSS). This allows the separation of gas and washed out solid from the liquid phase. The anaerobically-treated effluent will overflow into the second compartment for the aerobic biodegradation process. Two major components are involved in the second compartment. They are the liquid-solid separation in the settling compartment and a returned activated sludge (RAS) system. The implementation of RAS system will recycle the settling solids in the settling compartment into the aerobic compartment. This step is vital to maintain the preferred biomass concentration to ensure an efficient aerobic degradation process. Challenges viz. scum formation, foaming and clog are often encountered in the UASB and UASFF reactors but not the IAAB system (Chan et al., 2012). This shows that the packing media in the middle compartment helps to retain as much microorganisms as possible due to the large surface area that allows better contact with the incoming wastewater. Study conducted by Chan et al. (2012) showed that the IAAB successfully achieved a high $COD_{removal}$ and $TSS_{removal}$ of more than 99% and a methane composition of 64% in the treatment of POME at an OLR of 10.5 gCOD/L.day. The IAAB system is able to treat POME to meet the country's environmental standards consistently. As reported by Chan et al. (2012), the treated effluent leaving the settling compartment has BOD < 100 mg/L, COD < 500 mg/L, TSS < 45 mg/L and pH ~ 7.4. Biogas is generated from POME in the anaerobic compartment. The IAAB system developed is a high-rate reactor, and is able to control and stabilise the quality of treated POME. In addition, the IAAB system has a small footprint and requires shorter retention time, compared to the conventional biogas technologies (covered lagoon and tank system) that produce the same amount of energy. In this system, POME is fully treated and recovered. The treated POME meets the environmental discharge limit steadily and the effluent may be used for recycling and be reused in the palm oil mill.

2.5.2 Pilot-Scale Integrated Anaerobic-Aerobic Bioreactor (IAAB) System

Given the excellent performance of the laboratory-scale IAAB reported by Chan et al. (2012), upscaling the laboratory-scale IAAB to a larger volume has been done, termed as pilot-scale IAAB. This allows us to directly compare both the laboratory-scale and pilot-scale IAABs in terms of the performance evaluation, whereby the results obtained may be useful for further upscaling into commercial scale. Upscaling the laboratory-scale IAAB into pilot-scale has been carried out with an increase of volume from 55L (laboratory-scale IAAB) into $1.8m^3$ (pilot-scale IAAB). The pilot-scale IAAB will be used in this study for the purpose of long-term evaluation to complete POME treatment based on the process kinetics and optimised operating parameters, for the production of effluent that abides by the regulatory discharge limit.

It is essential to conduct a thorough study on the pilot-scale IAAB at various loading rates (in increments of COD concentration) to obtain optimal operating conditions for POME treatment and kinetic coefficients that can aid in commercialising the IAAB to combat the massive volumes of POME, coupled with constant fluctuation in characteristics of POME.

The pilot-scale IAAB is operated in a continuous system. Figure 2.17 shows the process flow diagram (PFD) of the POME treatment system using pilot-scale IAAB. Before filling in the 200 L Feed Tank (T101), the wastewater (POME) is manually filtered to remove large solid materials. From the Feed Tank (T101), the POME is pumped into the second Feed Tank (T102) of 200 L using a mechanical diaphragm pump (P101). During the start-up of the pilot-scale IAAB, POME is pumped into the bottom of the anaerobic compartment of the pilot-scale IAAB via a mechanical diaphragm pump (P102). The anaerobic compartment is equipped with pH and temperature sensors. There are six sampling points along the anaerobic compartment, namely SP1-SP6. A recirculation system, termed as W1, is installed in the anaerobic compartment to allow homogeneous mixing between the wastewater in the middle with the wastewater in the bottom of the anaerobic compartment. In the anaerobic compartment, the wastewater flows upward and overflows into the second compartment, which is the aerobic compartment. Oxygen is supplied into the aerobic compartment through a membrane diffuser. Inside the aerobic compartment, it is equipped with pH, temperature and dissolved oxygen (DO) sensors. A sampling point known as SP7 is located along the aerobic compartment of the pilot-scale IAAB. The aerobically-treated effluent overflows into the third compartment, i.e. settling compartment. It is subdivided into two sections by a baffle with a hopper at the bottom for settling purposes. The baffle has a 45° turn-out angle aiding the sedimentation process to result in proper separation of effluent suspended solid (settled into the hopper), whereas the clarified effluent rises to the discharge point (Discharge 1). A portion of the solid in the hopper is recycled into the aerobic compartment whilst the remaining solids are removed from the system.

Legend
T101: Feed Tank 1 (200L)
T102: Feed Tank 2 (200L)
P101: Influent Pump for raw POME
P102: Feed Pump
P103: Recirculation Pump
P104: Returned activated sludge Pump
P105: Waste activated sludge Pump
SP: Sampling Point
R1: Anaerobic recirculation inlet point
W1: Anaerobic recirculation outlet point
R2: Returned activated sludge inlet point
W2: Returned activated sludge outlet point

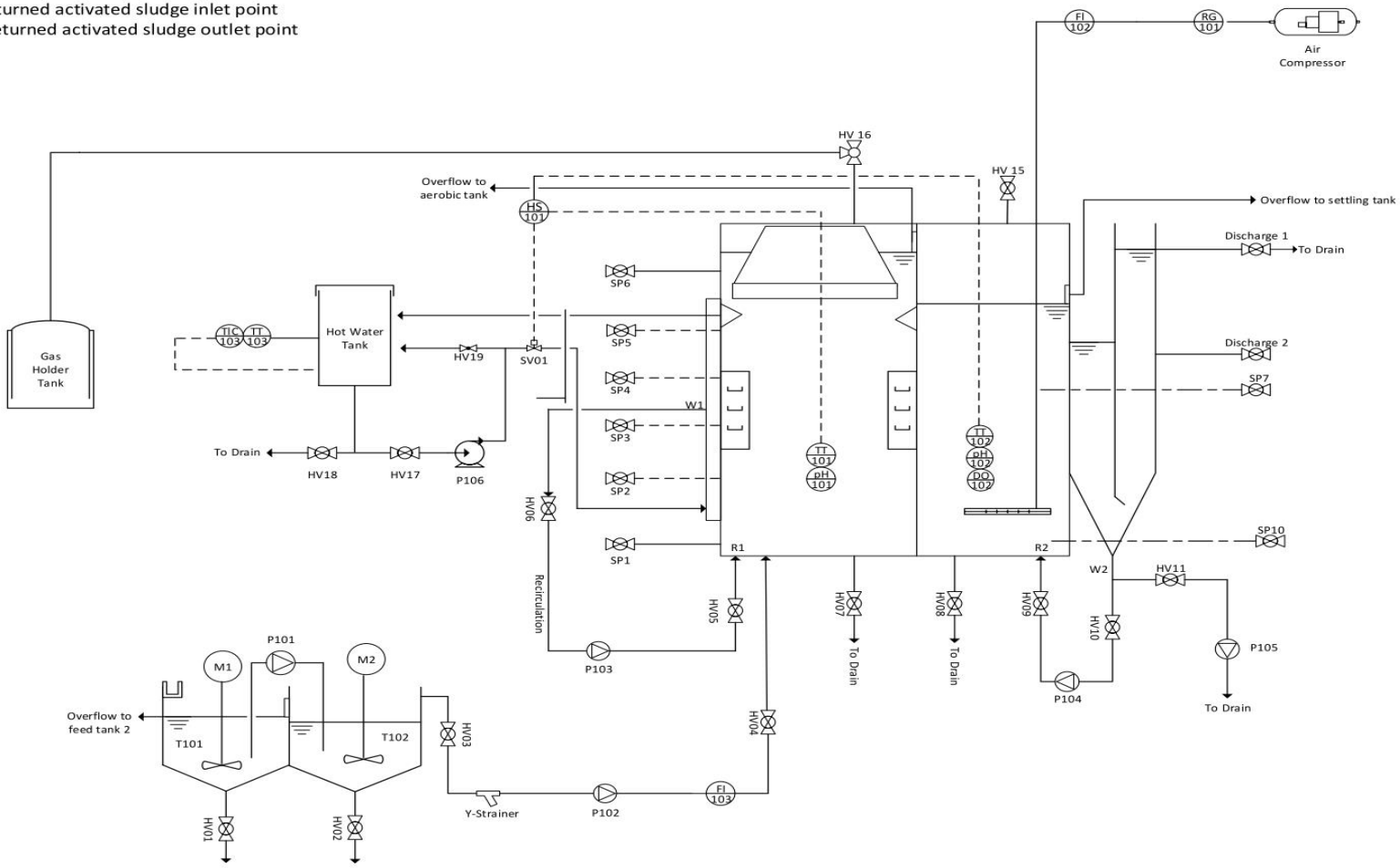


Figure 2.17: The Process Flow Diagram (PFD) of the pilot-scale IAAB for POME treatment

The pilot-scale IAAB aims to treat high strength wastewater, viz. POME, which gives a higher potential for a viable wastewater treatment technology. The attractive parts of the pilot-scale IAAB are well-explained in Table 2.6.

Table 2.6: Key features of the pilot-scale IAAB

Able to biodegrade POME in a short period of time	The concept of UASFF has been incorporated into the anaerobic compartment of the pilot-scale IAAB. It allows the development of sludge bed at the bottom of the compartment whereby it allowed homogenous mixing with the POME incoming from the bottom of the anaerobic compartment. Hence, at least 90% of the organic matter can be removed.
Able to capture the release of methane gas	GLSS has been installed on top of the anaerobic compartment of the pilot-scale IAAB. It allows the separation of gas from the anaerobically-treated effluent, which can be used for power generation. The methane gas released can be the secondary source of income for palm oil millers based on the incentive offered by government, which can be used to offset the installation cost.
Reduction of carbon footprint	The pilot-scale IAAB system only consist of three compartmentalisations in a single unit of bioreactor. Additionally, the compartments are purposely shaped in a rectangular and share common walls. The biogas produced during anaerobic digestion produced consist of mixture of methane and carbon dioxide gases have the potential to be compressed into 100% pure methane (Kapdi, et al., 2005), which will not release any carbon dioxide into the atmosphere. Hence, reducing the carbon footprint.
Ease of operation problems	Similar the laboratory-scale IAAB, a vertically-hollow channel is placed in the middle of the packing media. This acts a place for the anoxic microbial cells to continuously grow and replicate whilst actively degrading the organic matter in the wastewater. Additionally, it prevents the occurrence of clogging, which often faced by most of the UASFF bioreactors.
No emission of odour	The pilot-scale is an enclosed system. There wouldn't be any trace of odour emission during the active anaerobic digestion. Unlike those conventional ponding systems for POME treatment (without the facility of methane gas capturing), the trace of odour for the anaerobically-treated POME can be detected easily.

2.6 Design Procedure for Pilot-Scale IAAB

The design of the pilot-scale IAAB used in this study is mainly based on the results of the laboratory-scale IAAB study conducted by Chan et al. (2012). The results obtained from Chan et al. (2012), showed that the laboratory-scale IAAB has successfully been used to treat POME at OLR_{an} of 21.5 gCOD/L.day with overall $COD_{removal}$, $BOD_{removal}$, and $TSS_{removal}$ of more than 92.5%. OLR can be a yardstick for useful comparison between historical data and typical observed operation conditions. Hence, OLR as an independent parameter is used as a basis of the design operation of the pilot-scale IAAB.

In this design, the first priority is to maintain a maximum possible $COD_{removal}$ of the reactor by selecting the optimised OLR conditions for the desired $COD_{removal}$, and then to maintain the bioreactor at its best operating condition. In order to reduce the occurrence of shock loading in the bioreactor, a step-wise increment of the OLR is carried out.

During the increment of the OLR, the stability of the bioreactor is closely monitored to ensure that the microbial cells in each of the compartment are fully acclimatised to the continuous increment of the loading rate. Hence, this will provide a set of optimum operating condition that is able to treat POME effectively, whilst producing final treated effluent with BOD of less than 20 mg/L.

2.6.1 Summary of the design parameters

A summary of the design parameters of the pilot-scale IAAB for the treatment of POME is presented in Table 2.7. The detailed descriptions on these parameters will be presented in the following sections.

Table 2.7: Design Parameters of the Pilot-Scale IAAB

Process	Design Parameter	Average Value	Range	Unit
Anaerobic[a]	Temperature	-	30.0-33.0	°C
	V_{an}	1,000	1,000	L
	OLR_{an}	-	10.5-32.5	gCOD/L.day
	HRT_{an}	2.8	-	Day
	Q_{in}	360	-	L.day
	$MLVSS_{an}$	36,550	-	mg/L
	pH	-	6.71-7.45	-
	Q_r	5,280	-	L/day
	$R_{recirculation}$	15	-	-
Aerobic[a,b]	Temperature	-	30.0-33.0	°C
	V_a	600	600	L
	OLR_a	-	1.1-3.3	gCOD/L.day
	HRT_a	1.7	-	Day
	$MLVSS_a$	18,000	-	mg/L
	pH	-	8.30-8.98	-
	DO	-	1.95-3.10	mg/L
	Q_{RAS}	648	-	L/day
	R_{RAS}	-	2.00	-
	Q_{WAS}	216	-	L/day
	R_{WAS}	-	0.50-0.60	-

[a]: Literature data obtained from Chan et al. (2012)
[b]: Literature data obtained from Chan et al. (2010)

2.7 Design Consideration of Anaerobic Process

Anaerobic process is the first step for POME treatment to ensure maximum removal of organic matter whilst producing biogas. The essential design such as (a) wastewater characterisation; (b) loading rate; (c) bacteria concentration, and; (d) biogas capturing system are needed to be taken into consideration when maximising the performance of the anaerobic compartment of the pilot-scale IAAB. These will be discussed in details in Sections 2.7.1, 2.7.2, 2.7.3, and 2.7.4, respectively.

2.7.1 Wastewater Characterisation

In order to ensure a proper anaerobic treatment system, characterisation of the wastewater is the most crucial step. This allows us to identify the potential operational problems that may arise during the collection of samples. It is commonly known that the usage of high-rate anaerobic bioreactors or the conventional ponding system for the treatment of POME often faced a few operational problems, viz. scum formation, sludge flotation and clogging whenever a high loading rate was applied into the system (Najafpour et al., 2006; Zhang et al., 2008; Chan et al., 2009). This may be contributed by the lack of updated data on the characteristics of the POME especially on the manner used to produce POME (waste product formed during the production of CPO) as the study was conducted in both high and low crops seasons. Additionally, the high concentration of TSS and O&G in the POME will lead to a drastic scum formation if the POME is exposed to high temperatures. To fully understand the nature of the POME, a thorough characterisation study on the POME was conducted in three different palm oil mills (Table 2.8). The average values of POME characteristics were used as a yardstick for the pilot-scale IAAB study to ensure that the pilot-scale IAAB is able to combat the constant fluctuation of the POME used. This is to ensure that the pilot-scale IAAB is not under- or over-performing.

Table 2.8: Summary of measured parameters of POME collected from three different palm oil mills

Parameter	Palm Oil Mill A		Palm Oil Mill B		Palm Oil Mill C	
	Average	Range	Average	Range	Average	Range
pH	4.95 ± 0.09	4.86-5.04	4.27 ± 0.16	4.11-4.43	3.78 ± 0.34	3.44-4.12
BOD (mg/L)	52,433 ± 6,954	45,479-59,387	40,717 ± 2,181	38,536-42,898	73,412 ± 1,566	71,846-74,978
COD (mg/L)	86,692 ± 3,329	83,363-90,021	104,850 ± 2,966	101,884-107,816	122,094 ± 24,268	97,826-146,362
TSS (mg/L	45,500 ± 3,500	42,000-49,000	161,833 ± 68,199	93,634-230,032	44,957 ± 8,860	36,097-53,817
VSS (mg/L)	39,167 ± 2,255	36,912-41,422	74,833 ± 11,471	63,362-86,304	28,486 ± 13,000	15,486-41,486
O&G (mg/L)	13,117 ± 1,589	11,528-14,706	11,650 ± 433	11,217-12,083	12,589 ± 468	12,121-13,057
BOD:COD	0.61 ± 0.09	0.52-0.70	0.39 ± 0.03	0.36-0.42	0.50 ± 0.00	0.50
VSS:TSS	0.86	-	0.46	-	0.63	-

2.7.2 Organic Loading Rate

OLR is a useful indicator for direct comparison between historical data and typical observed operating conditions. It is essential to operate the reactor at an optimum loading rate as low OLR is known to restrict the capacity and investment of treatment facility, whereas high OLR may results in producing effluent with poor quality and higher oxygen requirement. In this study, the manner used to design the anaerobic system is mainly based on the OLR. This is because influent POME has a COD of more than 15,000 mg/L, which is classified as high strength wastewater. OLR is defined as the amount of organic matter added daily to reactor per unit of volume. Since the pilot-scale IAAB will be operated only at mesophilic range of temperature (20°C-45°C), the recommended OLR will be around 1.5 gCOD/L.day to 15 gCOD/L.day. However, maintaining the degradation capacity of the anoxic microbial cells is utmost important dependant parameter when selecting the optimum loading rate. As reported by Najafpour et al. (2006), operating the UASFF bioreactor at OLR 23.0 gCOD/L.day is able to achieve a $COD_{removal}$ of at least 90% for the treatment of POME. This allow us to postulate that the anaerobic compartment of the pilot-scale IAAB is able to withstand high loading rates, while maintaining the $COD_{removal}$ of at least 90%. Hence, in this design, the pilot-scale IAAB is designed to withstand high OLR of at least 30.0 gCOD/L.day at mesophilic condition while maintaining the degradation rate (as reflected on the removal efficiency).

The equation of OLR calculation is given as below:

$$OLR_{an} = \frac{Q_{in}.COD_{in}}{V_{an}} \quad (2.1)$$

Where OLR_{an} is the OLR applied in the anaerobic compartment, Q_{in} is the influent flow rate (L/day), COD_{in} is average inlet COD concentration (mg/L), and V_{an} is the volume of the anaerobic reactor (L).

2.7.3 Bacteria Concentration

It is essential to maintain an optimum concentration of microbial cells in the anaerobic compartment to maintain high degradation activity of the anoxic microbial cells, as well as to prevent the formation of dead zone in the bottom of the UASFF reactor. According to the recommended operating condition of the anaerobic compartment during the optimisation study for the treatment of POME (Chapter four, Results and Discussion), the required $MLVSS_{an}$ to achieve $COD_{removal}$ of 92% to 96% are within the range of 39,497 mg/L to 68,234 mg/L. In order to maintain the high activity of the anoxic microbial cells, the pilot-scale IAAB must be operated within this range of the $MLVSS_{an}$ concentration to avoid excessive concentration of bacteria in the anaerobic compartment, which may result in reactor failure. Additionally, the $MLVSS_{an}$ concentration used in this study was higher than the $MLVSS_{an}$ concentration used in the laboratory-scale IAAB ($MLVSS_{an}$ of 40,000 mg/L).

This may be due to the larger volume of the POME is pumped into the bottom of the anaerobic compartment of the pilot-scale IAAB than the laboratory-scale IAAB, which requires a higher concentration of bacteria to effectively biodegrade the organic or inorganic matter in the POME to obtain $COD_{removal}$ of more than 90%. In order to withstand high organic loading rates and short hydraulic retention times using UASB concept of bioreactor to treat POME, formation of granules, their structure and their stability are utmost important to withstand the effects of hydraulic shear and liquid up-flow velocity (Quarmby and Forster, 1995). The middle part of the anaerobic compartment of the pilot-scale IAAB consists of bio-ceramic rings that act as biomass immobilisers for the anaerobic microbial cells to continuously grow and replicate, which may produce extracellular polymers that may aid in the granulation process (Quarmby and Forster, 1995). Hence, contributing to the stability of the anaerobic compartment.

The equation of MLVSS calculation is given as below:

$$MLVSS_{an} = \frac{volume\ of\ sludge\ mass\ of\ each\ sampling\ point\ (mg)}{Voume\ of\ sampling\ point\ (L)} \quad (2.2)$$

2.7.4 Biogas Capturing System

One of the important features in anaerobic process is the ability to capture the biogas produced. In order to completely capture the biogas produced without releasing into the atmosphere, a proper and efficient system that allows proper separation of solid and liquid from gas has to be installed as part of the anaerobic compartment. A gas-liquid-solid separator (GLSS) has been installed into the anaerobic compartment of the pilot-scale IAAB to collect the biogas produced during the anaerobic digestion process. The separator is installed to allow the formation of a liquid-gas interface inside the gas collector sufficient to allow the easy release of the gas entrapped in the sludge (Figure 2.17). The anaerobically-treated effluent will overflow into the aerobic compartment for further polishing treatment. Additionally, the separator is aimed to avoid biomass loss and sludge flotation. The solid and liquid (anaerobically-treated effluent) will still be remained in the settling zone of the GLSS once the biogas is escaped from the gas collector. This shows that the GLSS has the potential in maintaining the solid concentration in the anaerobic compartment without the aid of external energy and control device. The ability of the solid and liquid to be settled in the GLSS system has totally eliminated the needs for an additional compartment for sludge settling. Compared to the covered lagoon system, the cost of installing a geomembrane is relatively more expensive than the GLSS system. Separation of gas from the mixture of anaerobically-treated effluent does not occur in the geomembrane of the covered lagoon system. As a result, an additional pond may be required for storage of solids before being subjected to the next series of treatment. Hence, it is more economically viable to install a GLSS system for the purpose of biogas capturing as minimal cost is involved, as the palm oil industry is currently moving towards the era of smaller footprint and greener image.

2.8 Design Consideration for Aerobic Process

Aerobic process is considered as the polishing step for the treatment of anaerobically-treated POME. Aerobic granule is a self-immobilised microbial aggregate with regular shape, excellent settleability, compact structure and diverse microbial functions (Wu et al., 2018). Due to its attractive nature, the aerobic granulation technology has been used widely for treating different kinds of wastewater that contained organics, nitrogen, phosphorus and toxic substances. However, several reasons were found to affect the stability of the aerobic granules. They are: (a) outgrowth of filamentous organisms; (b) hydrolysis of the anaerobic core; (c) loss of functional strains, and; (d) change of the roles of extracellular polymeric substances (EPS) (Lee et al., 2010; Wu et al., 2018). It is essential to identify the optimum operating conditions that allow the achievement of high removal efficiency of at least 95%, as well as to produce good quality final treated effluent that abides by the regulatory discharge limit. This is aimed to preserve the biological activity of the aerobic microbial cells at their highest level to maximise their degradation activity. The important parameters that need to be taken into consideration during the design of activated sludge are: (a) loading rate for aerobic process; (b) bacteria concentration; (c) returned activated sludge system; (d) waste-activated sludge system; (e) oxygen requirement, and; (f) food-to-microorganism (F/M) ratio.

2.8.1 Loading Rate

The aerobic compartment of the pilot-scale IAAB was designed to achieve and maintain $COD_{removal}$ of more than 90%. A study was conducted by Chan et al. (2010) where sequencing batch reactor (SBR) was used to treat the anaerobically-treated POME. Promising results were obtained where a lower $COD_{removal}$ (average 85%) was observed in the thermophilic aerobic system, compared to the mesophilic system (average 95%). The former operating condition with an average $COD_{removal}$ of 85% was achieved at lower OLR_a of 2.5 gCOD/L.day, whereas higher OLR_a of 4.0 gCOD/L.day allowed the achievement of $COD_{removal}$ of 95%. This allows us to conclude that a higher OLR_a is required to achieve high $COD_{removal}$. The result obtained during the start-up of the laboratory-scale IAAB was not consistent with the SBR study conducted by Chan et al. (2010); Chan et al. (2012). The $COD_{removal}$ obtained from the laboratory-scale IAAB ranges from 95 - 97% with OLR_a of 0.74 – 3.50 gCOD/L.day. Lower OLR_a was subjected for the treatment of anaerobically-treated POME in the laboratory-scale IAAB, as compared to the SBR, to allow the achievement of maximum degradation of biodegradable organic matter. This allow us to postulate that aerobic process is highly influenced by the anaerobic process, where the determination of the OLR_a is based on the COD of the anaerobically-treated POME. The result obtained from the laboratory-scale IAAB was used as a yardstick during the design of the pilot-scale IAAB study. Hence, the operation of the aerobic compartment of the pilot-scale IAAB was designed within the range of 2.6 – 3.5 gCOD/L.day with the aim of minimising the compromise in the treatment efficiencies.

2.8.2 Bacteria Concentration

Proper aerobic process is able to produce high quality final treated effluent where it is the polishing step for the anaerobically-treated effluent. To achieve that, maintaining the biological activity of the aerobic microbial cells is of utmost importance. This allows them to actively biodegrade the organic matter in the anaerobically-treated POME. The bacteria concentration of the aerobic compartment is measured daily, and it is expressed as MLVSS concentration. Based on the study conducted by Chan et al. (2010), the $MLVSS_a$ concentration of 17,000 to 23,000 mg/L was used as a yardstick for the initial stage of the pilot-scale IAAB evaluation. The $COD_{removal}$ obtained in the pilot-scale IAAB with similar $MLVSS_a$ concentration as abovementioned was lesser than 85% (Chapter four, Results and Discussion). Instead a higher range of $MLVSS_a$ (20,580 to 23,621 mg/L) was used in the aerobic compartment of the pilot-scale IAAB to allow the achievement of $COD_{removal}$ ranges from 96 to 96%. Compared with the typical $MLVSS_a$ concentration used in the industry (1,000 to 3,400 mg/L) (Tchobanoglous et al., 2014), the $MLVSS_a$ concentration adopted in the pilot-scale IAAB was in fact higher. This may be due to the allocated hydraulic retention time (HRT) in each of the system used for the treatment of anaerobically-treated POME. The HRT_a allocated in the industry is longer (> 20 days) as compared to the laboratory-scale IAAB (3.2 – 6.5 day), which will affect the size of the treatment system. The size of the treatment for the former HRT_a is as big as a football field, whereas a smaller size reactor (24 L) was used for the treatment system of the latter HRT_a. This shows that designing the aerobic compartment of the pilot-scale IAAB with a volume of lesser than the football field (approximation of 8,152 m^2) is able to reduce the HRT_a allocated for the aerobic microbial cells to actively degrade the biodegradable organic matter in the anaerobically-treated POME whilst maintaining high $COD_{removal}$. The maximum $MLVSS_a$ concentration maintained in the aerobic compartment of the pilot-scale IAAB is constrained by the settling time of the activated sludge [reflected as sludge volume index (SVI)] to obtain a clear separation of supernatant effluent that abides by the discharge limit set by the regulatory department of Malaysia. The range of $MLVSS_a$ concentration (20,580 - 23,621 mg/L) adopted in the mesophilic condition is acceptable as it is able to produce final treated effluent with BOD of less than 20 mg/L and are still compatible with the sludge settling characteristics. Thus, the bacteria concentration in the aerobic compartment is maintained within the range of 20,580 - 23,621 mg/L for long-term performance of the pilot-scale IAAB.

2.8.3 Returned Activated Sludge (RAS) System

In the aerobic compartment of the pilot-scale IAAB, returned activated sludge (RAS) system was installed. This is aimed to maintain the biomass concentration in the aerobic compartment of the pilot-scale IAAB by recycling sludge from the settling tank. This allows the creation of mass balance analysis around the aerobic compartment, where the solids entering the aeration compartment will be equal to the solids leaving the tank. Since this study is aimed to produce final treated effluent with BOD less than 20 mg/L, the concentration of solid remained in this aerobic compartment is utmost important.

This can be controlled by manipulating the RAS ratio (R_{RAS}), where a certain volume of solid will be pumped from the settling tank to the aerobic compartment of the pilot-scale IAAB. Direct comparison can be made between laboratory-scale and pilot-scale IAABs, as both the bioreactors have similar configurations. As a result, the R_{RAS} range of 0.47 – 0.89 reported by Chan et al. (2012) has been used as a guideline for the operation of the pilot-scale IAAB to maintain the $MLVSS_a$ at 18,000 mg/L (corresponding to $MLSS_a$ of 24,000 mg/L). Despite maintaining the R_{RAS} during the operation of the pilot-scale IAAB, it is unable to maintain the $COD_{removal}$ (more than 95%) similar to the one reported by Chan et al. (2012). Instead, rapid sludge wash out and drastic increase of the solid concentration in the final treated effluent occurred. This may be rectified by increasing the solid concentration in the aerobic compartment of the pilot-scale IAAB by increasing the R_{RAS}. To the best of my knowledge, the combination of anaerobic and aerobic processes for the treatment of POME in terms of pilot-scale study does not give a positive result as compared to the laboratory-scale study in terms of the $COD_{removal}$ of the aerobic compartment (as shown in Chapter Four, Results and Discussion; Table 4.14). This may be due to the absence of recirculation system installed in the wastewater treatment system, or that the designed R_{RAS} may not be sufficient to retain the solid concentration in the aerobic compartment. This will affect the biological activity of the microbial cells in the aerobic compartment, which will lead to excessive, uncontrolled growth of different types of filamentous bacteria (Stypka, 1998). Hence, by operating the reactor with optimum R_{RAS}, it ensures sufficient volumes of activated sludge will be recycled into the aerobic compartment to maintain the biological activity of the microbial cells in the aerobic compartment.

The returned activated sludge ratio, R_{RAS} is calculated as:

$$R_{RAS} = \frac{Q_{RAS}}{Q_{in}} \tag{2.3}$$

where R_{RAS} is the ratio of the returned activated sludge, Q_{in} is the influent flow rate (L/day), and Q_{RAS} is RAS flow rate (L/day).

The returned activated sludge flow rate, Q_{RAS} is calculated as:

$$Q_{RAS} = \frac{(Q_{in})(MLVSS_a)}{MLVSS_{RAS} - MLVSS_a} \tag{2.4}$$

where Q_{RAS} is the flowrate of the returned activated sludge (L/day), Q_{in} is the influent flow rate (L/day), $MLVSS_a$ is the biomass concentration in the aerobic compartment (mg/L), and $MLVSS_{RAS}$ is the biomass concentration of the returned activated sludge (mg/L).

2.8.4 Waste Activated Sludge (WAS) System

Excess activated sludge accumulated in the settling tank has to be removed in a daily manner to maintain the effectiveness of the wastewater treatment. This is controlled by maintaining the sludge retention time (SRT) of the reactor. Currently, the most common practice is to remove sludge from the sludge return line or withdrawing mixed liquor directly from the aeration tank. The latter is not feasible to be practised in the industry due to the large size of the treatment pond. The former is widely used in the palm oil industry as it can be removed using a smaller waste sludge pump as the texture of RAS is more concentrated than the WAS. The removal of sludge from the pilot-scale IAAB could not be conducted manually due to the high waste flow rate, Q_{WAS} (23,239 L/day). In this scenario, a WAS pump is used for efficient sludge removal to sustain the desired biomass concentration in the aerobic compartment.

The R_{WAS} can be calculated as below:

$$R_{WAS} = \frac{Q_{WAS}}{Q_{in}} \tag{2.5}$$

where R_{WAS} is the ratio of the waste activated sludge, Q_{in} is the influent flow rate (L/day), and Q_{WAS} is WAS flow rate (L/day).

The Q_{WAS} can be calculated as below:

$$Q_{WAS} = \frac{(MLVSS_a)(V_a)}{MLVSS_{WAS} - SRT} \tag{2.6}$$

where Q_{WAS} is the flowrate of the waste activated sludge (L/day), $MLVSS_a$ is the biomass concentration in the aerobic compartment (mg/L), $MLVSS_{WAS}$ is the biomass concentration of the waste activated sludge (mg/L), and V_a is the volume of the aerobic compartment (L).

2.8.5 Oxygen Requirement

Oxygen is provided to the activated sludge microorganisms by dissolving it in the wastewater. The effectiveness of the aerobic process mainly depends on the amount of oxygen available. Hence, it is important to determine the amount of gaseous oxygen dissolved in the wastewater for aerobic biodegradation of carbonaceous material. It can be determined from a method known as Winkler Test where the amount of dissolved oxygen (DO) is directly proportional to the titration of iodine with thiosulfate solution (Rahim and Mohamed, 1978). Oxygen is mainly used for endogenous respiration, and the amount used depends on the system SRT. The most common practice used to determine the amount of oxygen dissolved in the wastewater is by mechanical means.

In the aeration tank, homogeneous and sufficient mixing is required to disperse DO throughout the basin and to provide reasonably uniform solid concentration throughout the liquid. If solid is not uniformly concentrated throughout the liquid, this may lead to the deposition of suspended solids in the bottom of the aeration tank, termed as dead zone. In the industry, oxygen is provided into the aeration tank via a blower. It is designed in such a manner as to: (a) satisfy the biodegradation of COD of the anaerobically-treated wastewater; (b) create an environment that satisfies the endogeneous respiration; (c) provide adequate mixing between the incoming anaerobically-treated wastewater with the activated sludge; (d) maintain a minimum DO concentration throughout the aeration tank; (e) prevent excessive aeration; (f) save energy, and; (g) sustain high activity of the aerobic microorganism. In order to achieve these criteria, the installation of proper aeration system is important. In this study, the aerobic compartment of the pilot-scale IAAB is installed with submerged fine bubble diffusers. Air is provided to the diffusers provided by a blower. It is then dispersed into fine bubbles, which will rise to the surface of the liquid in the reactor. The DO in the aerobic compartment can be determined by using a DO meter. To control the DO concentrations in the aerobic compartment, an air flow meter is used to control flowrate of oxygen gas going into the compartment.

The concentration of DO in the aeration tank(s) in an activated sludge process is an important process control parameter that has a great effect on the treatment efficiency, operational cost and system stability. It is essential to maintain the optimum DO concentration in the aeration tank. As the DO drops, the quantity of these filamentous microorganisms increases, hence adversely affecting the settleability of the activated sludge. If DO continues to drop, even low dissolved-oxygen filamentous microorganisms will not be present in the mixed liquor and the treatment efficiencies will be seriously affected. In this scenario, effluent turbidity will increase and the treatment will deteriorate rapidly. Even though higher DO is often a target, it is not practical in reality as this will increase the energy cost for mixing. If DO is within the optimum level, there is a good chance that dead zones are minimal since normal currents and mixing will transport the oxygenated mixed liquor throughout the reactor. However, if the DO is excessive, then there could be problems in the settling of sludge due to the shearing of flocs and re-suspension of inert materials (Du et al., 2018). A high DO concentration also makes the denitrification less efficient. Due to the complex nature of the microbial activities in the aeration tank, even a small change introduced into the system can affect the biodegradation process of the inert material in the anaerobically-treated wastewater. Hence, it is essential to maintain the DO concentration within the optimum level to prevent the occurrence of instability of the reactor.

2.8.6 Food-to-Microorganism (F/M) ratio

Food to microorganism (F/M) ratio is one the significant factors in biological wastewater treatment which affects biomass growth, sludge settleability and pollutant removal. In order to determine the stability of the aerobic granular activated sludge process, optimal range of F/M ratio must be determined. Janga et al. (2007); Wu et al. (2018) reported that the increasing F/M ratio from 0.13 gCOD/gSS.day to 0.29 gCOD/gSS.day increases the concentration of EPS excreted by the aerobic microbial cells. This is because at higher F/M ratios, protein and polysaccharides from the diluted synthetic wastewater could be more adsorbed by the sludge EPS matrix, resulting in increased turbidity of the effluent produced. Medina and Neis, (2007); Wu et al. (2018) reported that total nitrogen removal efficiency ($TN_{removal}$) increases from 40.82 ± 11.22% to 65.96 ± 11.9% in the symbiotic algal bacterial system as the F/M ratio decreases from 0.56 gCOD/gSS.day to 0.15 gCOD/gSS.day. This shows that a low F/M ratio (corresponding to high SRT) is able to promote the growth of stable and healthy aerobic granules, which is also observed in the study reported by Tay et al. (2004). It is crucial to maintain a low F/M ratio (less than or equal to 0.2 gCOD/gSS.day) to downgrade the EPS secretion level, as well as to suppress the superficial hydrophocity of the aerobic granules (Wu et al., 2018). This is aimed to promote good settleability (as reflected as SVI reading) and high pollutant removal efficiency demonstrated by the aerobic microbial cells enclosed within the granular activated sludge. Hence, the F/M ratio of the aerobic compartment of the pilot-scale IAAB was designed within the range of ≤ 0.2 gCOD/gSS.day with the aim of minimising the compromise in treatment efficiency whilst producing good quality final treated effluent. The F/M ratio can be calculated as below:

$$\frac{F}{M} = \frac{(Q_{in})(COD_{out,an})}{(V_a)(MLVSS_a)} \quad (2.7)$$

Where Q_{in} is the influent flowrate (L/day), $MLVSS_a$ is the biomass concentration in the aerobic compartment (mg/L), $COD_{out,an}$ is the average outlet COD concentration (mg/L), and V_a is the volume of the aerobic compartment (L).

2.9 Kinetic Studies on Biological Treatment of POME

In order to evaluate the performance of each biological treatment of POME, kinetic study is carried out by determining the respective kinetic coefficient. Kinetic modelling is used to depict the treatment efficiency of conventional ponding, covered-lagoon and full-scale IAAB systems using the same operational conditions in terms of substrate utilisation during the anaerobic digestion process. There are two factors that must be considered during the application of kinetic model. The model must be complex enough to be realistic, but simple enough that fitted parameters can be easily interpreted and used for design purposes, as well as being consistent with the observed experimental behaviour.

The unstructured kinetic models are mostly being employed for modelling microbial systems due to their simplicity. In literature reviews, there are a number of kinetic models used to describe the anaerobic and aerobic systems. They are Contois model (Contois, 1959; Chen et al., 1980; Hu et al., 2002), Monod model (Cecchi et al., 1990; Borja et al, 1994), First order substrate removal (Borja et al., 1993; Işik and Sponza, 2005), Stover-Kincannon model (Hosseiny and Borghei, 2002), and Grau Second-order model (Grau et al., 1975). The most frequently used models to describe the substrate removal kinetics and verification of the validity of the models are Monod, Stover-Kincannon and Grau-Second models. These are performed by comparing the experimental data with the predicted data at decreasing HRT, thereby used to describe the treatment efficiency of each system. Additionally, Stover-Kincannon and Grau second-order models are widely applicable in describing the kinetic constants in ponding, covered lagoon and IAAB systems. These systems are classified as immobilised system as they are unable to be moved during the whole treatment process.

2.9.1 Monod Model

Monod model is applicable to soluble substrate under conditions where the involvement of microorganisms can be evaluated. It is used to describe the microbial growth and substrate utilisation in continuously operating systems. Additionally, the use of Monod model in a continuous system that requires effluent recycle will not change the substrate removal rate, as well as the microbial cell concentration. This is due to the application of mass balance analysis, where the same effluent that leaves the reactor returns to the reactor and kinetic study will only be conducted when the system is in steady-state conditions. For a continuous system without biomass recycle, the rate of change of biomass in the anaerobic system (ponding, covered-lagoon and IAAB systems) can be expressed as:

$$\frac{dX}{dt} = \frac{Q_{in}}{V_{an}} X_{in} - \frac{Q_{in}}{V_{an}} X_{out.an} + \mu X_{an} - K_d X_{an} \quad (2.8)$$

where Q_{in} is the flow rate (L/day); V_{an} is the volume of the anaerobic system (L); X_{in} is the influent biomass concentration; $X_{out,an}$ is the anaerobically-treated effluent biomass concentration; X_{an} is the biomass concentration ($MLSS_{an}$); μ and K_d are the specific growth rate and death rate constant, respectively.

It is assumed that the influent biomass concentration is negligible, hence at steady-state $\frac{dX}{dt}$ is equivalent to zero. SRT, or mean-cell residence time, is defined as the average time that the microbial cells spend in the system. It is represented by the ratio of total biomass in the reactor to biomass wasted in a given time. The SRT of the system is calculated using the formula below:

$$SRT = \frac{V_{an} X_{an}}{Q_{in} X_{out,an}} \quad (2.9)$$

Hence, the relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Monod model equation:

$$\mu = \frac{(\mu_{max})(COD_{out,an})}{(K_S + COD_{out,an})} \quad (2.10)$$

The constants μ_{max} and K_S represent the maximum growth rate of microbial cells when the influent is being used at their maximum rate and the level of substrate concentration at half of the maximum specific substrate utilisation rate μ_{max}, respectively. It is assumed that the concentration of influent biomass is negligible, $X_{in} = 0$ and the rate of change of biomass in terms of time at steady-state condition, $\frac{dX}{dt} = 0$. Hence, equation 2.8 can be simplified as follows:

$$\frac{Q_{in}}{V_{an}} X_{out,an} = X_{an}(\mu - K_d) \quad (2.11)$$

$$\mu = \frac{(Q_{in})(X_{out,an})}{V_{an} X_{an}} + K_d \quad (2.12)$$

By substituting equation (2.9) into equation (2.12), the following is obtained:

$$\mu = \frac{1}{SRT} + K_d \quad (2.13)$$

The Monod model equation (2.10) is introduced into equation (2.13), thereby yielding:

$$\frac{(\mu_{max})(COD_{out,an})}{K_S + COD_{out,an}} = \frac{1}{SRT} + K_d \quad (2.14)$$

Equation (2.14) is a non-linear equation due to its inability to give a straight line when a graph is drawn. In order to draw a straight line on a plotted graph, it is essential to transform the equation into a linearised form. Additionally, it could be used to determine the constants involved, which are μ_{max} and K_s.

$$\frac{SRT}{1 + SRT(K_d)} = \frac{K_S}{\mu_{max}}\left(\frac{1}{COD_{out,an}}\right) + \frac{1}{\mu_{max}} \quad (2.15)$$

The constants (μ_{max} and K_s) can be calculated from the intercept and slope of the best fit line by plotting graph of equation (2.15), respectively.

For a continuous system without biomass recycle, the rate of change of substrate in the anaerobic system (ponding, covered lagoon and IAAB systems) can be expressed as:

$$-\frac{dS}{dt} = \frac{Q_{in}}{V_{an}} COD_{in} - \frac{Q_{in}}{V_{an}} COD_{out,an} - \frac{(\mu)(X)}{Y} \quad (2.16)$$

Under steady-state conditions, the rate of change of substrate is considered negligible, hence, $\frac{dS}{dt}$ is equivalent to zero. HRT and Y can be defined as volume of anaerobic system divided by influent flow rate and cell yield coefficient, respectively.

$$HRT = \frac{V_{an}}{Q_{in,an}} \quad (2.17)$$

Hence, equation 2.16 can be simplified as followed:

$$\frac{(\mu)(X_{an})}{Y} = \frac{Q_{in}}{V_{an}}(COD_{in} - COD_{out,an}) \quad (2.18)$$

By substituting equation (2.17) into equation (2.18):

$$\mu = \frac{(Y)(COD_{in} - COD_{out,an})}{(HRT)(X_{an})} \quad (2.19)$$

The Monod Model can be used to calculate the anaerobically-treated effluent concentration, $COD_{out,an}$ by introducing equation (2.10) into equation (2.19), thereby giving:

$$\frac{1}{SRT} + K_d = \frac{Y(COD_{in} - COD_{out,an})}{(HRT)(X_{an})} \quad (2.20)$$

Equation (2.20) is a non-linear equation due to its inability to give a straight line when a graph is drawn. In order to draw a straight line on a plotted graph, it is essential to transform the equation into a linearised form. Additionally, it could be used to determine the constants involved, which are Y and K_d by calculating the slope and intercept of the line.

$$\frac{COD_{in} - COD_{out}}{(HRT)(X_{an})} = \frac{1}{Y}\left(\frac{1}{SRT_{an}}\right) + \frac{1}{Y}K_d \quad (2.21)$$

Hence, the anaerobically-treated effluent biomass concentration, $COD_{out,an}$ can be predicted by rearranging equation (2.14) to:

$$COD_{out,an} = \frac{K_s(K_d - SRT_{an} + 1)}{SRT_{an}(\mu_{max} - K_d) - 1} \quad (2.22)$$

2.9.2 Modified Stover-Kincannon Model

One of the unique features of Stover-Kincannon model is that the substrate utilisation rate is expressed as a function of the organic loading rate (OLR) using monomolecular kinetics (Aydinol and Yetilmezsoy, 2010). In this model, the substrate removal rate is considered as a function of substrate loading rate at steady-state, shown in Equation 2.23.

$$\frac{dS}{dt} = \frac{Q}{V}(S_i - S_e) \quad (2.23)$$

where $\frac{dS}{dt}$ is the substrate removal rate; Q is the flow rate; V is the anaerobic system volume; S_i and S_e are the influent and effluent substrate concentrations, respectively.

The original Stover-Kincannon Model is designed for rotating biofilm reactors, expressed as:

$$\frac{dS}{dt} = \frac{Q(S_o - S_e)}{V} = \frac{U_{max}(\frac{QS_o}{A})}{K_B + (\frac{QS_o}{A})} \quad (2.24)$$

where A represents the total disc surface area on which biomass is immobilised; U_{max} is the maximum substrate removal rate, and; K_B is the saturation value constant. The suspended biomass concentration is considered negligible compared to the attached biomass on the rotating biofilm reactor. Hence, modification is being carried out by replacing total discs surface area with total organic loading rates (OLR), as shown below:

$$\frac{dS}{dt} = \frac{Q(S_o - S_e)}{V} = \frac{U_{max}(OLR_i)}{K_B + (OLR_i)} \quad (2.25)$$

In order to draw a straight line on a plotted graph, it is essential to transform the equation into a linearised form. Additionally, it could be used to determine the constants involved, which are U_{max} and K_B by calculating the intercept and slope of the line, respectively.

$$\frac{V}{Q}(COD_{in} - COD_{out}) = \frac{K_B}{U_{max}}\left(\frac{1}{OLR_{an}}\right) + \frac{1}{U_{max}} \quad (2.26)$$

Where COD_{in} and COD_{out} are influent and effluent COD, respectively; and OLR_{an} is equivalent to QS_i/V.

The remaining substrate left in the system can be written as:

$$COD_{in} = COD_{out} + V\frac{dS}{dt} \quad (2.27)$$

Substituting equation (2.25) into (2.27) gives:

$$COD_{in} = COD_{out} + [\frac{(U_{\max})(OLR_{an})}{K_B + OLR_{an}}]V \quad (2.28)$$

2.9.3 Grau Second-Order Multicomponent Substrate Removal Model

The general equation of a second-order model is shown as below (Grau et al., 1975):

$$-\frac{dS}{dt} = k_s X_{an}(\frac{COD_{an,out}}{COD_{in}})^2 \quad (2.30)$$

where k_s is the multicomponent substrate removal rate constant.

Equation (2.30) is integrated within the boundary condition (COD=COD_{in} to COD_{out}, and t=0 to HRT) and then linearised, equation shown below will be obtained:

$$\frac{(COD_{in})(HRT)}{COD_{in}-COD_{out}} = \frac{COD_{in}}{(k_s)(X_{an})} + HRT \quad (2.31)$$

If the first term of the right side of the equation is considered as a constant, Equation 2.31 can be modified as follows:

$$\frac{(COD_{in})(HRT)}{COD_{in}-COD_{out}} = a + HRT \quad (2.32)$$

where a is equivalent to $\frac{COD_{in}}{(k_s)(X_{an})}$ (2.33)

Rearranging Equation (2.33) shows that k_s (substrate removal rate) is dependent on the substrate removal kinetics for each unit of microorganism. Additionally, b is a constant, which is greater than unity, thereby allowing $\frac{(COD_{in}-COD_{out})}{COD_{in}}$ to be replaced by E_{an}. It is represented by anaerobic substrate removal efficiency. Hence, equation (2.33) can be written as:

$$\frac{HRT}{E_{an}} = bHRT + a \quad (2.34)$$

The constants a & b can be determined by plotting Equation (2.34) from the intercept and slope of the best fit line.

In order to develop a formula for predicting anaerobic effluent substrate concentration ($COD_{out,an}$), one can use the influent substrate concentration (COD_{in}), HRT and substituting the values of constant a & b into Equation (2.32):

$$COD_{out} = 1 - (\frac{a+bHRT}{HRT}) \quad (2.35)$$

2.9.4 Contois Model

The well-known Contois equation expressed in Equation (2.36) was developed by Contois in 1959 (Contois, 1959) to describe an inverse relationship between microbial concentration and their specific growth rate.

$$\mu = \frac{\mu_{max} COD_{out,an}}{BX + COD_{out,an}} \quad (2.36)$$

where μ is the specific growth rate; μ_{max} is the maximum specific growth rate; $COD_{out,an}$ is the anaerobically-treated effluent biomass concentration, B is a growth coefficient of the Contois function; X is the microbial concentration.

According to Equation (2.36), the specific growth rate of a microbial cell is not only a function of the limiting substrate concentration as predicted by the classical Monod Equation (2.10) but also depends on the microbial cell concentration. Microbial cell concentration is inversely proportional to the specific growth rate. By substituting Equation (2.36) instead of Monod equation into Equation (2.8) gives

$$\frac{\mu_{max} COD_{out.an}}{BX + COD_{out,an}} = \frac{1}{HRT} + K_d \quad (2.37)$$

Equation (2.37) is a non-linear equation due to its inability to give a straight line when a graph is drawn. In order to draw a straight line on a plotted graph, it is essential to transform the equation into a linearised form. Additionally, it could be used to determine the constants involved, which are μ_{max} and K_d.

$$\frac{HRT}{1+SRT(K_d)} = \frac{B}{\mu_{max}}\left(\frac{1}{COD_{out}}\right) + \frac{1}{\mu_{max}} \quad (2.38)$$

The values of μ_{max} and B can be obtained by plotting Equation (2.38). The value of μ_{max} can be calculated from the intercept of the straight line, while B could be obtained from the slope of the line.
Under steady-state conditions, the rate of change in substrate concentration is negligible and by a similar technique to that used for the substrate concentration, Equation (2.8) can be reduced to

$$\frac{1}{HRT}(COD_{in} - COD_{out}) = \frac{X_{out}}{Y}(\frac{1}{HRT} + K_d) \quad (2.39)$$

The above equation can then be rearranged to estimate the effluent biomass concentration under steady-state condition as follows:

$$X_{out} = \frac{Y(COD_{in} - COD_{out})}{1+(K_d)(HRT)} \quad (2.40)$$

By substituting Equation (2.40) into Equation (2.37) and then rearranging it, the effluent substrate concentration at steady-state conditions can be expressed as:

$$COD_{out} = (\frac{(B)(Y)}{HRT(\mu_{max} - K_d) \times HRT + B \times Y - 1})(COD_{in}) \quad (2.41)$$

To date, none of the reports has been carried out on comparing the performance of conventional ponding with modified anaerobic system (listed in Table 2.5) by carrying out kinetic study. The behaviour of each POME treatment system in both the mills will be determined by carrying out a simple kinetic study. The determination of kinetic coefficients of each system using different kinetic models, i.e. Monod model, Stover-Kincannon model and Grau second-order multicomponent substrate removal model, allows one to predict some estimated parameters, e.g. $COD_{removal}$ and methane composition at a given OLR and HRT. The behaviour of the same system can be predicted as well either by increasing or decreasing the MLVSS concentration of the substrate use, which is represented by X. The fixed conditions for Monod model are COD_{in}, HRT and X. For Stover-Kincannon model, the variable that will result in changes of the regression analysis is the anaerobic OLR, denoted as OLR_{an}. The Grau second-order multicomponent substrate removal model is used to determine the effect of substrate removal rate, which depends on the MLVSS (X) and COD_{in}. The regression analysis obtained in each model for anaerobic and aerobic systems will be compared with the pilot IAAB, which will allow one to determine the most efficient system used to treat POME. Performance evaluation of the pilot-scale IAAB together with the proposed treatments will be revaluated using the same kinetic models.

2.10 Alternative to Anaerobic Mono-Digestion: Co-digestion

Anaerobic co-digestion, the simultaneous anaerobic digestion of two or more substrates, is a feasible option to overcome the drawbacks of mono-digestion (Table 2.9) and to improve the economic viability of the anaerobic digestion plants due to the higher methane production (Alvarez et al., 2014). From a research perspective, anaerobic co-digestion is focused on mixing more than a type of substrates to enhance positive interactions, i.e. macro- and micronutrient equilibrium, moisture balance as well as diluting inhibitory or toxic compounds (Choong et al., 2018). This allows us to conclude that the addition of more than one type of substrate to be co-digested under the anaerobic manner is able to enhance the production of methane. However, the reserved mind set of industry players in accepting new treatment methods for the improvement in methane production, as well as the overall treatment system, has halted the development of co-digestion in the current treatment means. According to the review by Alvarez et al. (2014), it shows that the most frequent main substrates used are animal manures (54%), sewage sludge (22%), and organic fraction of municipal solid waste (11%). Additionally, the substrates used for co-digestion are industrial waste (41%), agricultural waste (23%), and municipal waste (20%). The additive of greenery biomass in the anaerobic co-digestion directly increases the OLR of the entire anaerobic treatment system. These are the main ameliorated factors by the strategy of co-digestion. Hence, co-digestion is able to improve the overall anaerobic digestion performance, and the biogas production effectively (Yen and Brune, 2007; Bouallagui et al., 2009; Choong et al., 2018).

For this large industry to prosper, innovative and creative solutions are very much sought after to allow the generation of different bio refinery options to overcome challenges, as well as to produce 'green' industries (Loh et al., 2017).

Table 2.9: Advantages and disadvantages of co-digestion technology (Wu, 2007)

Advantages	Disadvantages
Improved nutrient balance and digestion	Increased digester effluent COD
Equalisation of particulate, floating, settling and acidifying wastes, through dilution by wastewater	Additional pre-treatment needed
Additional biogas collection	Increased mixing requirement
Economic advantages derived from the sharing of equipment	Wastewater treatment requirement
Additional fertiliser (soil conditioner) reclamation	High utilisation degree required
Renewable biomass (Energy Crops) disposable for digestion in agriculture	Decreasing availability and rates
Easier handling of mixed wastes	Hygienisation requirement
Synergistic effect	Restriction of land use for digestates
	Economically-critical; dependant on crop cost and yield

In the palm oil industry, it is difficult for the palm oil millers to adapt new treatment methods that may possibly improve the existing POME treatment method, due to the high capital cost requirement and the lack of manpower. Due to the stringent discharge limit set by DOE, where the BOD of the final discharge effluent need to be less than 20 mg/L, existing POME treatment methods (conventional ponding and covered lagoon systems) need to be improved. The large volumes of POME produced from the 454 palm oil mills operating in Malaysia shows that there is a gap between the industry and R&D. Innovative R&D needs to be continued, new technologies are to be exploited, and current technologies need to be improved to achieve more stringent sustainability requirements for POME treatment. In order to improve the current POME treatment method, co-digestion may act as an alternative for the mono-digestion due to the large amount of waste being generated during the processing of FFB into CPO. With the advancement in technology, the current POME treatment method (conventional ponding or covered lagoon systems) can be converted into using high-rate bioreactors. The co-substrates that had been co-digested with POME are EFB (Kim et al., 2013), sewage sludge (Sivasankari et al., 2013), cow manure (Sidik et al., 2013), and a mixture of EFB and microalgae (Ahmad et al., 2014; Ahmad et al., 2015). The improvement in terms of biogas or methane production for these studies are shown in Table 2.10.

Table 2.10: Biogas production performance of POME co-digestion

Co-substrate	Reactor Temperature	Composition	C/N Ratio	Biogas production	Reference
Oil palm EFB	10L digester, 35°C	0.25 to 0.31 (EFB/POME COD basis)	57-59	Methane production rate = 50.8 mL CH_4.$gVSS^1d^{-1}$	(Kim et al., 2013)
Sewage sludge	Batch 5L digester, 29°C	70% POME + 30% SS	20.0	Cumulative biogas production = 28.2 L	(Sivasankari et al., 2013)
Cow manure	Batch 1.25L digester, 28-34°C	60% POME + 40% Cow Manure	n/a	Cumulative biogas production = 0.864 L	(Sidik et al., 2013)
		70% POME + 30% Cow manure	n/a	Cumulative biogas production = 1.875 L	
		80% POME + 20% Cow manure	n/a	Cumulative biogas production = 1.504 L	
EFB, and microalgae: *Tetraselmis suecica*	Batch reaction vessel, 48°C	EFB: 0. 12g.$mLPOME^{-1}$; *Tetraselmis suecica*: 2mL.$mLPOME^{-1}$	n/a	Methane yield = 3,900.8 $mLCH_4$.$LPOME^{-1}d^{-1}$	(Ahmad et al., 2014)
EFB: and microalgae: *Nannochloropsis oculata*		EFB: 0. 12g.$mLPOME^{-1}$; *Nannochloropsis oculata*: 2mL.$mLPOME^{-1}$	n/a	Methane yield = 5,018.0 $mLCH_4$.$LPOME^{-1}d^{-1}$	(Ahmad et al., 2015)
EFB, and microalgae: *Chlorella* sp.		EFB: 0. 12g.$mLPOME^{-1}$; Chlorella sp: 2mL.$mLPOME^{-1}$	n/a	Methane yield = 5,295.8 $mLCH_4$.$LPOME^{-1}d^{-1}$	(Ahmad et al., 2014)
Refined glycerine wash water	500m^3 digester	99% POME + 1% refined glycerine wash water	n/a	Methane yield = 0.15 $kgCH_4/kgCOD_{removed}^{-1}$	(Sulaiman et al., 2009)
		98% POME + 2% refined glycerine wash water		Methane yield = 0.13 $kgCH_4/kgCOD_{removed}^{-1}$	
		97% POME + 3% refined glycerine wash water		Methane yield = 0.10 $kgCH_4/kgCOD_{removed}^{-1}$	
		96% POME + 4% refined glycerine wash water		Methane yield = 0.10 $kgCH_4/kgCOD_{removed}^{-1}$	

Refined glycerine wash water	500 m^3 digester	95% POME + 5% refined glycerine wash water	n/a	Methane yield = 0.09 $kgCH_4/kgCOD_{removed}^{-1}$	(Sulaiman et al., 2009)
		94.75% POME + 5.25% refined glycerine wash water		Methane yield = 0.08 $kgCH_4/kgCOD_{removed}^{-1}$	
Oil Palm EFB	Batch assay of 20L polyethylene (PE) tank, 27-30 °C	76% POME + 24% EFB	45	Cumulative methane production = 2.03 L CH_4	(Nurliyana et al., 2015)
Moringa Oleifera	500 mL conical flask	90% POME + 10 % *M. oleifera* extract	18-25	Methane yield = 24.3 mL $CH_4/gCOD_{removed}$	(Yap et al., 2021)
Biodiesel waste glycerine	10 L reactor	n/a	n/a	Methane yield = 0.53 LCH_4/gVS_{added}	(Rabii et al., 2019)
Maize straw	n/a	50% cow manure + 50% maize straw	28.31	Methane yield = 613.8 mL CH_4/g VS	(Wei et al., 2019)
		25% sewage sludge + 75% maize straw	12.50	Methane yield = 472.3 mL CH_4/g VS	
Cow Manure	n/a	25% sewage sludge + 75% cow manure	11.43	Methane yield = 470.3 mL CH_4/g VS	(Wei et al., 2019)

The data shown in Table 2.10 allow us to conclude that selecting an appropriate co-substrate and mixing proportion is of utmost importance in favouring synergy and optimising methane production. For instance, co-digestion of potato waste and pig waste maximises the generation of methane, while co-digestion of Cow manure and chicken waste yields minimum methane under mesophilic state (Siddique and Wahid, 2018). The sewage sludge with high total nitrogen will lower the C/N ratio within the anaerobic treatment system. On the other hand, lignocellulosic co-substrate such as EFB (Kim et al., 2013; Alvarez et al., 2014; Nurliyana et al., 2015) with high carbon content will increase the C/N ratio. Hence, this will improve the methane production by approximately 20% due to the increase in loading rate, as well as contributing to the system stability, nutrient balancing, synergistic effects of microorganisms, and processing cost (Henard et al., 2017; Siddique and Wahid, 2018).

Two important aspects, i.e. biogas capture and utilisation and maintaining the final discharge standard to comply with the regulation will move the palm oil milling industry towards the direction of creating a zero discharge or zero-emission state. The new discharge limit of BOD 20 mg/L has been gradually introduced nationwide, therefore research needs to be expedited and workable R&D solutions have to be showcased commercially. The rate of return on investment can be increased if a more productive biogas production from POME can be realised. As reported by Demirel and Scherer (2008); Sidik et al. (2013), the volume of biogas produced during the anaerobic mono-digestion of substrate fluctuated due to the large surface area of the treatment methods used. This is one of the major issues in the POME treatment. From an industrial standpoint, the improvement of methane production is mainly due to the increase in the OLR rather than the synergism, all kinds of mixtures are considered and being used. Hence, anaerobic co-digestion may be used as an alternative means to address the issues faced by palm oil millers.

2.10.1 Biogas Generation in Malaysia

Globally, anaerobic co-digestion system has become a more attractive feature for the generation of biogas as a source of renewable energy. Such technology is commonly used in developed countries, e.g. Germany (Weiland, 2010), Italy (Bacenetti et al., 2013), Canada (White et al., 2011), the United States (Shen et al., 2015), Denmark (Bojesen et al., 2015) and Norway (Venkatesh and Elmi, 2013). The use of biogas to produce heat, mechanical energy, and electrical energy is not commonly practised in Malaysia. At present, biogas is considered to have great potential for improving sustainable waste management and as a reliable source of energy.

Malaysia has a variety of different sources of raw material for biogas production, e.g. POME (Loh et al., 2017), animal waste (manure, blood, and rumen) (Abdeshahian et al., 2016), sewage sludge, food waste (Kumaran et al., 2016). The easily-available resources in Malaysia show that our country has a great potential for utilising such substrates for biogas production. Despite so, Malaysia remains in the initial stage of implementing the anaerobic co-digestion process for biogas production. In terms of social consideration, the public will definitely protest the establishment of anaerobic co-digestion plans near their respective residential areas due the possibility of pollution (Bong et al., 2017). Factors that halted such development are lack of local technology and grid access for biogas power plants. Most substrate-generating sources are located far from the interconnection points, whereas the distance should be within 10km to minimise power losses from transmission (Chin et al., 2013). Electricity generation from biogas requires high investment. The Malaysian government has launched various financial schemes for the improvement of renewable energy. However, shortage of skilled and expert personnel to maintain, operate and manage the anaerobic co-digestion process may slow down the establishment of biogas plants in Malaysia. In order to resolve these issues, government and educational institutions should take part in spreading knowledge, and arranging workshops on anaerobic digestion and biogas production, to develop such expertise.

2.10.2 Ways to overcome Methanogen Inhibition: Co-digestion

Anaerobic co-digestion can be considered as the instantaneous digestion of two or more substrate and co-substrate mixtures. Schematic diagram of the anaerobic co-digestion process is illustrated in Figure 2.18.

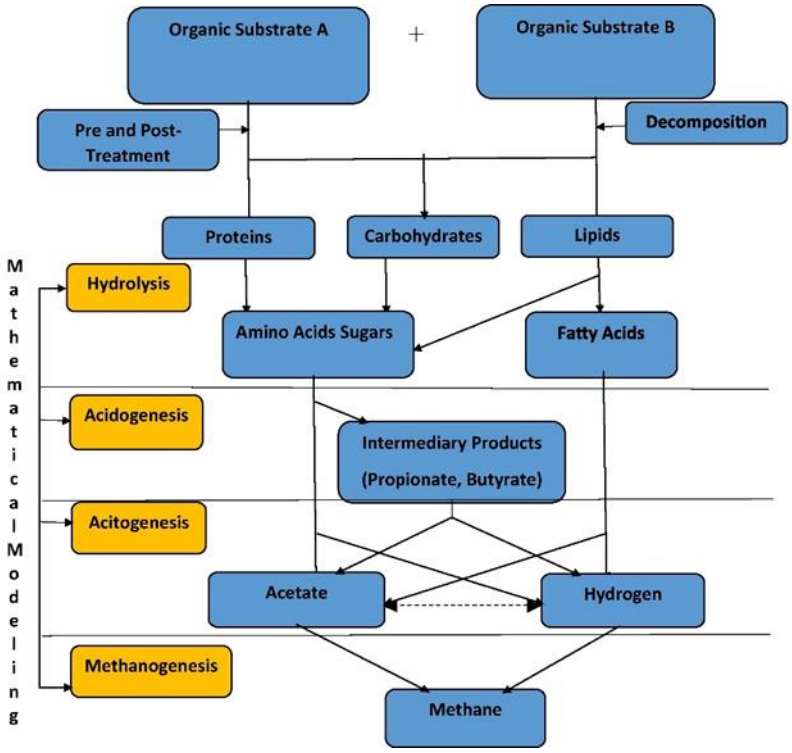


Figure 2.18: Flow diagram of anaerobic co-digestion and modelling application (Siddique and Wahid, 2018)

Typically, anaerobic processes are tailor-made for single substrates, but this can be further improved by using a variety of substrates that will make the process more stable. Primarily, anaerobic co-digestion is aimed at improving biogas and methane generation. Additionally, by performing anaerobic co-digestion, it is able to contribute to process stability, nutrient balance, synergistic effects of microorganisms, reduce greenhouse gas emission, and reduce processing cost (Henard et al., 2017; Siddique and Wahid, 2018). The anaerobic co-digestion technology uses different types of feed wastes to increase the methane generation, as shown in Table 2.11.

Table 2.11: Comparison of anaerobic co-digestion of various substrates and methane yields

Substrate	Condition of digester (°C)	Methane yield (L/gVS_{added})	Reference
Pig manure and Food waste	Mesophilic (37)	0.52	(Dennehy et al., 2016)
Potato waste and Pig waste	Mesophilic (37)	0.42	(Monou et al., 2008)
Cow manure and Fish waste	Mesophilic (37)	0.36	(Callaghan et al., 1999; Siddique and Wahid, 2018)
Cow manure and Brewery waste	Mesophilic (37)	0.30	(Callaghan et al., 1999; Siddique and Wahid, 2018)
Cow manure and yogurt waste	Mesophilic (37)	0.26	(Callaghan et al., 1999; Siddique and Wahid, 2018)
Potato waste and Abattoir waste	Mesophilic (37)	0.24	(Monou et al., 2008; Siddique and Wahid, 2018)
Cow manure and fruit & vegetables wastes	Mesophilic (37)	0.22	(Callaghan et al., 1999; Siddique and Wahid, 2018)
Cow manure and Chicken waste	Mesophilic (37)	0.13	(Callaghan et al., 1999; Siddique and Wahid, 2018)
POME and EFB	Mesophilic (27-30)	0.59	(Nurliyana et al., 2015)

Co-digestion of POME and EFB generates the maximum amount of methane, while co-digestion of Cow manure and Chicken waste yields the minimum amount of methane under mesophilic state. The selection of suitable types of co-substrates may improve the methane yields, reduce digestion lag times and improve the volatile solid (VS) removal. These remarkable improvements may be due to the high carbon content and buffering capacity (alkalinity) of the co-substrate complementing the low C/N ratio (17.93 ± 5.39) and lack of micronutrients in the POME (Nurliyana et al., 2015). Additionally, the high carbon content in pig/cow manure contributed to the high methane yield. This may be attributed to the low C/N ratio, high alkalinity and the presence of trace metals in pig manure complementing the high C/N ratio and lack of micronutrients in the organic waste residues (Alvarez et al., 2014). The addition of two different types of substrates will complement the missing nutrients and stabilise the C/N ratio, which suggests that sufficient acclimatisation period of the biomass will overcome all the possible factors that could cause methanogenesis inhibition. These positive synergistic effects are considered providing potential for higher methane yields. Therefore, anaerobic co-digestion between manures and C-rich wastes overcome these problems by maintaining a stable pH within the methanogens range, due to their inherent high buffering capacity, and by reducing the ammonia concentration by dilution, while enhancing methane production (Astals et al., 2012). It is worthy to understand the current progress of co-digestion with different substrates and comparison of biogas yield to be taken into consideration during the scaling of the digester used (Table 2.12).

Table 2.12: Current progress in co-digestion with different substrates

Feed stock	Condition	Comparison of biogas yield	Reference
Corn straw and blue microalgal biomass	Mesophilic	Co-digestion of these two substrates at C/N proportion of 21:1 enhanced the biogas generation remarkably, producing a specific methane yield of 235 mL/gVS at an OLR of 7gVS/L.day. A methane generation rate of 1,403 mL/L.day was attained by co-digestion that was more than mono-digestion.	(Zhong et al., 2013)
Manure and olive mill waste	Thermophilic	When olive mill waste was co-digested with cattle manure at C/N proportion of 20:1, it enhanced the methane production up to 17%, producing approximately 178 mLCH_4/gVS_{added}.	(Goberna et al., 2010)
Petrochemical wastewater and cattle manure	Mesophilic	When Petrochemical wastewater was co-digested with cattle manure at C/N of 22:1, it enhanced the methane production up to 50% without VFA accumulation.	(Siddique et al., 2015)
Arthrospira platensis and barley straw	Mesophilic	Co-digestion of *A. platensis* and carbon-rich feedstock such as barley straw, at C/N of 24:1, enhanced biogas production compared to the mono-digestion. Methane production rate at 324 mL/gVS at an OLR 2.0 gVS/L.day, which was achieved by co-digestion. This was 16% more than the mono-digestion.	(Herrmann et al., 2016)
Olive mill wastewater and poultry manure	Mesophilic	Co-digestion of olive mill wastewater and poultry manure with mixing ratio of 25:75 and C/N of 22:1 increased biogas generation by 20-25%.	(Gelegenis et al., 2007)
Food waste and pig manure	Mesophilic	Food waste was co-digested with pig manure at a mixing ratio of 1:4 resulted in the highest specific methane yield of 521 ± 29 mL CH4/gVS, as compared to the mixing ratios of 3:2 and 2:3.	(Dennehy et al., 2016)
Cassava pulp and pig manure	Mesophilic	Cassava pulp was co-digested with pig manure at a mixing ratio of 60:40 and a C/N ratio of 33, producing a specific methane yield of 306 mL/gVS_{added} at an OLR of 3.5 kgVS/m^3.day and a HRT of 15 days.	(Panichnumsin et al., 2010)
Swine manure and corn stalks, oat straw and wheat straw	Mesophilic	Co-digestion of swine manure with three different agricultural residues resulted in vast difference in methane amount produced at a C/N of 20:1. Corn stalks gave the highest methane production rate of 41.6 ± 12.1 L CH_4/day, followed by oat straw (35.0 ± 4.3 LCH_4/day), and lastly by wheat straw (16.6 ± 4.3 LCH_4/day).	(Wu et al., 2010)
POME and EFB	Mesophilic	Co-digestion of these two substrates at C/N of 45:1 enhanced the biogas generation remarkably, producing a cumulative methane yield of 0.5932 ± 0.02 mL/gVS. This was 8% more than the mono-digestion.	(Nurliyana et al., 2015)
POME and EFB	Thermophilic	Co-digestion of these two substrates under 55°C has enhanced the biogas production at a mixing ratio of 0.4:1, producing methane yield of 340 mL CH_4/gVS_{added}.	(O-Thong et al., 2012)
POME and *Moringa Oleifera*	Mesophilic	Co-digestion of these two substrates at C/N of 18-19 has enhanced the biogas generation, resulting in the highest methane yield of 24.3 mL CH_4/g$COD_{removed}$ as compared to mono-digestion of POME (19.1 mL CH_4/g$COD_{removed}$).	(Yap et al., 2021)

Municipal wastewater sludge and biodiesel waste glycerin	Mesophilic	Biodiesel waste glucerin was co-digested with municipal wastewater at 1.25% by volume has enhanced the biogas production and methane yield of 12.2 L/day and 0.53 $LCH_4/g\ VS_{added}$ as compared to mono-digestion of 8.2 L/day and 0.34 $LCH_4/g\ VS_{added}$, respectively.	(Rabii et al., 2019)
Cow manure and maize straw	Mesophilic	Co-digestion at these two substrates at C/N of 28.31 has enhanced positively the biogas production, producing methane yield of 613.8 mL CH_4/gVS_{added} as compared to C/N of 23.99 (534.8 mL CH_4/gVS_{added}), 25.21 (587.8 mL CH_4/gVS_{added}), 32.82 (603.5 mL CH_4/gVS_{added}), 7.33 (336.0 mL CH_4/gVS_{added}), 9.19 (395.0 mL CH_4/gVS_{added}), 12.5 (472.3 mL CH_4/gVS_{added}), and, 17.82 (352.3 mL CH_4/gVS_{added}). This was 28% more than the mono-digestion.	(Wei et al., 2019)
Sewage sludge and cow manure	Mesophilic	Sewage sludge was co-digested with cow manure at C/N of 11.43 has enhanced the total biogas production and methane yield of 7,055 mL CH_4 and 470.3 mL CH_4/gVS_{added}.	(Wei et al., 2019)

The dramatic increase in publications related to anaerobic co-digestion in the last decade reflects the viability and suitability of anaerobic co-digestion process in the improvement of biogas generation, ecological sustainability and stability of the process. It is essential to understand the characteristics of the feedstock, co-substrate used as well as the environmental conditions, as these are the factors that are needed to be taken into consideration during the designing of universal digesters, and lastly to optimising the anaerobic co-digestion process (Alvarez et al., 2014; Siddique and Wahid, 2018). Identifying the optimum ranges of each of the independent parameters (as discussed in Section 2.11) are equally essential, as they will indirectly influence the stability of the process, which will in turn affect the methanogenesis pathway. Grouping substrates of the chemical properties of the wastewater by biochemical composition may be useful to assess their biodigestibility and bioavailability. The three main components in substrates that will complement each other in different levels of anaerobic digestion are carbohydrates, protein and high-fat-content organic materials. The breakdown of carbohydrates will accelerate the production of VFA via acidogenesis. Protein and high-fat-content organic materials will produce relatively large amounts of methane in biogas process via methanogenesis. However, excessive concentration of these macronutrients will give an inhibitory effect on the growth of the methanogen. Temperature is the second independent parameter that is required to be taken into consideration during the design of the anaerobic process. It will drive the functions of the microorganisms in the process of anaerobic digestion. Mesophilic process is more stable compared to thermophilic process, as there are a wider variety of microorganisms that favours mesophilic, compared to thermophilic, conditions. Even though rates of gas production will increase along with the increase in temperature, it will reduce the content of methane produced. Additionally, it will affect the solubility of the CO_2, which will indirectly disturb the alkalinity of the digester. To prevent the usage of additional cost in the treatment of high-strength wastewater, the temperature ranges of 32°C - 35°C will be used as a design parameter to ensure continuous and stable production of methane, as well as to enhance the growth of microbial community in the anaerobic digester.

The overall pH of the anaerobic digester will affect its stability, as pH has a direct relationship with alkalinity. The 4 different steps involved in anaerobic digestion require different optimal pH to carry out their enzymatic reaction. To accommodate the enzymatic reactions of the 2 different types of microbial community, it is suggested to maintain the pH between 6.8 to 7.2, except for hydrolysis and acidogenic microorganisms that requires a lower pH of 5.5 to 6.5 to produce VFA. This allows us to infer that initial pH of the raw substrate is essential important as it will determine the fate the anaerobic digestion process. Particle size of the co-substrate is important as this will determine the fate of the biodegradability of the wastewater used. Larger particles may clog and make the digestion process more difficult. On the other hand, smaller particles will increase the surface area, which will help the microorganism to work more effectively in the hydrolysis step. Agyeman and Tao, (2014) concluded that decreasing the food waste particle size from 8mm to 2.5mm increases the rate of methane production by 10%-29%.

However, study conducted by Silvestre et al. (2015) concluded that the reduction the of size of sewage sludge and organic fraction municipal solid waste from 20mm to 8mm does not cause a significant change in methane yield. This shows that the particle size of the co-substrate used must be maintained within 3 mm to prevent the occurrence of major operational problems.

C/N ratio is the fifth independent parameter that has to be taken into consideration during the designing of the anaerobic digestion process. It will affect the entire anaerobic co-digestion process (Reilly et al., 2016). Substrates with an optimal C/N ratio is able to provide sufficient nutrients for microbial cells to maximise biogas production. The anaerobic process is more stable when the C/N ratio ranges from 20 to 30, while too high or too low C/N ratio could cause acidification or ammonia inhibition in the anaerobic process, which would result in the failure of biogas production. Moreover, C/N can also be used to design the anaerobic co-digestion of more than two different types of biogas materials with a higher or lower C/N ratio than the recommended range of 20-30. For instance, the most stable C/N ratio for co-digestion of dairy manure, swine manure and wheat straw is from 25 to 30; when the C/N is 27, the biogas yield reaches a maximum. The highest biogas production is obtained when the C/N of the co-digestion of food waste and rice husk is 20. During anaerobic co-digestion process, co-substrates are added to reduce the C/N proportion in the digesters, which may aid in enhancing the biogas production (Moset et al., 2017). However, different studies conducted by (Romano and Zhang, 2008; Li et al., 2011; Marañón et al., 2012) showed that there are various ranges of C/N ratios of 15-30 for biogas production. This may be due to the different nutrient compositions of the substrates, where they are usually rich in recalcitrant components. Therefore, C/N may be considered as an important parameter to evaluate the anaerobic digestion substrates in biogas production and practical engineering. HRT is the final factor that governs the stability of the anaerobic process. Unsuitable HRT inhibits the metabolic activity of microorganisms. However, long HRTs can lead to the death of the microbial cells due to insufficient nutrients. For industrial-scale applications, a short HRT is recommended as it will aid in minimising the volume of the digester and the investment costs (Li et al., 2015), maximising biogas production (Grosser, 2017) and maximising the net electrical energy production (Di Maria et al., 2015). If the HRT is shorter than the microbial cell generation times, it will cause the washout of the microbes, thus leading to failure of the anaerobic digestion system (Dareioti and Kornaros, 2015). Xie et al. (2017) reported that HRT has a direct relationship with the concentrations of VFAs and alkalinity, where an increase in VFAs and alkalinity will increase the HRT and vice versa. Anbalagan et al. (2016) reported that within nine days of incubation, rapid biogas production was observed during the treatment of integration of freshwater microalgae and activated sludge.

2.11 Factors Affecting Anaerobic Co-Digestion

Similar to anaerobic mono-digestion, it is essential to identify the parameters that favours stable anaerobic digestion. This is to maximise the performance of the microbial cells for biogas production. Factors that may be considered to affect the performance of the anaerobic co-digestion are chemical properties of the wastewaters, temperature, pH value, size of the particle, C/N ratio, and HRT will be discussed in details in the next section.

2.11.1 Chemical Properties of the Wastewaters

It is essential to understand the chemical properties of different types of wastewaters as they act as a yardstick for selecting suitable wastewaters for anaerobic co-digestion. The exact chemical composition of the substrates is hard to identify, hence, grouping substrates by their respective biochemical composition may be an option to assess their biodegradability and bioavailability.

The most common component of all the substrates available is carbohydrates. Food waste is highly enriched in various sugars, which are the favoured substrates for acidogen to form VFA. High concentrations of sugar enhance the performance of the acidogen to produce VFA that will decrease the pH of the digester. However, excessive concentration of VFA due to the overloading of the digester may affect the performance of the methanogen as the biological activity of acidogen is faster than the methanogen, i.e. rate of consumption of methanogen for acetate, H_2 and CO_2 into CH_4 is slower than the production of VFA by acidogen. All plant-derived substrates are rich in carbohydrates, which is difficult to be degraded by the anoxic microbial cells. In plant cell wall, polysaccharides are the most abundant organic compounds found in nature, consisting of cellulose, hemicelluloses, pectin, and phenolic polymer lignin (de Souza, 2013). These are the components that contribute to the high complexity and rigidity to the plant cell wall. Cellulose, being the major component of plant cell wall, is packed together by hydrogen bonds to form highly turgid and insoluble structures, known as microfibrils. However, cellulose shows a great potential for biogas production. Starch is easily degraded by the anoxic microbial cells for biogas generation. The same observations were observed (Li et al., 2009; Neves et al., 2009a; Neves et al., 2009; El-Mashad and Zhang, 2010; Kumar et al, 2010; Nayono et al., 2010; Borja et al., 1994; Kim and Oh, 2011; Kim et al., 2011; Zhang et al., 2011; Marañón et al., 2012; Wang et al., 2012; Zhang et al., 2012; Brown and Li, 2013; Dai et al., 2013; Rajagopal et al., 2013; Wang et al., 2013; Zhang et al., 2013) where substrates with higher starch content can be easily degraded by the anoxic microbial cells as it will contribute to the high buffering capacity, nutrient balance, supplement of lipid and trace element, as well as less VFA accumulation. Since plant cell wall is made up of cellulose, co-digestion of wastewater that contained high carbon content will greatly impact the production of methane. Thus, selection of substrates that contained sufficient amount of carbohydrates may increase the treatment efficiency as compared to mono-digestion.

Another important element that gives contribution during the anaerobic digestion process is protein. Substrates with rich-protein content can contribute to the production of large amounts of methane in the biogas process (Murovec et al., 2015; Siddique and Wahid, 2018). During the degradation of proteins by anoxic microbial cells, ammonium ions are released. However, the undesirably high concentrations of ammonium ions (NH^{+}_{4}) are the foremost inhibitors to the anaerobic digestion process. As reported by Murto et al. (2004), bacterial involvement in the degradation of protein will undergo rapid fermentation with a retention time of less than a day. Under this scenario, the last degradation step is often the rate limiting step since methanogens grow more slowly than the acidogens upstream in the degradation chain. The release of ammonium ions during the breakdown of protein acts as a potent inhibitor for the methanogen especially if it is available at high concentrations. This will reduce the percentage of methane produced. As reported by Yenigün and Demirel, (2013), the total ammonia nitrogen (mixture of NH_3 and NH_4 gasses) concentration between 5,000 and 7,500 mg/L is able to produce maximum specific methanogenic activity of 0.10-0.15 kg COD/kgVS.day and an average sludge content of 40 kg VS/m^3.

The positive results obtained in the batch study of different kinds of wastewater may be applicable in the high-rate bioreactor with continuous mode of operation. In this mode of operation, proper acclimatisation of microbial cells is important as this will affect the degradation efficiency of the anoxic microbial cells. To ensure proper acclimatisation of the microbial cells on the high concentration of the total ammonia nitrogen, slow feeding of the substrate into the system is required. This allows us to postulate that in order to acclimatise the microbial cells with high concentrations of the total ammonia nitrogen in the high-rate bioreactor, a gradual increase of OLR and total ammonia nitrogen must be carried out to prevent the reactor from experiencing instability.

Substrates with high-fat-content organic material may act as a solution to increase the biogas production during the anaerobic digestion. This is because these components present abundantly in the wastewater is readily degraded. Lipids have a higher methane production rate than protein and carbohydrates, and the lipids at proper proportions could significantly improve the anaerobic digestion process of organic fraction of municipal waste and sludge by enhancing methane production (Liu et al., 2012). The same observations were observed in study reported by Ye et al. (2013) where co-digesting rice straw with 79.1 g of kitchen waste is able to generate methane yield of 383.9 LCH_4/kg VS_{added} as compared to co-digestion of rice straw that does not contain kitchen waste with methane yield of 350.3 LCH_4/kg VS_{added}. Similar observation was obtained by Xie et al. (2012), where the methane content for degradation of lipids, protein and carbohydrates were 67-74%, 50-58%, and 50%, respectively.

It is worthy to note that substrates with high lipid content may be a suitable source to increase methane yield. Despite the promising results obtained from various researches, the high lipid concentrations can create a nuisance to the anaerobic digester, such as blockages, adsorption into biomass and inhibition of the biological activities of the anoxic cells. Although higher quality of biogas is produced from lipid, it is hampered by excessive OLR or long chain fatty acids (LCFAs) (Stabnikova et al., 2005; Wu et al., 2015; Dasa et al., 2016). The anaerobic digestion of the LCFAs is often halted by an interruption of the balance between the metabolic activities that the anaerobic digestion process comprises, because these LCFAs are potential inhibitors to many of the bacteria involved in the anaerobic digestion process (Koster and Cramer, 1987). The mechanism for the inhibition effect of LCFAs is the adsorption of LCFAs onto the microbial cell wall or the membrane halted the microorganism's transport and protective functions (Dasa et al., 2016). The accumulation of LCFAs in the digester stops the process of methane formation from acetate-digesting acetogens, thus reducing the production of biogas.

Another challenge in the anaerobic digestion of lipid-containing wastes is the occurrence of washout of the methanogen at high OLRs. As a matter of fact, methanogen tends to grow very slowly and is sensitive in the harsh process conditions or slight changes in the operating conditions. As a result, a longer retention time is needed for the methanogen to be adapted to the new operating condition. As reported by (Sousa et al., 2013; Dasa et al., 2016), methanogens responsible for biogas production in the anaerobic digestion of lipid-containing wastes are highly sensitive to the LCFAs (if the concentration of LCFAs in the digester is beyond the optimum limit). Under this scenario, there will be a great reduction of methanogen population (sludge washout), which will reduce the methane production. In addition, at high temperatures, LCFAs can cause foaming (Nguyen et al., 2017; Siddique and Wahid, 2018). Prior to deciding the type of substrate to be co-digested with wastewater that contained low C/N ratio, determination of the chemical component of the substrate is vital, especially the protein, carbohydrate and lipid content, as these are the components that affect the biogas production.

2.11.2 Temperature

Another factor that govern the anaerobic digestion process is temperature. Anaerobic digestion can be carried out in three different sets of temperatures - (a) psychrophilic [25°C]; (b) thermophilic [approximately 55°C], and; (c) mesophilic [approximately 35°C]. The most commonly used temperature in designing anaerobic digestion process is mesophilic and thermophilic. Operating the digester at mesophilic temperature seems to be more stable than thermophilic temperatures, despite the promising results as reported by Koster and Lettinga, (1984); Chan et al. (2012); Poh and Chong, (2014) in terms of higher conversion (and growth) rates and higher efficiency in pathogen reduction. In the palm oil mill industry, POME is discharged at high temperature ranging from 80°C - 90°C (Poh et al., 2010). According to Nkemka and Hao, (2018) and Siddique and Wahid, (2018), instability will occur if the digester is operated at high temperatures. Even though the occurrence of anaerobic digestion stability can be rectified by the addition of $NaHCO_3$, it may not be feasible to operate high-rate bioreactor in terms of the pilot- or pre-commercialisation scales in thermophilic temperature due to the involvement of sophisticated technology needed to control the system, which leads to the involvement of high cost. The process of anaerobic digestion and methane forming bacteria works best in the temperatures between 29°C to 41°C or between 49°C to 60°C, and pressures of about 1.1 to 1.2 bars absolute (Sorathia et al., 2012). These bacteria multiply best in these two different temperature ranges, but at high temperatures the bacteria are more prone to ambient influences. As a result, reduction of methane was observed in the biogas produced although the gas production rate increases proportionally with temperature (Dai et al., 2015; Siddique and Wahid, 2018). As reported by Sorathia et al. (2012), temperatures between 32°C-35°C are found to be the most efficient for stable and continuous production of methane. Biogas produced outside this range will have a higher percentage of carbon dioxide and other gases. It is worth to note that temperatures of the digester is relatively important to ensure continuous and high production of methane gas.

2.11.3 pH Value

The determination of pH value has an influence in the anaerobic digestion system, as this will affect the solubilisation of the organic matters present in the substrates (Feng et al., 2015; Siddique and Wahid, 2018). The biological activity of the microbial cells in the digester is very much dependent on the pH. This is to avoid the occurrence of reactor failure, which will indirectly affect the biological activity of the methanogenesis responsible to produce biogas. Since anaerobic digestion is a series of complex process as it involves different group of microbial cells responsible for different processes, they have their respective optimum pH values to maximise their biodegradation efficiency. Theoretically, most of the anoxic microbial cells prefer to maintain in neutral pH conditions. However, many researches (Lemmer et al., 2017 ; Siddique and Wahid, 2018) reported that maximum achievement of methane yield can be obtained when the pH of the digester is maintained between 6.8 and 7.2. Hydrolysis and acidogenesis are the key steps in anaerobic digestion, where the incoming substrates will be broken down into smaller constituents (simple sugars, amino acids, and fatty acids) to produce substrates for methanogenesis. In the former process, acetate and hydrogen are produced, which can then be consumed by methanogens directly. Other molecules, viz. VFA's with chain length greater than acetate, must first be catabolised into compounds by acidogen. The end products for the latter process are VFAs, ammonia, carbon dioxide and hydrogen sulphide. To continuously maintain the biological activity of the methanogen as it is dependent on the abovementioned processes, it is essential to maintain the pH values within the range of 5.5-6.5 (Kusch et al., 2011). This allow us to conclude that ability to maintain the optimal pH concentration is one of the key reasons to ensuring that the biological activities of each microbial cells are between the exponential and stationary phases of the bacterial growth curve. During the initial operating stages, there will be an occurrence of pH reduction due to the continuous production of VFAs produced from the breakdown of large organic polymers. As a result, inhibition of methanogenesis occurs due to the slow growth rate of the methanogen. To overcome this problem, the substrates must be introduced into the digester in a stepwise manner. This is to ensure sufficient time has been allocated for the microbial cells to be acclimatised to the new environment.

2.11.4 Particle Size

The size of the particle of the substrates to be co-digested will also affect the process of biogas production. Substrates with large particles will tend to clog and make the entire digestion much harder to handle and operate. On the other hand, substrates with smaller particles are easier to be colonised by the microbial cells responsible for hydrolysis to secrete extra-cellular enzymes. It seems that the size of the substrate, plays an important role in the first step of the anaerobic digestion process. As reported by Wall et al. (2016), larger dry solids that act as substrates for co-digestion (> 3 cm) caused a major operational problem without the continuous addition of rumen fluid supplement. To overcome this problem, crop substrate with high lignocellulosic content are more suitable to be subjected to two-phase digestion.

Study reported by Agyeman and Tao, (2014), an increase in specific methane yield from 0.47 LCH_4/gVS to 0.63 LCH_4/gVS was obtained when the size of the food waste was reduced from 8mm to 2.5mm. In the context of selecting substrate for co-digestion, it seems that the rate of methane production is a function of particle size.

2.11.5 Carbon to Nitrogen (C/N) Ratio

The determination of carbon to nitrogen (C/N) ratio of the substrate mixture subjected to anaerobic co-digestion process is important because it will cause a great impact on the overall anaerobic digestion process. Mixture of substrate subjected to co-digestion with optimal C/N ratio is able to maximise biogas production. On the other hand, lower C/N ratio leads to higher ammonia concentration due to the overloading of the digester with high COD concentration of the substrate. This will hinder the growth of the microbial cells in the digester, which in turn will reduce the performance efficiency of the anaerobic digestion process. When the C/N ratio is greater than the optimal value in the anaerobic digestion process, large amount of VFAs are produced. Maintaining the optimal C/N ratio within the optimal value for fermentation process is an essential technique used to enhance the biogas production in anaerobic co-digestion process. The C/N ratio for each substrate as reported by Guillaume and Lendormi, (2014); Siddique and Wahid, (2018) is shown in Table 2.11.

Table 2.11: List of substrates characterised into lower and higher value of C/N ratio

Comparatively lower C/N value materials	C/N ratio	Comparatively higher C/N value materials	C/N ratio
Cattle manure	15 – 26	Rice straw	50 – 68
Poultry manure	4 – 16	Wheat straw	51 – 151
Pig manure	7 – 15	Sugar cane waste	139 – 151
Sheep manure	20 – 34	Corn waste	51 – 57
Horse dung	19 – 26	Oats straw	47 – 51
Kitchen waste	26 – 30	Algae	74 – 101
Vegetable wastes	8 – 36	Sawdust	199 – 501
Food wastes	2 – 18		
Peanut waste	19 – 32		
Grass cutting wastes	11 – 15		
Slaughterhouse waste	21 – 36		
Goat manure	9 – 18		
POME	13 - 23		

Cattle by-products are classified under the lower C/N value materials mainly due to the lack of nutrient concentrations, which reduces the biological activity of the anoxic microbial cells. Normally, organic wastes are mainly used in biogas production as they are rich in lignocellulose-type resistant materials. However, in anaerobic process, with the absence of oxygen, it is not possible to biodegrade the lignocellulosic materials in the substrate. To rectify this issue, pre-treatments are required for such wastes to make the cellulose accessible to hydrolysis to produce VFAs. As a result, there will be a significant concentration of "invalid carbon" which will deviate the actual C/N ratio of the substrate. The C/N ratios shown in Table 2.11 may only act as a reference for the general characteristics of the organic waste materials available for co-digestion. For anaerobic mono-digestion, the most optimum C/N ratio ranges from 20 to 30 for a stable digestion process. The C/N ratio for anaerobic co-digestion is lower than mono-digestion. As reported by Sidik et al. (2013), the optimum C/N ratio for anaerobic co-digestion process is between 16 to 19 taken into consideration the difficulty in biodegrading the lignin content in the substrate used. To unify a specific range of the C/N ratio, including the existing carbon of the easily degradable part, and excluding the carbon that is not specifically affected by microorganisms, an available carbon/nitrogen ratio is proposed as reported by Wang et al. (2017). By maintaining the C/N ratio within the range suggested by Sidik et al. (2013), it is able to enhance the methane generation by approximately 3.8 and 1.5 fold as compared with perennial ryegrass alone and waste activated sludge alone, respectively (Dai et al., 2015).

2.11.6 Hydraulic Retention Time (HRT)

Hydraulic retention time (HRT) is defined as the amount of time needed for any microbial cells to consume and biodegrade the substrate. If the HRT is not being controlled properly, it will affect the anaerobic digestion process by suppressing the growth of the anoxic microbial cells. If the digester is designed to operate in long HRTs, it may lead to the death of the anoxic microbial cells due to shortage of nutrients. In this scenario, formation of dead zones will occur. As reported by Ganidi et al. (2009), creation of dead zones may be contributed by the occurrence of foaming where there will be an inverse solids profile in the anaerobic digester. This will reduce the active volume of the digester, hence resulting in the sludge not receiving the same degree of stabilisation. To adopt the concept of anaerobic co-digestion into industrial-scale applications, a short HRT is recommended. This will reduce the volume of the digester and the capital cost (Li et al., 2015). Furthermore, operating the industrial-scale digester at a shorter HRT is able to enhance and maximise biogas production (Grosser, 2017). It is expected that the gas production and biogas production rates at short HRT will be at least 46% higher in comparison to the results obtained at the longest HRT. We can conclude that the proposed solution to enhance the biogas production seems to be an interesting option as it offers a unique opportunity for industrial-scale wastewater treatment plants to improve their overall net electrical energy production by enhancing maximum energy recovery from sludge.

Operating the wastewater treatment plants at a short HRT is able to allow the industry players to fully utilise the existing infrastructure, hence allowing them to explore new potential for alternative treatment for organic industrial waste, viz. fat-rich materials and food waste.

As reported by Siddique and Wahid, (2018), addition of water to the substrate is able to reduce the HRT, which may not be an attractive solution for the industry players as this will reduce the organic or inorganic matter present in the wastewater. This may reduce the methane yield produced. Operating the industrial-scale anaerobic digester at a short HRT may be an attractive solution provided that the generation times of the microbial cells are shorter than the designed HRT. Sludge washout from the anaerobic digester may occur if the designed HRT is shorter than the microbial cells generation time (Dareioti and Kornaros, 2015; Siddique and Wahid, 2018). Another advantage of operating the anaerobic digester in a shorter HRT is to prevent the increase in the concentration of VFAs and alkalinity. Too high or low of the VFAs and alkalinity in the anaerobic digester will fail the anaerobic digestion, which is reflected by the sudden drop of the pH, rapid sludge washout and formation of scum. It is found that the optimal HRT may vary for different co-digestion processes due to the desirable temperature limits and nature of the microbial cells. Maximum methane production rate of 0.90 L/L_Rd was observed during the co-digestion of mixture of food waste and lignocellulosic biomass with liquid cow manure at HRT of 16 days (Dareioti and Kornaros, 2015). Besides methane production rate, optimal HRT will influence the total nitrogen removal efficiency ($TN_{removal}$) and the total phosphorus removal efficiency ($TP_{removal}$). Anbalagan et al. (2016) reported that operating the anaerobic digester for the treatment of microalgae and activated sludge at an HRT of 6 days has increased the $TN_{removal}$ and $TP_{removal}$ from 64.6 to 81.5% and 56 to 80%, respectively. By taking into consideration the HRT needed for efficient co-digestion process, this allows sufficient time for the microbial cells to biodegrade the organic matter available in the substrate to maximise the production of biogas.

2.11.7 Sludge Retention Time (SRT)

The process of anaerobic digestion is able to reduce the amount of materials and produced biogas, which can be used as a source of energy. However, it tends to produce large amounts of solid after the capturing of biogas and treating of the wastewater. The solid accumulated in the treatment system may come from the wastewater or inoculum. It is essential to maintain a certain concentration of solid in the digester to maintain the efficiency of the microbial cells in biodegrading the organic or inorganic matter in the wastewater. Theoretically, it is essential to allow the solid to retain in the digester for a longer period of time (> 30 days) to provide sufficient methanogenic activity at the prevailing conditions, i.e. longer solid retention time (SRT). As defined, high SRTs allow the enrichment of slowly growing bacteria and consequently, the establishment of a more diverse biocoenosis with broader physiological capabilities compared to the digester operating in low SRTs. As reported by Clara et al. (2005) by increasing sludge age, enhanced removal is observed for most substances.

Methanogenesis starts at SRT between 5 and 15 days at 25 °C and between 30 and 50 days at 15 °C (Abdelgadir et al., 2014). On the other hand, maximum hydrolysis starts at 75 days SRT (Abdelgadir et al., 2014). The time frame given by the previous author on the maximum activity of the hydrolysis and methanogenesis allow us to conclude there is a correlation between SRT and the growth of microbial cells responsible for these processes. It is essential to keep the anaerobic system balanced as the methanogenic bacteria use the acid intermediate as rapidly as they appear. However, if the methane bacteria are not present in suitable numbers or are being slowed down by unfavourable environmental conditions, they will not use the acid as rapidly produced by the acidogen, and the volatile acids will increase in concentration. This shows that an increase in acid concentration act as yardstick for the indication of methane formers are not in balance with the acid formers (Abdelgadir et al., 2014). Not only providing proper operating condition for methanogen, keeping the slow growing methanogen within the sludge in the anaerobic digester is also important as this will enhance the maximum production of biogas.

Based on literature review as thoroughly discussed in Section 2.11, it is essential to determine the optimum condition for each of the abovementioned factors. Table 2.12 summarises the important operating parameters that can be used a guideline for the planning of the subsequent anaerobic co-digestion study.

Table 2.12: The important operating parameters for anaerobic co-digestion study

Parameter	Unit	Range
Temperature	°C	32 – 35
pH	-	5.5 – 6.8
Particle Size	mm	~ 3
C/N	-	16 – 19
HRT	Day	6 – 16
SRT	Day	5 - 15

2.12 Selection of Substrate

To date, the organic fraction of household waste, food waste, sewage sludge, manure, and energy crops are principally used for biogas production. However, there are a wide range of other organic substrates that can be used for biogas production, viz. lignocellulosic materials. It has a great potential in biogas production. The incorporation of lignocellulosic materials into the anaerobic digestion process seems to be an attractive feedstock for biogas production due to: (a) the source of the substrate is abundantly available in the worldwide, and; (b) the carbohydrate-rich content. For agricultural by-products, the process of co-digestion is considered more economic than pre-treatments and to be a promising method to improve the biogas production. The co-digestion of nitrogen-rich substrates, viz. animal manure (e.g. chicken waste and pig manure) with carbon-rich biomass (e.g. rice straw, *Moringa Oleifera*, EFB) may balance the C/N ratio as well as increase the volumetric biogas production and biogas yield (Bhatia et al., 2007a; Bhatia et al., 2007b; Comino et al., 2010; Ye et al., 2013). An anaerobic system with highly diversified microorganisms for the treatment of organic matter in the mixture of two or different types of substrates allow the creation of more stable methanogenesis process despite the constant fluctuation of environmental conditions (Kallistova et al., 2014). Additionally, the mixing of two different substrates improves the nutrients in the influent substrate (as reflected on the C/N ratio), increases the growth rate of microorganisms as well as enhances the metabolic regulation of the system (Hoelzle et al., 2014; Hagos et al., 2017). Continuation studies have shown that co-digestion of pig manure with cultivated algae (Astals et al., 2015), dairy wastewater with cattle manure (Jihen et al., 2015), sewage sludge with glycerol (Jensen et al., 2014), POME with *M. oleifera* (Bhatia et al., 2007a; Bhatia et al., 2007b), and POME with EFB (Nurliyana et al., 2015) in different operating conditions confirmed that the biogas production is improved compared with single substrate feed. This technology seems to enhance nutritional balance in the system as well as reduces the possibility of lipids and ammonia inhibition in the digestion environment. Some characteristics of food waste like low C/N ratio and high biodegradability are the most serious features in the process of anaerobic digestion which will lead to inhibitions to the whole system. Hence, co-digestion with different substrates is a good way to balance the C/N ratio in an anaerobic system. Mixing with cellulosic waste is a common way to deal with such challenges.

The C/N ratio of POME ranges between 13 – 23 (Table 2.11), which can be classified as a type of heavy strength wastewater with low content of carbon and vice versa for their nitrogen content. The use of POME as the sole feedstock in anaerobic digestion is able to generate methane but the efficiency in methane production may be limited due to the improper C/N ratios exhibited by the substrate used. This can be improvised by the addition of substrate with high carbon content, i.e. *M. oleifera* based on the positive results reported by Bhatia and her co-workers. This is aimed to balance up the C/N ratio in the mixture of POME with *M. oleifera* that may accelerate the methane production and improve the breakdown of the organic/inorganic matter in the POME (as reflected on the removal efficiency of the tested parameters). The selection of *M. oleifera* as a substrate to be digested with POME will be discussed in detail in Section 2.13.2.1.1.

2.13 Coagulation-Flocculation Process

In order to effectively treat high strength wastewater, the incorporation of coagulant/flocculant agent may be required. The functions of these agents are destabilistaion of colloids, removal of inorganic and organic matter (particulate and/or dissolved), removal of metals and anions (arsenic, phosphate etc.), and removal of pathogen microorganisms (Tzoupanos and Zouboulis, 2008). POME is a type of high strength wastewater due to its high concentration of solid (24,846 mg/L – 30,920 mg/L). As a result, it requires the incorporation of coagulant/flocculant agent to ease the settling process. There are two major types of materials suitable for the use in coagulation/flocculation processes (Dobias and Stechemesser, 2005; Renault et al., 2009).

1. Inorganic and organic coagulants including mineral additives (lime, calcium salts, etc.), hydrolysing metal salts (aluminium sulphate, ferric chloride, ferric sulphate, etc.), pre-hydrolysed metals (poly-aluminium chloride, poly-aluminosilicate sulphate, etc.) and polyelectrolytes (coagulant aids);

2. Organic flocculants including cationic and anionic polyelectrolytes, non-ionic polymers, amphoteric and hydrophobically-modified polymers, and naturally-occurring flocculants (starch derivatives, guar gums, tannins, alginates, etc.)

Coagulation is mainly induced by inorganic metal salts, e.g. aluminium and ferric sulphates and chlorides. Without the aid of coagulant agent, it may not be possible to keep the slow-growing methanogen in the anaerobic digester. Slow growth of methanogen is often due to the change in the environment. On the other hand, acidogen is less sensitive towards environmental change than methanogen. In the absence of coagulant agent, the solid will not clump together, instead the solid will be in the colloidal dispersion form. It is usually mixtures (either heterogeneous or homogenous) in which the particles are invisible to the naked eye, cannot be removed by filtration manner, but can be contained within a semi-permeable membrane. This can be solved by adding coagulant agent into the wastewater to allow the agglomeration of solid.

Coagulation is the destabilisation of colloidal particles. These particles are essentially coated with a chemically sticky layer that allow them to flocculate (agglomerate) and settle in a reasonable period of time. Naturally occurring compounds from starch to iron and aluminium salt can accomplish coagulation. Additionally, synthetic cationic, anionic, and non-ionic polymers are very effective coagulants than the natural coagulant, but will contribute to the high capital of cost to the overall treatment system.

Coagulation- flocculation is a simple and efficient physico-chemical method for wastewater treatment that is widely used to treat POME (Ahmad et al., 2005), textile wastewater (Meriç et al., 2005) and abattoir wastewater (Amuda et al., 2006). In terms of wastewater treatment, the application of coagulation-flocculation is able to enhance the removal of solids in highly concentrated wastewater (high strength wastewater) that contain significant amounts of settleable solids, which is usually a more cost-effective treatment compared to the other treatment methods. It is commonly known that most of the suspended particles in wastewater carry negative charge in aqueous medium. The metal salts of the coagulant will hydrolyse rapidly in wastewater at isoelectric point to form cationic species, which are then adsorbed by the negatively charged colloidal particles. Hence, occurrence of simultaneous surface charge reduction and formation of micro-flocs (Souza 2013; Lee et al., 2014). The efficiency of coagulation-flocculation process depends on several factors such as type and dosage of coagulant/flocculant (Hu et al., 2006), pH (Miller et al., 2008), mixing speed and time (Gurses et al., 2003), as well as temperature and retention time (Howe et al., 2006). Hence, the optimisation of these factors may significantly increase the process efficiency. There are three classes of chemical coagulants, (a) hydrolysing metallic salts; (b) Pre-hydrolysing metallic salts; (c) Synthetic cationic polymers that can be used as a coagulant. These will be discussed in details in the next section.

2.13.1 Chemical Coagulant

Chemical coagulation is a complex phenomenon involving various inter-related parameters; hence, it is very critical to define how well coagulants will function under given conditions. Chemical coagulants can be categorised in three parts as illustrated in the following Figure 2.19.

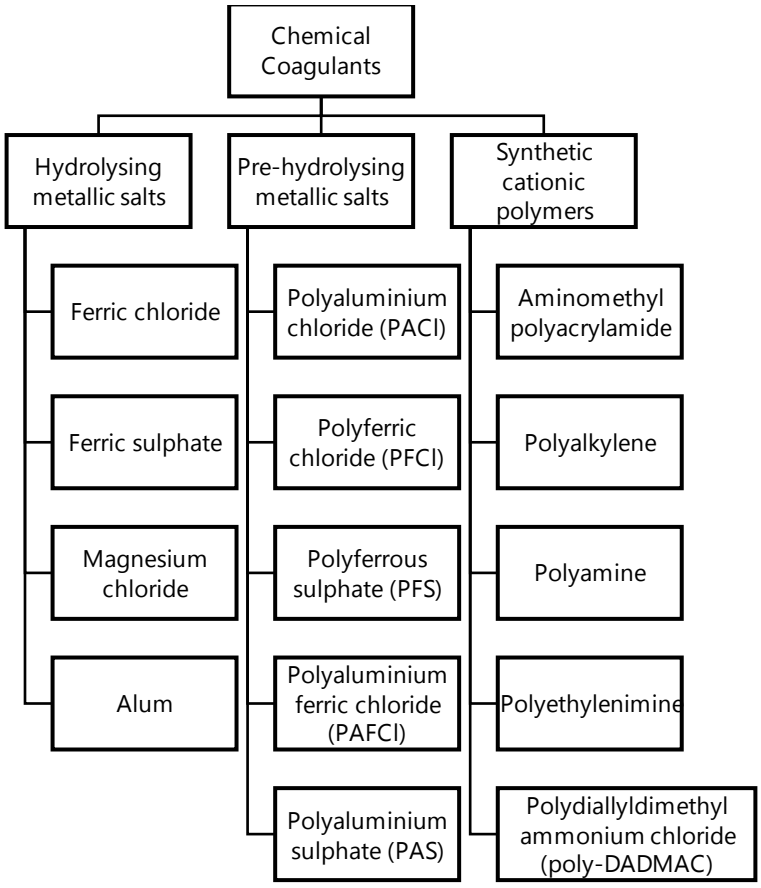


Figure 2.19: Categorisation of chemical coagulants according to their effectiveness (Verma et al., 2012)

It has been reported in the literature that pre-hydrolysed metallic salts are found to be more effective than the hydrolysing metallic salts, viz. aluminium sulphate (alum), ferric chloride and ferric sulphate, which are readily soluble in water. As reported by Verma et al. (2012), pre-hydrolysed coagulants such as Polyaluminium chloride (PACl), Polyaluminium ferric chloride (PAFCl), Polyferrous sulphate (PFS), and Polyferric chloride (PFCl) seem to provide better colour removal even at low temperatures and may also produce lower sludge volumes. Gregory and Rossi, (2001) also reported that pre-hydrolysed coagulants of PACl products exhibited more rapid flocculation and stronger flocs than alum at equivalent dosage. This may be due to the fact that these coagulants are pre-neutralised, have smaller effect on pH of water and so reduce the need of pH correction. Wastewater used in all industries are of negatively charged and hence cationic polymer is preferred over anionic and non-ionic polymers due to the better removal efficiency in terms of COD and TSS.

Different coagulants affect different degrees of destabilisation. The higher the valence of the counter ion, the more its destabilising effect and less dosage is required for coagulation. If pH is below the isoelectric point of metal hydroxide while precipitation of colloids by different coagulants supported by suitable polymer, then the positively-charged polymers can destabilise the negatively-charged colloids via the neutralisation pathway. Above the isoelectric point, anionic polymers will predominate where particle destabilisation may take place through adsorption and bridge formation. If high dosage of metal ions (coagulant) is subjected to wastewater treatment, a sufficient degree of oversaturation occurs to produce a rapid precipitation of a large quantity of metal hydroxide, enmeshing the colloidal particles which are termed as sweep flocs (Verma et al., 2012). For instance, when Fe (III) salts are used as coagulants, monomeric and polymeric ferric species are formed, the formation of which is highly pH dependent (Abo-Farha, 2010; Verma et al., 2012). Reported chemical coagulation technology and their performance are summarised in Table 2.13.

Table 2.13: Effectiveness of different chemical coagulants studied by different researches

Name of coagulant	Optimised dosage (mg/L)	Coagulant aids (if any)	Optimum pH	Optimum Results		References
Steel industry wastewater	N/A	N/A	4.3	COD	94% removal	(Anouzla et al., 2009)
				Colour	99% removal	
Potassium ferrate	100	Polyamine based polymer	6.5-8.5	COD	40% removal	(Ciabatti et al., 2010)
				Turbidity	80% removal	
				Colour	90% removal	
Polyaluminium Chloride (PACl)	10	N/A	7.2	Colour	99.9% removal	(Choo et al., 2007)
Polyepichlorohydrin-diamine	20	N/A	7.0	Colour	95% removal	(Kang et al., 2007)
Alum	200	Polyacrylamide based polymer (Cytec)	5.3	Colour	94% removal	(El-Gohary and Tawfik, 2009)
				COD	44% removal	
Alum	5,000	Copper sulphate as catalyst	4.0	COD	58.57% removal	(Kumar et al., 2008)
				Colour	74% removal	
Alum	20	Commercial cationic flocculant (Colfloc-RD-Ciba)	Near to neutral	COD	60% removal	(Golob et al., 2005)
				TOC	58% removal	
				BOD	50% removal	
Alum	7×10^4	N/A	5.7-6.5[a]	COD	72.6% removal	(Patel and Vashi, 2010)
				BOD	67.5% removal	
				Colour	74.5% removal	
Ferrous Sulphate	200	Polyelectrolyte	9.4	COD	90% removal	(Bidhendi et al., 2007)
				Colour	90% removal	
				TSS	80% removal	
Ferric chloride	400	N/A	8.3	COD	85% removal	(Bidhendi et al., 2007)
				Colour	Almost 100% removal	
Ferric chloride	293	N/A	6.0	COD	70.5% removal	(Kim et al., 2003)
				Colour	80.6% removal	
Ferric chloride	56	Cationic Polymer	4.0	COD	50% removal	(Suksaroj et al., 2005)
				Colour	90% removal	

Magnesium chloride	400	Polyelectrolyte (Koaret Reactive PA 3230)	11.0	COD	88% removal	(Tan et al., 2000)
				Colour	> 90% removal	
				TSS	95% removal	
Magnesium chloride	120	Lime	11.0	COD	40-50% removal	(El-Gohary and Tawfik, 2009)
				Sludge Production	1.71 kg/m3	
				Colour	97-100% removal	
Magnesium chloride	800	Hydrated lime	12	Colour	98% removal	(Gao et al., 2007)
Polyaluminium chloride (PACl)	0.1	Poly acrylamide-seed gum	8.5	Colour	80% removal	(Sanghi et al., 2006)
Polyaluminium chloride (PACl)	800	Anionic polyacrlamide Exerfloc 204	7.5	COD	65.4% removal	(Baraoidan et al., 2007)
				TSS	67.5% removal	
				TDS	35.84% removal	
				Chromium	44.92% removal	
				Colour	75.49% removal	
Ferrous sulphate	400	Lime and Cationic polymer	12.5	COD	50-55% removal	(Georgiou et al., 2003)
				Colour	80-90% removal	
Ferrous sulphate	1,000	Anionic polyelectrolyte (Henkel23500)	9.5	COD	59% removal	(Selcuk, 2005)
				Toxicity	80% removal	
				Colour	50% removal	
Ferrous sulphate	7×10^4	N/A	5.7-6.5	COD	80.2% removal	(Patel and Vashi, 2010
				BOD	74.0% removal	
				Colour	84.9% removal	
Ferric sulphate	7×10^4	N/A	5.7-6.5	COD	55.2% removal	(Patel and Vashi, 2010
				BOD	51.4% removal	
				Colour	57.9% removal	
Moringa Oleifera	500	N/A	3.97	COD	68.2 removal	(Yap et al., 2021)
				BOD	91.4% removal	
				TSS	86.4% removal	

[a]: Experiments were carried out at original pH of the raw wastewater

The studies made by various researches as described in Table 2.13 show that the natural pH of ferric chloride solution is more towards the trend of acidity. However, effective degradation of mixture of the inorganic and organic matter is achievable when the pH is maintained near neutral. The same observation was obtained for the colour removal. Addition of base, e.g. lime or NaOH, may be the prime requirement to ensure maximum removal of these matter in the wastewater. However, the use of the former base may result in the production of excessive sludge. This may result in excessive solid build-up as well as carryover of scum from the anaerobic digestion section to the aerobic or facultative digestion sections. On the other hand, the addition of polyelectrolyte as a coagulant does not aid in improving the performance of the coagulant. As shown in Table 2.13, lower $COD_{removal}$ was achieved despite the pH for the alum is near neutral or acidic. However, the opposite was obtained for colour removal efficiency, where higher colour removal efficiency was obtained at pH near to neutral. As reported by Verma et al. (2012), the use of alum as a coagulant agent is often associated with production of large amounts of sludge, which make this an unattractive option.

To date, the use of alum as a coagulant agent can be replaced by PACl products. It is an aluminium-based coagulant, which share many similarities with alum. The major differences between alum and PACl are: (a) partially pre-neutralised (higher basicity than alum); (b) contain Cl^- instead of SO_4^{2-}; (c) contains three times more aluminium content, and; (d) rapid aggregation velocity, bigger and heavier flocs. By comparing colour and COD removal efficiencies, PACl illustrated a better colour removal efficiency (more than 70%) in a wider pH range of 7-10. The $COD_{removal}$ achieved by PACl (Table 2.13) within the same pH range was less than 70%. This allows us to conclude that the biodegradation of the organic or inorganic matter in the wastewater is not influenced by pH instead the opposite was observed for the removal of colour. If the concentration of polyelectrolyte exceeded the optimum level, this will lead to the occurrence of increase in turbidity and reduce the SVI. As reported by Bidhendi et al. (2007); Verma et al. (2012), the concentration of polyelectrolyte added should not be more than 2mg/L to avoid the undesirable effects in the anaerobic digestion section.

The optimum pH for magnesium chloride varies between 9 and 12, as reported by Gao et al. (2007); El-Gohary and Tawfik, (2009). It gives very good $COD_{removal}$ if used with polyelectrolyte. The same observation was obtained as well for colour removal performance if it is used with lime. However, the drawbacks of using lime is the generation of large amounts of sludge. As a result, large amounts of capital will be required due to the disposal of large amount of sludge. Generally, the use of alum and magnesium chloride as coagulant may not be considered as an attractive alternative due to the production of large amounts of sludge and production of basic effluent after treatment. Both the ferric chloride and alum give high efficiency of $COD_{removal}$ at high concentration as reported by Kim et al. (2004); Golob et al. (2005); Bidhendi et al. (2007). However, the performance of the coagulant experienced a significant drop when ferric chloride or alum used with small amounts of cationic polymer (Table 2.13) despite the use of low concentration.

The opposite observation was obtained for colour removal efficiency, where the mixture of cationic polymer with alum or ferric chloride significantly increases the colour removal efficiency performance.

Another attractive coagulant will be polyferrous sulphate (PFS). PFS is an attractive coagulant because it is: (a) practically soluble in water, and; (b) able to form large amounts of polynucleic complex ions, viz. $(Fe_2(OH)_3)^{3+}$, $(Fe_2(OH)_2)^{2+}$, $(Fe_8(OH)_{20})^{4+}$, which allows the occurrence of solid flocculation. The advantages of using PFS as a coagulant aider are: (a) fast settling of flocs; (b) broad pH compatibility; (c) low iron contamination; (d) high heavy metal removal rate, and; (e) easy dehydration of sludge. The coagulation mechanism for PFS is similar to PACl, i.e. adsorption and neutralisation. At high turbidity, the mechanism of coagulation may be the sweep coagulation, where it is defined as addition of relatively large dosage of coagulant to the water that the water becomes saturated and the coagulant precipitates out. Hence, the particles get trapped in the precipitant as it settles down (Tchobanoglous et al., 2014).

Researchers have also investigated the application of polyaluminium ferric chloride (PAFCl) for the decolourisation of petrochemical wastewater as well as toxicity reduction in leather tanning wastewater (Gao et al., 2003; Lofrano et al., 2018). Both the studies concluded that PAFCl gives better turbidity and toxicity removal, which may be contributed by the quick formation of the flocs. The ability of quick formation of the flocs, and superior colour and toxicity removal efficiencies, may be contributed by the chemical composition of the coagulant, where it is made up of mixture of aluminium and iron salts. Hence, the addition of PAFCl has accelerated the sedimentation process, due to the bulky flocs formation. Other than PAFCl, potassium ferrate has also been utilised as a coagulant agent. It has been reported by Ciabatti et al. (2010) which showed that potassium ferrate has demonstrated its effectiveness as effective turbidity removal and COD reduction. Since ferrate (VI) ion is a strong oxidant in the entire pH range, hence after reduction to Fe (III) ion or ferric hydroxide during oxidation process, it possesses the ability to act as a coagulant agent. The unique dual function demonstrated by potassium or PAFCl allows us to conclude that chemical reagent can be an effective alternative coagulant to improve the current approaches for water and wastewater treatment. As reported by Gao et al. (2003), the mechanism of PAFCl is adsorption and charge neutralisation. The addition of coagulant into solution with large amounts of solid, i.e. POME, textile wastewater may result in destabilisation of colloids and aggregation of flocs. Colloids are very small particles that have large surface area, resulting in colloidal suspensions with negligible gravitational effects and predomination of surface phenomena. The colloids in the solution is removed via neutralisation of charges due to the opposite charges exhibited by the coagulant and the wastewater prior its removal by sedimentation/filtration. Other than PACl, ferrate (VI) has been found to be excellent in colour removal and COD reduction due to its strong oxidant in all the pH range.

Ferrate has two different functions (oxidant and coagulant), where chemical reagent can be an effective alternative to treat water and wastewater treatment. The attractive features attributed by these chemical reagents show that they can be effective alternatives to combat the large volumes of wastewater produced daily in various industries.

2.13.2 Natural Coagulant

Chemical coagulation with the aid of the above-discussed coagulants (as mentioned in Section 2.13.1) may act as an alternative method for the treatment of wastewater before being sent for biological treatment, if required. Even though the treatment efficiency using chemical coagulant has attracted researches to apply this concept to solve real life wastewater issues, the treatment efficiency is easily affected by extreme pH environment and low or very high temperature. Under this scenario, the coagulant is unable to coagulate the solid in the wastewater resulting in poor sedimentation, where there will be no clear separation of solid and wastewater. The fragile flocs may be easily ruptured under any type of physical forces which will affect the treatment efficiency. These can be solved using coagulants with high molecular weight number polymers sourced from synthetic or natural origins. They can function in different means: (a) as coagulant in itself, or; (b) as coagulant aids/bio flocculants, depending on the characteristics of the wastewater and polymers. The complex structure of the polymers either functions by destabilising the charged stable particles via the process of adsorption and neutralisation, or work as coagulant aids by attaching the destabilised particles with the functional groups via interparticle bridging.

As mentioned in Section 2.12, both the inorganic and organic coagulants can be used as coagulants, but the latter ones are more advantageous than the inorganic ones, i.e. the ability to produce large, dense and compact flocs. As a result, the flocs produced are stronger and have good settling characteristics (Renault et al., 2009). It is more beneficial to use organic polymer as compared to traditional coagulant, e.g. alum, due to the following reasons: (a) lower coagulant dosage; (b) maximum treatment efficiency at low temperatures, and; (c) produces small volumes of sludge. The disadvantages of using inorganic polymers and chemical coagulants are involvement of higher treatment cost, less biodegradability and toxicity. For instance, acrylamide is more toxic and gives severe neurotoxic effects (Dobias and Stechemesser, 2005). Due to their non-environmental nature, the use of natural polymers in the treatment of wastewater has sparked the interest of many researches due to its non-toxicity to the environment and biodegradability. Additionally, effluent treated after the natural polymer may be easily subjected to biological means, if needed, as it may not cause any harm to the biological organism in the treatment method used. Despite the large volumes of sludge produced, it can be disposed of safely as soil improver due to its abundant concentration of organic and inorganic nutrients and non-toxicity nature.

The function of coagulants to coagulate the solid in wastewater may allow us to demonstrate the same technique in POME treatment as the current POME treatment is laboriously intensive. Hence, these is a need to establish the use of natural low-cost polymers for POME treatment.

Many researches have studied the effectiveness of various natural coagulants extracted from plants or animals for the treatment of heavy industry wastewater. The effectiveness of these natural coagulants can be proved if it is used together with chemical coagulants. The natural coagulants used can be classified under the category of polysaccharides, hence also known as polymeric coagulants. Natural coagulants can be divided based on their source of origin, as shown in Figure 2.20.

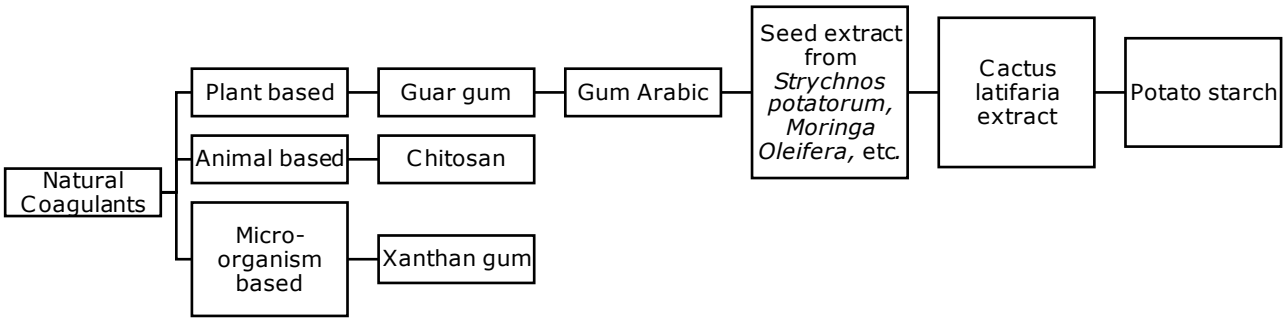


Figure 2.20: Categorisation of natural coagulants with their examples

Natural coagulants can be classified as polysaccharides or proteins. Even though polymers are known as non-ionic, they are not necessarily absent of charged interaction. There may be interactions between the polymer and a solvent within a solution environment, as the polymer may contain partially charged groups including –OH along its chain. This shows that there is a need to understand the coagulation mechanism associated with these natural coagulants to allow researchers to fully understand their usage. Aggregation of particulates in a solution can occur via four different pathways: (a) double layer compression; (b) sweep flocculation; (c) adsorption and charge neutralisation, and; (d) adsorption and interparticle bridging (Yin, 2010). The presence of coagulants with salt components causes compression of the double layer, resulting in destabilisation of the particulates (Packham and Division, 1965; Yin, 2010). Sweep flocculation occurs when coagulant traps the precipitant in a soft colloidal floc. Adsorption and charge neutralisation can be defined as neutralisation of two particulates with oppositely charged ions, whereas interparticle bridging occurs when a coagulant provides a polymeric chain which allow the particulates to bind to its adsorption site (Miller et al., 2008).

The existence of adsorption and charge neutralisation between wastewater and polysaccharides is due to the interaction between the anionic ions on the fine aggregate particles and cationic ions of the polysaccharides (Figure 2.21) (Pillai, 1997). In order to bring these particles together, these surface charges needed to be neutralised. Coagulation is the process of charge neutralisation and bonding of particles to form microfloc particles. Charge neutralisation can be obtained by addition of a coagulant, where it will neutralise the negative surface charge by its positive charge. The coagulated particles are then aggregated to larger particle sizes and settled by the addition of a flocculant.

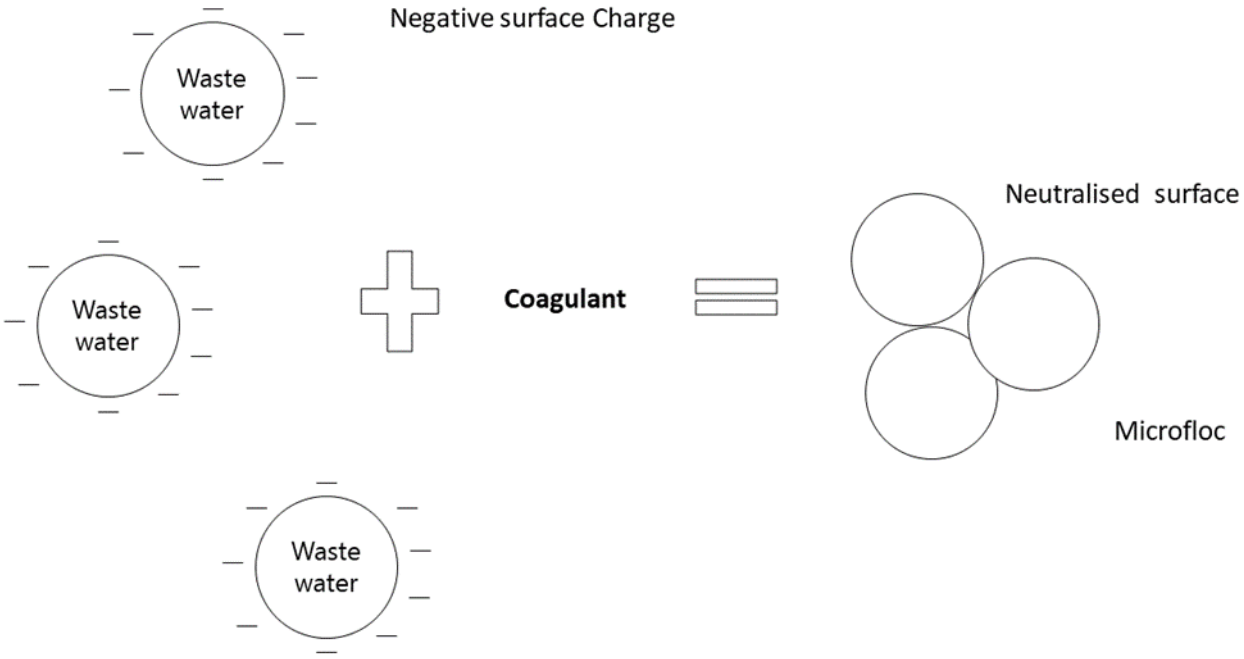


Figure 2.21: The mechanism of coagulation in wastewater

2.13.2.1 Natural plant-based polymers as coagulants

Polymeric coagulants are associated with mechanisms (a) adsorption and charge neutralisation; (b) adsorption and inter-particle bridging. This may be due to the increased number of unoccupied adsorption sites on the long-chained structures (especially polymers with high molecular weights). As discussed thoroughly by Yin, (2010), both these mechanisms are the underlying principles of plant-based coagulants. Electrolytes in aqueous solution are able to facilitate the coagulating effect of polymeric coagulants, as there is lesser electrostatic repulsion between particles. Although many plant-based coagulants have been reported, only four types are generally well-known within the scientific community, viz. nirmali seeds (*Strychnos potatorum*), *Moringa Oleifera*, tannin, and cactus.

2.13.2.1.1 *Moringa Oleifera*

Moringa Oleifera, a perennial deciduous tropical plant belonging to the Moringaceae family, is rich in many bioactive compounds. It is a tropical plant that is native to India. It has been widely used as a vegetable, functional food, and medical plant with a rich nutritional composition and diverse pharmacological activities (Villaseñor-Basulto et al., 2018; Ma et al., 2019). The typical lipid content (fatty acid and triglycerides) in the *M. oleifera* seeds is shown in Table 2.14.

Table 2.14: Lipid content in *Moringa Oleifera* seed (Abdulkarim et al., 2005; Villaseñor-Basulto et al., 2018)

Fatty Acids	Values (%)
Lauric acid (C12:0)	0.1
Myristic acid (C14:0)	0.1
Palmitic acid (C16:0)	7.8
Palmitoleic acid (C16:1)	2.2
Stearic acid (C18:0)	7.6
Oleic acid (C18:1)	67.9
Linoleic acid (C18:2)	1.1
Linolenic acid (C18:3)	0.2
Archidic acid (C20:0)	4.0
Eicosenoic acid (C20:1)	1.5
Behenic acid (C22:0)	6.2
Lignoceric acid (C24:0)	1.3

As reported by Kayode and Afolayan, (2015); Aly et al. (2016); Villaseñor-Basulto et al. (2018), large amount of fatty acids is determined from Egyptian *M. oleifera* seeds – mainly omega 9 (76%) and saturated fatty acids (viz. palmitic, stearic and arachidic acid) (12%). Additionally, the oil extracted from the *M. oleifera* seeds does not exhibit any toxic effects. Saponins, flavonoids, steroids, terpenoids, phenols, and triterpenoids are the materials that are commonly available in plants, which can be determined using the assay of gel electrophoresis (Nordmark et al., 2016; Villaseñor-Basulto et al., 2018). However, the lipid content in the *M. oleifera* seed is affected with the harvesting practice or weather condition leading to the variations in the range of 30% to 42% (Okuda et al., 1999). As reported by Nordmark et al. (2016), the presence of fatty acids in the *M. oleifera* seeds will not affect the coagulation activity. Oil extraction is not required prior to the coagulation-flocculation processes (Camacho et al., 2017), which may not incur any additional cost in the overall wastewater and/or water treatments. As reported by Lee et al. (2017), retaining 0.01% of fatty acids (e.g. palmitoleic, oleic, linoleic, linolenic, cis-11-eicosenoic, and cis-11,14-eicosadienoic acid) in the *M. oleifera* seed oils may significantly prevent the formation of *Staphylococcus aureus* biofilm. It is possible to extract an edible quantity of oil from the *M. oleifera* seeds before using it as coagulant. However, as reported by Bhatia et al. (2007) the presence of oil in the seeds may inhibit the formation of flocculation due to the inhibition of the surface of reaction.

The extracted oil could be utilised as a source of edible oil for human consumption, which may act as a potential substitute for high monounsaturated oils, viz. olive oil in diets (Muyibi and Alfugara, 2003). Hence, incorporation of *M. oleifera* (natural coagulant) into wastewater treatment will bring more economic benefits for the producing countries.

According to Ndabigengesere et al. (1995); Villaseñor-Basulto et al. (2018), the active agents of coagulation of *M. oleifera* extracts are dimeric cationic proteins, having molecular weight ranging from 6 to 16 kDa and isoelectric points between 10 and 11. The mechanism exhibited by *M. oleifera* extract is adsorption and neutralisation of the colloidal charges between the anionic charges (wastewater) and cationic charges (*M. oleifera* extract). The latter was reported to contain an active bio-coagulation compound as reported by Camacho et al. (2017) whereby it can reduce high turbidity and microorganisms in water and wastewater. The action of *M. oleifera* extract as a coagulant is mainly due to the presence of water-soluble cationic proteins in the seeds. Ndabigengesere et al. (1995) was the first research reported that *M. oleifera* extract is an effective natural coagulant which can be used in water treatment in two forms: (a) shelled dry seeds, and; (b) non-shelled dry seeds. The advantage of incorporating *M. oleifera* extract in wastewater treatment, such as POME treatment, is that chemical cost could be reduced when used for pH adjustment (Bhatia et al., 2007a). As reported by Bhatia et al. (2007b), pre-treatment of POME with *M. oleifera* seeds after oil extraction is able to produce a removal of 95% suspended solids and a 52.2% reduction in the COD.

The presence of primary aliphatic amines as one of the functional groups in the *M. oleifera* contributed to the coagulation process of the former parameter with optimum dosage. Other than POME, *M. oleifera* has been used to treat dairy wastewater with a 55.6% reduction in COD at an optimum pH between 7 to 9 at an optimum dosage of 300 mg/L (Pallavi and Mahesh, 2013). Nkurunziza et al. (2009) demonstrated that the use of *M. oleifera* (3%) extracted using 1% of NaCl (1M) resulted in 88.6% and 99.8% of the turbidity removal from the initial turbidity of 500 NTU and 450 NTU, respectively. Similar effect was obtained where *M. oleifera* was subjected to remove the initial turbidity of waters of 450-550 NTU (Madrona et al., 2010). Based on the studies conducted, this allows us to conclude that *M. oleifera* seeds was more effective in treating waters with high turbidity with at least 99.7% turbidity removal. Moreover, the incorporation of *M. oleifera* into wastewater treatment produces lower sludge volumes as compared to chemical coagulant (alum) with the added advantage of biodegradability and does not affect the pH of the treated wastewater (Camacho et al., 2017). Despite its efficiency in coagulating solid in wastewater, the use of *M. oleifera* will contribute to the increase in organic matter in the treated water, which is reflected in the increase on the COD values (Ndabigengesere et al., 1995; Okuda et al., 2001; Camacho et al., 2017). In order to resolve this issue, extracting the active coagulant component from the *M. oleifera* seed may not contribute much to the total amount of organic material of the treated wastewater as reported by Ghebremichael et al. (2005).

2.13.2.1.2 Nirmali Seed

Strychnos potatorum (nirmali seed) is a moderate-sized tree located in Southern and Central parts of India, Sri Lanka and Burma and it is predominantly used in the industry of traditional medical extract (Yin, 2010). Sanskrit writings from India reported that the seed powder was used for clearing muddy water in the rural community. Adinolfi et al. (1994) reported that polysaccharides extracted from *S. potatorum* can effectively reduce up to 80% turbidity of kaolin solution. This may act as evidence to prove that *S. potatorum* was the first reported plant-based coagulant for water treatment.

Nirmali seed extract is an anionic polyelectrolyte that can destabilise the particles in the water via inter-particle bridging mechanism (Tripathi et al., 1976; Yin, 2010; Maruthi et al., 2013). Studies reviewed by Tripathi et al. (1976) and Yin, (2010) demonstrated that the seed extract contained lipids, carbohydrates and alkaloids containing -COOH and free -OH on the surface groups. These compounds enhance the extracts' coagulation capability. Mixture of polysaccharide fraction extracted from *S. potatorum* seeds comprises 1:1.7 mixture of galactomannan and galactan, which is capable of reducing the turbidity of kaolin solution up to 80% (Adinolfi et al., 1994; Yin, 2010). The galactomannan is made up of a main chain of 1,4-linked β-D-mannopyranosyl residues bearing terminal α-D-galactopyranosyl units linked at the 0-6 position of some mannose residues (Corsaro et al., 1995; Yin, 2010). The coagulation mechanism associated with *S. potatorum* seeds may be interparticle bridging effects due to the presence of copious amount of -OH groups along the chains of galactomannan and galactan that provides weakly, but abundant, adsorption sites. Deshmukh et al. (2013) suggested that the seed extract may function as a particulate, colloidal and soluble polymeric coagulant, as well as a coagulant aid. As suggested by Vijayaraghavan and Sivakumar, (2011), the ionic (-COO-H^+) and non-ionic (galactomannan) substances are present in the *S. potatorum* extract, the author claimed that it may be a premature anionic polyelectrolyte. So far, there are not any studies that provide detailed elucidation of *S. potatorum* seeds responsible for coagulation and percentage composition of the extract. Due to the aforementioned reasons, further studies are required in this aspect.

As reported by Babu and Chaudhuri, (2005), 1.0-2.0 mg/L of *S. potatorum* seed is sufficient for clarification of water when the initial water turbidity is 18-21, 38-42 or 130-135 NTU. The same author also suggested that virus reduction of 3 log was demonstrated on the raw water spiked with poliovirus [2,370,000 – 5,000,000 plaque-forming units per litre (PFU L^{-1})] when subjected with *S. potatorum* seed. The concentration of *S. potatorum* seed ranges from 250 to 1,000 mg/L are able to reduce synthetic wastewater turbidity of 100 to 500 NTU, resulting in turbidity removal of 80.2%-90.6% (Muthuraman and Sasikala, 2014). Moreover, maximum turbidity removal efficiency of 80%, 89% and 96% is obtained when a mixture of synthetic wastewater and *S. potatorum* seed is subjected to 12 hours of retention time in the settling column for wastewater with initial turbidity of 100 NTU, 250 NTU, and 500 NTU, respectively.

Deshmukh et al. (2013) reported that *S. potatorum* seed can effectively reduce the turbidity of the wastewater to 94.08% (initial turbidity: 1,000-1,060 NTU), 99.01% (initial turbidity: 2,200-2,260 NTU) and 98.35% (initial turbidity: 3,400 NTU) at a concentration of 4.0 mg/L, 15 mg/L, and 6.0 mg/L, respectively. This allow us to postulate that *S. potatorum* seed is suitable to treat wastewater with turbidity concentration not exceeding 3,000 NTU. Seeds of *S. potatorum* contain materials that are effective as coagulant, and these may be used effectively for home water treatment in rural areas of developing countries.

2.13.2.1.3 Tannin

Tannin – is the general name given to the large polyphenol compounds obtained from natural materials, viz. organic material extracted from bark and wood of trees such as Acacia, Castanea, or Schinopsis (Graham et al., 2008; Vijayaraghavan and Sivakumar, 2011). Tannin is a type of polymer with molecular weights ranging from 100 to 10,000 and has traditionally been used as a tanning agent in the leather industry. In terms of human health, it has been reported that food rich in tannin are low in nutritional value and decreases the efficiency in converting the absorbed nutrients to new body substances (Chung et al., 1998; Yin, 2010). As a result, this has portrayed a negative light that may have limited its application as a natural coagulant for wastewater treatment. Despite its disadvantages, such application is still a preferred research area for many researches. (Özacar and Şengil, 2000; Özacar and Şengil, 2003; Choubey and Thakur, 2014; Sánchez-Martín et al., 2010; Aboulhassan et al., 2016; Singh et al., 2016) have been studying the application of tannin as either a standalone coagulant or a coagulant aid for wastewater treatment. The tannin used in these studies is obtained either from valonia or Acacia catechu, which is obtained either from the corn cup of the oak that grows in Asia Minor or found in the leaves, fruits, roots, and wood of trees that grows in central and northern part of India, respectively. As reported by Graham et al. (2008), the source of chemical responsible for coagulation is an aqueous extract of *Acacia mearnsii* bark, which consists of polyphenolic tannins, simple sugars and polymeric carbohydrates (hydrocolloid gums). The sources of tannin used by these researches concluded that it can be used as a substitute to chemical coagulants.

Various studies conducted by Özacar and Şengil, (2000); Özacar and Şengil, (2003); Sánchez-Martín et al. (2010); Choubey and Thakur, (2014) ; Aboulhassan et al. (2016) reported that the use of tannins as coagulant in wastewater treatment has demonstrated that the effectiveness of tannin mainly depends on the chemical structure of tannins that have been extracted from the plant and the degree of tannin modification. The presence of amine and phenolic groups for wastewater treatment clearly indicates its anionic nature as it is a good hydrogen donor. Figure 2.22 illustrates the schematic representation of basic tannin structure in aqueous solution and possible molecular interactions that induce coagulation.

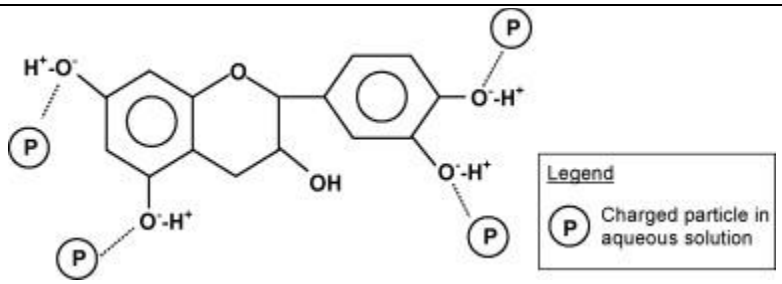


Figure 2.22: Schematic representation of basic tannin structure in aqueous solution and its possible molecular interaction (Yin, 2010)

Phenolic group is commonly known to easily deprotonate to form phenoxide, which is stabilised via resonance. It is due to the act of delocalisation of electrons within the aromatic ring which increases the electron density of the oxygen atom. This denotes that the effectivity of coagulation capability is govern by the numbers of phenolic groups in a tannin structure (Yin, 2010). Studies conducted by Graham et al. (2008); Yin, (2010); Choubey and Thakur, (2014) suggested that application of commercial tannin that contained amine and phenolic groups for wastewater treatment denotes that the tannin used is cationic in nature. Such effective nature of the commercial tannin used in these studies may be due to the presence of a single tertiary amine group per monomer, resulting in a charge density of approximately 3 mequiv.g^{-1}. The commercial tannin used in these studies also exhibited amphoteric nature as a consequence of presence of phenolic groups (Vijayaraghavan and Sivakumar, 2011).

As reported by Özacar and Şengil, (2000), wastewater treated with tannin has increased the filtered matter quantity from 0.03 to 0.53 kg/m^3 coupled with the increase of turbidity removal from 75% to 94% as compared to AN913 (synthetic anionic polyelectrolyte). This denotes that tannin could act as an effective agent for conditioning of sludge. Besides that, tannin has been used in the textile industry for the treatment of wastewater containing dye, where the largest removal of colour of approximately 96% was achieved in the presence of 0.6 mg/L of tannin (Costa et al., 2018). Study conducted by Choubey and Thakur, (2014) demonstrated that there is a direct relationship between coagulant dosage with residual turbidity and residual total suspended solids. As the coagulant dosage of tannin (extracted from Acacia catechu) increases from 1-3 mL/L, the residual turbidity of wastewater sample decreases from 200 NTU to 18 NTU (91% turbidity removal). The residual turbidity of wastewater sample increases from 34 NTU to 43 NTU when the coagulant dosage increases from 4mL/L to 5mL/L. Moreover, maximum removal of residual total dissolve solid of 57.3% was obtained when 4 mL/L of coagulant dosage was added into the wastewater sample. With the recommended dosage of tannin by Choubey and Thakur, (2014), the suspended particulates (inorganic or organic salts) can be coagulated resulting in decreasing the turbidity of the wastewater and solid content.

As reported by Heredia et al. (2012), the incorporation of tannin extracts from Silvafloc, Tanfloc, Acqualpol C1, and S5T are able to coagulate the long-chain anionic surfactants in aqueous solution resulting in removal efficiency of 60%, 62%, 66%, and 36%, respectively. The aforementioned studies allow us to denote that tannin extracted from plant have been recognised as the active agents in promoting coagulating activity in wastewater.

Besides treating water with low turbidity, tannin has been used in treating municipal wastewater. Study reported by Heredia and Martín, (2009) tannin-based coagulant and flocculant agent (TANFLOC) demonstrated high effectiveness in turbidity removal (almost 100%, dependent on the dosage) and around 50% of BOD_5 and COD removal efficiencies. TANFLOC flocculant product is a trademark that belongs to TANAC (Brazil). It is a tannin-based product, where it is modified by a physico-chemical process and demonstrate a high flocculant power. It is obtained from the bark of *Acacia mearnsii*. The tree is commonly found in Brazil and has a high concentration of tannins. TANFLOC is a vegetal water-extract tannin, mainly consisted of flavonoid structures with an average molecular weight of 1.7 kDa. More groups, viz. hydrocolloid gums and other soluble salts, are included in the TANFLOC structure. Chemical modification includes a quaternary nitrogen that gives TANFLOC the cationic character. The usage of 60-80 TANFLOC ppm dosage in the treatment of municipal wastewater resulted in almost 30% of anionic surfactants removal due to the mechanism of surfactant-turbidity adsorption (Heredia and Martín, 2009). The incorporation of TANFLOC, for the treatment of water and wastewater, is able to reduce anionic surfactants (harmful to the environment) and organic matter regardless of concentration. Despite its excellent performance in coagulating the organic matter in wastewater, its performance may be affected by pH. As reported by Özacar and Şengil, (2002), tannin is only effective at pH 11 with turbidity removal efficiency of 63% (10 FTU turbidities) and 80.5% (20 FTU turbidities). It is worth noting that at pH below 11, the phenomenon of destabilisation was observed by the final turbidity of the water. The aforementioned studies show that the efficiency of tannin-based coagulant is affected by the pH and initial turbidity of the wastewater tested (Özacar and Şengil, 2000; Özacar and Şengil, 2002; Heredia and Martín, 2009; Heredia et al., 2012; Choubey and Thakur, 2014; Costa et al., 2018). This allows us to deduce that the optimum condition for the wastewater to achieve efficient coagulation is at pH 7-11 and water turbidity of less than 200 NTU. Based on the suggested conditions, this shows that wastewater with pH less than 7 and turbidity of more than 200 NTU is not suitable to be treated using tannin-based coagulant.

2.13.2.1.4 Cactus (*Opuntia*)

Application of cacti species for water treatment is rather recent compared to other natural coagulants, viz. nirmali seeds or *M. oleifera*. The most commonly studied cactus genus for water treatment is Opuntia, which is colloquially known as "nopal" in Mexico or "prickly pear" in North America. This type of cactus has long been associated with its medicinal properties (Camacho, et al., 1983; Yin, 2010) and dietary food sources (Kossori et al., 1998; Yin, 2010). Besides *Opuntia*, other cactus species including Cactus latifaria have also been successfully used as natural coagulants (Diaz et al., 1999; Yin, 2010).

The high coagulation capability is likely attributed to the presence of mucilage in the *Opuntia*. It is a viscous and complex carbohydrate stored in the cactus inner and outer pads that has great water retention capacity (Sáenz et al., 2004). Previous studies have established that mucilage in cactus *Opuntia* consisting of carbohydrates viz. L-arabinose, D-galactose, L-rhamnose, D-xylose, and galacturonic acid (Trachtenberg and Mayer, 1981; Sáenz et al., 2004). The neutral sugars were found by Trachtenberg and Mayer, (1981); Wan et al. (2019) in the following composition – arabinose (24.6%), galactose (40.1%), rhamnose (13.1), and xylose (22.2%). The uronic acid was present as galacturonic acid, which was 10.7%-19.5% content of the purified mucilage. Miller et al. (2008) reported that galacturonic acid may be the active ingredient that contributed to the coagulation efficiency of *Opuntia* spp., though it should be noted that it only contributes to 50% of turbidity removal. Despite the low turbidity removal, this achievement may consider as a breakthrough for *Opuntia* spp., therefore this compound deserves further evaluation on its contribution to the overall coagulation capability of cactus. As reported by Wan et al. (2019) the predominant coagulation mechanism for *Opuntia* spp. is bridging and adsorption as shown in Figure 2.23.

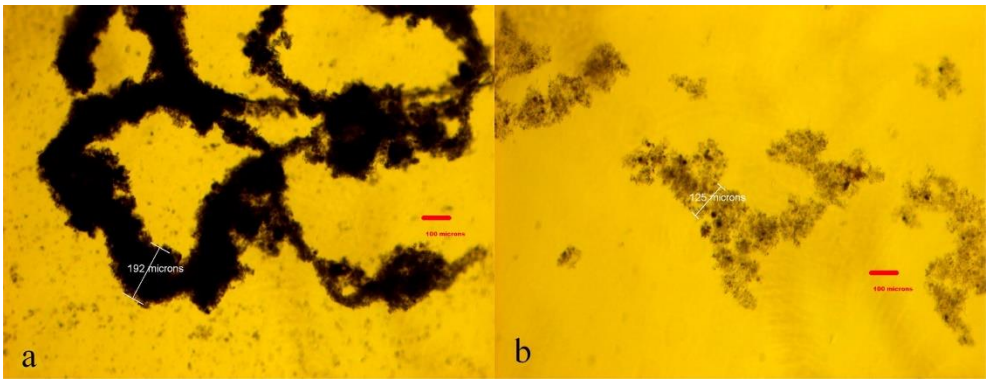


Figure 2.23: Tailings pond water (TPW) coagulation flocs formed by (a) *Opuntia* spp. (b) alum (Wan et al., 2019)

Unlike the flocs formed by alum, which are spherical in shape, the flocs formed by *Opuntia* spp. are long, distorted, threadlike, and heterogeneous in shape, as shown in Figure 2.23. As reported by Wan et al. (2019), the threadlike flocs start to form and become clearly visible within one-minute of rapid mixing (150 rpm) and grow in length and circumference during slow mixing. Additionally, the flocs formed by *Opuntia* spp. (Figure 2.23a) are denser than those formed by alum (Figure 2.23b).

The ultimate advantage of using bio-coagulant that is worth to be noted is the ability to settle faster, hence reducing the settling time, which is reflected by the average diameter of flocs formed by *Opuntia* spp. being greater than that from the use of alum. This allows us infer that mixing speed is one of the key points in contributing to the coagulation efficiency of the coagulant used.

The bridging and adsorption mechanism differ from the charge neutralisation, where the solution particulates do not have a direct contact with one another, but are bound to a polymer-like material that originates from the cactus species. Another group of researchers from Japan also claimed that galacturonic acid is present in natural microbial-based coagulants produced by *Enterobacter* sp. (Yokoi et al., 1997; Miller et al., 2008 ;Yin, 2010), *Pseudomonas* sp. (Yokoi et al., 1998; Miller et al., 2008; Yin, 2010) and *Klebsiella pneumoniae* (Nakata and Kurane, 1999; Miller et al., 2008; Yin, 2010). The studies conducted by different groups of researches allow us to highlight that galacturonic acid is one of the major active coagulating agents in plants, and hence require a further technical assessment for the long-term evaluation of the coagulation efficiency in treating low- and high- strength wastewater.

Although the use of cactus in wastewater treatment is not extensively reported in open literature, there is a high possibility that galacturonic acid (major constituent of pectin in plants) exists predominantly in polymeric form (polygalacturonic acid) that provides a platform for particles to adsorb on. Figure 2.24 shows the relevant dominant molecular interactions associated with adsorption and bridging in coagulation mechanism demonstrated by cactus (*Opuntia* spp.) (Manunza et al., 1997; Yin, 2010).

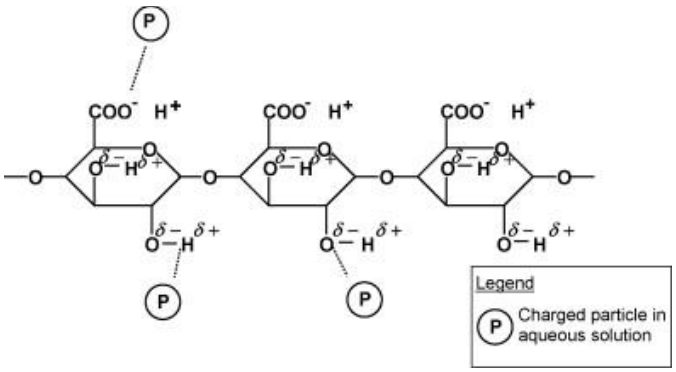


Figure 2.24: Schematic representation of polygalacturonic acid in aqueous solution and possible dominant molecular interactions associated with adsorption and bridging (Yin, 2010)

The polygalacturonic acid structure clearly demonstrates that its anionic properties are mainly attributed by the partial deprotonation of carboxylic functional groups throughout the chain of polygalacturonic acid. This allow us to denote that there is a possibility of occurrence of chemisorption between charged particles and –COO- with the aid of empirical substantiation. Besides that, the presence of –OH groups along the polymeric chain allow us to conclude that there is a possible intramolecular interaction that may distort the relative linearity of the chain. However, this is not extensively studied by Miller et al. (2008).

It is worth noting that the incorporation of *Opuntia* spp. into the treatment of tailings pond water resulted in significant arsenic removal of 64.05% (Wan et al., 2019). Such removal efficiency obtained may be due to the interaction between cactus mucilage and arsenate, where similar observation was reported by Fox et al. (2012). The protons on the –OH group on the mucilage polymers from either alcohol or carboxyl are attracted to the O-atom on the arsenate anion, which in turn induce the coagulation effect of the *Opuntia* spp. However, the treated tailings pond water using *Opuntia* spp. contained slight increase in the concentration of chromium and copper during coagulation. As reported by Kalegowda et al. (2015), the concentration of copper (5.21 to 0.45) and chromium (0.48 to 0.16) decreases from young, intermediate through to mature stages. As a result, there is a slight increase in the concentration of chromium and copper of the treated tailings pond water using *Opuntia* spp. Even though *Opuntia* spp. is unable to effectively remove chromium (-45.59%) and copper (-43.45%), the concentrations of both these metals are well below the United States Environmental Protection Agency (USEPA) discharge limit. Despite treating tailings pond water with 500 mg/L of *Opuntia* spp., it only contributed to a small percentage of phosphorus removal of 13.5% as compared to alum (77%) and ferric chloride (78%). *Opuntia* spp. does not exhibit such high phosphorus removal as compared to alum and ferric chloride. This may be due to the inability of the *Opuntia* spp. to react with soluble phosphate in the tailings pond water to form solid precipitates, unlike alum and ferric chloride commonly used in wastewater treatment. In the case of $COD_{removal}$, comparable performance was obtained for *Opuntia* spp. (~30%), alum (~32%), and ferric chloride (~28%).

Opuntia spp. has been used to treat vegetable oil refinery wastewater, where the COD, BOD, TSS, and O&G concentrations were 22,400 - 53,563 mg/L, 6,789 – 20,179 mg/L, and 504 – 6,542 mg/L, respectively (Dkhissi et al., 2018). Dkhissi et al. (2018) and his co-workers reported that the addition of 180 mL/L of cactus juice into the wastewater resulted in maximum turbidity, COD and colour removal of 85%, 62%, and 67%, respectively. An increase in the concentration of *Opuntia* spp. to 280 mL/L has increased the mean COD concentration of the treated water from 48,241 mg/L to 52,241 mg/L. It should be noted that the increasing-then-decreasing trends in the aforementioned parameters may be due to the usage of *Opuntia* spp. that cause larger amount of pollutant particles to agglomerate and settle. Optimum concentrations of *Opuntia* spp. in the suspension resulted in larger amounts of pollutant particles to aggregate and settle. If an excessive concentration of flocculent dosage (above the optimum value) is used to treat vegetable oil refinery wastewater, this would cause the aggregated particles to disperse and would also affect the settling of the particles. This would lead to hindrance in floc formation.

Such behaviour may be explained by the repulsive energy barrier occurring between the flocculent (*Opuntia* spp.) and the pollutant (vegetable oil refinery wastewater). The COD removal obtained in this study is similar to the other studies using another species of bio-flocculant and other natural coagulant for the removal of turbidity in water (Dkhissi et al., 2018).

Besides that, *Opuntia* spp. is used to treat oil sand process-affected water as well. It is worth noting that there is a direct relationship between initial turbidity with the determination of optimum dosages (Choudhary et al., 2019). When the initial turbidity of the model water is high (500 NTU), it required a higher amount of bio-coagulant and an optimum dosage of 1,500 mg/L was used to effect a turbidity removal of 97% (Choudhary et al., 2019). Such relationship allows us to infer that the higher dosage requirement can be inferred by the complex nature and relatively high turbidity of the oil sand process-affected water. It is a mixture of highly toxic organic and inorganic constituents, viz. naphthenic acids, toluene, benzene, polycyclic aromatic hydrocarbons, metals, and suspended particles. These pollutants have negatively-charged surface and it requires extra sites of bio-coagulant to get attached with to induce the coagulation effect (Shak and Wu, 2014). In order to continuously maintain efficient coagulation/flocculation process, it is vital to obtain an optimum sedimentation time to achieve high turbidity removal. Study reported by Choudhary et al. (2019) allows us to infer that the time required to attain the same level of turbidity removal (> 90%) was reduced (< 10 minutes of sedimentation time). Such observation can be explained by the collision and agglomeration rates between the particulates at high initial turbidity. This will enhance the formation of bulkier flocs, resulting in faster settling of the particulates in accordance to Stoke's law.

It is worth to compare all the four different types of plant-base coagulants commonly used in the wastewater treatment industry (as thoroughly discussed in details in Sections 2.13.2.1.1 – 2.13.2.1.4). The main function of these coagulants is to coagulate the organic matter in the wastewater to result in distinct separations of liquid and solid. The main benefit of applying these plant- base coagulants into wastewater treatment is the ability to reduce the overall cost of the wastewater treatment due to the unnecessity in extracting oil from these plant-base coagulants and pH adjustment without affecting their coagulation efficiency. Besides that, the least volume of sludge produced as compared to chemical coagulant is also another added advantage, which will not create extra burden for the industry players to allocate additional locations for sludge disposal that may contaminate the environment.

Initial turbidity and pH of the wastewater affect the coagulation efficiency of the plant-base coagulants. Out of the four types of plant-based coagulants, Nirmali seed, tannin and cactus are only able to treat wastewater with low turbidity with less than 2,000 NTU and pH of more than 7.0. When these coagulants were subjected to wastewater with high turbidity and acidic pH, this may cause the aggregated particles to disperse, and would affect the settling of the particles. Such behaviour may be explained by the repulsive energy barrier that occurred between the flocculant and the wastewater, which will hinder the formation of flocs. The aforementioned behaviour is not observed when *M. oleifera* is used in wastewater treatment.

Moreover, the coagulation mechanism demonstrated by each of the plant-base coagulant differs from each other. The coagulation mechanism demonstrated by *M. oleifera*, nirmali seed and cactus are: (a) adsorption and neutralisation; (b) interparticle bridging, and; (c) bridging and adsorption, respectively. For tannin, there is not any specific coagulation mechanism but it has been reported that the coagulation efficiency is dependent on the chemical structure and degree of chemical modification. The third difference exhibited by all the four different types of plant-base coagulants is the chemical structure that is responsible for coagulation. The chemical structure responsible for coagulation in *M. oleifera*, nirmali seed, tannin and cactus is cationic protein, polysaccharide (mixture of galactomannan and galactan), polyphenolic, and galacturonic acid, respectively.

By studying the similarities and differences exhibited by each of the plant-base coagulant, one can determine the most suitable plant-base coagulant for the treatment of wastewater. The criteria used to determine the suitability of the plant-base coagulant are the ability to coagulate wastewater with low pH and high turbidity, do not require to carry out any pH adjustment and oil extraction prior to coagulating activity, and able to produce distinct separation of solid and liquid. Based on the aforementioned criteria, *Moringa oleifera* seems to be the most suitable plant-based coagulant for the treatment of high strength wastewater. It will be used in the subsequent studies for the treatment of POME since POME is type of high strength wastewater with pH ranging from 3.3 – 5.7. Besides determining the suitable type of plant-base coagulant for wastewater treatment, it is important to determine the optimum condition(s) for efficient coagulation process.

2.13.3 Jar Test Parameters

Jar test is the most widely used method for evaluating and optimising the coagulation-flocculation processes (Ndabigengesere and Narasiah, 1998). Additionally, this regime can be used to evaluate the efficiency of a physical-chemical treatment, selection of coagulants and determination of the optimum operating conditions, i.e. pH and coagulant dosage (Clark and Stephenson, 1999; Aragonés-Beltrán et al., 2009). This assay consists of three stages: (a) rapid mixing stage; (b) slow mixing stage, and; (c) clarification. To have a set of constructive data, it is essential to determine the optimum rotation speed and duration of rapid and slow mixing stages.

2.13.3.1 Agitation Speed & Duration

The growth of floc is accomplished by gentle stirring coupled with the optimum mixing speed and duration. As reported by Rossini et al. (1999), the optimum mixing speed and duration will cohesively integrate the chemical (mixture of the wastewater and coagulant) and physical parameters (rapid mixing intensity) as it will affect the final results of the whole treatment. The selection of agitation and duration for rapid mixing and slow mixing stages are selected based on the turbidity removal efficiency and minimum values of suspended solids.

Various studies have been conducted to optimise the speed and duration of the mixing of different types of wastewater. High percentage of turbidity removal (> 90%) were achieved by different types of wastewater treatment, viz. tailings pond water, municipal wastewater, tap water, POME, textile wastewater, etc. (Ndabigengesere et al., 1995; Ozacar and Sengil, 2002; Ozacar and Sengil, 2003; Bhatia et al., 2007b; Bhatia et al., 2007a; Heredia and Martín, 2009;Choubey and Thakur, 2014; Bouaouine et al., 2019; Wan et al., 2019). The obtained results allow us to estimate the speed and duration required for rapid and slow mixing to achieve turbidity and suspended solid removals (> 90%). The proposed speed and duration for both the rapid and slow mixing is illustrated in Table 2.15.

Table 2.15: The recommended range of mixing speed and duration for different manner of mixing (Ndabigengesere et al., 1995; Ndabigengesere and Narasiah, 1998)

	Rapid Mixing	Slow Mixing
Speed (RPM)	150-250	25-40
Duration (minutes)	2-5	20-30

In order to ensure that there is an efficient formation of floc, it is essential to select the most optimum speed and duration of the rapid and slow mixing. Additionally, the texture of the POME has to be taken into consideration as insufficient mixing speed and duration may not allow maximum coagulation activity to occur between the substrate (POME) with the plant-base coagulant. In our study, the speed and duration for both the mixing manners are selected based on the study conducted by Bhatia et al. (2007a). This is because the pH of the POME used in this study fall within the range suggested by Bhatia et al. (2007a) that may be one of the contributing factors that reduces the efficiency of electrostatic patch mechanism (Gassenschmidt et al., 1995). To achieve similar outcomes, the speed and duration for rapid mixing and slow mixing are 150 rpm for 5 minutes and 30 rpm for 20 minutes, respectively.

The third step in conducting jar test is known as clarification, where there will be a distinct separation of solid and liquid. It is essential to determine the optimum time required for such occurrence. Study conducted by Bhatia et al. (2007a) indicates that the interaction between settling time and *M. oleifera* dosage on the reduction of suspended solid is greatly pronounced as observed, where the suspended solid concentration decreased when the settling time of the mixture of POME and *M. oleifera* dosage (3,000-4,000 mg/L) was maintained within 90-105 minutes. The optimisation analysis conducted by the same author demonstrated that the abovementioned observation agreed reasonably well with the experimental data with the optimum settling time of 114 minutes with *M. oleifera* dosage of 3,469 mg/L. Besides various dosages of *M. oleifera*, wider range of pH may also affect the settling time based on the parameter of percentage recovery of sludge. As inferred by Bhatia et al. (2007a), pH within the range of 4.5-5.5 will result in maximum recovery of sludge of 87.1% and suspended solids concentration of 181 mg/L with the settling time within 90-120 minutes. In order to adapt to such methods in this study, a settling time of 120 minutes is selected due to the same source of wastewater used with similar pH. The jar test parameters in terms of rapid mixing, slow mixing and clarification were determined based on the studies conducted by different researches (Table 2.15). The parameters used in the jar test study are: (a) Rapid Mixing: 150 rpm for 5 minutes; (b) Slow Mixing: 30 rpm for 20 minutes, and; (c) Clarification: 120 minutes.

2.14 Methanogen

Methanogenic archaea are vital in the anaerobic microbial degradation of organic waste with the resultant production of methane. It is a type of gas with potential for environmental harm, but also possible value as a non-fossil fuel. However, despite their key role as the terminal oxidisers in a complex microbial community, very little is unknown for controlling the flux of carbon through the anaerobic degradative pathway in the treatment of POME. Hence, an understanding of the microbial ecology in the treatment of POME is necessary to monitor the microbial activities. Methanogenesis is the final degradation process of organic matter in anoxic condition in every treatment of wastewater. Under favourable environment, organic matter is degraded into methanogenic substrate, viz. acetate, CO_2, H_2 and CH_4, by a diverse range of bacteria available in the wastewater. The CH_4 formed is released freely into the atmosphere (Watanabe et al., 2004). Methane gas is one of the greenhouse gases whose effect is estimated to be 25 times higher than CO_2 (Rodhe, 1990; Watanabe et al., 2004). The current commercialised POME anaerobic digestion technologies with biogas utilisation in Malaysia and Indonesia is listed in Table 2.16. Parameters that will inhibit the activity of methanogenesis (Section 2.14.1), application of molecular tools in the identification of methanogen (Section 2.14.2) and ways to alleviate methanogen inhibition (Sections 2.14.3 & 2.14.4) will be discussed thoroughly in the subsequent sections.

Table 2.16: Current commercialised POME anaerobic digestion technologies and biogas utilisation in Indonesia and Malaysia

Location of Anaerobic Digestion Technologies	Anaerobic Digestion Technology	Biogas Utilisation	Reference
Biogas and electricity generation in Kuala Sungai Baru, Malaysia	Closed anaerobic digester	Boiler	(CDM, 2012; Choong et al., 2018)
MY08-WWP-30, Methane Recovery in Wastewater Treatment, Pahang, Malaysia	Covered Pond	Flaring System	(CDM, 2010; Choong et al., 2018)
Biogas Project at Anson Oil Mill	Closed Anaerobic Digester	Boiler and Gas Engine	(CDM, 2012; Choong et al., 2018)
Kilang Minyak Sawit Tg. Tualang Mill Wastewater Biogas Recovery and Utilisation Project	Covered Pond	Boiler and Gas Engine	(CDM , 2012; Choong et al., 2018)
Magenko Renewables (Penang) Wastewater Methane Avoidance and Energy Generation Project, Malaysia	Closed Anaerobic Digester	Burner	(CDM , 2012; Choong et al., 2018)
Palm Oil Mill Effluent Methane Recovery & Utilisation System at QL Palm Oil Mill 1 at Tawau, Sabah	Closed Anaerobic Digester	Gas Engine	(CDM , 2012; Choong et al., 2018)
FELDA Biogas Plant (Methane Recovery and Utilisation at Sungai Tengi Palm Oil Mill, Malaysia)	Covered Pond	Gas Engine	(CDM , 2012; Choong et al., 2018)
Tian Siang Oil Mill (Air Kuning) Biogas Project	Closed Anaerobic Digester	Gas Engine and Thermal Heater	(CDM , 2013; Choong et al., 2018)
Biogas Recovery from Wastewater Treatment in PT. Umbul Mas Wisesa Palm Oil Mill	Closed Anaerobic Digester	Boiler	(CDM , 2015; Choong et al., 2018)
Methane Recovery and Electricity Generation from POME at Pelakar Mill, Jambi, Indonesia	Covered Pond	Gas Engine	(CDM , 2013; Choong et al., 2018)
Biogas Recovery in Siak	Closed Anaerobic Digester	Flaring System	(CDM , 2013; Choong et al., 2018)
PT Karya Mas Energi Biogas Bundled Project 3	Covered Pond	Gas Engine	(CDM , 2012; Choong et al., 2018)
Methane Recovery and Utilisation at PT Pinago Utama Sugihwaras Palm Oil Mill, Sumatera, Indonesia	Closed Anaerobic Digester	Burner	(CDM , 2012; Choong et al., 2018)

The domination of palm oil industry in both Indonesia and Malaysia together with the usage of anaerobic technologies to treat POME has pushed the research industry to move towards the era of understanding the mechanism of methanogenesis particularly in the ecology of methanogenic Archaea during the treatment of POME. Methanogen or methanogenic archaea belong to the Euryarchaeota together with halophilic (Halobacteriales) and thermophilic (Archaeoglobales, Thermocaccales and Thermoplasmatales) archaea to produce large amounts of atmospheric methane (Garrity and Holt, 2001; Watanabe et al., 2004). Currently, there are only five orders of methanogens: (a) *Methanosarcinales*; (b) *Methanopyrales*; (c) *Methanococcales*; (d) *Methanomicrobiales*, and; (e) *Methanobacteriales*; that have been described and each of them has a distinct lineage within the Euryarchaeota (Boone et al., 1993; Olsen et al., 1994; Garcia et al., 2000; Watanabe et al., 2004). Study reported by Yap et al. (2021) showed that methanogen under the class of *Methanomicrobiales* is commonly found in POME, granular sludge and anaerobically-treated POME.

The final step in biogas production is methanogenesis and the two group of bacteria involved in this process are known as acetotrophic and hydrogenotrophic bacteria (Lam and Lee, 2011; Ohimain and Izah, 2017). The former uses formate as electron donor for methane and carbon dioxide reduction, whereas the latter uses hydrogen as electron acceptor for methane production (Demirel and Scherer, 2008; Lam and Lee, 2011). Acetate, which is produced from acetic acid, can be used as a substrate for methanogens to produce biogas. There are two types of microbial classes that are involved in the process of methanogenesis, viz. CO_2-reducing methanogen and aceticlastic methanogen. Example of species involved in the conversion of hydrogen and CO_2 to methane is Methanobacterium (Ohimain and Izah, 2017). Besides that, Methanobacteria (*Methanococcus*) is involved in the conversion of acetate to CH_4 and CO_2 (Ohimain and Izah, 2017). Chin and Wong, (1983); Ohimain and Izah, (2017) reported that methanogenesis is the rate-limiting step in anaerobic digestion. Methane gas can be captured properly using high-rate anaerobic bioreactor as proposed by Chin and Wong, (1983); Borja and Banks, (1994). The metabolic activities of methanogens in POME result in the production of methane gas. As reported by Ohimain et al. (2013), approximately 66% of methane produced is formed via acetate decarboxylation, while the remaining 34% of methane is produced via carbon dioxide reduction mechanism by the activities of hydrogenophilic bacteria. Methanogen plays the final role in the anaerobic digestion process. It is essential to create an environment that is free from inhibitors for methanogens to carried out their metabolic activity to ensure the stability of the anaerobic digester, whilst being able to generate methane gas.

2.14.1 Methanogenesis Inhibitors

It is vital to identify inhibitors that will affect the anaerobic digestion process where antagonism, synergism, acclimation and complexing could significantly affect the phenomenon of inhibition of anaerobic digestion process. Ammonia is produced by the biological degradation of the nitrogenous matter, mostly in the form of ammonium ion (NH_4^+) and free ammonia (FA, NH_3) (Kayhanian, 1999; Chen et al., 2008).

Ammonia inhibition may result in the change in the intracellular pH, increase of maintenance energy requirement, and inhibition of a specific enzyme reaction due to the hydrophobic nature of the ammonia molecule that may diffuse passively into the cell, resulting in proton imbalance, and/or potassium deficiency (Sprott and Patel, 1986; Gallert et al., 1998; Chen et al., 2008). Methanogen is least tolerant to ammonia, and excessive concentration of ammonia will most likely cause the cessation of growth of methanogen during the anaerobic digestion process. As reported by Koster and Lettinga, (1988), the increased of ammonia concentration from 4,051 mg/L to 5,734 mg/L has reduced the methanogenic population activity to 56.5%. Study conducted by Gallert et al. (1998), had identified that *Methanospirillum hungatei*, *Methanosarcina barkeri*, *Methanobacterium thermoautotrophicum*, and *Methanobacterium formicicum* are the commonly-isolated methanogenic strains from the sludge digesters of sewage sludge. Out of the identified strains, *Methanospirillum hungatei* is the most sensitive with its methanogenic activity being inhibited due to the presence of 4.2 g/L (4,200 mg/L) of ammonia. It is essential to maintain the ammonia concentrations below 200 mg/L to prevent any reduction in methane production, as nitrogen is an essential nutrient for the anoxic microbial cells.

Ammonia has been known as the actual toxic agent in the anaerobic process, where it will increase the pH of the wastewater, which will affect the methane production. Study reported by Zeeman et al. (1985) showed that methane production was four times lower when the pH of the anaerobic digestion is at 7.5. This may be due to the increase in total ammonia concentration from 1.7 $kgNm^{-3}$ to 3.3 $kgNm^{-3}$ that increases the overall pH of the anaerobic digester to 7.5, coupled with the accumulation of VFA. Such occurrence may be due to the growth of methanogen outcompeting with acidogen due to the continuous supply of raw material. This condition allows us to infer that there is a direct relationship between VFA and pH resulting in low methane yield although the process is running stable (Angelidaki and Ahring, 1993; Angelidaki et al., 1993). The increase of the overall pH of the anaerobic digester also resulted in the accumulation of VFA. Such unfavourable condition does not permit the growth of methanogen, instead there will be continuous growth of acidogen. Study reported by Braun et al. (1981) showed an increase of pH to 8 resulted in accumulation of VFAs to 316 mg/L during the anaerobic digestion of liquid piggery wastewater, which is higher than the recommended value of 200 mg/L. The same observation was also reported by Wang et al. (2009); Yenigün and Demirel, (2013), where an increase in pH above 7.4 resulted in accumulation of VFAs, leading to the occurrence of reactor failure. Since VFAs are the mid-products in the production of methane, and their concentrations affect the fermentation efficiency, it is essential to monitor the concentration of ammonia during the anaerobic digestion process to achieve stable and efficient process.

H_2S is the toxic form of sulphide as it can be diffused into the cell membrane. In the cytoplasm, H_2S will denature the native protein by forming sulphide and disulphide cross-links between polypeptide chains (Chen et al., 2008; Amha et al., 2018). Such formation will interfere with the various coenzyme sulphide linkages as well as the assimilatory metabolism of sulphur. The presence of sulphate and organic matter will lead to the growth of sulphate-reducing bacteria (SRB).

In the sulphate-reduction microbial process, sulphate is used as the terminal electron acceptor, resulting in the formation of hydrogen sulphide (Ahmed and Rodríguez, 2018). SRB can utilise a wide variety of substrates as electron donor, e.g. H_2, ethanol, formate, lactate, pyruvate, succinate, short and long fatty acids, and some aromatic compound (Muyzer and Stams, 2008; Ahmed and Rodríguez, 2018). Several of these substrates are intermediates in the anaerobic digestion process, which allow competition to take place between SRB and other microbial groups in an anaerobic digester at acidogenic, acetogenic and methanogenic levels (Fedorovich et al., 2003; Ahmed and Rodríguez, 2018). Anaerobic digestion of sulphate-containing wastewater is problematic as the H_2S generated is malodorous, corrosive to equipment materials, highly toxic to humans and animals, and inhibitory to the anaerobic digestion microbial population including SRB (Barrera et al., 2013). In terms of bioenergy recovery purposes, the presence of SRB in the anaerobic digester is a great concern as this will lower the methane yields and leads to poor biogas quality due to the corrosive nature of the H_2S. In this case, the biogas produced requires pre-treatment before processing in energy conversion systems (Barrera et al., 2013; Muyzer and Stams, 2008; Ahmed and Rodríguez, 2018). Despite the mentioned disadvantages, anaerobic digestion of sulphate-containing wastewater will aid in pH neutralisation, heavy metal removal as metal sulphide precipitate and pathogen removal (Drury, 2000; Knobel and Lewis, 2002; Muyzer and Stams, 2008; van den Brand et al., 2015; Ahmed and Rodríguez, 2018). It is essential for us to maintain a feasible implementation of anaerobic digestion under the presence of sulphate-reducing bacteria by understanding the competition between sulphate-reducing bacteria with acidogenic, acetogenic and methanogenic to ensure continuous production of methane.

There are four types of competition that exist between the SRB with the microbial cells presence in different steps of anaerobic digestion process. The first type is known as competition between SRB and hydrolytic and acidogenic bacteria. SRB does not play a role in hydrolysis stage as it does not have the ability to degrade natural biopolymers, viz. starch, glycogen, protein and lipids. This shows that the SRB depends on the activity of other organisms for providing them with their favourable substrates (Hansen, 1993; Chen et al., 2008). Similar outcome was observed in the study conducted by O'Flaherty et al. (1999), where the addition of sulphate does not affect the degradation rate of glucose and lactose. This allow us to conclude that during the initial stage of anaerobic digestion, the SRB will not be able to outcompete the fast-growing fermentative microorganisms that are mainly involved in monomer degradation. The second type of competition is between SRB and acetogen. Acetogen is responsible for the generation of acetate from CO_2 and other electron source, e.g. H_2, CO, formate, etc., via the pathway known as reductive acetyl-CoA. Theoretically, SRB just outcompetes other anaerobes for substrates. In practice, other factors such as COD/SO_4^{2-} ratio, relative population of SRB and anaerobes, and sensitivity of SRB and anaerobes towards sulphide toxicity influence the competition. In this scenario, it is essential to monitor the concentration of propionate, butyrate and ethanol during the anaerobic digestion as they are substrates for the SRB.

Studies reported by (Laanbroek et al., 1984; , Overmeire et al., 1994; O'Flaherty et al., 1997; O'Flaherty et al., 1998) supported that SRB shows a higher affinity for these substrates by comparing the kinetic parameters of K_s and μ_{max} and ratio of COD/SO_4^{2-}. Such high affinity demonstrated by the SRB against the acetogen may be controlled by determining the kinetic parameters, which may aid as an effective tool to measure the growth of methanogen and SRB in the full-scale anaerobic digester, to reduce the competition for the same type of substrates.

The third type of competition is between SRB and hydrogenotrophic methanogen. By taking into consideration of thermodynamic and substrate affinity, H_2-oxidising SRB should effectively outcompete hydrogenotrophic methanogens under unfavourable condition (Zinder, 1993; Chen et al., 2008). This view was in-line with the previous studies reported by (Rinzema and Lettinga, 1988; Visser et al., 1993; Alphenaar et al., 1993; Harada et al., 1994; Uberoi and Bhattacharya, 1995; Omil et al., 1996; Colleran et al., 1998; O'Flaherty et al., 1999), where oxidation of H_2 is carried out exclusively by SRB. Even though methanogenesis and sulphate-reduction processes are not mutually-exclusive, methanogen could not compete for H_2 with the SRB despite the presence of H_2 stimulation (Oremland and Taylor, 1978; Chen et al., 2008). Selection of temperature profile (e.g. thermophilic and mesophilic) will create a great impact on the outcome of the competition between SRB and hydrogenotrophic methanogen (Colleran and Pender, 2002). Temperature will be considered as an important independent temperature during the planning of the operation of the anaerobic digestion, as it will indirectly affect the obtained methane yield. Hence, monitoring the growth curve of both the methanogen and SRB will allow us to maintain and optimise the biogas produced. Competition between SRB and aceticlastic methanogen is the fourth type of competition. Unlike the abovementioned methanogen-producing bacteria, aceticlastic methanogen has the ability to outcompete SRB for the same source of substrate, which is acetate. Studies conducted by (Rinzema and Lettinga, 1988; Arne Alphenaar et al., 1993 ; Stucki et al., 1993; Gupta et al., 1994; Chen et al., 2008) illustrated the successful competition of SRB. On the other hand, other studies reported the dominance of aceticlastic methanogen during the anaerobic digestion (Isa et al., 1986; Rinzema and Lettinga, 1988; Visser et al., 1993; Stefanie et al., 1994; Omil et al., 1996; Colleran et al., 1998; O'Flaherty et al., 1998; De Smul et al., 1999; Colleran and Pender, 2002; Chen et al., 2008). To resolve such contradicting discrepancies raised by different reseachers, it is vital to determine the parameters that may influence the growth of the aceticlastic methanogen and SRB in the presence of acetate. Parameters that can be measured, viz. ratio of COD/SO_4^{2-} and MPB/SRB, may act as useful yardstick for their respective predomination in anaerobic digestion (Choi and Rim, 1991; O'Flaherty et al., 1998). Besides that, the physical attribution of the high-rate bioreactor used also influenced the growth the aceticlastic methanogen and SRB. Practically, the superior attachment capability illustrated by the bioreactor used is able to prevent the washout of biomass that may act as source of food for other classes of anaerobic to carry out respective metabolic pathway. This may be attributed by the attainment of high solid retention times for good system efficiency and stability, with low HRT for system economy (Omil et al., 1996), which is able to overcome many of the problems historically associated with anaerobic processes.

The higher affinity of acetate illustrated by methanogen during sulphate-limiting conditions allows us to infer that acetate is the least favoured substrate for SRB (Colleran and Pender, 2002). Under such situation, there may be higher yield of methane produced. If the high-rate bioreactor is subjected to long HRT, it will lead to the occurrence of biomass washout. This will be attributed to the lower methane yield produced due to the low concentration of biomass available for the different levels of anaerobes. The performance of an anaerobic reactor is directly associated with the structure of the microbial community present within the reactor. The operational and environmental parameters, viz. pH, temperature, ratio of COD/SO_4^{2-} and MPB/SRB, will influence the fate of the microbial community coupled with the performance of the anaerobic digestion.

2.14.2 Identification of Methanogen: Applications of Molecular Tools

Inhibition of anaerobic digestion caused by perturbation due to substrate composition and/or operating conditions may affect the performance of the digester significantly. Such disturbance can be minimised by elucidating microbial community response to inhibitors and devising strategies to increase community resilience. To date, advanced molecular methods have been used as a tool in studying the complexity of the anaerobic digestion microbiome, which consists of a diverse community of microbial populations. Differences in microbial community structures make it particularly challenging to compare inhibition occurred during anaerobic digestion. To date, the majority of studies have only applied DNA-based methods to study the microbial community structure during anaerobic digestion (Li et al., 2015; Ma et al., 2015; Treu et al., 2016; Ziganshin et al., 2016; de Jonge et al., 2017). However, the drawback is relatively insensitive, especially in the condition of low biomass yield. Under this condition, it may impact DNA replication rates and DNA may still persist in the environment after a cell ceases activity (Amha et al., 2018). De Vrieze and Verstraete, (2016) explained that the occurrence of inhibition does not have a direct relationship with the microbial activity if their response is studied based on changes in salt. Such study can be carried out using RNA-based sequencing to determine the response of the microbial community towards high salt concentration. For instance, *Methanosaeta* remained the most abundant methanogen based on DNA-sequencing, but was strongly inhibited by high salt concentration according to RNA-based sequencing, corroborating performance observations. Similar result has been reported where addition of high volumes of fats, oils, and grease (FOG) further supported that RNA-based method is still the most suitable method used to study the anaerobic digestion inhibition (Amha et al., 2017).

The 16S rRNA gene is the most widely used biomarker and provides detailed phylogenetic information about Bacteria and Archaea from the mixed pool of microbial communities. Limitation of this approach should be noted. The number of copies of the 16S rRNA gene per genome vary from 1 to 15 and differences in relative abundances can often misrepresent the true quantity of genome population (Klappenbach et al., 2000). The number of copies of rRNA genes within the same organism will differ due to the different stages of development and in the metabolic state at the point of sampling, i.e. dormant, active or growing (Sukenik et al., 2012; Blazewicz et al., 2013; Amha et al., 2018).

The relative abundance information can also be misleading if the overall size of the community is changing constantly although quantitative sequencing methods have been widely used (Smets et al., 2016; Props et al., 2017). Despite the drawbacks illustrated by different researches, 16S rRNA gene sequencing is still being used widely for characterisation of microbial community.

Recently, molecular ecological methods, e.g. restriction fragment length polymorphism (RFLP), fluorescent in-situ hybridisation, etc., have been widely used for analysing microbial community structures in different environments. The denaturing gradient gel electrophoresis (DGGE) of small subunit rDNA is one of these methods. It allows us to identify the phylogenetic information on the microorganisms present and the community structural changes by analysing the bands that migrate separately on the DGGE gels. Methanogenic community have been investigated in paddy fields, landfills, oil reservoirs, anaerobic digestion, and sludge using the methods of clone library and terminal RFLP techniques (Großkopf et al., 1998; Chin et al., 1999; Richter et al., 2002; Lueders and Friedrich, 2000; Fey et al., 2001; Ramakrishnan et al., 2001; Weber et al., 2001) and not many studies have been conducted by cultivation methods (Asakawa et al., 1998). With the advance of technology that allows us to obtain real-live results, cultivation methods to study the methanogen community is not being practised anymore. It is time-consuming to cultivate the methanogen due to their fastidious growth requirements, viz. growing under completely anoxic environment and optimum proportion of organic/inorganic ions. Since methanogen only grow in condition of oxygen free, equipment such as anaerobic chamber or anaerobic jar have to be used. Daily addition of N_2 gas has to be added into this equipment to ensure that the condition is 100% oxygen-free to support the growth of methanogen. It is labour-intensive to perform such actions, as well as the involvement of high cost. Even though cultivation method may involve an enrichment step, it can introduce bias, or they are technically complex and time-consuming. This does not provide any accurate information on the composition of the methanogen communities present on the tested sample.

Our chosen alternative is to develop PCR-based techniques to investigate naturally occurring methanogen populations. Since many groups have used the amplification and analysis of 16S rRNA genes to unravel the complexities of methanogen populations in environmental samples, we have decided to adopt the approach suggested by Watanabe et al. (2004). Representatives of bands that were clear and high intensity observed on the DGGE gels can be subjected to sequencing. Such DNA sequences allow us to determine the strain present in the tested sample by subjected to DNA sequencing. As reported by Watanabe et al. (2004), the methanogenic *Archaea* found in paddy fields were mostly affiliated to Methanomicrobiales or Methanosarcinales, especially related to *Methanosaeta*. Besides that, Poh and Chong, (2010) also reported that the mixed culture of methanogenic archaea found in POME sample consisted of *Methanosaeta thermophila*, *Methanosarcina thermophilia*, *Methanobacterium thermoautotrophicum*, *Methanobacterium thermoformicicum*, and *Methanobacteriaum wolfei*. This allows us to infer that the possible methanogenic archaea present in the POME sample is similar to the one reported by Watanabe et al. (2004).

In order to obtain the DNA of these methanogenic archaea in the POME sample, the selection of primer is essentially important. Primer used in our study is known as 0357F (5'-CCC TAC GGG GCG CAG CAG-3') and 0691R (5'-GGA TTA CAR GAT TTC AC-3'), which were obtained from the same author due to the similarity of the methanogenic archaea found in the paddy soil sample. To provide more meaningful outcome of the identified methanogen strain, qPCR is used to for sequencing and quantification purposes. It is used to detect, characterise and quantify nucleic acids for numerous applications. In this assay, RNA transcripts are quantified by reserve transcribing them into cDNA first before proceeding with qPCR. DNA is amplified by three repeating steps: denaturation, annealing and elongation. It is followed by the step of amplification of DNA with the aid of fluorescent labelling. Dye-based qPCR, which is typically green in colour, is the fluorescent labelling agent that allows the quantification of the amplified DNA molecules by employing the use of a dsDNA binding dye. At each cycle, the fluorescence is measured. The fluorescence signal increases proportionally to the amount of replicated DNA and hence the DNA is quantified in real time. qPCR that uses fluorescence-based detection offers greater sensitivity and enables discrimination of gene numbers across a wider dynamic range than is found with end-point PCR; for example, two-fold changes in target concentration can be discriminated using qPCR (Smith and Osborn, 2009). Before the development of such reliable technology, competitive PCR (Diviacco et al., 1992) and limiting dilutions or most probable number (MPN)-PCR (Sykes et al., 1992) were the two alternative PCR-based methods for gene number quantification. However, these methods are time- and resource-consuming, requiring post-PCR analysis, and have now largely been replaced by fluorescence-based qPCR methods.

2.14.3 Ways to overcome Methanogen Inhibition: pH and Alkalinity

pH is the most important factor that governs the fate of anaerobic digestion of various types of wastewater. Methanogen, which is responsible for the production of biogas during the anaerobic digestion process, is very sensitive to pH changes. They are generally considered to have an optimum pH ranges of 6.8 to 7.2 (Poh and Chong, 2010; Hou et al., 2018). If the pH of the anaerobic digester is not within the recommended range, the methanogenic bacteria will be seriously inhibited, leading to the deterioration of the whole anaerobic digestion process. Various factors, viz. influent pH, water quality (organic matter, organic species, etc.), the biochemical reaction, acid-base balance, and gas-solid solution between the dissolution balance, should be taken into consideration during the initial design of the anaerobic digestion to maintain the optimum pH value throughout the study period (Fang and Liu, 2002; Li et al., 2010). Anaerobic system is a pH buffer system controlled by a carbonate system. It has a direct relationship with the fatty acid concentration, where an increase in VFA will consume the $-HCO_3$. This will result in an increase in the system's pH and the ratio of VFA/TA (Prakasham et al., 2009). Both the parameters' alkalinity and VFA play an important role in anaerobic system, where it will be an effective early warning of process instability. Li et al. (2014) reported that the threshold for VFA/TA of 0.35, and suggested that the use of relative changes in VFA concentrations is more feasible than absolute concentrations for reflecting process instability.

In addition to these conventional process parameters, innovative early warning indicators such as aromatic acids (Hecht and Griehl, 2009), VFA/Ca, PO_4/Ca (Kleyböcker et al., 2012) and the stable isotope composition of biogas (such as $\delta 13CH_4$ and $\delta 13CO_2$) (Lv et al., 2014; Polag et al., 2015) were being explored, but some studies have reported conflicted results. Acidification will occur if the pH of the anaerobic digestion does not fall within the suggested range, and it will take a long time to recover (Stavropoulos et al., 2016).

It is worth noting that the interaction between free ammonia, pH and VFA may lead to "suppressing steady-state", where it will run steadily but has a lower methane production (Dunfield et al., 1993; Bergman et al., 1998). The optimum pH control may also aid in reducing the toxicity of ammonia present in the anaerobic digester (Glatzel and Stahr, 2001). Horiuchi et al. (2002) reported that the acidification of crab wastewater improves the performance of UASB reactors, such as the lower COD concentration of effluent. pH adjustment within the range of 6.8 to 7.2 during the anaerobic digestion of cattle manure will increase the methane production four to five times (Zoetemeyer et al., 1982). During anaerobic digestion of pig manure, the VFA reached 320 mg/L when the pH of the anaerobic digester was 8. However, when the pH was adjusted to 7.4, VFA concentration was remarkably reduced to 20 mg/L. Such reduction in VFA concentration may be due to low pH conditions, which counteract with the ammonia inhibition (Dai et al., 2016). It is more worthy to note that both the methane and acid-producing microorganisms have their optimum pH for their respective metabolic pathway at different levels of anaerobic digestion.

2.14.4 Ways to overcome Methanogen Inhibition: Co-digestion

Co-digestion of various biosolid and plant wastes is an attractive approach for improving the efficiency of biotransformation by utilising the nutrients and bacterial diversity available in the wastes used (Wang et al., 2012). Many successful co-digestion processes using more than one type of substrates have shown large increases in the methane potential as compared to mono-digestion alone. Study reported by Umetsu et al. (2006) showed that co-digestion of 40% beet tops and dairy manure produced 1.49 times more methane as compared to 100% dairy manure mixture. The same observations were obtained by co-digestion of slaughterhouse waste with biowaste (Zhang and Banks, 2012), manure (Hejnfelt and Angelidaki, 2009) and mixture of biowaste and manure (Edström et al., 2003; Murto et al., 2004; Alvarez and Lidén, 2008; Cuetos et al., 2008). This allow us to infer that (a) waste composition (carbohydrates, protein and lipids); (b) C/N ratio are the crucial factors in reducing the inhibition of methanogen during the co-digestion process. It is essential to choose the best co-substrate and blend ration (C/N ratio) (a) to favor positive interactions, i.e. synergisms, macro- and micro-nutrient equilibrium and moisture balance; (b) dilute inhibitory or toxic compounds that may inhibit the methanogenesis activity; (c) optimise methane production; (d) enhance digestate stability (Mata-Alvarez et al., 2011; Astals et al., 2014).

To the author's knowledge, there is limited information available on how waste composition (carbohydrates, protein and lipids) influences anaerobic co-digestion performance or whether interaction between substrates enhance or attenuate inhibition thresholds, degradation rates, or biogas yields on individual substrates (Astals et al., 2014). The degradation of these waste composition occurred by different metabolic pathways, resulting in variation in methane yields (Angelidaki and Sanders, 2004), whereby excessive concentrations of these waste composition in the substrates may resulting in the inhibition of methanogenesis pathway. Such limited knowledge available in terms of the influence of waste composition on anaerobic co-digestion, allow us to infer that C/N ratio is an important indicator for controlling biological treatment systems. Studies show that crop residues containing low levels of nitrogen (high C/N ratio) are characterized by low pH substrate, poor buffering capacity, and possibility of high VFA accumulation in the anaerobic digestion process (Banks and Humphreys, 1998; Campos et al., 1999). Co-digestion of manure and other substrates able to overcome these problems by maintaining a stable pH suitable for methanogenesis range (6.8-7.2) due to their inherent high buffering capacity present in the anaerobic digestate. On the other hand, substrates that have low C/N ratios contain relatively high concentration of ammonia most probably inhibit anaerobic digestion process (Hansen et al., 1998; Prochazka et al., 2012; Wang et al., 2012). As reported by Astals et al. (2014), ammonia inhibition is often related to its capacity to diffuse into microbial cells and disruption of cellular homeostasis (Kayhanian, 1999), whereas LCFAs adsorb onto the cell membrane, interfering with membrane functionality (Chen et al., 2008; Palatsi et al., 2009). Such scenario may impair the growth of methanogen in the digestate as the growth of the methanogen is easily affected by external factors viz. high ammonia concentration (> 200 mg/L) (Chen et al., 2008; Yenigün and Demirel, 2013). The growth impairment of methanogen can be rectified by addition of crop materials with high carbon contents and coagulant effect will aid in decreasing the risk of ammonia inhibition in the digestate (Hashimoto, 1983; Wang et al., 2012). Hence, the incorporation of crop materials with these properties, viz. *Moringa Oleifera* (Section 2.13.2.1.1), Nirmali Seed (Section 2.13.2.1.2), Tannin (Section 2.13.2.1.3), and Cactus (Section 2.13.2.1.4) into the substrate – anaerobic co-digestion may be beneficial to overcome process instability and inhibition of methanogenesis process.

2.15 Challenges in Commercialisation of Natural Coagulants

Various indigenous plant extracts have been proven to possess coagulative properties in removing turbidity effectively (Sowmeyan et al., 2011). However, not many of the co-substrate used has reached the commercialisation phase due to constraints: (a) financial; (b) research and development; (c) market awareness, and; (d) regulatory approval. These have been thoroughly discussed for the indigenous plant extract of *M. oleifera* seeds (Sutherland et al., 2002). Besides, harvesting and processing of the indigenous plant extracts were also factors that halted the commercialisation of such product. Palm oil industry is one of the potential investors due to the abundant volume of POME produced daily, especially during the high crop season. However, palm oil millers are hesitant in incorporating new products to replace or improve the current POME treatment system. This may be due to the involvement of a large sum of money, and a lack of data to support such incorporation, that may or may not bring any beneficial improvement to the current system.

In order for the industry players to accept indigenous plant extracts, understanding the vulnerabilities and risk involved is the initial consideration required to ensure full utilisation of these natural coagulants as part of the wastewater treatment. Research and development serve as a platform and backbone for the identification of strengths, limitations, areas that require fine-tuning, as well as process improvements. The existing results are mostly conducted in the form of laboratory scale, where there may be a lack of information in terms of up-scaling for the use in the industry. The results that obtained during laboratory scale study may also be questioned by the industry players due to suitability of the natural coagulant under various working conditions. Additionally, the supply and demand of the natural coagulants in the market will affect the outlook and needs of natural coagulants, which may be a good source of replacement for the chemical coagulants. To date, chemical coagulants have been used widely in the treatment of wastewater. However, additional involvement of extra cost and the effect towards the environment has driven the industry to look for better alternatives. An evaluation on the economic, social and environmental aspects, which may be used as a sustainability indicator, would be a useful piece of data for the industry players to accept natural coagulant than chemical coagulant. Besides that, support from local government in terms of tax rebates and subsidy schemes may act as a form of support from the government bodies to motivate the industry players to adopt the use of natural coagulants as part of the wastewater treatment. The issue of commercialising natural coagulant will not be an overnight success; small steps have to be taken to bridge the gap and limitations encountered. Figure 2.25 shows the factors that must be taken into consideration for the successful commercialisation of natural coagulants.

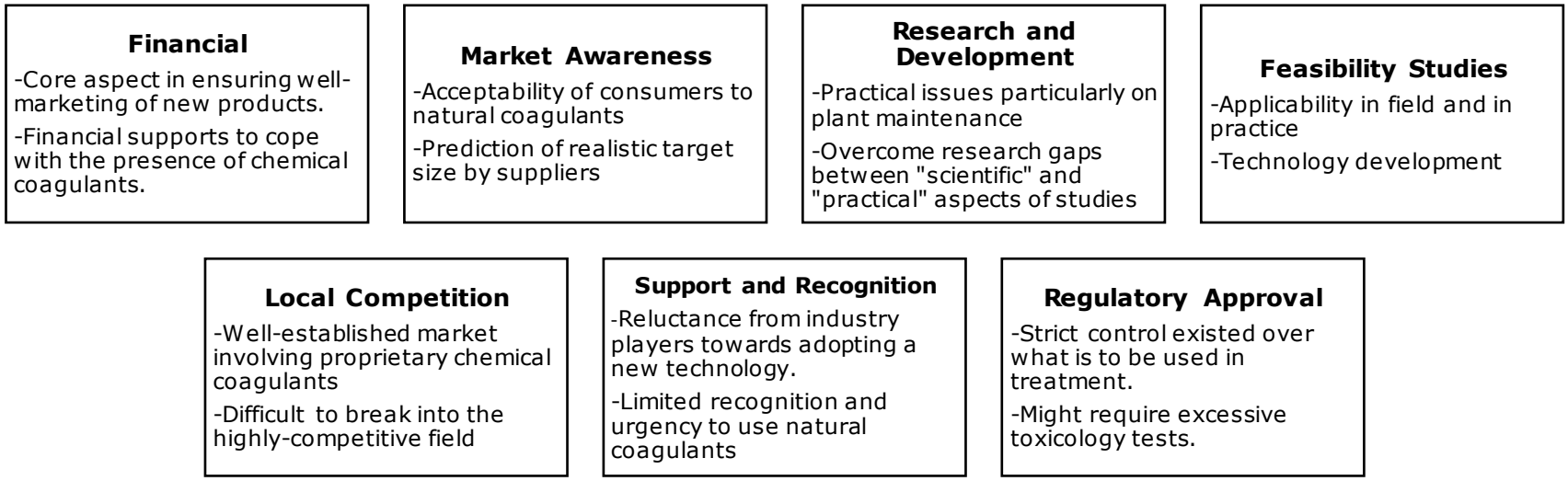


Figure 2.25: Challenges that faced during the commercialisation of natural coagulants (Choy et al., 2014)

2.16 Limitations and Further Improvements on the Current Natural Coagulant Studies

The use of natural coagulants in wastewater treatment are well known for their efficiency in reducing turbidity. However, little understanding is known about the characteristics and properties of the flocs formed. Thorough understanding on the flocs formed in terms of the floc strength, structure and compactness may be key in establishing a complete set of probable coagulation theory (Li et al., 2006). This piece of information may definitely attract the industry players to adopt such techniques into their wastewater treatment. With the advancement of technology, it is able to clearly view the flocs generated upon coagulation using various microscopy techniques, viz. transmission electron microscopy or scanning electron microscopy. The images generated will give us an insight on the structure and surface characteristics, which may lead to a better understanding on the bonding of the colloidal particles (Idris et al., 2012).

Limited studies are available to conclude the coagulation mechanism involved in each of the plant-based coagulants. The agglomeration of the colloidal particles that leads to water clarification may be due to more than one type of coagulation mechanisms. Additionally, the complexity of the process and synergistic effects that occurred among each component lead to the difficulty in determining the chemical constituents responsible for such occurrence. Additional work may be required, viz. isolation, purification and characterisation of the active agents, would be the answer for the underlying mystery of the involvement of coagulation in the wastewater. However, limited number of studies have been performed over the years to fully understand the active agent. Only a few plant-based coagulants have been thoroughly studied. They are the seeds of *M. oleifera* (Okuda et al., 2001; Okuda et al., 2001; Ghebremichael et al., 2005), *Moringa concanensis* (Sathiyabama, 2012), *Vigna unguiculata* and *Parkinsonia aculeate* (Marobhe et al., 2007), and the endosperm of *Cocos nucifera* (Fatombi et al., 2013).

Although natural coagulants are green in nature, the use of crude extract may result in the leaching of organic matters into treated water, which can be quantified in terms of total organic carbon (TOC), dissolved organic carbon (DOC) and other parameters. Crude extracts contain inorganic and organic molecules, e.g. lipids, which do not participate in active coagulation (Ghebremichael, 2007). If there is an increase in levels of organic matters in the treated water, it would incur an additional cost on the post treatment process using chlorine. This issue can be resolved by using purified active agents via ion exchange. Ghebremichael, (2007) reported that ion exchange has successfully eliminated the additional nutrient and organic loading present in the seeds of *M. oleifera*. Antov et al. (2010) reported partial purification of the active agents in legume, where the organic loading content has been successfully reduced to at least 16 times, as compared to the use of crude extracts. In order to ensure that industry players of various business entities accept the technology, extraction methods that are economically feasible, reliable and simple are required.

Even though the reviewed plant-based coagulants could be considered as potential alternatives to chemical coagulants, issues such as feasibility and sourcing of the plants could be the major challenge. For instance, the solid coagulated with *M. oleifera* seeds may bring harm to the environment if discharged into the riverways. One probable solution to counter for such harmful effects to the environment is to opt to the treated solid for the making of fertiliser. As certain fruits are seasonal, the availability of these plant-based coagulants throughout the year could be challenging. This would be difficult to collect the plant-based coagulants for mass consumption in real-time applications. Perhaps, finding ways to maintain the coagulation activity of the active agent of the plant-based coagulant may be a better alternative to resolve the seasonal issue. Additionally, the use of plant-based coagulants (such as cereal crops) that are available throughout the year with notable coagulation activities may be another alternative for industrial-scale applications. In some cases, the sole use of natural coagulants may not be enough to achieve the desired turbidity removal. The blending of more than one type of coagulant can reduce the dependence of the industry players towards chemical coagulants, while addressing the lower turbidity removal and unavailability of certain plant-based coagulants that may be seasonal.

Biogas generation through anaerobic co-digestion technology from different types of biodegradable organic substrates may be considered as a suitable alternative to fossil fuel usage. The development of the anaerobic co-digestion process by incorporating advanced technology will help to minimise the upcoming challenges that may be faced by the industry players. The initial stage of designing the anaerobic co-digestion technology has to be economically, environmentally feasible and sustainable for biogas generation, which can be in turn carried out in the laboratory and industrial scale. Studies that previously conducted to alleviate the mentioned challenges, viz. evaluating limiting factors, parameter calibration and characterisation, the dynamic behaviour of microbial cells, the characteristics of the organic matters, process stability and optimisation, need to be taken into consideration during the designing of methods to improve the existing anaerobic co-digestion technologies. Besides improving the technology, engaging the coagulation activity exhibited by the plant-based coagulant in treating wastewater may be the key in treating wastewater. For commercialisation purposes, the plant-based coagulant must be able to penetrate into the well-established market, highly governed by chemical coagulant players, starting from the local communities. In light with the health and environmental concerns of chemical coagulants, there is an urgent need for detailed research to use plant-based coagulant in treating wastewater produced in the palm oil industry as a sustainable alternative to chemical coagulants.

2.17 Summary

Based on the literature review on the various types of POME treatment system that are currently use in the palm oil industry, it can be concluded that the most suitable process flow for effective POME treatment is anaerobic process followed by aerobic. In order to resolve the issues of (a) shortage of land; (b) long HRT; (c) unable to treat POME at high OLR_{an}; (d) able to capture methane gas, combination of high-rate bioreactors seems to be the most suitable technology that can be used for POME treatment in the palm oil industry.

As discussed in Section 2.5.1, positive results (BOD < 100 mg/L, COD < 500 mg/L, TSS < 45 mg/L, pH ~ 7.4, and able to operate at high OLR_{an} 13.5 – 16.5 gCOD/L.day) were obtained when treating POME using laboratory-scale IAAB system designed by Chan, et al. (2012). This allow us to infer that the combination of anaerobic and aerobic processes in a single unit high-rate bioreactor can be an alternative for the existing POME treatment. In order to produce a set of reliable data that may convince the palm oil millers, the laboratory-scale prototype has been upscale to a larger volume (from 55L to 1.8 m^3). The upscale of the laboratory-scale IAAB is termed as pilot-scale IAAB (Section 2.5.2). During the entire study period, the pilot-scale IAAB is placed in a palm oil mill factory. This is to mimic the existing POME treatment system used in the palm oil industry, i.e. conventional ponding and combination of anaerobic pond and covered lagoon system as both the systems have been used to treat POME obtained during high- and low-crop seasons.

The installation of biogas capture system and utilisation of suspended carrier or packing medium will be used in the pilot-scale IAAB during the entire research study. With the methane yield (0.24 $LCH_4/gCOD_{removed}$) obtained by the laboratory-scale IAAB showed that the installed methane gas capturing system may seems to be the most suitable technology to capture the released of methane during anaerobic digestion process. This may be an attractive source of recovering energy whilst reducing the emission of greenhouse gases to the environment. Additionally, such technology may in the future lead to it being awarded FiT rates awarded by the Sustainable Energy Development Authority (SEDA) Malaysia. This will act as an additional source of revenue for the palm oil millers, which will shorten the payback period for such huge investment. This will definitely create a positive paradigm shift for the POME treatment management system in terms of maximising the hidden potential of "waste to wealth" monetary value.

The performance of the pilot-scale is evaluated at its maximum loading rate aiming to produce final treated effluent that abides the discharge limit set by DOE, Malaysia (BOD < 20 mg/L; TSS < 400 mg/L). Besides that, by maximising the performance of the pilot-scale IAAB can lower operating and capital cost. It is essential to analyse the kinetics of pilot-scale IAAB treating POME as the process kinetics can provide a rational basis for process analysis, control and design of the pilot-scale IAAB. For this purpose, Monod, Stover–Kincannon, Grau second-order, and Contois models, which are the most widely used models in the literature for the development of kinetic studies in anaerobic and aerobic digestion processes (Section 2.9) were chosen and would be applied to the pilot-scale IAAB used in this study.

Lastly, as discussed in section 2.12, the incorporation of plant-based coagulant/flocculant in the treatment of POME may seems to be another attractive approach for the palm oil millers. This is because these plant-based coagulant/flocculant plays the same role of the chemical-based coagulant/flocculant that is available in the market. Such attractive approach will be carried out in two different scales, i.e. laboratory-scale and pilot-scale IAAB based on the optimum operating conditions discussed in Section 2.11. For the latter prototype scale, the addition of plant-based coagulant/flocculant will be carried out at the maximum applicable loading rate. This is also aimed to produce final treated effluent with BOD of less than 20 mg/L, whereby direct comparison between mono- and co-digestion assays can be made.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental flow chart

The experimental works in the present research include sample analysis, co-digestion studies and pilot scale IAAB studies, as illustrated in the flowchart as shown in Figure 3.1.

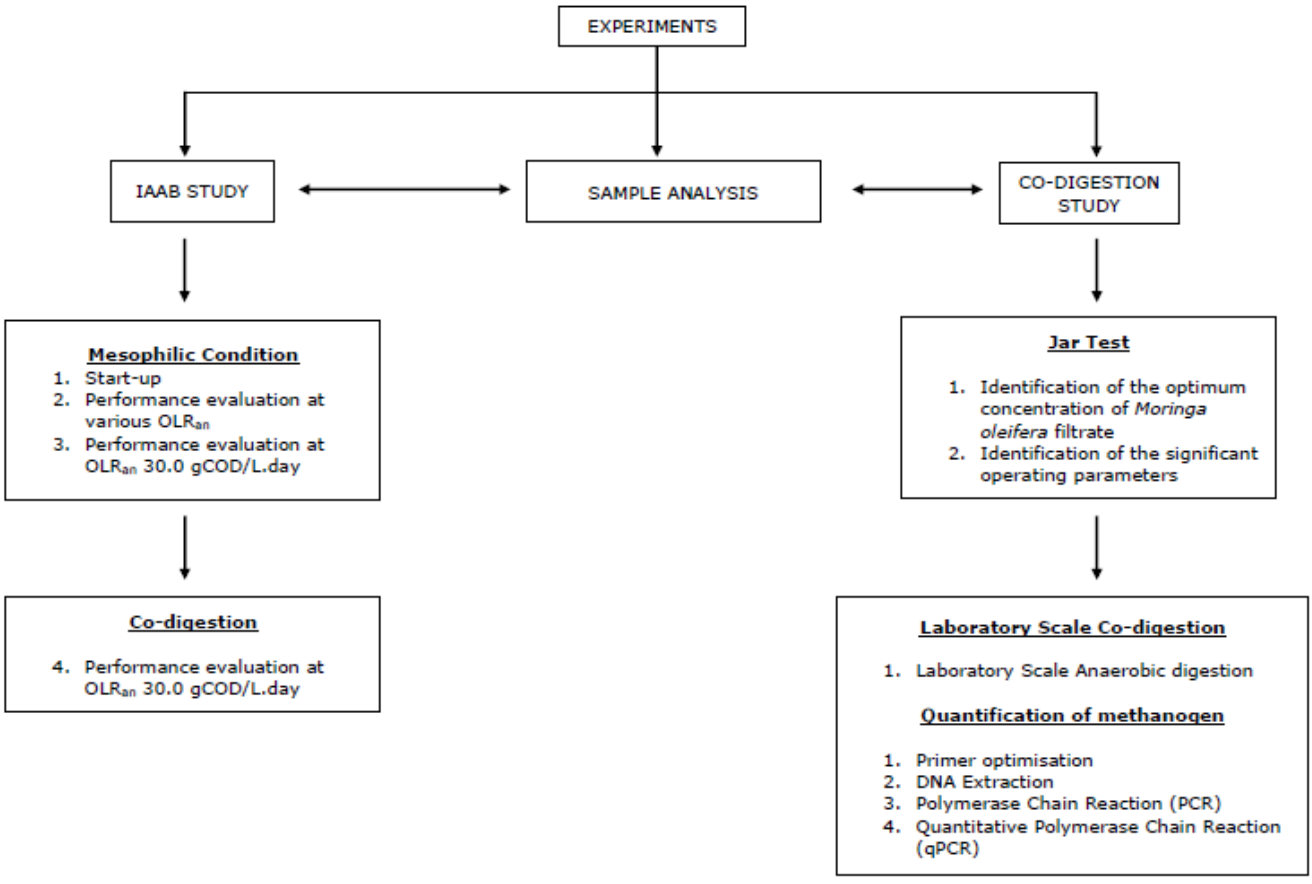


Figure 3.1: Flowchart of overall experimental activities involved in the present research

The first section of this study was to evaluate the performance of existing industrial scale anaerobic treatment system by determining the removal efficiencies of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solid (TSS), total volatile solid (TVS), and, lignin. The most suitable type of POME treatment system will be used as a reference for further improvising the POME treatment system. The second section evaluated the efficiency of *Moringa Oleifera* extract by co-digesting with POME. This was initially carried out in the form laboratory scale under two different feeding modes: (a) mono-digestion; (b) co-digestion. Their performance was evaluated by comparing the removal efficiencies of COD, BOD, TSS, oil & grease (O&G), and methane yield. The daily collected samples for both the feeding modes were subjected to quantitative polymerase chain reaction (q-PCR) to determine the concentration of methanogen in each of the collected samples.

The performance evaluation of the pilot-scale integrated anaerobic-aerobic bioreactor (IAAB) was carried out in the third section of this study aiming to produce final treated effluent with BOD of less than 20 mg/L under mesophilic condition. The performance of the pilot-scale IAAB was evaluated by determining the removal efficiencies of COD, BOD, TSS, total phosphate (TP), total nitrogen (TN), and lignin. Methane yield and methane composition were determined as well. The evaluation of the pilot-scale IAAB was divided into four different strategies: (a) start-up; (b) performance evaluation at various OLRs; (c) performance evaluation at OLR_{an} 30.0 gCOD/L.day (mono-digestion); (d) Co-digestion of POME with *M. Oleifera* extract. All analysis was carried out in duplicate to obtain the average data.

3.2 Materials

The chemicals and reagents used in this study are tabulated in Table 3.1.

3.3 Analytical Methods

Table 3.2 shows the analytical methods as well as the equipment used in determining the characteristics of all the samples collected in this study. The samples are raw POME, anaerobically-treated POME, aerobically-treated POME, and final treated effluent collected from palm oil mill. All the analysis was carried out in duplicates to obtain an average data.

3.3.1 Chemical Oxygen Demand (COD)

The COD for each sample is determined using the dichromate digestion method of HACH 8000. 2mL of the diluted sample is added into HACH high range COD digestion vial. The vial is then digested at 150°C for 2 hours using the HACH DRB 200 reactor. The digested sample is then allowed to cool at room temperature prior to being subjected for analysis using the HACH DR 2800 Spectrophotometer.

3.3.2 Biochemical Oxygen Demand (BOD)

Dilution water is prepared by adding BOD nutrient buffer pillow. 4mL of sample is placed in a 300mL of BOD bottle (Wheaton, US) consisting of unseeded, oxygen-saturated dilution water. The BOD bottle is placed in an incubator for 3 days at 30°C.

3.3.3 Total Solid (TS)

The concentration of TS in each sample is determined in accordance with the Standard Methods for the Examination of Water and Wastewater (Tchobanoglous, *et al*., 2014) (2540B). 10mL of sample is pipetted into a petri dish of known mass and placed in an oven (Memmert, Germany) at 105°C for 1-hour. TS is represented by the increase in mass as compared to the initial mass of the petri dish.

3.3.4 Total Suspended Solid (TSS)

TSS is determined using the photometric method (HACH Method 8006). 10mL of the sample is poured into a sample cell and the TSS concentration in each sample is analysed using HACH DR 2800 spectrophotometer.

3.3.5 Total Volatile Solid (TVS)

The analysis of TVS is determined using the Standard Methods of 2540E. The dried sample from the TS analysis is placed into a muffled furnace (Nabertherm) for 7 minutes. The solid that remained on the glass microfibre filter of 2.1cm (Whatman) represents the fixed solid, whereas the decrease in weight of the petri dish is the volatile solid.

Table 3.1: List of chemicals and reagents used

Chemicals/Reagents	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
Test 'N Tube™ Total Nitrogen Reagent Set	Total Nitrogen (TN) analysis	HACH, Loveland, CO
Test 'N Tube™ Total High Range Phosphorus Reagent Set	Total Phosphorus (TP) analysis	HACH, Loveland, CO
Volatile Acid Reagent Set	Volatile Fatty Acid (VFA) analysis	HACH, Loveland, CO
Tannin and Lignin Reagent Set	Lignin analysis	HACH, Loveland, CO
Ammonia Nitrogen Set	Ammoniacal Nitrogen (AN) analysis	HACH, Loveland, CO
Sulphuric Acid	Total Alkalinity (TA) analysis	Merck, Germany
n-Hexane 99.6%	Oil & grease (O&G) analysis	Merck, Germany

Table 3.2: List of parameters and analysis methods used

Parameters	Reference No.	Analytical Methods	Equipment used
pH	-	-	pH meter (HACH Sension1, Loveland, CO)
Temperature (T)	-	-	pH meter (HACH Sension1, Loveland, CO)
BOD	-	3-Day BOD Test	BOD Incubator (HACH, Loveland, CO)
COD	HACH 8000	Reactor Digestion Method	HACH DRB200 Reactor, DR2800 Spectrophotometer
TSS	HACH 8006	Photometric Method	HACH DR2800 Spectrophotometer
TS	APHA 2540 B	Total Solids, dried at 103-105°C	Oven (Memmert, Germany)
TVS	APHA 2540 E	Volatile Solids, ignited at 550°C	Muffled Furnace (Nabertherm, United States)
TN	HACH 10071	Persulfate Digestion Method	HACH DRB200 Reactor, DR2800 Spectrophotometer
TP	HACH 10127	Molybdovanadate Method with Acid Persulfate Digestion	HACH DRB200 Reactor, DR2800 Spectrophotometer
VFA	HACH 8196	Esterification Method	HACH DR2800 Spectrophotometer
Lignin	HACH 8193	Tyrosine Method	HACH DR2800 Spectrophotometer
O&G	USEPA Method 1664	n-Hexane gravimetric Method	Rotary evaporator (Buchi, Switzerland), Soxhlet extractor (Electrothermal, United Kingdom)
TA	APHA 2320 B	Titration Method	pH meter (HACH Sension1, Loveland, CO)
Biogas Composition	-	-	Biogas analyser (Geotechnical Instruments, GA5000)

APHA, American Public Health Association (Standard Methods for the Examination of Water and Wastewater); USEPA, United States Environmental Protection Agency

3.3.6 Total Nitrogen (TN)

The concentration of TN is each sample is determined using the persulfate digestion method of HACH Method 10071. Total Nitrogen Persulfate Reagent Powder Pillow is added into Total Nitrogen Hydroxide Reagent vial consisting of 2mL of diluted sample. The vial is placed into the DRB 200 reactor for digestion purposes for 30 minutes. TN Reagent A powder pillow is then added into the vial that has been cooled at room temperature. The vial is allowed to stand for 3 minutes. Subsequently, TN Reagent B powder pillow is added into the digestion vial and a 2-minute reaction timer is initiated. 2mL of the digested treated prepared sample is pipetted into TN Reagent C vial and is allowed to stand for 5 minutes. It is then used to determine the nitrogen content using the HACH DR 2800 Spectrophotometer.

3.3.7 Total Phosphorus (TP)

The phosphorus content in each sample is determined using the molybdovanadate method with acid persulfate digestion. 5mL of the sample is added into a Total Phosphorus Test 'N Tube Vial. Next, Potassium Persulfate Powder Pillow is added into the vial. It is then placed into the HACH DRB 200 Reactor for 30 minutes at 105°C. 2mL of 1.54N of sodium hydroxide and 0.5mL of Molybdovanadate Reagent are added into the digested cool vial. The vial is left for 7 minutes prior to being analysed by HACH DR 2800 Spectrophotometer.

3.3.8 Volatile Fatty Acid (VFA)

The VFA content in each sample is measured using the esterification method of HACH 8196. Initially, 0.5mL of filtered sample is placed into a dry 25mL sample cell. 1.5mL of ethylene glycol and 0.2mL of 19.2N Sulphuric acid standard solution are then added into the sample cell. The mixture is swirled and placed into boiling water bath for three minutes. 0.5mL of hydroxylamine hydrochloride solution is added into the cool solution followed by 2mL of 4.5N sodium hydroxide solution, 10mL of ferric chloride sulphuric acid and 10mL of distilled water. The sample cell is left for 3 minutes prior to transferring to a dry 10mL sample cell. It is then analysed using the HACH DR 2800 Spectrophotometer.

3.3.9 Lignin

The lignin content in each sample is determined using the tyrosine method of HACH 8193. 25mL of sample is pipetted into 50mL of beaker consisting of 0.5mL of TanniVer^{TM} 3 Tannin-Lignin Reagent and 5mL of sodium carbonate solution. The solution is mixed and left for 25 minutes for reaction purposes. 10mL of the reacted sample is then poured into a 10mL square cell to be analysed using the HACH DR 2800 Spectrophotometer.

3.3.10 Oil and Grease (O&G)

The analysis of O&G is carried out according to USEPA Method 1664, n-Hexane gravimetric method. Initially, an empty petri dish with filter paper is weighed. 10mL of the sample is pipetted into the petri dish and the

wet weight is determined. It is then placed on top of a water bath (Daihan Scientific, Japan) for the removal of moisture. The dried sample is placed into an oven (Memmert, Germany) for 1 hour at 103°C. The sample is then allowed to cool at room temperature prior to inserting the sample into extraction thimble (Whatman, United Kingdom). 400mL of n-hexane is poured into a weighed Florentine flask. The Soxhlet extractor is turned on for at least 8 hours until no more yellow solution is extracted into the Florentine flask. The flask is then allowed to cool down before being installed into a rotary evaporator (Buchi, Switzerland). The water bath is set at 80°C to allow the removal of excess n-hexane. The dried Florentine flask is then placed into an oven (Memmert, Germany) for 1 hour at 60°C to drive off any residual n-hexane. The cooled Florentine flask is weighed again to determine the weight increase of the Florentine flask, where it is represented by the O&G content in the sample.

3.3.11 Total Alkalinity (TA)

TA is determined according to the Standard Methods using the titration method of APHA 2320 B. 5N of sulphuric acid is added to 20mL of sample in a measured amount until the final pH of the effluent becomes 4.3.

3.4 Comparison of different industrial scale palm oil mill effluent (POME) anaerobic systems in degradation of organic contaminants and kinetic performance

Three different palm oil mills were selected to compare their overall performance of the POME treatment. The operation parameters used by these palm oil mills will act as a reference for determining the optimised operational parameters for the pilot-scale IAAB.

3.4.1 Site Location and Process

Three sampling sites were selected among the palm oil mills in Malaysia for on-site analysis and POME samples collection. The three identified sites chosen in this study are denoted as: (1) Palm Oil Mill A, (2) Palm Oil Mill B and (3) Palm Oil Mill C. The deployed POME treatment methods are conventional open ponding without covered lagoon (control), added covered lagoon with biogas mixing and covered lagoon with hydraulic mixing, respectively (Figure 3.2).

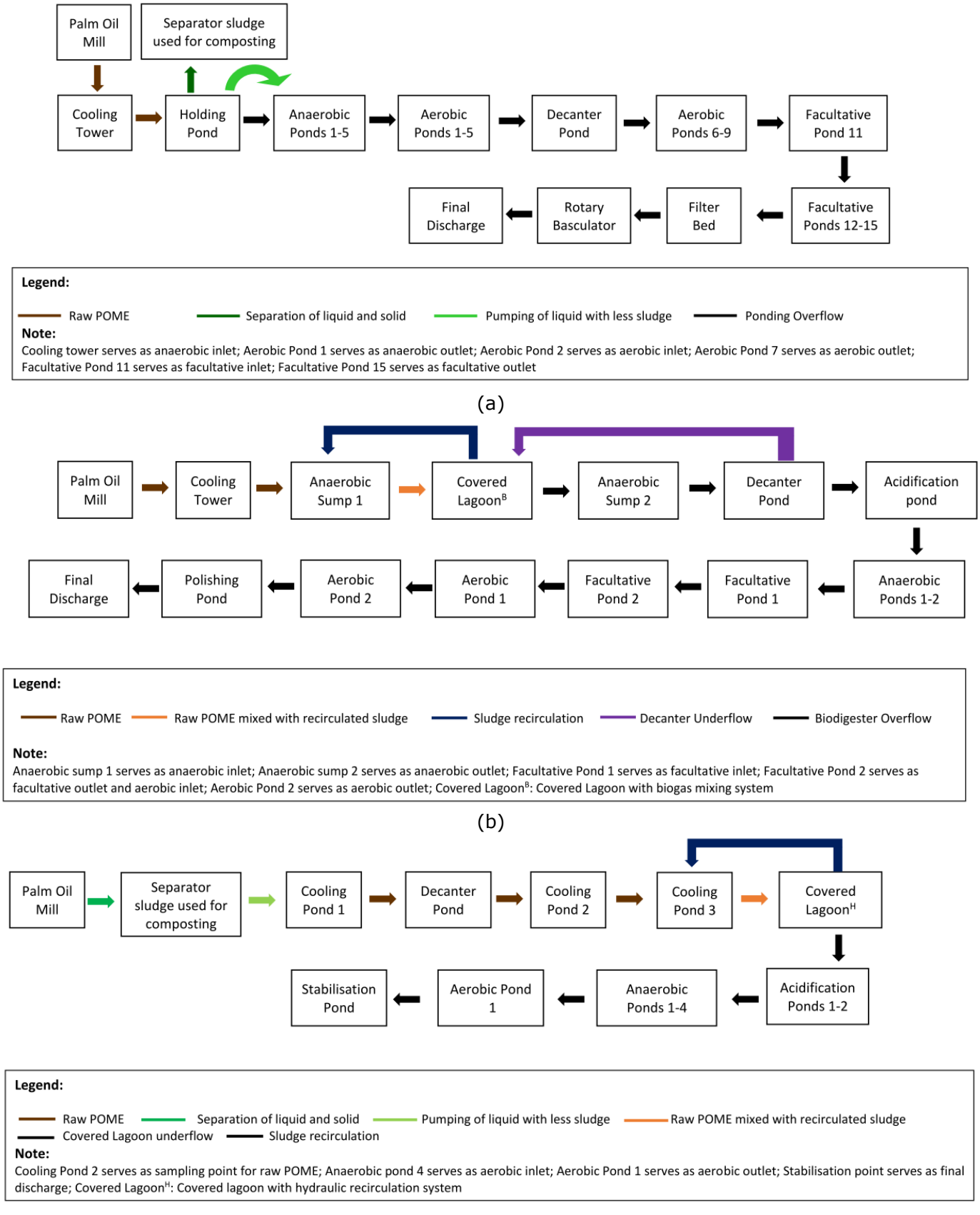


Figure 3.2: Process flow diagram (PFD) of POME treatment for: (a) conventional open ponding, (b) added covered lagoon with biogas mixing and (c) covered lagoon with hydraulic mixing

Figure 3.2(a) shows the process flow of a conventional open ponding system at a mill capacity of 60 tonnes fresh fruit bunches processed per hour. The discharged raw POME at 80°C is pumped into a cooling tower to reduce temperature to 40°C. The cooled POME is transferred to a holding tank that contains a rotary basculator to retain the solids to be used later as fertiliser while the filtrate is discharged into the first anaerobic pond. The POME is treated in series by overflowing through 5 ponds containing anaerobic microbial cells for a HRT of 45 days. Then, the anaerobically-treated effluent overflows into 9 aerobic ponds with a decanter pond located between the 5th and 6th aerobic pond at a HRT of 126 days. However, all the aerobic ponds do not have an aeration system and the bacteria sludge concentration is not monitored and controlled. The aerobically-treated effluent overflows into 5 facultative ponds equipped with surface aerators. The treated effluent from the decanter pond will flow into aerobic ponds 6-9 followed by facultative ponds, where the effluent will be retained for 14 days in the latter pond before being discharged into a river. Prior to that, the treated effluent will pass through a polishing pond that comprises a filter bed and rotary basculator. The filter bed will retain the untreated solid content before going through the rotary basculator. The solid that cannot be trapped by the filter bed will be trapped by the rotary basculator prior to discharging the POME. The dimensions of each anaerobic and aerobic pond are 43.3m × 19.5m × 6m and 43.3m × 10.36m × 6m (length × width × depth), respectively. The dimensions for facultative and polishing pond are 70.1m × 7.7m × 6m and 45.7m × 7.6m × 6m (length × width × depth), respectively. There are 15 ponds in total with a land area of 67,831.27 m^2.

Figure 3.2(b) shows similar POME treatment with an added geomembrane covered lagoon to capture the released biogas during anaerobic process, and used as a mixing device to replace the existing anaerobic ponds. In this system, the raw POME is mixed with recycled sludge from the covered lagoon for pH adjustment and further temperature reduction. A diffuser is installed in the covered lagoon, and biogas is used to circulate the substrate mixture. The decanter pond prior to the facultative ponds is used to retain and recycle the sludge into the covered lagoon so that the desired bacteria concentration can be maintained. The HRT of each anaerobic, facultative and aerobic pond are 22, 19 and 23 days, respectively. The aerobically-treated effluent will be retained in the polishing pond for 19 days before being discharged into a river. The volumes for each of the anaerobic, facultative and aerobic pond are 32,000 m^3, 18,750 m^3 and 23,580 m^3, respectively. All in all, the treatment has 7 ponds and a covered lagoon in a land area of 218,600 m^2.

Figure 3.2(c) shows process flow of POME treatment that uses covered lagoon as a pre-treatment step prior to open ponding system to treat POME. The raw POME undergoes filtration for solids removal in the sludge. The filtered raw POME is pumped into a cooling pond for temperature reduction. It is then pumped into a decanter pond, where oil is removed before being cooled further. The installation of hydraulic recirculation system in the covered lagoon allows constant mixing of anaerobically-treated POME with the incoming fresh POME produced from the palm oil mill, to enhance the activity of the microbial cells.

The ponding system comprises two acidification, four anaerobic, one aerobic and one stabilisation ponds. Subsequently, the anaerobically-treated POME is pumped through 2 acidification ponds with HRT of six days each. The anaerobically-treated POME from the acidification ponds will flow into four anaerobic ponds with a total HRT of 120 days. Later, the treated POME overflows into an aerobic pond with a HRT of 15 days without the use of aerator system. Successively, the aerobically-treated effluent is pumped into the stabilisation pond that has an aeration system with a HRT of two days. A continuous supply of oxygen for the microbial cells in the pond further enhances breakdown of organic components. In addition, the stabilisation pond that also acts as a final discharge outlet aids in holding the non-degraded solid content before discharged POME into a river. The dimensions of anaerobic, aerobic and stabilisation ponds are 170m x 30m x 5m, 110m x 30m x 5m and 75m x 30m x 3m (length x width x depth), respectively. There are 8 ponds and a covered lagoon with the land area of 130,050 m^2.

In order to evaluate the POME performances by the three selected palm oil mills, a comprehensive characterization study of raw POME and several effluent samples from different sampling points was conducted. The sampling points, sampling duration and sampling date for each of the palm oil mill are summarised in Table 3.3.

Table 3.3: The sampling date, sampling duration and sampling points for respective palm oil mill

Characteristics	Palm Oil Mill		
	A	B	C
Sampling Date	12/08/2015-14/08/2015	07/09/2015-09/09/2015	04/06/2016-01/07/2016
Sampling Duration	Hourly	Hourly	Daily
Sampling Points	• POME Holding pond • Cooling Tower • Aerobic Pond 1 • Aerobic Pond 2 • Aerobic Pond 7 • Facultative Pond 11 • Facultative Pond 15 • Final Discharge	• Cooling Tower • Anaerobic Sump 1 • Anaerobic Sump 2 • Facultative Pond 1 • Facultative Pond 2 • Aerobic Pond 2 • Final Discharge	• Cooling Pond 1 • Cooling Pond 2 • Anaerobic Sump 2 • Anaerobic Pond 4 • Aerobic Pond 1 • Stabilisation Pond

3.4.2 Sample Collection and Parameter Analysis

POME samples from various sampling points (Table 3.3) of the first two sites - open ponding system without covered lagoon and added covered lagoon with biogas mixing - were collected hourly for three consecutive days. For the covered lagoon with hydraulic mixing, samples from various sampling points were collected daily starting April till June 2016. At every one-hour interval, 500mL grab sample was collected from a sampling point for on-site analysis. Composite sample from a sampling point was formed by combing 100mL of all the grab samples collected over one day and then stored in a polyethylene sampling container for off-site analysis. All samples were preserved at temperature < 4°C (but above its freezing point) to prevent

POME from undergoing further biodegradation by the present microbial communities. Table 3.4 shows the list of parameters and test methods for characterisation of POME while the details of methodologies used for each of the analysis are listed in Section 3.4.3.

Table 3.4: The on- and off-site parameters used in three palm oil mills

On-Site Parameters	Off-Site Parameters
pH	COD
Temperature	BOD
Flow Rate	TSS
Weather	VSS
Sample Texture	Lignin
	TP
	TN
	O&G
	VFA

3.4.3 Analytical Method

Temperature and pH were determined by using a pH meter (HACH Sension1, Loveland, CO) with a temperature probe. The volumetric flow rates, Q_{POME}, for Palm oil mill A and Palm oil mill B were determined by measuring the time taken for POME to fill up a 1-L bucket as the POME produced from both the mills were discharged into an open drainage system. The time taken was recorded and repeated 5 times to obtain an average. The Q_{POME} for palm oil mill C could not be measured directly due to the closed piping system; hence an estimation was made on the basis of daily FFB processed based on Eq. 3.1. Lastly, the weather during sample collection time was observed and recorded. Any physical change in the grab sample was recorded.

$$Q_{POME} = (POME{:}FFB) \times FFB \quad (3.1)$$

Where Q_{POME} is the volumetric flow rate of POME processed each day (m^3/hour), FFB is the amount of fresh fruit bunches processed (t/day) and ratio of POME to FFB (POME:FFB) equals 0.6. All collected composite samples were subjected to off-site analysis as shown in Table 3.4. The method used to measure the COD, BOD, TSS, VSS, TN, TP, VFA, lignin, and O&G were summarised in Sections 3.3.1 to 3.3.10.

3.4.4 Performance Study

The performance of the mills for each process was evaluated based on Eqs. 3.2 to 3.6 for removal efficiency of each contaminant (BOD, COD, TSS, lignin, Q&G) considered in this study (Table 3.5). Eq. 3.7 determines the organic loading rate (OLR) while Eq. 3.8 measures the HRT of POME during anaerobic digestion.

Table 3.5: The parameters monitored in this study

No	Symbol	Unit	Description	Equation	Eq. No
1	$COD_{removal}$	%	COD removal efficiency	$\frac{COD_{in} - COD_{out}}{COD_{in}} \times 100$	3.2
2	$BOD_{removal}$	%	BOD removal efficiency	$\frac{BOD_{in} - BOD_{out}}{BOD_{in}} \times 100$	3.3
3	$TSS_{removal}$	%	TSS removal efficiency	$\frac{TSS_{in} - TSS_{out}}{TSS_{in}} \times 100$	3.4
4	$Lignin_{removal}$	%	Lignin removal efficiency	$\frac{Lignin_{in} - Lignin_{out}}{Lignin_{in}} \times 100$	3.5
5	$O\&G_{removal}$	%	O&G removal efficiency	$\frac{O\&G_{in} - O\&G_{out}}{O\&G_{in}} \times 100$	3.6
6	OLR_{an}	gCOD/L.day	OLR for anaerobic process	$\frac{(Q_{in}).(COD_{in})}{V_{an}}$	3.7
7	HRT_{an}	Day	HRT for anaerobic process	$\frac{V_{an}}{Q_{in}}$	3.8

COD_{in}: Influent COD concentration (mg/L); COD_{out}: Treated effluent COD concentration (mg/L); BOD_{in}: Influent BOD concentration (mg/L); BOD_{out}: Treated effluent BOD concentration (mg/L); TSS_{in}: Influent TSS concentration (mg/L); TSS_{out}: Treated effluent TSS concentration (mg/L); $Lignin_{in}$: Influent lignin concentration (mg/L); $Lignin_{out}$: Treated effluent lignin concentration (mg/L); $O\&G_{in}$: Influent O&G concentration (mg/L); $O\&G_{out}$: Treated effluent O&G concentration; Q_{in}: influent flow rate (L/day); V_{an}: volume of the anaerobic compartment (L)

3.5 Co-Digestion of POME with *Moringa Oleifera* (Laboratory Scale)

In order to obtain a set of optimised operating parameters for the digestion of *M. oleifera* with POME, a jar test is used to evaluate the performance of different concentration of *M. oleifera* to treat raw POME.

3.5.1 Wastewater Preparation and Preservation

POME and anaerobic seed sludge were collected from Havys Oil Mill, Mukim Bera, Pahang. The characteristics of the POME are determined by analyses, such as pH, TS, TSS and COD. In order to prevent the collected influent from undergoing biodegradation due to the action of the microbial cells, it was preserved at temperature of less than 4°C, but above its freezing point. The required volume is thawed at room temperature of 28°C before use.

3.5.2 *Moringa Oleifera* Extract Preparation and Preservation

The matured and dried seed pods of *M. oleifera* seeds were obtained from Serdang, Malaysia (Figure 3.3a). The seed wings and coats from the pods were removed manually and older seeds (Figure 3.3b) were selected and ground to fine powders using Retsch Rotor Beater and Ultra Centrifugal Mills to a size of 0.08 µM. Crude *M. oleifera* water soluble extract was prepared by blending 2 g of finely ground *M. oleifera* in 200 mL of distilled water for 30 seconds to extract the active ingredient. The resulting suspension was filtered through a muslin cloth and the filtrate made up to 500 mL to give a stock solution of 10,000 mg/L. Different concentrations of *M. oleifera* extract, ranging from 2 to 1,000 mg/L, were prepared by diluting the extract with distilled water and were used in subsequent experiments. According to Jahn (1986), the coagulation efficiency of the *Moringa Oleifera* will be affected if the stock solution was stored for more than two days at room temperature. Hence, the stock solution was prepared fresh as and when needed to be used.

3.5.3 Jar Test

A series of jar test of different dosages of *M. oleifera* extract will be conducted to determine the optimum dosage based on the dependent parameters of suspended solid and organic matter removal efficiencies. Additionally, pH and temperature of the wastewater before and after the addition of the various dosages of *M. oleifera* extract will be determined. Jar test was performed using 300 mL of raw POME and 50 mL of *M. oleifera* extract of different concentrations (Table 3.6) in a 500-mL beaker. The mixture in each beaker was allowed to coagulate uniformly at 150 rpm for 5 mins (rapid mixing) and then followed by 30 rpm for 20 mins (slow mixing) (as discussed in Section 2.13.3) without any pH adjustment under a flocculator (PUDAK Scientific, Indonesia). The mixture was then left to settle for 2 hours and the supernatant at the top layer removed for further analysis such as COD, O&G and TSS. The optimum concentration of *M. oleifera* extract for co-digestion was identified based on the highest reduction rates of COD, O&G and TSS.



(a)



(b)

Figure 3.3: (a) *Moringa Oleifera* b) *Moringa Oleifera* Dried Seeds

Table 3.6: Concentration of M. oleifera extract prepared from the stock solution (10,000 mg/L)

Label	Sample Concentration (mg/L)	Dilution Factor
A	1,000	10
B	500	20
C	250	40
D	125	80
E	36	280
F	18	556
G	16	625
H	8	1,250
I	4	2,500
J	2	5,000

3.5.4 Anaerobic Digestion (AD)

The AD was conducted in a 500-mL conical flask closed system with an effective volume of 450 mL. The organic loading rate (OLR) for the AD was maintained at 5.0 gCOD/L.day by feeding daily a predetermined amount of raw POME or *M. oleifera* extract with a known COD concentration. In order to maintain high biomass activity, anaerobic sludge with a final mixed liquor volatile suspended solid (MLVSS) concentration of 40,000 mg/L was maintained in the conical flask during the start-up. The conical flask was connected to another conical flask containing 0.2 N NaOH which was then connected to a water displacement setup via a rubber tubing. CO_2 formed during AD was absorbed by NaOH to concentrate the methane. Biogas released was thus concentrated and flowed via the rubber tubing (Figure 3.4). The NaOH in the conical flask was changed at every 3-day interval due to turbidity of the solution caused by CO_2 absorption. Anaerobically-treated raw POME was collected daily by displacing an equal volume to the feed, allowed to settle down for 30 mins before supernatant collected for analysis of BOD, COD, TSS and O&G. This experiment was conducted for a period of 30 days.

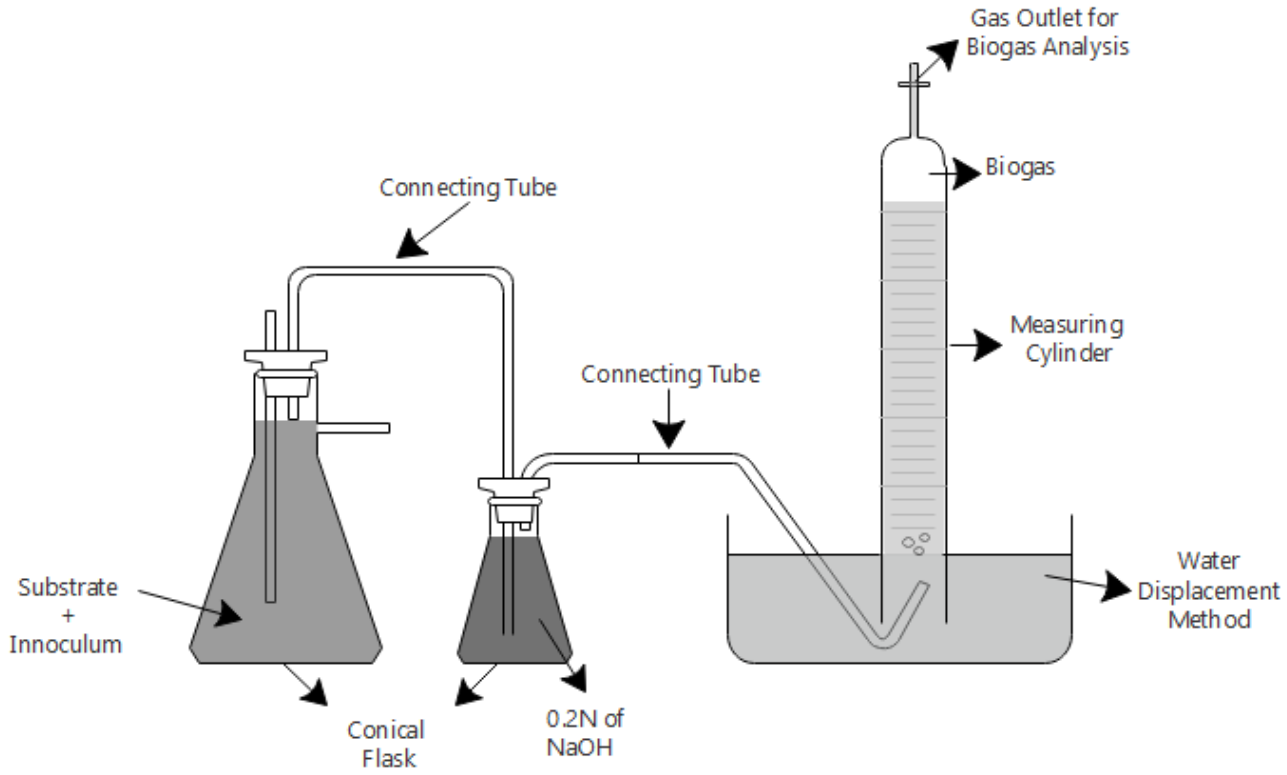


Figure 3.4: Setup of anaerobic digestion

Mono-digestion in this study refers to AD of a single substrate. Two mono-digestion setups, labelled T1 (raw POME alone) and T2 (*M. oleifera* extract alone), were used as controls. The co-digestion was carried out as in T3 and T4 as shown in Table 3.8. For T3, 50 mL of *M. oleifera* extract with the optimum concentration determined in jar test was added daily into the 500-mL conical flask (daily feed). For T4, 50 mL of the *M. oleifera* extract with the same concentration was added into the 500-mL conical flask only once on day 1 of the study (one-time feed). Two different assays were carried out to compare the effectiveness of adding *M. oleifera* extract at different mode for the co-digestion study. All experimental runs had a similar setup as in Figure 3.3 and all collected samples were subjected to the analysis of COD, TSS, O&G and BOD.

Table 3.8: The treatment condition involved in this study

No	Run	Function	Mode	Treatment condition
1	T1	Mono-digestion[c]	NA	Raw POME + Anaerobic Sludge
2	T2			*M. oleifera* extract + Anaerobic Sludge
3	T3[a]	Co-digestion	One-time feed (day 1)	Raw POME + Anaerobic Sludge+ *M. oleifera* extract
4	T4[b]		Daily Feed	Raw POME + Anaerobic Sludge+ *M. oleifera* extract

[a]50 mL of *M. oleifera* extract is added daily; [b]50 mL of *M. oleifera* extract is added during day 1 of the study; [c]digestion with POME or *M. oleifera* extract alone as specified as control.

3.5.5 Analysis

The performance of the laboratory scale co-digestion of POME with *M. oleifera* extract was monitored and analysed in terms of the BOD, COD, O&G, and TSS. All analyses on COD, BOD, TSS, and O&G were measured in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA 1992). The methods used to determine the COD, BOD, TSS, and O&G were summarised in Section 3.31, Section 3.32, Section 3.3.4, and Section 3.3.10, respectively. A water displacement system was used to determine the methane productivity during AD period. The $COD_{removal}$, $BOD_{removal}$, $TSS_{removal}$, $O\&G_{removal}$, and methane yield of each treatment were calculated based on formulas in Table 3.9.

Table 3.9: The parameters monitored in the present study

No	Symbol	Description	Unit	Formula	Eq No.
1	$COD_{removal}$	COD removal efficiency	%	$\frac{COD_{in} - COD_{out}}{COD_{in}} \times 100$	3.9
2	$BOD_{removal}$	BOD removal efficiency	%	$\frac{BOD_{in} - BOD_{out}}{BOD_{in}} \times 100$	3.10
3	$TSS_{removal}$	TSS removal efficiency	%	$\frac{TSS_{in} - TSS_{out}}{TSS_{in}} \times 100$	3.11
4	$O\&G_{removal}$	O&G removal efficiency	%	$\frac{O\&G_{in} - O\&G_{out}}{O\&G_{in}} \times 100$	3.12
4	Methane Yield	Methane Yield	mL $CH_4/gCOD_{removed}$	$\frac{Cumulative\ volume\ of\ CH_4}{COD_{in} - COD_{out,an}}$	3.13

3.5.6 Identification of Methanogen in Anaerobic Digester

As discussed in Section 2.14.2, PCR-based techniques and gel extraction of DNA were adopted in this research to investigate naturally occurring methanogen populations (Figure 3.5). The daily collected samples (50 mL) from Section 3.5.4 were stored in a 50-mL falcon tube at -20°C to prevent the collected samples from undergoing biodegradation by microbial cells. The samples were thawed at room temperature before used.

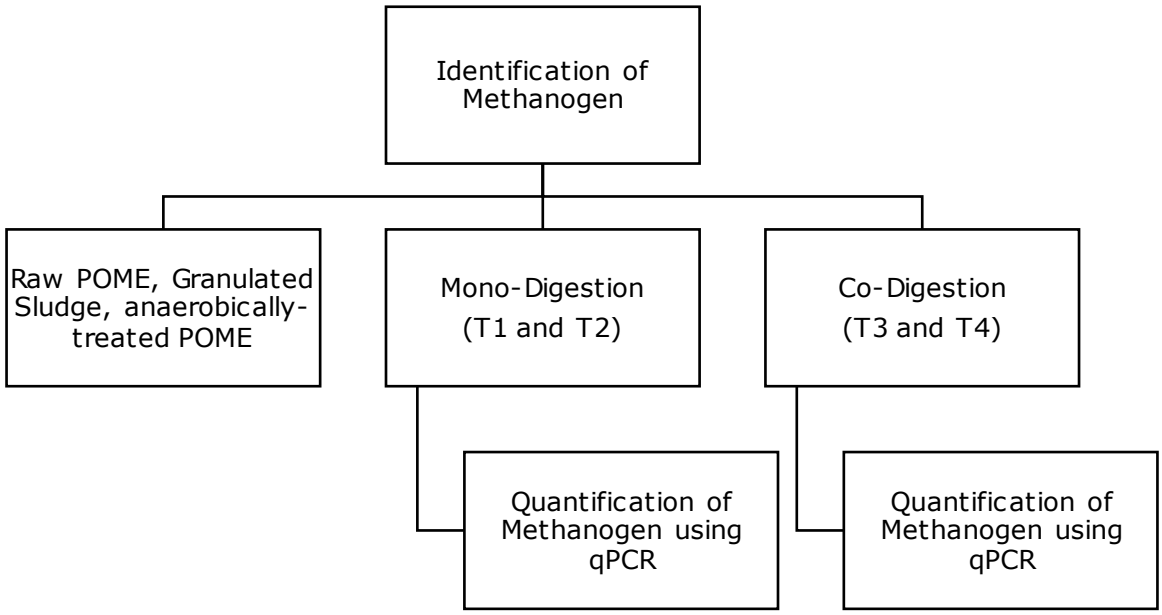


Figure 3.5: Experimental flowchart for identification and quantification of methanogen in this study

3.5.6.1 DNA Extraction

DNA was extracted using DNeasy PowerSoil Kit (Qiagen, Germany) from anaerobically-treated samples collected from T1, T2, T3, and T4. Prior to DNA extraction, all samples were centrifuged at 10,000 rpm for 20 mins at 4°C to remove supernatant. Total DNA from POME was used throughout this study as DNA standard. The semi-solid sludge remaining in the digester was used for DNA extraction. The final elution of 50 µL was used to determine DNA concentration and purity using Epoch Microplate Spectrophotometer (BioTek, USA) and software Gen5-Take3 (Biotek, USA). The quantity of DNA was measured in ng μL^{-1} by the ratio of absorbance value at 260/280 nm. The reading of A260/280 ratio of approximately 1.8 to 2.0 was considered acceptable quality (purity).

3.5.6.2 DNA Sequencing

Prior to DNA sequencing, polymerase chain reaction (PCR) was conducted using Mastercycler Nexus Gradiet (Eppendorf). PCR amplified DNA fragment specific for methanogen using primers METHF and METHR (Table 3.10). Nuclease free water was used as a template for negative control.

Table 3.10: Primers used for PCR Optimisation

Primers	Forward(F)/Reverse(R)	Sequence	Source of primer
METHF	F	5'-CCC TAC GGG GCG CAG CAG-3'	Watanabe *et al*., 2004
METHR	R	5'-GGA TTA CAR GAT TTC AC-3'	Watanabe *et al*., 2004

PCR protocol for METH primers were 94°C for 3 mins followed by 40 cycles of initial denaturation at 94°C for 1 min, 52°C for 45 secs and 72°C for 1 min 30 secs of annealing and elongation of the targeted gene. Final extension was carried out at 72°C for 5 mins and ended on hold at 4°C. PCR products were separated in 1.5% (w/v) agarose gel (1st Base) prepared with a 0.5X Tris-Borate EDTA (TBE) buffer and 1X SYBR™ Safe DNA Gel Stain (Invitrogen). 5µl of KAPA Universal Ladder (KAPA Biosystems) was used as a DNA ladder. Gel electrophoresis was run at 80 Volts (V) for an hour in a submerged horizontal electrophoresis system (Wide Mini-Sub® Cell GT, Biorad). Agarose gel products were visualized and recorded using ChemiDoc™ MP Imaging Syetem with Image Lab™ Software (Biorad).

DNA was recovered from agarose gel using GF-1 Gel DNA Recovery Kit (Vivantis) and was sent for Sanger sequencing (First Base Asia, Serdang). DNA sequenced was analysed using the Sequence Scanner 2.0 software (Thermo Fisher) and determined for similarity using Basic Local Alignment Search Tool (BLAST) and GENBANK software tools to obtain the sample identification.

3.5.7 Quantification of Methanogen using Real Time Quantitative Polymerase Chain Reaction (qPCR)

3.5.7.1 Absolute Quantification of Methanogen

DNA samples from Section 3.6.1 were diluted 10-fold with nuclease free water to ensure that the final concentration of DNA templates in qPCR amplification was between 15-20 ng/µL. A standard curve of 10-fold dilution series (1×10^{1}, 1×10^{0}, 1×10^{-1}, 1×10^{-2}, and 1×10^{-3}) of methanogen DNA was prepared in 100 µL. A volume of 90 µL of nuclease free water was aliquoted into each dilution to prepare a final volume of 100 µL.

qPCR amplification was conducted using Eco 48-well plates (Illumina) in a total volume of 20 µL using an Eco Real-Time PCR System (Illumina). Each reaction mixture consisted of 10 µL of 1X KAPA SYBR FAST qPCR Kit Master Mix (2X) Universal (Kapa Biosystems), 0.4 µL of METHF and METHR primers each, 2 µL of diluted sample DNA template and 7.2 µL of nuclease free water. Non-template control (NTC) reactions contained the same mixtures, except 2 µL of nuclease free water was used to replace the diluted sample DNA templates. Reaction mixtures were pipetted into Eco 48-well plates with the support of Eco loading dock (Illumina) and sealed with Eco adhesive seals (Illumina). The set up was centrifuged (Eppendorf, 5810R) at 2,500 rpm, 4°C for 3 mins before running the Eco Real-Time polymerase chain reaction (PCR) System.

Thermal cycling conditions for DNA template amplifications were 95°C for 3 mins, followed by 40 cycles at 95°C for 5 secs and 60°C for 30 secs to detect and quantify the fluorescence at a temperature above the denaturation temperature of primer-dimers.

A melting curve temperature profile was programmed for one cycle at 95°C for 15 secs, 55°C for 15 secs and 95°C for 15 secs. Technical repeats were carried out with four individual reactions for each biological samples of samples DNA templates respectively. C_q values were calculated by Eco Real-Time PCR v5.0 software (Illumina) to show the significant fluorescence signals that rise above the threshold level during early cycles of the exponential phase of qPCR amplifications. A standard curve was obtained by plotting the C_q value versus logarithm of the concentration of each 10-fold dilution series of methanogen DNA. The relationship between C_q and DNA concentration was determined by correlation analysis. Total amount of *Methanogen* spp. DNA was quantified by comparing C_q values to the crossing point values of the linear regression line of standard curve and was expressed as methanogen. DNA equivalent in mg/g. The standard curve was generated by Eco Real-Time PCR v5.0 software (Illumina) using the C_q values of the DNA extracted from the POME samples as described in Section 3.6.1.

In order to verify that single qPCR product was amplified, the reactions were analysed by 1.5% (w/v) agarose gel electrophoresis using 0.5X TBE buffer at 80 V (Wide Mini-Sub® Cell GT, Biorad) for 1 hour. DNA bands on the agarose gel were visualised and recorded using ChemiDoc™ MP Imaging System with Image Lab™ Software (Biorad).

3.5.8 Statistical Analysis

All data were analysed statistically by one-way analysis of variance (ANOVA) using GraphPad Prism software version 5.02. Significant differences among the treatments at $P < 0.05$ were determined by Turkey multiple comparison tests using Prism software.

3.6 Pilot Plant Integrated Anaerobic-Aerobic Bioreactor (IAAB) Study

3.6.1 Wastewater Preparation and Preservation

POME was obtained from Havys Oil Mill, Mukim Bera, Pahang, Malaysia and their characteristics were presented in Table 3.11. POME was collected on a weekly basis and was kept at room temperature (28°C) before feeding into the anaerobic compartment.

Table 3.11: The characteristics of POME

Parameter	Units	Average	Range
pH	-	3.90 ± 0.34	3.30-4.80
BOD	mg/L	73,412 ± 10,058	63,354-83,470
COD	mg/L	146,824 ± 24,268	75,000-200,000
TSS	mg/L	29,138 ± 8,860	8,300-58,000
TN	mg/L	1,126 ± 123	1,020-1,375
TP	mg/L	455 ± 146	220-794
VFA	mg/L	11,874 ± 5,358	7,140-20,915
O&G	mg/L	4,633 ± 488	4,000-5,874

3.6.2 Operation of the Pilot-Scale IAAB

Figures 3.6 and 3.7 show the actual view and process flow diagram (PFD) of the pilot scale IAAB. POME as the substrate for this system is filtered in feed tank 1 (T101) before pumping into feed tank 2 (T102) with the aid of influent feed pump, P101 (Pompe Dosatrici, Italy). The filtered POME from feed tank 2 is pumped into the bottom of the anaerobic compartment using a diaphragm feed pump (P102) (Pompe Dosatrici, Italy). The wastewater is recirculated from W1 to the bottom of the anaerobic compartment, R1, to allow equal distribution of substrate to the sludge bed. It is done by using a mechanical diaphragm pump (Pompe Dosatrici, Italy) designated as P103. There are six sampling points (SP1-SP6) along the anaerobic compartment to facilitate the sampling of sludges at various height of the anaerobic compartment. A biogas analyser (Biogas 5000, Geotech, UK) is used to measure the biogas concentration via the designated gas sampling port (HV 16). The Gas-Liquid-Solid Separator (GLSS) is connected to a water displacement system to allow the measurement of volume of biogas.

The wastewater flow upwards and overflow into the aerobic compartment for further breakdown. Air is supplied into the aerobic compartment via a membrane diffuser, which is connected directly into an air compressor (Swan Air Compressors, Taiwan). The aerobically-treated effluent will overflow into the settling tank for further separation of sludge. This effluent containing high concentrations of suspended solids will flow down the settling tank as directed by the baffle. As a result, there will be distinct separation of solid and liquid. The latter phase is released out the sampling point of discharge 1 as final discharge effluent. Sludge blanket is formed at the bottom of the settling tank due to the high volume of suspended solid coming directly from the aerobically-treated effluent.

A portion of the settled sludge is discharged from the bottom of the settling tank, W2. Additionally, the remaining sludge is recycled back into the bottom of the aerobic compartment, R2 with the aid of returned activated sludge pump, P104 (Pompe Dosatrici, Italy). Sludge sample from aerobic compartment is collected from SP7 (Figure 3.5) to determine the mixed liquor suspended solid (MLSS).



(a) (b)

Figure 3.6: Actual views of the pilot-scale IAAB: (a) Back view; (b) Front view

Legend
T101: Feed Tank 1 (200L)
T102: Feed Tank 2 (200L)
P101: Influent Pump for raw POME
P102: Feed Pump
P103: Recirculation Pump
P104: Returned activated sludge Pump
P105: Waste activated sludge Pump
SP: Sampling Point
R1: Anaerobic recirculation inlet point
W1: Anaerobic recirculation outlet point
R2: Returned activated sludge inlet point
W2: Returned activated sludge outlet point

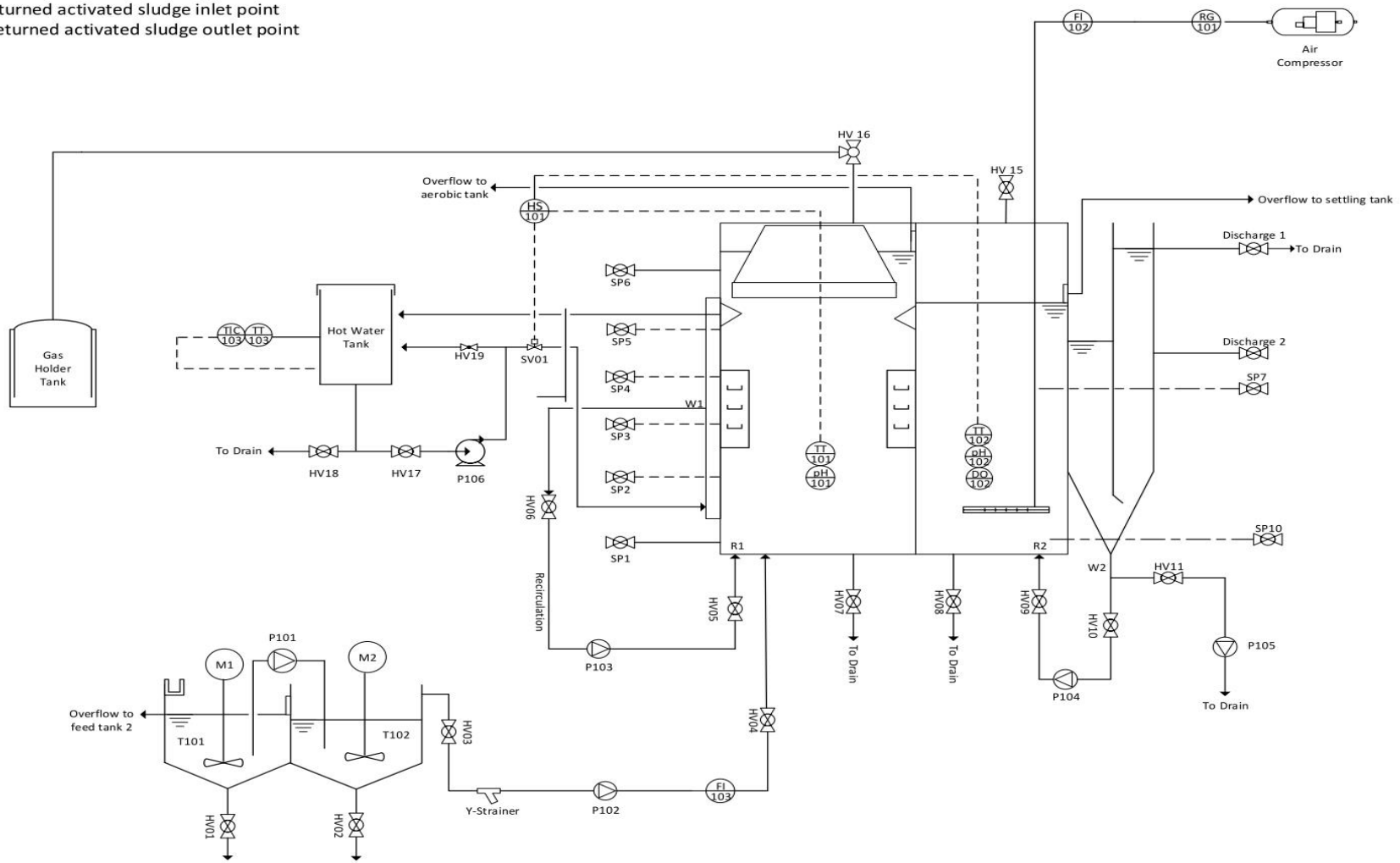


Figure 3.7: The PFD of the Pilot-Scale IAAB

3.6.3 Seed Sludge

Anaerobic sludge obtained from an anaerobic pond treating POME in Pahang, Malaysia was added into the anaerobic compartment. Acclimatised activated sludge obtained from an aerobic pond that treats anaerobically-treated POME in Klang, Malaysia was added into the aerobic compartment as well. The characteristics of the anaerobic and aerobic sludge are shown in Table 3.12.

Table 3.12: Quality of anaerobic and aerobic sludge inoculated into the pilot-scale IAAB

Parameter	Unit	Anaerobic Sludge		Aerobic Sludge	
		Average	Range	Average	Range
TSS	mg/L	81,279 ± 1,845	79,000-83,120	29,325 ± 540	28,000-29,860
VSS	mg/L	47,142 ± 958	46,000-48,000	24,926 ± 520	24,000-25,450
VSS/TSS	-	0.58 ± 0.03	0.55-0.62	0.85 ± 0.03	0.82-0.88

3.6.4 Performance of the Bioreactor and Stability Parameters

The performance of the pilot-scale IAAB system was monitored and analysed in terms of the $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, methane production rates, methane composition, and methane yields. The stability of the reactor was monitored as well by determining the pH, VFA concentration, TA, ratio of VFA:TA, mixed liquor volatile suspended solid (MLVSS) concentration, and F/M ratio. The main parameters involved in this study are shown in Table 3.13 together with their calculation methods.

Table 3.13: The parameters monitored in this study

No	Symbol	Unit	Description	Equation	Eq. No
1	OLR_{an}	gCOD/L.day	OLR for anaerobic process	$\frac{Q_{in}.COD_{in}}{V_{an}}$	3.14
2	OLR_a	gCOD/L.day	OLR for aerobic process	$\frac{Q_{in}.COD_{out,an}}{V_{an}}$	3.15
3	$COD_{removal}$	%	Overall COD removal efficiency	$\left(\frac{COD_{in}-COD_{out}}{COD_{in}}\right)\times 100$	3.16
4	$BOD_{removal}$	%	Overall BOD removal efficiency	$\left(\frac{BOD_{in}-BOD_{out}}{BOD_{in}}\right)\times 100$	3.17
5	$TSS_{removal}$	%	Overall TSS removal efficiency	$\left(\frac{TSS_{in}-TSS_{out}}{TSS_{in}}\right)\times 100$	3.18
6	$TP_{removal}$	%	Overall TP removal efficiency	$\left(\frac{TP_{in}-TP_{out}}{TP_{in}}\right)\times 100$	3.19
7	$TN_{removal}$	%	Overall TN removal efficiency	$\left(\frac{TN_{in}-TN_{out}}{TN_{in}}\right)\times 100$	3.20
8	$Lignin_{removal}$	%	Overall lignin removal efficiency	$\left(\frac{Lignin_{in}-Lignin_{out}}{Lignin_{in}}\right)\times 100$	3.21
9	HRT_{an}	Day	HRT for anaerobic process	$\frac{V_{an}}{Q_{in}}$	3.22
10	HRT_a	Day	HRT for aerobic process	$\frac{V_a}{Q_{in}}$	3.23

11	R	-	Recirculation ratio	$\frac{Q_r}{Q_{in}}$	3.24
12	R_{RAS}	-	Returned activated sludge ratio	$\frac{Q_{RAS}}{Q_{in}}$	3.25
13	Q_{in}	L/day	Influent Flow Rate	$\frac{OLR.V_{an}}{COD_{in}}$	3.26
14	$MLVSS_{an}$	mg/L	Biomass concentration in the anaerobic compartment	$\frac{\sum V_i.MLVSS_i}{V_{an}}$	3.27
15	rCH_4	LCH_4/L.day	Volumetric Methane production rate	$\frac{Q_{CH_4}}{V_{an}}$	3.28
16	Y_M	$LCH_4/gCOD_{removed}$	Methane Yield	$\frac{Q_{CH_4}}{Q_{in}(COD_{in}-COD_{out})}$	3.29
17	Q_{RAS}	L/day	Returned activated sludge flow rate	$\frac{Q_{in}.MLVSS_a}{MLVSS_{RAS}-MLVSS_a}$	3.30
18	Q_{WAS}	L/day	Waste activated sludge flow rate	$\frac{MLVSS_a.V_a}{MLVSS_{WAS}-SRT}$	3.31
19	F/M	gCOD/gMLVSS.day	Food to Microorganism Ratios	$\frac{(Q_{in})(COD_{in})}{(V_a)(MLVSS_a)}$	3.32

Where Q_{in} is the influent flow rate (L/day); V is the volume of the bioreactor (L); $COD_{out,an}$ is the COD concentration of the anaerobically treated POME (mg/L); $MLVSS_i$ is the biomass concentration at sampling port, I (mg/L); V_i is the volume of section i; COD_{in} is the influent COD concentration (mg/L); BOD_{in} is the influent BOD concentration (mg/L); BOD_{out} is the treated effluent BOD concentration (mg/L); TSS_{in} is the influent TSS concentration (mg/L); TSS_{out} is the treated effluent TSS concentration (mg/L); TP_{in} is the influent TP concentration (mg/L); TP_{out} is the treated effluent TP concentration (mg/L); TN_{in} is the influent TN concentration (mg/L); TN_{out} is the treated effluent TN concentration (mg/L); $Lignin_{in}$ is the influent lignin concentration (mg/L); $Lignin_{out}$ is the treated effluent lignin concentration (mg/L); QCH_4 is the methane production rate (L/day) ; SRT is the time taken for the activated sludge to be in the aerobic compartment (day); V_a is the volume of the aerobic compartment (L); and $MLVSS_a$ is the biomass concentration in the aerobic compartment (mg/L).

The COD_{in} is measured before determining the true Q_{in} by using Eq. (3.26). The anaerobic compartment is divided into six sectional volumes, where each compartment serves as a sampling point (Figure 3.7) to allow the estimation of the total biomass concentration in the anaerobic compartment, $MLVSS_{an}$, using Eq (3.27) (Borja and Banks, 1994; Chan et al., 2012). The production of biogas is determined using water displacement method with a 10L inverted water-filled cylinder for a collection period of 3 minutes, four times a day. The average value for the production of biogas of a particular day is calculated. Additionally, the volumetric methane production rate, rCH_4, is calculated by determining the biogas yield at standard room & temperature (STP) using ideal gas law and the effective volume of the anaerobic compartment [Eq (3.28)). The methane yield, Y_M, was also calculated by determining the flow rate of CH_4, (QCH_4) and the difference between influent COD and effluent COD (Eq (3.29)).

3.6.5 Bioreactor Start-up

The start-up of the pilot-scale IAAB is at mesophilic conditions at various OLRs. Throughout the start-up period, the performance of the pilot-scale IAAB was closely monitored in terms of $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, methane gas production and methane yields. Additionally, the stability of the bioreactor was assessed by determining its pH, VFA concentration, TA, ratio of VFA:TA, and TSS concentration. The start-up of pilot-scale IAAB was considered successful once 85% of $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$ were achieved at both the anaerobic and aerobic compartments for 3 consecutive days. Lastly, the gradual increase of $MLVSS_{an}$ (Eq (3.27)) and $MLVSS_a$ (MLVSS in aerobic compartment) were taken as one of the options for the successful start-up of pilot-scale IAAB at each stage.

3.6.5.1 Mesophilic Condition

The start-up of the pilot-scale IAAB was initiated at mesophilic conditions, where operation of the anaerobic compartment was initiated followed by aerobic and settling compartments provided that stability has been achieved at the anaerobic compartment.

3.6.5.2 Anaerobic Process

As summarised in Table 3.14, the start-up of anaerobic compartment is divided into five stages. Initially, ceramic ball was filled into the anaerobic compartment. 200L of anaerobic sludge, which represented 20% of the effective volume of the anaerobic compartment was added into the anaerobic compartment. Nitrogen gas was purged to replace the air inside the anaerobic compartment. This was aimed to displace oxygen in the compartment. As shown in Table 3.14, no feed was added into the anaerobic compartment of pilot-scale IAAB. The anaerobic sludge was allowed to stabilise for a period of 24 hours to ensure gradual adaption to ambient temperatures. Concurrently, the recirculation pump (P103) as shown in Figure 3.7 was initiated at a recirculation ratio (R) of 15 (Eq (3.24)) equivalent to recirculation flow rate, (Q_r) of 6,216 L/day to create homogeneous mixing in the anaerobic tank. On the second day, the anaerobic compartment was fed with POME various influent COD concentration of 2,985, 7,441, 14,925, and 24,461 mg/L at stage 2, 3,4, and 5 respectively (Table 3.14). This was aimed to reduce the impact of the organic loading towards the anaerobic microorganisms present in the anaerobic sludge. At each stage, the pilot-scale IAAB was closely monitored in terms of $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, rCH_4 (Eq. 3.28), Y_M (Eq. 3.29), VFA concentration, TA, and, ratio of VFA/TA. The OLR_{an} was increased by increasing the influent COD concentration when the $COD_{removal}$ remained relatively constant for three consecutive runs and the VFA concentration was less than 500 mg/L. The operation of the aerobic process begun when the anaerobic compartment had reached its steady state at stage 5 and the $COD_{removal}$ remained relatively constant at 85% for 3 consecutive days.

3.6.5.3 Aerobic Process

The required OLR_a (Eq. 3.15) during the start-up period was low to ensure gradual adaptation of the activated sludge to the integrated biological treatment system. The anaerobically-treated effluent was used as the substrate for the aerobic compartment due to the integration configuration of the bioreactor system. As a result, the OLR_a was influenced by the COD of the anaerobically-treated effluent. Prior to initiating the aerobic process, a total of 200L of acclimatised activated sludge was added into the aerobic compartment. The anaerobically-treated effluent overflowed into the aerobic compartment at a flow rate of 317 L/day. A period of 2-days was required to fill up the 600L tank and 1-day to fill up the 200L of settling tank. This shows that the final discharge effluent can be obtained after 3-days of operation. The dissolved oxygen (DO) concentration in the aerobic compartment was maintained at approximately 2 mg/L. The performance of the aerobic compartment during the start-up period was closely monitored by determining the $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, and MLSS concentration. The start-up of the aerobic compartment was considered stable once the effluent COD and TSS concentrations reached constant values with $COD_{removal}$ of at least 70% and the MLVSS concentration was increasing gradually.

Table 3.14: The start-up strategy of anaerobic and aerobic compartments of the pilot-scale IAAB

Operating Mode	Stage	Time (day)	Dilution Factor of POME	Influent COD (mg/L)	Influent Flow Rate (L/day)	Anaerobic Digestion Average OLR_{an} (gCOD/L.day)	Anaerobic Digestion HRT_{an}[a] (day)	Aerobic Digestion Average OLR_a[b] (gCOD/L.day)	Aerobic Digestion HRT_a[c] (day)
Start-up of anaerobic compartment	1	1	No Feed	No Feed	-	-	-	-	-
	2	2-6	29	2,985	360	1.0	2.78	-	-
	3	7-11	10	7,441	360	3.0	2.78	-	-
	4	12-16	5	14,925	317	5.0	3.15	-	-
	5	17-31	2	24,461	317	8.0	3.15	-	-
Start-up of aerobic compartment	6	17-31	2	24,461	317	-	-	0.82	1.99

[a]Calculated based on Eq.3.20; [b]Dependent on the COD concentration remaining in the anaerobically-treated effluent (Eq. 3.13); [c]Calculated based on Eq. 3.21

3.6.6 Performance and Kinetic Evaluation of the Pilot-Scale IAAB at various OLRs

After the successful accomplishment of the start-up of the pilot-scale IAAB, it was then operated at various OLR_{an} as shown in Table 3.15. The increase of OLR_{an} from 8.0 to 32.5 gCOD/L.day was achieved by increasing the influent flow rates from 299.58L/day in stage 1 to 419.58L/day in stage 8 at influent COD concentration ranging from 25,228 to 76,601 mg/L. It should be noted that the OLR_a was influenced by the anaerobically-treated POME COD and the corresponding OLR_a at each stage (Eq. 3.15) will be presented in Chapter 4 (Results and Discussion). The performance of the pilot-scale IAAB was monitored and analysed in terms of the overall anaerobic and aerobic $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, methane production rate, methane yield and methane composition at various OLRs. The stability of the reactor was determined based on pH, VFA concentration, TA, VFA:TA ratio, MLVSS concentration, and F/M ratio. Kinetic analysis by using Stover-Kincannon, Grau second-order multicomponent substrate removal, Monod, and Contois as discussed in Chapter Two (Literature Review), Section 2.9 would be carried out based on the values obtained from the stable performance stages. Table 3.16 depicted various kinetic models applied to both the anaerobic and aerobic processes of the pilot-scale IAAB.

Table 3.15: The schedule of the pilot-scale IAAB during the steady-state performance at various OLRs

Stage	Time (day)	Average Influent COD (mg/L)	Influent Flow Rate (L/day)	Anaerobic Digestion		Aerobic Digestion	
				OLR^{an} (gCOD/L.day)	HRT_{an} (day)	OLR_a* (gCOD/L.day)	HRT_a (day)
1	1-15	25,228	299.58	8.0	3.31	0.82	1.99
2	16-30	37,690	265.80	10.5	3.82	0.66	2.29
3	31-45	43,237	307.60	13.5	3.26	0.90	1.96
4	46-60	44,427	351.13	15.0	2.86	0.93	1.72
5	61-75	50,416	363.33	18.0	2.77	1.03	1.66
6	76-90	59,551	340.73	20.0	2.94	1.08	1.76
7	91-105	59,157	372.20	22.0	2.69	1.93	1.61
8	106-120	67,893	412.53	28.0	2.43	2.13	1.46
9	121-135	78,888	393.60	30.0	2.56	2.26	1.54
10	136-150	67,866	441.27	32.0	2.27	5.80	1.36
11	151-165	76,601	419.58	32.5	2.36	7.50	1.42

* It depends on the COD concentration of the anaerobically-treated effluent calculated using (Eq.3.13), as shown in Table 3.13

Table 3.16: Mathematical formula for different kinetic model

Kinetic Model	Equation	Eq No.
Monod	$\frac{COD_{in} - COD_{out,an}}{HRT_{an} \times X_{an}} = \frac{1}{Y}\left(\frac{1}{SRT}\right) + \frac{1}{Y}K_d$	3.33
	$\frac{SRT_{an}}{1 + (K_d \times SRT_{an})} = \left(\frac{K_S}{\mu_{max}}\right)\left(\frac{1}{COD_{out,an}}\right) + \frac{1}{\mu_{max}}$	3.34
Modified Stover-Kincannon	$\frac{V_{an}}{Q_{in}(COD_{in} - COD_{out,an})} = \frac{K_B}{\cup_{max}}\left(\frac{1}{OLR_{an}}\right) + \frac{1}{\cup_{max}}$	3.35
Grau second-order multicomponent substrate removal	$\frac{HRT_{an}}{E_{an}} = bHRT_{an} + a$	3.36
	$a = \frac{COD_{in}}{k_s \times X_{an}}$	3.37
Contois	$\frac{COD_{in} - COD_{out,an}}{HRT_{an} \times X_{an}} = \frac{1}{Y}\left(\frac{1}{SRT}\right) + \frac{1}{Y}K_d$	3.38
	$\frac{SRT_{an}}{1 + (K_d \times SRT_{an})} = \left(\frac{\beta}{\mu_{max}}\right)\left(\frac{X_{an}}{COD_{out,an}}\right) + \frac{1}{\mu_{max}}$	3.39

3.6.7 Mono-Digestion Study

Based on the results obtained in Section 4.3.2 (Chapter 4, Results and Discussion), it is apparent that the pilot-scale IAAB is able to operate at high OLR_{an} of 30.0 gCOD/L.day and able to produce final treated effluent with BOD of less than 20 mg/L. The corresponding OLR_a (Eq. 3.15) influenced by the anaerobically-treated POME COD will be presented in Chapter 4 (Results and Discussion). The biomass concentration in both the anaerobic and aerobic compartments ($MLVSS_{an}$ and $MLVSS_a$, respectively), and the F/M ratio of the aerobic compartment are the most vital operating parameters that determine the performance of both the compartments. Hence, they were selected as the independent parameters for the mono-digestion study under mesophilic condition.

The performance of the pilot-scale IAAB was monitored and analysed in terms of the overall $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, methane production rate, methane yield, and methane composition for anaerobic compartment. For aerobic compartment, the overall $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, final treated effluent BOD and TSS were closely monitored. The stability of the reactor for both the processes were determined based on pH, VFA concentration, TA, VFA:TA ratio, MLVSS concentration, and F/M ratio. The main parameters involved in this study are shown in Table 3.13 together with their calculation methods. Kinetic models used in this study were Contois, Stover-Kincannon, and Grau second-order multicomponent substrate removal as discussed in Chapter Two (Literature Review), Section 2.10 would be carried out as well. Table 3.16 showed the mathematical formula used for each of the kinetic model.

3.6.8 Co-Digestion Study

Based on the result obtained in Section 4.3.3 (Chapter 4, Results and Discussion), the methane yield produced by the pilot-scale IAAB at OLR_{an} of 30.0 gCOD/L.day was not comparable to the commercial scale POME treatment. Based on the laboratory scale study conducted in Section 3.5 (Co-digestion of POME with *Moringa Oleifera*), it allows us to infer that the incorporation of *M. oleifera* filtrate into POME able to improve the methane yield as compared to mono-digestion. Such co-digestion treatment was incorporated into the pilot-scale IAAB to attain the same outcome as the laboratory scale co-digestion study (Section 3.5, Co-digestion of POME with *Moringa Oleifera)*.

3.6.8.1 *M. Oleifera* Seeds Powder & *M. Oleifera* Filtrate Preparation

The *M. oleifera* seeds used in this study was similar to the one used in Section 3.5.2. The way used to produce *M. oleifera* seeds powder and *M. oleifera* filtrate were similar to the one mentioned in the same section. In this study, 500 mg/L of *M. oleifera* filtrate was prepared by diluting the filtrate with distilled water and was used in the subsequent experiment. Such concentration was used as it was obtained from the previous study conducted in the laboratory-scale (Section 3.5).

3.6.8.2 Operation of the Pilot-Scale IAAB

The pilot-scale IAAB was operated under the same manner as described in Section 3.6.2. In this study, freshly prepared *M. oleifera* filtrate was added into the feed tank (labelled as T101) that consist of POME during day 1 to day 4 of the study period. With the help of the stirrer (labelled as M1), the mixture of the POME and *M. oleifera* extract will be mixed homogeneously for 4 hours prior to initiating feed pump (labelled as P101) (Pompe Dosatrici, Italy).

3.6.8.3 Bioreactor Performance and Stability Parameter

In this study, the pilot-scale IAAB was operated at OLR_{an} 30.0 gCOD/L.day. This was obtained based on the outcome of the study in Section 4.3.2 (Performance and Kinetic Evaluation of the Pilot-Scale IAAB at various OLRs). Additionally, the study carried out in Section 4.3.3 (Mono-digestion study) clearly denote that the pilot-scale IAAB able to produce final treated effluent with BOD and TSS of less than 20 mg/L and 400 mg/L, respectively at OLR_{an} 30.0 gCOD/L.day. The corresponding OLR_a (Eq. 3.15) will be presented in Chapter 4 (Results and Discussion). The performance of the co-digestion study using the pilot-scale IAAB was monitored and analysed in terms of the overall $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, methane production rate, methane yield, and methane composition for anaerobic compartment. For aerobic compartment, the overall $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, final treated effluent BOD and TSS were closely monitored. The stability of the reactor for both the processes were determined based on pH, VFA concentration, TA, VFA:TA ratio, MLVSS concentration, and F:M ratio. The main parameters involved in this study are shown in Table 3.13 along with their calculation methods.

3.7 Statistical Analysis

All the experiments and analysis were performed in duplicate and the average values were presented in the form of tables and figures. The results were expressed as mean value ± standard deviation of the carried-out analysis for all the experiments (as shown in Chapter 4, Results and Discussion). Furthermore, analysis of variance was performed to find the significance differences between the results.

CHAPTER FOUR

RESULTS AND DISCUSSION

The findings and the results obtained throughout the current research are discussed as followed. This chapter is divided into three main sections: (a) Comparison of the existing industrial scale anaerobic systems used for the treatment of POME; (b) Co-digestion of POME with *Moringa Oleifera* extract (laboratory scale); (c) Evaluation on the performance of the pilot-scale integrated anaerobic-aerobic bioreactor (IAAB). The first section of this chapter thoroughly compares the existing industrial scale anaerobic systems used for the treatment of POME. This is to identify the most suitable type of industrial scale anaerobic system and optimum parameters for the treatment of POME for the production of final treated effluent that abides the discharge limit set by DOE, Malaysia. This may be a way to combat the issue of constant fluctuation of the characteristics of POME. The results of the characterisation of POME, anaerobically-treated POME and aerobically-treated POME determined based on the analysis methods were presented in the first part of the first section of this chapter. The last part of Section 1 discussed the determined kinetic parameters of the industrial scale anaerobic system using several established mathematical models, viz. Modified Stover-Kincannon, Monod, Contois, and Grau Second-Order kinetic models.

The second section of this chapter discussed the anaerobic co-digestion of POME with *M. oleifera* extract conducted in laboratory scale. The optimum concentration of *M. oleifera* extract to coagulate the solids in POME was carried out using the concept of jar test. The determined optimum concentration of *M. oleifera* extract was then applied to the anaerobic laboratory-scale study aiming to compare the performance of mono- and co-digestion of POME with or without *M. oleifera* extract. The third section of this chapter presented the start-up, steady state performance, kinetic evaluation, mono-digestion, and co-digestion using the pilot-scale IAAB under the mesophilic condition. The detailed start-up procedure for each of the compartments, i.e. anaerobic, aerobic and sedimentation were presented and discussed in the first part of Section 3. The maximum loading capacity and kinetic parameters of the pilot-scale IAAB using several established mathematical models, viz. Modified Stover-Kincannon, Monod, Contois, and Grau Second-Order kinetic models were determined and discussed in the second part of Section 3.

The performance of the pilot-scale IAAB were carried out for the mono- and co-digestion assay at its most optimum anaerobic OLR. The performance of the pilot-scale IAAB at mono- and co-digestion assay was compared to identify the best treatment approach for the achievement of BOD 20 mg/L was presented in the last part of Section 3.

4.1 Comparison of different industrial scale palm oil mill effluent (POME) anaerobic systems in degradation of organic contaminants and kinetic performance

As noted previously, the current research is aimed to produce final discharge effluent with BOD 20 mg/L using the pilot-scale IAAB for POME treatment. The current POME treatment, viz. conventional open ponding and covered lagoon systems are still currently used that requires large footprint with low organic removal efficiencies. This leads to the issues of shortage of lands, emission of greenhouse gases, and difficulty in producing effluent that abides the discharge limit. In order to alleviate these issues, it is essential to compare different industrial scale of POME anaerobic systems that has been widely used in the existing palm oil industry. The comparison results will be used as a reference for determining the optimum operational parameters for the pilot-scale IAAB used in this study, which is the stepping stone towards full commercial scale of IAAB. Furthermore, it can provide the palm oil millers an alternative choice of POME treatment in terms of efficient and cost-effective POME management.

4.1.1 Characteristics of Raw POME

Table 4.1 shows the characteristics of raw POME collected from three different palm oil mills in comparison with the typical characteristics of POME published in the literature (Ma and Ong, 1985; Oswal et al., 2002; Ahmad et al., 2003; Zinatizadeh et al., 2006; Choorit and Wisarnwan, 2007; Wu et al., 2007; Poh et al., 2010; Loh et al., 2017).

Table 4.1: The characteristics of POME obtained from the three sampled palm oil mills coupled with the typical characteristic of POME

Parameter	Palm Oil Mill A	Palm Oil Mill B	Palm Oil Mill C	Typical characteristic Loh et al. (2017)
FFB Processed (t/day)	60	60	60	-
BOD (mg/L)	52,433 ± 6,954	40,717 ± 2.181	73,412 ± 1,566	31,826 ± 15,131
COD (mg/L)	86,692 ±3,329	104,850 ± 2,966	122,094 ± 24,268	104,545 ± 10,221
BOD:COD	0.61 ±0.09	0.39 ± 0.03	0.50 ± 0.00	0.34 ± 0.17
TSS (mg/L)	45,500 ±3,500	161,833 ± 68,199	44,957 ± 8,860	84,097 ± 38,868
VSS (mg/L)	39,167 ±2,255	74,833 ± 11,471	28,486 ± 13,000	47,495 ± 14,012
VSS:TSS	0.86	0.46	0.63	0.56
TP (mg/L)	1,432±205	2,465 ± 235	3,270 ± 311	1,949 ± 850
TN (mg/L)	3,867 ±102	4,934 ± 32	5,145 ± 33	4,401 ± 1,823
O&G (mg/L)	13,117 ±1,589	11,650 ± 433	12,589 ± 468	12,384 ± 5,073
Lignin (mg/L)	5,000 ±1,235	4,633 ± 420	3,567 ± 355	4,400 ± 430
VFA (mg/L)	25,368 ±3,364	14,256 ± 764	16,285 ± 347	34,000 ± 4,509
COD:TN:TP	250:11:4	250:12:6	250:11:7	250:11:5
pH	4.95 ± 0.09	4.57 ± 0.16	3.78 ± 0.34	4.5

Values are means ± standard deviation (n=4). [1]

[1] Values are means ± standard deviation (n=4)

All the measured parameters (Table 4.1) except for O&G, lignin and COD:TN:TP from the three different palm oil mills deviate 22-67% from those published in the literature. As data was collected during high crop season and the palm oil mills were operating at their maximum capacity, higher Q_{POME} (25.11-42.88 m^3/hour) were accomplished compared to that reported by Poh et al. (2010). Poh and co-workers reported different measured Q_{POME} between low and high crop seasons, deviating from 20% to 50%. The texture of the collected POME from the palm oil mills could contribute to a large deviation of the measured parameters. High Q_{POME} coupled with maximum operating capacity would produce a concentrated and thicker texture of POME which in turn could affect POME properties such as O&G, BOD, COD, TSS and VSS due to an increased organic and inorganic matters during oil extraction process. Hence, conducting a comprehensive and complete POME characteristics study especially during high crop season will ensure proper process design and equipment sizing accomplished to prevent the treatment plant from operating over its designed capacity.

The quality data of POME for palm oil mill B has the least deviation. The amount of FFB processed was lower than the other two mills. Lower crops yield was due to the haze and lack of sunlight. Since the palm oil mill does not operate in a continuous manner, fresh FFB might be stored prior to processing which not only affects oil extraction rate, but also contributes to a much higher solid content in POME due to the rotten oil palm fruitlets. Tendency of mixing freshly-produced and old-processed POME is high too which might increase the organic and inorganic matters of POME (Chin et al., 2013). As a result, the COD, TSS and VSS concentrations have doubly increased. The POME quality from palm oil mill C deviates the most from literatures. Its 24-hour palm oil milling operation requires large amount of water, e.g. during sterilisation, threshing, extraction and purification processes, and ended up as the largest POME sources with high concentrations of organic and inorganic matters i.e. COD (122,094 mg/L) and BOD (73,412 mg/L) as shown in Table 4.1.

It is vital to determine the digestibility of POME substrate as biological treatment is the cheapest and the most convenient manner in treating large amount of wastewater regardless of high or low organic strength. The ratio of BOD:COD is one of the parameters to determine POME suitability to be treated biologically. As shown in Table 4.1, the ration of BOD:COD for raw POME from palm oil mills A and C are higher than 0.5 except for palm oil mill B. This implies that the raw POME from these two sites is suitable for biological treatment. Typical high strength wastewater contains high concentration of fats, O&G coupled with other organic components with BOD and COD of at least >10,000 mg/L (Pipeline, 2003). The COD and BOD concentrations for raw POME collected in this study are >10,000 mg/L (Table 4.1) meeting the criteria as a high strength wastewater. Biological treatment (both anaerobic and aerobic) of POME can be identified based on macronutrients ratio (COD:TP:TN). This ratio (Table 4.1) was higher than the recommended one (COD:TP:TN = 250:5:1) for all 3 palm oil mills, implying that the concentrations of nitrogen and phosphate in POME were sufficient to support microorganisms growth as well as anaerobic degradation.

However, a longer retention time is required to prevent eutrophication due to high concentration of phosphate in POME during anaerobic treatment to ensure maximum utilisation of the nutrient by microbial cells. The high concentration of nitrogen, on the other hand, is required to synthesise proteins, amino acids, DNA and RNA; hence will allow the microbial cells to replicate and ensure POME to be degraded efficiently. Consequently, the concentration of nitrogen in the POME will be reduced.

The ratio of VSS:TSS can determine digestibility of the substrate. As shown in Table 4.1, palm oil mill B has the lowest ratio of VSS:TSS compared to palm oil mills A and C. The TSS concentration in the POME is dictated by the sampling point. For palm oil mills A and C, POME was collected after the decanter pond (Figures 3.2a and 3.2c, Chapter 3, Materials and Methods) whereby some of the solids had settled down prior to further treatment. For palm oil mill B, the POME generated flowed from the POME inlet to the cooling tower via an underground piping. The cooling tower did not allow settling of solids due to a continuous mixing of fresh and existing POME. As a result, the POME from the cooling tower exhibited the highest TSS concentration (161,833 mg/L) and rendered the VSS:TSS ratio the lowest implying that a longer retention time is required to digest the POME.

The pH of the POME as well as O&G concentration during anaerobic digestion are monitored for any inhibitory effects. VFAs are important intermediate compounds derived from metabolic pathway of methane fermentation which affect biological treatment of POME due to acidity caused (Buyukkamaci and Filibeli, 2004; Poh et al., 2010; Loh et al., 2019). Acetic and propionic acids are the major VFAs present during anaerobic bio-degradation (Loh et al., 2019). These VFAs indicate cell metabolic for hydrogen-producing acetogen and acetoclastic methanogen. The pH of raw POME ranging from 3.78 to 4.95 (Table 4.1) is lower than the optimum pH for methanogenesis (6.8-7.2) to occur. This pH range must be improved and maintained throughout anaerobic digestion as it affects growth of microbial cells and methanogen as well as VFAs concentration in POME. Biological activity (methanogenesis) will be much reduced if pH drops to below 6.2; hence, maintaining a suitable pH during anaerobic digestion is key to prevent accumulation of inhibitory VFAs. The high VFA concentrations of raw POME from all 3 palm oil mills (>2,000 mg/L) might give some inhibitory effect to methanogen (Loh et al., 2019). High concentration of VFAs can be remedied by maintaining an optimum alkalinity level (2,000-5,000 mg/L) in the anaerobic digester (Tchobanoglous, et al., 2014). In this study, recirculation of treated effluent back into the system (in palm oil mills B and C) might be a practical way to maintain pH (5.98-6.32) and alkalinity of POME.

Formation of scum during anaerobic digestion is problematic during operation. POME contains high concentrations of TSS and O&G, typically ranged 28,846 mg/L-30,920 mg/L and 5,614 mg/L-8,812 mg/L, respectively (Loh et al., 2017). The ranges are 44,957-161,833 mg/L and 11,650-13,117 mg/L, respectively for the 3 mills studied (Table 4.1). The high TSS in POME especially for palm oil mill B causes least contact between the solids and the anoxic microbial cells suspended in the anaerobic sludge. Additionally, the O&G in POME solidify once temperature drops to <40^{o}C. As a result, a layer of scum will form on top of the liquid surface of the anaerobic digester/pond.

The scum tends to hinder decomposition of organic and inorganic matters in POME. Consequently, this will limit transportation of soluble substrate to the bacterial biomass, and creates a stressful environment. Although the formation of scum can be minimised by creating an internal recirculation and transferring the solids into another pond (e.g. in palm oil mills B and C), this might not be a long-term solution as more dumping sites will be opened instead of reducing solids concentration. If the TSS and O&G can be coagulated and settled, maximum contact of the solids and the anoxic microbial cells can be established. This will prolong the retention time to digest the complex matters into simpler soluble matters, hence the formation of scum can be minimised or eliminated.

4.1.2 Comparison of Different Anaerobic Treatment Methods

The anaerobic treatment of POME for palm oil mill A (open ponding without covered lagoon, palm oil mill B (added covered lagoon with biogas mixing mode) and palm oil mill C (covered lagoon with hydraulic mixing mode) was assessed. Figure 4.1 shows major POME treatment components involved and their functions in producing a final treated effluent discharge. Their operating conditions are shown in Table 4.2. The respective anaerobic treatments differ in their capability to perform: (a) solids content reduction of pre-treated POME, (b) internal recirculation system, (c) mixing mode of substrate, and (d) biogas capturing. Dependent on biogas capturing system performance, high $COD_{removal}$ and $BOD_{removal}$ efficiencies as well as high biogas yield at low HRT have been achieved (Loh et al., 2017). Factors such as (1) adequate mixing system and (2) sufficient time allocated for the anoxic microbial cells to continuously digest and consume the solids, whereby the sludge retention time (SRT) and microbiological retention time (MRT) are longer than the HRT, would affect biogas system performance. Though covered lagoon system has a longer HRT compared to closed digester, both techniques show comparable performances in term of $COD_{removal}$ and volume of biogas generated.

Figure 4.2 shows their COD, BOD, TSS, TVS and lignin removal efficiencies. The open ponding system (palm oil mill A) shows the lowest removal efficiency particularly for COD and BOD though with the longest HRT (45 days) (Table 4.2), probably due to absence of homogenous mixing of sludge and the incoming raw POME. A limited transportation of soluble substrate to the bacterial biomass was anticipated. Furthermore, sludge accumulation took place at the bottom of the ponds and in many cases, the beds became shallow over time due to the lack of routine desludging. This causes a short circuit as the incoming raw POME escapes into the subsequence ponds before the bacteria is able to break the components down. This problem becomes more apparent as the O&G content of the raw POME in palm oil mill A was reported to be the highest (Table 4.1). This O&G forms a thick layer of scum at the surface of the ponds. The scum layer is formed when the O&G, mainly composed of crude palm oil, solidifies at temperature below 40°C and floats to the top of the surface due to the absence of mixing. This scum layer hinders any further breakdown of these compounds (Poh et al., 2010). The hardened scum layer has worsened the problem of short circuit as it forms a barrier for perforation of the raw POME to the bottom of the ponds.

The inlet feeding point of the raw POME is usually placed on top of the pond and is withdrawn overflow after treatment. As such, some portion of the incoming raw POME will be retained at the top layer of the scum and then escapes from the pond before it is being treated. All these have eventually led to the lowest $COD_{removal}$ and $BOD_{removal}$ reported in Palm oil mill A.

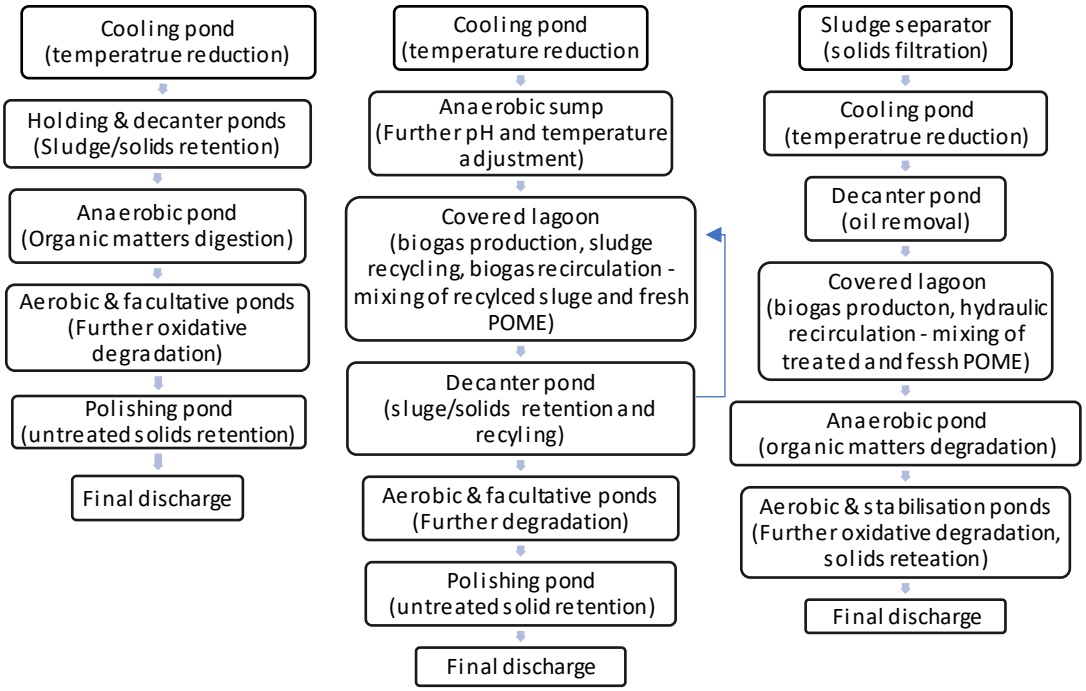


Figure 4.1: Major components of (a) open ponding without covered lagoon, (b) added covered lagoon with biogas mixing mode and (c) added covered lagoon with hydraulic mixing mode

Table 4.2: The operating condition for each treatment system

Parameter	Unit	Palm Oil Mill A	Palm Oil Mill B	Palm Oil Mill C
Treatment System	-	Conventional open ponding system	Covered Lagoon + Open Ponding Systems	Covered Lagoon + Open Ponding Systems
Mixing Substrate	-	-	Biogas	Hydraulic
Volume	m^3	5,066	32,000	25,500
Temperature	°C	40.76 ± 2.77	40.97 ± 1.29	39.98 ± 4.57
Effluent pH	-	7.27 ± 0.27	7.17 ± 0.15	7.30 ± 0.20
OLR	$gCOD/m^3.day$	1.95	1.85	0.61
HRT	Day	45	22	30
Flow Rate	$m^3/hour$	27.05 ± 0.79	42.88 ± 9.16	25.11 ± 5.49
Recirculation Flow Rate	$m^3/hour$	-	10.38 ± 0.10	-

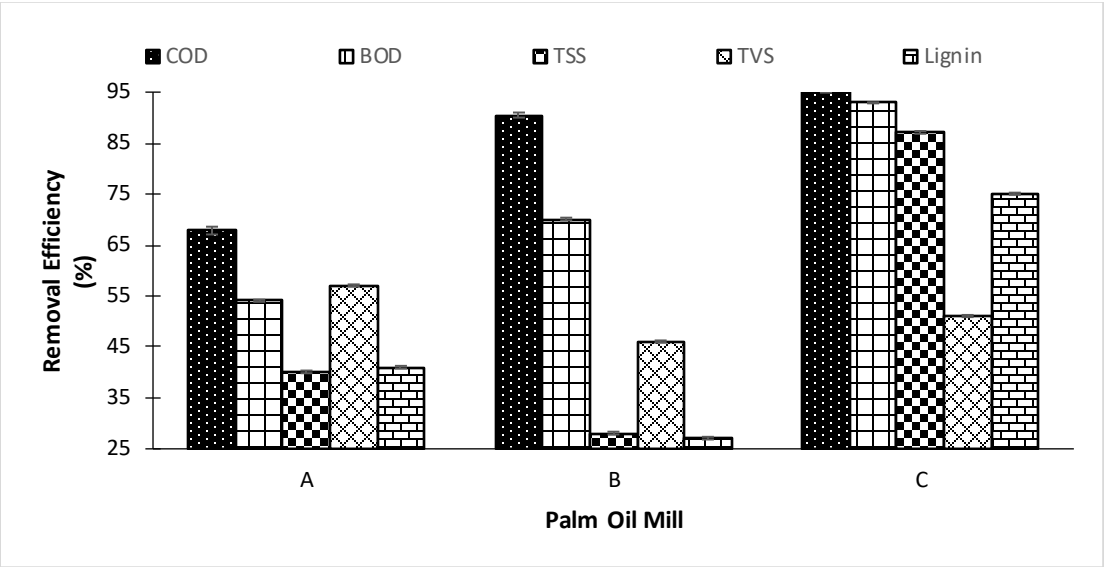


Figure 4.2: The COD, BOD, TSS, TVS, and lignin removal efficiencies after each anaerobic treatment

Biogas recirculation can provide effective mixing and intimate contact between the wastewater and anaerobic sludge. Study has shown that gas-liquid mixing using recycled biogas is beneficial for many digesters and provides better stability during digestion process, entailing more enhanced biogas output consistency (McMahon et al., 2001). It has been implemented in covered lagoon system during continuous treatment of POME with intermittent mixing duration. As reported by Ako and Kitamura (2015), intermittent mixing of gas for 45 min/h has accelerated methane production from H_2/CO_2 mixture compared to continuous mixing. Similar biogas recirculation period was adopted by palm oil mill B for POME treatment, resulting in higher $COD_{removal}$ and $BOD_{removal}$ than that of palm oil mill A. Palm oil mill B had a higher concentration of H_2S (>2,000 mg/L) from the biogas produced compared to palm oil mill C. In the presence of sulphate, methanogenic bacteria will compete with sulphate-reducing bacteria for hydrogen and acetate, which is pH-dependent. At alkaline pH at palm oil mill B, methanogen activity of the anaerobic sludge was inhibited in the presence of H_2S. This might have caused some inhibitory effect to the *acidogenic and acetogenic* bacteria in the POME and suppressed their biodegradation activities (Visser et al., 1993). Besides, the high concentration of the remaining solids as reflected by the lowest removal efficiencies of TSS (28%) and TVS (46%) (Figure 4.2) and the high loading rate of palm oil mill B were indicative of an inappropriate biogas recirculation period which might possibly disrupt the syntrophic relationships and spatial juxtaposition (Visser et al., 1993). Additionally, low removal efficiencies of TSS and TVS translate to low biodegradability of solids in the anaerobically-treated POME. The solids were retained in a decanter pond after the covered lagoon system for further polishing with an aerobic system (Figure 4.1). The recirculation of solids from the decanter pond back into the covered lagoon gave rise to an excessively high concentration of solids which might cause an adverse effect to the anaerobically-treated POME due to incomplete hydrolysis.

The amount of suspended and volatile solids should not pose any operational problems, viz. clogging, scum formation or inhibition to granular sludge formation provided that a longer HRT period (>22 days) is accomplished to allow maximum contact between solids and biomass for complete solids hydrolysis. These circumstances will allow higher amount of CH_4 as well as lower concentration of H_2S to be produced in palm oil mill B.

Based on Figure 4.2, it can be concluded that covered lagoon with hydraulic mixing mode (palm oil mill C) is the most effective anaerobic treatment for POME. It exhibits the highest removal efficiencies for BOD, COD, TSS and lignin. A geo-membrane canvas is used to cover an anaerobic pond to trap the biogas released during anaerobic digestion of POME. The use of anaerobically-treated POME to recirculate the substrate mixture in the system has allowed a maximum contact of the influent with the sludge beneath the system. The recirculated anaerobically-treated POME can prevent the system from overloading. The mixing of anaerobically-treated POME with raw POME would increase the pH and alkalinity of the POME for methanogenesis to occur. The optimum pH and alkalinity for methanogenesis is 6.8-7.2 and 2,000-5,000 mg/L, respectively (Chen et al., 2008; Poh et al., 2010). The low loading rate (0.61 gCOD/m^3.day), a HRT of 30 days and a continuous recirculation mode within the covered lagoon have enabled continuous activation of microbial cells to degrade organic matters efficiently in producing anaerobically-treated effluent suitable to be polished via subsequent aerobic system.

The anaerobically-treated POME for all the 3 types of treatments still contained high COD and BOD concentrations. It is vital to subject these effluents to a suitable post-treatment, viz. aerobic treatment involving aeration (refer Section 4.1.3) before discharging to meet the regulatory limits. The microbial cells in the aerobic stage responsible for the breakdown of carbonaceous contaminants from the effluent require nitrogen and phosphorus for their growth and reproduction. The minimal nutrient condition for their growth is BOD:TN:TP = 100:5:1 (Chan et al., 2010; Tchobanoglous, et al. 2014). The anaerobic treatment of raw POME as the primary treatment step has managed to reduce its organic strength substantially in preparing for aerobic treatment. The BOD:TN:TP of the anaerobically-treated POME for palm oil mills A, B, and C are 100:42:12, 100:640:26 and 100:188:120, respectively. These ratios meet the nutrient balance criteria for the activated sludge to endure aerobic metabolic activities. More importantly, there is no additional cost involvement as no nutrients are added into the system for the post-treatment of anaerobically-treated POME.

4.1.3 Subsequent Treatment for Anaerobically-Treated POME

The anaerobically-treated POME was further treated aerobically to breakdown its remaining organic matter. Oxygen is provided for each of the POME treatment system with surface aerators installed at different type of ponds. Table A1 (Chapter 7, Appendices) shows the characteristics of the final discharge from palm oil mills A, B and C using different treatment methods. Figure 4.3 shows further contaminants removal capabilities via aerobic degradation for each studied site. Direct comparison is not possible as each mill utilises different treatment steps (Figure 3.2, Chapter 3, Materials and Methods) and (Figure 4.1).

As shown in Table 4.3, the pH and organic loading rate (OLR) of the final discharge and aerobically-treated POME, respectively do not differ significantly from one another and fall within the recommended range, averaging at 8.65 and 2.05 gCOD/m^3.day, respectively.

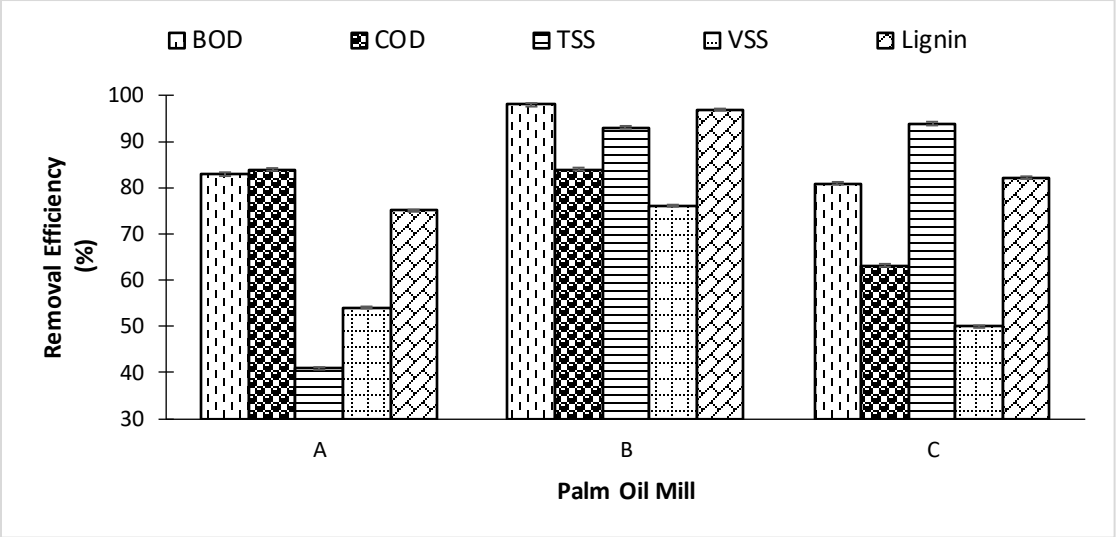


Figure 4.3: Aerobic treatment performance efficiency in the three palm oil mills

The decanter pond at palm oil mill A between the aerobic and facultative ponds has allowed better settling of solids that have not been anaerobically degraded. However, the settled solids are not recycled back into the anaerobic ponds but are removed and dewatered instead. The effluent with lesser concentration of sludge is then treated in the facultative ponds assisted by a built-in aeration system. The $COD_{removal}$, $BOD_{removal}$, $TSS_{removal}$, $VSS_{removal}$ and $lignin_{removal}$ were 83%, 84%, 41%, 54% and 75%, respectively (Figure 4.3). The removal efficiencies via aerobic treatment stage were higher than those at anaerobic stage for the open ponding system. The mixed liquor volatile suspended solids:mixed liquor suspended solids i.e. MLVSS:MLSS (0.69) of the sludge in the facultative ponds is within the recommended value of 0.63-0.88 by Chan et al. (2010). The provision of a 24-hour air supply further reduced the contaminants in the anaerobically-treated POME especially for lignin (Higuchi, 2004; Martínez *et al*., 2005).

In palm oil mill B, the decanter pond located between the anaerobic sump 2 and acidification pond is used to retain and recycle the sludge from the anaerobically-treated POME back into the covered lagoon to maintain the bacteria concentration. In the facultative ponds, 19 days has been allocated for each pond to allow conversion of anoxic microbial cells into aerobic microbial cells without stressing them. The effluent with lesser concentration of sludge is then treated in the aerobic pond with aeration. The homogenous mixing of effluent in the aerobic pond with sufficient air supply activates the microbial cells. As shown in Figure 4.3, the $COD_{removal}$, $BOD_{removal}$, $TSS_{removal}$, $VSS_{removal}$ and $lignin_{removal}$ were 98%, 84%, 93%, 76% and 97%, respectively. These were higher than those obtained through anaerobic treatment probably due to the presence of higher concentration of aerobic bacteria as reflected by the high MLVSS concentration of 3,822 mg/L and MLVSS:MLSS of 0.67. This ratio is also within the recommended value by Chan et al. (2010). The same observation between palm oil mills A and B proved that aeration provided in both the facultative pond (palm oil mill A) and aerobic pond (palm oil mill B) had resulted in a MLVSS:MLSS ratio within the recommended value for optimal cell growth. The high quantity of active aerobic microbial cells requires large amount of oxygen (provided by the aerator installed) to break down the organic matters that cannot be anaerobically degraded previously.

In palm oil mill C, the presence of acidification followed by anaerobic ponds after the covered lagoon allow settling of solids that cannot be broken down anaerobically. Effluent with lesser concentration of solids overflows into the aerobic pond to allow better conversion of microbial cells from anoxic to aerobic mode without causing any stress to the microbial cells at a HRT of 15 days. As shown in Figure 4.3, the $COD_{removal}$, $BOD_{removal}$, $TSS_{removal}$, $VSS_{removal}$ and $lignin_{removal}$ were 63%, 81%, 94%, 50% and 82%, respectively. These performance efficiencies except for TSS and lignin were lower than those by palm oil mills A and B. However, the BOD, TSS and lignin contents of the final discharge for palm oil mill C are the lowest compared to the other 2 mills (Table 4.3). The typical MLVSS:MLSS for conventional activated sludge and extended aeration activated sludge ranges from 0.70 - 0.85 to 0.60 – 0.75, respectively (Tchobanoglous et al., 2014). The MLVSS concentration of the aerobic pond is 7,938 mg/L whereas the MLVSS:MLSS is 0.92 which is higher than the recommended ratio. The high $TSS_{removal}$ and $lignin_{removal}$ of 94% and 82%, respectively might be due to unexpected settling of more organic matters in the aerobic pond itself instead of being degraded.

It is vital to produce a final discharge with consistent characteristics that is suitable to be released into the river ways due to huge volume produced annually. In palm oil mill A, filter bed and rotary basculator are used to treat the aerobically-treated effluent, which act as a post-treatment step. They play the same role; whereby large quantities of liquid are filtered to trap the solid content that cannot be degraded in both the biological treatment modes. For palm oil mill B, high population of aerobic microbial cells have the tendency

to flocculate and settle the organic matters in the pond. However, high concentrations of suspended solids (3,722 mg/L) and VSS (2,444 mg/L) have remained in the final discharge. This shows that settling of solids in the aerobically-treated effluent is insufficient even at a HRT of 19 days. The quality of the final discharge can be improved by increasing settling efficiency of the polishing pond to allow optimum stabilisation of the organic matters during solid-liquid separation. In palm oil mill C, the presence of pure aerobic microbial cells in extended aeration system and sufficient retention time in the aerated stabilisation pond produced effluent with the lowest COD, BOD, TSS, TVS and lignin concentrations (Table A1, Chapter 7, Appendices). However, a combined covered lagoon with the existing activated sludge system is still unable to reduce the BOD of the final discharge to below 20 mg/L. Overall, the final discharge from all the 3 mills do not comply with the discharge limit set by DOE in terms of BOD, COD and TSS.

Therefore, further POME treatment study needs to be carried out, not limiting to activated sludge system. To date, measures for first step POME treatment focus on (a) trapping the CH_4 released during anaerobic digestion and (b) substrate used for mixing. In this study, the former has been established using digester tank or covered lagoon technology. The latter allows appropriate mixing of anaerobically-treated POME for high removal efficiencies of COD (95%), BOD (93%), TSS (87%), VSS (51%) and lignin (75%). Based on the findings, the covered lagoon with hydraulic mixing mode is the most efficient first step in POME treatment. The performance efficiency of the covered lagoon system might be improved based on the following suggestions: (a) maintain OLR_{an} at the range of 0.60-0.80 gCOD/m^3.day, (b) HRT – 28-30 days, (c) mixing feature – mixture of anaerobically-treated POME with fresh raw POME, (d) temperature – 39-41°C and (e) settling of sludge – downstream allocation of decanter pond to allow settling of anaerobically-treated solids prior to recycling back into the system. However, the use of a covered lagoon requires large land area, which is deemed not a long-term solution.

4.1.4 Kinetic Modelling

Four different kinetic models, namely Stover-Kincannon (Senturk et al., 2010), Monod (Monod, 1949), Contois (Contois, 1959) and Grau second order (Grau et al., 1975) were applied to the most performed anaerobic POME treatment system i.e., covered lagoon with hydraulic mixing (palm oil mill C. The operating data (330 days) were subjected to kinetic modelling based on Equations 4.1, 4.3, 4.6, and 4.8 (Table 4.3) to evaluate POME treatment performance for (1) the specific parameters used and (2) the relationships between variables.

Table 4.3 The mathematical formula for different kinetic model

Kinetic Model	Equation	Eq. No
Modified Stover-Kincannon (Senturk, et al., 2010; Chan et al., 2012)	$COD_{out,an} = COD_{in} - \frac{769.23 COD_{in}}{824.77 + OLR_{an}}$	4.1
Monod (Monod, 1949; Abdurahman, et al., 2011)	$COD_{out,an} = \frac{120.91(\frac{1}{SRT_{an}} + 0.00483)}{0.493 - \frac{1}{SRT_{an}} - 0.000483}$	4.2
	$X_{an} = \frac{0.098 SRT_{an}(COD_{in} - COD_{out,an})}{HRT\,(1 + 0.00483 SRT)}$	4.3
Contois (Contois, 1959; Chen and Hashimoto, 1980)	$COD_{out,an} = \frac{(0.6119 X_{an})(\frac{1}{SRT_{an}} + 0.00483)}{\frac{1}{SRT} - 0.0542 - 0.00483}$	4.4
Grau second-order (Grau, et al., 1975; Sanchez, et al., 2001)	$COD_{out,an} = COD_{in}\left(1 - \frac{HRT}{11.687 + 1073.5 HRT}\right)$	4.5

$COD_{out,an}$: anaerobically-treated effluent COD concentration; COD_{in}: influent COD concentration; OLR_{an}: organic loading rate for anaerobic compartment; SRT_{an}: solid retention time for anaerobic compartment; X_{an}: anaerobically treated effluent biomass concentration.

4.1.4.1 Regression of Kinetic Model

A graphical method and method of least squares linear regression were employed to obtain the line of best fit for kinetic evaluation on COD basis. Figure 4.4 shows the linear regression results for the reciprocal of total organic loading removal rate, $[V_{an}/(Q_{in}(COD_{in}\text{-}COD_{out,an})]$ against reciprocal of total OLR, $V_{an}/(Q_{in} \times COD_{in})$ based on modified Stover-Kincannon model. Due to the resulted linear plot, linear regressions (least squares method) was used to determine the intercept ($\frac{1}{U_{max}}$) and the slope ($\frac{K_B}{U_{max}}$). Saturation value constant (K_B) and maximum substrate removal rate (U_{max}) were calculated from the line plotted in Figure 4.4.

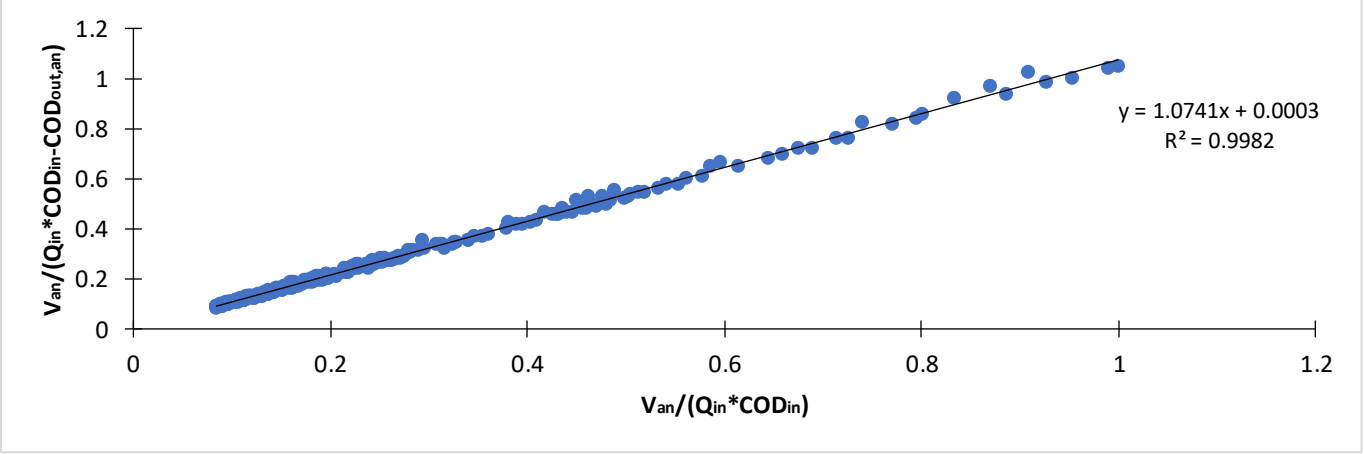


Figure 4.4: The linearised plot of Stover-Kincannon Model for the determination of maximum substrate removal rate (U_{max}) and saturation value constant (K_B) in anaerobic system

The kinetic coefficients, μ_{max} and K_s, for the Monod model was determined by plotting Eq. 3.34 (Table 3.16, Chapter 3, Materials and Methods), as shown in Figure 4.5. Their values were calculated from the intercept and slope of the straight line, respectively. Due to the resulted linear plot, linear regressions (least squares method) was used to determine the intercept ($\frac{1}{\mu_{max}}$) and the slope ($\frac{K_S}{\mu_{max}}$). Maximum specific growth rate (μ_{max}) and half-velocity constant (K_S) were calculated from the line plotted in Figure 4.5.

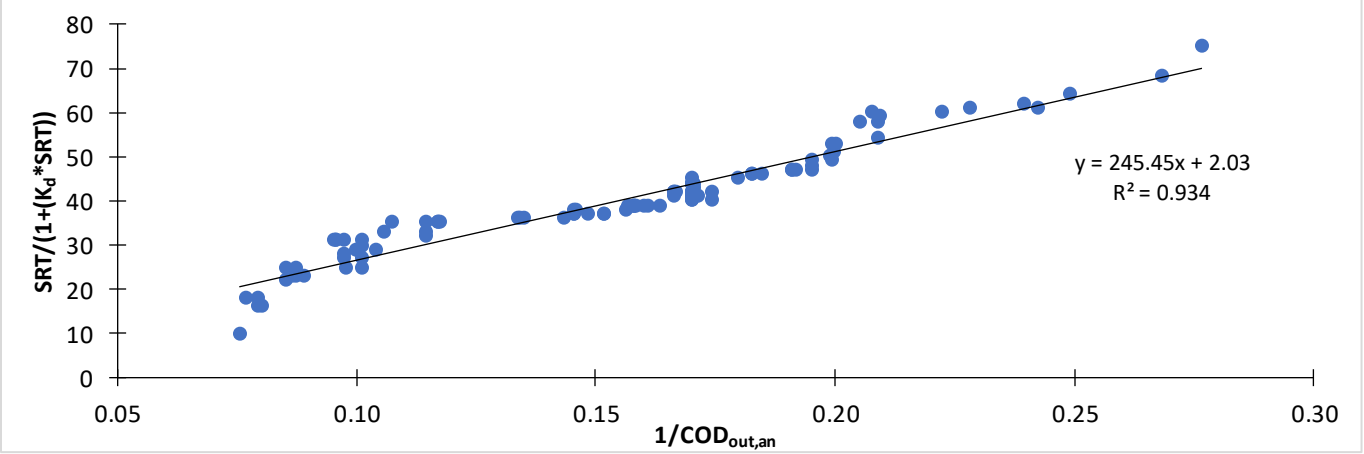


Figure 4.5: The linearised plot of Monod model for the determination of maximum specific growth rate (μ_{max}) and half-velocity constant (K_s) in anaerobic system

In order to determine the kinetic coefficients (β and μ_{max}) for the Contois model, Eq. 3.39 (Table 3.16, Chapter 3, Materials and Methods) was plotted in Figure 4.6. Similarly, their values were determined from the slope and intercept of the line, respectively. Due to the resulted linear plot, linear regressions (least squares method) was used to determine the intercept ($\frac{1}{\mu_{max}}$) and the slope ($\frac{\beta}{\mu_{max}}$). Maximum specific growth rate (μ_{max}) and growth parameter (β) were calculated from the line plotted in Figure 4.6.

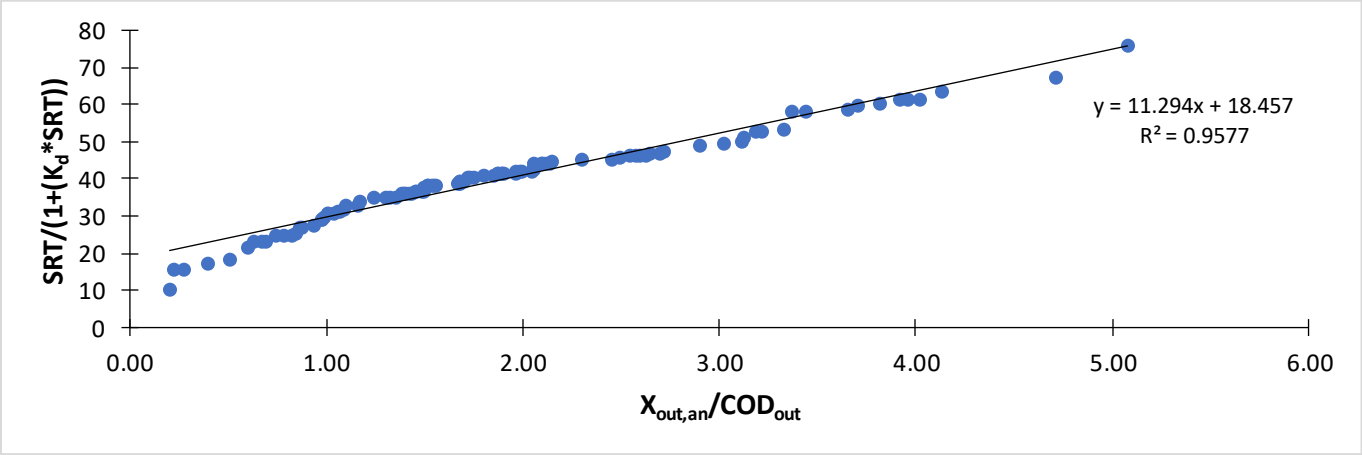


Figure 4.6: The linearised plot of Contois model for the determination of maximum specific growth rate (μ_{max}) and growth parameter (β)

To determine the kinetic coefficients (a and b) for the Grau second-order model, Eq. 3.36 (Table 3.16, Chapter 3, Materials and Methods) was plotted, as shown in Figure 4.7. Due to the resulted linear plot, linear regressions (least squares method) was used to determine the Grau second-order kinetic coefficients (a and b) and they were calculated from the line plotted in Figure 4.7.

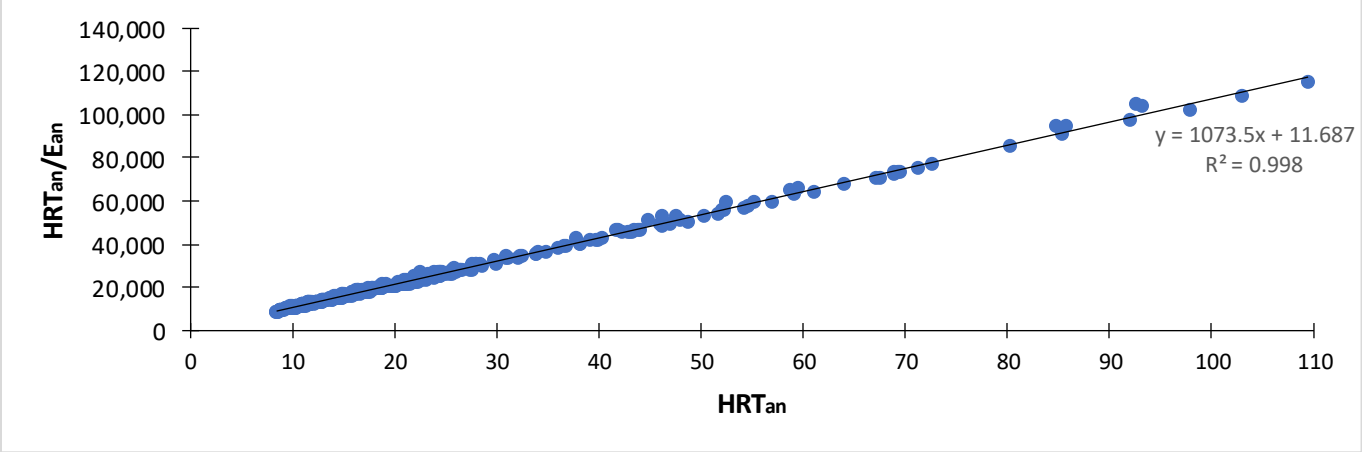


Figure 4.7: The linearised plot of Grau Second-Order model for the determination of kinetic coefficients (a and b)

4.1.4.2 Evaluation of Kinetic Models

Correlation coefficient (R^2) is used to select the most suitable model to represent a system undertaken. In this study, all the models used had high R^2 of 0.9982, 0.934, 0.9577 and 0.998 for Stover-Kincannon, Monod, Contois and Grau second-order models, respectively (Figures 4.4-4.7), thus were insignificant to describe the performance of the covered lagoon system with hydraulic mixing (palm oil mill C). Further evaluation was done by comparing the constants obtained with other studies having similar process and the models used were also validated by comparing the predicted data with an independent set of experimental data. The COD of the anaerobically-treated POME for modified Stover-Kincannon, Monod, Contois and Grau second-order models can be predicted using Eq. 4.1, Eq. 4.2, Eq. 4.4 and Eq. 4.5, respectively. The biomass concentration can be estimated using Monod model via Eq. 4.3. Figure 4.8 shows the predicted values of $COD_{out,an}$ for each model and the independent set of data obtained from palm oil mill C. Based on the findings, the modified Stover-Kincannon model was the most suitable in describing the performance of the system due to its highest R^2 of 0.9767 as well as low standard deviation. It postulates that OLR_{an} is the most important parameter for estimating and optimising the performance of the anaerobic covered lagoon system with hydraulic mixing mode. The result concurs with those findings by Chan et al. (2012) reporting that the modified Stover-Kincannon model was a more appropriate model to describe the kinetic performance of an anaerobic system for POME.

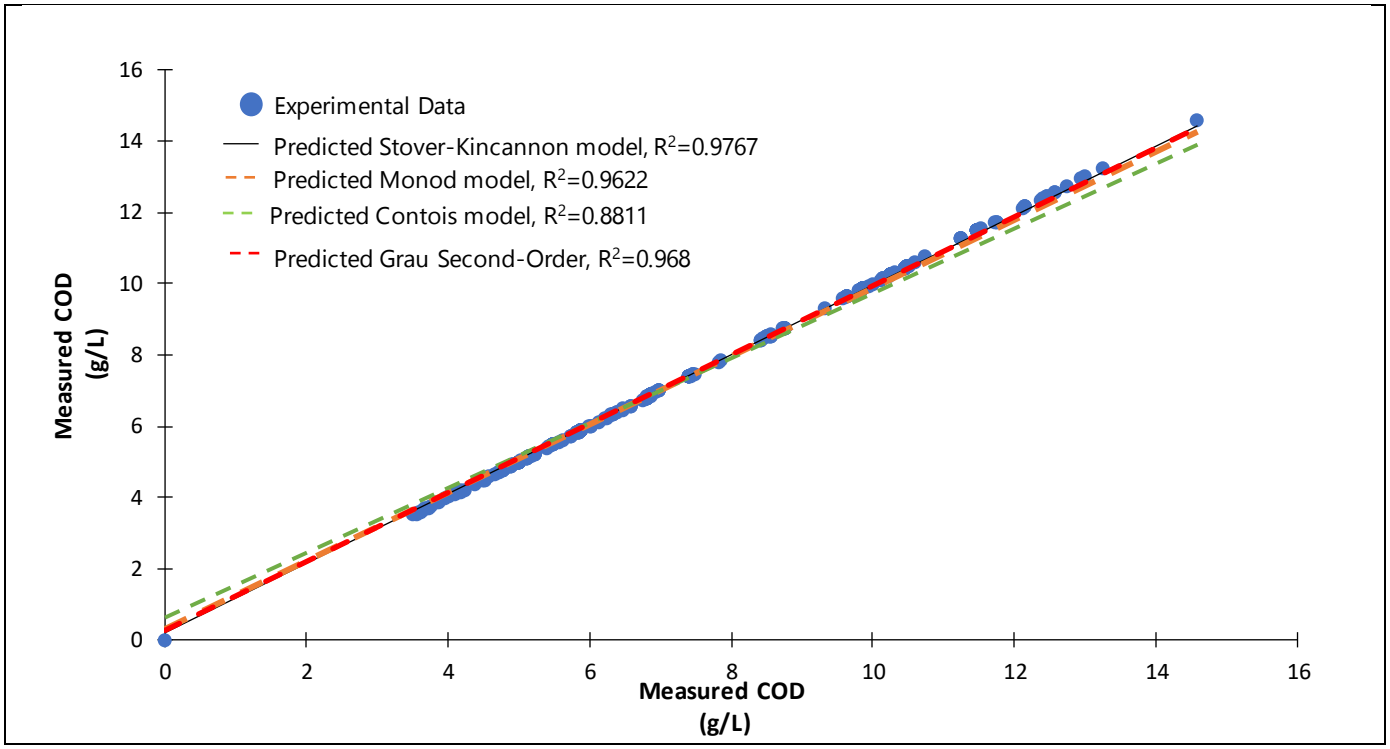


Figure 4.8: Comparison between predicted and the actual effluent data from the covered lagoon with hydraulic mixing system

Table 4.4 Comparison of kinetic constants for different wastewater treatment using the four kinetic models in the kinetic models

Kinetic Models	Substrate	Reactor Type	Influent COD (mg/L)	OLR (gCOD/L.day)	HRT (day)	Kinetic Parameters				References
						K_B gCOD/L.day	U_{max} gCOD/L.day			
Modified-Stover Kincannon	Soybean ww	AF	7,520-11,450	2.06	1.0-1.45	85.50	83.30			(Yu et al., 1998)
	Simulated starch ww	UAF	100-4,000	1.15-16.70	0.25-1.00	49.80	50.60			(Ahn and Forster, 2000)
	Molasses	AHR	2,000-15,000	1.00-10.00	0.5-2.0	186.23	83.30			(Buyukkamaci and Filibeli, 2002)
	Simulated ww	UASB	4,214	1.00-15.80	0.25-4.16	8.20	7.50			(Işik and Sponza, 2005)
	Papermill ww	UAF	1,972-3,536	1.08-11.38	6-24	104.15	86.21			(Yilmaz et al., 2008)
	Slaughter-house ww	UAF	6,000-6,500	3.1-6.1	24-48	120.88	99.01			(Padilla Gasca and Lopez-Lopez, 2010)
	POME	IAAB	68,550-79,800	10.50-21.50	2.3-6.5	14.70	23.10			(Chan et al., 2012)
	POME	CL	77,075-168,741	1.00-4.37	14.0-68.0	3,580.33	3,333.33			This study
Monod						Y (mgVSS/mgCOD)	K_d (per day)	μ_{max} (per day)	K_S (mg/L)	
	Ice-cream ww	CSTR	5,500	ND	2.99-7.45	0.2116	0.0131	0.784	0.4028	(Hu et al., 2002)
	Simulated ww	UASB	4,214	1.00-15.80	0.25-4.16	0.125	0.0065	0.105	>4,000	(Işik and Sponza, 2005)

Monod	POME	IAAB	68,550-79,800	10.50-21.50	2.3-6.5	0.196	0.038	0.103	8,168	(Chan et al., 2012)
	POME	CL	77,075-168,741	1.00-4.37	14.0-68.0	0.0982	0.0048	0.493	120.91	This Study
Contois						Y (mgVSS/mgCOD)	K_d (per day)	μ_{max} (per day)	β (mgCOD/ mgVSS)	
	Ice-cream ww	CSTR	5,500	ND	2.99-7.45	0.2116	0.0131	0.930	0.4818	(Hu et al., 2002)
	Simulated ww	UASB	4,214	1.00-15.80	0.25-4.16	0.125	0.0065	0.105	0.465	(Işik and Sponza, 2005)
	POME	CL	77,075-168,741	1.00-4.37	14.0-68.0	0.0982	0.00483	0.054	0.612	This Study
Grau Second-Oder							k_s (day^{-1})	a (day^{-1})	b^c	
	Textile ww	UAFB	243-1973	1.0-8.21	9.6-23.76		252.0	0.915	5.138	(Sandhya and Swaminathan, 2006)
	Synthetic ww	AHR	2,000-15,000	1.0-10.0	0.5-2.0		10.810	0.033	1.192	(Büyükkamaci and Filibeli, 2002)
	Synthetic textile ww	UASB	4,214	1.0-15.8	0.25-4.17		0.337	0.562	1.095	(Işik and Sponza, 2005)
	Synthetic ww	UASB	3,000-4,000	6.0-34.0	0.083-0.833		0.26	0.029	0.011	(Sponza and Ulukoy, 2008)
	Synthetic ww	UASB	300-4,000	0.9-20.1	0.167-0.333		0.058	0.083	0.006	(Bhunia and Ghangrekar, 2008)
	Food processing ww	MACR	5,250-5,750	1.1-5.0	1.06-5.11		-	0.260	1.025	(Senturk et al., 2010)

Grau Second-Order	POME	IAAB	68,550-79,800	10.50-21.50	2.3-6.5		0.655	3.000	0.633	(Chan et al., 2012)
	POME	CL	77,075-168,741	1.00-11.92	14.0-68.0		0.539	11.687	1073.5	This Study

AHR: anaerobic hybrid reactor; IAAB: Integrated anaerobic-aerobic bioreactor; CL: Covered lagoon; AF: Anaerobic filter; UASB: Upflow anaerobic sludge reactor; UAF: Upflow anaerobic filter; UAFB: Upflow anaerobic fixed bed reactor; MACR: Mesophilic anaerobic contact reactor; ww: Wastewater

The Stover-Kincannon model is diverse/robust as it can accommodate different substrate utilisation rate and reactor configuration under anaerobic treatment (Table 4.4). For the covered lagoon system used in this study, the U_{max} (3,333.33 gCOD/L.day) and K_B (3,580.33 gCOD/L.day) values were way higher than those previously studied, probably due to the large OLR_{an} range of 1.00 to 11.92 gCOD/L.day applied in the system. High lignin content (3,394 mg/L) in POME has also contributed to low biodegradation, causing much lower digestibility condition in the anaerobic condition deployed (Ho et al., 1984; Wu, et al., 2009; Chan et al., 2012). Contrarily, very low U_{max} (7.50 gCOD/L.day) and K_B (8.20 gCOD/L.day) values are achievable by the simulated wastewater using an UASB system (Table 4.4), despite a wide range of OLR (1.00-15.80 gCOD/L.day) is applied. This could be explained by 1) low influent COD concentration of 4,214 mg/L; 2) relatively small effective volume of the system i.e., 2.5 L and; 3) short HRT of 0.25-4.16 days. The U_{max} value in this study was found to be higher than those (23.10 gCOD/L.day) using the laboratory scale IAAB previously studied by Chan et al. (2012), probably due to lower HRT (2.3-6.5 days) and influent COD concentration (68,550-79,800 mg/L) from the latter. For an industrial scale anaerobic treatment such as the one used for this study, a long HRT coupled with low OLR_{an} allow complete digestibility and breakdown of organic compounds, especially the lignin components. Not much can be referred and compared though as those predicted performance data of other systems in Table 4.4 are at laboratory-scale. The kinetic coefficients obtained in this study showed that there is relationship between the size of the treatment system with the kinetic coefficients obtained. Besides that, the influent COD concentration subjected to each of the reactor types allow us to determine the suitable type of bioreactor configuration for high strength wastewater. The kinetic parameters obtained in this study can be a quick reference to predict the performance efficiency in terms of $COD_{removal}$ of newly built anaerobic treatment prototype for POME or other wastewater types. The results can be used to estimate treatment efficiency of full-scale bioreactors for POME. The comparison made in Table 4.4 will act as a guideline for other researchers to improve the existing the POME treatment system by incorporating suitable technology (i.e. moving towards the direction of high-rate bioreactor system) to produce final treated effluent that able to abide the stringent discharge limit set by Department of Environment (DOE), Malaysia (DOE, 1977). This is aimed to reduce the (a) carbon footprint of the existing POME treatment system and (b) size of the treatment system.

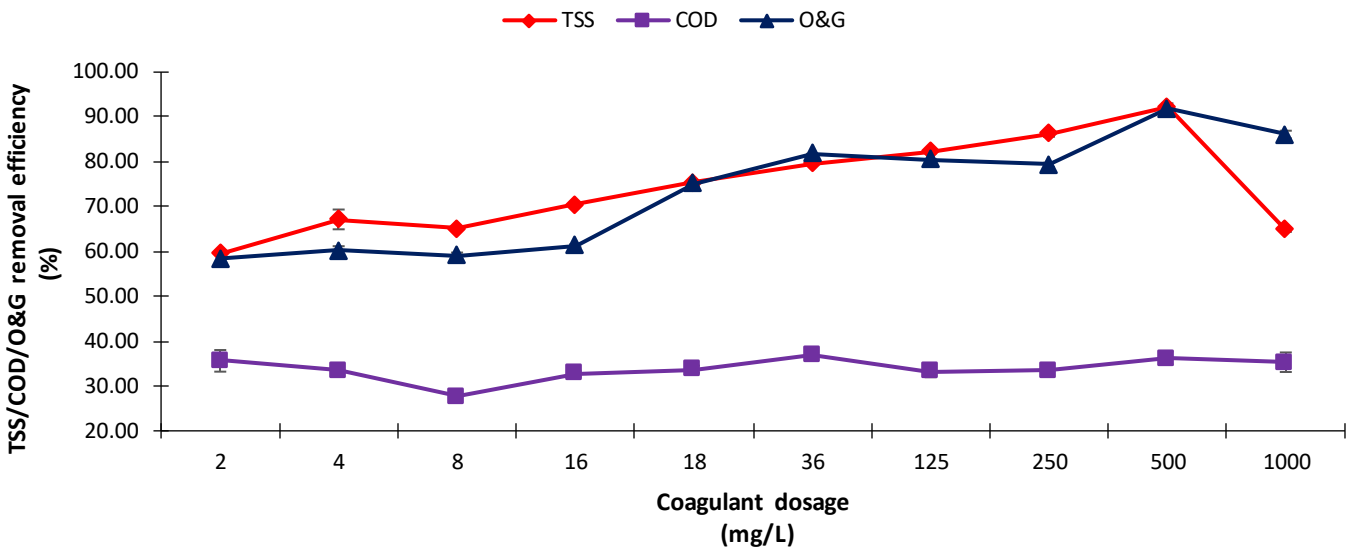
4.2 Synergistic effect of Anaerobic Digestion of Palm Oil Mill Effluent (POME) with *Moringa Oleifera* Extract

4.2.1 Effect of *Moringa Oleifera* Extract as Coagulant on POME

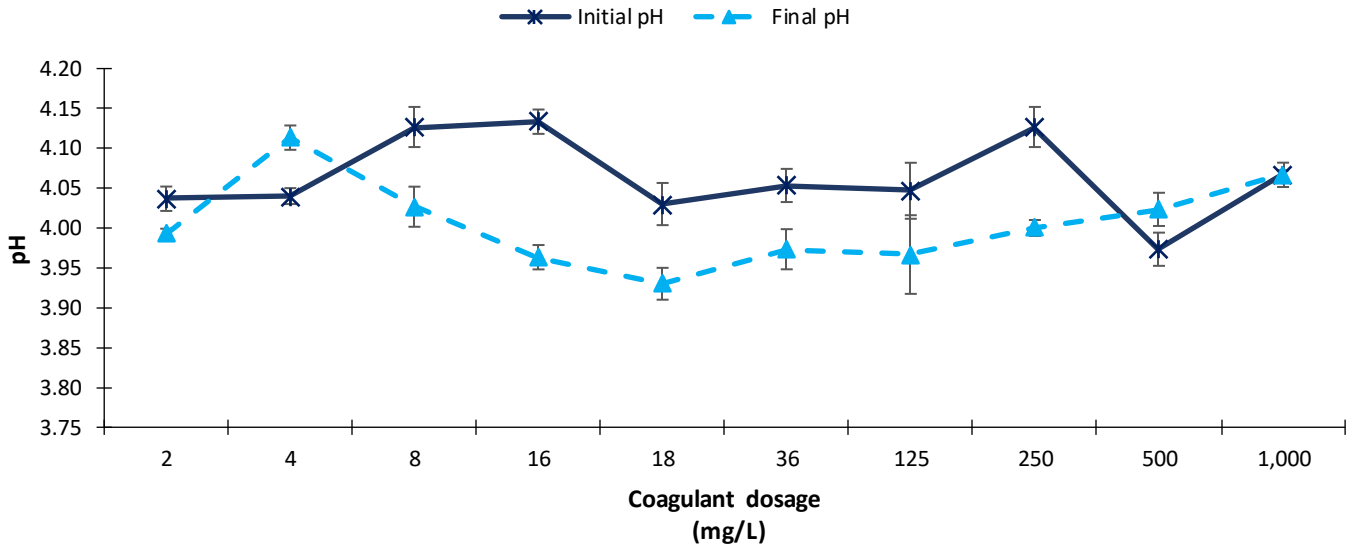
Moringa Oleifera was used in this study as a substrate for co-digestion due to its unique capability in coagulating the solid in the wastewater by the mechanism of adsorption and neutralisation as discussed in Section 2.13.2. This will reduce the overall turbidity of the wastewater subjected to co-digestion by the formation of large flocs. The activity of *M. oleifera* extract to coagulate with POME in forming large flocs through reduction in COD, O&G and TSS of POME was examined. The COD, O&G and TSS of raw POME were 64,250±884 mg/L, 24,700±325 mg/L and 23,100±5,860 mg/L, respectively (Table 4.5). The COD, O&G and TSS exhibited by *M. oleifera* extract had negligible effects on the treated supernatant as the volume used was small in comparison to the total volume used. The effect of dosing *M. oleifera* extract on the pH of the treated supernatant was also monitored throughout the experiments to check on its influence on pollutant removal. The initial pH of the diluted *M. oleifera* extract at various concentration is shown in Table 4.5. The initial pH of the diluted *M. oleifera* extract is shown in Table 4.5. The COD, TSS and O&G concentrations of each of the pre-treated POME after the addition of different concentrations of *M. oleifera* extract are also depicted in Table 4.5.

Table 4.5: Quality of POME dosed with *M. oleifera* extract before jar test experiments

***M. oleifera* extract**			**Raw POME + *M. oleifera* extract**			
Diluted Concentration (mg/L)	**Dilution factor**	**Initial pH**	**Initial pH**	**COD (mg/L)**	**TSS (mg/L)**	**O&G (mg/L)**
0	0	0	3.87	64,250 ± 884	23,100 ± 860	24,700 ± 325
2	5,000	7.40	4.04	43,050 ± 212	9,389 ± 16	10,250 ± 20
4	2,500	7.45	4.04	42,575 ± 106	7,913 ± 124	9,992 ± 25
8	1,250	7.32	4.13	45,150 ± 71	8,058 ± 4	10,258 ± 18
16	625	7.28	4.13	43,750 ± 71	6,820 ± 255	9,525 ± 15
18	556	7.25	4.03	42,700 ± 141	5,659 ± 58	6,232 ± 8
36	280	7.20	4.05	40,600 ± 141	4,758 ± 4	4,515 ± 3
125	80	7.02	4.05	43,650 ± 354	4,175 ± 35	4,832 ± 10
250	40	7.08	4.13	43,150 ± 71	3,215 ± 7	5,235 ± 15
500	20	6.81	3.97	41,900 ± 141	1,635 ± 7	2,012 ± 25
1,000	10	6.47	4.07	42,700 ± 141	8,170 ± 42	3,512 ± 30



(a)



(b)

Figure 4.9: Effect of *M. oleifera* extract dosage on (a) TSS/COD/O&G reduction and (b) Initial/Final pH in jar test POME pre-treatment

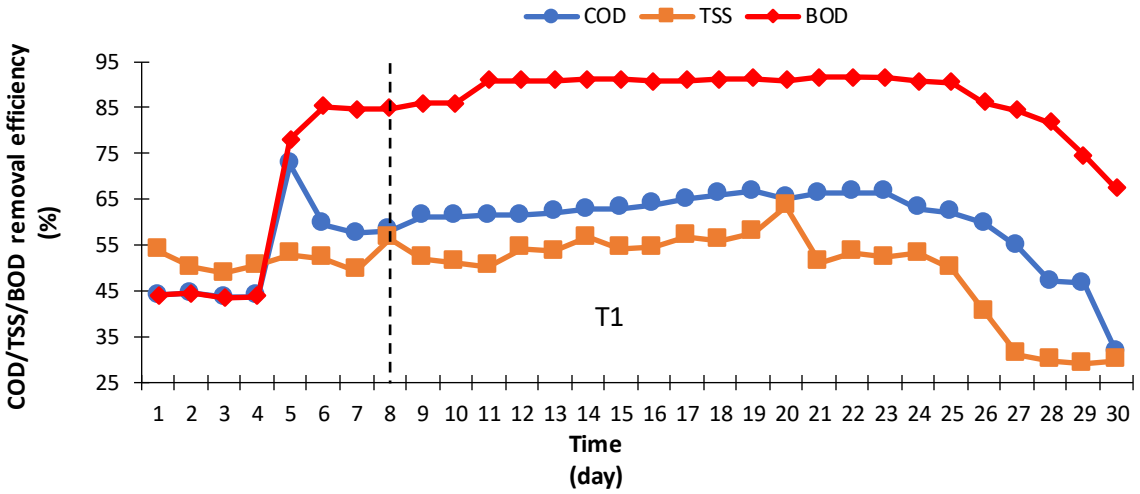
Figure 4.9(a) shows a general increasing trend of $COD_{removal}$, $TSS_{removal}$ and $O\&G_{removal}$ with increasing dosage of *M. oleifera* extract from 2 mg/L to 500 mg/L with more prominent removal observed for $TSS_{removal}$ and $O\&G_{removal}$ from 58% to 92%, respectively. Thereafter, drastic reduction occurred when the *M. oleifera* extract dosage reached 1,000 mg/L. Lower concentrations of *M. oleifera* extract (2-16 mg/L) were insignificant in removing COD, TSS and O&G; more so for COD. Too low a concentration of *M. oleifera* extract caused very little and incomplete flocculation, resulted in difficulty for the small flocs to settle down. This finding corresponded to Muyibi and Okuofu (1995), where low doses of polymer increase water turbidity. It is noteworthy that the $COD_{removal}$ from the jar test experiment was less than $TSS_{removal}$ and $O\&G_{removal}$ which concurred with Bhatia et al. (2007a). This probably was caused by the remaining organic matters present in *M. oleifera* after the coagulation process (Bhatia et al., 2007a). In addition, high dosage of *M. oleifera* extract (1,000 mg/L) had resulted in the lowest $TSS_{removal}$; hence did not aid in clarification. This ineffective overdosing occurred due to over-saturation of *M. oleifera* causing re-stabilisation of SS in POME. Therefore, the optimum dosage was 500 mg/L of *M. oleifera* extract which allowed distinct separation between SS and supernatant leading to the highest $TSS_{removal}$ (92.24%) and $O\&G_{removal}$ (91.90%). Protein (36.7%) having dimeric cationic charges present in the *M. oleifera* extract (Ndabigengesere et al., 1995) was responsible for coagulating the solid of POME (anionic charge) by effectively doubled up in clumping the solids and forming thicker sludge which settled down via gravitational force.

Figure 4.9b shows the initial and final pH of the pre-treated POME at different dosages of *M. oleifera* extract. The pH of POME was important in affecting the effectiveness of the electrostatic patch mechanism (surface phenomena) governing adsorption-neutralisation charges that had occurred between POME and *M. oleifera* extract (Bhatia et al., 2007b). The initial pH (immediately after addition of different concentration of *M. oleifera* extract) ranged between 3.97 and 4.13 (Table 4.5). The low pH had caused the anionic organic molecules (solids of POME) to react directly with the cationic species containing in *M. oleifera* extract; allowing the destabilised particles to form larger flocs that settled easily by gravitational force. Different dosage of coagulant used in this study showed potential solid removal of POME right from the pond at the beginning of low initial pH value. This encouraging fact indicates that pH adjustment on raw POME in actual treatment system should be omitted. Right after the coagulation process, the measured pH of the pre-treated POME was found not significantly changed (Figure 4.9b) implying that the use of environmentally-friendly coagulant did not affect the POME pH much before and after coagulation. Since coagulation takes place effectively around pH 3.0-4.0, the use of *M. oleifera* extract in POME treatment will be economical as there is no need to adjust the pH during treatment. Interestingly, as the effective pH range for coagulation by *M. oleifera* extract is much lower compared to Fe/Al coagulant which usually occurs at around 5.5 - 6.5 (Bell-Ajy et al., 2000), its effect on coagulation mechanism and efficiency is worth pursuing in future study.

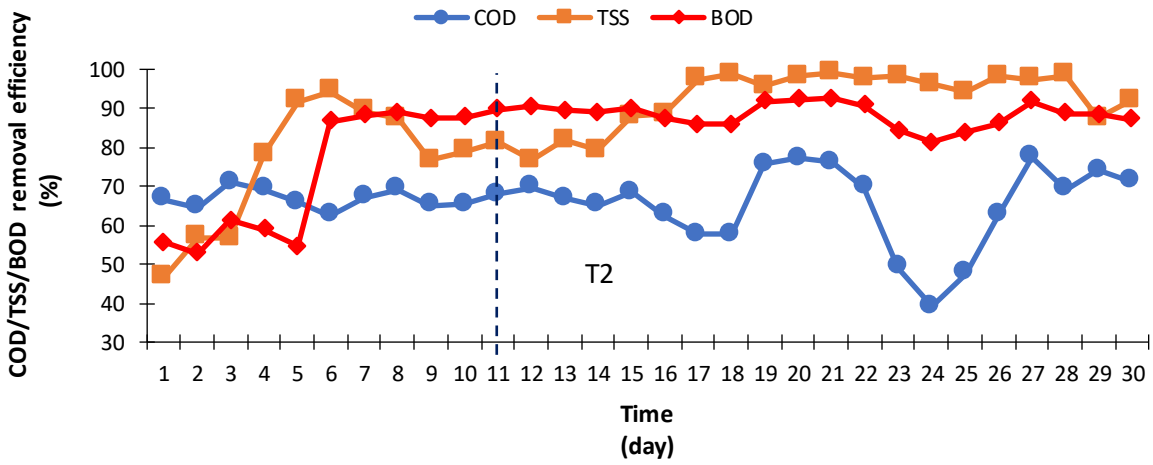
4.2.2 Performance of Mono- and Co-Digestion of POME with *Moringa Oleifera*

4.2.2.1 BOD, COD and TSS Removal Efficiencies

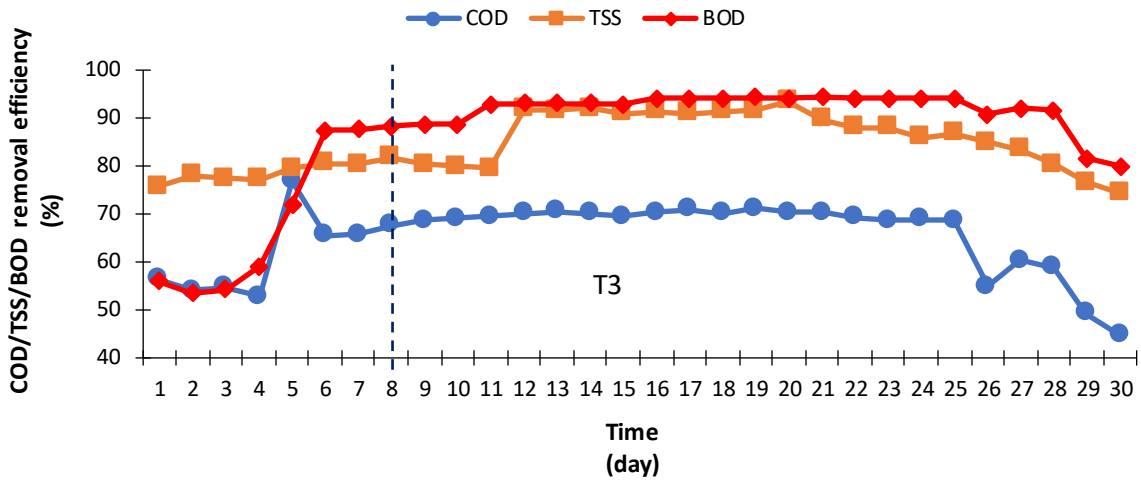
The AD performances of T1, T2, T3 and T4 over 30 days were evaluated. Initial COD, TSS and BOD of the raw POME were 80,030±15,658, 25,080±3,154 and 40,015±11,715 mg/L while for *M. oleifera* extract, 4,350, 6,250 and 2,175 mg/L, respectively. These values were different from those in section 4.2.1 as the tested POME were sampled at two different timing. The coagulated solids would not be removed from the digester. Instead, it would be the nitrogen source and immobilisation media for the anoxic microbial cells. The $COD_{removal}$, $TSS_{removal}$ and $BOD_{removal}$ throughout the study were calculated and compared (Figure 4.10).



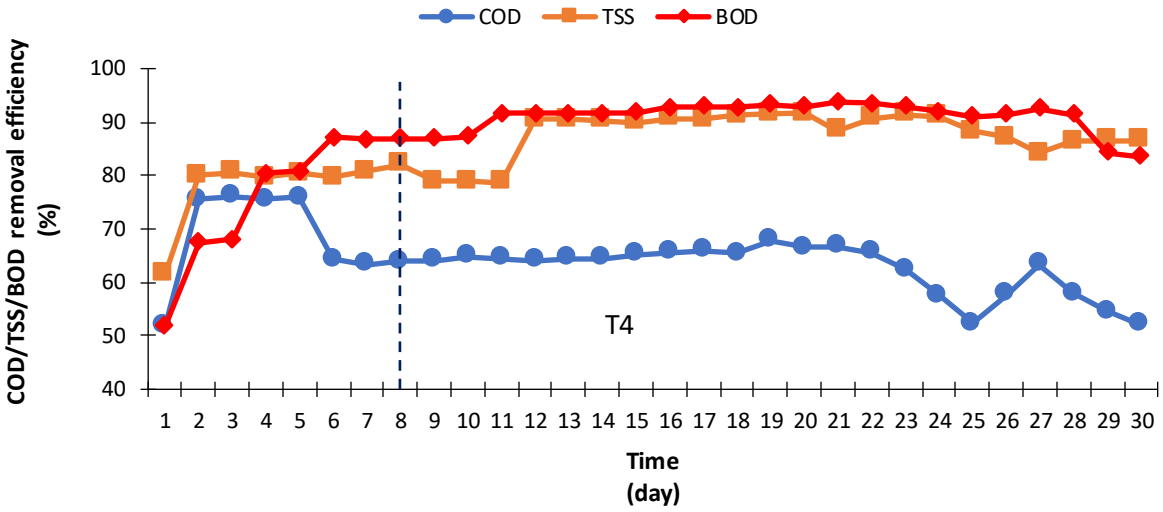
(a)



(b)



(c)



(d)

Figure 4.10: COD/TSS/BOD removal efficiencies profiles for (a) T1, (b) T2, (c) T3 and (d) T4. The start-up period for each study is indicated in blue dotted line. T1: mono-digestion of POME; T2: mono-digestion of *M. oleifera* extract; T3: co-digestion of POME-*M. oleifera* (daily feed) and T4: co-digestion of POME-*M. oleifera* (one-time feed – day 1).

The start-up AD performance was evaluated by determining the removal efficiencies of COD, TSS and BOD in a daily basis. Once all efficiencies determined had remained constant for three consecutive days, the start-up was considered stabilised where bacteria had acclimatised and ready to perform. The start-up AD performance took 8 days for T1, T3, and T4. However, T2 required 11 days to stabilise the start-up performance of the AD (Figure 4.10b). This may be explained by the absence of suitable type of organic/inorganic matter for the anaerobic microbial cells present in the anaerobic sludge. As a result, the anaerobic microbial cells required a longer period of time to acclimitise to the new source of substrate (*M. oleifera* extract). During initial start-up period for all experiments, the $COD_{removal}$ and $BOD_{removal}$ were highly dependent on the pH of the system. A drop in pH was quickly rectified by the addition of $NaHCO_3$. At this situation, the $COD_{removal}$ and $BOD_{removal}$ were mostly low. During the 8-day start-up period for mono-digestion of raw POME (T1), both $COD_{removal}$ and $BOD_{removal}$ maintained at around 43-44% as the pH gradually increased from 6.40 to 6.66. The pH of the system must be maintained between 6.8 and 7.2 for optimum AD. For mono-digestion of only *M. oleifera* extract (T2) without POME, the $COD_{removal}$ fluctuated while $BOD_{removal}$ maintained at around 52% to 89% during the start-up period where the pH was increased to 7.41. *M. Oleifera* provided an improved $COD_{removal}$, $TSS_{removal}$ and $BOD_{removal}$ for both T3 and T4 compared to T1 when co-digested with POME during the start-up period. The $COD_{removal}$, $TSS_{removal}$ and $BOD_{removal}$ of T3 showed a constant increase from 53-77%, 77-82%, and 53-88%, respectively from day 1 to 8. However, drastic increase of $COD_{removal}$ (52-76%), $TSS_{removal}$ (61-82%) and $BOD_{removal}$ (52-87%) was observed at day 2 of T4 treatment. Daily feed of *M. Oleifera* though boosted the removal efficiency within very short period but the effect did not prolong once it was saturated. Most of the set-ups (T1, T3 and T4) were able to reach steady state within 8 days. T2 required 11-days to reach steady state.

After achieving steady-state, the $COD_{removal}$, $TSS_{removal}$ and $BOD_{removal}$ of T1 ranged 61-67%, 50-63% and 86-92% while those of T2 showed 58-76%, 77-99% and 86-93%, respectively. Mono-digestion using *M. Oleifera* alone showed higher COD and BOD removal than POME alone as shown by the higher $COD_{removal}$ and $BOD_{removal}$ of T2 than T1. As T2 exhibited higher VSS: TSS ratio (0.92) than T1 (0.87), the organic matters can be better degraded by the readily available microbial cells present in the anaerobic sludge. Another plausible reason might be the absence of inhibitory compounds, viz. phenol commonly found in wastewater treatment. The mono-digestion of POME and *M. oleifera* extract, T1 and T2, as shown in Figures 4.10(a) and 4.10(b) indicated that the use of *M. oleifera* extract could assist AD of POME to achieve a higher $TSS_{removal}$ compared to that without the extract. In addition, the flocs produced had a higher resistance to breakage caused by intense mixing usually practiced. The low molecular weight coupled with high charge density (dimeric cationic behaviour) of the protein present in *M. oleifera* were responsible for coagulation to happen (Gassenschmidt et al., 1995). Although slightly better $COD_{removal}$ and $BOD_{removal}$ were observed for POME-*M. oleifera* co-digestion (T3 and T4) over POME mono-digestion (T1), their organic pollutant removal was mainly attributed to the resulting coagulating effect on the seed sludge itself where the initial COD and BOD concentrations were found too low for efficient AD.

The coagulated solids were intentionally remained in the digester without being separated as they played an important role during AD by stabilising the growth of the anoxic microbial cells. They act as an immobilisation media for the microbial cells to grow, thereby allowing for occurrence of entrapment of the microbial cells as well as facilitating biofilm formation. The latter prevents the loss of bacteria to maintain the biomass concentration in the digester.

The POME-*M. oleifera* co-digestion as shown in Figures 4.10(c) and 4.10(d) was compared with mono-digestion in Figures 4.10(a) and 4.10(b). The $COD_{removal}$ (T1: 63.59 ± 3.59%; T3: 65.51 ± 5.51%; T4: 68.16 ± 4.51%), $TSS_{removal}$ (T1: 54.18 ± 3.24%; T3: 86.04 ± 5.19%; T4: 86.41 ± 5.04%) and $BOD_{removal}$ (T1: 88.99 ± 3.76%; T3: 88.50 ± 7.11%; T4: 91.36 ± 4.77%) for T3 and T4 were higher than T1. Among the parameters assessed, $TSS_{removal}$ showed the largest difference between mono- and co-digestion, at 60% and at least 90%, respectively. The dimeric cationic protein in *M. Oleifera* was responsible to adsorb and neutralise the colloidal charges of POME solids so that the solids could be coagulated when adequate residence time was given (Ndabigengesere et al., 1995). This helped increasing the surface contact between the microbial cells of the anaerobic sludge and the organic matters of POME which were made readily available in the form of larger and denser flocs. The addition of *M. oleifera* extract did not contribute to the reduction of COD and BOD as shown by almost similar removal efficiencies exhibited for both treatments. Hence, their removal efficiencies came from the breakdown of POME by the anaerobic microbes instead of *M. oleifera* extract. Interestingly, the anaerobic treatment of POME and *M. oleifera* extract had turned the colour of POME mixture from light brown to dark brownish. According to Poh et al. (2010) and Nurliyana et al. (2015), the darkened colour might be due to the presence of lignin compound as well as other non-biodegradable compounds. In this study, the coloured pigments were believed to come from the *M. oleifera* extract itself as it is a plant material. In addition, the usual unpleasant sour odours of POME caused by acidic compounds present had also reduced. This corresponded well to the changes of pH of the co-digested POME mixture over a 30-day period from acidic (4.30) to neutral (7-8). This allows us to postulate that the use of *M. oleifera* extract could help maintaining the pH of POME through charge neutralisation (Ndabigengesere et al., 1995), probably caused by the ability of the protonated protein to degrade the nitrogen compound (NO_3^- and NO_2^-) into ammonia, and PO_4^{3-} into phosphate salt, in POME. This phenomenon is capable of overcoming the acidity in POME mainly due to volatile fatty acids generated during degradation of carbohydrates and fats/lipids. The resulting effluent with a pH near neutral has thus reduced the electrostatics attraction of TSS making them more stable (Azmi et al., 2014; Ng et al., 2019) with less floating concentration in POME matrix; which then could reduce the TSS concentration to prevent formation of effluent with high turbidity.

The fed-in frequency/mode of *M. oleifera* extract for the co-digestion study was evaluated (T3 and T4). In Figures 4.10(c) and 4.10(d) where *M. oleifera* extract was either added daily into the digester (T3) or just once during day 1 of the study (T4), the $COD_{removal}$, $TSS_{removal}$ and $BOD_{removal}$ ranges were almost similar. This implies that dosing mode of *M. Oleifera* does not affect co-digestion performance, and can be flexibly used to increase POME AD treatment efficiency for solids (TSS) removal. This result confirms other previous findings showing that *M. oleifera* has excellent coagulation property in treating water and wastewater (Ndabigengesere et al., 1995; Othman et al., 2008). Other studies (Muyibi and Evison, 1995; Ndabigengesere and Narasiah, 1998; Okuda et al., 2001; Bhatia et al., 2007a; Bhatia et al., 2007b; Adam and Ali, 2017) have also shown that *M. Oleifera* is able to produce effluent with low TSS concentration and turbidity. The positive development of POME AD treatment by using natural and biodegradable coagulants would be a paradigm shift in searching for cost-effective management of POME.

From the economic and commercial points of view, inclusion of *M. oleifera* extract for POME AD is not yet matured and requires further exploitation before it can be accepted as the solution for the palm oil industry. Large volume of POME produced daily and drastic fluctuation of the characteristics of POME might affect performance efficiency and consistency. The efficiency of digesting POME with this substrate in a large-scale prototype will be developed to allow simultaneous digestion of *M. oleifera* extract with POME to produce convincing commercial data. Figure 4.11 compares removal efficiencies of COD, BOD and TSS of POME mono-digestion and POME-*M. oleifera* co-digestion.

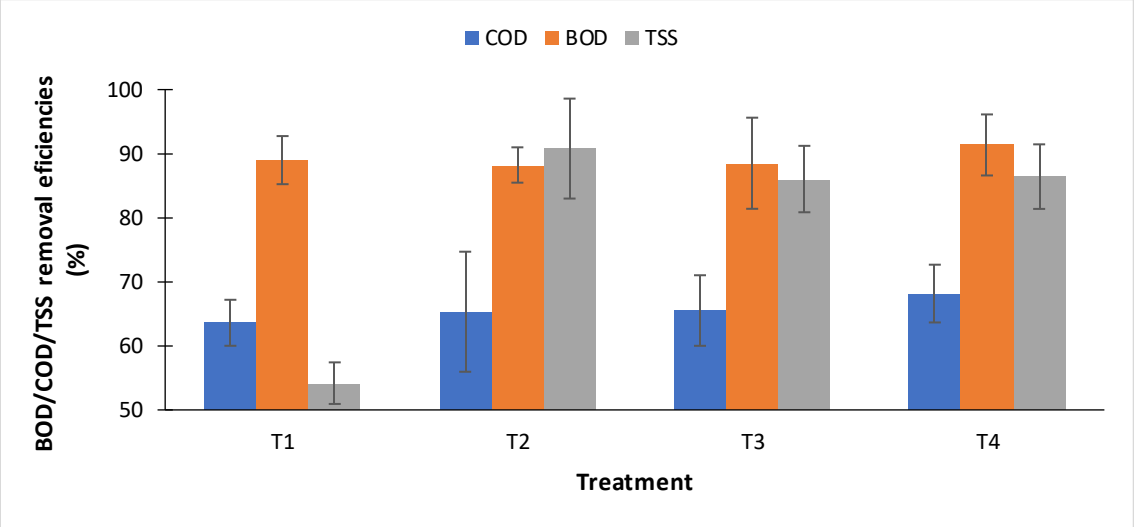


Figure 4.11: Average COD/TSS/BOD removal efficiencies of POME anaerobic digestion treatment during steady state period. T1: mono-digestion of POME; T2: mono-digestion of *M. oleifera* extract; T3: co-digestion of POME-*M. oleifera* (daily feed) and T4: co-digestion of POME-*M. oleifera* (one-time feed – day 1)

The addition of *M. oleifera* extract had successfully improved the $COD_{removal}$, $BOD_{removal}$ and $TSS_{removal}$ of POME mono-digestion. In particular, the effect of *M. oleifera* extract was more prominent for $TSS_{removal}$ showing huge improvement up to 59-60% in T3 and T4, respectively as compared to T1. The protein present in the *M. oleifera* extract was able to effectively coagulate the solids in the POME, thereby increased the retention time between POME and methanogenic bacteria, instead of being carried over to the outlet of the anaerobic digester as solid washout (refer to Section 4.2.2.2).

4.2.2.2 Methane Productivity

POME treated via AD will produce biogas (methane). In this study, T1, T3 and T4 were monitored for methane productivity while the involvement of T2 in AD was negligible. As mentioned, (Section 4.2.1.), while it seemed that COD removal was relatively high in T2 (Figure 4.11), the fact that it exhibited a very low initial COD content (Section 4.2.2.2.1.) had translated to a very small absolute COD removal, hence hardly any detectable methane in T2 as shown in Figure 4.12. The pH of T1, T3 and T4 prior to AD were 4.30±0.29, 4.30±0.29 and 4.30±0.29. These pH values differed from those in section 4.2.1 due to different sampling time. After dosing with *M. oleifera* extract, their final pH was 7.03±0.59, 7.03±0.45 and 7.00±0.36, respectively. These pH values were similar as they contained mainly POME of similar characteristics. As the anoxic microbial cells continuously consumed the protein-rich organic matter from *M. oleifera* extract, alkaline condition was created. During POME AD by hydrolytic, acidogenic and acetogenic bacteria, organic acids were produced and then degraded by the methanogenic archaea to produce methane (Nurliyana et al., 2015).

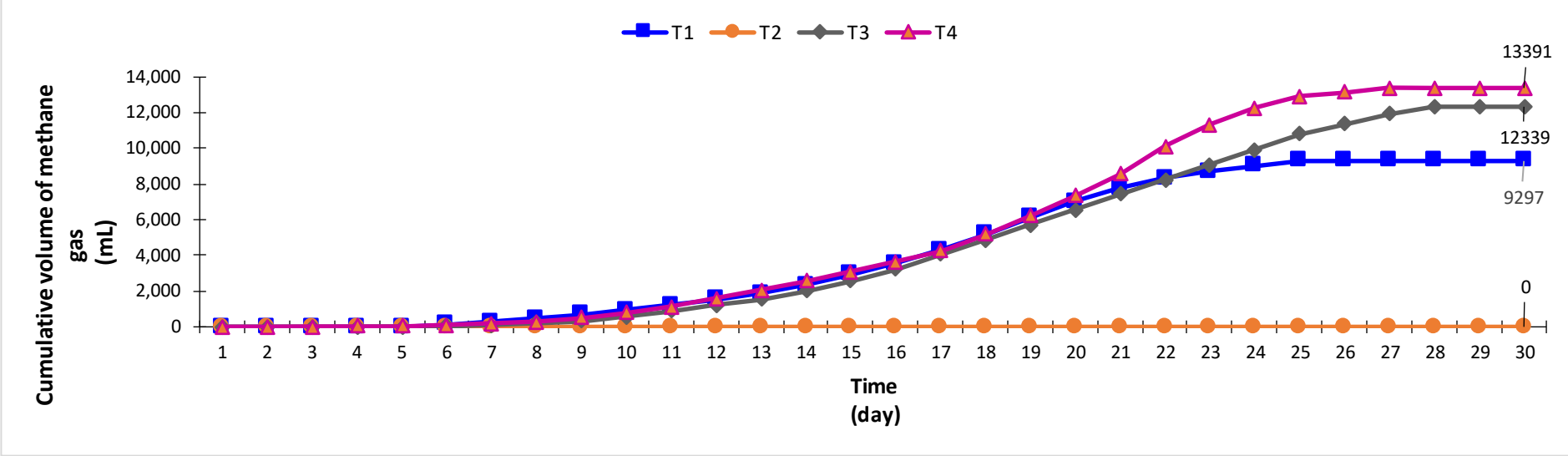


Figure 4.12: Cumulative volume of methane from each treatment over a period of 30 days. T1: mono-digestion of POME; T2: mono-digestion of *M. oleifera* extract; T3: co-digestion of POME-*M. oleifera* (daily feed) and T4: co-digestion of POME-*M. oleifera* (one-time feed – day 1).

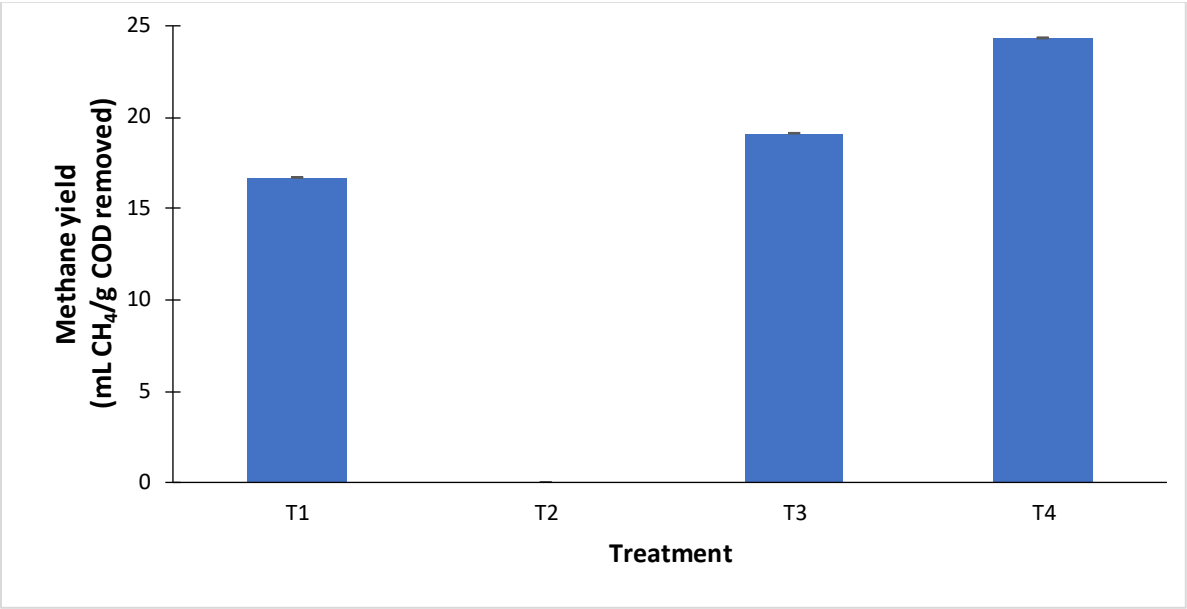


Figure 4.13: Methane yield from each treatment. T1: mono-digestion of POME; T2: mono-digestion of *M. oleifera* extract; T3: co-digestion of POME-*M. oleifera* (daily feed) and T4: co-digestion of POME-*M. oleifera* (one-time feed – day 1).

Figure 4.12 shows a cumulative volume of methane produced from each POME AD treatment for a 30-day period. Methane production for T1, T3 and T4 begun at day 6, 5 and 3, respectively. Dosing of *M. oleifera* extract as co-digestion substrate (T3 and T4) in POME had assisted methane production at an earlier time than that of T1 and accumulated more methane over the same period. In addition, the methane production of POME-*M. oleifera* co-digestion was further enhanced and its period of production prolonged till the 27th and 28th day than that of POME alone which stopped on the 25th day. This study also proved that *M. oleifera* alone (T2) is definitely not a suitable substrate for AD but can be used to enhance methane production of other organic substrates via AD, such as POME in this study due to its excellent coagulating property.

POME-*M. oleifera* co-digestion (T4, one-time feed) at day 1 was able to produce 1.5 times higher methane yield (46% improvement) compared to POME mono-digestion (T1), and the production was ceased at day 27. On the other hand, POME-*M. oleifera* co-digestion (T3, daily feed) produced 1.14 times higher methane yield (14% improvement) compared to POME mono-digestion, and the production was stopped at day 28. This showed that one-time feed mode of *M. oleifera* extract in POME was sufficient to improve the methane production and yield. T4 gave the highest methane productivity i.e., 13.391 L CH_4 followed by T3, 12.339 L CH_4, over 30 days; both of which were higher than that of POME mono-digestion (T1), 9.297 L CH_4 over the same period. For T3 and T4, methane production was initially slow on the 4th day but rapidly increased after coagulation took place. For T1, methane was produced continuously starting from day 6 to day 21.

The findings showed an improvement in methane generation using *M. oleifera* as a co-digestion substrate. The relatively high content of protein (36.7%) exhibited by *M. oleifera* extract has probably altered and improvised the initial C/N ratio of POME critical for microorganism rejuvenation (Ndabigengesere et al., 1995). During AD, a relatively high concentration of nitrogen source from the substrate used is much anticipated to enable the microbial cells to continuously replicate among themselves and be in tip-top condition to perform organic degradation. Table B1 (Chapter 7, Appendices) shows the weekly ratio of C/N exhibited by the pure and mixed substrates, T1–T4. The C/N ratio after dosing with *M. oleifera* extract has significantly decreased, and is approaching C:N ratio for adequate microbial function. It is vital to maintain a balanced C/N ratio to allow optimum POME AD performance in getting more methane. A low C/N ratio will result in an increased ammonia concentration causing inhibition of methanogenic archaea (Nurliyana et al., 2015; Astals et al., 2018) while a high C/N ratio will disrupt the formation of protein and reduce the energy and structural metabolism of the microorganisms, which will then create an imbalance proportion between viable and death microbial cells (Deublein and Steinhauser, 2008; Nurliyana et al., 2015).

Methane yield in Figure 4.13 was the total volume of methane gas produced per mass of COD removed. From the obtained results, T4 showed the highest methane yield of 24.3 mL CH_4/g COD removed compared to T3 and T1. Daily feed of *M. oleifera* extract (T3) did not increase the methane yield further compared to one-time feed mode (T4). Both feeding modes gave an improved optimum C/N ratio ranged 18-20 for anaerobic co-digestion in this study (Table B1, Chapter 7, Appendices), and is within the range recommended by literature i.e., 16-19 (Sidik et al., 2013). It is essential to maintain the C/N ratio of POME in this range to provide a balanced food-to-microorganism ratio for optimum AD performance.

Another factor that might contribute to an increased methane yield is solid retention time (SRT) of the anaerobic sludge in the digester. SRT is crucial to allow sufficient time to be spent by microorganisms for metabolism and reproduction among themselves. Practically, the SRT must be longer than the regeneration time of a particular organism to allow for occurrence of microbial cells proliferation (Smith et al., 2013). Otherwise, it will be washed out of the system. Generally, the SRT for AD must be >20 days at mesophilic condition to ensure trouble free operation and better system performance.

Nges and Liu (2010) showed that reduction of SRT from 35 days to 30 days under both thermophilic and mesophilic conditions has increased methane yield and composition of methane in biogas from 0.307 m^3 (STP) CH_4 kg VSS added/day to 0.392 m^3 (STP) CH_4 kg VS added/day and 54% to 59%, respectively. Methane yield had increased in both the co-digestion systems (T3 and T4), i.e., 19.12 mL $CH_4/gCOD_{removed}$ and 24.34 mL $CH_4/gCOD_{removed}$ compared with T1, 16.70 mL $CH_4/gCOD_{removed}$. The addition of *M. oleifera* extract had allowed the solids of the incoming POME to stay longer (SRT determined in this study >30 days) and perform the desirable coagulation in the digester. This high SRT enabled an enrichment of slowly growing methanogen bacteria for establishment of a more diverse biocoenosis with broader physiological capabilities compared to that without *M. oleifera* extract (T1). As a result, it aided and accelerated the methane production. Throughout the entire study, the $TSS_{removal}$ of T1 was unable to reach up to an expected maximum of 90%, instead, the highest was only 63%. This removal was lower compared to those achieved maximally via T2 (99%), T3 (92%) and T4 (92%). The solids were unable to be retained in T1 for a longer period for maximum biodegradation activity due to the absence of coagulating agent. Contrarily, the protein in the *M. oleifera* extract was able to coagulate the solids in T3 and T4 forming flocs (Gassenschmidt et al., 1995) that had facilitated biodegradation activity due to disposal of larger surface areas as compared to the non-agglomerated solids in T1. A maximum contact between the microbial cells and the larger flocs (i.e., immobilisation media) thus enhanced biodegradation activity in producing more methane at the end of AD.

The methane production for T1, T3 and T4 gradually increased and reached maximum levels before decreasing gradually to reach a stabilised phase. Probably, the production at this point faced limited readily available biodegradable materials for consumption; hence a restricted biological activity occurring. Most of the remaining complex materials e.g., lignin would have been entrapped within the complicated cell walls of the digestate and are largely not easily accessible to the intended microbial cells.

Moringa seeds are one of the most promising natural coagulants discovered so far as they are essentially effective for treatment of high turbidity water and have coagulation effects comparable to alum (Muyibi and Evison, 1995; Anwar et al., 2007). Based on the findings, co-digestion of POME with *M. oleifera* extract having a concentration of 1,000 mg/L at one-time feed mode (day 1) is recommended. Its addition aids high $TSS_{removal}$ and methane production offering AD system a better performance compared to conventional system.

Literature search reveals that the protein content of *M. oleifera* seed is affected by both genetic and environmental factors (Abdulkarim et al., 2005). In 2016, Leone et al. (2016) conducted a first critical review focusing on composition of *M. oleifera* seeds and oil. The disclosed main chemical compositions (g/100 g of dry weight) are fat (36.7), protein (31.4), carbohydrate (18.4) and fibre (7.3). In Zhao et al. (2019), the protein contents of *M. oleifera* seeds from different regions - China (38.1%), India (36.2%) and Pakistan (28.5-34%) (Anwar et al., 2004) - are close to that from Malaysia (38.3%) (Abdulkarim et al., 2005). On average, the protein content is affirmed to be >30%. The ability of *M. oleifera* seeds to function as a flocculating agent has been well documented as early as 1995 by Gassenschmidt et al. (1995) and Ndabigengesere et al. (1995), through sequencing of the ion-exchange chromatographic isolated flocculating proteins from the seeds of *M. oleifera* Lam. and determination of the positive charges of the active cationic behaviour responsible for coagulation. Leone et al. (2016) also disclosed the presence of a short cationic protein, known as the *M. oleifera* cationic protein that causes bacterial cell damage through rapid flocculation and cell fusion. From these studies, it is clear that the protein present in *M. oleifera* exhibits flocculating activity due to the cationic nature. The cationic nature of the flocculating proteins may be attributed to the containing amide or -N=C- group and nitrile or C≡N group (Faizi et al., 1994), which we figure that they exhibit electron lone pairs that can be donated to any potential receptor, in this case the TSS; hence offering the binding capability. This could further lead to association of two such proteins to bind and form dimeric cationic protein (Ndabigengesere et al., 1995) due to the unshared electron lone pairs present.

Undeniably, the increase of biogas/methane productivity in the co-digestion might also be contributed by the carbohydrate in the *M. oleifera* extract, which appears quite substantially varying from 5% (Ndabigengesere et al., 1995) to 18% (Leone et al., 2016). Together with the approximated carbon content of 55% (Ndabigengesere et al., 1995), *M. oleifera* is a rich carbon source for microbial rejuvenation. In addition, *M. oleifera* seed also contains minerals such as K (256-563), Ca (124-314), Fe (3.5-5.9) (mg/100 g), and others (Zhao et al., 2019). K and Ca are the two main minerals present. As water was used to extract *M. oleifera* seed in this study, there is possibility that these inorganic elements could have been readily dissolved and may further contribute as well in coagulating TSS or O&G of POME. Variation exists in minerals content from literature data so it will be more rationalise to carry out extract characterisation in future study so as to derive the extent (degree) of their contribution in coagulation beside the flocculating protein as determined in this study.

4.2.3 Identification of Methanogen in Mono- and Co-Digestion

In order to gain more insights into biodegradation of co-digestion system, the amount of methanogen present in each of the treatment system was quantified. Such identification has direct correlation with the methane yield obtained (Section 4.2.2).

4.2.3.1 Raw POME, Granulated Sludge and Treated POME

Little is known regarding the diversity and presence of methanogens in POME. Nurul Adela et al. (2014) initiated a study to look into the morphological and microscopic behaviours of the microbial communities in a digester. The study stressed a need to use molecular technique to identify the microbial communities responsible for organic degradation. Tabatabaei et al. (2009) and Meesap et al. (2012) used *E.coli* to clone the plasmid vector that contained the purified 16S rDNA to quantify methanogens of POME. The purified 16S rDNA was obtained via PCR-based DGGE method. In this study, the 16s RNA was targeted to extract complementary DNA (cDNA) which is technically more advantageous to allow identification of methanogen in POME samples via PCR and sequencing. DNA extracted from POME was used as standard for qPCR assay. The gel electrophoresis image (Figure 4.14) shows DNA purity and concentration in raw POME, granular sludge and anaerobically-treated POME. Since these samples are decaying organic matters, one of the many compounds they degrade into is humic acid which is known to be an inhibitory compound in PCR (Tebbe and Vahjen, 1993; Amha et al., 2018 and Yap et al., 2018). Although POME contains high amount of organic matters where the high humic acid content is a potential PCR inhibitor, it is not encountered in this study.

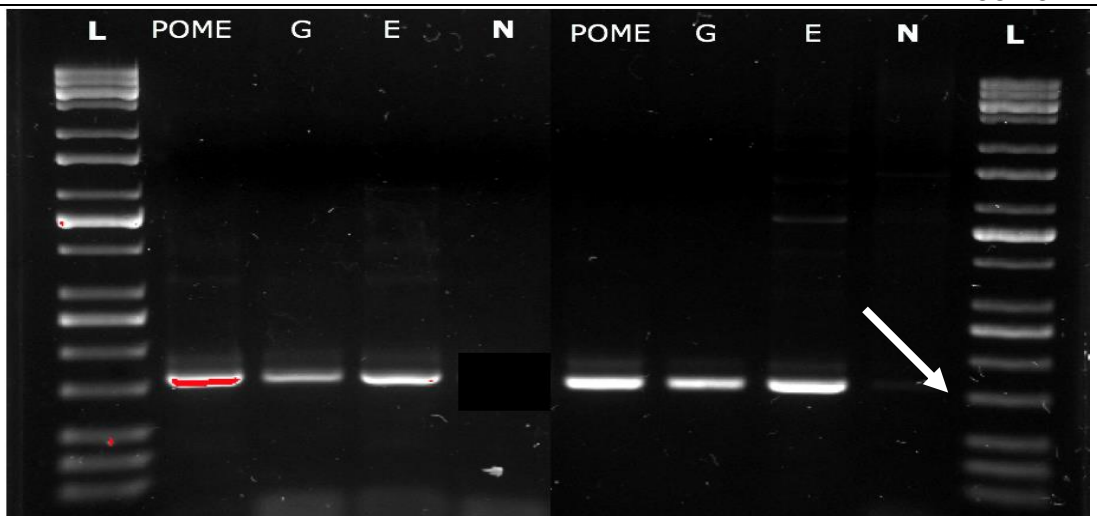


Figure 4.14: Gel electrophoresis image of raw palm oil mill effluent (POME), granular sludge (G) and anaerobically-treated POME (E) using protocol A (left) and B (right) with negative control (N) and METH primers

The METHF and METHR primers used in this study showed high specificity towards the five orders of known methanogenic archaea, thus was used in cloning library for archaeal communities analysis and PCR-single strand conformation (Watanabe et al., 2004). The preliminary results showed that most of the methanogens present in the DNA of raw POME, granular sludge and anaerobically-treated POME were *Methanoculleus* spp. and *Methanomicrobiaceae* spp. (Table B2, Chapter 7, Appendices). Although the results obtained were similar to Watanabe et al. (2004), an additional species previously reported in paddy fields, oil reservoirs, AD and sludge (Dianou et al., 2001; Imachi et al., 2008; Sakai et al., 2011; Tian et al., 2009 and Watanabe et al., 2004), *Methanolinea* spp., was reported. POME and its anaerobically-treated sample had the highest probability of *Methanoculleus* spp. while granular sludge was most likely a *Methanolinea* spp.

4.2.3.2 Mono- and Co-Digested POME Samples

The total DNA concentrations (consisting of mixtures of methanogen and non-methanogen) for T1, T2, T3 and T4 were evaluated as shown in Figure 4.15. On the other hand, Figure 4.16 shows methanogen DNA only. The extracted DNA was analysed for concentration and purity. The concentrations of DNA in all samples were lower in the first 7 days (below 50 ng/µL) compared to days 22 to 24 (250-450 ng/µL) of the treatment Figure 4.15). POME mono-digestion (T1) experienced a significantly increased amount of methanogen DNA from week 1 to week 3 in the treatment but reduced in week 4 (Figure 4.15). The source of methanogen in T1 was possibly from the existing sludge in the cooling pond where the POME was extracted as well as from the sludge inoculum used. Storage of POME overtime as in T1 could increase the population of methanogen. However, overloaded substrate in the digester might create a toxic environment inhibiting growth and survival of the overpopulated methanogen as seen in week 4 of T1. Hence, high microbial activity must be balanced with population to maintain and sustain sufficient quantity of methanogen in treating POME. Chen et al. (2008) showed that sulphate reducing bacteria would outcompete with native methanogen for the same source of organic/inorganic substrates which then could probably cause reduction in population size. Temperature can be one of the contributing factors that leads to predominance of methanogen population in the anaerobic digester. Sulphate reducing bacteria will be dominant when temperature of the digester is higher at 37°C or under thermophilic conditions (Chen et al., 2008; Moestedt et al., 2013). The average temperature employed throughout the experimental period for all the four treatments was 28.43°C (mesophilic), therefore methanogen population size maintained for AD. It is essential to maintain temperature at <37°C to avoid creating unnecessary stressful competition for the same source of substrates, viz. acetate, hydrogen, propionate and butyrate which are the important fermentation intermediates during AD.

DNA concentration of methanogen in T2 showed an increasing trend starting from week 2 to week 4 (Figures 4.15 & 4.16. The growth of methanogen was higher in *M. oleifera* extract (T2) compared to POME (T1). The results showed that *M. oleifera* extract alone had provided a more suitable environment for growth of methanogen and other microorganisms as compared to just POME alone. In particular, there was no inhibitory substances such as phenolic compounds in T2 unlike that in POME (T1) (Mamimin et al., 2012; Saifuddin et al., 2014; Chantho et al., 2016). T2 had also provided an alkaline pH (7.55±0.20) favouring the growth of methanogen. Previously, Hernandez and Edyvean (2008) encountered a significant reduction of biogas production due to presence of high amount of phenolic compounds in anaerobic sludge. Generally, plant-based nutrients are well-digested by microorganisms. A metagenomics study in cow rumen has identified microbes that have genes in degrading cellulosic materials, allowing digestion of plant biomass (Hess et al., 2011). Another similar study by Klocke *et al.* (2008) managed to use plant biomass in a two-phase biogas reactor. These results so far prove similar methanogens as found in our study that are able to degrade plant biomass present in POME. Based on these findings, *M. oleifera* can be a suitable media to sustain methanogen and other microorganism growth. However, if it is digested alone, its COD concentration

(4,350 mg/L, Section 4.2.2.2.1) is too low to generate enough biogas to meet the economic target for renewable energy production. Failure of T2 to produce biogas might be also due to absence of favourable substrates for the production of volatile fatty acids (i.e. intermediate product for methane production) by acidogen and acetogen.

Figure 4.16 shows that methanogen required more time to be acclimatised in co-digestion compared to mono-digestion as it was only detected in week 2 for T3 and T4. As shown, although there were significant methanogen DNA concentrations detected at week 1 for T3 and T4, the amount was more or less similar to that in T1. Hence, they might be from the same substrate used in this study and not derived from additional substrate - *M. oleifera* extract added due to acclimatisation. This was also evidenced by the production of biogas starting from day 8 (beginning of week 2) for T3 and T4 (Figure 4.12). The methanogen concentration was found to be higher in T4 compared to T3 by weeks 3 and 4. Such results explained the higher methane productivity (Section 4.2.2.2) and better removal efficiencies of COD, TSS and O&G (Section 4.2.2.1) for T4.

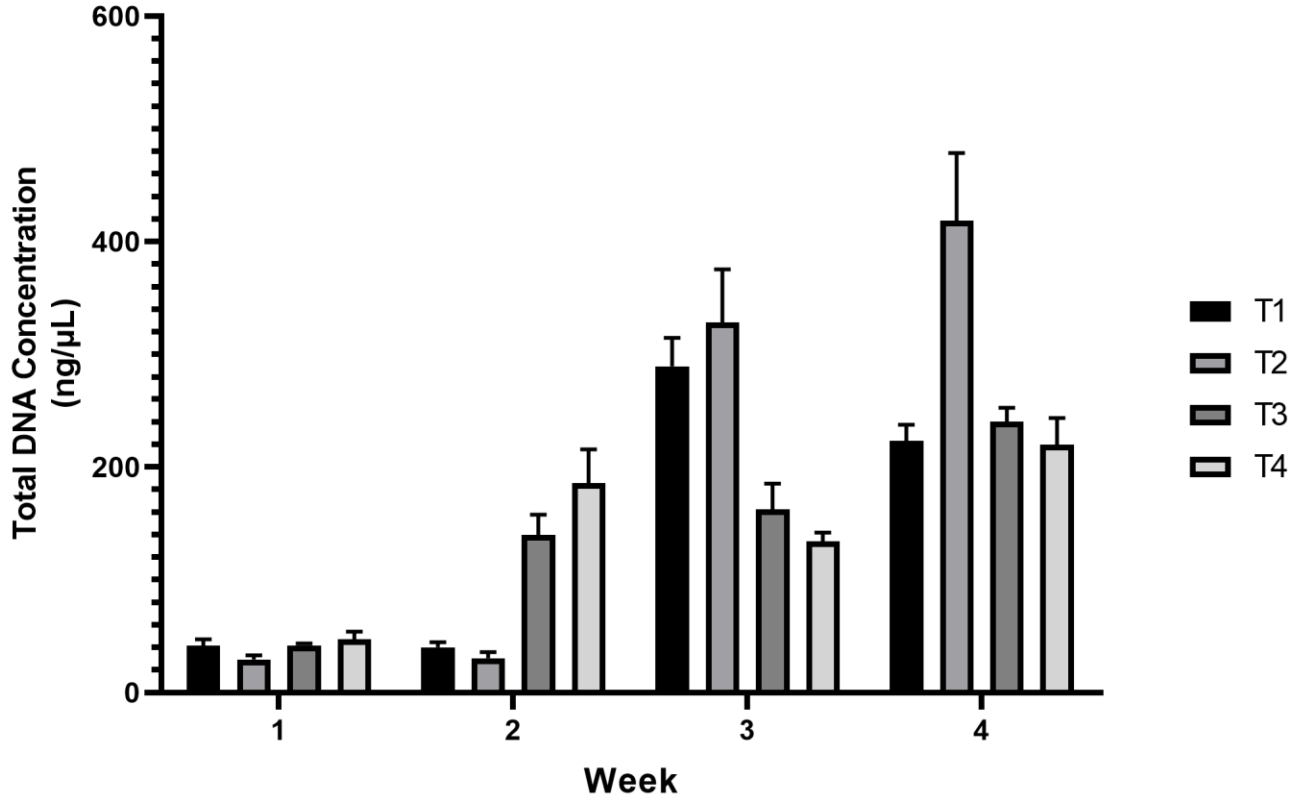


Figure 4.15: Total DNA concentration against time based on qPCR on a weekly basis. T1: mono-digestion of POME; T2: mono-digestion of *M. oleifera* extract; T3: co-digestion of POME-*M. oleifera* (daily feed) and T4: co-digestion of POME-*M. oleifera* (one-time feed – day 1).

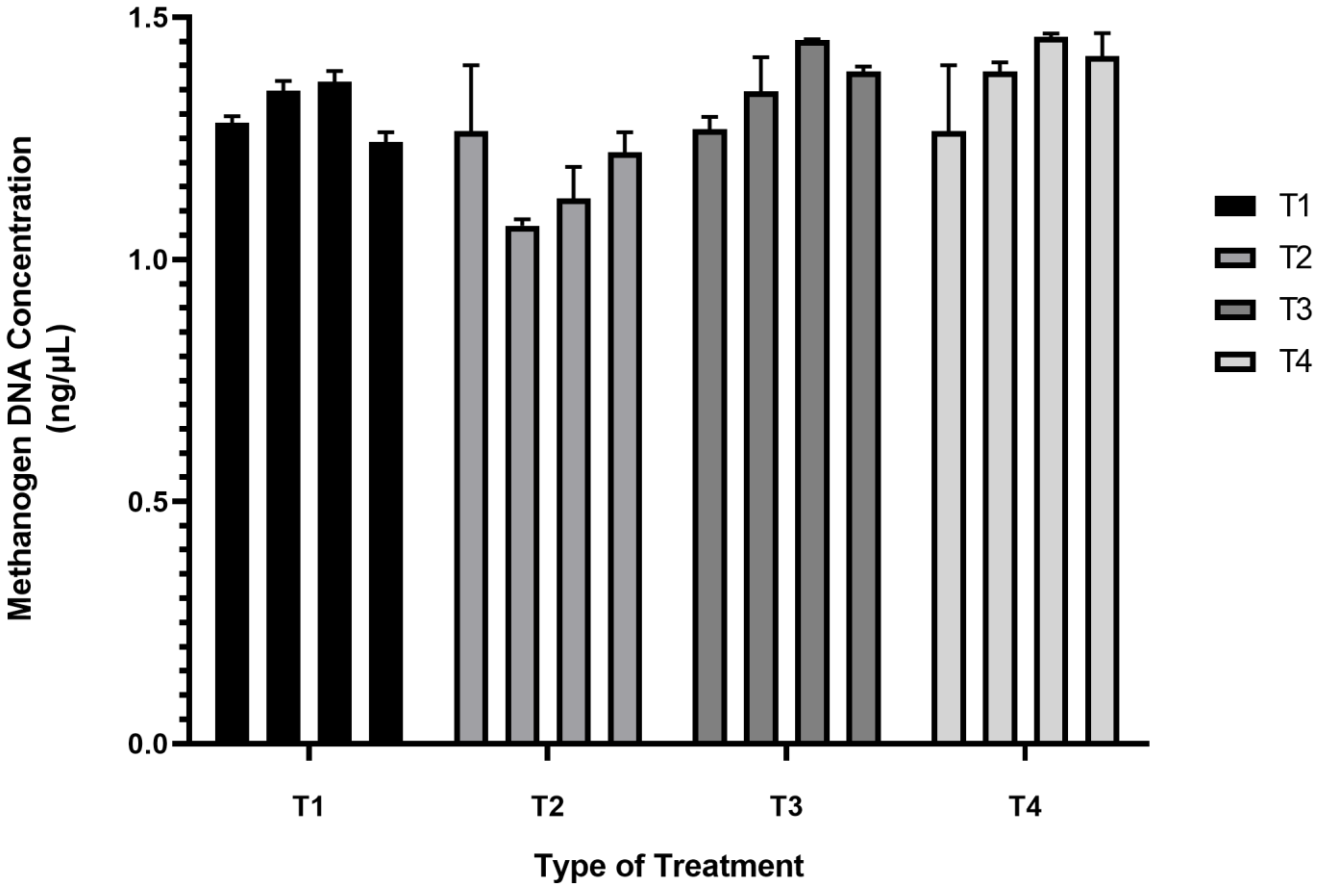


Figure 4.16: Quantification of methanogen DNA concentration of treatment samples subjected to qPCR on a weekly basis. T1: mono-digestion of POME; T2: mono-digestion of *M. oleifera* extract; T3: co-digestion of POME-*M. oleifera* (daily feed) and T4: co-digestion of POME-*M. oleifera* (one-time feed – day 1).

Methanogen DNA from T1, T2, T3 and T4 was further analysed using gel electrophoresis. T2 exhibited single band throughout the study indicating presence of only one type of DNA. T1, T3 and T4 had more than one type of methanogen DNA, hence they were sequenced separately for the identity of methanogen (Table B3, Chapter 7, Appendices). The METH primers adapted from Watanabe et al. (2004) exhibited high specificity towards *Methanobrevibacter* spp., *Methanoculleus* spp. or *Methanosarcina* spp. However, T2 had the highest similarity to *Methanosaeta* spp., while the rest of the samples showed a mix of *Methanosaeta* spp. and *Methanobrevibacter* spp.

Methanosaeta spp. could be the most dominant methanogenic archaea in the nature due to its ability to thrive in a vast ecosystem probably due to its uniqueness in undergoing aceticlastic methanogenesis pathway whereby they utilise acetate as a carbon source and electron donor (Smith and Ingram-Smith, 2007). *Methanobrevibacter* spp. on the other hand especially *Methanobrevibacter smithiii* is the most studied as it is found inhabiting the human gut. *M. smithii* is the most dominant methanogenic archaea in the human gut microbiota of about 10% and is also found in our faeces (Samuel *et al.*, 2007). Based on the finding by Watanabe *et al*. (2004) in paddy field soil, *Methanosarcinales and Methanomicrobiales* are closely related to *Methanosaeta.* However, in this study *Methanobrevibacter* spp. and *Methanosaeta* spp. were identified using the same primer as Watanabe et al. (2004). This explains that wild methanogens are present in a diverse environment; they are also diverse in their speciation.

4.2.3.3 Quantification of Methanogen in Mono- and Co-Digested POME

There are also other similar studies using qPCR-based method to quantify the number of methanogens present and methane gas production mostly from poultry such as chickens (Saengkerdsub et al., 2006; Saengkerdsub et al., 2007). Other methanogen quantification methods employed such as methanogen biomass is calculated by quantitation of coenzyme M (Elias et al., 1999) and by oligonucleotide probe hybridization (Raskin et al., 1994). This is the first reported study of methanogen identification and quantification using two-step cDNA and qPCR assay for production of biogas from POME. Compared to PCR-based DGGE method using 16S rDNA by Tabatabaei et al. (2009) and Meesap et al. (2012), the method used in this study is simpler, flexible and highly sensitive for low sample volume with more stable cDNA pool for multiple use, and allow parallel and sensitive quantification of absolute amounts of transcripts. The advantage of using qPCR in this study is real time automated identification of organism present in POME without post amplification analysis which saves time (Smith and Osborn, 2009). The knowledge gained about methanogen taxonomy and population growth in POME could be beneficial in designing wastewater treatment for efficient biogas production in a large-scale POME treatment plant. The total DNA concentration of T4 was significantly different amongst the treatments employed but insignificant for the methanogen DNA except for T2 on a weekly basis (Figures 4.15 & 4.16).

The quantity of methanogen in the inoculum (1 mg of anaerobic sludge) was determined for all the 4 treatments (Figure 4.17) at the end of the experiment based on the methanogen DNA concentration quantified by the qPCR. Both the POME-*M. oleifera* co-digestion at different feed mode (T3 & T4) demonstrated a significant difference in the quantity of methanogen obtained in the inoculum. No difference was obtained during the quantification of methanogen DNA concentration using qPCR method (Figure 4.16). This allow us to conclude that addition of *M. oleifera* extract as a substrate for co-digestion is able to enhance the AD by increasing the nitrogen content in the substrate (POME) as reflected by the resulted C/N (Table B1, Chapter 7, Appendices). Despite POME being the source of biogas production (proven in T1), it lacks sufficient amount of nutrients for consumption by methanogen to increase biogas production. In this study, the incorporation of *M. oleifera* extract into POME AD has drastically increased biogas production as well as enhanced organic degradation without imparting any inhibitory effect for the methanogens to grow and replicate for the generation of biogas. The outcomes obtained in this study allow us to infer that *M. oleifera* is suitable to be co-digested with POME due to the high removal efficiencies of TSS of more than 90% (Figure 4.10). Such unique approach of the T4 treatment (one-time-feed - day 1) will be used in the subsequent study using a larger prototype placed in an existing palm oil mill with constant fluctuation of the characteristics of POME (refer to Section 4.3.4).

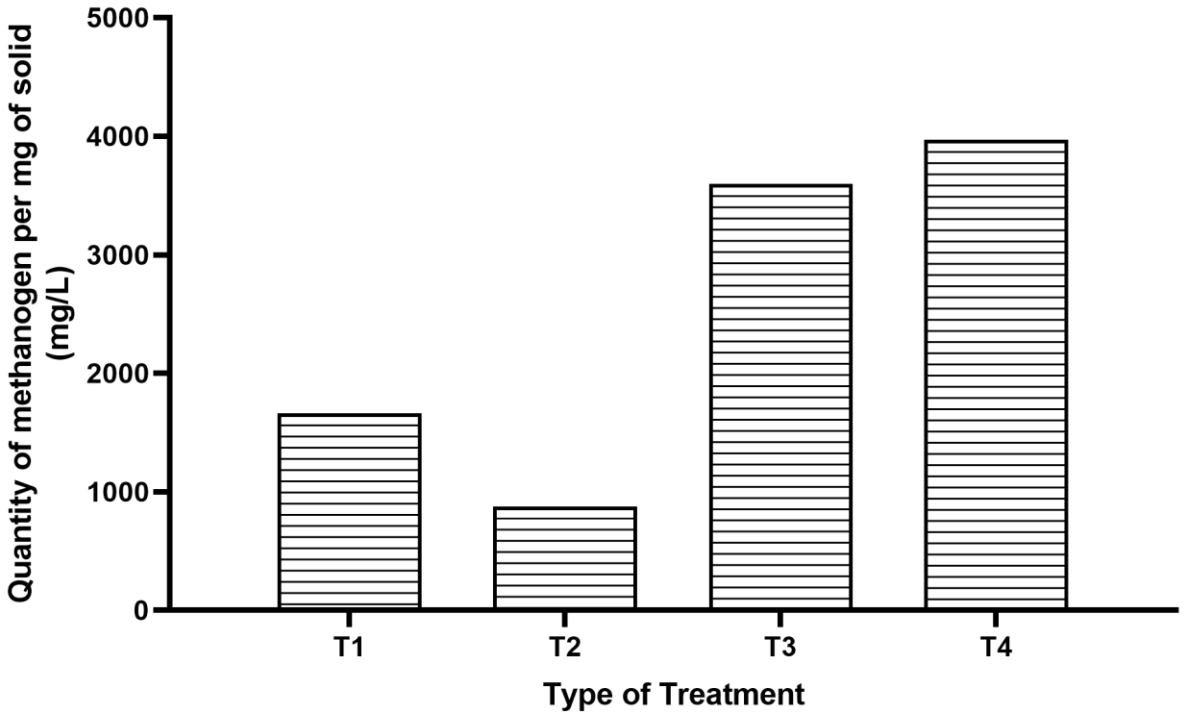


Figure 4.17: Quantity of methanogen present in the anaerobic sludge at the end of the experiment for respective treatment. T1: mono-digestion of POME; T2: mono-digestion of *M. oleifera* extract; T3: co-digestion of POME-*M. oleifera* (daily feed) and T4: co-digestion of POME-*M. oleifera* (one-time feed – day 1).

4.3 Pilot-Scale Integrated Anaerobic-Aerobic Bioreactor (IAAB) Study

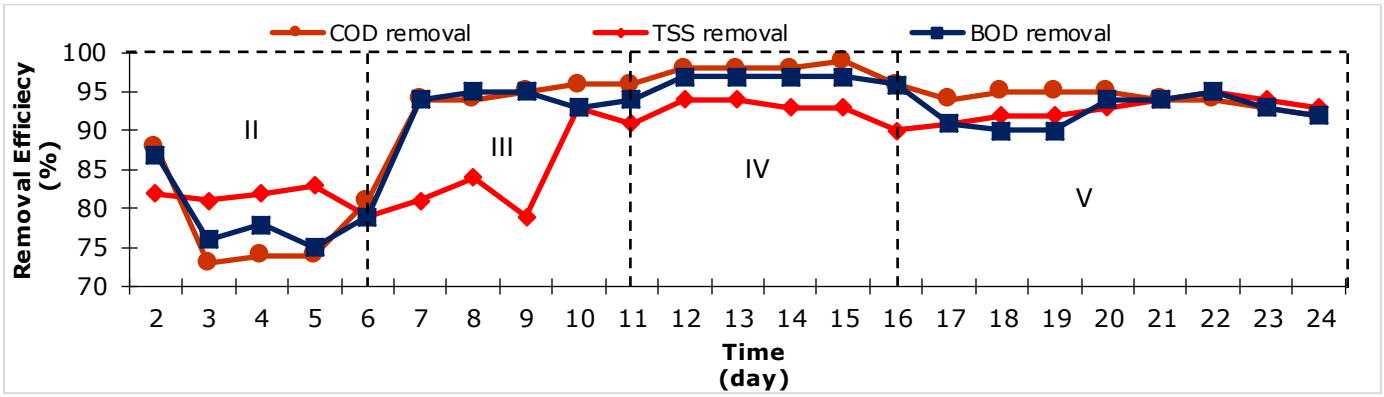
4.3.1 Start-up of the Pilot-Scale IAAB

The start-up of the pilot-scale IAAB was divided into two parts; first, the initialisation of the anaerobic compartment and then followed by the aerobic and settling compartments of the pilot-scale IAAB once the anaerobic process has shown stable operation.

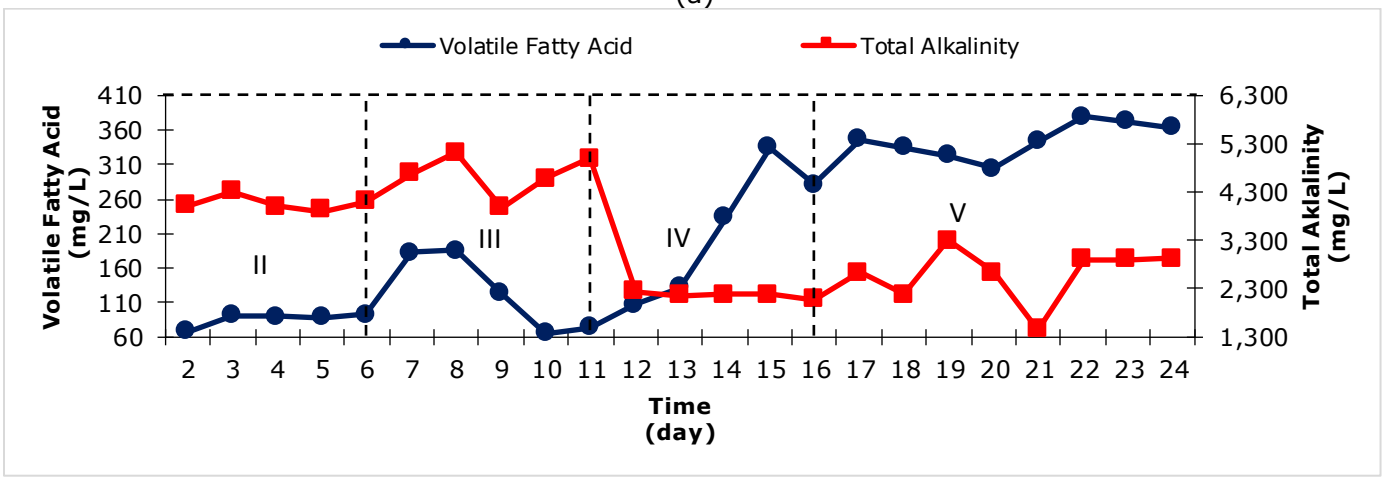
4.3.1.1 Anaerobic Process

Adapting a suitable start-up procedure for the pilot-scale IAAB is relatively essential to prevent the build-up of unnecessary stress in the anaerobic compartment. It is aiming to grow, build up and retain sufficient concentration of active and well-balanced biomass. The start-up of the pilot-scale IAAB was carried out following the procedure used by Chan et al. (2012a) to allow direct comparison between the laboratory scale IAAB and pilot-scale IAAB. The approach used in the start-up of the pilot-scale IAAB was step wise increment of OLR to produce most rapid biomass development. Table C1 (Chapter 7, Appendices) showed the general operating conditions used to run the pilot-scale IAAB during the start-up period. The performance of the pilot-scale IAAB during the start-up period was monitored closely in terms of COD, BOD, and TSS removal efficiencies, rCH_4, Y_M, VFA concentration, TA, VFA/TA ratio and MLSS concentration.

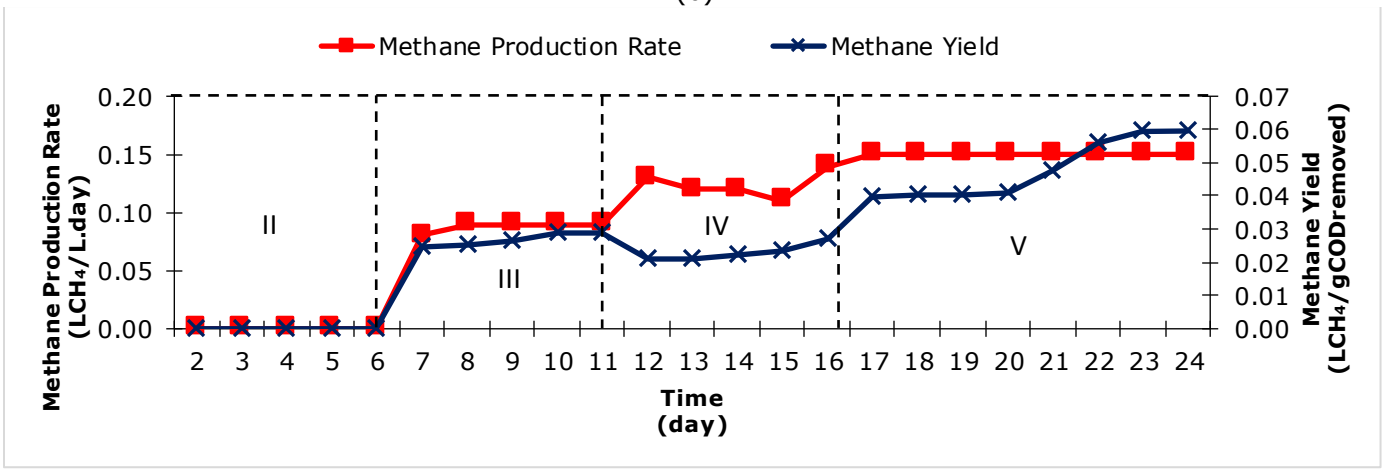
4.3.1.1.1 BOD, COD and TSS Removal Efficiencies



(a)



(b)



(c)

II: Stage 2 III: Stage 3 IV: Stage 4 V: Stage 5

Figure 4.18: The Profiles of (a) COD/TSS/BOD Removal Efficiencies (b) Effluent VFA and TA (c) Volumetric methane production rate and methane yield during the start-up period.

The COD, TSS and BOD profiles during the start-up period is shown in Figure 4.18a. At stage I, POME was not added into the anaerobic compartment of pilot-scale IAAB and the anaerobic sludge was allowed to stabilise for a period of 24 hours (as mentioned in Section 3.6.5.2). During stage II (days 2-6), the OLR of the anaerobic compartment is 1.0 gCOD/L.day equivalent to influent COD and TSS of 2,985 and 989 mg/L, respectively. The effluent can be collcted on day 4 from the outlet of the anaerobic compartment (SP6). The $COD_{removal}$, $TSS_{removal}$, and $BOD_{removal}$ on day 4 are 74%, 82%, and 78%, respectively. This may be due to the good quality of the anaerobic sludge with MLVSS of 85,097 mg/L. At the end of stage II, the achieved $COD_{removal}$, $TSS_{removal}$, and $BOD_{removal}$ are 81, 79, and 79%, respectively. The influent COD concentration is increased to 8,520 mg/L, which corresponded to OLR 3.0 gCOD/L.day.

The effluent COD, BOD, and TSS concentrations was stable at days 2-4 as it is not affected by the increased of OLR_{an}. On day 9, the $TSS_{removal}$ and $BOD_{removal}$ showed a slight drop. This may be due to the increased in COD concentration. However, this observation is not being seen in the determination of $COD_{removal}$. It remained relatively stable throughout the stage III despite the increase of OLR_{an}. This may probably due to the presence of microbial cells in the anaerobic compartment that actively digest the inorganic compound presence in the raw POME. After a day of slight drop, the $TSS_{removal}$ and $BOD_{removal}$ increased to 93%. This shows that the microbial group in the anaerobic compartment is able to adapt well enough to the sudden increase of COD concentration at a relatively short HRT of approximately 3 days coupled with high MLVSS of 79,720 mg/L. Towards the end of stage III, the achieved $COD_{removal}$, $TSS_{removal}$, and $BOD_{removal}$ are 96, 91, and 94%, respectively.

At stage IV, the COD concentration increases to 15,721 mg/L that corresponded to OLR 5.0 gCOD/L.day. The increase of OLR coupled with HRT of 3.11 days does not affect the effluent COD, TSS, BOD concentrations. It may be probably due to the stability of the anaerobic compartment that has been achieved in the previous OLR_{an} of 3.0 gCOD/L.day. As a result, the $COD_{removal}$, $TSS_{removal}$, and $BOD_{removal}$ fluctuated between 96-99%, 90-94%, and 96-97%, respectively. The less fluctuation of each parameter shows that the anaerobic compartment is relatively stable at OLR 5.0 gCOD/L.day despite the increase of COD concentration. At the end of stage IV, a satisfactory $COD_{removal}$, $TSS_{removal}$, and $BOD_{removal}$ of 96, 90, and 96%, respectively are obtained.

POME with COD and TSS concentrations of 27,564 and 10,793 mg/L, respectively is added into the anaerobic compartment that gives OLR_{an} of 8.0 gCOD/L.day at the end of stage V. Similar observation as stage IV, the $COD_{removal}$, $BOD_{removal}$, and $TSS_{removal}$ does not fluctuate much (Figure 4.18a) despite the increased in OLR and short HRT (3.23 days). This may be probably due to the self-regulation capability present in the biological system itself (Chan et al., 2012a). As a result, the microbial cells in the anaerobic compartment able to acclimatise themselves to the rapid increase in COD concentration. At the end of stage V, the $COD_{removal}$, $TSS_{removal}$, and $BOD_{removal}$ are 92, 93, and 92%, respectively. A satisfactory start-up of the bioreactor is obtained at OLR 8.0 gCOD/L.day, whereby the obtained removal efficiency for each parameter is at least 90%.

4.3.1.1.2 Volatile Fatty Acid (VFA) and Total Alkalinity (TA)

The effluent VFA and TA profiles throughout the start-up period are illustrated in Figure 4.18b. The stability of the anaerobic process can be judged based on the ratio of VFA:TA to prevent the decrease in pH, ultimately leading to failure of the digester (Buyukkamaci and Filibeli, 2004). Prior to increasing the OLR, extra care is taken to ensure that the effluent VFA is less than 500 mg/L, pH is greater than 7.0, and VFA:TA is between 0.3 and 0.4 (Poh et al., 2010; Tchobanoglous et al., 2014) . At stage III, the increased in OLR_{an} has resulted in producing effluent with maximum VFA and TA of 185 and 5,125 mg/L at day 8. This is probably due to the excessive concentration of VFA produced by acidogenic bacteria. As a result, the methanogen and acetogenic bacteria are unable to rapidly consume the produced VFA. The pH of the effluent collected on day 8 is 6.99, which is considered stable for anaerobic process despite the absence of alkalinity to neutralise the increase in VFA. On day 10, a new balance is obtained whereby a reduction of effluent VFA and TA is observed. At the end of stage III, the VFA and TA of the collected effluent is 74 and 5,000 mg/L with ratio of VFA:TA of 0.01.

An increase in VFA concentration is observed in stage IV, whereby the peak concentration is 335 mg/L at day 15. Eventually the VFA concentration decreases to 281 mg/L at day 16, as shown in Figure 4.18b. However, the same observation is not seen in the TA profile. At stage IV, the TA fluctuates between 2,076 to 2,250 mg/L. The less fluctuation of TA seen in stage IV suggests that portion of the alkalinity has been utilised to counteract the effect of VFA increment thereby maintaining the pH within the range for anaerobic digestion to occur effectively. As observed, the pH of the collected effluent throughout stage IV fluctuates from 6.93 to 7.17. At the end of stage IV, satisfactory VFA and TA of 281 and 2,076 mg/L, respectively were obtained.

As shown in Figure 4.18b, a drastic decreased of TA to 1,450 mg/L was observed on day 21. Despite the sudden drop of the TA, the $COD_{removal}$, $TSS_{removal}$ and $BOD_{removal}$ were not affected and their removal efficiencies remained as high as 94% (Figure 4.18a). This implies that the bacteria community (acidogenic and acetogenic) in the anaerobic compartment is in the balance proportion and able to withstand the low alkalinity level (1,450 mg/L). As a result, the bacterial cells able to rapidly convert the produced VFA into organic matter (as reflected on the obtained $COD_{removal}$, $TSS_{removal}$ and $BOD_{removal}$) despite the sudden increased of VFA concentration (305 mg/L to 343 mg/L). The alkalinity produced in the previous stage (Stage IV) is required to neutralise the effect of increase in VFA, which has lower down the pH below the recommended level for methanogenesis. The observed TA was below the suggested lower limit of anaerobic process alkalinity (2,000 mg/L) (Tchobanoglous et al., 2014). When the stability of the anaerobic process recovered, the final concentration of the effluent TA was 2,925 mg/L, resulting in VFA:TA ratio of 0.12. At the end of stage V, the obtained effluent VFA was 364 mg/L. The effluent VFA obtained in stage V was higher than stage II (93 mg/L), stage III (74 mg/L), and stage IV (281 mg/L).

The obtained result was similar to the one reported by Buyukkamaci and Filibeli (2002); Chan et al. (2012a), whereby the source of wastewater and increased in COD concentration influenced the concentration of VFA. The effluent VFA concentration and alkalinity level were below 500 mg/L and 3,000 mg/L, respectively during the start-up period. Additionally, the VFA/TA ratio during the entire start-up period was within the range of 0.01-0.24, which is less than the optimum VFA/TA ratio as suggested by Tchobanoglous et al. (2014).

4.3.1.1.3 Volumetric Methane Production Rate, Methane Gas Composition, and Methane Yield

The methane production rate and methane yield during the start-up period are presented in Figure 4.18c. Biogas production was detected on the 7th day with methane production of 0.08 LCH_4/L.day and methane content of 32%. Towards the end of stage III, the methane production rate and methane content were improved to 0.09 LCH_4/L.day and 41%, which is equivalent to a methane yield of 0.0291 $LCH_4/gCOD_{removed}$.

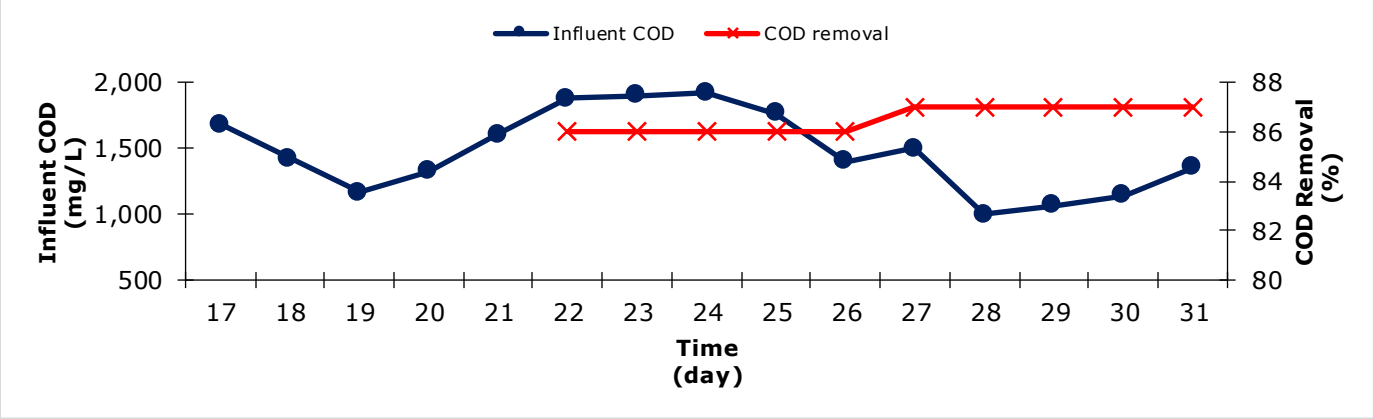
A proportional increase of methane production, methane content and methane yield were observed at stages IV and V coupled with the increased of the OLRs. This may due to the higher concentration of readily available organic matter being converted rapidly to biomass. At the end of stage V, the methane production rate and methane yield were 0.15 LCH_4/L.day and 0.0593 $LCH_4/gCOD_{removed}$. The biogas was found to have 58% of methane gas. The methane yield obtained at this stage was not close to the theoretical maximum that was obtained using glucose as a carbon source (0.35 $LCH_4(STP)/gCOD_{removed}$) (Lawrence and McCarty, 1969; Chan et al., 2012). The methane yield and methane content obtained in this study were lower than the laboratory scale IAAB (0.24 $LCH_4/gCOD_{removed}$ and 64%, respectively at OLR of 10.5 gCOD/L.day) (Chan et al., 2012a). This may be due to the higher concentration of sulphate (beyond the optimum range of 5 mg/L) that accumulated due to the high influent flow rate of 317 L/day, which gives a low hydraulic retention time of 3.15 days (Zinatizadeh et al., 2006). The short retention time does not allow enough time for the anoxic microbial cells to be in contact with the biomass. As a result, the sulphate reducing bacteria competed with methanogen for the same source of organic or inorganic substrates, which supressed the methane production (Chen et al., 2008). Additionally, the high lignin content (1,840 mg/L) and high specific gravity than water may probably contributed to the low methane yield as these matters settled to the bottom of the reactor (Ho et al., 1984; Zhang et al., 2008).

4.3.1.2 Aerobic Process

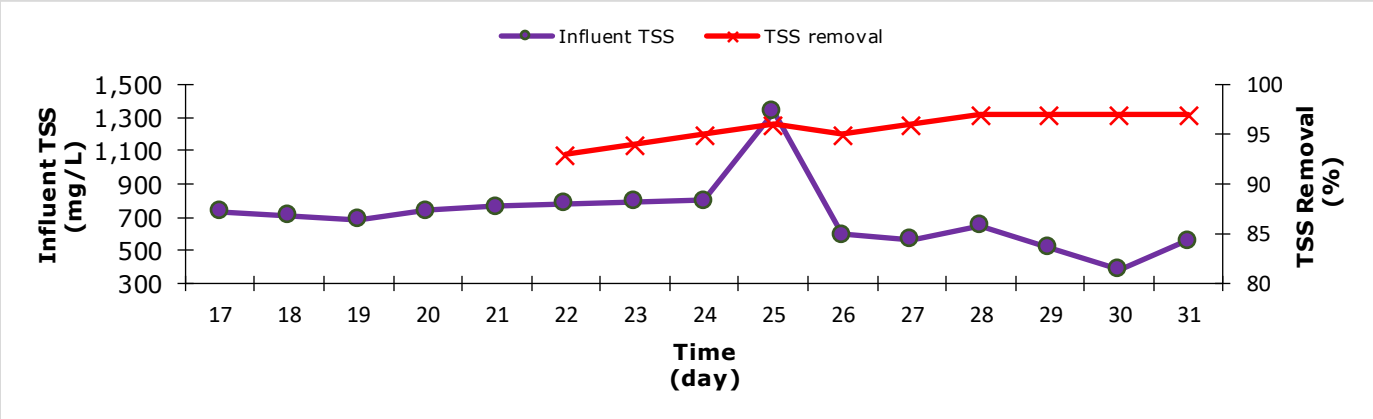
The operation of the aerobic compartment was begun once the anaerobic compartment has reached steady state. The general operating conditions of the aerobic process were shown in Table C1 (Chapter 7, Appendices). The performance of the aerobic compartment during the start-up period was evaluated by determining the $BOD_{removal}$, $COD_{removal}$, and $TSS_{removal}$ of the final treated effluent together with the $MLSS_a$ concentration.

4.3.1.2.1 COD, TSS and BOD Removal Efficiencies

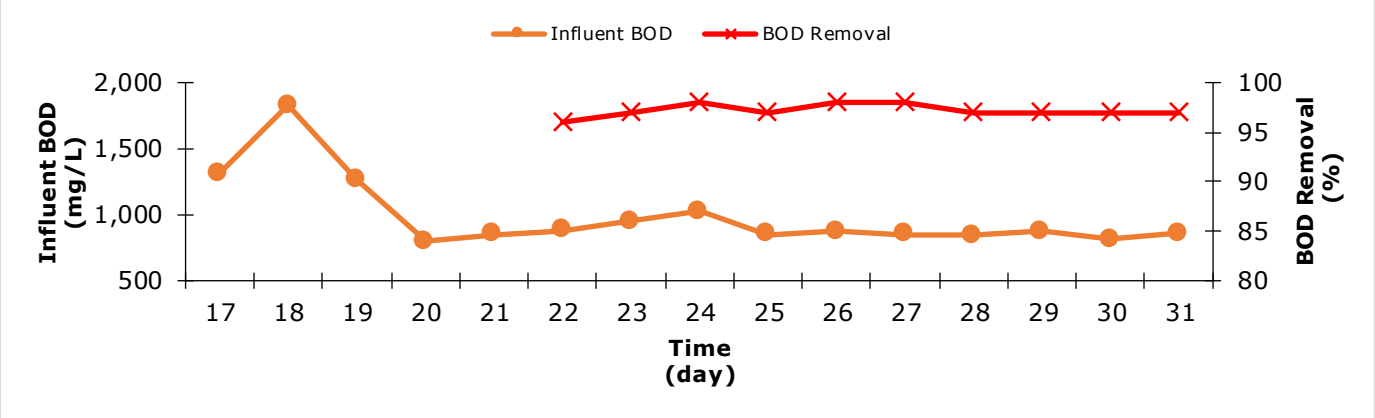
The influent COD of the aerobic compartment was approximately 1,680 mg/L, which is equivalent to OLR_a of 0.90 gCOD/L.day and hydraulic retention time of 1.89 days on the first day of the operation (Day 17). On day 22, settled aerobically treated effluent emerged at the outlet of the settling compartment and was collected for analysis. Figure 4.19 illustrates the COD, TSS, and BOD removal efficiencies of the aerobic treatment system during the start-up period from days 17-31.



(a)



(b)



(c)

Figure 4.19: The start-up period of the aerobic compartment (a) $COD_{removal}$ (b) $TSS_{removal}$ (c) $BOD_{removal}$

The COD, TSS and BOD removal efficiencies showed a similar trend when plotted against operation day. The COD, TSS and BOD removal efficiencies achieved on day 22 were 86%, 93%, and 96%, respectively. Satisfactory results were obtained on day 22 with effluent COD, TSS and BOD of 670 mg/L, 129 mg/L, and 33 mg/L, respectively. An increased in the removal efficiencies of these parameters were observed in day 28. The COD, TSS and BOD removal efficiencies were 87%, 97%, and 98%, respectively. This implies that it is vital to use acclimatised activated sludge obtained from an aerobic pond that treats anaerobically-treated POME as well to give a stable aerobic process. The high bacteria concentration with MLVSS of 19,627 mg/L was able to acclimatise to the new bioreactor configuration in a fairly short period of time (15 days).

As shown in Table C2 (Chapter 7, Appendices), the anaerobic and aerobic compartments of the pilot-scale IAAB exhibited a stable process. Additionally, the quality of the final treated effluent obtained is below the discharge limit set by DOE, Malaysia. Towards the end of the start-up period, the overall COD, BOD, and TSS removal efficiencies obtained by the pilot-scale IAAB were 93.04%, 99.80%, and 99.24%, respectively at OLR 8.0 gCOD/L.day. The overall BOD and TSS removal efficiencies obtained in this study were similar to the laboratory scale IAAB reported by Chan et al., (2012a) except for the overall $COD_{removal}$. This may be due to the lower influent COD concentration used in this study (24,470 mg/L) as compared to the laboratory scale IAAB (68,550 mg/L), which is equivalent to OLR 8.0 and 10.5 gCOD/L.day, respectively. These results indicate that the start-up of the pilot-scale IAAB was accomplished successfully and had reached a steady state.

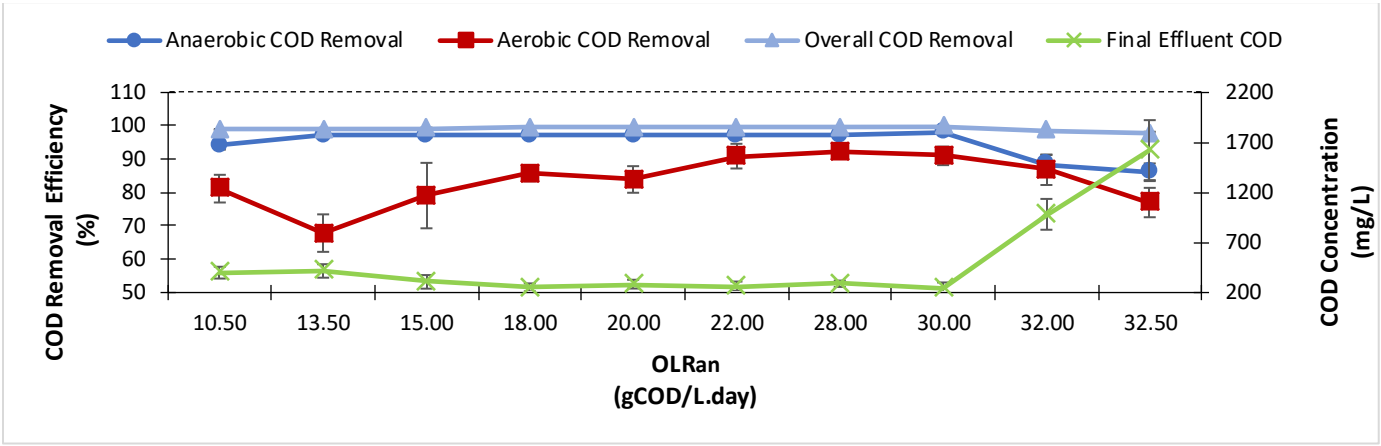
The start-up of the pilot-scale IAAB was achieved at a lower OLR_{an} (8.0 gCOD/L.day) as compared to the laboratory scale IAAB (OLR_{an} 10.5 gCOD/L.day). Additionally, Chan and co-workers also infer that the laboratory scale IAAB able to treat POME at a higher OLR_{an} (maximum OLR_{an} of 22.5 gCOD/L.day). In order to demonstrate the maximum performance of the pilot-scale IAAB, the same approach carried out by Chan and co-workers was applied in this study. This is to allow direct comparison can be made between the different volume of the similar prototype, i.e., laboratory-scale and pilot-scale. Hence, further studies on evaluating the maximum applicable OLR_{an} of the pilot-scale IAAB will be presented in the next section (Section 4.3.2).

4.3.2 Steady-State Performance at various OLRs

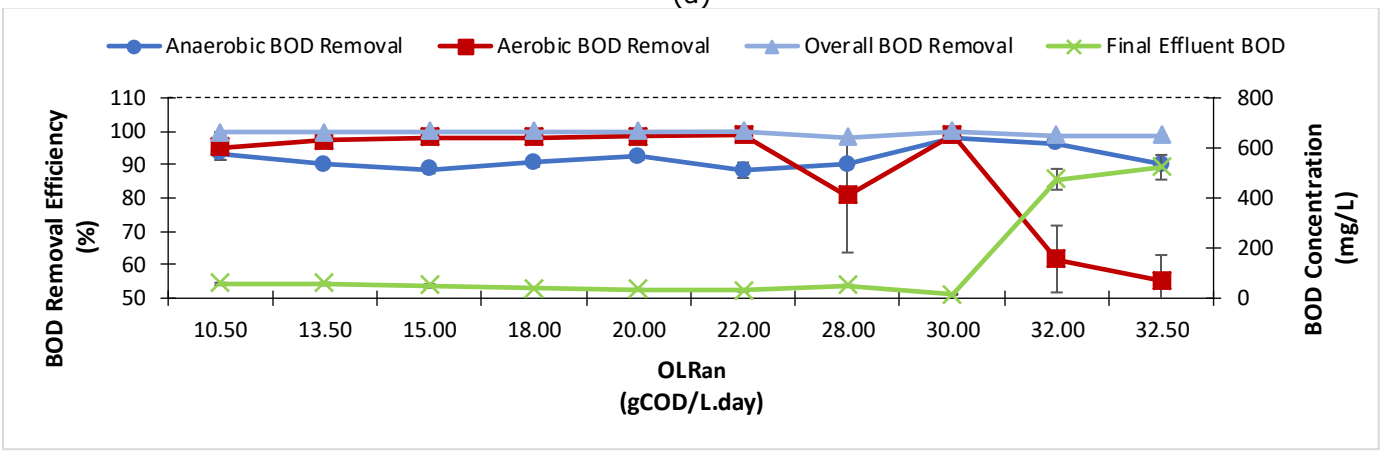
The pilot-scale IAAB was operated at different OLRs to evaluate the maximum applicable OLR and kinetic coefficients using different mathematical models. The OLR_{an} was gradually increased from 8.0 gCOD/L.day to 32.5 gCOD/L.day in 11 stages to minimise the occurrence of overloading at both the anaerobic and aerobic compartments due to the sudden increase in loading rate. General operating conditions applied to the pilot-scale IAAB is summarised in Table C3 (Chapter 7, Appendices). The performance of the pilot-scale IAAB during the steady state period was monitored closely in terms of COD, BOD, and TSS removal efficiencies, rCH_4, Y_M, VFA concentration, TA, VFA/TA ratio, MLSS concentration and F/M ratio.

4.3.2.1 COD, BOD and TSS Removal Efficiencies

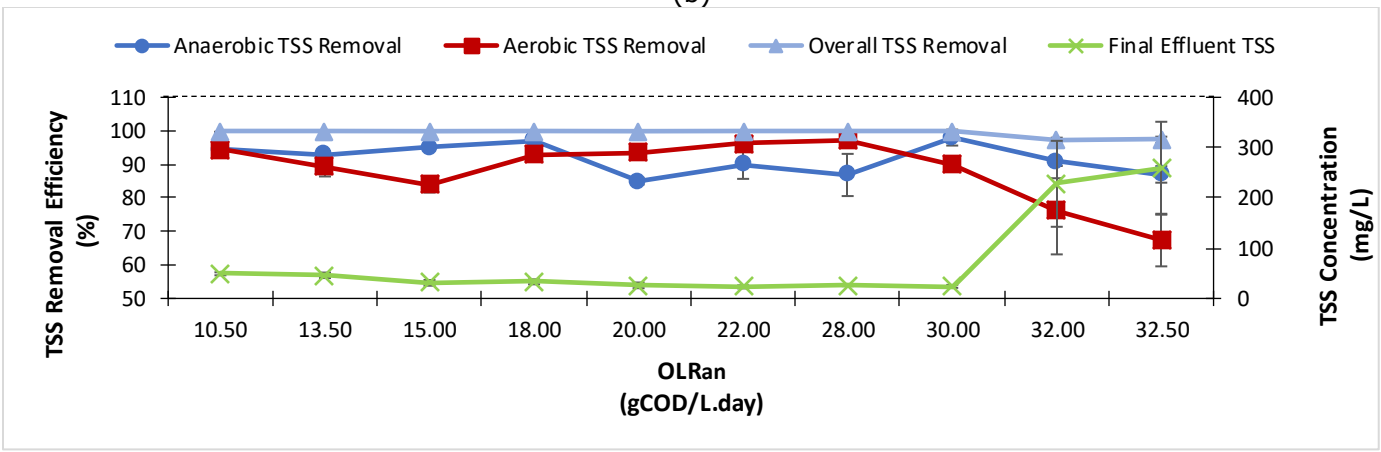
The performance of the pilot-scale IAAB was evaluated at various OLRs by determining the BOD, COD, and TSS removal efficiencies, as illustrated in Figure 4.20. Similar to start-up period, the system was able to adapt to the rapid increased of the COD concentration at each stage in a relatively short period of time (~ 3-4 days) whilst maintaining the removal efficiencies of at least 95% for each parameter.



(a)



(b)



(c)

Figure 4.20: (a) COD (b) BOD (c) TSS removal efficiencies at various OLRs. The error bars were the standard deviation.

Figure 4.20a shows the average $COD_{removal}$ at different OLRs. As can be seen, a trend of increment of the average $COD_{removal}$ coupled with the increasing of the loading rate for the anaerobic treatment up to an OLR value of 30.00 gCOD/L.day. The same observation was obtained for the study conducted by Rincón et al. (2008) for the treatment of olive mill solid residue using the continuous stirred tank reactor under mesophilic condition. However, the obtained result does not correspond to the previous studies reported for POME. At stages 2 and 3, increased of the $COD_{removal}$ from 94% to 97% was observed during the increased of the loading rate. The $COD_{removal}$ was maintained at 97% at 13.50 gCOD/L.day (HRT of 1.96 days), 15.00 gCOD/L.day (HRT of 1.72 days), 18.00 gCOD/L.day (HRT of 1.66 days), 20.00 gCOD/L.day (HRT of 1.76 days), 22.00 gCOD/L.day (HRT of 1.61 days), and 28.00 gCOD/L.day (HRT of 1.46 days). At stage 9, which corresponded to OLR 30.0 gCOD/L.day, the highest $COD_{removal}$ of 98% was achieved. Even though the anaerobic compartment was at high COD concentration ranges from 37,690 mg/L to 78,888 mg/L in stages 2 to 9, the average $COD_{removal}$ was still as high as 97%. Additionally, the pH (6.71-7.45) and VFA:TA (0.01-0.02) were lower than the failure limit value. This is a marked improvement over the study on POME treatment using a UASB and UASFF reactors by Borja and Banks (1994); Najafpour et al. (2006) whereby the former author achieved a $COD_{removal}$ of more than 90% at OLR of 1.27 gCOD/L.day whereas the latter author achieved a $COD_{removal}$ of 89.5% at OLR of 23.15 gCOD/L.day. The simultaneous increased of OLR_{an} and $COD_{removal}$ was not observed in the laboratory scale IAAB as well (Chan et al., 2012b). This implies that the pilot-scale IAAB able to tolerate higher ranges of OLR (10.50-30.00 gCOD/L.day) as compared to laboratory scale IAAB (13.5-16.5 gCOD/L.day). This may be due to the entrapment of bacteria in the packing section as well as development of biofilm that prevent the loss of bacteria resulting in maintaining high biomass concentration in the anaerobic compartment (MLVSS of 36,129 mg/L) of the pilot-scale IAAB.

A significant decrease in $COD_{removal}$ of 88% ± 2.93 was observed when the OLR_{an} was increased to 32.0 gCOD/L.day. Despite the low $COD_{removal}$ obtained at this stage 10, the performance of the reactor does not deteriorate significantly. However, further increase of the OLR_{an} to 32.5 gCOD/L.day resulted in obtaining a minimum $COD_{removal}$ value of 86% ± 2.64. At this stage, slight reduction of MLVSS was observed from 32,084 mg/L (OLR_{an}: 32.0 gCOD/L.day) to 30, 233 mg/L (OLR_{an}: 32.5 gCOD/L.day). The F/M ratio obtained at both the stages were 1.274 ± 0.205 gCOD/g.MLVSS.day (OLR_{an}: 32.0 gCOD/L.day) and 1.088 ± 0.159 gCOD/g.MLVSS.day (OLR_{an}: 32.5 gCOD/L.day). This allow us to postulate that the high F/M ratio may reduce the degradation performance of the anaerobic compartment of the pilot-scale IAAB (Aziz et al., 2019). The performance of the anaerobic compartment was slightly affected due to the increased in VFA:TA (1.06), which is higher than the optimum working range for anaerobic digestion. At this stage, failure of the anaerobic compartment was detected.

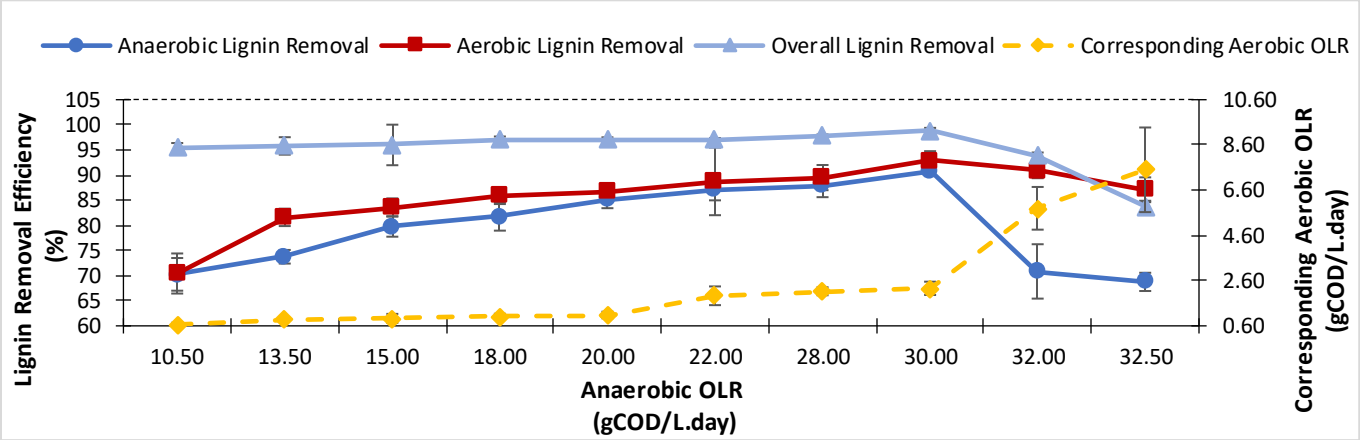
The average $COD_{removal}$ of the aerobic compartment is shown in Figure 4.20a. An increasing trend of the $COD_{removal}$ was observed from 81%±4.15 to 93%±2.78 during the increment of OLR_{an} from 10.5 to 30.0 gCOD/L.day, which corresponded to the average OLR_a of 0.66±0.06, 0.90±0.08, 0.93±0.20, 1.03±0.15, 1.08±0.11, 1.93±0.41, 2.03±0.29, and 2.26±0.18 gCOD/L.day in stages 2, 3, 4, 5, 6, 7, 8, and 9, respectively. The highest $COD_{removal}$ of the aerobic compartment of 93% was obtained in stage 9, which implies that a higher OLR_a is required to give a higher $COD_{removal}$. At this stage, the activity of the aerobic microbial cells can be improved as reflected on the F/M ratio of 0.114 gCOD/gMLVSS.day, which is within the range of 0.06-0.18 gCOD/gMLVSS.day reported by Chan et al., (2010). However, the $COD_{removal}$ of the aerobic compartment started to decrease to 88±4.57% and 77±4.41% when the OLR_{an} was increased to 32.0 gCOD/L.day (average corresponding OLR_a of 5.80±0.94 gCOD/L.day) and 32.50 gCOD/L.day (average corresponding OLR_a of 7.50±1.87 gCOD/L.day) in stages 10 and 11, respectively. This implies that the biomass activity of the aerobic microbial cells undergoes an inhibition period at stage 11 with the lowest $COD_{removal}$ of 77±4.41%. This shows that the pilot-scale IAAB can be operated up to OLR_{an} of 30.0 gCOD/L.day without any severe inhibition to both the anaerobic and aerobic processes, whilst maintaining the pH within the range for methanogenesis to occur. The maximum loading rate of the pilot-scale IAAB was higher than the laboratory scale IAAB at mesophilic (21.0 gCOD/L.day) and thermophilic (28.0 gCOD/Lday) reported by Chan et al. (2012b). It is worth noting that the maximum loading rate obtained in this study is higher than the other integrated anaerobic-aerobic bioreactors (OLR of 0.39-26.80 gCOD/L.day; influent COD of 341-65,700 mg/L) (Garbossa et al., 2005; Chan et al., 2009).

Figures 4.20b and 4.20c showed the BOD and TSS removal efficiencies, which followed a similar pattern to Figure 4.20a ($COD_{removal}$). This further confirmed that the increased in the COD, BOD, and TSS removal efficiencies are mainly due to the increased in influent COD concentration. Throughout the stages of 2-9, high $TSS_{removal}$ of 85.0 to 97.0% were achieved at OLR_{an} of 10.5 to 30.0 gCOD/L.day. This shows that the installation of gas-liquid-solid separator (GLSS) on top of the anaerobic compartment able to provide a further polishing step for the anaerobically-treated POME by separating the solid from liquid as well as preventing washout of sludge into the aerobic compartment. The results obtained in this study were similar to those reported by Chan et al. (2012b). The effluent BOD concentrations obtained in this study were lesser than 100 mg/L starting from stages 2 to 8. It is worth noting that at stage 9, which is corresponded to OLR_{an} of 30.0 gCOD/L.day and OLR_a of 2.26 gCOD/L.day was able to produce effluent with BOD of less than 20 mg/L. This postulates that the treatment of POME using pilot-scale IAAB is able to produce effluent that abides the discharge limit set by DOE, Malaysia. A significant increase on the BOD of the final treated effluent was observed at OLR_{an} of 32.0 gCOD/L.day in stage 10 and 32.5 gCOD/L.day in stage 11, with effluent BOD of 475 mg/L and 523 mg/L, respectively. The final treated effluent produced at both the OLRs has exceeded the discharge limit. Rapid sludge washout was observed at both the stages, which implies that the aerobic system was unable to withstand the high OLR_a of 5.80 gCOD/L.day and 7.50 gCOD/L.day. Hence, the system was overloaded (Zheng et al. 2006).

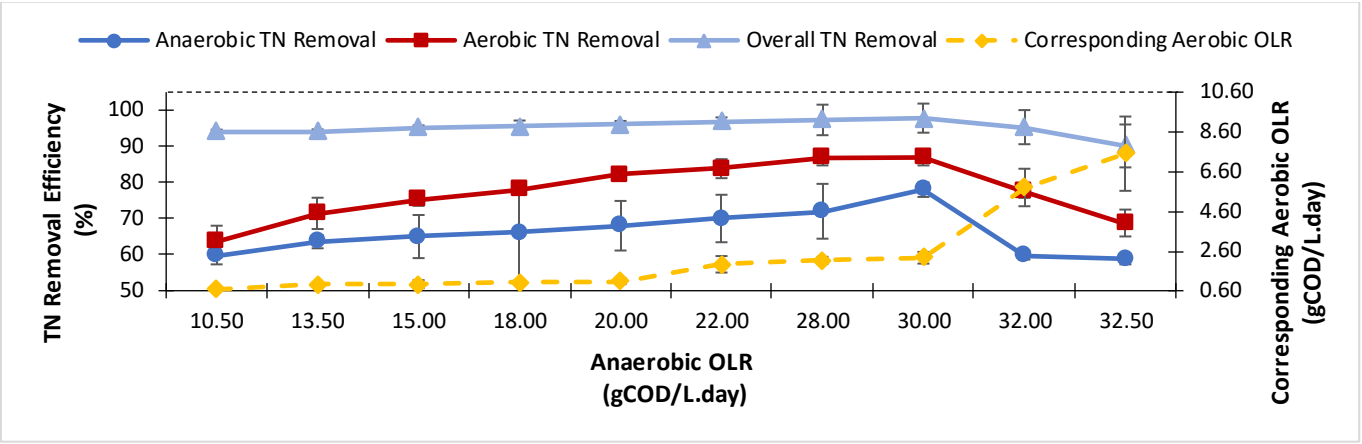
It is essential to compare the contribution of each of the anaerobic and aerobic processes at each stages as both the processes were carried out in a single unit of bioreactor. The same observation was obtained from stages 2-9 whereby anaerobic digestion contributed the most to the total $COD_{removal}$ followed by aerobic digestion. Anaerobic digestion contributed 96.04 ± 1.19%, 95.99 ± 2.61%, 97.25 ± 0.85%, 96.63 ± 0.49%, 97.85 ± 0.56%, 95.88 ± 1.24%, 95.55 ± 0.52%, and, 96.12 ± 0.88% for stages 2-9 (corresponding to OLR_{an} of 10.5-30.0 gCOD/L.day), respectively to the total $COD_{removal}$. Aerobic digestion contributed 3.96 ± 1.19%, 4.00 ± 2.61%, 2.75 ± 0.85%, 3.37 ± 0.49%, 2.15 ± 0.56%, 4.12 ± 1.24%, 4.45 ± 0.52%, and 3.88 ± 0.88% for stages 2-9 (correspnding to average OLR_a of 0.66-2.26 gCOD/L.day), respectively to the total $COD_{removal}$. The same observation was obtained from study conducted by Chan et al. (2013), anaerobic and aerobic digestion contributed 94.4 ± 1.2% and 5.1 ± 0.3%, respectively to the total $COD_{removal}$ during the operation of the laboratory scale IAAB at OLR_{an} 15.0 gCOD/L.day. The same observation obtained for both the laboratory and pilot-scale IAABs has allow us to confirm that anaerobic compartment plays a major role in biodegrading organic content in the raw POME, whilst the aerobic compartment acts as further polishing system to produce final treated effluent that meet the discharge limit set by the DOE, Malaysia.

4.3.2.2 Lignin, TN and TP Removal Efficiencies

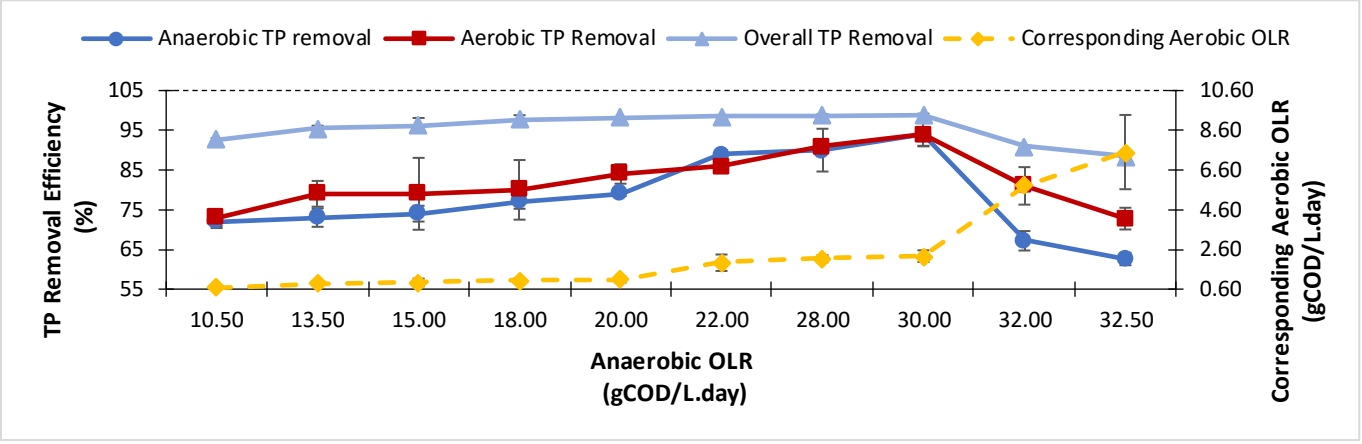
The performance of the pilot-scale IAAB was evaluated at various OLRs by determining the lignin, TN, and TP removal efficiencies, as illustrated in Figure 4.21.



(a)



(b)



(c)

Figure 4.21: (a) Lignin (b) TN (c) TP removal efficiencies at various OLRs. The error bars were the standard deviation.

Figures 4.21a, 4.21b and 4.21c showed an increment of the average lignin, TN and TP removal efficiencies coupled with the increasing of the loading rate for the anaerobic and aerobic treatments. It is worth noting that the graphs obtained for these parameters were similar to the BOD, COD and TSS removal efficiencies. This further confirmed that high concentrations of biomass ($MLVSS_{an}$ of 36,129 mg/L; $MLVSS_{a}$ of 24,976 mg/L) are relatively vital in overcoming the effect of high loading rate (Razi and Noor 1999; Zheng et al., 2006).

Lignin, TN and TP are the parameters that were not often quantified in literature to describe the performance of the system in terms of anaerobic and aerobic processes. The concentration of TN and TP determined the amount of nutrient available in these processes to ensure high treatment efficiency. The adequate removal of lignin from these processes may probably produce effluent with less odour as well as safe to be discharged into the environment (Poh et al., 2010). It is vital to ensure high removal efficiencies of these parameters to prevent the occurrence of eutrophication due to rapid depletion of dissolved oxygen in the bodies of water. Additionally, the high lignin content may lead to the production of effluent with dark brownish colour and high concentration of dissolved organic matter. It is essential to determine the contribution of each compartment i.e., anaerobic and aerobic on the removal of these parameters.

An increasing trend on the average $lignin_{removal}$ from 70.0±3.99% to 93.0±0.69% during the increment of OLR_{an} from 10.5 gCOD/L.day (average OLR_a of 0.66±0.06 gCOD/L.day) to 30.0 gCOD/L.day (average OLR_a of 2.26±0.18 gCOD/L.day). The highest $lignin_{removal}$ of the aerobic compartment of 93% was obtained in stage 9, which implies that a higher OLR_a and sufficient dissolved oxygen concentration (>2 mg/L) able to enhance the breakdown of lignin content in the anaerobically-treated POME. This is postulated that oxygen is needed for the activation of the lignin degrading enzyme that may be secreted by the microbial cells in the aerobic compartment (Hatakka, 1994; Guillen et al., 2000). Study reported by Wu et al. (2005) showed that the used of lignin degrading enzyme able to remove 71% of lignin from a pulp mill wastewater. Even though the concentration of lignin is not considered in the discharged limit set by DOE, it is worth noting that the lignin concentration in the final treated effluent produced during OLR_{an} 30.0 gCOD/L.day is less than 100 mg/L. However, the average $lignin_{removal}$ of the aerobic compartment started to decrease to 91±0.50% and 87±1.09% coupled with the increased of OLR_{an} to 32.0 gCOD/L.day (average corresponding OLR_a 5.80±0.94 gCOD/L.day) and 32.50 gCOD/L.day (average corresponding OLR_a of 7.50±1.87 gCOD/L.day), respectively. The high lignin concentration in the final treated effluent produced at OLR_{an} 32.0 gCOD/L.day and 32.5 gCOD/L.day were 102 mg/L and 291 mg/L resulted in dark brownish colour. This postulates that the presence of dissolved organic matter contributed to the appearance of the final treated effluent (Bajpai et al., 1993; Kakahi et al., 2011).

Anaerobic digestion contributed 89.98 ± 1.99%, 86.19 ± 12.45%, 90.14 ± 1.76%, 84.29 ± 1.86%, 83.56 ± 2.08%, 73.81 ± 3.80%, 75.65 ± 1.19%, and 76.82 ± 2.88% for stages 2-9 (corresponding to OLR_{an} of 10.5-30.0 gCOD/L.day), respectively to the total $lignin_{removal}$. Aerobic digestion contributed 10.02 ± 1.99%, 13.81 ± 12.45%, 9.86 ± 1.76%, 15.71 ± 1.86%, 16.44 ± 2.08%, 26.19 ± 3.80%, 24.35 ± 1.19%, and 23.18 ± 2.88% for stages 2-9 (corresponding to average OLR_a of 0.66-2.26 gCOD/L.day), respectively to the total $lignin_{removal}$. The total $lignin_{removal}$ contributed by the anaerobic and aerobic processes increases with the increased of OLR_{an} from 10.5-15.0 gCOD/L.day (corresponding to average OLR_a of 0.66-0.93 gCOD/L.day). However, the same observation was not observed as the OLR_{an} increases from 18.0 to 30.0 gCOD/L.day (corresponding to average OLR_a of 1.03-2.26 gCOD/L.day). This allow us to infer that oxygen is required to degrade the lignin content in the POME at high OLR_{an} (18.0-30.0 gCOD/L.day) and OLR_a (1.03-2.26 gCOD/L.day). By comparing the overall total $lignin_{removal}$ determined for both the anaerobic and aerobic processes, anaerobic compartment of the pilot-scale IAAB contributed the most in the degradation of lignin. The same observation was obtained for the total $COD_{removal}$ (Section 4.3.2.1). This again confirmed that degradation of lignin mainly carried out in the anaerobic process whereas aerobic process only act as a polishing system due to the continuous provision of oxygen in the aerobic compartment of the pilot-scale IAAB, which may be the contributing factor for producing final treated effluent with low lignin concentration (< 100 mg/L).

Figures 4.21b and 4.21c showed the TN and TP removal efficiencies, which give a similar pattern as lignin removal efficiency (Figure 4.21a). This further confirmed that the breakdown of nitrogen and phosphorus occurred mainly in the aerobic compartment of the pilot-scale IAAB whereby nitrification and enhanced biological phosphorus removal (EBPR) required oxygen to remove these organic compounds from the anaerobically-treated POME (Marvast and Jashni, 2015). Throughout the stages of 2-9 of the anaerobic compartment low $TN_{removal}$ of 60.0% to 78.0% were achieved at OLR_{an} of 10.5 gCOD/L.day to 30.0 gCOD/L.day. Despite the low $TN_{removal}$ achieved in the anaerobic compartment of the pilot-scale IAAB, accumulation of ammonia was not observed. Study reported by Chen et al. (2008) showed that *Methanospirillum hungatei*, *Methanosarcina barkeri*, *Methanobacterium thermoautotrophicum*, and *Methanobacterium formicicum* were inhibited at ammonia concentration ranges between 4.2 g/L to 10.0 g/L. The concentration of ammonia in the anaerobic compartment throughout the stages ranges from 187.50 to 301.18 mg/L, which is lower than the failure limit for the aforementioned methanogens. The average $TP_{removal}$ obtained in the anaerobic compartment from stages 2-9 ranges from 73% to 94%. Additionally, the pH of the anaerobically-treated effluent (6.83-7.50) that is within the range for methanogenesis to occur (Poh et al., 2010).

The same trend was observed in the aerobic compartment as well for both the TN and TP removal. The average TN and TP removal efficiencies increases from 63.6% to 86.9% and 73.0% to 94.0%, respectively during the increment of OLR_{an} from 10.50 gCOD/L.day (average corresponding OLR_a of 0.66±0.06 gCOD/L.day) to 30.0 gCOD/L.day (average OLR_a of 2.26±0.18 gCOD/L.day).The increased in biomass concentration in the aerobic compartment showed that the nutrient content in the anaerobically-treated POME in terms of BOD:TN:TP is sufficient to support the growth of the microbial cells as nitrogen and phosphorus are the main building block for their growth and reproduction. The installation of recirculation system (settling compartment back into aerobic compartment) further enhances the nitrification and removal of phosphorus processes (Watari et al., 2017). The result obtained showed that the removal of phosphorus and nitrogen happened effectively in the aerobic compartment of the pilot-scale IAAB (Yeoman et al. 1988; Barr et al. 2010). As reported by Yeoman et al. (1988), the high nutrient concentration in terms of BOD:TN:TP (100:6:1.5) coupled with aerator in the activated sluge in the aerobic compartmet was able to enhance the growth of the microbial cells in the aerobic compartment resulting in further removal of nitrogen and phosphorus.

Literature search reveals that removal of nitrogen also occurred in aerobic condition due to the presence of nitrifying bacteria (NH_4^+ to NO_3^-) and denitrifying bacteria (NO_3^- to N_2) in the aerobic compartment - silmultaneous nitrification and denitrification (SND). The co-existence of two different groups of bacteria in one compartment is aimed to achieve near complete removal of nitrogen under aerobic conditions as reported by Ruan et al. (2006) ; Seifi and Fazaelipoor (2012). The formation of EPS in the activated sludge system will act as a backbone for the nitrfying and denitrifying bacteria. It is the main component of flocs, biofilm and granular sludge, in which they act as a network structure through chemical bonding and physical composing to promote the microbial aggregates formation (Zhu et al., 2012). Such aggregation will increase the bacteria concentration in the system coupled with the sufficient nutrient concentration.

In the activated sludge system, the ammonium and nitrite oxidisers are autotrophic bacteria that requires oxygen as electron acceptor to carry out nitrifcation process (Seifi and Fazaelipoor, 2012). The NO_3^- produced by the nitrifying bacteria will act as an electron acceptor for a group of heterotrophic bacteria to reduce NO_3^- to N_2. Denitrifiers are mostly facultative anaerobic whereby they can use both O_2 and NO^-_3 as electron acceptors. When the concentration of O_2 is sufficient (reflected on the DO concentration) in the activated sludge system, denitrifiers prefer to use O_2. SND may seems to be feasible to be carried out as this can be done by controlling the oxygen concentration in the aerobic compartment of the pilot-scale IAAB. This allow us to postulate that DO concentration of at least 2 mg/L is sufficient for denitrifiers to reduce nitrate (NO_3^-) to nitrogen (N_2) in the anaerobically-treated POME.

The increased in OLR_{an} from 30.0 gCOD/L.day (average OLR_a of 2.26±0.18 gCOD/L.day) to 32.0 gCOD/L.day (average corresponding OLR_a 5.80±0.94 gCOD/L.day) resulted in drastic reduction of TN and TP removal efficiencies for anaerobic (from 94.0% to 67%) processes. However, such observation was not observed in the aerobic process with slight reduction of $TN_{removal}$ (94.0% to 81.0%) and $TP_{removal}$ (86.9% to 77.5%) (Figures 4.21b and 4.21c). This allow us to postulate that the oxygen concentration in the aerobic compartment (dissolved oxygen concentration was maintained at around 2 mg/L) is sufficient to enhance the nitrification and phosphorus removal despite the high loading rate (average corresponding OLR_a 5.80±0.94 gCOD/L.day). However, the same observation was not observed during the increment of OLR_{an} from 32.00 gCOD/L.day (average corresponding OLR_a 5.80±0.94 gCOD/L.day) to 32.50 gCOD/L.day (average corresponding OLR_a of 7.50±1.87 gCOD/L.day). The increase in loading rate has resulted in a decreased in TN and TP removal efficiencies for both the anaerobic and aerobic processes (Figures 4.21b and 4.21c). The nitrogen concentration in the final treated effluent for OLR_{an} 32.0 gCOD/L.day (average corresponding OLR_a 5.80±0.94 gCOD/L.day) and 32.50 gCOD/L.day (average corresponding OLR_a of 7.50±1.87 gCOD/L.day) were 585 mg/L and 720 mg/L, respectively, which were higher than the recommended concentration of 45 mg/L of nitrate (State Water Resources Control Board, 2017).

The phosphorus concentrations in the final treated effluent for both the OLRs were 125 mg/L and 252 mg/L, respectively. This allow us to postulate that a higher energy requirement may be needed to effectively enhance the nitrification and phosphorus removal, which will increase the overall treatment cost. The high concentration of the nitrogen and phosphorus in the final treated effluent that is usually released into the river ways may lead to the occurrence of eutrophication (Smith and Schindler, 2009).

The nitrogen concentration in the final treated effluent produced at OLR_{an} 10.5 gCOD/L.day (average corresponding OLR_a of 0.66 ± 0.08 gCOD/L.day), 13.5 gCOD/L.day (average corresponding OLR_a of 0.90 ± 0.05 gCOD/L.day), 15.0 gCOD/L.day (average corresponding OLR_a of 0.93 ± 0.19 gCOD/L.day), 18.0 gCOD/L.day (average corresponding OLR_a of 1.03 ± 0.11 gCOD/L.day), 20.0 gCOD/L.day (average corresponding OLR_a of 1.08 ± 0.15 gCOD/L.day), 22.0 gCOD/L.day (average corresponding OLR_a of 1.93 ± 0.41 gCOD/L.day), 28.0 gCOD/L.day (average corresponding OLR_a of 2.13 ± 0.18 gCOD/L.day), and 30.0 gCOD/L.day (average corresponding OLR_a of 2.26 ± 0.29 gCOD/L.day) were 87 ± 28 mg/L, 84 ± 14 mg/L, 82 ± 1.78 mg/L, 129 ± 7.27 mg/L, 58 ± 24 mg/L, 81 ± 5.06 mg/L, 84 ± 3.32 mg/L, and, 95 ± 1.82 mg/L, respectively. On the other hand, the nitrogen concentration in the final treated effluent produced at OLR_{an} 32.0 gCOD/L.day (average corresponding OLR_a of 5.80 ± 0.94 gCOD/L.day) and 32.5 gCOD/L.day (average corresponding OLR_a of 7.50 ± 1.87 gCOD/L.day) were 585 ± 4.23 mg/L and 720 ± 3.23 mg/L, respectively. The obtained result showed that the nitrogen concentration in the final treated effluent produced at OLR_{an} 10.5 to 30.0 gCOD/L.day abided the POME discharge limit (200 mg/L) as reported by Loh et al. (2009) except for OLR_{an} 32.00 and 32.50 gCOD/L.day. The BOD and TSS of the final treated effluent produced at OLR_{an} 10.5-30.0 gCOD/L.day (corresponding to average OLRa of 0.66-2.26 gCOD/L.day) were below 50 mg/L. This allow us to confirmed that sufficient nutrient concentrations coupled with aerator in the aerobic compartment able to produce final treated effluent way below the discharge limit determined by MPOB and DOE.

4.3.2.3 Regression of Kinetic Models

Based on the results obtained in Section 4.3.2, it can be concluded that the pilot-scale IAAB can be operated up to OLR 32.0 gCOD/L.day with acceptable stability and free of operational problems. Due to the instability of the pilot-scale IAAB at OLR 32.50 gCOD/L.day in stage 11, only ten steady state performance data sets obtained from stages 1 to 10 were used to determine the kinetic parameters required for all the kinetic models. For the evaluation of kinetic parameters on a COD basis, a graphical method and least square linear regression were used to obtain line of best fit.

Table 4.6: Kinetic parameters of the pilot-scale IAAB that treats POME

Kinetic Models	Kinetic Parameters	Unit	Anaerobic		Aerobic	
			Values	R^2	Values	R^2
Modified Stover-Kincannon	K_B	gCOD/L.day	440.83	0.9843	0.6573	0.8407
	$\cup_{max}$	gCOD/L.day	416.67		2.8209	
Grau second-order	a	per day	0.5261	0.7884	0.4134	0.4061
	b	-	0.8875		0.5709	
	k_s	Per day	14.591		3.3629	
Monod	Y	mgVSS/mgCOD	0.1949	0.7060	0.0718	0.7373
	k_d	per day	0.1088		0.0154	
	μ_{max}	per day	0.1764	0.6984	0.2465	0.8811
	K_S	mg/L	9,411		500.76	

Table 4.7: Prediction of: (a) COD anaerobically-treated effluent; (b) COD final treated effluent; (c) biomass concentration anaerobically-treated effluent; (d) biomass concentration of final treated effluent

Treatment	Kinetic Model	Equation	Eq. No.
Anaerobic	Modified Stover-Kincannon	$COD_{out,an} = COD_{in} - \frac{416.67 COD_{in}}{440.83 + OLR_{an}}$	4.10
	Grau second-order	$COD_{out,an} = COD_{in}(1 - \frac{HRT_{an}}{0.5261 + 0.8875 HRT_{an}})$	4.11
	Monod	$COD_{out,an} = \frac{9{,}411(\frac{1}{SRT_{an}} + 0.1088)}{0.1764 - \frac{1}{SRT_{an}} - 0.1088}$	4.12
		$X_{out,an} = \frac{0.1949(COD_{in} - COD_{out,an})}{HRT_{an}(1 + 0.1088 SRT_{an})}$	4.13
Aerobic	Modified Stover-Kincannon	$COD_{out} = COD_{out,an} - \frac{2.8209 COD_{out,an}}{0.6573 + OLR_a}$	4.14
	Grau second-order	$COD_{out} = COD_{out,an}\left(1 - \frac{HRT_a}{0.4134 + 0.5709 HRT_a}\right)$	4.15
	Monod	$COD_{out} = \frac{500.76\left(\frac{1}{SRT_a} + 0.0154\right)}{0.2465 - \frac{1}{SRT_a} - 0.0154}$	4.16
		$X_{out} = \frac{0.0718(COD_{out,an} - COD_{out})}{HRT_a(1 + 0.0154 SRT_a)}$	4.17

4.3.2.3.1 Modified Stover-Kincannon Model

Based on the experimental results, the regression of the Modified Stover-Kincannon kinetic parameters (K_B and $\cup_{max}$) were carried out using linearised form of Eq. (3.35) (Table 3.16, Chapter 3, Materials and Methods). The predicted K_B and $\cup_{max}$ were determined by plotting Eq. 3.35 as shown in Figure C4 (Chapter 7, Appendices). The R^2 obtained from Eq. (3.35) for anaerobic and aerobic digestion of the pilot-scale IAAB were high (>0.96), which showed that it can be applied. The kinetic parameters are summarised in Table 4.6 with regression coefficients.

Figure C4 (Chapter 7, Appendices) illustrated the plots of reciprocal of organic loading removal rate [$V/Q_{in}(COD_{in} - COD_{out})$] against the reciprocal of OLR [$V/(Q_{in}.COD_{in})$] in the both the compartments of anaerobic and aerobic. The obtained values of $\cup_{max}$ and K_B for both the processes are shown in Table 4.6. The high regression coefficients of 0.9843 and 0.9703 for both anaerobic and aerobic compartments respectively proved the validity of the modified Stover-Kincannon model. The parameter of $\cup_{max}$ shows the maximum substrate removal rate whereas K_B shows the saturation value constant. The rate constant ($\cup_{max}$) obtained for aerobic compartment was higher than that of the anaerobic compartment. From these results, the anaerobically-treated and final treated effluent COD concentrations of the pilot-scale IAAB can be predicted from Eq. (4.10) and Eq. (4.11), respectively, as shown in Table 4.7.

4.3.2.3.2 Grau Second-Oder Multicomponent Substrate Removal Model

In order to determine the kinetic coefficients (a, b and ks), Eq. (3.36) (Table 3.16, Chapter 3, Materials and Methods) was plotted in Figure C5 (Chapter 7, Appendices). The regression coefficients for anaerobic and aerobic processes are 0.7884 and 0.4061, respectively. The a and b constant values were found from the intercept and slope of the best fit line. The calculated a, b and k_s are shown in Table 4.6.

From the obtained results, the anaerobically-treated and final treated effluent COD concentrations of the pilot-scale IAAB can be predicted from Eq. (4.11) and Eq. (4.15), respectively (Table 4.7).

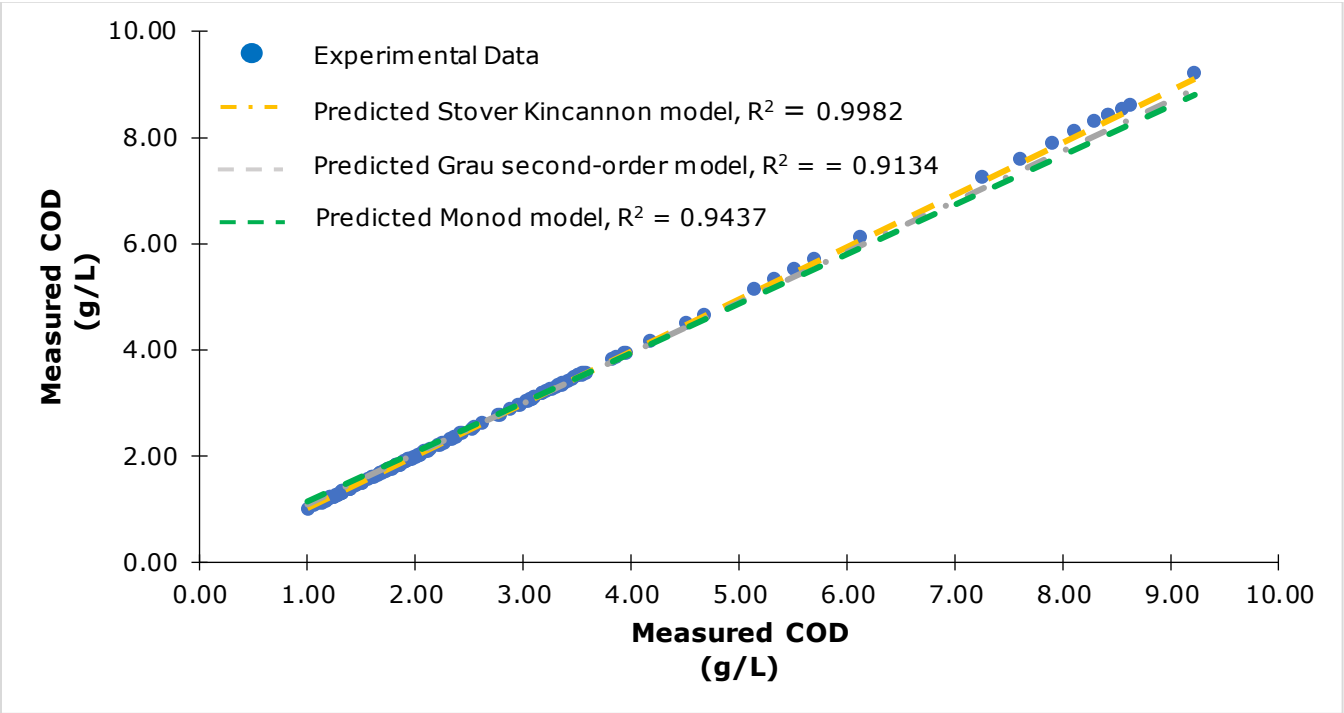
4.3.2.3.3 Monod Kinetic Model

Linearised form of Eqs. (3.33) and (3.34) (Table 3.16, Chapter 3, Materials and Methods) were plotted as shown in Figures C6 and C7 (Chapter 7, Appendices) to determine the Monod kinetic parameters of Y, k_d, μ_{max} and K_s for both the anaerobic and aerobic processes. The obtained kinetic coefficients are shown in Table 4.6.

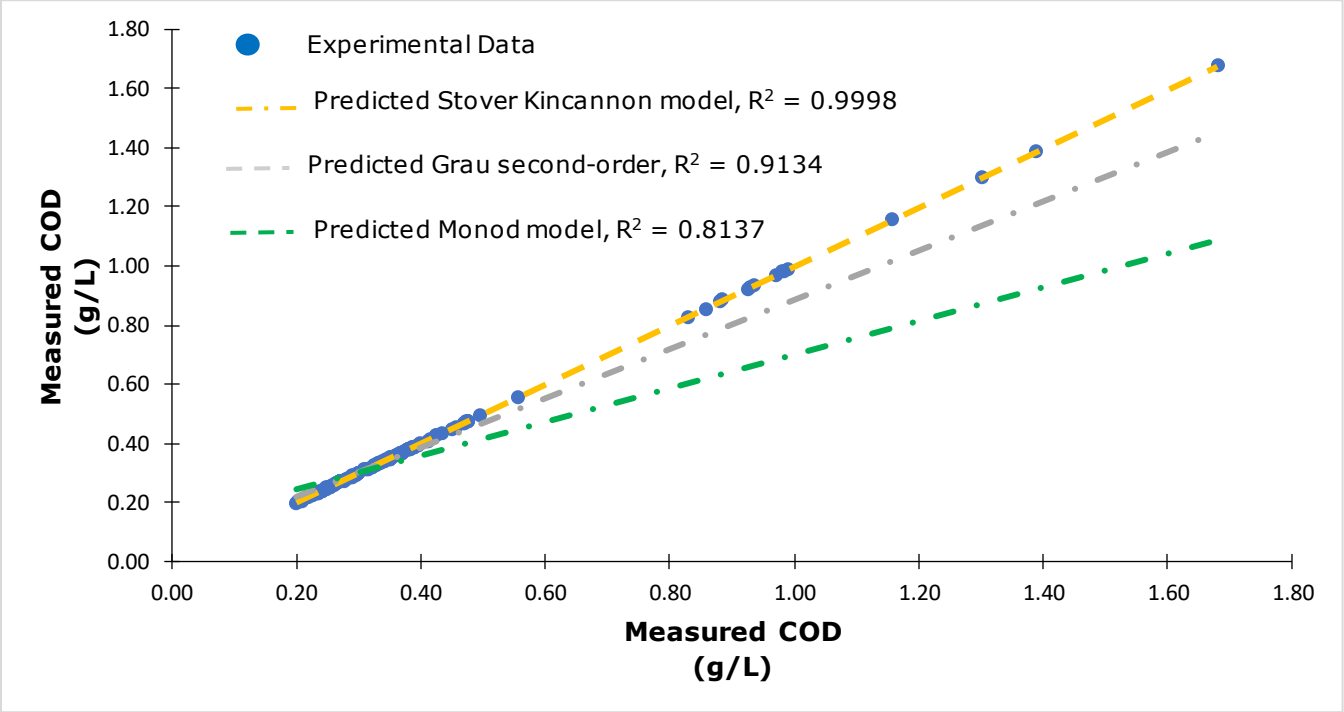
Parameter Y shows the growth of the microbial cells whilst k_d determined the decay of the microbial cells in the anaerobic and aerobic processes. The obtained results for both the processes showed that the values of Y were higher than the k_d, which implies that the growth of the microbial cells outweigh the death of the microbial cells. Additionally, μ_{max} demonstrated the active biomass concentration in each of the processes. Low value of μ_{max} of 0.1764 day^{-1} and 0.2465^{-1} were achieved in the anaerobic and aerobic compartment, respectively. Low μ_{max} obtained in both the compartments showed that high concentration of active biomass was obtained in these compartments (Zinatizadeh et al., 2006). The μ_{max} value obtained in the anaerobic compartment was lower than the aerobic compartment. This agreed with the laboratory scale IAAB conducted by Chan et al. (2012b) regardless in mesophilic and thermophilic conditions (Anne et al., 2006). The K_S values obtained in the anaerobic (9,411 mg/L) and aerobic (500.76 mg/L) were lower than the influent COD concentration. This postulated that the biomass concentration in these compartments were sufficient to allow rapid breaking down of the substrate from the influent, thereby giving high $COD_{removal}$ of at least 95%. Additionally, the absence of substrate accumulation in these compartments showed that the designed HRT had created a synergistic effect towards the growth of the microbial cells. The obtained results were in agreement with other types of wastewaters using the same type of bioreactors (Talaiekhozani et al., 2015). From the obtained results, the anaerobically-treated and final treated effluent COD concentrations of the pilot-scale IAAB can be predicted from Eq. (4.12) and Eq. (4.16), respectively (Table 4.6). The biomass concentration in both the anaerobic and aerobic compartments can be predicted using Eq. (4.13) and Eq. (4.17), respectively (Table 4.7).

4.3.2.3.4 Evaluation of Kinetic Models

Figures 4.22a and 4.22b showed the predicted COD_{out} in the anaerobic and aerobic compartment of the pilot-scale IAAB, respectively, which were obtained based on the Grau second-order multicomponent, Monod and Stover Kincannon kinetic models. The calculated COD_{out} for both the compartments was compared to the obtained experimental data of various OLR_{an}. The applicability of the kinetic models in predicting the performance of the pilot-scale IAAB was justified based on the R^2, whereby it needed to be at least 0.80.



(a)



(b)

Figure 4.22: The comparison between the predicted COD_{out} based on the Grau second-order multicomponent, Monod and Stover Kincannon kinetic models and experimental data for the (a) anaerobic (b) aerobic compartments of the pilot-scale IAAB

The anaerobically-treated effluent and aerobically-treated effluent COD concentrations can be predicted using Stover Kincannon due to the obtained R^2 of 0.9982 and 0.9998, respectively. This shows that OLR_{an} and OLR_a should be taken into consideration in estimating and optimising the performance of both the compartments.

Table 4.8 summarises the coefficients determined from the models applied in the previous studies and compared with the coefficients obtained in this study. The kinetic model of Stover Kincannon has been widely used for determining the kinetic constants in immobilised systems, viz. anaerobic filter, upflow anaerobic filter, upflow anaerobic sludge bed, integrated anaerobic-aerobic bioreactor, and covered lagoon regardless of anaerobic and aerobic systems. For the anaerobic system, the K_B (440.83 gCOD/L.day) and U_{max} (416.67 gCOD/L.day) values obtained in this study were higher than the values in the other studies (Table 4.8) except for the POME wastewater treatment using covered lagoon system. This may be due to higher rates of substrate utilisation in the anaerobic compartment using POME despite the high lignin concentration of 2,343 mg/L. This was further proven by the achievement of high $COD_{removal}$ of at least 96% for OLR_{an} ranging from 10.5 to 30.0 gCOD/L.day (Xu et al., 2013). This allow us to postulate that the high lignin content in POME does not inhibit the biological activity of the microbial cells in the anaerobic compartment. This can be carried out by maintaining high recirculation ratio between 12-26, which is vitally important to maintain high $COD_{removal}$ and accelerates the digestibility in the anaerobic compartment of the pilot-plant IAAB (Lomas et al., 2000 and Chan et al., 2012a). The K_B (0.6573 gCOD/L.day) and U_{max} (2.8209 gCOD/L.day) values achieved in the aerobic compartment were lower than the values in other studies as illustrated in Table 4.8. This shows that the anaerobically-treated POME despite the low lignin concentration (549 mg/L) does not attributed to the high rate of substrate utilisation. The results obtained for this study contradicted with the laboratory scale IAAB reported by Chan et al. (2012b), as shown in Table 4.8. It can be speculated that the performance of the two different processes in an integration system will outweigh one another (Edstrom et al., 2003; Wang et al., 2005). For Monod kinetic constants for the anaerobic system as shown in Table 4.8, a wide range of Y and K_d values ranging from 0.053 to 0.2116 mgVSS/mgCOD and 0.0038 to 0.093 per day, respectively were obtained for the treatment of ice-cream wastewater, simulated wastewater, synthetic wastewater, distillery spend wash, and POME. The Y value (0.1949 mgVSS/mgCOD) for the anaerobic process obtained in this study fell within the abovementioned range. The k_d (0.1088 per day) value obtained in this study was higher than that of most previous works. This may be due to the small size granules (<1.7 mm) in the anaerobic compartment resulting in limiting substrate could not be transferred throughout the granule mass (Jijia et al., 2015). Hence, occurrence of partial cell starvation, death and cell lysis that outweigh the new cell generation. On the other hand, Table 4.8 shows that the Y (0.0718 mgVSS/mgCOD) and k_d (0.0154 per day) were the lowest among all the other wastewaters. The big size granules (>1.7mm) in the aerobic compartment gave the lowest deadth rate constant value (k_d) that implies that the is no limitation of substrate transfer into the granule mass (Jijia et al., 2015).

As reported by Sponza and Ulukoy, (2008), the Monod kinetic constants of Y should be larger than k_d. In this study, the Y value is larger than the k_d for both the anaerobic and aerobic processes, which signify that the concentration of viable microbial cells is larger than the death microbial cells in both the compartments. This may be contributed to the usage of recirculation system, which allow the creation of homogenous mixing in both the compartments. In the anaerobic system, the μ_{max} value (0.176 day^{-1}) was comparable to those reported in Table 4.8, ranging between 0.058 and 0.784 day^{-1}. However, it was lower than the μ_{max} value (0.493 day^{-1}) determined by the treatment of POME using covered lagoon system, despite the same type of wastewater was being used in both the studies. This may be explained by the volume of the system with higher concentration of viable microbial cells is present in the anaerobic system. As compared to the similar type of system, the μ_{max} value in this study was found to be higher than the laboratory scale IAAB (Chan et al., 2012b) (0.103 day^{-1}). This may be probably due to the lower $MLVSS_{an}$ range (14,560-40,100 mg/L) in the laboratory scale IAAB. This shows that the volume of the system will affect the μ_{max} value as it demonstrated the concentration of active biomass in the system itself. The μ_{max} value obtained in the aerobic system was lower than the treatment of pharmaceutical (0.770 day^{-1}) and synthetic (11.520 day^{-1}) wastewater. This may be due to the higher COD concentration of the anaerobically-treated POME used in this study, which slow down the biodegradation efficiency. The μ_{max} value obtained in this study was higher than the laboratory scale IAAB, as shown in Table 4.8. This may be probably due to the high MLVSS concentration in the aerobic compartment of the pilot-scale IAAB (20,847 mg/L) as compared to the laboratory scale IAAB (18,000 mg/L) (Chan et al., 2012a). The K_s value obtained in this study was higher than those reported in Table 4.8 except for the covered lagoon system. This may be probably due to the insuffienct retention time allocated for the microbial cells to biodegrade the continuous incoming of raw POME resulting in accumulation of substrate in the system (Santos et al.,2017). The K_s value for the aerobic system was relatively similar to the one obtained by Chan et al. (2012a). This might be due to the use of similar feed and similar type of system, which were anaerobically-treated POME and integrated anaerobic-aerobic bioreactor (IAAB). The values of K_s and μ_{max} are highly dependant on the COD concentration of the substrate as well as the volume of the system. The achievement of low μ_{max} and high K_s signifies that raw POME cannot be biodegraded easily as compared to the other generic wastewater treatment (Truax et al., 1995; Chan et al., 2012b).

For Grau second order kinetic model, the k_s value for the anaerobic system was in a wide range of 0.058 day^{-1} to 252.0 day^{-1}. The k_s value obtained in this study (14.591 day^{-1}) fell within these ranges but significantly lower than k_s value (252 day^{-1}) of textile wastewater by Sandhya and Swaminathan (2006). The k_s value obtained in this study was higher than the POME wastewater conducted by Chan et al., (2012b) despite the use of the same type of reactor. The high k_s value (14.591 day^{-1}) obtained in this study correlated with the study reported by Borghei et al. (2008), whereby the increased in substrate removal depending on the microorganisms concentration in the reactor. The high removal efficiencies of COD and TSS of at least 95% in the anaerobic compartment may be probably due to the sufficienct concentration of bacteria (MLVSS of 36,129 mg/L).

The anaerobic compartment was relatively stable throughout the entire OLR_{an} (10.5-30.0 gCOD/L.day), which is reflected on the VFA:TA of 0.07 to 1.02 . The ability to maintain high MLVSS concentration (24,200-48,238 mg/L) in the anaerobic compartment mainly due to the entrapment of bacteria and development of biofilm on the packing media. These will prevent the occurance of sludge washout, hence maintaining the MLVSS concentration in the anaerobic compartment. Meanwhile, the k_s value obtained in the aerobic system was comparable to those of synthetic wastewater with low initial substrate concentration (750-2,250 mg/L) and low OLR_a range (0.75-4.50 gCOD/L.day) using upflow aerobic immobilised biomass (UAIB) (Borghei et al., 2008). The k_s value (3.363 day^{-1}) obtained in this study was almost similar to the laboratory scale IAAB (3.295 day^{-1}) reported by Chan et al., (2012b). This shows that the aerobic system of the IAAB regardless of laboratory or pilot scales were much superior than the UAIB, which may be due to the higher $MLSS_a$ concentration and metabolic activity of the aerobic microbial cells in both the IAABs. Retaining high concentration of solid (as reflected on the MLVSS) in the aerobic compartment may probably increased the microbial activity, which actively degrade the organic matter present in the anaerobically-treated POME.

The major conclusion that can be attained from this section is that the difference in the kinetic coefficiencts are mainly due to the variations in the characteristics of the wastewater, OLR, bacteria concentration (MLVSS), reactor configuration used in these studies (Table 4.8). Direct comparison of the kinetic parameters mentioned in Table 4.8 cannot be made with this study due to the abovementioned independent factors instead it can be used a reference only. Kinetic analysis on the same type of wastewater treatment system is vitally essential to attain a better understanding and prediction of the bioreactor's performance for future study. Hence, kinetic parameters obtained for this study using different kinetic models (Table 4.8) can act as a yardstick for the planning of the subsequent phase of the study as OLR_{an} 30.0 gCOD/L.day is the maximum applicable OLR_{an} of the pilot-scale IAAB (Sections 4.3.2.1 & 4.3.2.2).

During the operation of the pilot-scale IAAB at high OLR (up to 32.5 gCOD/L.day) not much operational problems were faced, viz. adjustment of pH was not needed, frequent scum formation, sludge washout and low $TSS_{removal}$. This allow us to infer that the pilot-plant IAAB system is feasible to be up-scaled into a commercial scale. As reported by Chan et al. (2021), a pre-commercialised IAAB is built at one of the palm oil mills in Malaysia with the scale-up factor of 1,667 and total removal efficiency of 99% for COD and BOD. The unique design in the packing section of the pilot-scale IAAB has allowed retention of biomass with high μmax (0.1764 day^{-1}) able to mitigate the clogging problem frequently faced by most of the UASFF bioreactors. However, the limitation of the IAAB technology (regardless of laboratory-scale or pilot-plant) is the possibility of experiencing biomass washout if the HRT is lower than 2.5 days if operating at OLR higher than 30.0 gCOD/L.day. Positive outcomes of this research will act as a stepping stone in providing sustainable energy solution in the palm oil industry. Hence this will allow the palm oil millers to play a vital role in conserving water quality as well as combating the issue of climate change.

Table 4.8: Comparison of kinetic constants of both the anaerobic and aerobic systems using the Monod, modified Stover -Kincannon and Grau second-order kinetic models under mesophilic condition

System	Kinetic Models	Substrate	Reactor Type	Influent COD (mg/L)	OLR (gCOD/L.day)	Kinetic Parameters				References
						K_B (gCOD/L.day)	U_{max} (gCOD/L.day)			
Anaerobic	Modified Stover Kincannon	Soybean ww	AF	7,520-11,450	2.06	85.50	83.30			(Yu et al., 1998)
		Simulated starch ww	UAF	100-4,000	1.15-16.70	49.80	50.60			(Ahn and Forster, 2000)
		Molasses	AHR	2,000-15,000	1.00-10.00	186.23	83.30			(Buyukkamaci and Filibeli, 2002)
		Simulated ww	UASB	4,214	1.00-15.80	8.20	7.50			(Işik & Sponza, 2005)
		Synthetic ww	AF	2,000-4,000	1.15-16.7	50.6	49.8			(Ahn et al., 2007)
		Papermill ww	UAF	1,972-3,536	1.08-11.38	104.15	86.21			(Yilmaz et al., 2008)
		Milk permeate ww	MBBR	8,610-23,230	2.0-28.0	102.3	89.3			(Wang et al., 2009)
		Slaughterhouse ww	UAF	6,000-6,500	3.1-6.1	120.88	99.01			(Padilla Gasca and Lopez-Lopez, 2010)
		POME	IAAB	68,550-79,800	10.50-21.50	14.70	23.10			(Chan et al., 2012b)
		POME	CL	77,075-168,741	1.00-4.37	3,580.33	3,333.33			(Yap et al., 2020)
		POME	$IAAB^P$	25,228-76,601	8.0-32.5	440.83	416.67			This study

						Y (mgVSS/mgCOD)	K_d (per day)	μ_{max} (per day)	K_S (mg/L)	
Anaerobic	Monod	Ice-cream ww	CSTR	5,500	ND	0.2116	0.0131	0.784	0.4028	(Hu et al., 2002)
		Simulated ww	UASB	4,214	1.00-15.80	0.125	0.0065	0.105	>4,000	(Işik and Sponza 2005)
		Synthetic ww	UASB	3,000-4,000	6.0-34.0	0.780	0.093	0.213	560	(Sponza and Ulukoy 2008)
		Distillery spend wash	AHR	90,000-130,000	4.5-11.1	0.053	0.004	-	-	(Kumar et al., 2007)
		Synthetic ww	UASB	300-4,000	0.9-20.1	0.083	0.006	0.058	226	(Bhunia and Ghangrekar 2008)
		POME	IAAB	68,550-79,800	10.50-21.50	0.196	0.038	0.103	8,168	(Chan et al., 2012b)
		POME	CL	77,075-168,741	1.00-4.37	0.0982	0.0048	0.493	120,910	(Yap et al., 2020)
		POME	IAAB[P]	25,228-76,601	8.0-32.5	0.1949	0.1088	0.1764	9,411	This study
						k_s (day $^{-1}$)	a (day $^{-1}$)	b[a]		
	Grau second-order	Synthetic ww	AHR	2,000-15,000	1.0-10.0	10.810	0.033	1.192		(Buyukkamaci and Filibeli 2002)
		Synthetic textile ww	UASB	4,214	1.0-15.8	0.337	0.562	1.095		(Isik and Sponza 2005)
		Textile ww	UAFB	243-1,973	1.0-8.21	252.0	0.915	5.138		(Sandhya and Swaminathan 2006)
		Synthetic ww	UASB	300-4,000	0.9-20.1	0.058	0.083	0.006		(Bhunia and Ghangrekar 2008)
		Synthetic ww	UASB	3,000-4,000	6.0-34.0	0.26	0.029	0.011		(Sponza and Ulukoy 2008)

Anaerobic	Grau second-order	Food processing ww	MACR	5,250-5,750	1.1-5.0	-	0.260	1.025		(Senturk et al., 2010)
		POME	IAAB	68,550-79,800	10.5-21.5	0.655	3.000	0.633		(Chan et al., 2012b)
		POME	$IAAB^P$	25,228-76,601	8.0-32.5	14.591	0.5261	0.8875		This study
Aerobic	Modified Stover Kincannon					K_B (gCOD/Lday)	U_{max} (gCOD/Lday)			
		Synthetic ww	MBBR	750-4,500	0.75-4.5	9.4	8.3			(Hosseiny and Borghei 2002)
		Synthetic ww	UAIB	750-2,250	0.75-4.5	106.8	101			(Borghei et al., 2008)
		AN POME	IAAB	5,000-24,000	0.72-7.2	327	312			(Chan et al., 2012b)
		AN POME	$IAAB^P$	1,000-16,100	0.44-11.14	0.6573	2.8209			This study
	Monod					Y (mgVSS/mgCOD)	k_d (per day)	μ_{max} (per day)	K_S (mg/L)	
		Synthetic ww	CSTR	2,600	5.2	0.500	0.096	11.520	9	(Vogelaar et al., 2003)
		Pharmaceutical ww	ASR	1,500-7,000	0.3-3.5	0.480	0.045	0.770	2,980	(Suman Raj and Anjaneyulu 2005)
		AT OMSR	BR	660-13,100	-	-	-	0.230	160	(Sanchez et al., 2007)
		AN POME	IAAB	5,000-24,000	0.7-7.2	0.418	0.054	0.148	311	(Chan et al., 2012b)

Aerobic	Monod	AN POME	IAAB^{P}	1,000-16,100	0.44-11.14	0.0718	0.0154	0.2465	501	This study
	Grau second-order					k_s (day $^{-1}$)	a (day $^{-1}$)	b^{a}		
		Synthetic ww	MBBR	750-4,500	0.75-4.5	0.337	0.562	1.095		(Hosseiny and Borghei 2002)
		Synthetic ww	UAIB	750-2,250	0.75-4.5	3.582	0.047	1.007		(Borghei et al., 2008)
		AN POME	IAAB	5,000-24,000	0.7-7.2	3.295	0.415	0.975		(Chan et al., 2012b)
		AN POME	IAAB^{P}	1,000-16,100	0.44-11.14	3.363	0.413	0.571		This study

[a]: Dimensionless

AF: Anaerobic filter; UAF: Upflow anaerobic filter; AHR: anaerobic hybrid reactor; UASB: upflow anaerobic sludge bed; MBBR: moving bed biofilm reactor; IAAB: integrated anaerobic-aerobic bioreactor; CL: covered lagoon; IAAB^{P}: Pilot Plant Integrated Anaerobic-Aerobic Bioreactor; CSTR: Continuous stirred tank reactor; UAFB: upflow anaerobic fixed bed; MACR: Mesophilic anaerobic contact reactor; UAIB: upflow aerobic immobilised biomass; ww: wastewater; AN POME: Anaerobically-treated POME

4.3.3 Mono-Digestion of Palm Oil Mill Effluent (POME)

The findings and the results obtained throughout the current study are presented and discussed as follows:

1. Performance evaluation of the pilot-scale IAAB at OLR 30.0 gCOD/L.day is vital to determine a set of operating parameters that allow the achievement of high removal efficiency and final discharge effluent that abide by the discharge limit set by Department of Environment (DOE) of Malaysia. In this section, the performance of the pilot-scale IAAB is evaluated in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solid (TSS) removal efficiencies.

2. The stability of the anaerobic compartment plays a vital role in allowing rapid and healthy growth of anoxic microbial cells. This is to ensure maximum degradation of the organic and inorganic substrate of palm oil mill effluent (POME), whilst producing biogas.

3. The achievement of BOD 20 mg/L for the final treated effluent is vitally important as it will be released into the watercourse. Important dependant parameters, viz. mixed liquor volatile suspended solid (MLVSS) and food-to-microorganisms ratio (F/M), are important in allowing the aerobic microbial cells to perform maximum degradation of the organic matter in the anaerobically-treated POME.

4. Kinetic model, viz. Stover Kincannon, Grau Second-Order and Contois were used to gauge the performance of the pilot-scale IAAB at OLR_{an} 30.0 gCOD/L.day. The kinetic model will be selected based on the obtained correlation coefficients (R^2 > 97%). The kinetic parameters obtained will allow us to predict the performance behaviour or the design of the biological reactors for future upscaling purposes.

4.3.3.1 Reactor Performance

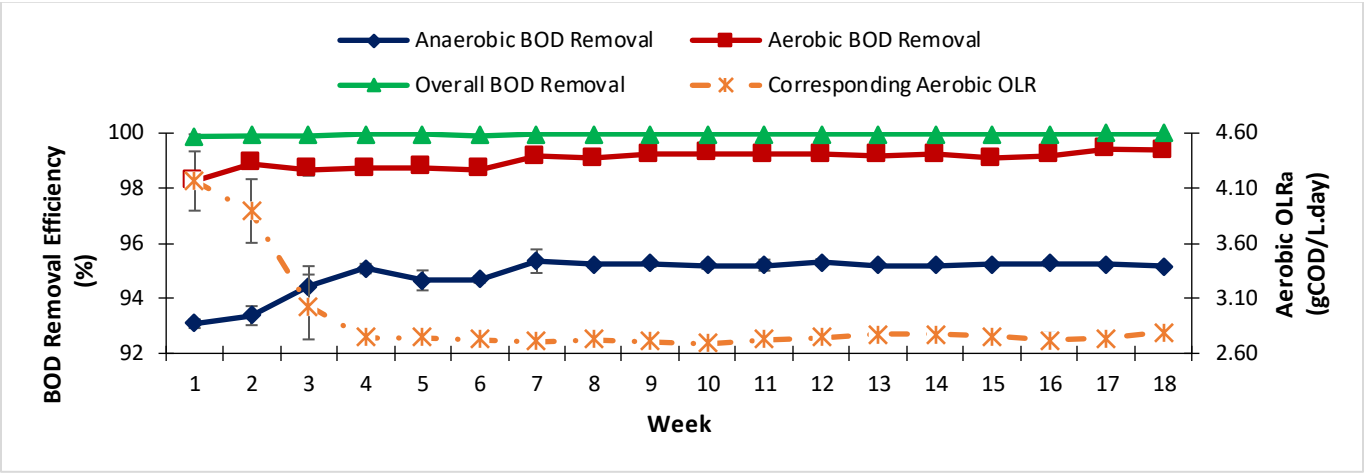
The pilot-scale reactor was operated on 25th April 2016 and has been operated for 348 days with 14 different anaerobic organic loading rate (OLR_{an}) (1.0-32.5 gCOD/L.day). Prior to this study, the pilot-scale reactor was operated at various OLRs to determine the most optimum OLR_{an} that is able to produce high removal efficiency for the tested parameters and produce final treated effluent that abides the discharge limit set by the DOE, Malaysia. Additionally, the pH value of the pilot-scale reactor was maintained within the range of 6.51-7.28, which is within the appropriate range for mesophilic digestion and methanogenesis. The pilot-scale reactor was initially operated at OLR_{an} 30.0 gCOD/L.day, using the operating condition at the most optimum OLR_{an} (30.0 gCOD/L.day). The operating condition of the pilot-scale IAAB operated in the mode of mono-digestion at OLR_{an} 30.0 gCOD/L.day is shown in Table D1 (Chapter 7, Appendices).

4.3.3.2 Performance Evaluation of Mono-Digestion

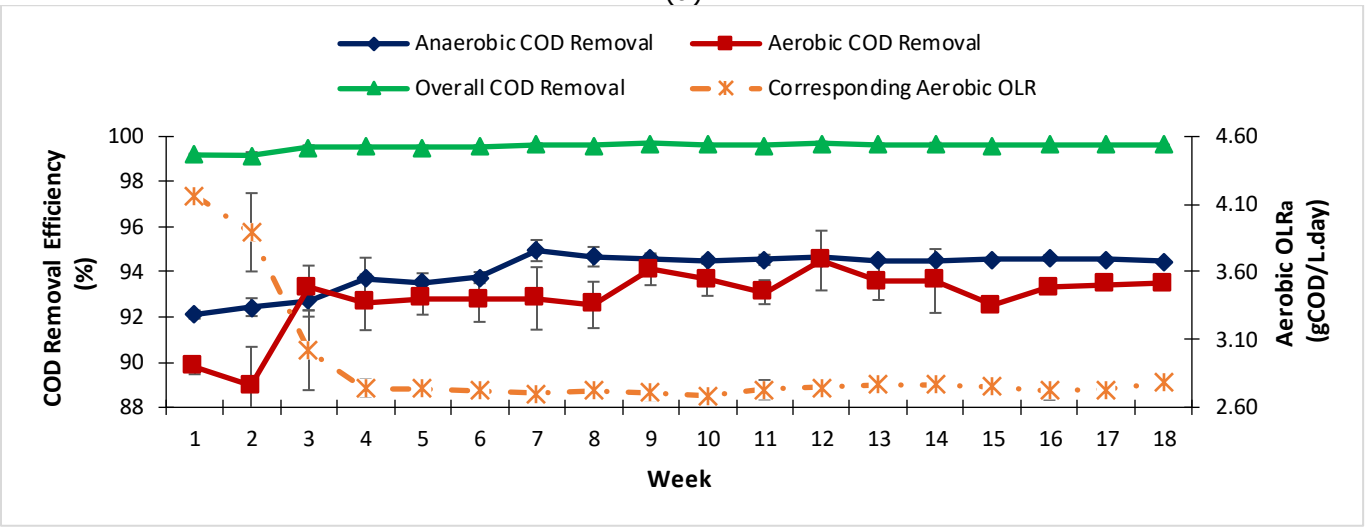
Based on the previous steady-state performance study (Section 4.3.2) carried out by varying the OLR_{an} ranging from 10.5 gCOD/L.day to 32.5 gCOD/L.day, it was reported that OLR_{an} 30.0 gCOD/L.day gave the highest overall removal efficiencies of BOD (99.9%), COD (99.7%) and TSS (99.8%), methane yield (0.0566 $LCH_4/gCOD_{removed}$), methane purity (58%), and, BOD of the final treated effluent (79% of the data obtained has BOD of less than 20 mg/L within the study period). Such promising data obtained during this study period has been put into a longer study period (18 weeks) to obtain a set of data that is comparable with the current industry mode of treatment method. The pilot-scale IAAB was operated under the mode of mono-digestion whereby only POME was added into the reactor at a continuous manner. The performance of the pilot-scale IAAB was evaluated in terms of $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$, which is the same dependent parameters as the laboratory-scale IAAB. This will allow us to compare the performances of both the IAABs at two different scales. In this study, the BOD, COD and TSS of the raw POME used were 73,412 mg/L, 146,824 mg/L and 29,138 mg/L, respectively.

4.3.3.2.1 Anaerobic Process

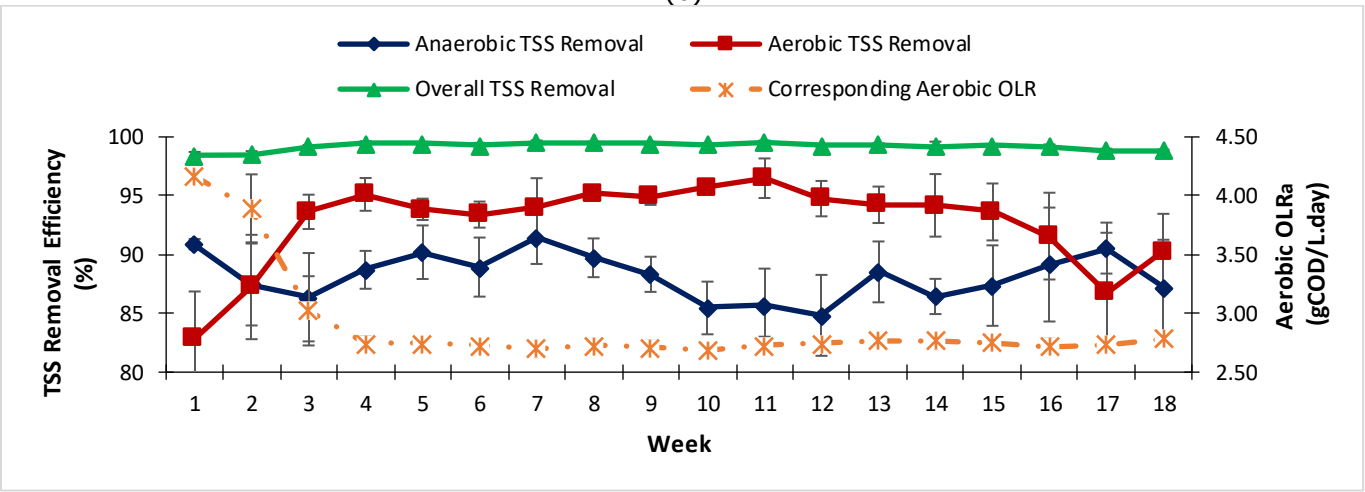
Figures 4.23a, 4.23b and 4.23c show the average BOD, COD and TSS removal efficiency profiles throughout the entire study period.



(a)



(b)



(c)

Figure 4.23: (a) BOD, (b) COD, (c) TSS, removal efficiencies of OLR 30.0 gCOD/L.day. The error bars are the standard deviations.

As observed, a similar trend of weekly average $BOD_{removal}$ and $COD_{removal}$ were observed with $BOD_{removal}$ of 92 – 95% and $COD_{removal}$ of 93 – 95% were obtained from Week 1 to Week 6 (OLR_{an} 30.0 gCOD/L.day and average HRT_{an} of 2.65 ± 0.22 day) for the anaerobic treatment. Starting from Week 7 till Week 18, constant $BOD_{removal}$ and $COD_{removal}$ were obtained with removal efficiencies of at least 95% (shown in Figures 4.27a and 4.27b) at average of HRT_{an} of 2.82 ± 0.17 day. It is worth noting that the anoxic microbial cells in the anaerobic sludge able to continuously biodegrade the high concentration of the organic matter present in the POME despite operating the pilot-scale IAAB at various OLR_{an} (10.5 gCOD/L.day to 32.5 gCOD/L.day) for a period of 348 days prior to operating it at OLR_{an} 30.0 gCOD/L.day. This shows that the pilot-scale IAAB is robust enough to handle different scenario, viz. operating under various range of OLR_{an} and handling of the constant fluctuation of the characteristics of POME due to the high or low crop of FFB. Additionally, from Weeks 1-3 showed a slow increment of the $BOD_{removal}$ and $COD_{removal}$ for the anaerobic treatment with removal efficiencies of 93 – 94% and 92 – 93%, respectively. During this period of time the anoxic microbial cells were acclimatising to the sudden changes in the operating condition in terms of reduction of OLR_{an} (from 32.5 gCOD/L.day to 30.0 gCOD/L.day) and increment of HRT_{an} (from 2.33 ± 0.13 day to 2.76 ± 0.20 day). This allow us to deduce that the anoxic microbial cells required a period of 21 days to adapt themselves to the sudden changes in the operating condition. On the other hand, the same trend as the $BOD_{removal}$ and $COD_{removal}$ was not observed in the $TSS_{removal}$ profile (Figure 4.23c). Fluctuation of the $TSS_{removal}$ was observed throughout the 18 weeks of study period ranges from 85% to 91%. Lowest and highest $TSS_{removal}$ was achieved on week 12 (84.8%) and week 7 (91.4%), respectively, as shown in Figure 4.23c. This may be explained by the poor settling of sludge separated from the anaerobically-treated effluent. Additionally, the rate of recycling of sludge from the bottom of anaerobic compartment to the middle-section of the anaerobic compartment labelled as SP3 (Figure 3.7, Chapter 3, Materials and Methods) may also affect the biodegradation activity of the anoxic microbial cells due to the texture of the anaerobic sludge. The applied R_r of 2.30 – 3.77 (Table D1, Chapter 7, Appendices) may not be sufficient to allow homogenous mixing of the mixture at the bottom of the compartment (consisting of fresh POME) with the mixture at the middle-section of the compartment (consisting of POME and anaerobic sludge). This has resulted in least concentration of organic matter being exposed to the anoxic microbial cells, which may affect the $TSS_{removal}$ of the anaerobic compartment. Besides altering the R_r, sludge removal from the compartment may also aid in reducing the texture of the anaerobic sludge. In this scenario, a lower R_r may be used for recycling purposes, which will increase the substrate concentration for the anoxic microbial cells to biodegrade.

The $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ obtained during the study period was satisfactory with removal efficiencies of more than 85%. This may be contributed by good inoculum seed (MLVSS of 48,000 mg/L), which resulted in good microbial growth. The second factor is the configuration of the pilot-scale IAAB. As reported by Chan et al. (2012a), the anaerobic compartment of the IAAB (regardless of laboratory-scale or pilot-scale) comprises of two different types of bioreactor design, i.e. UASFF and UASB bioreactors. The usage of packing media fixed in the hollow structure of the middle compartment of the anaerobic compartment able to retain the biomass by allowing the anoxic microbial cells to remain in the pores of the packing media. The position of the packing media is parallel to the recirculation outlet (Figure 3.7, Chapter 3, Materials and Methods), which will increase the contact between the microbial cells with the wastewater recirculated via the bottom of the anaerobic compartment (labelled as R1 in Figure 3.7, Chapter 3, Materials and Methods). Besides being able to hold the biomass in the packing media with the aid of the hollow structure in the middle of the anaerobic compartment, HRT (2.30-3.13 days) may be the contributing factor towards the achievement of satisfactory removal efficiencies. Study reported by Chelliapan et al. (2011) demonstrated that an increase in HRT (from 2 to 4 days) for the treatment of brewery wastewater has resulted in an increased $COD_{removal}$ from 82% to 92%. The same observation was obtained in this study, whereby an increase in $COD_{removal}$ was observed when the HRT was increased from 2.70 to 2.90 days. This allow us to deduce that maximum retainment of the biomass and HRT of the anaerobic compartment are the major factors that may affect the removal efficiencies of the tested parameters. The concept of the UASFF bioreactor during the designing of the anaerobic compartment has proven that treatment of POME can be carried out at high OLR_{an} and short HRT of 2.76 ± 0.20 day. The same observation was obtained by the study reported by Zinatizadeh et al. (2006). Zinatizadeh and co-workers showed that the UASFF bioreactor was able to treat POME at high OLR_{an} (23.15 gCOD/L.day) and short HRT (1.5 days). This allow us to deduce that the UASFF bioreactor may be suitable to be used for the treatment of high strength wastewater at high OLR_{an} and short HRT for the achievement of removal efficiencies of at least 85% for the tested parameters.

Even though the pilot-scale IAAB was operated at high OLR_{an} (30.0 gCOD/L.day) for a period of 118 days, sudden decrease in $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ were not observed. As reported by Mamun and Idris (2008), a decrease in $BOD_{removal}$ and $COD_{removal}$ of 91.4% to 64.7% and 85% to 65.2%, respectively, were observed when the loading rate increased from 1.5 kgBOD/m^3.day to 6.12 kgBOD/m^3.day. However, the same trend was not observed in this study. This again confirmed that the adaptation of UASFF technology in the anaerobic compartment of this pilot-scale IAAB is able to withstand high loading rates whilst maintaining the removal efficiencies at least 85%. As reported by Emadian et al. (2015), the use of recirculation system in the anaerobic compartment has further enhanced the degradation process as well.

This may be due to the continuous exposure of the incoming influent to the anoxic microbial cells that resides between the pores of the packing media. It is worth noting that the application of high OLR_{an} (30.0 gCOD/L.day) in the treatment of high strength wastewater with organic removal efficiencies of more than 85% has been not been reported so far.

4.3.3.2.2 Aerobic Process & Settling Tank

Microorganisms involved in the removal of carbonaceous contaminants from wastewater require sufficient concentration of nitrogen and phosphorus for maximal growth and reproduction. The provision of sufficient concentration of nutrients (expressed in terms of BOD:TN:TP) is essential to stimulate the microbial cells to perform their respective synthesis reaction. Low nutrient concentration will promote the growth of filamentous bacteria over floc formers that affect the settling of the activated sludge. So as to prevent the occurrence of poor settling of activated sludge, the determination of BOD:TN:TP of the anaerobically-treated POME is essential in this study. The recommended BOD:TN:TP of 100:6:1.5, coupled with aerator in the activated sludge, is able to enhance the growth of microbial cells in the aerobic process to produce effluent with BOD and TSS of less than 50 mg/L. The anaerobic treatment of the POME reduces its organic strength drastically and results in an effluent with BOD:TN:TP of 100:39:43. This meet the nutrient balance criteria for activated sludge process for sustaining the aerobic metabolic activity. This infers that anaerobic treatment is the most suitable initial treatment for POME to produce anaerobically-treated effluent for activated sludge process without any additional cost of nutrient addition into the system. Similar trend was observed at the removal efficiencies profiles (Figures 4.27a - 4.27c) with the occurrence of slow increment of removal efficiencies during the study period of week 1 – week 3. During these 3 weeks, the microbial cells in the activated sludge may be still in the recovery phase as reactor failure was observed during OLR_{an} 32.5 gCOD/L.day (corresponding to average OLR_a of 6.73 ± 1.73 gCOD/L.day) as reported in the previous study (Section 4.3.2). The pilot-scale IAAB was operated at various OLR_{an} ranging from 10.5 gCOD/L.day to 32.5 gCOD/L.day, prior to operating the pilot-plant IAAB at OLR_{an} 30.0 gCOD/L.day (corresponding to average OLR_a of 2.86 ± 0.37 gCOD/L.day). The low concentration of microbial cells in the aerobic compartment as reflected on the average MLVSS concentration of 22,431 ± 416 mg/L may also contributed to the slow increment of removal efficiencies. The average F/M ratio obtained for week 1, week 2 and week 3 were 0.202 ± 0.001 gCOD/gMLVSS.day, 0.193 ± 0.001 gCOD/gMLVSS.day and 0.139 ± 0.008 gCOD/gMLVSS.day, respectively (as illustrated in Figure 4.24). At these conditions, the average BOD of the final treated effluent for week 1, week 2 and week 3 were 44.33 ± 27.47 mg/L, 27.14 ± 0.89 mg/L, and 28.14 ± 3.44 mg/L, which is higher than the targeted BOD of 20 mg/L. This further confirmed that sufficient concentration of biologically active portion of the microbial cells plays a major role in degrading the organic matter available in the anaerobically-treated POME to produce final treated effluent that abides the discharge limit set by DOE Malaysia.

Alteration was carried out on the returned activated sludge (RAS) and waste activated sludge (WAS) ratios. A decrease in R_{RAS} from 5.47 to 1.99 was carried out. Additionally, an increase in R_{WAS} from 0.88 to 2.92 was implemented as well. This is to remove the activated sludge located at the bottom of the hopper of the settling tank that may contained inactive microbial cells from the previous study. The former act was carried out to slow down the rate of recycling of activated sludge from the bottom of the hopper of the settling tank to the aerobic tank to minimise the mixing of inactive microbial cells (in the settling tank) with the active microbial cells (in the aerobic compartment). By performing such act, it is able to reduce the occurrence of shock loading in terms of overgrowing of filamentous bacteria over the floc formers and foam formation. The changes on the RAS and WAS ratios had resulted in great improvement on the performance of the pilot-scale IAAB in terms of the $BOD_{removal}$ (improved by 1%), $COD_{removal}$ (improved by 3%) and $TSS_{removal}$ (improved by 12%), as illustrated in Figures 4.27a-4.27c. Alteration on the RAS and WAS ratios may be the key operating parameters governing the performance of the aerobic compartment. Performance improvement in terms of the removal efficiencies for the tested parameters was agreeable with outcomes reported by Hosseini, et al. (2008). During this period of time, the concentration of the microbial cells increases from 22,000 mg/L to 23,000 mg/L (as reflected on the MLVSS reading). This allow us to infers that the aerobic compartment of the pilot-scale IAAB required at least 21 days to fully recover from the occurrence of shock loading due to the application of high OLR_{an} (32.5 gCOD/L.day) in the previous study.

Starting from Weeks 4 – 18 onwards, an increased in $BOD_{removal}$ and $COD_{removal}$ were observed except for $TSS_{removal}$ (as shown in Figure 4.23a-4.23c). This shows that MLVSS concentration of more than 22,000 mg/L but less than 23,000 mg/L and sufficient dissolved oxygen (DO) concentration of 2.03 – 5.21 mg/L were adequate for the microbial cells in the aerobic compartment of the pilot-scale IAAB to carry out their respective biological activity effectively. Additionally, the RAS and WAS ratios were modified as well. The former parameter was increased from 1.99 to 2.09 whereas the latter parameter was reduced from 2.92 to 2.29. Under such operating condition, the $BOD_{removal}$ and $COD_{removal}$ obtained during Weeks 4-18 ranges from 98%-99% and 92%-94%, respectively. This again allow us to confirm that the aerobic compartment of the pilot-scale IAAB had fully recovered from the occurrence of shock loading and it was operated at OLR_{an} 30.0 gCOD/L.day (corresponding to average OLR_a of 2.73 ± 0.05 gCOD/L.day) instead of the previous OLR_{an} 32.5 gCOD/L.day (corresponding to average OLR_a of 3.58 ± 0.55 gCOD/L.day). Maintaining the operational parameters (Table D1, Chapter 7, Appendices) throughout the study period will allow the achievement of removal efficiencies of at least 90% for the tested parameters.

However, the $TSS_{removal}$ profile does not give the similar trend as the $BOD_{removal}$ and $COD_{removal}$ profiles. An increment of $TSS_{removal}$ was achieved during Weeks 4 - 16 at the range of 92% to 96% (Figure 4.23c). Towards the end of the study period (Weeks 17-18), a drastic drop in the $TSS_{removal}$ was observed (Figure 4.23c). The average $TSS_{removal}$ obtained during Week 17 and Week 18 were 86.76 ± 5.10%, and 90.26 ± 3.20%, respectively. At Weeks 17-18, sludge bulking and formation of foam was detected in the aerobic compartment and settling tank of the pilot-scale IAAB. This may be explained by the existence of competition between filamentous bacteria and floc formers for the same source of food. The reduction of R_{RAS} (from 2.02 to 1.87) and increment of DO concentration (from 2.00 to 2.73) has affected the homogenous mixing of the fresh- (aerobic compartment) and non-fresh activated sludge (settling tank). Despite the increased of the R_{WAS} (from 1.82 to 2.73), but the sludge in the settling tank was difficult to be removed due to the thick texture of the solid itself. As a result, accumulation of inactive microbial cells occurred in the settling tank. Higher amount of energy was required by the Pump 105 (responsible for the discharging of solid from the settling tank to the drain) (Figure 3.7, Chapter 3, Materials and Methods). By explaining in terms of the integrated design of the pilot-scale IAAB, the poor performance of the aerobic compartment will affect the settling tank and anaerobic compartment as well. The high biomass concentration in the settling tank may create risks for the activated sludge process to deteriorate, due to the low sludge age and the high concentrations of suspended solids in the supernatant. The high concentrations of suspended solids in the supernatant may also be caused by rising sludge, due to the of nitrogen gas via the pathway of biological denitrification (Wilen 1995). The poorly soluble nitrogen gas will adhere to the activated sludge flocs and float them to the surface of the settling tank, resulting in the flowing of activated sludge to the external environment. The final treated effluent produced during the Week 17 consist of mixture of solid and supernatant, i.e. poor separation of solid from supernatant. Daily rectification, viz. Foam removal from the surface of the aerobic compartment and removal of aged sludge from the settling tank does not seems to aid in increasing the $TSS_{removal}$ to at least 92%. Instead, the $TSS_{removal}$ obtained during the Weeks 17 and 18 were 86.76 ± 5.10 % and 90.26 ± 3.20 %, respectively. At this stage, the aerobic compartment of the pilot-scale IAAB may be experiencing partial inhibition. Such occurrence may be rectified either by removing the sludge from the settling tank manually or adjustment of pH using caustic, lime or magnesium hydroxide if the pH is less than 6.5 (Jenkins et al., 1993).

4.3.3.2.3 Food-to-Microorganisms (F/M) Ratio

The operation of the pilot-scale IAAB at OLR_{an} 30.0 gCOD/L.day (corresponding to average OLR_a 2.86 ± 0.37 gCOD/L.day) was carried out in a continuous manner for a period of 126 days. It is vital to determine the daily F/M ratio, which will act as an indicator for (a) maintaining the optimum biomass concentration in the aerobic compartment; (b) determine the amount of biomass needed to be discharged daily from the settling tank. It is worth to note that 70% of the total study periods had a final treated effluent with BOD of less than 20 mg/L (Figure 4.24).

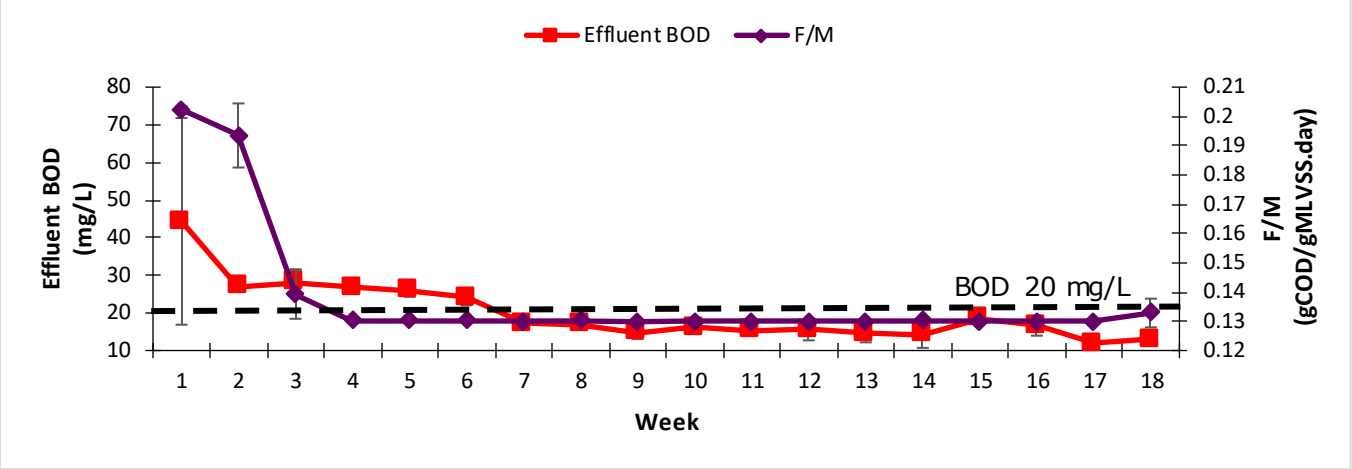


Figure 4.24: Profile of F/M and Final Treated Effluent BOD of the Pilot-Scale IAAB at mono-digestion mode (Aerobic Compartment)

At the initial period of the study, the pilot-scale IAAB was able to produce final discharge effluent with an average BOD of 44 ± 27.47 mg/L and F/M ratio of 0.202 ± 0.001 gCOD/gMLVSS.day. Despite being able to produce final discharge effluent that abides by the discharge limit set by the DOE Malaysia, it is important to develop a prototype that consistently produce final discharge effluent with BOD of less than 20 mg/L at high OLR_{an}. This is to minimise the occurrence of diluting POME or shorten the retention period of the treatment at the aerobic or polishing pond. A drastic drop in the effluent BOD was observed in week 2 with an average BOD of 27 ± 0.89 mg/L with a slight drop in F/M ratio and MLVSS of 0.19 ± 0.011 gCOD/gMLVSS.day and 22,183 ± 854 mg/L, respectively. Modification on the R_{RAS} (decrease from 5.47 to 3.86) and R_{WAS} (increase from 0.88 to 0.89) allow us to denote that F/M ratio governs the bacterial concentration in the aerobic compartment. This has resulted in slight improvement on the performance efficiency of the aerobic compartment in terms of the $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ (as shown in Section 4.3.3.2.3). Accumulation of solid was observed in the aerobic tank at week 2 (F/M ratio of 0.19 ± 0.011 gCOD/gMLVSS.day; average OLR_a of 3.89 ± 0.289 gCOD/L.day). Under this scenario, a slight drop in MLVSS and sludge volume index (SVI) from 22,200 mg/L to 22,183 mg/L and SVI of 95, respectively. The SVI obtained allow us to infer that the texture of the sludge in the aerobic compartment of the pilot-scale IAAB may be old and over-oxidised. To rectify this issue, large amounts of solid regardless of active or non-active activated sludge was discharged from the designated waste outlet (via P105, Figure 3.7, Chapter 3, Materials and Methods). The same observation was reported by Abdullah, et al. (2013), where the microbial cells have been washed out from the reactor, as reflected on the low MLSS concentration. The poor settling of the activated sludge allows us to postulate that filamentous network may not be formed within each of the microbial cells if the activated sludge is observed under the microscope (Abdullah et al., 2013).

In order to promote the formation of the filamentous network, which is the backbone for the build-up of bioflocs or bioparticles in the activated sludge system, the RAS and WAS ratios were maintained at the range of 1.73 to 3.99 and 3.43 to 4.50. This is to increase the concentration of microbial cells in the aerobic compartment of the pilot-scale IAAB by ensuring homogenous mixing in the aerobic tank with the aid of the installed compressor that sends oxygen directly into the tank. At the same time, sludge was rapidly discharged from the settling tank to prevent the formation of dead zones. Under this scenario, this will enhance the growth of microbial cells by allowing them to replicate themselves, which will increase the number of bacteria cells (as reflected in the MLVSS concentration).

The reduction of average Q_{in} from 409 ± 24.66 L/day (Week 2) to 369 ± 26.34 L/day (Week 3) responsible for the pumping of influent from the feed tanks (T101 and T102) (Figure 3.7, Chapter 3, Materials and Methods) had reduced the F/M ratio from 0.193 ± 0.011 gCOD/gMLVSS.day to 0.139 ± 0.008 gCOD/gMLVSS.day, whilst maintaining the BOD of the final discharge effluent at 28 ± 3.44 mg/L (Figure 4.24). Additionally, the increase of the bacteria concentration from 22,183 ± 854 mg/L to 22,912 ± 561 mg/L (Figure 4.25) has also resulted in a decrease F/M ratio. Lowest fluctuation on the F/M ratio (0.130 ± 0.0007 gCOD/gMLVSS.day) was observed from week 4 to week 18 (Figure 4.24). This is achievable by controlling the WAS ratio between 3.43 to 4.50 to maintain the bacteria concentration in the aerobic tank within 22,900 mg/L and 23, 350 mg/L, as reflected on the measured MLVSS concentration (Figure 4.25). The low F/M ratio of 0.130 ± 0.0007 gCOD/gMLVSS.day obtained in this study was not observed in the other aerobic treatment of the anaerobically-treated POME. As reported by Rahman (2017), the recommended F/M ratio for aerated activated sludge system is 0.2 gCOD/gMLVSS.day with MLVSS concentration of 1,000 to 2,000 mg/L. However, the low F/M ratio (0.130 ± 0.0007 gCOD/gMLVSS.day) observed in this study is mainly due to the high MLVSS concentration (22,000 to 23,350 mg/L) in the aerobic compartment of the pilot-scale IAAB. The recommended F/M ratio found in this study may not be suitable for treatment of anaerobically-treated POME using open ponding system. This may be explained by the high Q_{in} (11.92 ± 41.5 m^3/hour – 40.4 ± 15.15 m^3/hour) used to pump influent into the ponding system, which will increase the F/M ratio (Poh et al., 2010). The recommended F/M ratio may be more suitable for the treatment of anaerobically-treated POME in bioreactor system. It consists of one or two integrated or separated compartmentalisation used for the treatment of POME. The two different systems used in the treatment of POME has affected the allocated retention time, as it depends on the Q_{in} of the influent. As a result, the obtained F/M ratio in this study (0.130 ± 0.0007 gCOD/gMLVSS.day) is lower than the recommended F/M ratio (0.2 gCOD/gMLVSS.day), as the latter ones was obtained from samples treated using the open ponding system.

Theoretically, the obtained F/M ratio in our study indicates that the microorganisms concentration outweighs the amount of food in the aerobic compartment of the pilot-scale IAAB, where the microbial cells will tend to clump together to form a dense floc that will settle well in the clarifier (Tchobanoglous et al., 2014). The act of homogenous mixing of the activated sludge with the incoming anaerobically-treated effluent and mixing of fresh and recycled activated sludge were carried out using the installed compressor that sends oxygen directly into the aerobic tank, which may prohibit the formation of clumped microbial cells and further enhance the degradation process. The oxygen concentration in the aerobic compartment of the pilot-scale IAAB can be monitored via the installed DO meter. The provision of sufficient air (as reflected on the DO reading) is able to increase the productivity of the microbial cells synthesis by further degrading the organic matter in the anaerobically-treated effluent. Hence, a reduction of BOD in the final treated effluent was obtained. On Week 7, the BOD of the final treated effluent was 17.14 ± 1.68 mg/L, while the F/M ratio and MLVSS concentration obtained were 0.129 ± 0.0003 gCOD/gMLVSS.day and 22,921 mg/L, respectively. This allows us to denote that the bacteria concentration and F/M ratio measured in Week 7 in the aerobic compartment of the pilot-scale IAAB may act as a parameter to produce final treated effluent with BOD of less than 20 mg/L. From Weeks 8 to 18, the bacteria concentration and F/M ratio in the aerobic compartment of the pilot-scale IAAB were maintained at a range of approximately 23,000 mg/L (Figure 4.25) and 0.129 - 0.130 gCOD/gMLVSS.day (Figure 4.24), respectively. The average BOD of the final treated effluent obtained for week 8, week 9, week 10, week 11, week 12, week 13, week 14, week 15, week 16, week 17, and week 18 were all well below 20 mg/L, i.e., 16.86 ± 0.69 mg/L, 14.86 ± 1.77 mg/L, 16.14 ± 0.90 mg/L, 15.45 ± 0.59 mg/L, 15.49 ± 2.80 mg/L, 14.60 ± 2.43 mg/L, 14.13 ± 3.43 mg/L, 16.61 ± 2.66 mg/L, 16.61 ± 2.66 mg/L, 12.00 ± 0.00 mg/L, and 13.00 ± 1.00 mg/L, respectively.

4.3.3.2.4 MLVSS Concentration

Throughout the study period (18 weeks), it should be noted that there is no any addition of activated sludge into the aerobic compartment of the pilot-scale IAAB. Throughout the study period, the MLVSS concentration demonstrated a relatively stable profile (Figure 4.25).

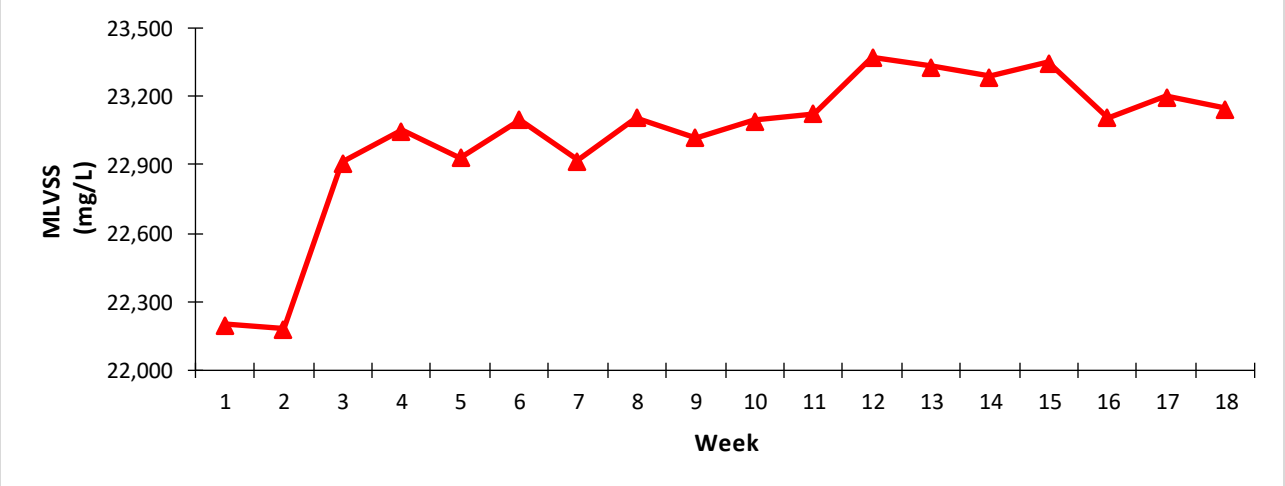


Figure 4.25: MLVSS Concentration of the aerobic compartment of the pilot-scale IAAB at mono-digestion mode

Throughout the 18 weeks, the MLVSS concentration ranges between 22,000 mg/L and 23,000 mg/L, which denotes that high concentration of active growth microbial cells is present in the aerobic compartment of the pilot-scale IAAB. The lowest and highest MLVSS concentrations were observed in Week 2 (22,183 ± 854 mg/L) and Week 15 (23,350 ± 383 mg/L), respectively. This shows that by maintaining the RAS (1.73 - 3.99) and WAS (3.43 – 4.50) ratios responsible for the recycling of sludge from the settling tank back to the aerobic tank and removal of sludge from the settling tank, respectively, will be able to proliferate and maintain the growth of the active microbial cells in the aerobic compartment. The MLVSS concentrations in Week 1 (22,200 ± 176 mg/L), Week 2 (22,183 mg/L ± 854 mg/L) and Week 3 (22,912 ± 561 mg/L) were the lowest as compared to the other weeks (4-18) (Figure 4.25). The performance of the aerobic compartment was also affected in terms of the low $BOD_{removal}$ (Week 1: 98.26%; Week 2: 98.91%; Week 3: 98.68%), $COD_{removal}$ (Week 1: 89.81%; Week 2: 88.97%; Week 3: 93.29%) and $TSS_{removal}$ (Week 1: 82.83%; Week 2: 87.24%; Week 3: 93.63%). The bacteria concentration in the aerobic compartment showed a slight increment in Week 4 with MLVSS of 23,051 ± 452 mg/L (Figure 4.25) with improved removal efficiencies of the tested parameters (Figures 4.27a-4.27c). This allow us to further confirmed that the aerobic compartment of the pilot-scale IAAB required at least 21 days to be fully recovered from the previous OLR_{an} (32.5 gCOD/L.day).

An increase in $BOD_{removal}$ (98.70% to 99.08%), $COD_{removal}$ (92.65% to 94.50%) and $TSS_{removal}$ (93.39% to 96.49%) were observed when the MLVSS concentration in the aerobic compartment was maintained in the range of 22,000 – 23,350 mg/L. A slight increase in the MLVSS concentration in Week 6 (23,099 ± 299 mg/L) as compared to Week 5 (22,933 ± 487 mg/L). The $BOD_{removal}$ and $COD_{removal}$ were affected slightly with reduction of removal efficiencies from 92.84% to 92.76% and 98.74% to 98.68%, respectively. Significant reduction was observed on the $TSS_{removal}$ from 95.11% to 93.39%. The SVI of the activated sludge in the aerobic compartment of the pilot-scale IAAB was 70 (less than preferred SVI range of 100-200). The TSS of the final treated effluent in Week 6 was 58.86 ± 17.63 mg/L as compared to Week 5 (51.86 ± 5.08 mg/L). This shows that the synthesis reaction of the aerobic microbial cell is highly sensitive towards slight modification on the operating parameter, in terms of decreasing the WAS ratio to 1.48. As such, the inactive microbial cells may still remain in the settling tank or being recirculated into the aerobic compartment. This allows us to infer that maintaining the operating condition of the pilot-scale IAAB (Table D1, Chapter 7, Appendices), viz. maintaining the RAS ratio (1.73 – 3.99) and WAS ratio (3.43 – 4.50) played relatively important roles in maintaining optimum concentrations of biologically active portion of the microbial cells in the aerobic compartment and settling tank. This in turn will directly affect the degradation activity of the aerobic microbial cells, as reflected in the removal efficiencies of the tested parameters.

This finding concurred with the study reported by Rahman (2017), where sufficient aeration, RAS ratio, WAS ratio, and consistent feeding of anaerobically-treated effluent into the aerobic compartment are the major dependant parameters that required to be controlled and monitored during the operation period without affecting the removal efficiencies of the tested parameters. In this study, the pilot-scale IAAB was able to successfully produce final treated effluent with BOD less than 20 mg/L by maintaining optimum MLVSS (22,200 mg/L – 23,350 mg/L), DO concentration (2.03 mg/L – 5.21 mg/L), RAS ratio (1.73 – 3.99) and WAS ratio (3.43 – 4.50).

4.3.3.3 Stability of Mono-Digestion

The stability of the anaerobic compartment is determined by the ratio of VFA/TA. For example, when the ratio is less than 0.3-0.4, the process is considered as favourable (Borja et al., 2004; Chen et al., 2008; Chan et al., 2012a). Since the VFA/TA ratio, where VFA concentration represents the factor influencing system stability and TA concentration represents the buffering capacity, can properly reflect the result of the interaction between them. However, the parameter of VFA/TA ratio may not be feasible to evaluate the stability of the anaerobic system for high strength wastewater, viz. sewage sludge or POME. VFA accumulation, biogas production and ammonia concentration are the main factors that needed to be taken into consideration during the evaluation of real stability degree of the anaerobic system. As reported by Duan et al. (2012), system stability of the high-solid anaerobic system was affected by VFA concentration and successful recovery was proved at high OLR (2.0 kgVS/m^3.d^3) with VFA accumulation up to 10 g/L when the VFA/TA ratio was near to 1.0. The stability of the pilot-scale IAAB in the mono-digestion mode is presented in Figure 4.26.

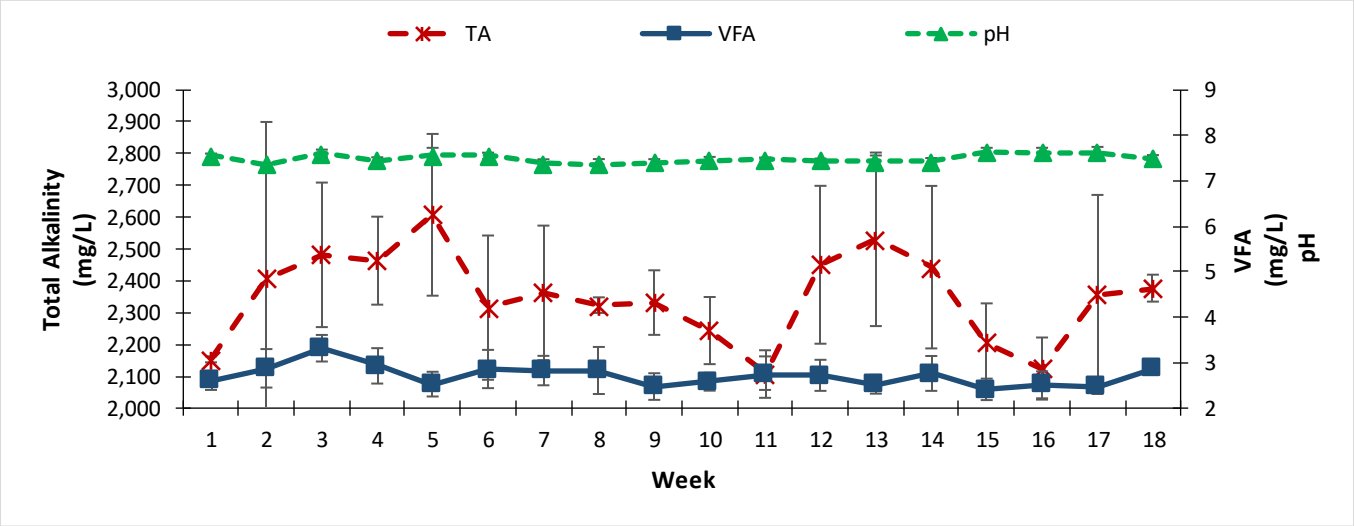


Figure 4.26: Stability of the anaerobic compartment of the pilot-scale IAAB throughout the mono-digestion study

The VFA concentration and TA obtained from weeks 1 – 18 were between 241 – 332 mg/L and 2,109 -2,607 mg/L, respectively. The VFA concentration obtained throughout the study period of 18 weeks was more than the suggested optimum range of less than 200 mg/L (Tchobanoglous, et al., 2014). In this study, the criteria set for the VFA concentration of the anaerobic compartment has to be below 500 mg/L, which was similar to the one reported by Chan et al. (2012a). Based on the criteria set in this study, the VFA concentration throughout the study period was below 500 mg/L (as acetic acid). There may be some accumulation of unused volatile acids produced in the reactor as affected by the high OLR_{an} applied. This allow us to deduce that the metabolic activity of the methanogen is slower than the acidogen, which may affect the overall methane production. Under high OLR_{an}, it is essential to maintain the buffering capacity of the anaerobic system with the presence of adequate amount of alkalinity. The experimental data obtained in this study showed that the TA in the range of 2,109 -2,607 mg/L as $CaCo_3$ was sufficient to maintain the pH of the anaerobic compartment within the range of 7.26-7.67 (which is above the optimum pH for methanogenesis). The VFA/TA ratio obtained throughout the study period of 18 weeks was between 0.10 – 0.13.

Despite operating the pilot-scale IAAB at high OLR_{an} (30.0 gCOD/L.day), the anaerobic compartment of the pilot-scale IAAB was relatively stable with no undesirable wash-out of slow-growing methanogenic bacteria or scum formation throughout the study period. This stability may be explained by the carbonate/bicarbonate buffering, which is produced by the generation of CO_2 during the anaerobic digestion process. Additionally, the excessive TA present from the previous loadings as the pilot-scale IAAB has been operated at various OLRs may be the contributing factor in maintaining the buffering capacity of the anaerobic compartment of the pilot-scale IAAB at favourable level throughout the study period (Borja et al., 2004). Under such condition, the strong buffering exhibited by the anaerobic compartment of the pilot-scale IAAB able to guard against possible acidification reactor giving a pH of the same order as the optimal pH for methanogenic bacteria (Borja et al., 2004). Ammonia accumulation was not observed in this study as well with no rapid increase of VFA concentration, which do not require the use of salts for pH control (Yenigün and Demirel, 2013). The optimum operating condition of pH and VFA:TA under OLR_{an} of 30.0 gOD/L.day aids in reducing the ammonia toxicity whereby no accumulation of VFA (271 ± 22 mg/L) is observed throughout the 18 weeks of study (Chen et al., 2008). This allow us to postulate that the anoxic microbial cells are able to survive in a high loading rate condition, while effectively degrading the organic and inorganic matter present in the POME (as shown in Section 4.3.3.2.1). The operating condition of the pilot-scale IAAB at OLR_{an} 30.0 gCOD/L.day able to create a conducive environment for the growth of different types of anoxic microbial cells that are responsible for the anaerobic process.

4.3.3.3.1 Methane Yield, Methane Production Rate and Methane Composition

During the degradation process by hydrolytic, acidogenic and acetogenic bacteria, organic acid is produced and then be degraded by the methanogenic archaea to produce methane gas. The biogas produced throughout the period of 18 weeks is measured in terms of the methane yield, methane production rate and methane composition.

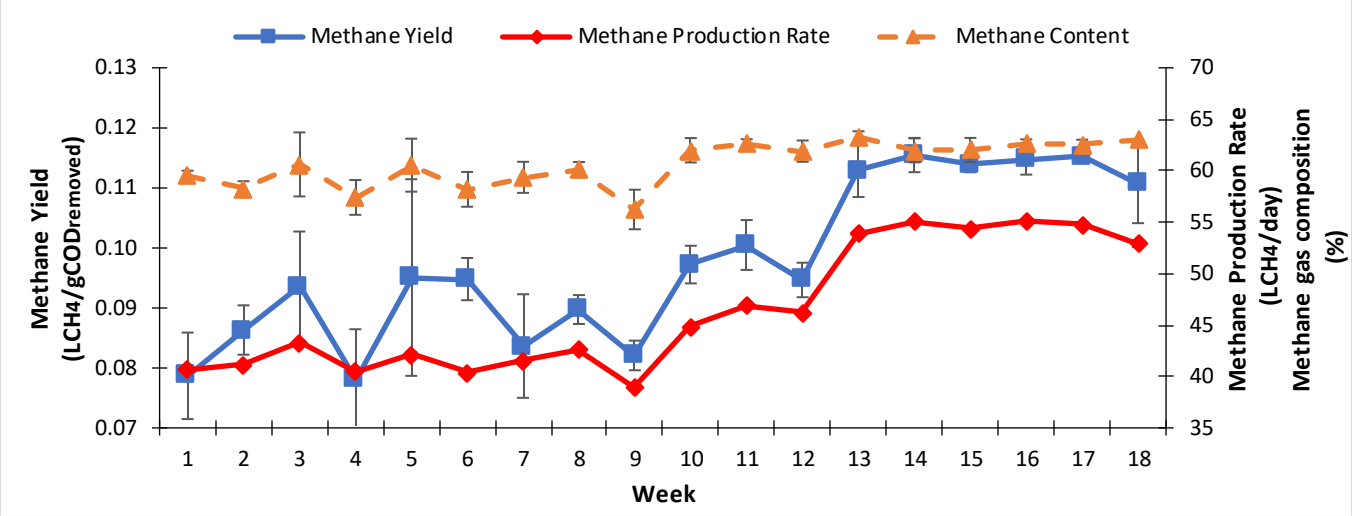


Figure 4.27: Volumetric methane production rate and methane yield throughout the period of 18 weeks

The operation of the pilot-scale IAAB at high OLR_{an} of 30.0 gCOD/L.day does not affect the methanogenesis process, as illustrated in Figure 4.27. Methane yield showed the total volume of methane gas produced per mass of COD removed. The volumetric methane production rate is a function of OLR, where the volume of the methane produced will increase proportionaly with the increase in OLR. From the obtained result, the methane yield and methane composition starting from weeks 1 to 9 fluctuated from 0.079 to 0.095 $LCH_4/gCOD_{removed}$ and from 57.4% to 60.6%, respectively. However, a proportional increase of methane yield (0.082 $LCH_4/gCOD_{removed}$ to 0.113 $LCH_4/gCOD_{removed}$) and methane composition (56.2% to 63.2%) were observed starting from weeks 10 to 13. By comparing the results of weeks 1-9 and weeks 10-13, it can be observed that higher methane yield and methane composition were achieved in the latter weeks. This is because the anaerobic sludge has been continuously fed with the same type of substrate (POME) for a long period of time (344 days). The same observation was also reported in the study of co-digestion of solid potato waste and sugar beet leaves (Parawira et al., 2008). This allows us to postulate that the source of sludge used to start-up the anaerobic process plays a relatively important role for the growth of anoxic microbial cells as the formation of biofilm will protect the sensitive methanogen from toxic shock and high loading rate. The methane yield and methane composition obtained from weeks 14 to 18 fluctuated between 0.115 $LCH_4/gCOD_{removed}$ to 0.111 $LCH_4/gCOD_{removed}$ and from 62% to 63%, respectively.

The least fluctuation for both the parameters at weeks 14 to 18 may allow us to deduce that the growth rate of acid formation bacteria outgrowth the methanogen. During the period of weeks 14-18, the anaerobic compartment of the pilot-scale IAAB was relatively stable with no occurrence of sudden sludge washout or scum formation. The experimental data obtained during this period of time (weeks 14-18) showed that the VFA concentration (241 mg/L – 287 mg/L), ammonia concentration (252 mg/L – 285 mg/L), VFA/TA ratio (0.105 – 0.121), TA concentration (2,124 mg/L – 2,443 mg/L), and pH (7.44 – 7.63) were beyond the optimum condition that impaired the methanogenesis activity. The least increase of the methane production that occurred during the weeks of 14-18 may be explained by the fact that the effluent COD in the anaerobic compartment of the pilot-scale IAAB at higher OLR_{an} is largely produced by the unused VFA (Borja et al., 2004). The same observation was obtained for the degradation activity of the POME carried out by the anoxic microbial cells with $BOD_{removal}$ (constant achievement of 95%), $COD_{removal}$ (constant achievement of 95%) and $TSS_{removal}$ (86%-91%) (as shown in Figures 4.27a - 4.27c). This again confirmed that the growth phase of the anoxic microbial cells during the weeks of 14-18 has changed from exponential to stationary phases.

Throughout the long-term performance study of the pilot-scale IAAB at OLR_{an} 30.0 gCOD/L.day, the methane yield obtained was lower than the laboratory scale IAAB (0.24 $LCH_4/gCOD_{removed}$) as reported by Chan et al. (2012a). This may be due to the rapid changes of the external surrounding. During the study period, the pilot-scale IAAB was placed in a palm oil mill with rapid external changes, viz. weather, constant fluctuation of POME characteristics and short period of cut off electricity which may exert unnecessary pressure to the anoxic microbial cells in the anaerobic compartment of the pilot-scale IAAB especially the methanogen. The relatively low biogas yield in the pilot-scale IAAB study compared to that obtained in the laboratory scale study was most likely the result of the unintentional variation in temperature as methanogenesis is usually more sensitive to changes in temperature than hydrolysis (Kaparaju et al., 2009). Another possible reason that contributed to the vast difference in methane yields for both the laboratory scale and pilot-scale studies of the IAAB was the allocated retention time. The retention time for anaerobic process for both the laboratory scale and pilot-scale IAAB per cycle are 6.5 and 2.8 days, respectively. The longer retention time in the laboratory scale IAAB gave a more complete degradation of organic material resulting in higher methane yield than the pilot-scale IAAB. However, it may seem not plausible to operate the pilot-scale IAAB at a longer retention time during the operation of the high OLR_{an}. This may be explained by the inversely proportional relationship between influent flow rate and retention time. The mono-digestion treatment concept for POME treatment may seems unstable due to the low C/N and presence of inhibitory compound that may affect the overall methane production (Nurliyana et al., 2015). It is essential to examine an alternative approach for anaerobic digestion of POME. In this case, co-digestion possibly can overcome the deficiencies of mono-digestion in terms of alleviating the inhibitory effect of high ammonia (>4.2 g/L) and sulphide (>2 g/L) concentration and exhibit a more stable biogas production due to the improved buffer capacity (Omil et al., 1996; Nayono et al., 2010; Zhang et al., 2013).

4.3.3.4 Optimum Operating Condition – OLR, MLVSS and F/M

The optimum operating conditions to produce final treated effluent with BOD 20 mg/L for the treatment of POME using the pilot-scale IAAB were obtained in this present study and summarised in Table 4.9.

Table 4.9: Operating conditions for optimum pilot-scale IAAB performance in the mono-digestion treatment

Parameter	Unit	Optimum Condition
OLR_{an}	gCOD/L.day	30.0
OLR_a	gCOD/L.day	2.60 – 3.55
MLVSS	mg/L	22,000 – 23,350
F/M_a	gCOD/gMLVSS.day	0.129 – 0.202

Throughout the study, a satisfactory result was obtained for the anaerobically-treated effluent and final discharge effluent (Table 4.10). The pilot-scale IAAB with UASFF reactor configuration (anaerobic compartment), aerated aerobic compartment and gravity settling of the settling tank showed that it has the potential to produce final treated effluent that abides by the discharge limit set by the Malaysian DOE.

Table 4.10: Optimum pilot-scale IAAB performance in the treatment of anaerobically-treated POME and final treated effluent with its respective discharge limit by the Malaysian Department of Environment (DOE).

Parameter[a]	Anaerobically-treated Effluent	Final Treated Effluent	Overall Removal Efficiency (%)	DOE Standards
COD	4,800 ± 517	343 ± 102	99.58 ± 0.15	-
BOD[b]	2,066 ± 191	18 ± 5	99.95 ± 0.02	20
TSS	996 ± 274	64 ± 30	99.24 ± 0.33	400
TN	558 ± 68	95 ± 5	95.12 ± 0.82	200
Lignin	542 ± 135	67 ± 35	98.47 ± 1.54	-
NH_3-N	454 ± 128	28 ± 3	98.08 ± 2.43	150

[a]: All parameters in mg/L
[b]: Sample incubated for 3-days at 30°C

It is worth noting that in comparison with the laboratory-scale IAAB as shown in Table 4.11, the achievement of producing final treated effluent with BOD 20 mg/L was achieved at a higher OLR_{an} and MLVSS concentration in this present investigation. Additionally, by comparing with other type of treatment system (Table 4.11), a higher $COD_{removal}$ was obtained at a higher OLR_{an} in this present study. A higher $COD_{removal}$ was obtained in this present study as compared to the laboratory scale IAAB, which is the scaled-up version of the laboratory scale IAAB with the similar type of system. This allows us to postulate that there is improvement on the performance of the pilot-scale IAAB in terms of COD, BOD and TSS removal efficiencies of the anaerobically-treated effluent and to consistently produce final treated effluent with BOD less than 20 mg/L (at least 70% from the total number of experiment days) and TSS of less than 400 mg/L (100% from the total number of experiment days). Additionally, the present study also demonstrated that the pilot-scale IAAB may be a suitable prototype to treat large volumes of POME at a lower retention time and higher loading rate as compared to the conventional ponding system. This study shows that the configuration of integrated system of anaerobic and aerobic processes may be an alternative to replace the current POME treatment system, where the quality of the produced final treated effluent was better than the conventional POME treatment system in terms of achievement of BOD less than 20 mg/L and TSS less than 400 mg/L, consistently.

Table 4.11: Anaerobic-aerobic systems using high-rate bioreactors

No	Type of system	Scale	Type of wastewater	Influent COD (mg/L)	OLR_{an} (gCOD/L.day)	Anaerobic	Aerobic	References
						COD Removal (%)	COD Removal (%)	
1	UASB + CSTR	Laboratory	Wool acid	499 – 20,000	-	51 – 84	-	(Isik and Sponza, 2006)
2	UASB + CSTR	Laboratory	Cotton Textile mill	604 – 1,038	-	9 – 51	-	(Isik and Sponza, 2004)
3	UASB + CSTR	Laboratory	Simulated textile	4,214	1.01 – 15.84	-	-	(Isik and Sponza, 2008)
4	2UASBs + CSTR	Laboratory	Municipal solid food waste	5,400 – 20,000	4.3 – 16	58 – 79	85 – 89	(Agdag and Sponza, 2005)
5	UASB + CSTR	Laboratory	Pulp and paper industry effluent	5,500 – 6,600	16	85	-	(Tezel et al., 2001)
6	UASB + CSTR	Laboratory	Pharmaceutical Industry	3,000	3.6	68 – 89	71 – 85	(Sponza and Demirden, 2007)
7	UASB + AS	Laboratory	Olive mill + municipal wastewater	1,800 – 4,400	3.7	70 – 90	> 60	(Gizgis et al., 2006)
8	UASB + AS	Laboratory	Starch industry wastewater	20,000	15	77 – 93	64	(Vladimir et al., 2003)
9	UASB + AS	Pilot	Municipal wastewater	386 – 958	-	69 – 84	43 – 56	(Sperling et al., 2001)
10	UASB + AFB	Laboratory	Synthetic Textile wastewater	2,700	4.8	50	60	(Yu et al., 2000)
11	RBC + SBR	Laboratory	Cheese whey + dairy manure	37,400 – 65,700	5.2 – 14.1	46.3 – 62.6	93 – 95	(Po and Liao, 1989)
12	RBC + SBR	Laboratory	Dairy Cattle	39,900 – 40,100	8.2 – 26.8	18.7 – 29	86 – 87	(Lo and Liao, 1986)
13	FFB + FFB	Laboratory	Poultry Slaughterhouse	400 – 1,600	0.39	-	-	(Pozo and Diez, 2003)
14	EGSB + Aerobic biofilm reactor	Pilot	POME	35,000	10	93	22	(Zhang et al., 2008)

15	UBF + MBR	Laboratory	Synthetic wastewater	6,000 – 14,500	7.2	98	-	(Ahn et al., 2007)
16	UASB + Aerobic solid contact system	Pilot	Municipal	341	2.6	34	-	(Motto et al., 2007)
17	UASB + RBC	Pilot	Domestic sewage	640	-	35 – 47	52 – 56	(Castillo et al., 1999)
18	CSTR + AS	Laboratory	Green olive debittering wastewater	23,500	0.47	37.4 – 48.9	73.6	(Aggelis et al., 2001)
19	AFFFBR + AS	Laboratory	Purified terephthalic acid (PTA)	5,000	4.0 – 5.0	62 – 64	90	(Pophali et al., 2007)
20	AnFB + Air lift suspension reactor	Laboratory	Complex industrial wastewater	3,800	25.0 – 30.0	-	60 – 65	(Heijnen et al., 1990)
21	Hybrid bioreactor + AS	Laboratory	Oil shale dump leachate	2,000 – 4,600	-	20 – 40	60 – 80	(Kettunen and Rintala, 1995)
22	Packed column reactor + AS	Pilot	Textile industry wastewater	800 – 1,200	-	30 – 65	40 – 90	(Kapdan and Alparslan, 2005)
23	Covered lagoon + OPS	Industrial	POME	90,000 – 105,000	0.61	95	63	(Yap et al., 2020)
24	IAAB	Laboratory	POME	13,700 – 68,500	10.5	92.1	95.1	(Chan et al., 2012a)
25	IAAB	Pilot	POME	75,000 – 200,000	30.0	94.0	93.0	This study

[a] Types of reactor: UASB, Upflow Anaerobic Sludge Blanket; CSTR, Continuous stirred-tank reactor; AS, Activated Sludge; AFB, Aerobic fluidised bed; RBC, Rotating biological contactors; SBR, Sequencing batch reactor; FFB, Fixed-film bioreactor; EGSB, Expanded granular sludge bed; UBF, Upflow bed filter; MBR, Membrane Bioreactor; AFFFBR, Anaerobic fixed-film bed reactor; AnFB, Anaerobic fluidised bed; OPS, Open ponding system

4.3.3.5 Evaluation of Kinetic Modelling

Three different kinetic models, namely Contois, Stover-Kincannon, and Grau second order were applied to the anaerobic and aerobic processes of the pilot-scale IAAB in mono-digestion mode of POME treatment (Contois, 1959; Chen and Hashimoto, 1980; Şentürk et al., 2010; Chan et al., 2012b; Grau et al., 1975; Sánchez et al., 2010). The operating data (18 weeks) were subjected to kinetic modelling based on Eqs. 3.35, 3.36, and 3.38 (Table 3.16, Chapter 3, Materials and Methods) to evaluate POME treatment performance for (1) the specific parameters used and (2) the relationships between variables. The predicted COD of the anaerobically-treated effluent and final treated effluent was shown in Table 4.12.

Table 4.12: Prediction of: (a) COD anaerobically-treated effluent; (b) COD final treated effluent; (c) biomass concentration of anaerobically-treated effluent; (d) biomass concentration of final treated effluent

Kinetic Model	Equation	Eq No.
Contois (Contois, 1959; Chen and Hashimoto, 1980)	$COD_{out,an} = \frac{0.005X_{out,an}(\frac{1}{SRT_{an}} + 0.044)}{\frac{1}{SRT_{an}} - 3.364 - 0.044}$	4.18
	$COD_{out} = \frac{7.647X_{out,an}(\frac{1}{SRT_a} + 0.002)}{\frac{1}{SRT_a} - 0.5365 - 0.002}$	4.19
Stover-Kincannon (Şentürk et al., 2010; Chan et al., 2012b)	$COD_{out,an} = COD_{in} - \frac{454.55COD_{in}}{453.41 + OLR_{an}}$	4.20
	$COD_{out} = COD_{out,an} - \frac{20{,}000COD_{out,an}}{18 + OLR_a}$	4.21
Grau second-order multicomponent substrate removal (Grau et al., 1975; Sánchez et al., 2001)	$COD_{out,an} = COD_{in}\left(1 - \frac{HRT_{an}}{0.146 + 1.009HRT_{an}}\right)$	4.22
	$COD_{out} = COD_{out,an}\left(1 - \frac{HRT_a}{0.051 + 1.042HRT_a}\right)$	4.23

Based on the results obtained in Section 4.4.2, it can be concluded that the pilot-scale IAAB can be operated up to OLR_{an} 30.0 gCOD/L.day with acceptable stability and free of operational problems. Steady state performance data at OLR_{an} 30.0 gCOD/L.day was used to determine the kinetic parameters required for all the kinetic models. For the evaluation of kinetic parameters on a COD basis, a graphical method and least square linear regression were used to obtain line of best fit. The R^2 obtained from Eqs. 3.38, 3.39, 3.35, and 3.36 (Table 3.16, Chapter 3, Materials and Methods) for both the anaerobic and aerobic processes of the pilot-scale IAAB were at least more than 0.96, which showed that it can be applied to predict the performance of the pilot-scale IAAB in both the processes. The kinetic parameters are summarised in Table 4.13 together with the regression coefficients.

Table 4.13: Kinetic parameters of the pilot-scale IAAB in mono-digestion mode

Kinetic Models	Kinetic Parameters	Unit	Anaerobic		Aerobic	
			Values	R^2	Values	R^2
Modified Stover-Kincannon	K_B	gCOD/L.day	453.41	0.8575	0.6573	0.8407
	$\cup_{max}$	gCOD/L.day	454.55		2.8209	
Grau second-order	a	per day	0.1464	0.9901	0.0511	0.9809
	b	-	1.0087		1.042	
	k_s	Per day	10.484		3.3629	
Contois	Y	mgVSS/mgCOD	0.0672	0.0834	0.0749	0.9804
	k_d	per day	0.0436		0.2722	
	μ_{max}	per day	0.2973	0.0152	0.5361	0.9696
	β	mg/L	0.0052		0.7647	

4.3.3.5.1 Contois Kinetic Model

In order to determine the kinetic coefficients (β, μ_{max}, Y and k_d) for the Contois model, Eqs. 3.38 and 3.39 (Table 3.16, Chapter 3, Materials and Methods) were plotted in Figures D2 and D3 (Chapter 7, Appendices) for both the anaerobic and aerobic processes. Their values were determined from the slope and intercept of the line, respectively. Maximum specific growth rate (μ_{max}), growth parameter (β), Y and k_d were calculated from the line plotted based on the linear regressions (least squares method) for the determination of the intercept ($\frac{1}{\mu_{max}}$ and $\frac{1}{Y}k_d$) and the slope ($\frac{\beta}{\mu_{max}}$ and $\frac{1}{Y}$).

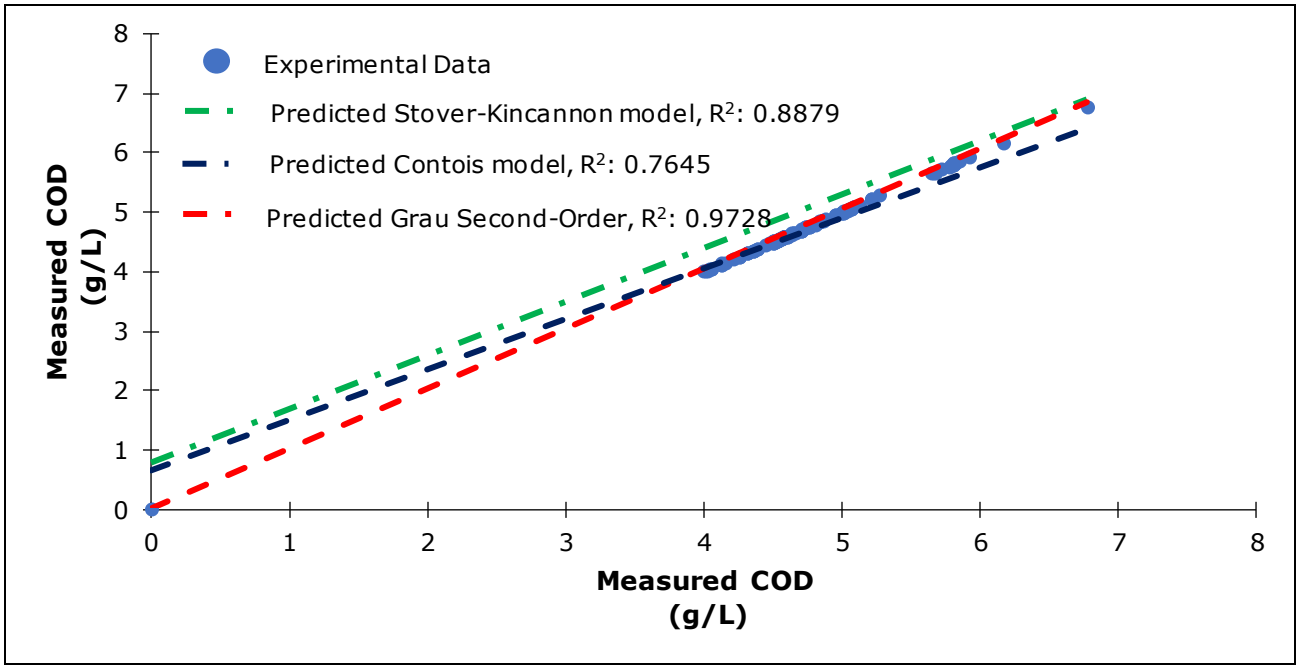
4.3.3.5.2 Grau Second-Order Multicomponent Substrate Removal Model

To determine the kinetic coefficients (a and b) for both the anaerobic and aerobic processes for the Grau second-order model, Eq. 3.36 (Table 3.16, Chapter 3, Materials and Methods) was plotted. Linear regressions (least squares method) was used to determine the Grau second-order kinetic coefficients (a and b) and they were calculated from the line plotted in Figure D4 (Chapter 7, Appendices).

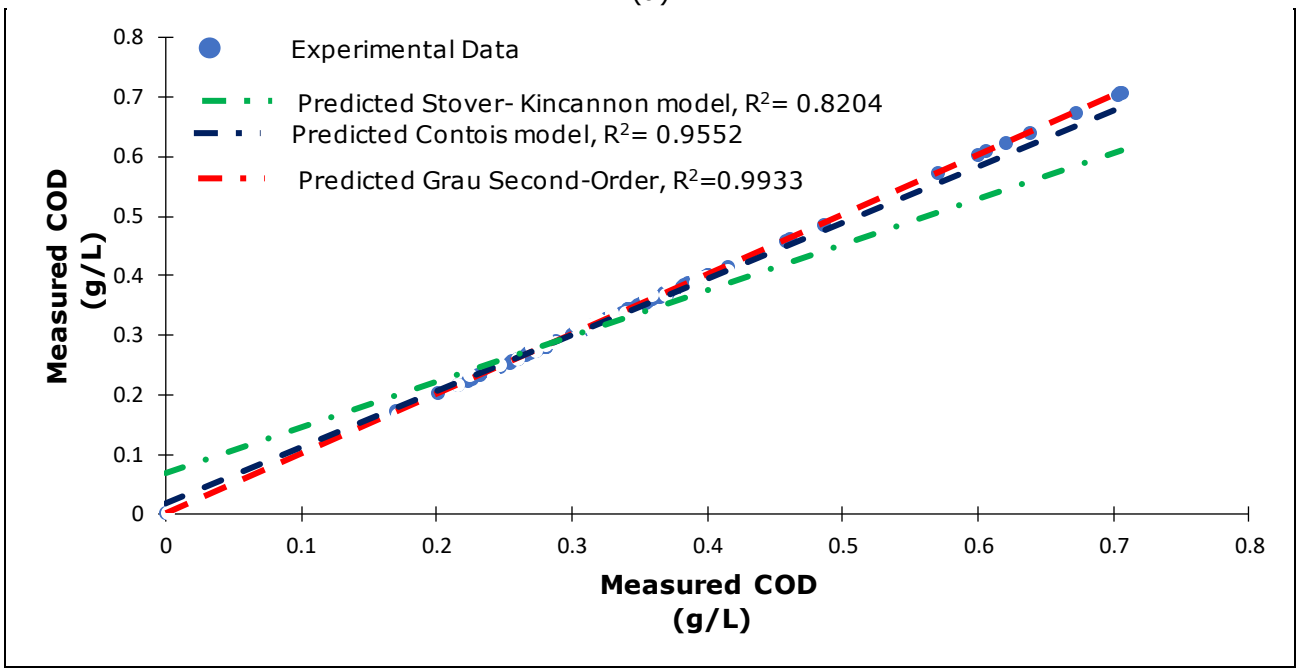
4.3.3.5.3 Modified Stover-Kincannon Model

A graphical method and method of least squares linear regression were employed to obtain the line of best fit for kinetic evaluation on COD basis. Linear regression in terms of the reciprocal of total organic loading removal rate, $[V_{an}/(Q_{in}(COD_{in}-COD_{out,an})]$ against reciprocal of total OLR, $V_{an}/(Q_{in} \times COD_{in})$ based on modified Stover-Kincannon model for both the anaerobic and aerobic processes was used to determine saturation value constant (K_B) and maximum substrate removal rate (U_{max}) based on the intercept ($\frac{1}{\cup_{max}}$) and the slope ($\frac{K_B}{\cup_{max}}$) (Figure D5, Chapter 7, Appendices).

Selection of the most suitable kinetic model to describe the performance of the pilot-scale IAAB during mono-digestion mode will be based on the correlation coefficient (R^2). In this study, all the models used for the anaerobic process had high R^2 of 0.8575 and 0.9901 for Modified Stover-Kincannon and Grau second-order models, respectively. The R^2 obtained for Contois model for the anaerobic process was 0.0152. Additionally, the R^2 obtained for aerobic process of the pilot-scale IAAB for Modified Stover-Kincannon, Grau second-order, and Contois models were 0.8407, 0.9809, and 0.9696, respectively. This allow us to denote that Grau Second-Order kinetic model may be used to represent the performance of the pilot-scale IAAB based on the R^2 obtained (Figures D2-D5 (Chapter 7, Appendices)). Further evaluation was done by comparing the constants obtained with other studies having similar process and the models used were also validated by comparing the predicted data with an independent set of experimental data. The COD of the anaerobically-treated POME can be predicted using Eq. 4.18, Eq. 4.20, and Eq. 4.22 for Contois, Stover-Kincannon, and Grau second-order models, respectively. Additionally, the COD of the final treated effluent can be determined via Eq. 4.19, Eq. 4.21, and Eq. 4.23 for Contois, Stover-Kincannon, and Grau second-order models, respectively. The expected values of $COD_{out,an}$ and COD_{out} for each kinetic model and the measured data of $COD_{out,an}$ and COD_{out} was illustrated in Figure 4.28.



(a)



(b)

Figure 4.28: Comparison between the predicted and the actual chemical oxygen demand (COD) data of (a) anaerobically-treated effluent (b) final treated effluent of pilot-scale IAAB

The findings obtained as shown in Figure 4.28 for both the anaerobic and aerobic processes allow us to denote that Grau Second-Order was the most suitable model in describing the performance of the system due to its highest R^2 of 0.9728 (Anaerobic Process) and 0.9933 (Aerobic Process) as well as low standard deviation. Table 4.14 summarises the coefficients determined from the models applied in the previous studies and compared with the coefficients obtained in this study for both the anaerobic and aerobic processes of the pilot-scale IAAB at mono-digestion mode. The Grau Second-Order model can be used to predict the performance of different types of reactor configuration under anaerobic and aerobic treatments. For the pilot-scale IAAB used in this study, the k_s value for the anaerobic system was within the wide range of 0.058 day^{-1} to 252.0 day^{-1}. Even though the k_s value (1.0087 day^{-1}) obtained in this study fell within these ranges, significantly lower than the k_s value (252.0 day^{-1}) for the textile wastewater reported by Sandhya and Swaminathan, (2006). This may be explained by the low influent COD concentration of 243 mg/L – 1,973 mg/L used in the textile wastewater treatment as compared to this study. The k_s value determined in this study was lower than the previous pilot-scale IAAB study in terms of the various OLR_{an}. The k_s value determined in the former study was 1.0087 day^{-1} whereas the latter study was 14.591 day^{-1} despite the same type of reactor used for both the studies. Supposedly, the k_s value in the former study should be higher than the latter study due to the high COD concentration of the POME used (75,000 mg/L – 200,00 mg/L). However, the high k_s value obtained in the previous study may be affected by the high microorganism concentration and stability in the anaerobic compartment as reflected on the MLVSS concentration of 24,200 mg/L – 48,238 mg/L and VFA:TA ratio of 0.07 to 1.02, respectively. The entrapment of the microbial cells on the packing media placed in the hollow centre of the anaerobic compartment has aided the development of the biofilm to prevent the occurance of sludge washout. These will maintain the MLVSS concentration in the anaerobic compartment and ensuring high removal efficiencies of COD (83% - 97%) and TSS (77% - 99%) as microbial cells are the key players in ensuring high removal efficiencies of COD (83% - 97%) and TSS (77% - 99%). As reported by Işik and Sponza, (2005), the k_s value will increase as the substrate removal rate increased depending on the initial substrate concentration and microorganism concentration in the reactor. However, such observation was not observed in our study. This allow us to denote that the k_s value is inversely proportional to the initial substrate concentration.

By comparing with the similar type of system but with a different volume, the k_s value (1.0087 day^{-1}) obtained in this study was higher than the laboratory scale IAAB (0.655 day^{-1}) (Chan et al., 2012b). The $COD_{removal}$ (92% - 96%) and $TSS_{removal}$ (85% - 96%) obtained in the pilot-scale IAAB was higher than the laboratory scale IAAB with $COD_{removal}$ (85% - 92.1%) and $TSS_{removal}$ (90.5% - 95.3%). This may be explained by the higher microorganism concentration in the pilot-scale IAAB of 47,537 ± 14,191 mg/L as compared to the laboratory scale IAAB of 39,150 ± 675 mg/L and higher OLR_{an} in the pilot-scale IAAB (30.0 gCOD/L.day) and as compared to the laboratory scale IAAB (10.5 gCOD/L.day). Such observation obtained during the performance evaluation for both the pilot-scale IAAB (Section 4.3.3) and laboratory scale IAAB allow us to infers that the volume of the system may affect the k_s value besides the initial substrate and microorganism concentrations in the reactor.

This allow us to further confirmed that there are several factors that affect the k_s value in the anaerobic system, viz. initial substrate concentration, concentration of microbial cells in the reactor and the volume of reactor used that has the same type of system. Meanwhile, the k_s value obtained in the aerobic system was comparable to those of synthetic wastewater with low initial substrate concentration (750-2,250 mg/L), low OLR_a range (0.75-4.50 gCOD/L.day), MLVSS concentration (790 mg/L – 1,470 mg/L), and $COD_{removal}$ (88.9% - 97.3%) using upflow aerobic immobilised biomass (UAIB) (Borghei et al., 2008). The k_s value (3.363 day^{-1}) obtained in this study was higher than the laboratory scale IAAB (3.295 day^{-1}) reported by Chan et al., (2012b). This may be explained by the higher MLVSS concentration (20,580 mg/L – 23,661 mg/L) and COD concentration of the anaerobically-treated POME (4,000 mg/L – 6,775 mg/L) used in this study as compared to the laboratory scale IAAB. The k_s value obtained in this study was similar to the previous study conducted using the similar prototype but at wider range of OLR_{an} (10.5 gCOD/L.day – 32.5 gCOD/L.day). This allow us to denote that the aerobic compartment of the pilot-scale IAAB able to maintain high solid concentration (as reflected on the MLVSS concentration ranges from 22,000 mg/L to 23,000 mg/L) resulting in high removal efficiencies of COD and TSS of more than 90% in both the studies. The aerobic system of the pilot-scale IAAB was much superior than the UAIB and laboratory scale IAAB with the ability to retain high concentration of solid (as reflected on the MLVSS) in the reactor to actively degrade the organic matter present in the anaerobically-treated POME.

The major outcome attained from this kinetic study is that loading rate plays an important role in determing the performance of the pilot-scale IAAB for the achievement of final treated effluent that abides the discharge limit set by DOE, Malaysia. The performance of the pilot-scale IAAB at anaerobic and aerobic treatments under mono-digestion mode can be predicted using the Grau second-order (Figures 4.36a & 4.36b), respectively. For the steady-state performance at various OLRs, Stover Kincannon model was the most suitable kinetic model to describe the performance of the pilot-scale IAAB at anaerobic and aerobic treatment (Figures 4.28a & 4.28b), respectively. Both the kinetic models showed that loading rate plays a relatively important role in determining the performance of the pilot-scale IAAB. Additionally, the kinetic models used to predict the performance of the pilot-scale IAAB at two different scenarios, i.e. Steady-State Performance at various OLRs (Section 4.3.2.3) and Mono-digestion of POME (Section 4.3.3) showed that aerobic system of the pilot-scale IAAB is suitable to be used for the production of final treated effluent that abides the discharge limit set by DOE, Malaysia. Despite operating the pilot-scale IAAB at high loading rate (OLR_{an}: 30.0 gCOD/L.day; OLR_a: 2.86 ± 0.37 gCOD/L.day), the methane yield obtained was lower than the laboratory scale IAAB (0.24 $LCH_4/gCOD_{removed}$) and this may be resolved by adjusting the C/N using the approach of co-digestion. The results obtained during the laboratory-scale of co-digestion of POME with *M. oleifera* Extract (Section 4.2) will be used as a yardstick for the improvement of the methane yield. Hence, further study on evaluating the performance of the pilot-scale IAAB under co-digestion mode using *Moringa Oleifera* as a co-substrate to be digested with POME will be discussed in the next section.

Table 4.14: Comparison of kinetic constants for different wastewater treatment using the three kinetic models

System	Kinetic Models	Substrate	Reactor Type	Influent COD (mg/L)	OLR (gCOD/L.day)	Kinetic Parameter				References
						K_B (gCOD/Lday)	U_{max} (gCOD/Lday)			
Anaerobic	Modified Stover-Kincannon	Soybean ww	AF	7,520-11,450	2.06	85.50	83.30			(Yu et al., 1998)
		Simulated starch ww	UAF	100-4,000	1.15-16.70	49.80	50.60			(Ahn and Forster, 2000)
		Molasses	AHR	2,000-15,000	1.00-10.00	186.23	83.30			(Büyükkamaci and Filibeli, 2002)
		Simulated ww	UASB	4,214	1.00-15.80	8.20	7.50			(Işik and Sponza, 2005)
		Synthetic ww	AF	2,000-4,000	1.15-16.7	50.60	49.80			(Ahn et al., 2007)
		Papermill ww	UAF	1,972-3,536	1.08-11.38	104.15	86.21			(Yilmaz et al., 2008)
		Milk permeate ww	MBBR	8,610-23,230	2.0-28.0	102.30	89.30			(Wang et al., 2009)
		Slaughter-house ww	UAF	6,000-6,500	3.1-6.1	120.88	99.01			(Padilla-Gasca and López López, 2010)
		POME	IAAB	68,550-79,800	10.50-21.50	14.70	23.10			(Chan et al., 2012b)
		POME	CL	77,075-168,741	1.00-4.37	3,580.33	3,333.33			(Yap et al., 2020)
		POME	$IAAB^P$	25,228-76,601	8.0-32.5	440.83	416.67			Previous study (Section 4.3.2.3)
		POME	$IAAB^P$	75,000-200,00	30.0	453.41	454.55			This study

Anaerobic	Contois					Y (mgVSS/mgCOD)	K_d (per day)	μ_{max} (per day)	β (mgCOD/mgVSS)	
		Ice-cream ww	CSTR	5,500	-	0.212	0.013	0.930	0.482	(Hu et al., 2002)
		Simulated ww	UASB	4,214	1.00-15.80	0.125	0.007	0.105	0.465	(Işik and Sponza, 2005)
		POME	MAS	61,660	-	-	-	25.71	533.52	(Nour et al., 2015)
		POME	CL	77,075-168,741	1.00-11.92	0.0982	0.00483	0.054	0.612	Under review
		POME	IAAB[P]	75,000-200,000	30.0	0.0672	0.0436	0.297	0.0052	This study
	Grau second-order					k_s (day^{-1})	a (day^{-1})	b^a		
		Synthetic ww	AHR	2,000-15,000	1.0-10.0	10.810	0.033	1.192		(Büyükkamaci and Filibeli, 2002)
		Synthetic textile ww	UASB	4,214	1.0-15.8	0.337	0.562	1.095		(Isik and Sponza, 2005)
		Textile ww	UAFB	243-1,973	1.0-8.21	252.0	0.915	5.138		(Sandhya and Swaminathan, 2006)
		Synthetic ww	UASB	300-4,000	0.9-20.1	0.058	0.083	0.006		(Bhunia and Ghangrekar, 2008)
		Synthetic ww	UASB	3,000-4,000	6.0-34.0	0.260	0.029	0.011		(Sponza and Uluköy, 2008)
		Food processing ww	MACR	5,250-5,750	1.1-5.0	-	0.260	1.025		(Senturk et al., 2010)
		POME	IAAB	68,550-79,800	10.5-21.5	0.655	3.000	0.633		(Chan et al., 2012b)

Anaerobic	Grau second-order	POME	IAAB^{P}	25,228-76,601	8.0-32.5	14.591	0.5261	0.8875		Previous study (Section 4.3.2.3)
		POME	IAAB^{P}	75,000-200,000	30.0	1.0087	0.1464	1.0087		This study
Aerobic	Modified-Stover Kincannon					K_B (gCOD/Lday)	U_{max} (gCOD/Lday)			
		Synthetic ww	MBBR	750-4,500	0.75-4.5	9.4553	8.3402			(Borghei and Hosseiny, 2002)
		Synthetic ww	UAIB	750-2,250	0.75-4.5	106.8	101			(Borghei et al., 2008)
		AN POME	IAAB	5,000-24,000	0.72-7.2	327	312			(Chan et al., 2012b)
		AN POME	IAAB^{P}	1,000-16,100	0.44-11.14	0.6573	2.8209			Previous study
		AN POME	IAAB^{P}	4,000-6,775	2.55-4.21	0.6573	2.8209			This study
	Contois					Y (mgVSS/mgCOD)	k_d (per day)	μ_{max} (per day)	β (mgCOD/mgVSS)	
		Raw sewage sludge	MAS + UF	1,090-3,800	0.1-10.0	0.74	-	0.384	0.111	(Nour et al., 2010)
		Dairy ww	ASP	180-1,200	-	0.68	0.070	2.13	0.20	(Emerald et al., 2012)
		Raw sewage sludge	UMAS	-	-	0.391	0.012	0.287	0.212	(Adam et al., 2013)
		POME	IAAB^{P}	4,000-6,775	2.55-4.21	0.0749	0.2722	0.536	0.7647	This study

						k_s (day $^{-1}$)	a (day $^{-1}$)	b^a		
Aerobic	Grau second-order	Municipal ww	ASP	230-445	-	0.217	0.002	1.346		(Grau et al., 1975)
		Synthetic ww	MBBR	750-4,500	0.75-4.5	0.337	0.562	1.095		(Hosseiny and Borghei, 2002)
		Synthetic ww	UAIB	750-2,250	0.75-4.5	3.582	0.047	1.007		(Borghei et al., 2008)
		AN POME	IAAB	5,000-24,000	0.7-7.2	3.295	0.415	0.975		(Chan et al., 2012b)
		AN POME	IAAB^P	1,000-16,100	0.44-11.14	3.363	0.413	0.571		Previous study (Section 4.3.2.3)
		AN POME	IAAB^P	4,000-6,775	2.55-4.21	3.363	0.051	1.042		This study

[a]: Dimensionless

ww: wastewater; AF: Anaerobic filter; UAF: Upflow anaerobic filter; AHR: anaerobic hybrid reactor; UASB: upflow anaerobic sludge bed; MBBR: moving bed biofilm reactor; IAAB: integrated anaerobic-aerobic bioreactor; CL: covered lagoon; IAABP: Pilot Plant Integrated Anaerobic-Aerobic Bioreactor; CSTR: Continuous stirred tank reactor; UAFB: upflow anaerobic fixed bed; MACR: Mesophilic anaerobic contact reactor; UAIB: upflow aerobic immobilised biomass; AF: anaerobic filter; UAF: upflow anaerobic filter; MAS: Membrane anaerobic system; MAS+UF: Membrane anaerobic system + ultra-filtration; ASP: activated sludge process; UMAS: ultrasonic membrane anaerobic system; AHR: anaerobic hybrid reactor; AN POME: Anaerobically-treated POME.

4.3.4 Co-Digestion of Palm Oil Mill Effluent (POME) with *Moringa Oleifera*

4.3.4.1 Reactor Performance

The pilot-scale reactor was operated on 25th April 2016 and has been operated for more 400 days with 14 different anaerobic organic loading rates (OLR_{an}). In order to determine the suitable feeding frequency of *M. oleifera* filtrate, a laboratory-scale study of the co-digestion study was previously conducted. Considering the positive results obtained in the laboratory study of the co-digestion approach, the same approach was then applied to the pilot-scale reactor. Prior to this study, the pilot-scale reactor was operated with an OLR_{an} of 30.0 gCOD/L.day and a HRT of 326 days to support the microorganism adaptation. Additionally, the pH value was maintained within the range of 6.51-7.28, which is within the appropriate range for mesophilic digestion and methanogenesis. The pilot-scale reactor was then fed with a mixture of POME and *M. oleifera* filtrate at a ratio of 1.43 v/v% with the initial concentration of 500 mg/L, whilst maintaining the same operating conditions of the previous study. The operating condition of the pilot-scale IAAB operated co-digestion mode is shown in Table E1 (Chapter 7, Appendices).

The stability of the pilot-scale IAAB can be determined based on the concentration of the volatile fatty acid (VFA) as it is the metabolic status of an anaerobic degradation system (Li et al., 2011; Xie et al., 2011; Ye et al., 2013). However, the VFA level could not be specific indicator as the composition of the substrates and the operating conditions are different (Murto et al., 2004; Ye et al., 2013). Ahring et al. (1995); Ye et al. (2013) suggesting that the relative change in the VFA concentration could be used as an indicator. The stability of the pilot-scale IAAB in the co-digestion mode is presented in Figure 4.29.

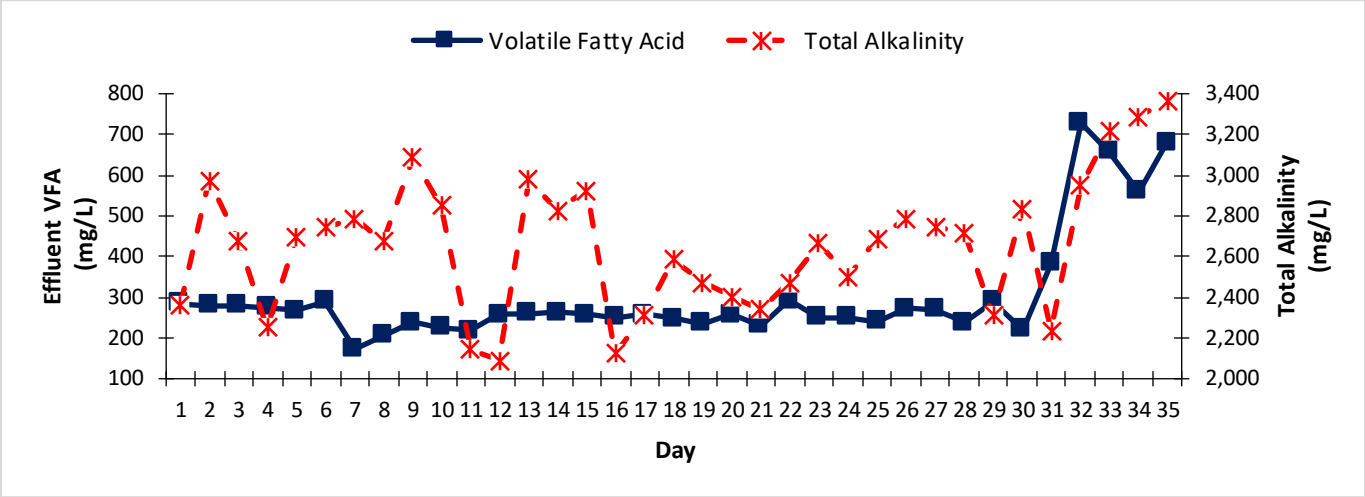


Figure 4.29: Stability of the anaerobic compartment of the pilot-scale IAAB throughout the co-digestion study

The VFA concentration and total alkalinity obtained from days 1 - 30 were between 173 - 291 mg/L and 2,092 - 3,088 mg/L, respectively. The maximum VFA concentrations were obtained on Days 31 - 35, which were 383 mg/L, 728 mg/L, 658 mg/L, 560 mg/L, and 681 mg/L, respectively. The VFA concentration obtained throughout the whole study period is more than the suggested allowable range of less than 200 mg/L (Tchobanoglous, et al. 2014). However, the accumulation of VFA does not inhibit the methanogenesis process. This may be contributed by the high total alkalinity concentration throughout the 35-day ranges of between 2,092 mg/L to 3,363 mg/L (Figure 4.29). The strong buffering capacity in the anaerobic compartment of the pilot-scale IAAB is able to create a balance between acid production and methane production without creating any harm on the anaerobes. This is reflected by the pH (6.84 - 7.14, which is the accepted pH range for methanogenesis) and VFA/TA ratio (0.06 - 0.25, which is lower than the failure limit of anaerobic digestion) (Borja et al., 2004; Chen et al., 2015).

It is worth noting that despite the high OLR_{an}, the anaerobic compartment of the pilot-scale IAAB is still able to degrade the incoming mixture of POME with *M. oleifera* filtrate effectively (as shown Section 4.3.4.2). Because the buffering capacity of the reactor configuration was maintained at favourable levels with excess total alkalinity present at all loadings, the rate of methanogenesis was not affected. The experimental data obtained in this study indicate that a total alkalinity in the range of 2,092 - 3,363 mg/L as $CaCO_3$ was sufficient to maintain the pH within 6.84 - 7.14 at a high OLR_{an} of 30.0 gCOD/L.day.

The stability of the anaerobic compartment of the pilot-scale IAAB may be attributed to the carbonate/bicarbonate buffering, which is produced by the generation of CO_2 during the digestion process that is not completely removed from the reactor as gas (Borja et al. 2004). The buffering guards against possible acidification of the reactor giving a pH optimal for methanogenic bacteria.

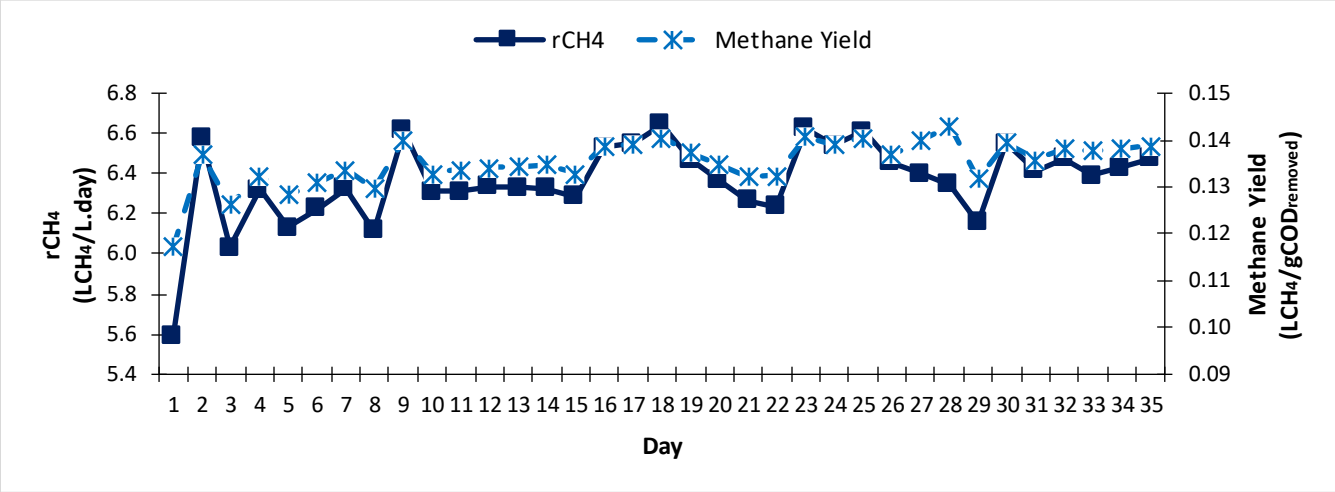


Figure 4.30: Methane yield profile for the pilot-scale IAAB during the co-digestion of POME with *M. oleifera* filtrate

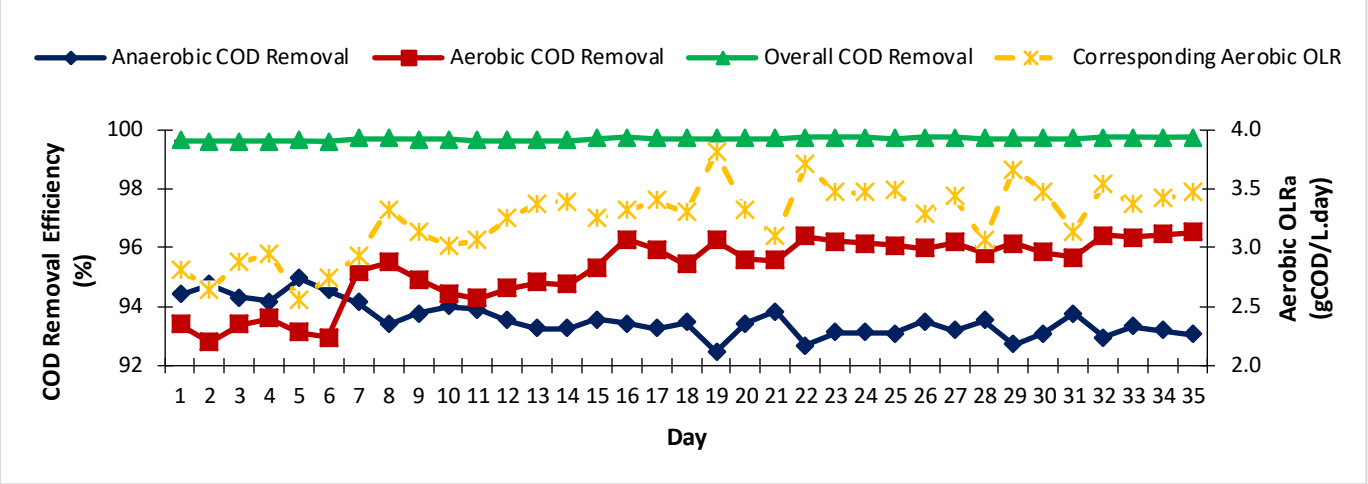
The operation of the pilot-scale IAAB at a high OLR_{an} of 30.0 gCOD/L.day does not affect the methanogenesis process, as shown in Figure 4.30. The volumetric methane production rate is a function of OLR, where the volume of the methane produced will increase proportionaly with the increase in OLR. In this study, an increasing trend of methane yield was observed throughout the study period, which concurs with the results obtained in the laboratory-scale study where a higher methane yield was obtained in co-digestion compared to mono-digestion. This allow us to postulate that the incorporation of *M. oleifera* filtrate as a substrate for co-digestion is able to initiate and accumulate the methane production more rapidly than mono-digestion for both the pilot- and laboratory-scales. The methane yield obtained ranges from 0.117 $LCH_4/gCOD_{removed}$ to 0.143 $LCH_4/gCOD_{removed}$. The methane yield obtained in this study shows that the activity of the methanogenic bacteria was not impaired by the high OLR_{an} of 30.0 gCOD/L.day. This may be due to the use of plant-based substrate (*M. oleifera* extract) that does not contain any inhibitor that impair the high activities of methanogenesis (Murto *et al*., 2004). Towards the end of the study, there was no significant difference in the methane yield obtained despite the increased VFA content from days 31 - 35 (Figure 4.30). This may be due to the adequate buffering capacity provided in the experimental system. As a result, it has the capability to balance the production of VFA and biogas without causing reactor failure as observed in most reactors whilst maintaining optimum conditions for anaerobic digestion to occur (Ahring et al, 1995; Borja et al., 2004; Murto et al., 2004; Li et al., 2011; Xie et al., 2011; Ye et al., 2013; Chen et al., 2015). Additionally, due to the coagulating effect by the protein in the *M. oleifera* filtrate, higher biomass hold-ups is observed in the reactor and this leads to a balanced C/N composition. It may be one of the factors that contributed to a better enrichment of the anoxic microbial cells in the system that allows the complete degradation of the complex and readily biodegradable materials, despite the presence of lignin

from the *M. oleifera* extract. Hence, this improved the biogas production during the co-digestion process between POME and plant-based substrates.

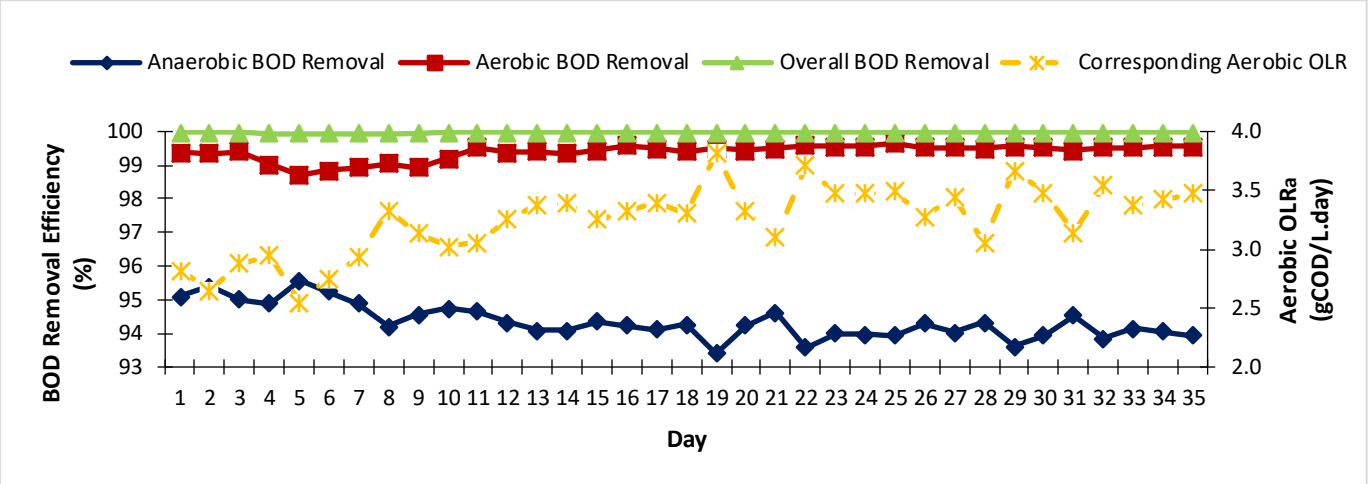
It is worth to note that the used of pilot-scale IAAB for this study has produced three-fold increase in methane yield as compared to laboratory scale (Section 4.2) (Laboratory scale: 0.0243 $LCH_4/gCOD_{removed}$; Pilot-scale IAAB: 0.143 $LCH_4/gCOD$removed). This can be explained by the design of the anaerobic compartment of the pilot-scale IAAB. The middle part of the pilot-scale IAAB serves as a biomass immobiliser whereby it helps to reduce the occurrence of sludge washout. As a result, the anoxic microbial cells will continue to proliferate in the fixed film bioreactor randomly packed with ceramic bio-rings. This will allow maximum degradation of the agglomerated solids by the anoxic microbial cells. Hence, increasing the methane yield in the pilot-scale IAAB.

4.3.4.2 Performance Evaluation

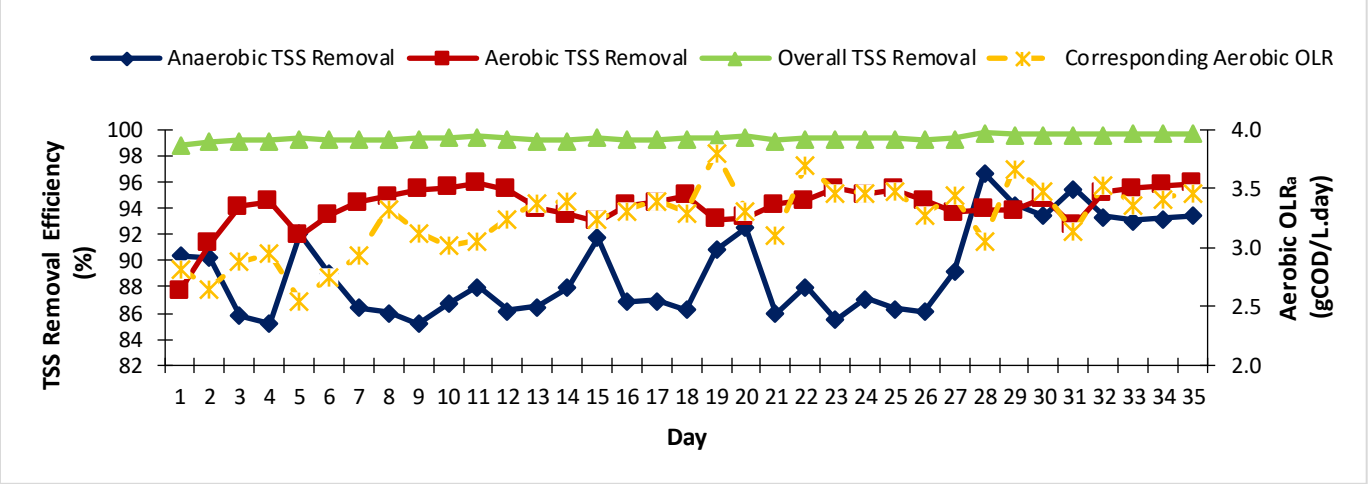
The performance of the pilot-scale IAAB over a period of 35 days was evaluated in terms of $COD_{removal}$, $BOD_{removal}$ and $TSS_{removal}$. In this study, the COD, BOD and TSS of the raw POME were 88,721 mg/L, 44,365 mg/L and 9,449 mg/L, respectively. The COD, TSS and BOD of the *M. oleifera* extract were 4,350 mg/L, 6,250 mg/L and 2,175 mg/L, respectively.



(a)



(b)



(c)

Figure 4.31: (a) COD (b) BOD (c) TSS removal efficiencies profiles for the pilot-scale IAAB

In order to evaluate the performance of the pilot-scale IAAB in terms of anaerobic and aerobic digestion processes, the removal efficiencies of the COD, BOD and TSS were evaluated daily for a period of 35 days. Figure 4.31 shows the COD, BOD and TSS removal efficiency profiles for all experimental runs throughout the study. COD, BOD and TSS removal efficiencies demonstrated a similar trend in the anaerobic process, where the obtained removal efficiencies for COD, BOD and TSS ranges from 93 - 94%, 94 - 95%, and 88 - 97%, respectively at HRT_{an} of 2.94 ±0.11 day. The anaerobic compartment of the pilot-scale IAAB comprises of two different technologies, i.e., upflow anaerobic sludge blanket fixed film (UASFF) and upflow anaerobic sludge blanket (UASB) bioreactors. These can be used to treat mixture of POME and *M. oleifera* filtrate at high OLR_{an} (30.0 gCOD/L.day). This is because the UASFF bioreactor is able to fix the biomass in the middle of the packing media with the aid of the vertically structured hollow channel to withstand the high loading rate and small HRT. Additionally, the increased population density on the packing media enhances the opportunity for cross-feeding, co-metabolism, and interspecies hydrogen and protein transfer. This results in the achievement of high removal efficiencies of the measured parameters in the anaerobic compartment (Borja et al. 2004). Similar observation was observed in the mono-digestion of POME, where no significant difference was observed between the COD and BOD removal efficiencies. This allows us to deduce that the removal efficiencies for both the parameters come from the breakdown of POME instead of *M. oleifera* extract. However, significant difference was observed when comparing the $TSS_{removal}$ of mono-digestion (93 ± 4%) and co-digestion (96 ± 5%). The dimeric cationic protein responsible for coagulating the solids in POME requires time to adsorb and neutralise the colloidal charges between the two substrates (Ndabigengesere et al., 1995). This increases the contact between the microbial cells in the anaerobic sludge with the organic matter, which is available in the form of large and dense flocs. Higher MLVSS concentration (53,000 - 64,000 mg/L) was obtained in co-digestion mode, allowing degradation efficiency of at least 90%. The packing media is able to uphold the biomass concentration, resulting in continuous stimulation of growth of micro colonies, hence maximising the $TSS_{removal}$ in the co-digestion mode.

In aerobic process, the provision of balanced nutrient and oxygen is the main criteria in the degradation of complex and readily biodegradable materials in the anaerobically-treated POME. This act as a post treatment to polish the effluent prior to discharge into waterways. However, the nutrient concentration and inhibitory substances (which are available in the activated sludge or the incoming anaerobically-treated effluent) are always the issue faced by the millers to prevent the growth of filamentous bacteria or floc formers, as well as creating an imbalance proportion of viable and death oxic microbial cells. In order to ensure efficient aerobic process, the determination of BOD:TN:TP of the anaerobically-treated POME is important. In this study, the BOD:TN:TP of the mixture of anaerobically-treated POME and *M. oleifera* extract is 100:10:9, which is higher than the recommended ratio of BOD:TN:TP (100:6:1.5).

The same observation was obtained in the mono-digestion. This has proven that the use of UASFF technology is able to carry out degradation activity in mono- and co-digestion to produce anaerobically-treated effluent with sufficient nutrient content, without the need of additional nutrient to sustain the aerobic metabolic activities. As shown in Figure 4.31, a drop in the measured removal efficiencies of COD, BOD and TSS was observed from days 1 - 6. This may be due to the high concentration of bacteria in the activated sludge as reflected on the MLVSS concentration ranges from 23,345 - 21,054 mg/L. Additionally, the F/M ratio obtained from days 1-6 ranges from 0.1378 to 0.1395 gCOD/gMLVSS.day (as illustrated in Figure 4.32). Under this condition, the BOD of the final treated effluent was above 20 mg/L (which is higher than the target BOD set for this study). This allows us to postulate that the proportion of substrate and bacteria concentration is relatively vital to treat mixture of anaerobically-treated effluent with *M. oleifera* filtrate to BOD less than 20 mg/L.

Starting from Day 4, alteration was carried out on the returned activated sludge (RAS) and waste activated sludge (WAS) ratios. A decrease in the RAS ratio from 2.10 to 1.88 was carried out. On the other hand, an increased in the WAS ratio from 2.78 to 4.51 was performed as well. This is to remove the biologically non-active microbial cells that are always located on the hopper of the settling tank. The high carbon content in the *M. oleifera* filtrate (54%) has proliferated the growth of aerobic microbial cells. The incoming anaerobically-treated POME acts as a substrate for the aerobic microbial cells, the former which provides sufficient DO to allow them to continuously degrade the organic matter. Additionally, the installed compressor that sends oxygen directly into the aerobic tank allow mixing of fresh and recycled activated sludge, which will further enhance the degradation process. The modification on the WAS and RAS ratio has improved the performance of the pilot-scale IAAB in terms of the $COD_{removal}$ (improved by 3-4%), $BOD_{removal}$ (improved by 1-2%, almost 100% removal) and $TSS_{removal}$ (improved by 5-6%) (as shown in Figures 4.39a-4.39c). The results obtained concurred with the study reported by Hosseini et al. (2008) where both the RAS and WAS ratios were the key operating conditions in improving the performance of the aerobic activated sludge system. The decreased RAS ratio has reduced the oxygen demand, which aids in the reduction of the overall cost of the aerobic treatment. Concurrently, reduction in MLVSS concentration from 29,550 mg/L to 25,200 mg/L was observed from days 4-8. As a result, low oxygen demand is observed in the aerobic compartment (as reflected sudden increase of DO reading) due to the reduction in the bacteria concentration. The aimed of treating the anaerobically-treated POME using activated sludge process is to reduce oxygen demand when the aerobic microbial cells utilise the organic compound to yield energy for growth (Parker, 2001). If the RAS ratio was not decreased, the biologically non-active microbial cells from the settling tank will compete with the proliferating aerobic microbial cells for oxygen. This will increase the DO reading of the aerobic compartment, which may create excessive foam formation. This may affect the performance of the aerobic and anaerobic compartments as the entire system is designed in the form of integrated system.

This shows that the introduction of a substrate with high levels of carbon content has provided an intermediate breakdown of the homogenous mixture to balance up the nutrient content in the raw substrate (POME), which will stabilise the anaerobic and aerobic processes. This can be explained by the integrated design of the pilot-scale IAAB, whereby the incoming POME containing mixture of *M. oleifera* filtrate with sufficient nutrient will be treated effectively within the bioreactor before being discharged into the river ways. In this scenario, the mixture of POME with *M. oleifera* extract will be treated evenly by the microbial cells in both the anaerobic and aerobic compartments of the pilot-scale IAAB. This is not observed in the current POME treatment whereby not all the raw substrate (POME) or treated effluent are subjected to proper treatment as the treated effluent overflow from one pond to the other pond. As a result, the produced final discharge may still contain mixture of treated and non-treated effluent. Hence, a better POME treatment system should be implemented to replace the existing treatment as a way to push the industry towards greener image.

Starting from days 8-35, an increased in the $COD_{removal}$, $BOD_{removal}$ and $TSS_{removal}$ were observed. This shows that with an adaptation period of at least 7 days, MLVSS concentration of less than 26,000 mg/L and an adequate DO concentration between 1.98 - 2.21 are sufficient for the microbial cells in the aerobic compartment to carry out the synthesis activity effectively. Under such conditions, this will contribute in maintaining at least 90% for the measured parameters. The $COD_{removal}$, $BOD_{removal}$ and $TSS_{removal}$ at days 8 - 35 ranges from 95 - 96%, 99 - 100% and 93 - 96%, respectively, were obtained by maintaining the operational parameters (shown in Table E1 (Chapter 7, Appendices)) during the continuous operation of the pilot-scale IAAB. In this study, F/M ratio was determined daily as it allows the determination of the optimum biomass concentration in the aerobic compartment and the amount of biomass needed to be wasted daily from the settling tank. Throughout the study, 74% of the total studies had a final treated effluent with BOD less than 20 mg/L (Figure 4.32).

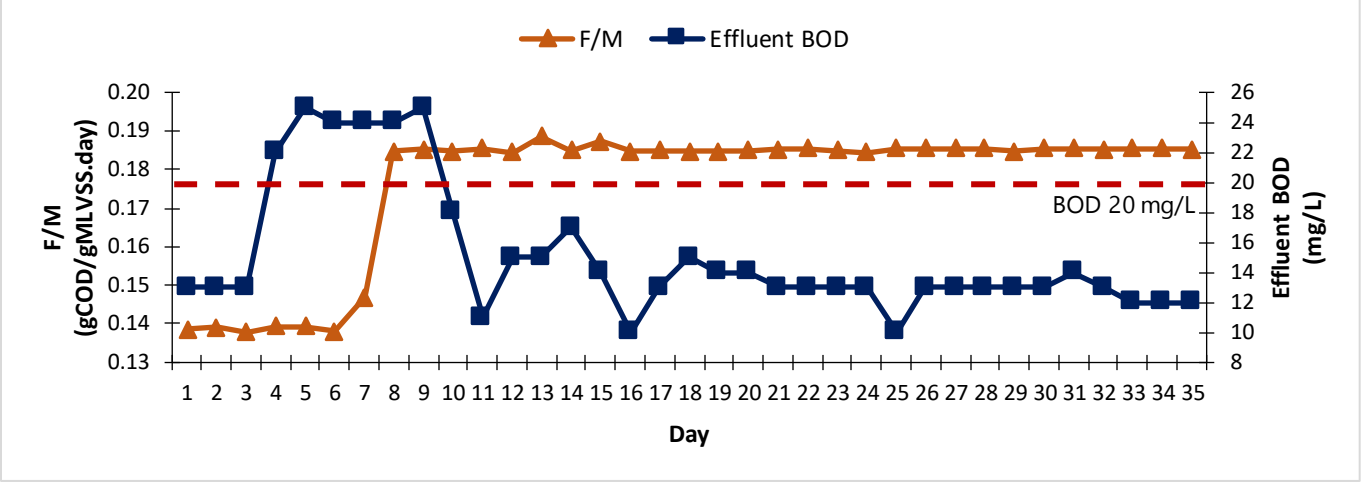


Figure 4.32: Effluent BOD and F/M ratio profile of the pilot-scale IAAB at co-digestion mode (Aerobic Compartment only)

At the initial period of the co-digestion study from Days 1 - 3, the BOD of the final discharge effluent and F/M ratio of the aerobic compartment were 13 mg/L and 0.137 - 0.139 gCOD/gMLVSS.day (Figure 4.32). The performance of anaerobic compartment remained stable, where the COD and BOD removal falling in a narrow range, i.e., 93-94% and 92-95% respectively throughout the 35 days as discussed previously. However, a sudden increment on the final discharge effluent BOD (22-25 mg/L), TSS (87-95mg/L) and SVI (>150) were observed on Days 4-9. Sludge bulking and formation of foam had occurred in the aerobic tank as well.

This may be explained by the formation of different types of filamentous bacteria that synthesise hydrophobic compounds, which is primarily affected by the occurrence of shock loading of the system due to the introduction of a new type of substrate (mixture of POME and *M. oleifera* extract) (Stypka et al., 1998; Almshawit, 2006). To rectify the issue of shock loading in the system, foam was removed from aerobic compartment manually to remove the excessive and uncontrolled growth of different types of filamentous bacteria. This is to prevent the introduction of foam into the anaerobic compartment, which will affect the biological activity of the anoxic microbial cells. Adjustment on the WAS and RAS ratios were carried out as well, where the ratio of the WAS has to be greater than RAS. This is to ensure solid accumulated at the bottom of the hopper of the settling tank is discharged daily, to prevent the formation of dead zones. From Day 10 onwards, a better quality of the final treated effluent was obtained from the sampling point labelled as Discharge 1 in terms of the BOD and TSS. The BOD and TSS of the final treated effluent on Day 10 were 18 mg/L and 62 mg/L, respectively. The operating condition of the aerobic compartment of the pilot-scale IAAB on Day 10 in terms of the MLVSS and F/M ratio were 18,012 mg/L and 0.185 gCOD/gMLVSS.day.

It is worth noting that the F/M ratio of the aerobic compartment of the pilot-scale IAAB was constantly maintained at a range of 0.185 - 0.188 gCOD/gMLVSS.day from Days 8 - 35 (Figure 4.32) with the MLVSS concentration of 18,000 - 20,000 mg/L. These stable reading of the parameters was achievable by maintaining the RAS and WAS ratios between 1.05 - 2.75 and 2.17 - 4.13, respectively (as shown in Table E1 (Chapter 7, Appendices)). The obtained F/M ratio in this study was within the recommended range of 0.06 - 0.18 gCOD/gMLVSS.day for the treatment of anaerobically-treated POME using activated sludge system (Chan, et al., 2010). However, the F/M ratio obtained in this study was lower than the typical F/M ratio in conventional activated sludge system, which ranges from 0.2 - 0.4 gBOD/gMLVSS.day. This is because the incoming influent (POME) contained relatively high concentrations of TSS (28,846 to 30,920 mg/L) and COD (15,000 to 100,000 mg/L), which requires adequate population of bacteria (as reflected by the high MLVSS concentration) to effectively degrade the organic matter.

In this study, we found out that operating the aerobic compartment at relatively stable F/M ratio (0.185 - 0.188 gCOD/gMLVSS.day) and maintaining the MLVSS concentration between 18,000 - 20,000 mg/L had allowed the production of good quality final treated effluent until the end of the study period at Day 35. The BOD and TSS of the final treated effluent from Days 10 - 35 were 10 - 18 mg/L and 35 - 97 mg/L, respectively. Despite operating the pilot-scale IAAB at F/M ratio of 0.185 gCOD/gMLVSS.day on Days 8 - 9, the BOD of the final treated effluent was still above 20 mg/L. This can be explained by the fact that the aerobic microbial cells require at least a period of 9 days to remedy the occurrence of shock loading experienced by the aerobic microbial cells, due to the introduction of new type of substrate, to allow the achievement of BOD less than 20 mg/L.

4.3.4.3 Optimum Operating Condition

The use of *M. oleifera* extract is able to create a positive synergistic relationship with POME by complementing the missing nutrients in the POME, which is reflected on the C/N ratio. The high carbon and protein sources in the *M. oleifera* of 54.8% and 36.7%, respectively, are able to increase the C/N ratio of the mixture of the POME and *M. oleifera* filtrate. Study conducted by Nurliyana et al. (2015), where the optimum C/N ratio for co-digestion of POME with empty fruit bunch (EFB) is 45, acts as a yardstick for creation of stability of anaerobic digestion. It is vital to create a stabilised anaerobic digestion process, as it plays a major role in degrading the incoming substrate (POME and *M. oleifera* extract) and simultaneously able to generate biogas before being subjected to further aerobically-polishing treatment. The optimum operating conditions for the co-digestion of POME with *M. oleifera* extract were attained from the present study and summarised in Table 4.15. By maintaining the pilot-scale IAAB at the proposed optimum conditions, a consistent final treated effluent quality that abides by the discharge limit set by DOE can be obtained (Table 4.16).

Table 4.15: Operating conditions for optimum pilot-plant IAAB performance in the co-digestion treatment

Parameter	Optimum Conditions
OLR_{an} (gCOD/L.day)	30.0
OLR_{a} (gCOD/L.day)	2.55 - 3.81
MLVSS (mg/L)	18,000 - 20,000
F/M_{a} (gCOD/gMLVSS.day)	0.185 - 0.188

$_{a}$:Aerobic Process; $_{an}$: Anaerobic Process

Table 4.16: Optimum pilot-scale IAAB performance during the co-digestion of POME with *Moringa Oleifera* Extract and its respective standard discharge limits by the Malaysian Department of Environment (DOE)

Parameters[a]	Anaerobically-Treated POME		Final Treated Effluent		DOE Standards
	Value	Removal Efficiency (%)	Value	Overall Removal Efficiency (%)	
Effluent BOD	2,511 ± 274	94 ± 1	15 ± 2	99.9 ± 0.01	100[b]
Effluent COD	5,725 ± 624	94 ± 1	265 ± 36	99.7 ± 0.05	-
Effluent TSS	628 ± 139	95 ± 3	56 ± 21	99.2 ± 0.35	400
Effluent Lignin	510 ± 108	69 ± 8	30 ± 4	99.1 ± 0.06	-
Effluent Ammoniacal Nitrogen	385 ± 128	85 ± 10	15 ± 2	99.7 ± 0.25	100
Effluent pH	7.37 ± 0.12	-	8.47 ± 0.12	-	5.0 - 9.0

[a]: All parameters in mg/L except pH; b: Sample incubated for 3 days at 30°C

4.3.4.4 Comparison between Mono-Digestion and Co-Digestion

In order to allow for a meaning comparison between mono- digestion and co-digestion, the inoculum used in the pilot-scale IAAB was obtained from the same source, which is from an existing anaerobic pond that has been used to treat POME. For mono-anaerobic digestion with POME only, the $COD_{removal}$, $BOD_{removal}$ and $TSS_{removal}$ of the system were 92 - 93%, 93 - 94% and 86 - 90%, respectively. *M. oleifera* provided an improved $TSS_{removal}$ (~1.6%), $COD_{removal}$ (~16%), and $BOD_{removal}$ (~18%) when it was co-digested with POME during the initial start-up period, compared to mono-digestion. The dimeric cationic protein is responsible for the formation of the large and dense flocs by coagulating the solids in POME. Such formation that occurred during the co-digestion process has allowed the microbial cells to easily biodegrade them. Hence, this allow us to postulate that the coagulation mechanism able to improve the $TSS_{removal}$ as compared to the mono-digestion.

Despite operating the pilot-scale IAAB at high OLR_{an} and the introduction of a new type of substrate to be co-digested with POME, temporary reactor failure of the acetoclastic and methanogenesis pathway was not observed throughout the study period. Additionally, the pH of the system throughout the study period for mono-digestion and co-digestion modes was within the range of 6.8 - 7.2, which is the optimum pH range for methanogenesis to occur. The concentration of NH_3-N from the final treated effluent shows a trend of gradual depletion for both the mono-digestion (reduction from 202 mg/L to 14 mg/L) and co-digestion (reduction from 250 mg/L to 10 mg/L) during the entire experiment period. It is worth noting that the pilot-scale IAAB is relatively stable despite the occurrence of inoculum washing-out in both the mono-digestion (instability on Days 1 - 2) and co-digestion (instability on Days 6 - 8). The measured NH_3-N from the final treated effluent produced from mono-digestion and co-digestion abides by the POME discharge limit set by the DOE of Malaysia (Table 4.18).

Regarding the biogas, co-digestion showed a 72% higher methane yield than mono-digestion. The same observation was obtained when conducting the same assay in the form of laboratory scale (Section 4.2), co-digestion showed 59% higher methane yield than mono-digestion. This can be explained by the different substrate compositions used for the digestion process. According to Janke, et al. (2016), carbohydrate-based substrate would produce around 52 - 63% (mono-digestion of POME only) of CH_4 in biogas, while a share of protein (mixture of POME and *M. oleifera* extract) could increase the CH_4 content up to 63 - 64%. Furthermore, the higher lignin content found in the anaerobically-treated POME produced from the mono-digestion (lignin concentration: 662 mg/L; $lignin_{removal}$: 71 ± 5.95%) might have been another factor that has influenced the significant differences in the methane yield and methane composition between the mono-digestion of POME and co-digestion of POME with *M. oleifera* extract (lignin concentration: 489 mg/L; $lignin_{removal}$: 79 ± 3.98%). The same observation was obtained in the study reported by Montgomery and Bochmann, (2014), where the recalcitrant presence of lignin may have hampered the hydrolysis of such fibrous materials, resulting in a lower conversion of the volatile solids into biogas. Additionally, the high carbohydrates content in the POME (29.55%) may have contributed to the low methane yield. This may be

explained by the slow growth rate experienced by methanogens as compared to the acidogens present in the upstream degradation chain (Murto et al., 2004; Ruiz et al., 2008).

For the case of co-digestion of POME with *M. oleifera* filtrate, the ability of the UASFF to hold high biomass concentrations (56,000 - 67,000 mg/L), coupled with the ability of the dimeric cationic protein in the shelled *M. oleifera* to coagulate the solid in the incoming mixture of POME, may have contributed to the higher methane yield (0.117 - 0.143 $LCH_4/gCOD_{removed}$). Besides the coagulant effect by the dimeric cationic protein of the *M. oleifera* extract, the high lipid (35%) and protein (37%) contents in the shelled *M. oleifera* reported by Ndabigengesere et al. (1995) could have further enhanced the methane production by methanogenesis. The same observation was reported by Wang et al. (2014) where anaerobic co-digestion of kitchen waste and fruit/vegetable waste with higher fat and protein content achieved higher methane productions (0.693 - 0.725 L/gVS) than the one with lower fat and protein content (0.620 L/gVS). Hence, selecting suitable type of substrate with high fat and protein content is able to improve the methane production in an anaerobic digestion process.

The final discharge level of BOD for East Malaysia (20 mg/L) and Peninsular Malaysia (100 mg/L) has been emphasised for the past few years to reduce the grey water footprint as designed by Hoekstra et al. (2011). Additionally, the issue of carbon footprint has been highlighted in the palm oil industry. In order to reduce the carbon footprint of the POME treatment system, the IAAB system by Chan et al. (2012) has been upscaled to pilot scale with a total volume of $1.8m^3$ (Section 2.5.2, Chapter 2, Literature Review) to ensure that the results obtained are comparable to the existing POME treatment system. Regarding the production of final treated effluent with BOD less than 20 mg/L, co- and mono-digestions showed that 74% and 70% of the data obtained has achieved BOD ranges from 10 - 18 mg/L and 10 – 19 mg/L, respectively. In this study, the important parameters that allow the production of BOD less than 20 mg/L have been identified and summarised in Table 4.17 for both the mono- and co-digestion processess.

Table 4.17: The important dependant parameters in the aerobic compartment for the achievement of BOD less than 20mg/L

Parameter	Unit	Mono-digestion	Co-digestion
OLR_a	gCOD/L.day	2.60 - 4.21	2.55 - 3.81
F/M_a	gCOD/gMLVSS.day	0.130 - 0.133	0.185 - 0.187
$MLSS_a$	mg/L	27,450 - 29,350	23,050 - 27,556
$MLVSS_a$	mg/L	20,580 - 23,621	18,012 - 20,000
R_{RAS}	-	1.73 – 3.99	1.05 – 2.75
R_{WAS}	-	3.43 – 4.50	2.13 – 4.13

The parameters listed in the table above showed a significant difference for both the digestion manners to achieve BOD less than 20 mg/L. Higher bacteria concentration was needed in mono-digestion to treat POME than co-digestion. The high MLVSS concentration of the activated sludge in the aerobic compartment of the pilot-scale IAAB has resulted a low F/M ratio as both the parameters are inveserly proportional to each other. However, the use of high biomass concentration may create risks for the activated sludge process to deteriorate, due to (a) low sludge age; (b) high concentrations of suspended solids in the supernatant. The latter one may also be caused by rising sludge (nitrogen gas via the pathway of biological denitrification) (Wilen 1995). The poorly soluble nitrogen gas will adhere to the activated sludge flocs and float them to the surface of the settling tank, resulting in the flowing of activated sludge to the external environment. Extra care has to be taken during the mono-digestion to prevent the occurence of rising sludge by maintaining the R_{WAS} (3.43 - 4.50) to allow the frequent discharging of solids that are accumulated at the hopper of the settling tank. At the same time, the rate of solid recycling (R_{RAS}) was maintained within 1.73 - 3.99 to ensure the solids in the settling tank are mixed evenly in the aerobic tank with the aid of the installed compressor that sends oxygen directly into the tank. It is essential to upkeep the activated sludge activity and the bacteria concentration by maintaining both the ratios to allow the continuous proliferation of the aerobic microbial cells for active degradation of the organic matter in the anaerobically-treated effluent. Priority was given to the R_{RAS} and R_{WAS} as both the parameters affect the MLVSS and F/M ratio in the aerobic compartment. Maintaining the F/M ratio (0.130 - 0.133 gCOD/gMLVSS.day) and $MLVSS_a$ (20,580 - 23,621 mg/L) are important to produce a final treated effluent from Discharge 1 (Figure 3.7, Chapter 3, Materials and Methods) with BOD within the range of 11 - 19 mg/L.

These observations were not observed during the co-digestion process. The addition of *M. oleifera* filtrate into the raw POME has brought a significant effect towards the entire POME treatment. In the anaerobic process, *M. oleifera* filtrate will increase the contact between the microbial cells in the anaerobic sludge with the organic matter, as it appears in the form of large and dense flocs. Additionally, the *M. oleifera* filtrate is able to provide nitrogen and carbon sources for the indigenous anoxic microbial cells to continuously replicate themselves. With these conditions in the anaerobic compartment, it is able to produce anaerobically-treated effluent that contained balanced C/N ratio and BOD:TN:TP that are sufficient for the aerobic microbial cells to carry out the degrading activities. By comparing the two digestion manner, the MLVSS concentration (18,000 - 20,000 mg/L) used in the aerobic compartment was lower than the mono-digestion method (Table 4.17). Additionally, the F/M ratio (0.185 - 0.187 gCOD/gMLVSS.day) used in the co-digestion study was higher than the mono-digestion manner. This is because an increase in BOD of the final treated effluent was observed from Days 4 - 9. This shows that the MLVSS concentration and F/M ratio are correlated with each other to produce a final treated effluent with BOD and TSS of less than 20 mg/L and 400 mg/L, respectively. In order to reduce the biomass concentration in the aerobic compartment, changes in the R_{RAS} (1.05 - 2.75) and R_{WAS} (2.17 - 4.13) were carried out. The R_{RAS} ratio applied in the aerobic compartment is able to withstand the texture of the thick sludge in the settling tank, as this will maintain a desired concentration of biomass in the settling tank. With the R_{WAS} applied in this study, the thick sludge in the bottom of the settling tank must be removed as frequently as possible to ensure removal of particulate-bound phosphorus in the activated sludge flocs to prevents the occurrence of the denitrification process (Wilen 1995). By maintaning the abovementioned operating conditions (Table 4.17), a final treated effluent with BOD less than 20 mg/L can be produced. Table 4.18 shows the quality of the final treated effluent for mono-digestion and co-digestion treatment method.

Table 4.18: The average BOD_3, COD, TSS, NH_3-N, and lignin of the final treated effluent

Parameter[a]	Mono-digestion	Co-digestion	Regulatory discharge limit
BOD_3	20 ± 31	15 ± 2	20.00
COD	343 ± 102	265 ± 36	No discharge limit
TSS	64 ± 30	56 ± 21	400.00
NH_3-N	75 ± 31	15 ± 2	100.00
Lignin	80 ± 36	30 ± 4	No discharge limit

[a]: All parameters in mg/L; BOD_3: Sample incubated for 3 days at 30°C



(a) (b)

Figure 4.33: Quality of the final treated effluent produced by two different treatment manners (a) mono-digestion (b) co-digestion

As shown in Table 4.18, the addition of *M. oleifera* extract as a substrate for co-digestion is able to produce a final treated effluent with the least concentration of degradable organic materials, where they are degraded by means of microbial cells that spontaneously form flocs. Carbon and nitrogen are usually the growth-limiting compound in industrial wastewaters. The provision of substrate with sufficient concentration of nitrogen and phosphorus is essential to allow continuous synthesis of cell material in the activated sludge process for active degradation of the incoming organic material. This is aimed to produce a final treated effluent that meets the regulatory discharge requirement. Final treated effluent produced by co-digestion process gave better clarity in effluent than the mono-digestion process, as illustrated in Figure 4.33. This allow us to postulate that a balanced proportion of microbial cells and substrate in the aerobic compartment is able to degrade the organic matter that cannot be degraded during the anaerobic process, hence producing supernatant that contained low concentration of solid. Additionally, the sludge in the settling tank is thick enough to allow efficient separation of solid and supernatant. The formation of large and dense flocs with the aid *M. oleifera* extract has allow the sludge to settle down easily as reflected on the average SVI of 34 (less than preferred range of SVI 100). The satisfactory operation of these two parameters in the settling tank allows the production of final treated effluent with better clarity and compliance to the regulatory discharge limit.

The results obtained showed that co-digesting POME with *Moringa Oleifera* extract is able to create a positive paradigm shift for the current POME treatment. The high performance of the pilot-scale IAAB operated in the co-digestion manner was found to be attributed to several factors, namely: (a) sufficient retention of MLVSS concentration in the anaerobic and aerobic compartments; (b) development of good settling activated sludge; (c) balanced proportion of microbial cells and substrate in the aerobic compartment; (d) sufficient recirculation ratio adopted in the anaerobic and aerobic compartments, and; (e) frequent discharging of biomass located at the hopper of the settling tank to prevent the development of inactive cells. The results obtained also proved that the pilot-scale IAAB is able to operate at high OLR_{an} of 30.0 gCOD/L.day, which is superior to the laboratory-scale IAAB (10.5 gCOD/L.day).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In the present research, pilot-scale IAAB has been proposed as an effective POME treatment system. It can act as an alternative for the current POME treatment manner used by the existing palm oil industry. The pilot-scale IAAB was able to achieve high overall BOD, COD and TSS removal efficiencies of more than 98%, high methane yield with at least 60% methane purity and able to produce final treated effluent that complies with the Environment Quality Act (EQA) 1974 requirement under mesophilic condition.

1. The effect of different anaerobic modes: (a) open ponding system without covered lagoon, (b) added covered lagoon with gas mixing mode and (c) covered lagoon with hydraulic mixing mode in overall POME treatment was evaluated. The crucial factors influencing the performance of each of the anaerobic POME treatment were constant removal of sludge and the deployed recirculation system. Further improvement was required for the system to be able to meet the stringent final discharge limit set, though continuous aeration system and appropriate retention time have been implemented.

    a) Results obtained showed that the covered lagoon with hydraulic mixing was the most efficient anaerobic POME treatment system with high $BOD_{removal}$, $COD_{removal}$, $TSS_{removal}$, and $Lignin_{removal}$ of 56%, 95%, 87%, and 75%, respectively.

    b) It was found that the modified Stover-Kincannon model was the most appropriate in predicting the performance of the covered lagoon system with hydraulic mixing with R^2 of 0.9767.

2. The present study allowed us to infer that the design of the pilot-scale IAAB was successfully deployed in the POME treatment installed in the palm oil mill located in Pahang, Malaysia. The performance of the pilot-scale IAAB regardless of high- or low crop seasons was evaluated by determining the removal efficiencies of the tested parameters and applying the suitable biokinetic models.

   a) The start-up of the pilot-scale IAAB under mesophilic condition was accomplished in 39 days (Anaerobic Compartment: 24 days; Aerobic Compartment: 15 days). Such achievement was accomplished by the successful development of high bacteria concentration in both the anaerobic and aerobic compartments of the pilot-scale IAAB. The development of high microbial activity in these compartments has led to the achievement of high removal efficiencies of the tested parameters. An increase in the $BOD_{removal}$ (Stage II: 76.42%; Stage III: 92.46%; Stage IV: 96.81%; Stage V: 98.68%), $COD_{removal}$ (Stage II: 74.93%; Stage III: 95.61%; Stage IV: 97.67%; Stage V: 98.04%) and $TSS_{removal}$ (Stage II: 79.94%; Stage III: 83.03%; Stage IV: 93.22%; Stage V: 95.76%) were achieved at Stages II, III, IV, and V.

   b) The start-up of the aerobic compartment was carried out once steady state has been achieved in the anaerobic compartment of the pilot-scale IAAB. At the end of the start-up period of the anaerobic compartment, Stage V (OLR_{an}: 8.0 gCOD/L.day; OLR_a: 0.90 gCOD/L.day), the overall $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ obtained were 99.80%, 98.56% and 99.24%, respectively. The average methane yield and methane purity obtained at Stage V were 0.025 $LCH_4/gCOD_{removed}$ and 56.34%, respectively. The stepped up OLR approach adopted in the start-up of the pilot-scale IAAB was proven to be efficient due to the shorter start-up period as compared to the laboratory-scale IAAB (45 days) and other studies in the literatures (56-150 days). Throughout the start-up period, supplementary of alkalinity was not necessary to sustain high microbial activity as well as preventing the occurrence of inhibition in both the anaerobic and aerobic compartments.

c) The maximum loading of the pilot-scale IAAB at various OLR_{an} (10.5 gCOD/L.day to 32.5 gCOD/L.day) was successfully investigated. The obtained results in the present research proved that the pilot-scale IAAB was able to handle high OLR_{an} up to 30.0 gCOD/L.day (corresponding to average OLR_a of 2.16 gCOD/L.day) under mesophilic condition without any operational problems. At this condition, the overall $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ were 99.79 ± 0.30%, 99.64 ± 0.09%, and 99.76 ± 0.03%, respectively. The average methane yield and methane purity achieved under the condition of OLR_{an} 30.0 gCOD/L.day (corresponding to average OLR_a of 2.16 gCOD/L.day) were 0.057 ± 0.007 $LCH_4/gCOD_{removed}$ and 57.68 ± 1.58%, respectively. The final treated effluent produced was in compliance with the discharge limit of BOD less than 20 mg/L (79% of the total study period) and TSS of less than 400 mg/L (100% of the total study period). Such promising results achieved by the pilot-scale IAAB were superior than the laboratory-scale IAAB except for the methane yield and methane purity. The obtained results showed that the highly effective design method used during the scale-up analysis of the laboratory-scale IAAB able to create a synergistic relationship with the designed OLR.

d) Under mesophilic condition, anaerobic digestion contributed 96.12 ± 0.88% to the total $COD_{removal}$ while aerobic digestion contributed 3.88 ± 0.88% at OLR_{an} 30.0 gCOD/L.day (corresponding to average OLR_a 2.16 ± 0.42 gCOD/L.day). The result obtained showed that anaerobic compartment played a major role in the COD removal whilst aerobic compartment act as a further polishing system in producing final treated effluent that abided the discharge limit set by the regulatory department. However, the role of anaerobic and aerobic compartments in the treatment of POME had become opposite during the increment of OLR_{an} from 30.0 gCOD/L.day to 32.0 gCOD/L.day and 32.5 gCOD/L.day. When the OLR_{an} increased to 32.0 gCOD/L.day (corresponding to average OLR_a 4.25 ± 1.72 gCOD/L.day), these percentages were decreased to 92.87 ± 3.89% and increased to 7.13 ± 3.89% in anaerobic and aerobic compartments, respectively. The increment of OLR_{an} to 32.5 gCOD/L.day (corresponding to average OLR_a 6.73 ± 1.73 gCOD/L.day) resulted in further decreased and increased in the total $COD_{removal}$ of anaerobic and aerobic compartments of 89.20 ± 3.31% and 10.80 ± 3.31%, respectively. This shows that the role of aerobic has become exceptionally essential at high OLR_{an} to reduce the organic matter concentration in the anaerobically-treated POME.

e) Three kinetic models namely Modified Stover-Kincannon, Grau Second-Order, and Monod models were applied successfully to the steady state performance data of the pilot-plant IAAB at OLR_{an} ranging from 10.5 gCOD/L.day to 32.0 gCOD/L.day under the mesophilic condition. Modified Stover-Kincannon is the most appropriate to estimate the performance of the anaerobic and aerobic compartment of the pilot-plant IAAB with R^2 >0.99.

3. The pilot-plant IAAB was operated under the conditions of high OLR_{an} (30.0 gCOD/L.day), high $MLVSS_{an}$ (48,000 mg/L) and high $MLVSS_a$ (22,993 mg/L) concentrations yielded the optimum conditions for the production of final treated effluent compliance with the discharge limit regulated by the DOE, Malaysia under mesophilic condition. This study is termed as mono-digestion.

a) At its optimum condition, the pilot-scale IAAB exhibited high overall $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ of 99.95 ± 0.02%, 99.58 ± 0.15% and 99.24 ± 0.33%, respectively. Good quality of the final treated effluent was obtained with average BOD and TSS of 18 ± 5 mg/L and 64 ± 30 mg/L and well below the discharge limit. Additionally, good stability was observed in the pilot-scale IAAB with average pH, TA and VFA/TA of 7.49 ± 0.13, 2,356 ± 246 mg/L and 0.12 ± 0.02 mg/L, respectively. The average methane yield and methane purity obtained during the operation of the pilot-scale IAAB at its optimum condition were 0.0978 ± 0.0140 $LCH_4/gCOD_{removed}$ and 60.64 ± 2.47%, respectively.

b) At the optimum condition for pilot-scale IAAB (OLR_{an} 30.0 gCOD/L.day; OLR_a 2.86 ± 0.37 gCOD/L.day), anaerobic digestion contributed 94.53 ± 0.75% whereas aerobic digestion contributed 5.47 ± 0.75% to the total $COD_{removal}$. The finding obtained was in agreement with the study conducted under the condition of operating the pilot-scale IAAB at various OLRs (10.5 gCOD/L.day to 30.0 gCOD/L.day).

c) Three kinetic models, viz. Modified Stover-Kincannon, Grau Second-Order and Contois models were applied successfully to the pilot-scale IAAB at its optimum condition (OLR_{an}: 30.0 gCOD/L.day; OLR_a: 2.86 ± 0.37 gCOD/L.day). Grau Second-Order is the most appropriate kinetic model for predicting the performance of the anaerobic compartment (R^2 = 0.9728) and aerobic compartment (R^2 = 0.9933) of the pilot-scale IAAB.

4. Co-digesting POME with *Moringa Oleifera* extract to achieve synergistic effects was carried out on the pilot-scale IAAB at its optimum condition. The operating conditions of the co-digestion assay were (a) OLR_{an} 30.0 gCOD/L.day (b) OLR_a 3.25 ± 0.30 gCOD/L.day (c) $MLVSS_{an}$ 58,500 mg/L (d) $MLVSS_a$ 19,766 mg/L. Such conditions have resulted in the production of the final treated effluent that complies with the discharge limit set by the regulatory department. This study is termed as co-digestion.

    a) During the operation of the co-digestion mode, the pilot-scale IAAB exhibited high overall $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ of 99.97 ± 0.01%, 99.70 ± 0.05% and 99.15 ± 0.35%, respectively. The quality of the final treated effluent produced by the co-digestion assay compliance with the discharge limit set by the DOE, Malaysia. The average BOD and TSS obtained under such operating mode were 15 ± 2 mg/L and 56 ± 21 mg/L, respectively. The incorporation of *M. oleifera* extract into the digestion of POME exhibited good stability in terms of the pH (7.37 ± 0.12), TA (2,660 ± 330 mg/L) and VFA/TA (0.11 ± 0.04). Additionally, the average methane yield and methane purity obtained during the co-digestion assay were 0.1353 ± 0.006 $LCH_4/gCOD_{removed}$ and 64 ± 0.44%, respectively.

    b) During the operation of the pilot-scale IAAB under the co-digestion assay, anaerobic digestion contributed 94.00 ± 0.66% whilst aerobic digestion contributed 6.00 ± 0.66% to the total $COD_{removal}$. Such finding was in agreement with the results obtained during the studies conducted at various OLR_{an} (10.5 gCOD/L.day to 30.0 gCOD/L.day) and mono-digestion.

5. An effective and complete POME treatment system using pilot-scale IAAB with two different sets of digestion assay operating conditions were proposed in this research.

    a) Based on the outcome of the present study allow us to infer that the pilot-scale IAAB able to handle high OLR_{an} of 30.0 gCOD/L.day. The pilot-scale IAAB was subjected to two different assays, namely mono-digestion and co-digestion. The performance evaluation in terms of the anaerobic and aerobic removal efficiency of BOD, COD and TSS does not exhibit any significant difference between the two digestion assays. The overall $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ obtained from both the assays were quite similar to each other. Mono-digestion and co-digestion assay operated under the optimum operating condition are chosen as the best options for POME treatment.

b) Operating the pilot-scale IAAB under mono- and co-digestion at OLR_{an} 30.0 gCOD/L.day able to treat POME with high COD concentration ranging from 67,825 mg/L to 94,750 mg/L. The overall $BOD_{removal}$, $COD_{removal}$ and $TSS_{removal}$ achieved at OLR_{an} 30.0 gCOD/L.day were more than 99.15%. However, the methane yield achieved by co-digestion mode was 72% higher than the mono-digestion mode. Additionally, the methane purity obtained by the co-digestion mode (64 ± 0.44%) was higher than the mono-digestion mode (60.64 ± 2.47). This shows that the addition of *M. oleifera* extract had enhanced the activity of methanogenesis due to the coagulating ability exhibited by the co-substrate used.

c) This allowed us to infer that anaerobic process is the primary step in POME treatment for producing anaerobically-treated effluent that is suitable for further biodegradation by the aerobic microbial cells. The combination of anaerobic and aerobic processes in the treatment of POME able to produce final treated effluent with BOD less than 20 mg/L and TSS of less than 400 mg/L in both the mono-digestion and co-digestion assay.

d) The present research showed that overall reduction of capital and treatment costs are highly anticipated due to the reduced volume of bioreactor system and able to produce renewable energy (methane gas). Additionally, palm oil millers (Feed-In Approval Holders) is eligible to enjoy the incentive provided by Sustainable Energy Development Authority (SEDA) under the Malaysia's Feed-In Tariff by selling the purified methane gas to the oblige Distribution Licensees.

6. The results obtained during the performances of the pilot-scale IAAB in mono- and co-digestion at high OLR_{an} 30.0 gCOD/L.day were promising. The crucial factors that governed the performance of the pilot-scale IAAB were (a) sufficient concentration of MLVSS in both the anaerobic and aerobic compartments (bacteria population) (b) adequate contact between the microbial cells and substrate (sufficient recirculation ratio in anaerobic and aerobic compartments) (c) development of activated sludge with good settling ability for the separation of liquid from solid (d) balance C/N composition to nourish the growth of anoxic microbial cells for the occurrence of maximum degradation.

7. The positive outcomes obtained throughout the entire study will benefit the society. It will act as a stepping stone for the palm oil industry to understand the importance of adopting suitable POME treatment method. This is to ensure that the quality of the treated POME comply with the discharge limit set by the Department of Environment (DOE), Malaysia. Additionally, this piece of data will expand their horizon with the most up-to-date POME treatment technology as these technologies are developed and improved based on the issues faced by the them. Hence, this will push the palm oil industry to move towards the direction of "green industry" and "technology savy".

5.2 Recommendations

1. A scale-up analysis of the pilot-scale IAAB is recommended in terms of the volume of bioreactor. A commercial plant can be installed in one of the palm oil mills in Malaysia to determine the long-term performance evaluation of the system on the constant fluctuation of the characteristics of POME during high- or low crop season as well as operating it at high loading rate. The data produced by the commercial plant will be another stepping stone for the palm oil millers to accept the approach of bioreactor system to replace the current POME treatment. This will act as a way in promoting palm oil industry as a "Green Industry" in the worldwide as well as reducing the carbon footprint of the treatment system.

2. Comparison can be made with the current treatment technology used by the existing palm oil mills. This piece of valuable data will be beneficial for the palm oil millers to consider adopting new treatment system for effective POME treatment system aiming to exhibit high treatment efficiencies of the new POME treatment technology. The comparison data produced will act as a reference for the cost benefit analysis to quantify its potential on a paradigm shift in managing wastewater treatment in a more beneficial and profitable manner.

3. The analysis was carried out in duplicate to obtain the average data. For duplicate values (e.g. x1, x2) the sample standard deviation is $sd = |x2 - x1| \sqrt{2}$. This statistic is able to give the sample range of the two values. However, with such manner resulted in a great deal of sampling variability. All the analysis will be subjected to mean deviation with only duplicate value is worth pursuing in future study. The formula of the mean deviation is as followed:

$$Mean\ Deviation = \sum |x - \mu| /N$$

CHAPTER SIX

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CHAPTER SEVEN

APPENDICES

Appendix A – Comparison of different industrial scale palm oil mill effluent (POME) anaerobic systems in degradation of organic contaminants and kinetic performance

Table A1: The characteristics of the final discharge effluent

Parameter	**Palm Oil Mill A** Average ± standard deviation (SD)	**Palm Oil Mill B** Average ± SD	**Palm Oil Mill C** Average ± SD	**Regulatory Discharge** (Loh et al., 2017)
Temperature (°C)	30.32 ± 0.18	27.16 ± 0.26	24.67 ± 0.12	-
pH	8.41 ± 0.003	8.77 ± 0.12	8.78 ± 0.024	5-9
OLR_a ($gCOD/m^3.day$)	2.03 ± 0.006	2.15 ± 0.003	1.98 ± 0.001	-
BOD (mg/L)	936 ± 7.12	103 ± 0.63	69.32 ± 4.03	20
COD (mg/L)	1,767 ± 27	848 ± 14	874 ± 23	-
BOD:COD	0.53 ± 0.009	0.12 ± 0.002	0.08 ± 0.006	-
TSS (mg/L)	6,667 ± 1,018	3,722 ± 1,314	108–± 20	400
VSS(mg/L)	4,111 ± 294	2,444 ± 111	83–± 23	-
Lignin (mg/L)	153 ± 16	57 ± 1	47.17 ± 1	-

OLR_a: Organic loading rate for aerobically-treated effluent

Appendix B – Synergistic effect of Anaerobic Digestion of Palm Oil Mill Effluent (POME) with *Moringa Oleifera* Extract

Table B1: C/N ratio (weekly basis) of each of the POME anaerobic digestion treatment

Week	T1	T2	T3	T4
1	25 ± 1.2	18 ± 0.5	18 ± 2.3	18 ± 0.8
2	24 ± 0.8	19 ± 1.2	20 ± 0.8	19 ± 0.5
3	24 ± 1.1	19 ± 0.8	20 ± 0.9	19 ± 0.6
4	25 ± 0.9	18 ± 1.2	19 ± 1.3	18 ± 0.6

Table B2: Identified methanogen in different types of palm oil mill effluent (POME) samples

Sample	Primer F/R	Accession No.	E value	% identity	Name of organism	Source Reference
POME	F	LT626036.1	9e-140	98%	*Methanoculleus* spp.	Anaerobic digestion, Belgium
G[1]	F	JF754559.2	1e- 90	91%	*Methanolinea* spp.	Petroleum reservoir production water, Shanghai, China
E[1]	F	LT626036.1	9e-135	98%	*Methanoculleus* spp.	Anaerobic digestion, Belgium

[1]G denotes granular sludge; E denotes anaerobically-treated POME

Table B3: Identity of Methanogen for respective treatment

Name of organism	Accession no.	Treatment on Day 24	Treatment mode	Treatment condition
Uncultured *Methanobrevibacter* spp.	AY454735.1	T1	Mono-digestion[c]	Raw POME + anaerobic sludge
Uncultured *Methanosaeta* spp.	HQ392680.1	T2		*M. oleifera* extract + anaerobic sludge
Uncultured mixture of *Methanobrevibacter* spp. and *Methanosaeta* spp.	AY454734.1 HQ392680.1	T3	Co-digestion	Raw POME + anaerobic sludge+ *M. oleifera* extract (daily feed)
Uncultured mixture of *Methanobrevibacter* spp. and *Methanosaeta* spp.	AY454734.1 HQ392680.1	T4		Raw POME + anaerobic sludge+ *M. oleifera* extract (one-time feed – day 1)

[c]: Digestion with palm oil mill effluent (POME) or *M. oleifera* extract alone as specified as control.

[1] G denotes granular sludge; E denotes anaerobically-treated POME

Table C1: General operating conditions of the pilot-plant IAAB during the start-up period

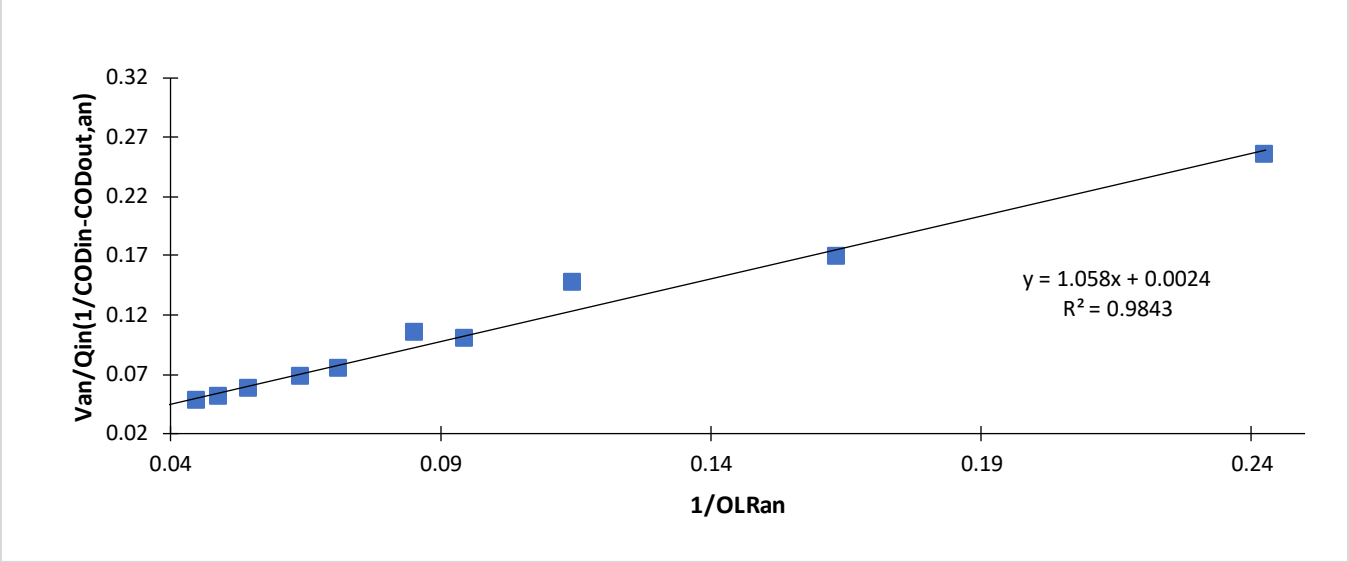
Process	Parameter	Unit	Average	Range
Anaerobic	Temperature	°C	-	30.0-33.0
	V_{an}	L	1,000	1,000
	OLR_{an}	gCOD/L.day	-	1.0-8.0
	HRT_{an}	Day	2.92	2.54-3.32
	Q_{in}	L/day	349.34	303-402
	$MLSS_{an}$	mg/L	52,915	40,647-62,294
	pH	-	-	7.04-7.62
	Q_r	L/day	5,449	4,775-6,091
	R_{RAS}	-	16	15-16
Aerobic	Temperature	°C	-	30.0-33.0
	V_a	L	600	600
	OLR_a	gCOD/L.day	0.78	-
	HRT_a	Day	1.34	0.27-0.90
	$MLSS_a$	mg/L	19,718	14,475-28,660
	pH	-	8.28	7.86-8.72
	DO	mg/L	-	1.95-3.10
	Q_{RAS}	L/day	131.36	90.00-194.20
	R_{RAS}	-	0.43	0.29-0.60

Table C2: The performance of the pilot-plant IAAB during the start-up period

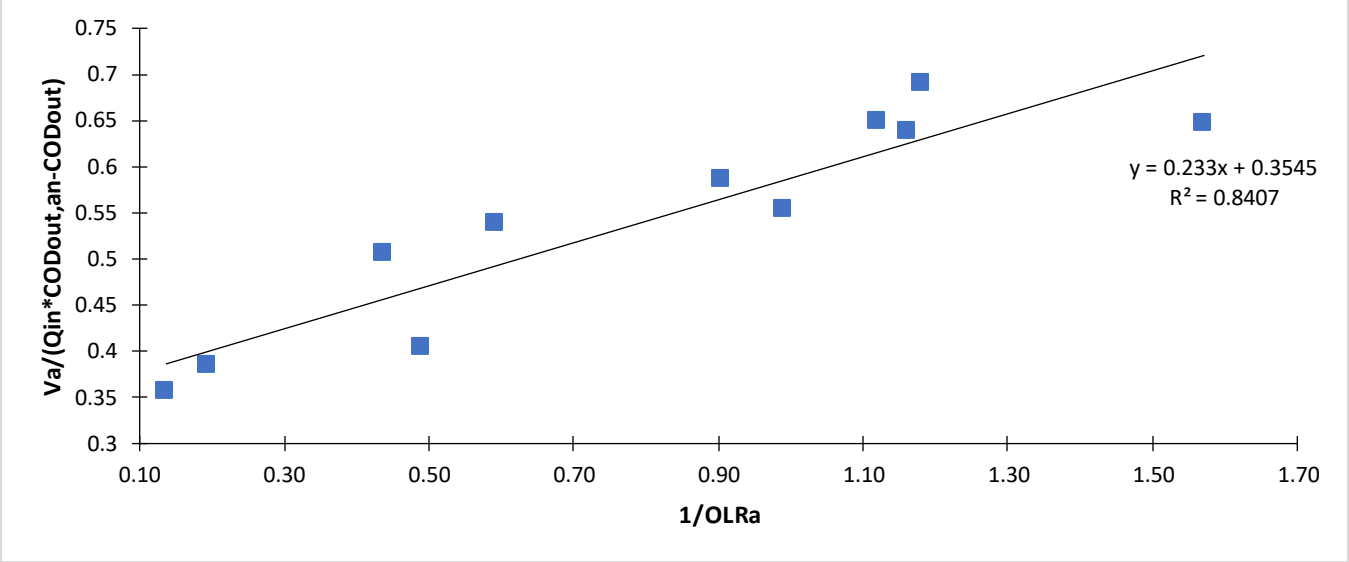
Parameters	Treated effluent	Average Overall Removal Efficiency (%)	Average Anaerobic Removal Efficiency (%)	Average Aerobic Removal Efficiency (%)	DOE Standards
Effluent BOD	25 ±4.27	99.80 ±0.04	93.68 ± 1.43	96.86 ±0.91	100
Effluent COD	357 ±64	93.04 ± 3.28	93.64 ± 2.17	93.26 ± 6.50	-
Effluent TSS	76 ± 16	99.24 ± 0.28	92.76 ±2.25	95.33 ±3.45	400
Effluent pH	8.25 ±0.17	-	-	-	5.0-9.0

Table C3: General operating conditions of the pilot-plant IAAB at various OLRs

Process	Parameter	Unit	Average	Range
Anaerobic	Temperature	°C	-	30.0-33.0
	V_{an}	L	1,000	1,000
	OLR_{an}	gCOD/L.day	-	10.50-32.50
	HRT_{an}	Day	-	2.15-4.12
	Q_{in}	L/day	-	243-467
	$MLSS_{an}$	mg/L		26,620-53,061
	pH	-	-	6.71-7.45
	Q_r	L/day		4,845-6,221
	R_{RAS}	-		12-26
Aerobic	Temperature	°C	-	30.0-33.0
	V_a	L	600	600
	OLR_a	gCOD/L.day		0.64-8.66
	HRT_a	Day		1.29-2.47
	$MLSS_a$	mg/L		12,353-40,550
	pH	-		8.38-8.98
	DO	mg/L	-	1.50-3.10
	Q_{RAS}	L/day		145-1,241
	R_{RAS}	-		0.12-4.66
	Q_{WAS}	L/day		162-740
	R_{WAS}	-		0.52-1.97

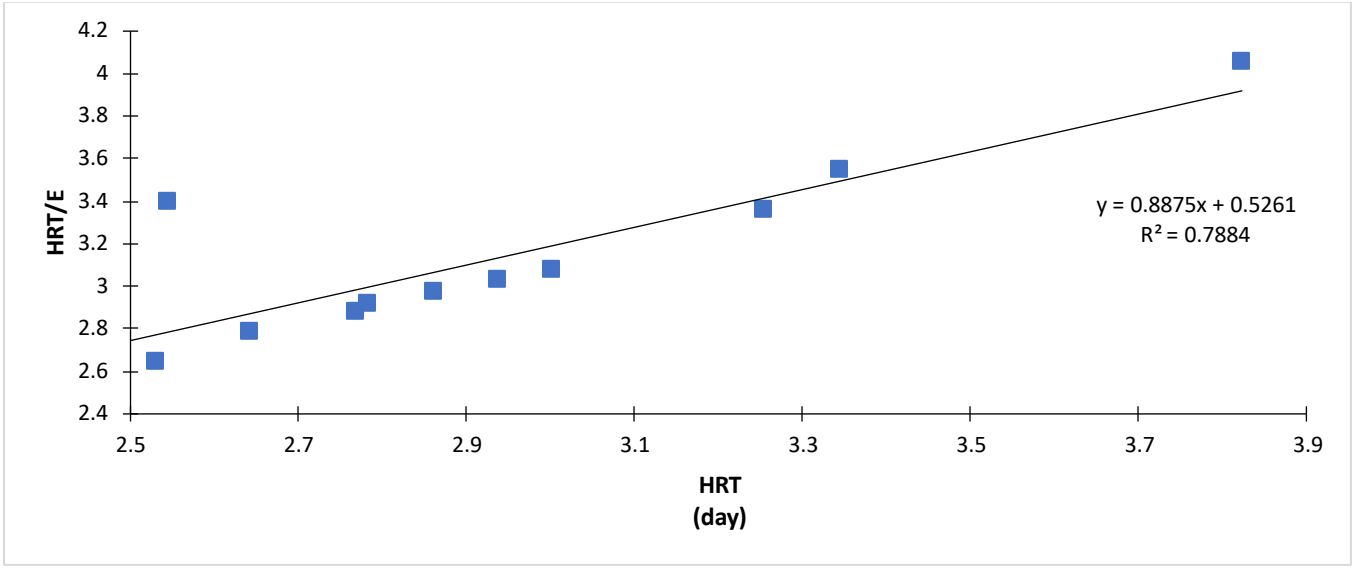


(a)

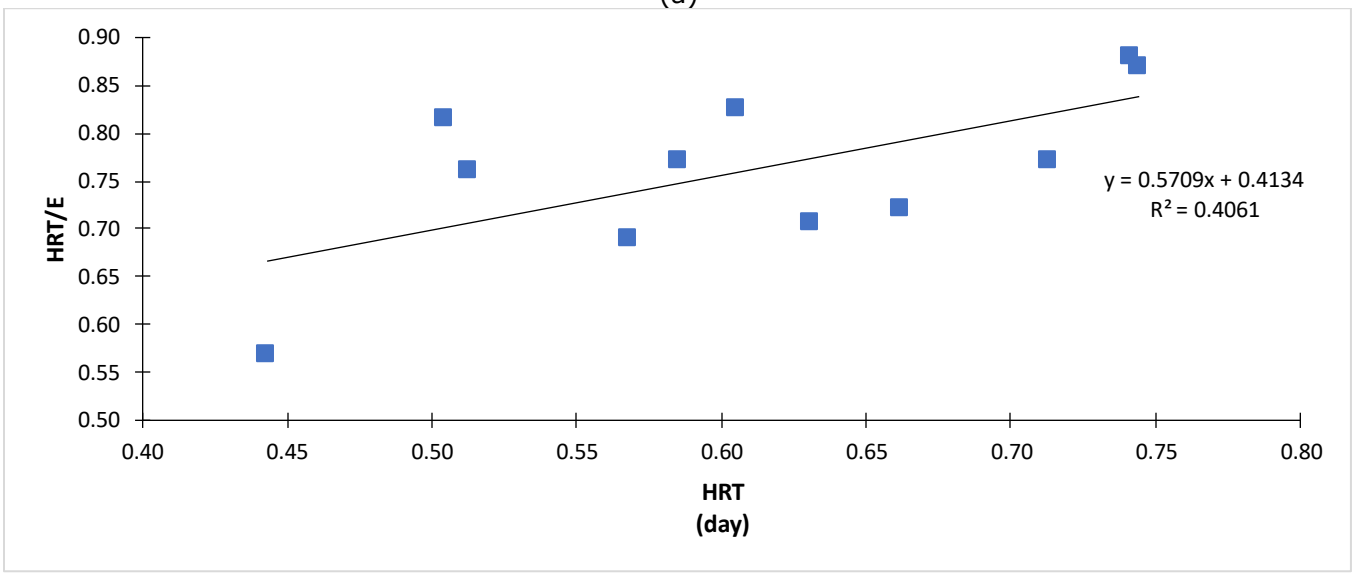


(b)

Figure C4: Linearised plots of modified Stover-Kincannon models in (a) Anaerobic Digestion; (b) Aerobic Digestion of the pilot-plant IAAB

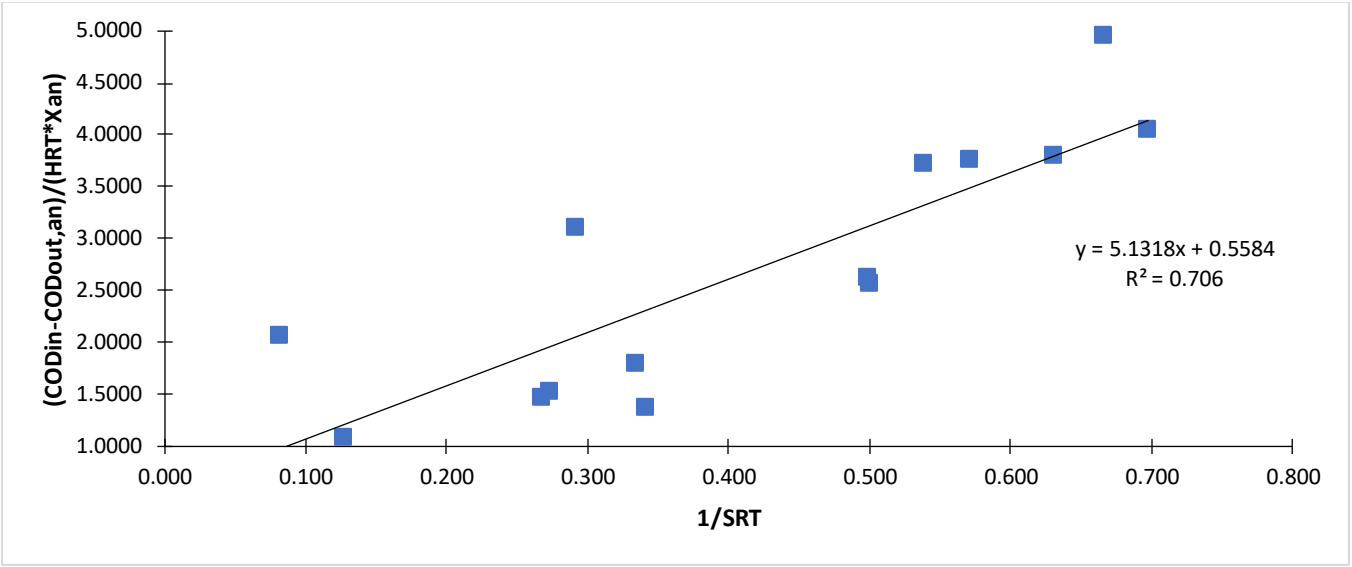


(a)

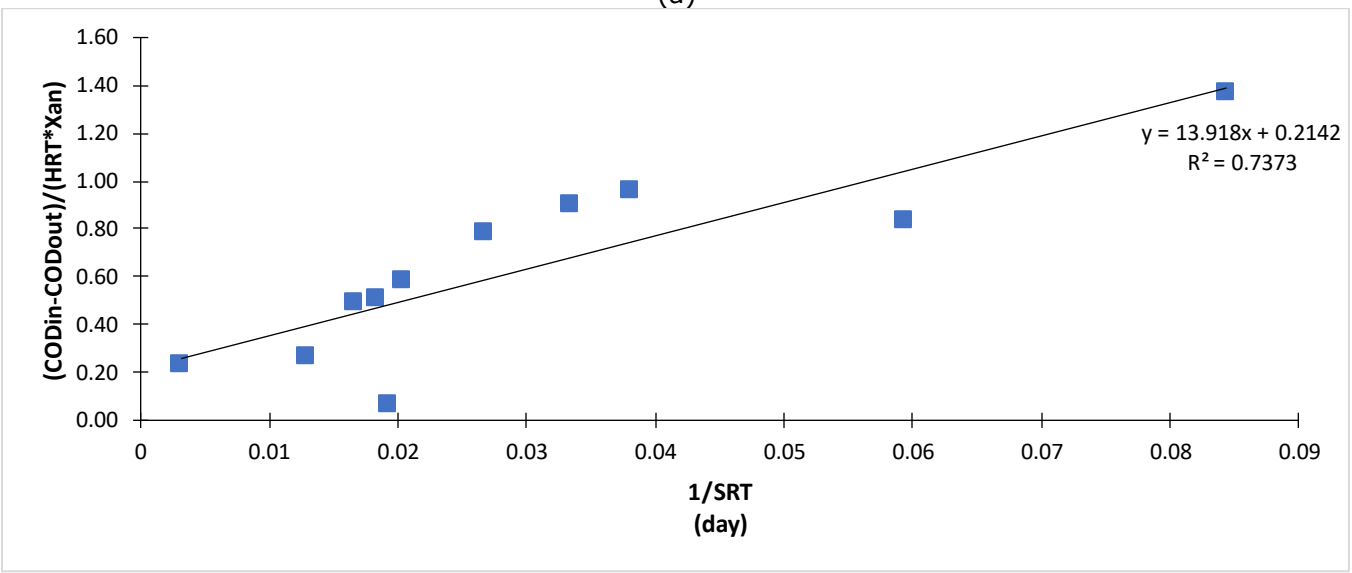


(b)

Figure C5: Linearised plots of Grau second-order multicomponent substrate removal models in (a) Anaerobic Digestion (b) Aerobic Digestion of the pilot-plant IAAB

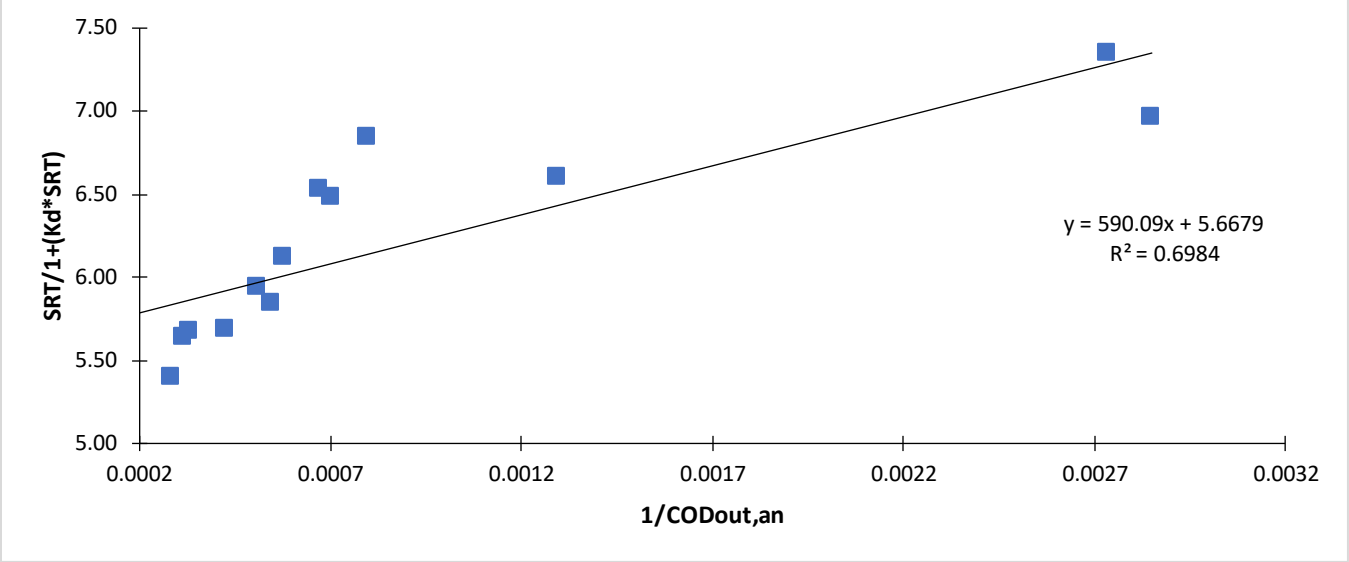


(a)

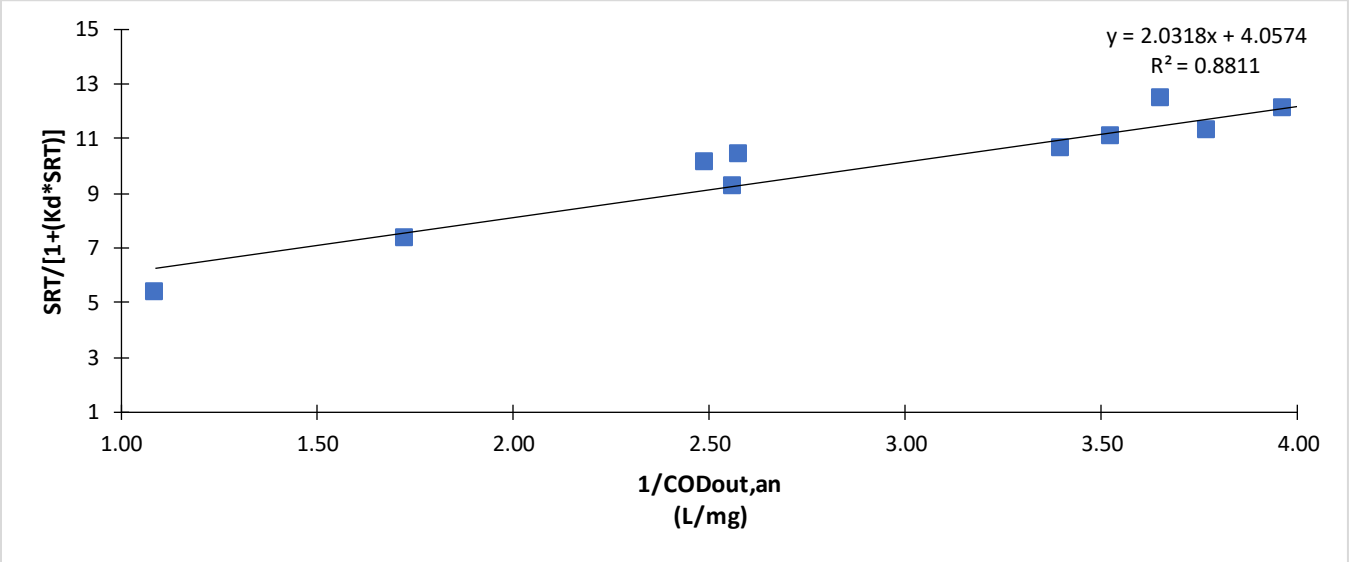


(b)

Figure C6: Linearised plots of Monod model for the determination of Y and k_d in (a) anaerobic digestion (b) aerobic digestion of the pilot-plant IAAB



(a)



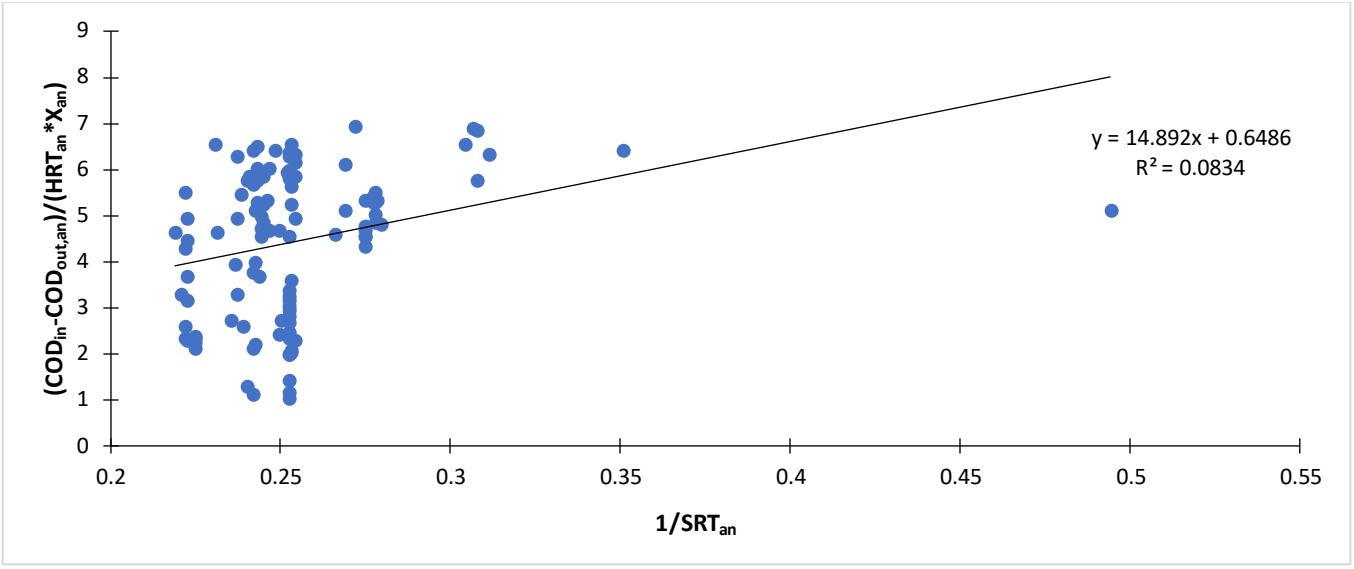
(b)

Figure C7: Linearised plots of Monod model for the determination of μ_{max} and K_s in (a) anaerobic digestion (b) aerobic digestion of the pilot-plant IAAB

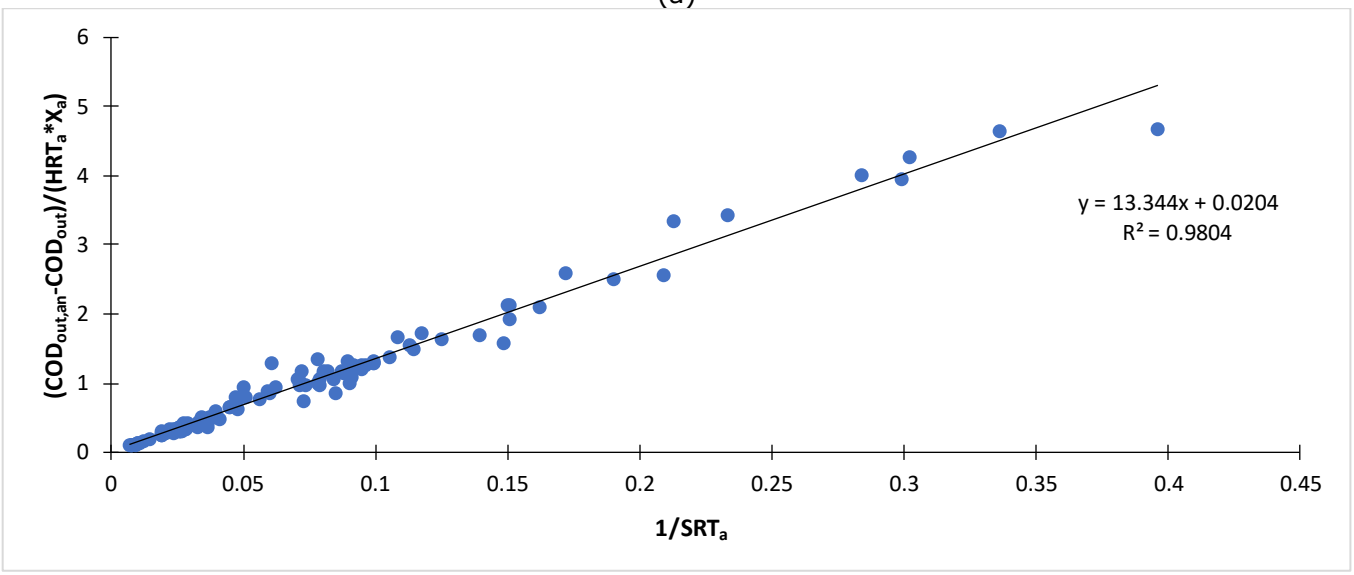
Appendix D – Mono-Digestion of Palm Oil Mill Effluent (POME)

Table D1: Operating conditions of the pilot-plant IAAB during the mono-digestion study

Process	Parameter	Unit	Range
Anaerobic	Temperature	°C	30.0 - 33.0
	V_{an}	m^3	1,000
	OLR_{an}	gCOD/L.day	30.0
	pH	-	6.51 - 7.28
	Q_{in}	L/day	319 - 445
	R_r	-	2.30 – 3.77
	HRT_{an}	Day	2.30 - 3.13
	F/M_{an}	gCOD/gMLVSS.day	0.248 - 1.434
	$MLSS_{an}$	mg/L	20,152 - 142,572
	$MLVSS_{an}$	mg/L	18, 320 - 129,613
Aerobic	V_a	m^3	600
	OLR_a	gCOD/L.day	2.55 – 4.21
	pH	-	10.09 – 10.85
	HRT_a	Day	1.38 – 1.88
	F/M_a	gCOD/gMLVSS.day	0.130 – 0.207
	Dissolved Oxygen	mg/L	2.03 – 5.21
	$MLSS_a$	mg/L	27,450 – 29,350
	$MLVSS_a$	mg/L	20,580 – 23,621
	R_{RAS}	-	1.73 – 3.99
	R_{WAS}	-	3.43 – 4.50

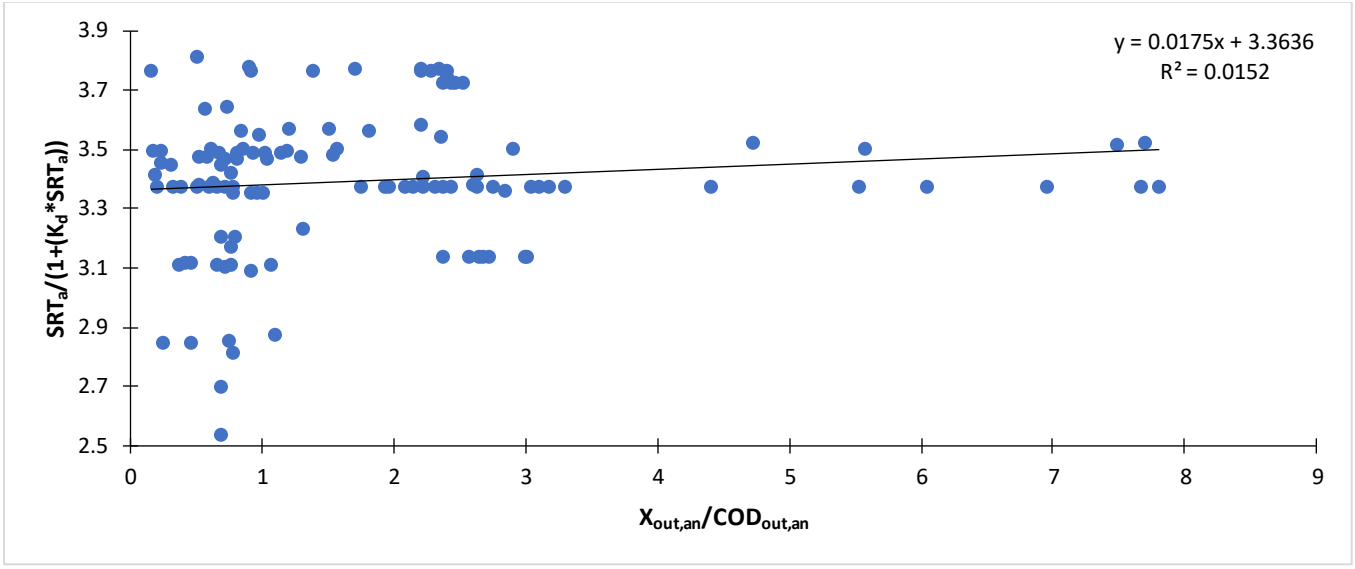


(a)

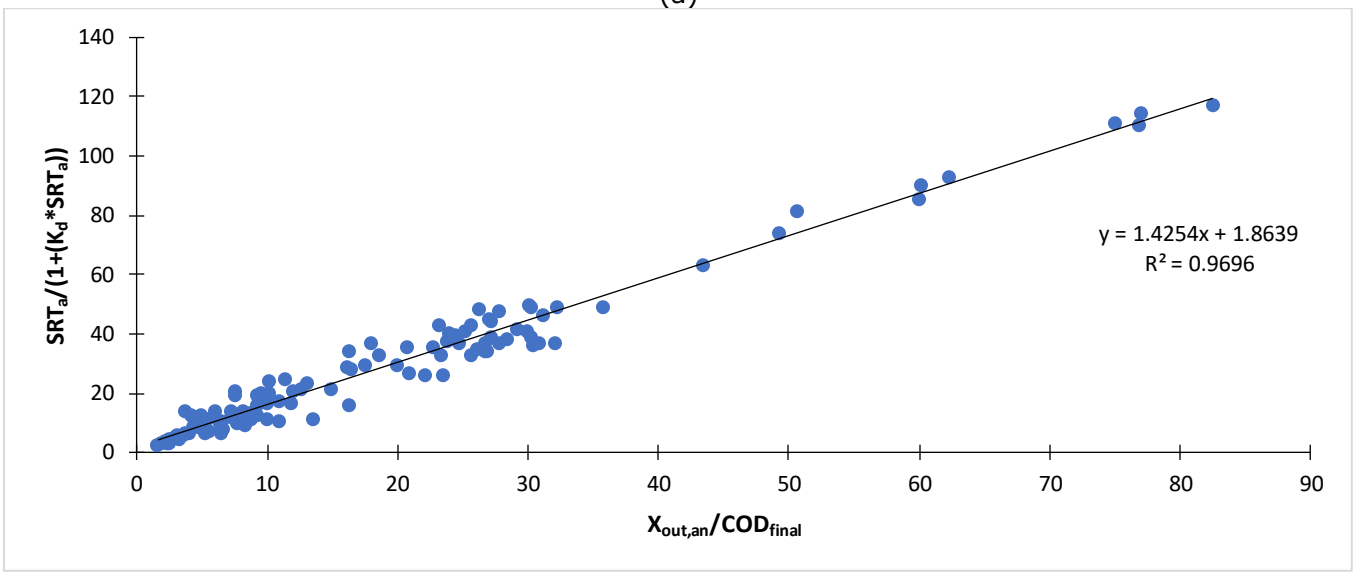


(b)

Figure D2: Linearised of Contois kinetic model for the determination of Y and k_d in (a) anaerobic process (b) aerobic process during mono-digestion mode of the pilot-plant IAAB

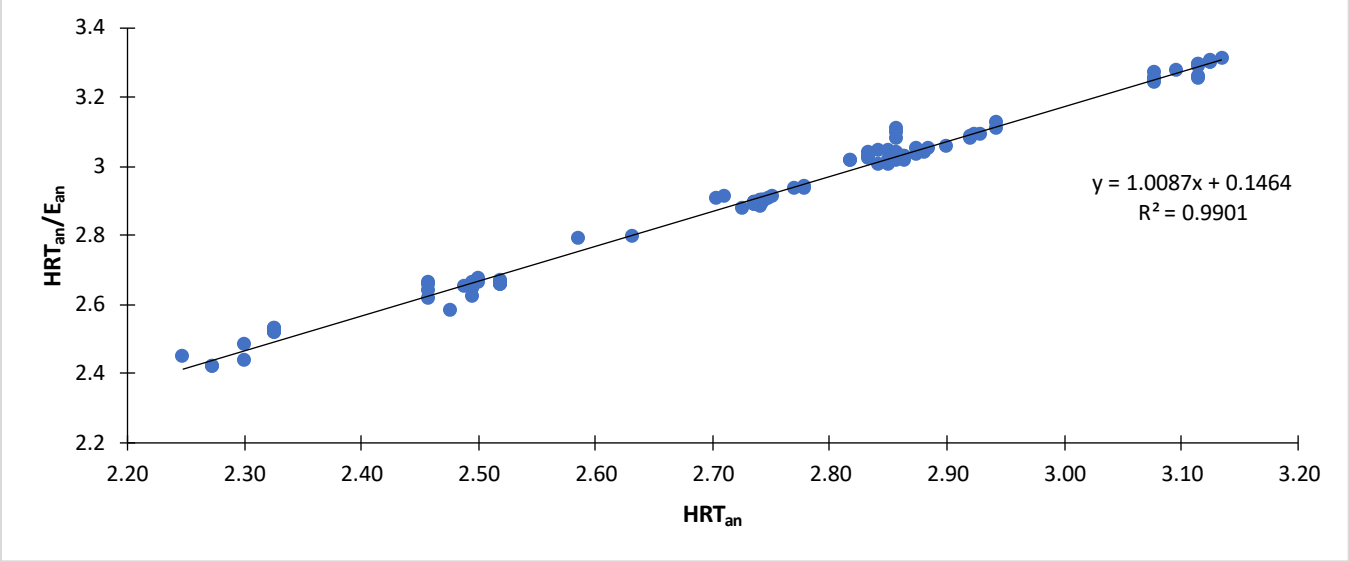


(a)

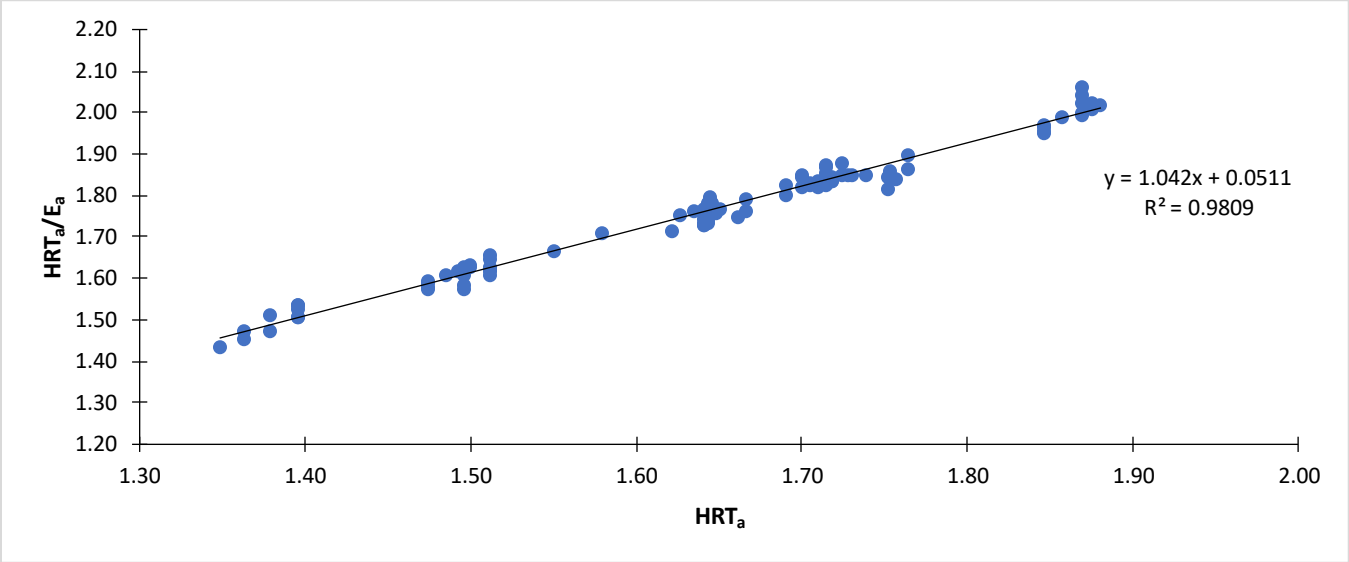


(b)

Figure D3: Linearised plots of Contois kinetic model for the determination of maximum specific growth rate (μ_{max}) and growth parameter (β) in (a) anaerobic Process (b) aerobic Process

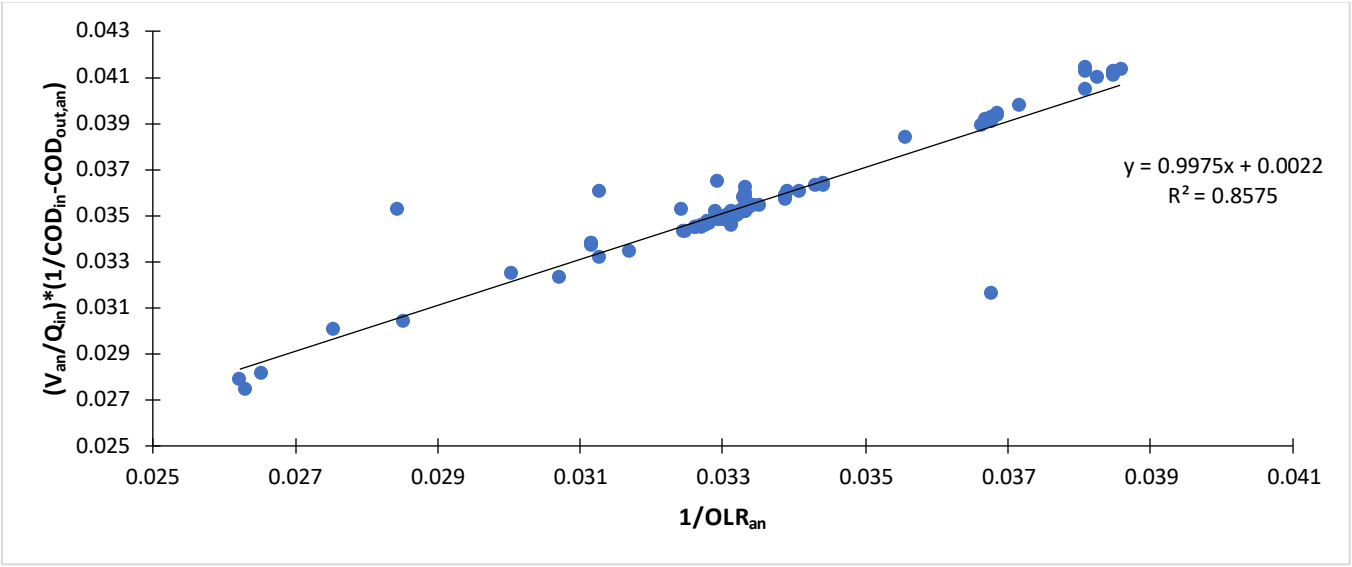


(a)

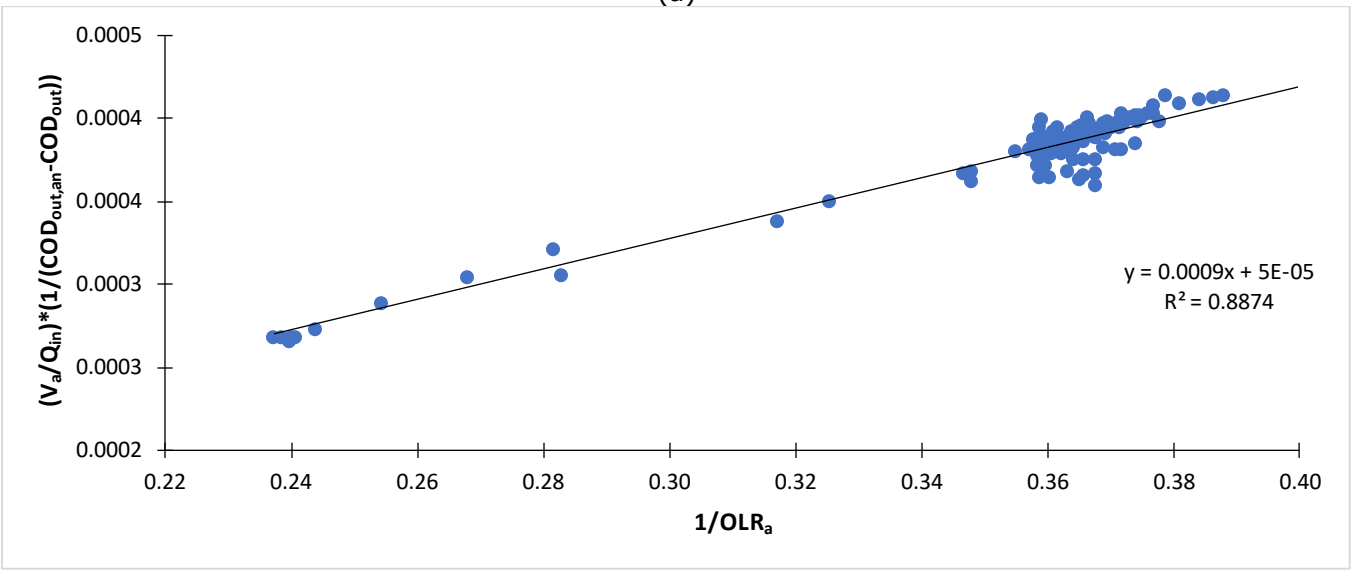


(b)

Figure D4: The linearised plot of Grau Second-Order model for the determination of kinetic coefficients (a and b) in (a) anaerobic process (b) aerobic process



(a)



(b)

Figure D5: The linearised plot of Stover-Kincannon model for the determination of maximum substrate removal rate ($\cup_{max}$) and saturation value constant (K_B) in (a) anaerobic Process (b) aerobic Process

Appendix E – Co-Digestion of Palm Oil Mill Effluent (POME) with *Moringa Oleifera*

E1: Operating condition of the pilot-plant IAAB during the co-digestion study

Process	Parameter	Unit	Range
Anaerobic	Temperature	°C	30.3 - 37.3
	V_{an}	m^3	1,000
	OLR_{an}	gCOD/L.day	30.0
	pH	-	6.84 - 7.14
	Q_{in}	L/day	320 - 350
	HRT	Day	2.86 - 3.12
	$MLSS_{an}$	mg/L	56,000 - 67,000
	$MLVSS_{an}$	mg/L	53,000 - 64,000
Aerobic	V_a	m^3	600
	OLR_a	gCOD/L.day	2.55 - 3.81
	pH	-	10.03 - 10.39
	HRT	Day	1.71 - 1.87
	F/M_a	gCOD/gMLVSS.day	0.185 - 0.187
	Dissolved Oxygen	mg/L	1.98 - 2.21
	$MLSS_a$	mg/L	23,050 - 27,556
	$MLVSS_a$	mg/L	18,000 - 20,000
	R_{RAS}	-	1.05 - 2.75
	R_{WAS}	-	2.13 - 4.13