



Faculty of Engineering

**Optimization of Factors affecting Chemical Oxygen Demand Reduction
from Palm Oil Mill Effluent using Anaerobic Digestion**

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Optimization of Factors affecting Chemical Oxygen Demand Reduction from Palm Oil Mill Effluent using Anaerobic Digestion

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A thesis submitted

In fulfilment of the requirements for the degree of Master of Engineering
(Mechanical and Manufacturing Engineering)

Faculty of Engineering
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2023

DECLARATION

I hereby declare that this thesis is original write-up from my research. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. The thesis has not been accepted for any degree, and not concurrently submitted in candidature of any other degree.

.....

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ABSTRACT

This thesis was the outcome of a research conducted on chemical oxygen demand (COD) of palm oil mill effluent (POME) which focused on improving the performance of an anaerobic reactor. This study has addressed COD in POME as the problem to be solved and accordingly, this research was designed to reduce COD level in POME before POME it was discharged to the environment. The study aimed to optimize the factors that affect COD reduction from POME, and it was divided into three specific objectives: (1) determine the significant factors affecting COD reduction from POME, (2) determine the impact of manipulating variables on COD reduction from POME and (3) determine the optimum value of factors that contribute to COD reduction from POME. The novelty of this research was using C/N enriched inoculum from banana peel in a two-stage anaerobic reactor to determine its effects on COD reduction performance of POME. A two-stage fermentation process and 350 L lab-scale reactors were used to treat POME. Five input factors were used which include pH, C/N, organic loading rate (OLR), hydraulic retention time (HRT), and sludge retention time (SRT). The Design Expert software was used for data analysis. The research findings found that the significant factors were pH, C/N, and OLR with p-values of 0.0001, 0.0227, and 0.0001 respectively. HRT and SRT also contributed to COD reduction although their contributions were not significant ($p > 0.05$). The impact of these five factors was able to contribute up to 80% COD reduction and the lowest value of COD reduction contributed by these five factors is 57%. The findings in surface response diagrams revealed the optimum value of factors that affect COD reduction; 6.5 for pH, 26 for C/N, 8.5 kg/d.m³ for OLR, 7 days for HRT, 13 days for SRT, and 77% for COD reduction. The developed model was validated, and the results gave an average error within 6.72% and standard deviation error of 3.79%. In this study, banana peel which would normally be discarded upon consumption

was used as inoculum to adjust the C/N to prepare substrate with POME. The findings demonstrated that the addition of banana peel achieved COD reduction up to 77%. Thus, this study justified that further research on POME should be done using different types of substrate and other contributing factors to achieve higher COD reduction from POME.

Keywords: Optimization, chemical oxygen demand reduction, palm oil mill effluent, anaerobic reactor, anaerobic digestion

Pengoptimuman Faktor yang mempengaruhi Pengurangan Permintaan Oksigen Kimia dari Efluen Kilang Kelapa Sawit menggunakan Pencernaan Anaerobik

ABSTRAK

Tesis ini adalah hasil kajian yang dilakukan terhadap permintaan oksigen kimia (COD) efluen kilang kelapa sawit (POME) yang memfokuskan pada peningkatan prestasi reaktor anaerobik. Kajian ini telah menangani COD di POME sebagai masalah yang harus diselesaikan dan dengan demikian, penyelidikan ini dirancang untuk mengurangkan tahap COD di POME sebelum POME dilepaskan oleh kilang. Kajian ini bertujuan untuk mengoptimumkan faktor-faktor yang mempengaruhi pengurangan COD dari POME dan ia dibahagikan kepada tiga objektif khusus: (1) menentukan faktor-faktor penting yang mempengaruhi pengurangan COD dari POME, (2) menentukan kesan manipulasi pemboleh ubah terhadap pengurangan COD dari POME dan (3) tentukan nilai optimum faktor yang menyumbang kepada pengurangan COD dari POME. Keberkesanan dalam penyelidikan ini adalah menggunakan inokulum diperkaya C/N dari kulit pisang dalam reaktor anaerob dua peringkat untuk menentukan kesannya terhadap prestasi penurunan COD POME. Proses penapaian dua peringkat dan reaktor skala makmal 350 L digunakan untuk merawat POME. Lima faktor input digunakan termasuk pH, C/N, laju pemuatan organik (OLR), waktu pengekal hidraulik (HRT), dan waktu penahan lumpur (SRT). Reka bentuk perisian eksperimen (DOE) digunakan untuk analisis data. Hasil kajian mendapati bahawa faktor yang signifikan adalah pH, C/N, dan OLR dengan nilai p masing-masing 0.0001, 0.0227, dan 0.0001. HRT dan SRT juga menyumbang kepada pengurangan COD walaupun sumbangannya tidak signifikan ($p > 0.05$). Kesan daripada lima faktor ini dapat menyumbang kepada pengurangan COD sehingga 80% dan nilai pengurangan COD terendah yang disumbangkan oleh lima faktor ini adalah 57%. Penemuan dalam

gambarajah tindak balas permukaan menunjukkan nilai optimum faktor yang mempengaruhi pengurangan COD; 6.5 untuk pH, 26 untuk C / N, 8.5 kg / d.m³ untuk OLR, 7 hari untuk HRT, 13 hari untuk SRT, dan 77% untuk pengurangan COD. Model yang dibangunkan disahkan dan hasilnya memberikan ralat rata-rata dalam 6.72% dan ralat sisihan piawai 3.79%. Dalam kajian ini, kulit pisang yang biasanya akan dibuang apabila dimakan digunakan sebagai inokulum untuk melaraskan C/N untuk menyediakan substrat dengan POME. Penemuan menunjukkan bahawa penambahan kulit pisang mencapai pengurangan COD sehingga 77%. Oleh itu, kajian ini membenarkan bahawa penyelidikan lebih lanjut mengenai POME harus dilakukan dengan menggunakan pelbagai jenis substrat dan faktor penyumbang lain untuk mencapai pengurangan COD yang lebih tinggi dari POME.

Kata kunci: *Pengoptimuman, pengurangan permintaan oksigen kimia, efluen kilang minyak sawit, reactor anaerob, pencernaan anaerobik*

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LIST OF ABBREVIATIONS

BOD	Biochemical Oxygen Demand
C/N	Carbon-to-Nitrogen Ratio
CCD	Central Composite Design
CPO	Crude Palm Oil
COD	Chemical Oxygen Demand
CV	Coefficient of Variation
DOE	Design of Experiment
GHG	Greenhouse Gas
HRT	Hydraulic Retention Time
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
OLR	Organic Loading Rate
pH	Potential of Hydrogen
POME	Palm Oil Mill Effluent
RSM	Response Surface Methodology
SRT	Sludge Retention Time
TS	Total Solid
TSS	Total Suspended Solid
VFA	Volatile Fatty Acid
VIF	Variance Inflating Factor
VS	Volatile Solid
VSS	Volatile Suspended Solid
WTE	Waste-to-Energy

LIST OF NOMENCLATURE

Variable	Description	Unit
t	Time	Day or hour or second
V	Volume	m ³ or L
Q	Flow rate	m ³ /hr
X _i	Mixed liquor suspended solids	mg/L
Q _x	Excess biosolids removal rate	m ³ /d
X _x	MLSS in the excess biosolids flow	mg/L
W	Volatile solid weight	kg
C	Organic carbon content	g/kg VS
N	Nitrogen content	g/kg VS
COD	Chemical oxygen demand	kg COD/m ³

CHAPTER 1

INTRODUCTION

1.1 Study Background

This thesis is an outcome of a research conducted to reduce chemical oxygen demand (COD) of palm oil mill effluent (POME). This work is experimental research which was conducted at operations research laboratory of Universiti Malaysia Sarawak (UNIMAS) by collecting raw POME from Bau palm oil mill. Two state anaerobic digester was operated with banana peel inoculum to decompose COD of POME. Optimization model was used for conducting research in reducing COD from POME.

The oil palm industry is a major agro-industry in Malaysia and is developing as one of the country's important industries. Malaysia is behind Indonesia in terms of the largest producer of this vegetable-based oil. Table 1.1 shows the production of crude palm oil (CPO) in the year 2018 and 2019 (MPOB, 2018, 2019).

Table 1.1: CPO Production in Malaysia for 2018 and 2019 (MPOB, 2018, 2019)

Months	2018 ('000 tonnes)	2019 ('000 tonnes)
January	1,586	1,737
February	1,342	1,544
March	1,574	1,672
April	1,558	1,649
May	1,525	1,671
June	1,332	1,510
July	1,503	1,740
August	1,620	1,821
September	1,853	1,842
October	1,964	1,795
November	1,845	1,538
December	1,808	1,333

It is reported that the plantation of oil palm amounted to 5.90 million hectares of land in 2019, a rise of 0.9% from 5.85 million hectares reported in 2018 (MPOB, 2018, 2019). 26.9% of the total Malaysian oil palm planted area is in Sarawak, while Sabah and Peninsular Malaysia accounted for 26.2% and 46.9% respectively.

In the extraction of CPO and other products, a hazardous effluent is produced known to be POME. The properties of POME are listed in Table 1.2.

Table 1.2: Properties of POME (Zainal et al., 2017)

Parameter	Average value concentration
Chemical oxygen demand (mg/L)	51000
Biochemical oxygen demand (mg/L)	25000
pH	4.2
Total suspended solids (mg/L)	18000
Total volatile solids (mg/L)	34000
Oil and grease (mg/L)	4000–6000
Ammoniacal nitrogen (mg/L)	35
Total nitrogen (mg/L)	750

Untreated POME is acidic in nature and contains an oily residue. The presence of oxygen is required to breakdown the oily mixture which is known as biochemical oxygen demand (BOD). Occasionally, the BOD of untreated POME is hundredfold of that of municipal wastewater (Madaki & Seng, 2013). Raw POME is made up of 95–96% water, less than 5% of total solids, and oil and grease. The production of 1 tonne of CPO produces about 1.0–2.5 m³ of POME (Ahmad et al., 2015; Jefferson et al., 2016). The temperature of POME when it enters the cooling pond is about 80–90°C. Although POME is a non-toxic effluent, it has an unpleasant odour which can cause nuisance to the neighbourhood of the mills (Chan & Chong, 2019).

Various anaerobic technologies are being used by palm oil mills to treat POME. The common types of anaerobic bioreactors used in treating POME include anaerobic fluidized bed-reactor (AFBR), anaerobic sequencing batch reactor (ASBR), continuous stirred tank reactor (CSTR), expanded granular sludge bed (EGSB), up-flow anaerobic filtration (ABF), up-flow anaerobic sludge blanket (UASB), and up-flow anaerobic sludge fixed film (UASFF) reactors (Poh & Chong, 2009; Ahmad et al., 2015). Due to the advancement in bioprocess engineering, various strategies had been undertaken to improve yields and efficiencies of bioreactors. This has led to the development of new types of reactors such as advanced anaerobic expanded granular sludge bed (AnaEG), anaerobic membrane reactor (AnMBR) and up-flow anaerobic sludge blanket-hollow centred packed beds (UASB-HCPB) (Choong et al., 2018; Aziz et al., 2019). According to Aziz et al. (2019), AnaEG, AnMBR and UASB-HCPB are still in the early stages and further research is required on their operational conditions and effectiveness in treating POME.

Information published on POME related to COD indicates that it is one of the top listed pollutants responsible to damaging water resources and greenhouse gas emission. It was also reported that the performance of anaerobic reactor with higher concentration of carbon-to-nitrogen (C/N) would be significantly higher compared to the traditional COD reduction process (Nurliyana et al., 2015; Raman et al., 2019). On this background, the COD of POME get priority to be reduced before it discharges to water bodies and environment. However, the fundamental theme of this study was to increase the performance of COD reduction from POME by the aid of a two-stage CSTR and C/N enriched inoculum.

1.2 Problem Statement

It is important to reduce the concentration of COD, BOD, and other pollutants in POME before it is discharged. At present, the current practices of POME treatment are of using waste stabilization pond (WSP) and anaerobic reactor. However, numerous studies have been conducted on POME treatment revealed that the performance of COD and BOD reduction remained insignificant (Nurul Adela et al., 2014; Shahidul et al., 2018). The reason for the poor performance is caused by the inadequate use of input factors such as pH, C/N, organic loading rate (OLR), hydraulic retention time (HRT), and sludge retention time (SRT) in POME digestion process (Kim et al., 2003; Mamimin et al., 2015; Ohimain & Izah, 2017; Shahidul et al., 2018). Ohimian and Izah (2017) stated that pH plays a vital role in maintaining the effectiveness of microorganisms during the digestion process of POME. Nurliyana et al. (2015) stated that C/N is an important element which provide food from carbon and enzyme from nitrogen for microbial growth. Mao et al. (2015) stated that an adequate OLR provides sufficient organic materials (food) for microorganisms to feed to reduce COD from POME. An adequate HRT increases the contact time between microorganisms and organic materials for better reduction of COD (Alrawi et al., 2011). An adequate SRT allows the microbial population to adapt to the environment inside the anaerobic reactor for the digestion of POME (Dvořák et al., 2016).

The pollutants of POME namely BOD, COD, TSS and biomass content are responsible for water, air, and soil contamination. However, COD is the highest-level pollutant compared to the others. When untreated POME is discharged directly to the environment, the high level of COD in POME contributes to the formation of greenhouse gas (GHG), damaging water quality by consuming dissolved oxygen from water, and polluting soil by absorbing nutrients. This causes the destruction of aquatic life, natural

ecosystem and increase biodiversity loss (Chan & Choong, 2019). POME is known as a highest level of toxic effluent compared to the other effluent (Ahmed, 2009). Due to this reason, COD in POME is identified as a problem to be solved. And accordingly, this research is designed to reduce COD level in POME to achieve a standard discharge limit by the Department of Environment, Malaysia which is below 50 mg/L.

For POME treatment, many types of inoculums are being used for reducing COD by increasing C/N ratio. However, research on the use of waste banana peel in treating POME is not published extensively. It was reported that the C/N ratio of banana peel is about 35 (Choi et al., 2013; Odedina et al., 2017; Sial et al., 2019). The use of banana peel waste as an inoculum with high C/N ratio is possible to use with POME to prepare the substrate for anaerobic digestion. This inoculum from banana peel will be used to increase C/N ratio of POME substrate from 7 to about 30 (Nurul Adela et al., 2014) to achieve optimum level of COD reduction. Concerning this research gap, study on POME treatment for reducing COD with banana peel-based inoculum is not available in the published journals. This background, a research gap exists in the POME treatment in terms of using biomass waste of banana peel to increase C/N to achieve optimum level of COD reduction.

In this background, the novelty of this research of using C/N enriched inoculum from banana peel in a two-stage anaerobic reactor to determine its effects on COD reduction performance of POME. In this aspect, this study is novel and original.

1.3 Research Question

The research questions based on the above problem statement are as follows:

- a) What are the significant factors that affect COD reduction from POME?

- b) What is the impact of manipulating variables on COD reduction from POME?
- c) What is the optimum value of factors that contribute to COD reduction from POME?

This purpose of this research is to answer these questions.

1.4 Research Hypothesis

Null hypothesis (H_0): High C/N ratio of banana peel will contribute to achieve high level of COD reduction.

Alternate hypothesis (H_A): High C/N ratio of banana peel will not contribute to achieve high level of COD reduction.

1.5 Research Objective

The main aim of this study is optimization of COD reduction from POME and the objective is divided into the following specific sections to achieve the research goal:

- a) To determine the significant factors affecting COD reduction from POME using anaerobic digestion.
- b) To determine the impact of manipulating variables on COD reduction from POME; and
- c) To determine the optimum value of factors that contribute to COD reduction from POME.

1.6 Scope of Study

This research is limited to the following scope of work to achieve the research goal. The experiment was conducted by using anaerobic reactor. The digestion process was conducted at mesophilic temperature (about 30–35 °C).

The study started by gathering information through literature reviews related to the latest study in POME treatment. The experiment setup was conducted with a two-stage fermentation and an anaerobic reactor. To achieve research objective one, Design Expert (Version-2018) is used for data analysis. The limits of variables and experimental runs were determined by central composite design (CCD) and design of experiment (DOE). The experimental data was analysed by Design Expert to determine the significance level.

The experimental data collected from objective one was analysed to determine the impact of manipulating variables on COD reduction. The results were justified and compared to another previous research.

The experimental data collected from objectives one and two were analysed by Design Expert to determine the optimum values of inputs and outputs. The results were presented in 3D plots obtained from the software.

1.7 Organization of Thesis

The structure for this thesis is shown in Figure 1.1.

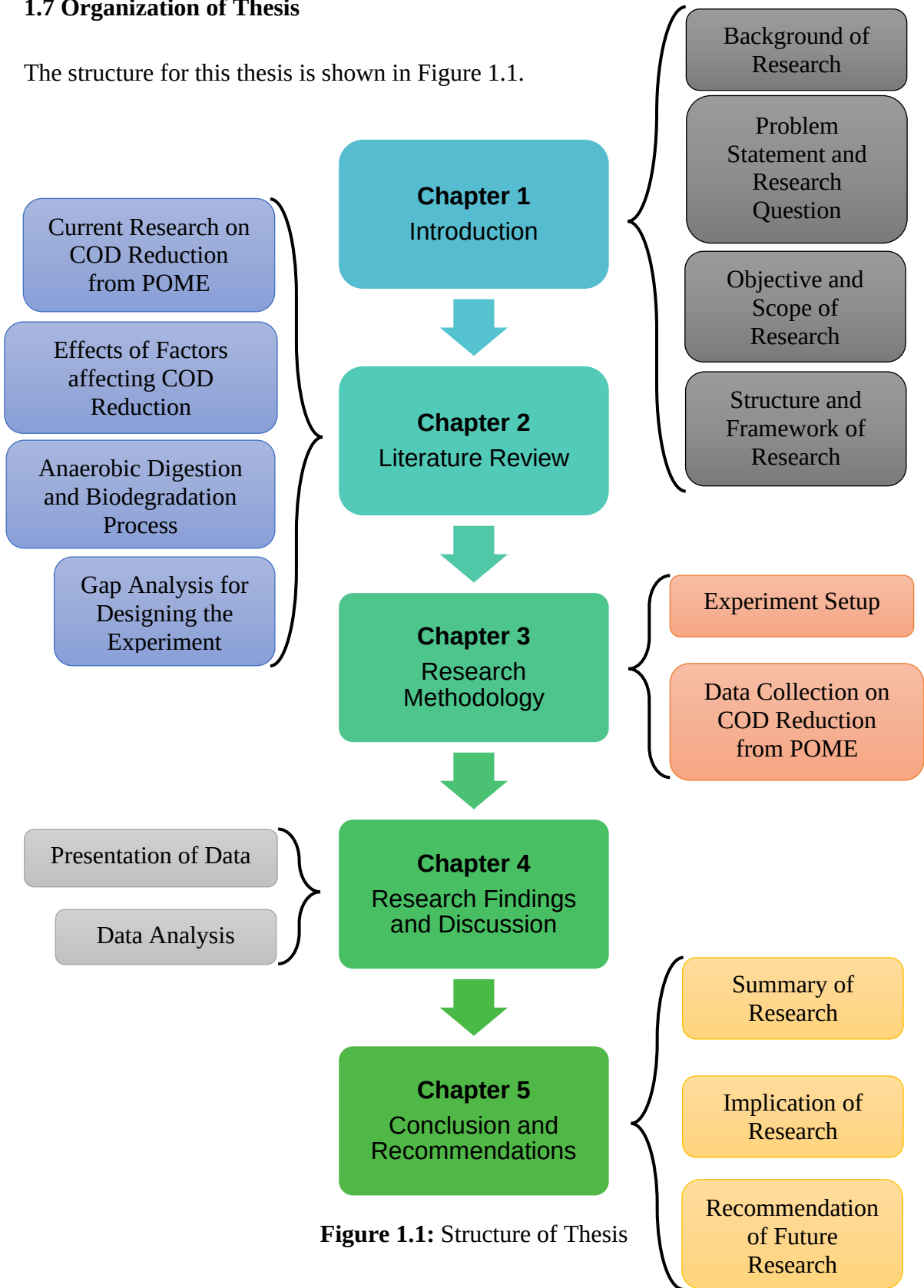


Figure 1.1: Structure of Thesis

To achieve the objectives of this study, the report is divided into five chapters. Chapter 1 provides a general introduction about the research covering background of study, problem statement, research question, research hypothesis, objective of research, scope of study and thesis structure. Chapter 2 includes literature review on current research in COD reduction from POME, factors affecting COD reduction, anaerobic digestion, and biodegradation processes. Chapter 3 presents the research methodology. This chapter provides the process and instrumentation of two-stage fermentation and, an anaerobic reactor. Chapter 4 comprises of experiments, data collection, data analysis and experimental results. Chapter 5 contains conclusion of study and recommendation for future research. At the end of the report, references and appendices are presented. Appendix A contains the list of papers published. Appendix B contains the experimental setup. Appendix C contains the result and data presentation.

CHAPTER 2

LITERATURE REVIEW

2.1 Chapter Overview

This chapter presented the review of literature related to the current research in COD reduction optimization from POME by the anaerobic digester. The specific objectives of the literature were to know the updated concept and models used in anaerobic digestion process for POME in COD reduction and its relevant process control parameters. The literature review was focused on source and procedure of POME generation, impacts of POME on environment and health, anaerobic digestion process, stages of anaerobic biodegradation, factors affecting anaerobic digestion performance and mechanics of optimization. This chapter was ended with findings of literature review, and research gap analysis based on literature review findings, and chapter summary.

2.2 Objectives of the Literature Review

This study attempted to review the literature on COD reduction optimization from POME by anaerobic digestion. Focused areas of this review were potential factors of anaerobic process that affected COD reduction at optimum level including pH, C/N, OLR, HRT, and SRT. Emphasis was given to reveal the operating condition of two-stage fermentation process and anaerobic reactor, inoculum preparation by using banana peel and stages involved in anaerobic digestion.

2.3 Strategic Focus on Studying Literature Review

This research focuses on the highly polluted palm oil mill effluent known as POME with aim to reduce COD for contributing to achieve sustainable development goal (SDG), and special attention was given to support SDG-6 (clean water production for all) and SDG-

13 (climate action). For achieving objectives of review, preliminary about 300 journals were studied on POME process, which were published between 2000 to 2019. About 100 papers were short-listed related to the objective of this research. The papers reviewed were published between the year 2010 to 2019. In addition to study journal articles, books, daily newspapers, and newsletters were also reviewed to update the latest research on POME process for COD reduction.

The review is narrowed down to peer reviewed journals or conferences. No description is imposed on the outlets' field to enable potential research findings from different fields. The search process was conducted in Google Scholar and Mendeley. The library is applied to provide a comprehensive and systematic methodology. Article titles and related keywords needed to include the following terms: pH, C/N, OLR, HRT, SRT, reduction of COD, and their combinations.

2.4 Source of Palm Oil Mill Effluent Generation

Palm oil is the source of POME. It is a polluted effluent generated during CPO production from sterilization of fresh fruit bunch (FFB) in combination with about 95% water (Kamyab et al., 2018). Clarification of extracted CPO contributed the highest to POME generation at 60% while sterilization of FFB contributed about 36%. The process of hydrocyclone contributed only 4% (Rupani & Singh, 2010). Various research has demonstrated that FFB process efficiency to produce CPO is a determinant of POME quality. Based on the POME's chemical and physical properties, POME treatment processing technique has also been selected by researchers (Gobi & Vadivelu, 2013).

2.5 Impacts of Palm Oil Mill Effluent on Environment, Health, and Economy

POME is a hazardous and toxic effluent (Kamarudin et al., 2015). POME alone can contribute to create different types of impacts on the quality of water, soil, and air. POME also affects economy and the quality of agricultural and fish products. The COD and BOD of POME are the main contaminants which are the proven pollutant affecting human health, biodiversity loss and water bodies (Kamarudin et al., 2015). COD is one of the main sources of methane (CH_4) and carbon dioxide (CO_2) emission, which are recognized as global warming potential agents. In that aspect, COD in POME must be eliminated before it is discharged into the environment (Tan & Lim, 2019).

2.6 Anaerobic Digestion of POME for COD Reduction

Anaerobic digestion was the most effective treatment method for POME (Choong et al., 2018). It was the most beneficial as it was cost effective and environmentally friendly. This process also reduced the quantity of sludge and converted COD to CH_4 (Khadaroo et al., 2019). In this process, anaerobic microorganisms digested the organic matter (pollutants) and thus, reduced both BOD and COD of POME. The complex polymers of POME were degraded by activities of hydrolytic, acetogenic, acidogenic, and methanogenic microorganisms. At the end of anaerobic digestion process, biogas was produced which consisted of 65% CH_4 , 35% CO_2 and some trace amounts of hydrogen sulphide (Zain & Mohamed, 2018).

Bio-methanation or methanogenesis was the formation of methane gas by microbes also known as microorganisms that decomposed biodegradable matter in an anoxic environment. There were several factors that were involved in the decomposition of palm oil biomass to CH_4 . These factors were described in the following sections.

2.6.1 Hydraulic Retention Time in POME Digestion

HRT is a measure of the average duration of time that a compound remained in a decomposing or biodegradation process. In a palm oil mill, HRT indicated the average time the POME resided in reactor during biogas production. The HRT could be measured with the following equation (Equation 2.1) (Poh & Chong, 2009):

$$HRT = \frac{\text{Volume of aeration tank}}{\text{Influent flow rate}} = \frac{V}{Q} \quad \text{Eq. 2.1}$$

HRT is retention time. It is usually stated in hours (h) or days (d). V is the volume of reactor or aeration tank (m^3) and Q is the flow rate of influent (m^3/h). In most bioreactor, the production of biogas decreased as the HRT increased. The properties of HRT could affect the production of other biogas such as bio-hydrogen. The HRT was closely related to the OLR and substrate concentration; thus, a good balance had to be achieved for good digester operation (Abdurahman, 2010).

2.6.2 Sludge Retention Time in POME Digestion

SRT is a key parameter which could affect biochemical and physical treatment of POME. It was a key factor in the success of up-flow anaerobic sludge bed (UASB) reactors, which determined the eventual performance of hydrolysis and methanogenesis in a UASB system. The SRT in the reactor was usually defined as the ratio of sludge mass to the discharge rate of sludge (d) (Poh & Chong, 2009) (Equation 2.2):

$$SRT = \frac{\text{Sludge mass in the digester}}{\text{Sludge discharge rate}} = \frac{X_i V_i}{Q_x X_x} \quad \text{Eq. 2.2}$$

Where, X_i is the mixed liquor suspended solids (MLSS) in each reactor (mg/L), V_i is the individual reactor volume (L), Q_x is the excess biosolids removal rate (m^3/d) and X_x is

the MLSS in the excess biosolids flow (mg/L). Methanogenesis at 25 °C started at SRT between 5 and 15 days while at 15 °C, it started at SRT between 30 and 50 days. The maximum performance was 51% at 25 °C; and at 15 °C it was 25% at 15. The maximum hydrolysis was 50% at SRT 75 days and 25 °C; and it was 24% at 15 °C (Halalsheh et al., 2005). The SRT and process temperature had important effects on the hydrolysis of proteins, carbohydrates, and lipids. The most substantial portion of the digestion of proteins, carbohydrates, and lipids occurred within the first 10 and 15 days at process temperatures of 35 °C and 25 °C, respectively (Mahmoud et al., 2004).

2.6.3 Carbon-to-Nitrogen in POME Digestion

C/N is known as the ratio of elemental carbon to the elemental nitrogen present in POME. The overall C/N could be altered when two different substrates were combined giving a new C/N. A high C/N would leave carbon still available and decreasing nitrogen caused microorganisms to starve and eventually die. However, too much nitrogen would result in leftovers at the end of digestion which would make the effluent becomes alkaline. The correct ratio of carbon-to-nitrogen would prevent the loss of methane content (Okonkwo et al., 2016). The C/N was calculated based on the chemical composition (Shanmugam & Horan, 2009). Several authors agreed on an optimal C/N of between 20–35 (Kayhanian, 1999; Habiba et al., 2009) for anaerobic digestion. In anaerobic digestion, the methanogenic bacteria required optimum C/N of 8–20 (Kossmann et al., 2011) and 20–30 (Yen & Brune, 2007) reflecting a moderate N concentration for sufficient metabolism. According to Khalid et al. (2011), a C/N between 22–25 seemed to be best for anaerobic digestion of fruit and vegetable waste, whereas the optimal C/N for anaerobic degradation of organic waste was 20–35. Nurul Adela et al. (2014) reported that the C/N of the POME was in the range of 10.08–11.44. The variation of the C/N values could affect the pH of a slurry. The

increased in carbon content would give rise to more carbon dioxide formation and lower pH value, while high value of nitrogen would enhance production of ammonia gas that could increase the pH to the detriment of the microorganisms (Dioha et al., 2013). The C/N could be determined by dividing the total organic carbon content by the total nitrogen content, according to the following equation (Equation 2.3) (Dioha et al., 2013):

$$\frac{C}{N} = \frac{W1 \times C1 + W2 \times C2}{W1 \times N1 + W2 \times N2} \quad \text{Eq. 2.3}$$

Where, W1 and W2 are the volatile solid (VS) weight (kg) in as single substrate in the mixture, C1 and C2 are the organic carbon content (g/kg VS) in each substrate and N1 and N2 are the nitrogen content (g/kg VS) in each substrate.

2.6.4 Organic Loading Rate in POME Digestion

The OLR in anaerobic digestion depended on the technology used in the palm oil industry. Anaerobic sludge blanket processes such as UASB, up-flow staged sludge bed (USSB), expanded granular sludge bed (EGSB), internal circulation (IC), and inverse anaerobic fluidized bed (IAFB) reactors could bear very high loading rates, up to 40 kg COD/m³.d (Chen et al., 2010). The higher OLR compared to the optimum level could cause operational problems such as flotation of sludge, formation of foams at the gas-liquid interface in the gas-liquid-solid (GLS) separator, and build-up of undigested ingredients. The efficiency of the treatment would be affected due to higher or lower level of OLR. OLR is the mass of organic material that is daily entered to the treatment system per unit volume of reactor. By changing the influent concentration and flow rate, values of OLR could be varied. Under the conditions of changing HRT and flow rate, OLR could be expressed in the following form (Equation 2.4):

$$OLR = \frac{Q \times COD}{V} \quad \text{Eq. 2.4}$$

Where, OLR is organic loading rate (kg COD/m³.d), Q is flow rate (m³/d), COD is chemical oxygen demand (kg COD/m³) and V is reactor volume (m³). OLR could be simplified into (Equation 2.5):

$$OLR = \frac{COD}{HRT} \quad \text{Eq. 2.5}$$

2.6.5 Chemical Oxygen Demand

Organic matters were usually quantified as BOD and COD while inorganic matters were mainly quantified as sulphate, chloride, ammonium, heavy metals, and others (Lee & Nikraz, 2014). COD was the amount of oxygen consumed by the organic compounds and inorganic matter which were oxidized in waste (Pujar et al., 2014). COD could be measured according to the APHA Standard Methods (Geerdink et al., 2017). COD test used a strong chemical oxidant in an acid solution (potassium dichromate in a 50% sulphuric acid solution) and heated to oxidize both organic and inorganic substances in wastewater sample to obtain CO₂ and H₂O. The most popular current testing method for COD involves using sealed and heated low range (3–150 ppm) or high-range (20–1500 ppm) prepared vials. It would change colour from orange to green based on the amount of oxidation and it could be read by using a laboratory calorimeter. The test data was available in few hours giving it a major advantage over the 5-day BOD test, as it provided faster water quality assessment and process control.

2.6.6 Biochemical Oxygen Demand

The BOD was the amount of oxygen consumed by the organic and inorganic compounds which were oxidized by biological-oxidation effect in a certain condition (Pujar

et al., 2014). BOD could be measured according to the APHA Standard Methods (Kumar et al., 2010). The BOD test was based on the principle that if sufficient oxygen was available, aerobic biological decomposition by microorganisms would continue until all waste was consumed. This test was known as BOD₅ because it measured the amount of dissolved oxygen at the beginning and end of a five-day period in which the sample was kept in a dark and incubated conditions. The change in concentration of dissolved oxygen over five days represents the demand for oxygen by the aerobic biological microorganism for respiration. Due to long period of time required to obtain the test data, the results provided historical data only. Therefore, the BOD test was not recommended for rapid water quality assessment or optimal process control.

Since BOD was mainly a biochemical parameter, it made the BOD:COD ratio a good indicator of the proportion of biochemically degradable organic matter to total organic matter (Kumar et al., 2010). Relatively, substrate COD strength had significant effect on the ultimate amount of biogas yield, as well as the methane content (Ghani & Idris, 2009). COD gave a precise estimation of the organic (degradable) material content of a given sample (Curry & Pillay, 2012). Therefore, higher values of COD were favourable for anaerobic digestion (Samuel et al., 2012). Table 2.1 summarizes the previous research done on HRT, SRT, C/N and OLR.

Table 2.1: Previous Research on HRT, SRT, C/N and OLR

Substrate	Control Parameters of Experiments (Manipulating Variables)	Type of reactor used and inoculum	Findings	References
POME TS: 11,900 mg/L sCOD: $\pm 8,000$ mg/L pH: 4.48	HRT: 5–10 d pH in first AFBR: 5–5.5 pH in second AFBR: 7 (adjusted by adding NaOH) Objective: To study the effectiveness of separating acidogenic and methanogenic processing on sCOD removal for acidogenic reactor and methane production for methanogenic reactor	Two-stage AFBR by feeding fresh POME intermittently eight times per day Material: Acrylic Natural zeolite as immobilization media Digested biodiesel waste (COD of 1,980 mg/L and pH of 7.53) as starter inoculum	HRT: 5–10	(Ramadhani et al., 2020)
Acidified POME COD: $63,600 \pm 6,500$ mg/L TSS: $16,280 \pm 3,340$ mg/L VSS: $12,990 \pm 3,440$ mg/L pH: 5.23 ± 0.18 Temperature: 36–37 °C	HRT: 1–10 d Operating temperature: 54 ± 1 °C pH maintained at 7.0–7.5 using 5 M KOH Reactor is filled with 2 L microflora and acclimatized for 1 day Stirring rate: 80 rpm Objective: To optimize HRT on thermophilic methane production performance and stability of ASBR	Lab-scale ASBR Inoculum: Anaerobic seed sludge from anaerobic pond, acclimatized and enriched with acidified POME and nutrients for 1 month Headspace of reactor was sparged with N ₂ gas after feeding	HRT: 1–10	(Abd Nasir et al., 2019)

Table 2.1 continued

POME COD: 100,000 mg/L TS: 65,000 mg/L VS: 54,000 mg/L SS: 35,000 mg/L	Operating temperature: 55 °C pH was adjusted to 6.8–7.2 with NaHCO₃ Stirring rate: 37.5 rpm HRT maintained at 6 days 34% of digested slurry was recycled back into digester Objective: To maintain short HRT and high degradation of POME by applying recycle sludge	Pilot scale CSTR with and without sludge recycling	HRT: 6	(Irvan et al., 2018)
POME pH: 5.1 ± 0.2 BOD: 3,500 ± 500 mg/L COD: 56,500 ± 300 mg/L TS: 32,000 ± 300 mg/L VS: 26,000 ± 400 mg/L VSS: 8,300 ± 200 mg/L	Operating temperature: 37 °C HRT: 10 d pH: 6.8 Stirring rate: 150 rpm Objective: The potential of dairy manure as inoculum to produce methane from POME with regulated pH and temperature	CSTR Material: Glass Inoculum: Dairy manure (COD of 28,585 ± 500 mg/L and pH of 7.2)	HRT: 10	(Krishnan et al., 2017)

Table 2.1 continued

POME pH: 4.42 ± 0.12 BOD: $33,000 \pm 2806$ COD: $62,000 \pm 7842$ TSS: $50,000 \pm 654$ VSS: $42,230 \pm 2806$	Operating temperature: 35 °C HRT: 4 d OLR: 1.5–5.0 kg-COD/m³/d Objective: Effect of CKD dosage on the process including VFA, alkalinity, COD removal, biogas production and sludge waste reduction	UASB CKD was used as an alkaline source to neutralize POME and as a degradable organic source	HRT: 4	(Ahmad, 2016)
POME pH: 3.9–4.6 COD: 41,000–99,000 TS: 28,000–64,700 VS: 23,200–53,900	HRT: 6 d SRT: 8–22 d Stirring rate: 250 rpm Operating temperature: 55 ± 1 °C Objective: To study the effect if recycle sludge on high degradation of POME to biogas by maintaining short retention time.	CSTR with and without sludge recycling	SRT: 8–22	(Irvan et al., 2017)

Table 2.1 continued

POME COD: $106,934 \pm 34,843$ C/N ratio: 17.93 ± 5.39 pH: 4.8 ± 0.02 Temperature: 70 ± 5.69 °C	POME samples were fixed at 100 ml while amount of EFB sample were adjusted to attain selected C/N ratio of 25, 30, 35, 40, 45, 50, and 55. Operating temperature: 27–30 °C Objective: To investigate the co-digestion of EFB and POME under facultative conditions for methane production	Polyethylene tank	C/N: 25–55	(Nurliyana et al., 2015)
Acidified POME pH: 5.08 ± 0.1 COD: $35,500 \pm 3,300$ TS: $31,000 \pm 3,600$ TSS: $24,100 \pm 4,200$ VSS: $19,000 \pm 5,000$	Operating temperature: 55 °C and 37 °C OLR: 2.4, 3.2, and 4.0 kg COD/m³/d HRT: 8 d Objective: To compare the performance and condition of anaerobic digestion between mesophilic and thermophilic conditions at three different OLR using acidified POME.	Anaerobic digester with water bath shaker of 80 rpm Inoculum collected from POME treatment pond with VSS of 22,300 mg/L and pH of 7.30.	OLR: 2.4–4.0	(Hamzah et al., 2020)

Table 2.1 continued

POME Temperature: 85 ± 10 °C COD: $92,730 \pm 15,470$ mg/L TS: $66,120 \pm 10,720$ pH: 4.56 ± 0.12	Operating temperature: 55 ± 5.0 °C HRT in reactor 1 (ASBR): 2 d Stirring rate in ASBR: 150 rpm pH in ASBR: 5.5 ± 0.2 OLR in ASBR: 27.5 kg COD/m³/d HRT in reactor 2 (UASB): 10 d pH in UASB: 7–8 OLR: 5.5 kg COD/m³/d Objective: To scale-up biohythane production system from POME with thermophilic mixed consortium and thermophilic methanogenic archaea at various HRT and OLR.	ASBR and UASB Inoculum in ASBR: Thermoanaerobacterium-rich sludge Inoculum in UASB: Sludge from laboratory scale reactor operating under thermophilic methane production from POME	OLR: 5.5	(Seengenyoun et al., 2019)
POME pH: 4.7 ± 0.1 Temperature: 55 °C COD: $60,000 \pm 3,002$ mg/L TS: $44,980 \pm 336$ TSS: $25,009 \pm 4,142$	Operating temperature: 55 °C pH: 7.53 ± 0.26 OLR: 2–10 kg COD/m³/d Objective: To evaluate the performance of a bench scale TAnMBR by using PTFE membrane for POME treatment	TAnMBR Inoculum: Seed sludge	OLR: 2–10	(Yee et al., 2019)

Table 2.1 continued

POME pH: 4.73 tCOD: 88,200 ± 200 mg/L sCOD: 64,400 ± 400 mg/L TS: 51,900 ± 300 mg/L SS: 26,500 ± 3,000 mg/L VS: 43,300 ± 100 mg/L VSS: 23,500 ± 2,400 mg/L	pH of POME was adjusted to 7 with 6 mol/dm³ NaOH HRT: 3.3 d Operating temperature: 55 °C OLR: 2 kg COD/m³/d and increased by a step of 2.1 ± 0.9 kg COD/m³/d Stirring rate: 78 rpm Objective: To investigate the effect of chitosan addition on reactor performance especially methane production at high OLR.	Modified CSTR Inoculum: Seed sludge	OLR: 2–26.5	(Khemkhao et al., 2016)
POME 4.73 ± 0.03 tCOD: 70,500 ± 917 sCOD: 39,067 ± 1,159 TS: 51,880 ± 310 SS: 26,547 ± 3,043 VS: 43,260 ± 140 VSS: 23,513 ± 2,486	pH was adjusted to 7 with 6 N NaOH Operating temperature: 55 °C HRT: 3.3 d OLR: 2 kg COD/m³/d and increased by a step of 2.1 ± 0.9 kg COD/m³/d Objective: To analyze the performance of CSTR in terms of stability and efficiency of the process in removing COD and in producing biogas.	CSTR Material: Glass Inoculum: Seed sludge from a domestic wastewater treatment plant (TS: 54,170 ± 440 mg/L, VS: 18,800 ± 30 mg/L, SS: 53,290 ± 290 mg/L, VSS: 18,670 ± 90 mg/L)	OLR: 2–19	(Khemkhao et al., 2015)

2.7 Anaerobic Biodegradation to Recover Resources from POME

The processes of hydrolysis, acidogenesis, acetogenesis, and methanogenesis were simplified in a schematic representation in Figure 2.1.

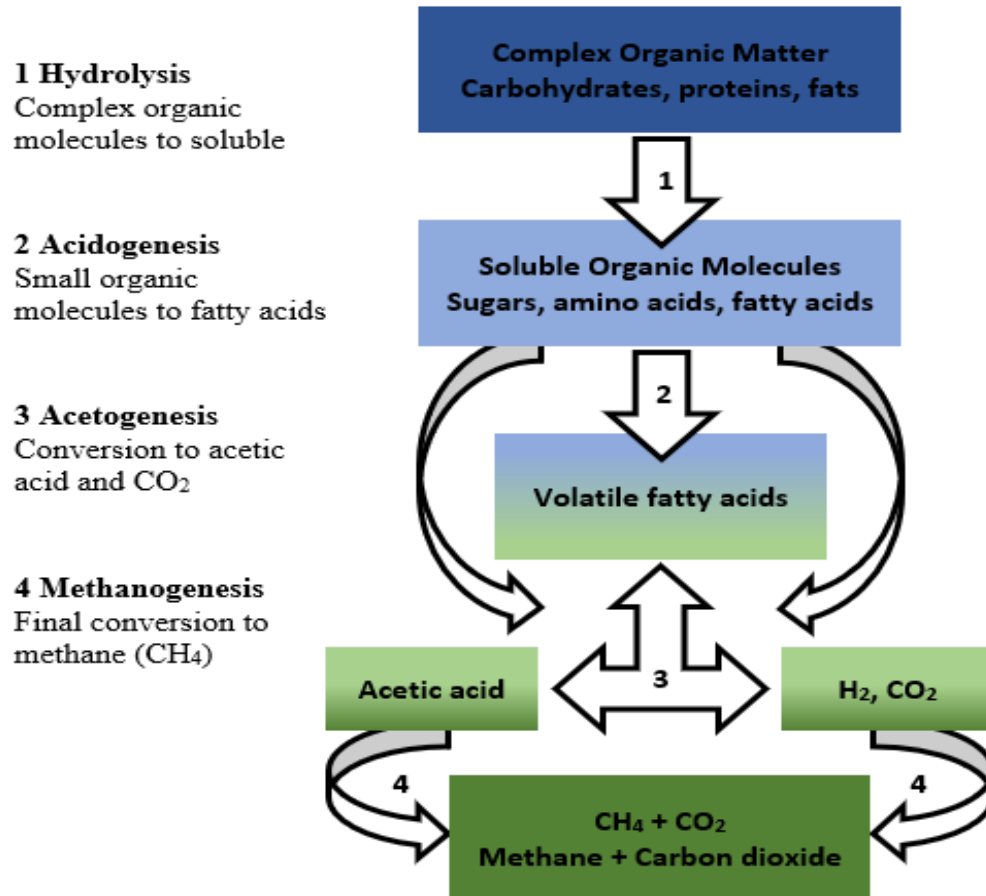


Figure 2.1: Schematic Representation of Anaerobic Biodegradation (Monge et al., 2013)

2.7.1 Hydrolysis for POME Digestion

In general, hydrolysis was a chemical reaction in which water was broke down to form H^+ cations and OH^- anions. It was the first step in anaerobic digestion process. This process involved enzymes which facilitated the conversion of organic polymers such as carbohydrates, proteins, lipids and nucleic acid into soluble monomers and dimers such as simple sugars, amino acids and fatty acids (Adekunle & Okolie, 2015). This wa carried out

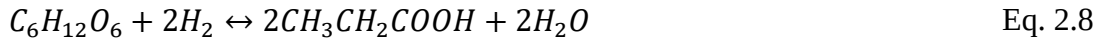
by several hydrolytic enzymes such as cellulases, cellobiase, xylanase, amylase, lipase, and protease secreted by hydrolytic microbes (Weiland, 2010). Hydrolysis was important because microorganisms were unable to absorb large organic polymers to be used as an energy source. Different types of organic polymers required different enzymes. Therefore, the rate of breakdown depended on the composition of the substrate. Usually, the decomposition rate of cellulose and hemicellulose occurred at a slower rate compared to decomposition rate of proteins (Schunurer & Jarvis, 2009). Many of the monomers, which were still large must be further broke down in the next step, which was acidogenesis in order to produce methane. Equation 2.6 shows the step of hydrolysis during anaerobic digestion (Verma, 2002; Ostrem, 2004; Haandel & Lubbe, 2012; Zupančič & Grilc, 2012; Biarnes, 2013).



2.7.2 Acidogenesis for POME Digestion

Acidogenesis or acidification phase was the second step in anaerobic digestion process. In acidogenesis, microorganisms such as facultative and obligatory bacteria further broke down the hydrolysed products into simpler organic products. The fermentative bacteria produced an acidic environment in the digestive tank to form organic products such as ammonia (NH₃), acetate, hydrogen (H₂), carbon dioxide (CO₂), hydrogen sulphide (H₂S), shorter volatile fatty acids, carbonic acids and alcohols (Adekunle & Okolie, 2015). Due to the interaction of different populations of microorganisms, the acidogenesis process was divided into hydrogenation and dehydrogenation (Ziemiński, 2012). Hydrogenation involved the addition of hydrogen while dehydrogenation was the removal of hydrogen (Sanfilippo & Rylander, 2000). Acetate, H₂ and CO₂ would soon become the precursors of biomethane formation. While some of the final products were still too large, they must

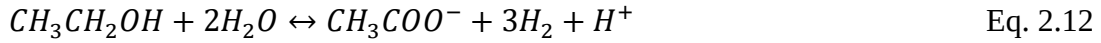
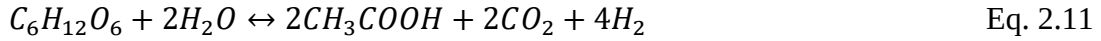
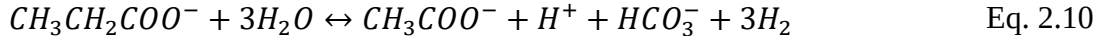
undergo the next process which was acetogenesis. Equation. 2.7, Equation 2.8 and Equation 2.9 showed the steps of acidogenesis during anaerobic digestion (Verma, 2002; Ostrem, 2004; Haandel & Lubbe, 2012; Zupančič & Grilc, 2012; Biarnes, 2013).



2.7.3 Acetogenesis for POME Digestion

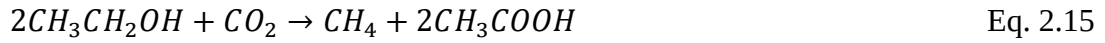
Acetogenesis was the formation of acetate, a derivative of acetic acid and the products during this phase were consumed by microorganisms. It was the third step in anaerobic digestion process. Methanogens converted products during acidogenesis into volatile fatty acid (VFA) and alcohols because these products were not able to be converted right into methane (Adekunle & Okolie, 2015). VFA would be oxidized to form acetate, H_2 and CO_2 . Meanwhile, VFA containing longer carbon chains that one unit underwent oxidation to form acetate and hydrogen (Seadi et al., 2008). During anaerobic oxidation, the production of H_2 was caused by the role of protons as the final electron acceptor. The oxidation process depended on the partial pressure of H_2 and would only occur if the partial pressure was low. The formation of H_2 during acetogenesis was known as a waste product and had toxic effects to the microorganisms carrying out this step. It hindered the work of acetogenic bacteria. However, hydrogen scavenging bacteria or methanogens utilized the H_2 produced and converted them to CH_4 (Seadi et al., 2008). This explains the importance of a cooperation between acetogens and methanogens in which transfer of H_2 occurs (Deublein & Steinhauser, 2010; Chandra et al., 2012; Nurliyana et al., 2015). Equation. 2.10, Equation

2.11 and Equation 2.12 showed the steps of acetogenesis during anaerobic digestion (Verma, 2002; Ostrem, 2004; Haandel & Lubbe, 2012; Zupančič & Grilc, 2012; Biarnes, 2013).



2.7.4 Methanogenesis for POME Digestion

Methanogenesis was the final step in anaerobic digestion. It utilized the substrates which were formed during the previous stages such as acetic acid, CO₂ and H₂ (Ziemiński, 2012). CH₄ was produced by two groups of methanogens, which were acetotrophic methanogens and hydrogenotrophic methanogens. Acetotrophic methanogens converted acetate into CH₄ and CO₂. Meanwhile, hydrogenotrophic methanogens utilized H₂ as electron donor and CO₂ as electron acceptor to produce CH₄ (Demirel & Scherer, 2008). The formation of CH₄ and CO₂ were performed by methanogens at strict anaerobic environments (Aslanzadeh, 2014). These methane-formers were susceptible to even small amount of oxygen present in the system. According to Seadi et al. (2008), methanogenesis was a vital step because the rate of biochemical reaction in this stage was the slowest in the entire process of anaerobic biodegradation. During this stage, the removal efficiency of COD and BOD increased greatly as most of the conversion process of organic matter into methane and carbon dioxide took place in methanogenesis. Therefore, the efficiency of methanogenesis corresponded to the removal efficiency of COD or BOD during the treatment process of anaerobic wastewater. Equation 2.13, Equation 2.14, and Equation 2.15 showed the steps of methanogenesis during anaerobic digestion (Verma, 2002; Ostrem, 2004; Haandel & Lubbe, 2012; Zupančič & Grilc, 2012; Biarnes, 2013).



2.8 Substrate from Banana Peel for Anaerobic Digestion

Banana was the second most widely cultivated fruit in Malaysia which was covering approximately 28,000 ha with a production of about 18.9 MT/ha (Tock et al., 2010). The banana peel was accounted for approximately 40% of the total weight of fresh bananas. Farmers often faced the challenges to dispose banana peels because it appeared to be health hazard (Abdullah et al., 2014). Banana peels were normally dumped into landfill and resulted in environmental problems due to carbon emission (CO₂ eq). The banana peels were also responsible for water and soil pollution (Ahmad & Danish, 2018; Vu et al., 2018).

Banana peel was a waste biomass enriched with organic materials that contained a large amount of carbohydrates, crude protein, crude fat, had high total sugar presence, vitamins, minerals, and other trace elements. Micronutrients were the potential parts of this biomass with iron (Fe), zinc (Zn) and carbon (C). The high C/N ratio and carbohydrate made banana peels a potential source to be used for various anaerobic process as an inoculum. Especially in POME digestion process, the C/N ratio and pH of banana peel-based inoculum would contribute to increase C/N and pH of the substrate (Hapsoh et al., 2015; Nathoa et al., 2014). The chemical properties of banana peel were listed in Table 2.2.

Table 2.2: Properties of Banana Peel (Odedina et al., 2017; Sial et al., 2019)

Parameters	Unit	Banana waste (peel)
Moisture	% Fresh wt.	82.3
Volatile solids	% Dry wt.	88.1
Fixed solid	% Dry wt.	11.9
Carbon	% Dry wt.	39.2
Hydrogen	% Dry wt.	5.3
Nitrogen	% Dry wt.	1.7
Sulphur	% Dry wt.	0.1
Oxygen	% Dry wt.	39.6
Sum CHNS-O	% Dry wt.	85.8
Cellulose	% Dry wt.	13.5
Hemicellulose	% Dry wt.	39.7
C/N ratio	-	23.1–35
pH*		7

Chemical properties listed in Table 2.2 demonstrated that banana peel was a potential substrate for microbial growth during fermentation phase of anaerobic process (Essien et al., 2005; Chooklin et al., 2014; Kadir & Rahman, 2016; Divyabharathi et al., 2017). Thus, banana peel-based inoculum could be an appropriate process for COD reduction of POME by anaerobic digestion (Bardiya et al., 1996; Nagao et al., 2012; Pisutpaisal et al., 2014).

Banana peel in the anaerobic digester as an inoculum with C/N about 30 and pH about 7 could potentially contribute to increase the performance of anaerobic process for POME and resulted in less HRT (Pisutpaisal et al., 2014). Indeed, the use of banana peel as inoculum for co-digestion with POME would give added novelty to any research for improving the performance efficiency of anaerobic reactor to convert COD and VSS in POME to biogas for capture.

In line with SDG-6 (clean water) to reduce the water pollution due to POME, the banana peel and its biomass were potential resources for reducing COD of POME. There

was a need to have sustainable management of these organic wastes to achieve environmental-friendly and economic benefits (Permpool et al., 2016).

2.9 Effect of Contributing Factors on Performance of Anaerobic Digestion

2.9.1 Effect of pH on POME Digestion

The pH value determined if the solution was acidic or alkaline. During anaerobic digestion, pH was an essential parameter which determined the performance and stability of the system for COD reduction. Methanogens, responsible for COD decomposing were sensitive to the change in pH. At pH range of 6.5–7.8, this bacteria was effective for breaking down of COD (Nayono, 2010). At pH levels below 6.6, the methanogenic activities decreased due to the slowed growth rate of methanogens (Zhang et al., 2009). Therefore, at pH lower than 6.5 and higher than 7.6 may resulted in the reduction of biogas production rate. A study conducted by Wong et al. (2013) reported that the concentration of methane increased as pH increased in the treatment of POME in a suspended closed anaerobic bioreactor (SCABR). Methanogenesis and acidogenesis had different optimal pH levels. As for acidogenesis, the optimal pH value was ranging between 5.5–6.5 (Kim et al., 2003). Due to different optimal pH values in both processes, a two-stage anaerobic digestion was the preferred method to separate the hydrolysis/acidogenesis (first stage) and acetogenesis/methanogenesis processes (second stage). From the above-mentioned findings, it could be concluded that pH had a positive effect on COD reduction from POME.

2.9.2 Effect of C/N on POME Digestion

By introducing a co-substrate into a digestion system to increase the C/N was known as co-digestion. The process of co-digestion provided few benefits to the anaerobic digestion system (Esposito et al., 2012). The presence of a co-substrate would reduce the concentration

of harmful compound available in the system. By adding a co-substrate, the pH and moisture content of a system could be improved as well. Besides that, co-digestion provided the required buffer capacity of a mixture. Co-digestion also improved the biodegradability of an organic matter present in an anaerobic system. Co-digesting two different substrates increased the variation of microbial population involved in the biochemical reaction process. An increased in COD removal efficiency and reduction of volatile solids in substrate, C/N played a vital role (El-Mashad & Zhang, 2010). This was due to the effective acclimatization of methanogens in the anaerobic system which produced an efficient removal of COD and volatile solids. A study conducted by Sidik et al. (2013) on the co-digestion of POME and cow manure (CM) by varying the C/N values. The highest removal efficiency of COD achieved by the author was 67% when digester was fed with 70% POME and 30% CM at C/N range of 25–30. Co-digestion of POME and EFB with a C/N of 45 carried out by Nurliyana et al. (2015) was able to reduce total volatile solids around 63 to 78% while mono-digestion of POME only achieved 57% reduction efficiency. The high removal efficiency from co-digestion was a result of balanced nutrition provided during the anaerobic digestion process. As for COD reduction, the removal efficiency decreased at increasing C/N. The amount of organic material in the system increased due to the addition of EFB to increase the C/N, resulting in the rise of COD value (Nurliyana et al., 2015). Thus, C/N has an effect on COD reduction from POME.

2.9.3 Effect of OLR on POME Digestion

The biogas production in an anaerobic reactor increased with increasing of OLR. However, there were consequences in increasing OLR as the stability and efficiency of digestion process could be affected. The addition of large volume of organic material could alter the environment inside the reactor and interrupted the fermentation process during the

early stages. An extremely high OLR lead to higher microbial activity during hydrolysis and acidogenesis, resulting in the rise of VFA production. Due to increasing VFA production, the pH inside the reactor would decrease. As a result, the efficiency of methanogenic bacteria to convert high amount of VFA to methane would become lesser and resulting in lower COD reduction (Mao et al., 2015). Besides that, high OLR could result in sludge bed flotation, extreme foaming, build-up of undigested substrate, and decreasing treatment effectiveness (Abdelgadir et al., 2014). Based on the various research outcomes, it could be concluded that OLR had an association with COD reduction from POME.

2.9.4 Effect of HRT on POME Digestion

The COD represented the organic matter under anaerobic conditions. During anaerobic digestion, COD was digested and converted into harmless end products which include methane, carbon dioxide, and water. An increased in methane production meant an increased in COD removal. A well-adapted microbial population in anaerobic condition lead to an increase in the rate of digestion of organic matters (Alrawi et al., 2011). HRT was one of the anaerobic conditions which provided an effect on COD reduction of effluent. The removal efficiency of COD anaerobically digested from-POME was 96.48% at 20 days HRT as conducted by Alrawi et al. (2011). A longer HRT was found to achieve better COD removal efficiencies and was suitable for digestion of POME. A short HRT could reduce the contact time between organic matter and bacteria resulting in the insufficient reproduction of methane-forming bacteria (Maroun & Fadel, 2007; Poh & Chong, 2009). The COD removal efficiency as conducted by Siddique et al. (2016) was increasing steadily from 68.2 to 98.5% as HRT increased from 3 to 9 days. The findings indicated that HRT was positively associated with COD reduction from POME.

2.9.5 Effect of SRT on POME Digestion

Generally, increasing the SRT improved the removal efficiency of COD (Dereli et al., 2014). SRT played an important role to reduce the COD contents in industrial wastewaters such as pharmaceutical wastewaters and saline wastewaters (De Vrieze et al., 2014; Ng et al., 2014; Shi et al., 2014). A high SRT performed the function of allowing microbial community to be acclimatized to the environment of the components available in these wastewaters (Dvořák et al., 2016). The effect of increasing SRT by sludge recycling improved the removal efficiency of COD in fermentation process. During the fermentation of POME, COD removal efficiency achieved 85% when sludge recycling was used to increase the effect of SRT (Irvan et al., 2017). Previous researchers had also conducted studies on COD removal efficiencies and they found that the removal efficiencies was higher with sludge recycling compared to that without sludge recycling (Busu et al., 2010; Gnanapragasam et al., 2010; Lee et al., 2010). A study conducted by Chen et al. (2012) found that the value of supernatant COD increased steadily from 5.0 mg/L at SRT of 30 days to as high as 210.2 mg/L at SRT of 50 days. When SRT increased, the microorganisms began to compete for nutrients resulting in the formation of antibiotics to prevent the growth of other microorganisms. As a result, certain microbial population would not survive and died. Dead cells of microorganism then dissolved into the bioreactor causing the concentration of soluble microbial particles to increase and hence, supernatant COD increased as well (Huang et al., 2001).

Sludge recycling through POME substrate involved separating biomass and returned it into the reactor gave better effect in reducing COD (Ali, 2012; Duan et al., 2012). The SRT should be prolonged if another complex substrate was added into the reactor. Besides

that, the high concentration of biomass could be maintained inside the reactor by increasing SRT which would reduce the HRT (Demirel et al., 2005; Leu et al., 2012).

2.10 Current Technology for Anaerobic Digestion

This section presents the current technologies that had been used in various research for COD reduction from POME.

2.10.1 Up-flow Anaerobic Sludge Blanket-Hollow Centre Packed Bed Reactor

A study was conducted by Poh and Chong (2014) on the performance of up-flow anaerobic sludge blanket-hollow centre packed bed (UASB-HCPB) reactor on POME treatment at thermophilic conditions. The aim of this study was to determine the performance of UASB-HCPB which was evaluated on the effects of HRT, OLR, and MLVSS concentration. Packed bed was used to stop the biomass from moving and reduced sludge washout. The biofilm formed by immobilised biomass provided a high surface area between biomass and substrate. Due to high surface area of biomass, treatment efficiency of POME was increased. Besides, the hollow channel allowed biogas and effluent to flow smoothly to the upper section of the reactor. The UASB-HCPB reactor is presented by Figure 2.2.

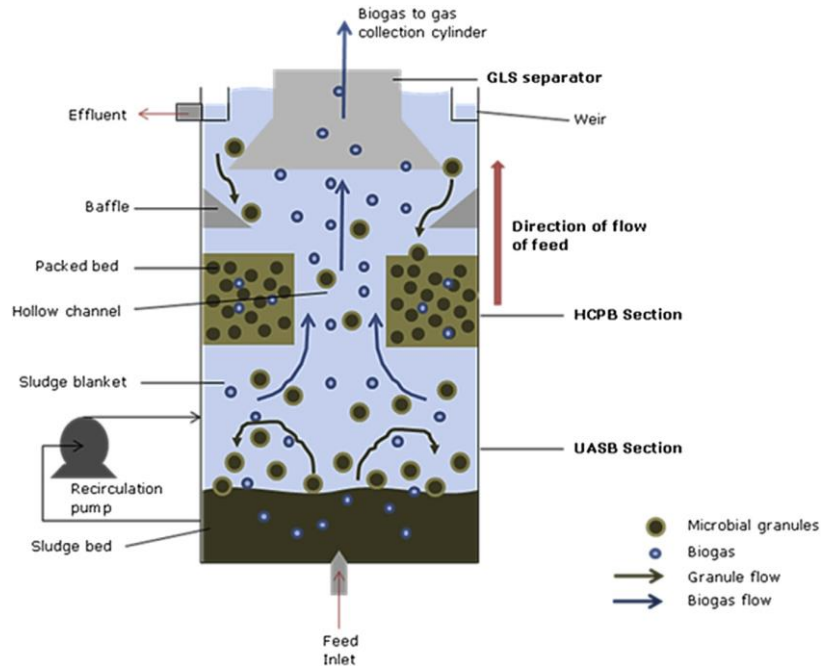


Figure 2.2: Design of UASB-HCPB Reactor (Poh & Chong, 2014)

The process of treating POME was conducted at temperature of 55 °C. To determine the effects of HRT, it was varied while feed and MLVSS concentration were maintained at a constant value. The effects of OLR were determined by changing the feed COD concentration while keeping HRT and MLVSS concentration constant. The effects of MLVSS concentration were determined by changing its concentration by adding or removing the inoculum while maintaining HRT at 5 days. The parameters which were monitored during the experiment are COD, BOD, TSS, alkalinity, methane concentration, and VFA. The experiment consisted of 13 runs. The effects of HRT and OLR were studied from runs 1–9. HRT was varied between 2–4 days.

The result showed a fluctuating behaviour from runs 1–9. They showed an increasing trend in overall COD and suspended solids (SS) removal, overall methane concentration, and biogas production rate. The quality of effluent from the UASB-HCPB improved as HRT decreased due to increasing OLR. The methane concentration was increasing up to run 5 at

low OLR (4.28–9.19 g/L.d) and reduced until run 9 due to high corresponding OLR (13.75–27.65 g/L.d). Runs 10–13 was conducted to study the effects of MLVSS concentration. HRT and OLR was kept constant at 5 days and 6.66 g/L.d respectively. Run 11 produced effluent with the highest quality. Run 11 conducted at OLR of 6.66 g/L.d, HRT of 5 days and MLVSS concentration of 14.98 g/L was the best performance of the UASB-HCPB. Due to the imbalance population of acidogens and methanogens in the reactor, which was related to HRT and OLR, it affected the performance of anaerobic digestion system. Besides, an increased in MLVSS concentration would lead to lower production of methane. HRT, OLR, and MLVSS concentration were important operating conditions which would determine the performance of an anaerobic digestion system.

2.10.2 High-Rate Continuous Stirred Tank Reactor

A study was conducted by Khemkhao et al. (2015) on the performance of a high-rate continuous stirred tank reactor (CSTR) in terms of COD removal and biogas production. The aim of this study was to investigate the performance of reactor and biodegradation of palm oil fibre and residual oil in the high-rate CSTR. Two types of CSTR were used in this study, which were the typical CSTR and a modified CSTR with a deflector installed at its upper section. The deflector was installed to promote the retaining of suspended solids inside the reactor. The experimental setup was shown in Figure 2.3.

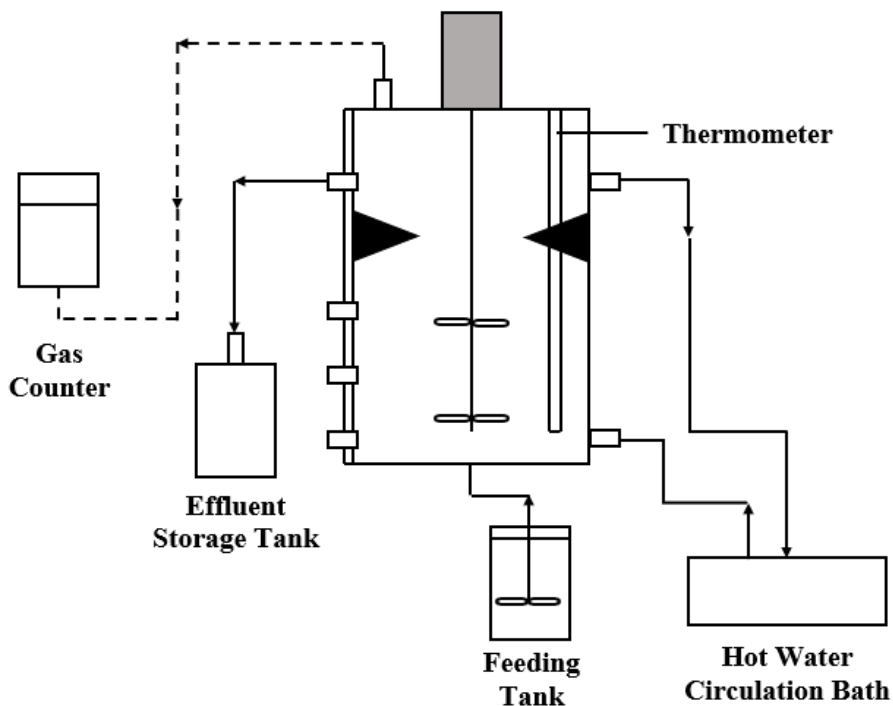


Figure 2.3: Diagram of a CSTR (Khemkhao et al., 2015)

Raw POME was used as the substrate for this study and neither physical nor chemical pre-treatment was done to remove the fibres and fat, oil, and grease (FOG). To determine the performance of the reactor, nine steps of OLR were performed starting from 2 to 19 g COD/L.d. The pH in the reactor was maintained between 7–8 which was suitable for methanogenesis.

Findings demonstrated that the biogas production increased with increasing OLR only to decline as the OLR reached 17.6 and 19.0 g COD/L.d. However, the amount of methane produced was between 60–72% giving an average of 68%. Throughout the experiment, the removal efficiency of soluble COD averaged at more than 90% while removal efficiency of total COD was around 82–93%. From this study, the modified CSTR could be operated at the highest OLR of 19.0 g COD/L.d with HRT of 3.3 days giving a COD removal efficiency of 82% and methane content of 68%. The biogas and methane

production as well as total COD removal responded rather fast as OLR was increasing. However, the growth rate of methanogens was relatively slow which could affect the methane content although the OLR was increased.

2.10.3 Expanded Granular Sludge Bed Reactor

A study was conducted by Wang et al. (2015) on the treatment efficiency of POME and stability in the expanded granular sludge bed (EGSB) reactor. The aim of this study was to determine the performance of EGSB reactor in terms of COD removal efficiency and biogas production. This study was conducted using a pilot-scale EGSB reactor which was operated at 9.8 days. The experimental setup was presented by Figure 2.4.

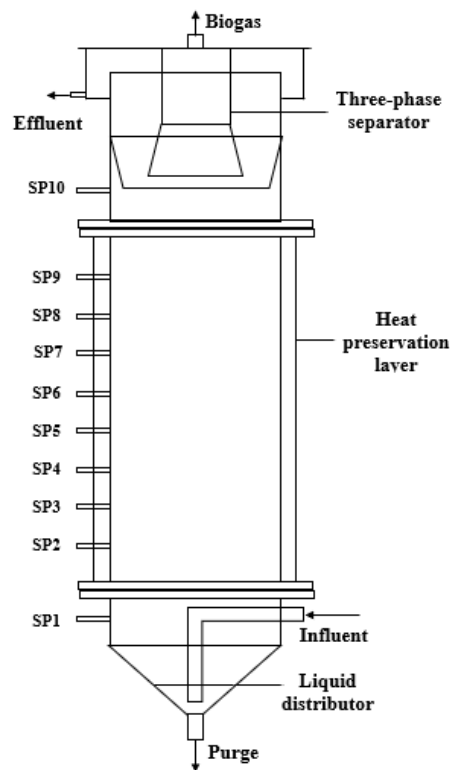


Figure 2.4: Schematic Diagram of EGSB Reactor (Wang et al., 2015)

The organic loading rate was at 6.45 kg COD/m³.d. The temperature of the reactor was maintained around 35 °C. A dissolved air flotation (DAF) was used to collect SS from

the effluent which was recycled back into the reactor. A dose of cationic polymer (PAM) was fed into the DAF to speed up the formation of sludge granules to reduce the start-up period.

The findings demonstrated that anaerobic EGSB reactor was able to reduce the COD of POME from 71,179 mg/L to 12,341 mg/L. The removal of SS by using DAF further reduced the COD to an average of 3,587 mg/L giving 94.89% COD removal efficiency. The biogas production from the reactor was about 27.65 m³/m³ POME. The methane content in the biogas was 65–70%. The stability of an anaerobic reactor could be maintained by sufficient mixing, biogas recirculation, and effluent recycling. Excessive mixing could result in a drop in pH inside the reactor which affected the proses of methanogenesis. Besides that, a disruption in the metabolic activities of microorganisms inside the reactor such as accumulation of VFA could reduce the pH resulting in lower production of methane.

2.10.4 Suspended Closed Anaerobic Reactor

A study was conducted by Wong et al. (2014) on the performance of a suspended closed anaerobic reactor (SCAR) for the anaerobic degradation process of POME. The aim of this study was to evaluate the performance of suspended growth anaerobic degradation process in terms of pH, COD reduction, and biogas production. The experiment was presented by Figure 2.5.

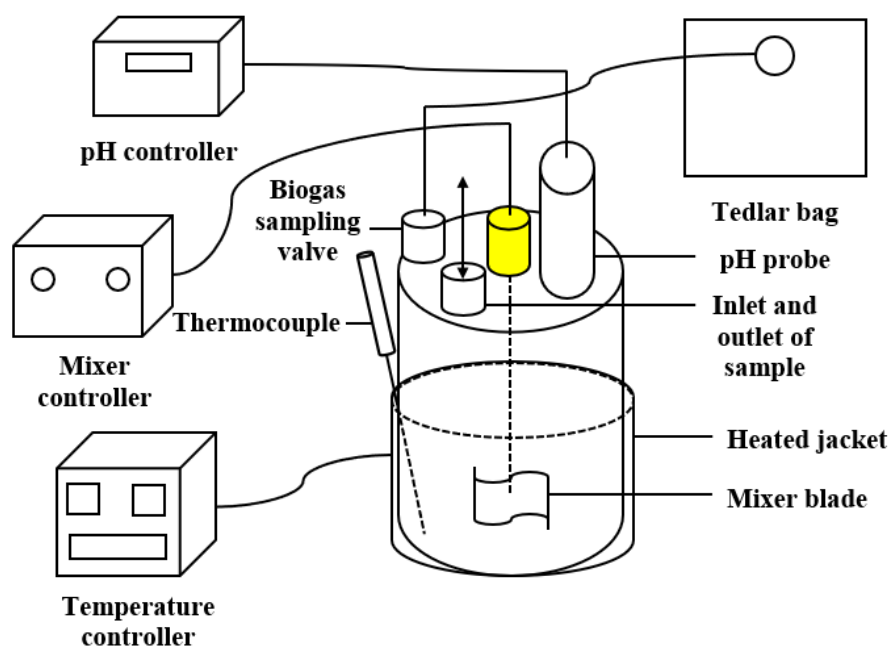


Figure 2.5: Schematic Diagram of SCAR (Wong et al., 2014)

Parameters which were analysed include pH, COD, soluble COD, microbial growth, VFA, total alkalinity, and biogas composition. In this study, SCAR was used, and it was operated at different HRT of 12, 10, 8, 6, and 4 days by using different feed flowrates of 375 ml, 450 ml, 560 ml, 750 ml, and 1,125 ml respectively. 16 hours of continuous stirring was conducted, and another 6 hours were used to allow active sludge to settle in the reactor. The temperature during the process of methanogenic degradation was maintained at 35 °C.

From this experiment, it was found that the pH in SCAR reduced steadily from 7.34 to 6.89 as HRT was decreasing. Meanwhile, the effluent COD increased from 28,120 to 37,650 mg/L as HRT was decreasing. The removal efficiency of COD was highest at 66.09% on HRT of 12 days while it was the lowest at 47.30% on HRT of 4 days. The biogas production rate of SCAR was found to be increasing from 1,097 to 3,977 ml biogas/day as HRT decreased. The methane content decreased from 48.50 to 19.27% as HRT varied from 12 to 4 days. The best performance of SCAR was obtained at HRT of 12 days by contributing

to removal efficiency of COD at 66.09% and methane composition of 48.05%. The pH value of an anaerobic system could affect the growth of methanogens which were responsible for the formation of methane. The rate of methane production increased with low OLR and longer HRT. pH not less than 7 and HRT more than 10 days were the operating conditions which were recommended to achieve a good performance in anaerobic degradation of POME.

2.10.5 Anaerobic Hybrid Reactor and Anaerobic Baffled Reactor

A study was conducted by Jeong et al. (2014) on the performance of anaerobic hybrid reactor (AHR) and anaerobic baffled reactor (ABF) in mesophilic and thermophilic conditions. The aims of the study were to compare and evaluate the performance of combined system of AHR and ABF under mesophilic and thermophilic anaerobic condition for POME treatment. The anaerobic digestion system consisted of AHR as the primary digester and ABF as the secondary digester. The AHR consists of UASB and anaerobic filter (AF). At the lower part of the reactor, POME was treated by microorganisms from the sludge bed. At the upper of the reactor, POME was treated by a biofilm formed on the filter media. Meanwhile, the ABF is a combination of anaerobic baffle reactor and AF. The experiment was presented by Figure 2.6.

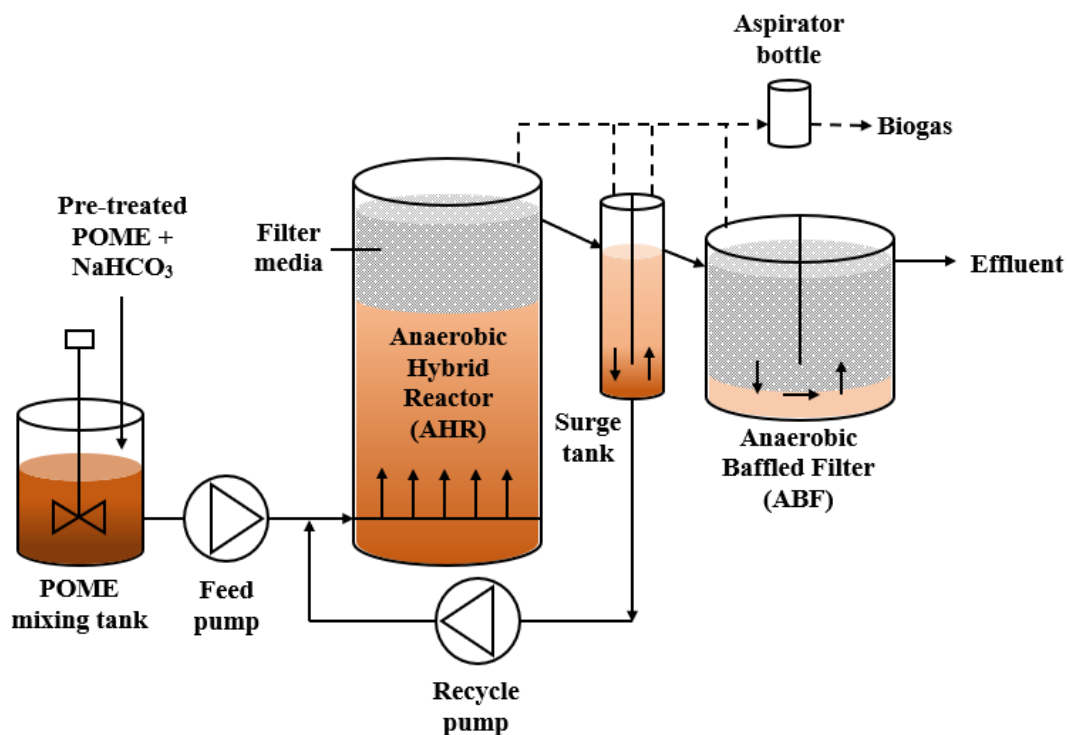


Figure 2.6: Schematic Diagram of AHR-ABF (Jeong et al., 2014)

A pre-treatment on raw POME was done by a screw decanter to remove the SS and residual oil. Temperatures of 37 °C and 55 °C were used in this experiment. The feed flow rates were fixed to 0.73 L/d and 0.77 L/d with OLR increased gradually from 2 to 15 kg COD/m³/d. HRT of the combined system were 5.8 and 6.2 days respectively. The parameters which were measured include COD values, TSS, VSS, and volume of biogas produced.

The results of the experiment showed that the combined system of AHR and ABF produced removal rates of COD between 90–93% and 93–95% respectively. The treatment of POME was better in thermophilic conditions compared to mesophilic. Based on the experiment, the rate of biogas production increased from 1.3 to 13.5 L/d and 1 to 20 L/d in mesophilic and thermophilic conditions respectively at high OLR. The ratio of specific methane production was higher in thermophilic reactors than those in mesophilic reactors. In this study, the thermophilic reactor performed better in COD removal and biogas

production compared to the mesophilic reactor. The high temperature in the reactor sped up the metabolic process of anaerobic digestion of POME resulting in higher COD removal and biogas production.

2.10.6 Anaerobic Sludge Blanket-Continuous Stirrer Tank Reactor

A study was conducted by Krishnan et al. (2016) on the performance of a two-stage UASB-CSTR under thermophilic conditions. The aim of this study was to evaluate various OLR on H_2 and CH_4 production. The first stage was using the UASB reactor which focused on the production of H_2 . While in the second stage, CSTR was used to focus on the production of CH_4 . The experiment setup was shown in Figure 2.7.

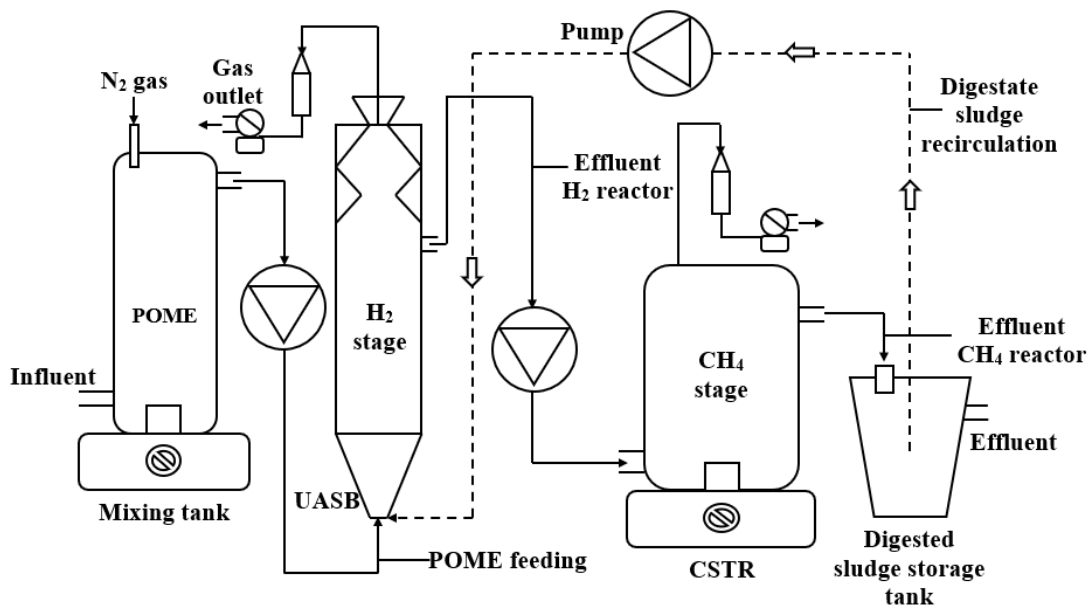


Figure 2.7: Schematic Design of UASB-CSTR (Krishnan et al., 2016)

The UASB-CSTR process was done at a constant temperature of 55 °C. Raw POME was sieved to remove coarse particles before it was used. NaOH was added before POME was fed into the UASB reactor to obtain the pH of 5.5. The OLR for the UASB reactor were varied at 25, 50, 75, 100, and 125 kg COD/m³.d while in CSTR were varied at 4, 8, 12, 16,

and 20 kg COD/m³.d. The HRT was kept constant at 6 hours. The mixing in CSTR was maintained at 120 rpm. The parameters measured consisted of hydrogen and methane production, VFA composition, MLVSS, and effluent VSS composition.

The experiment demonstrated that the rate of gas production and COD removal in the UASB unit were at maximum at 4.5 L/d and 40% respectively at OLR of 75 kg COD/m³.d. Both gas production rate and COD removal decreased as OLR was beyond 75 kg COD/m³.d. In the CSTR unit, the removal rate of COD was 85% at OLR of 12 kg COD/m³.d. The gas production rate was 3 times higher than the UASB unit. The best results were obtained at OLR of 75 kg COD/m³.d for the UASB unit and 12 kg COD/m³.d for the CSTR unit. The highest H₂ and CH₄ content were 35% and 68% respectively. The two-stage fermentation process was conducted to separate the microbial activities of acid formers and methane formers. Both acidogens and methanogens had different growth rate. Acidogens grew rapidly while methanogens had a slower growth rate.

2.10.7 Two-Stage Thermophilic Fermentation and Mesophilic Methanogenic Process

A study was conducted by Mamimin et al. (2015) on the performance of a two-stage thermophilic fermentation and mesophilic methanogenic process for biohythane production. The aim of this study was to develop a two-stage thermophilic fermentation and mesophilic methanogenic process for hydrogen and methane production from POME. Factors affecting process stability and microbial community were determined. The experiment setup was pictured in Figure 2.8.

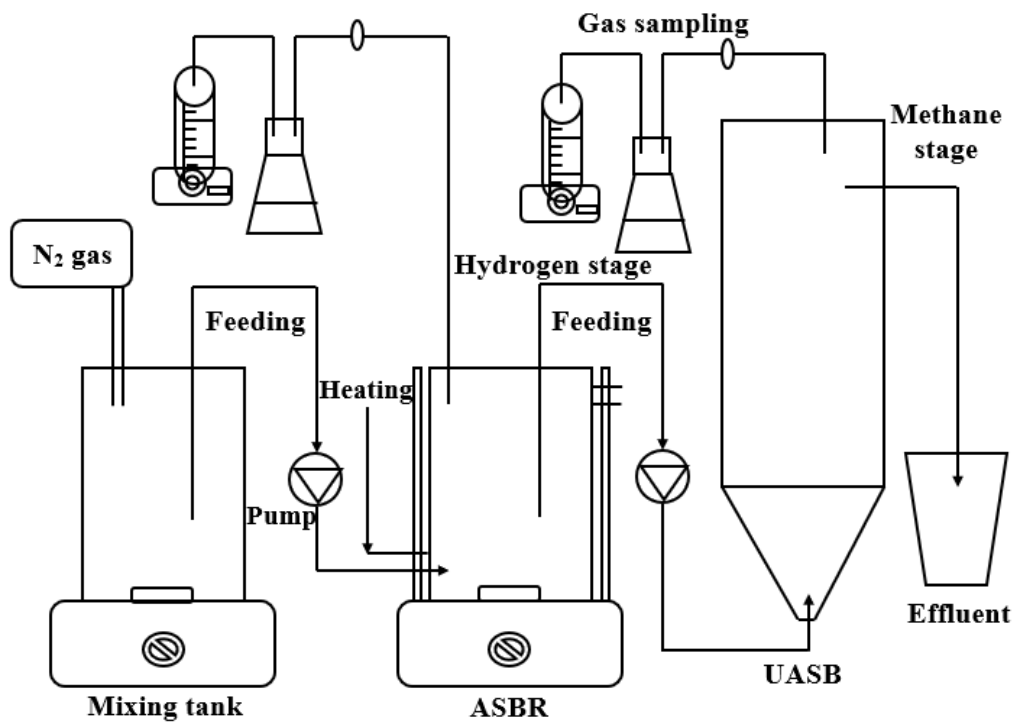


Figure 2.8: Schematic Diagram of Two-stage Hydrogen and Methane Process (Mamimin et al., 2015)

The thermophilic fermentation process was using anaerobic sequencing batch reactor (ASBR) for hydrogen production. It was operated at temperature of 55 °C, pH of 5.5 and HRT of 2 days. The mesophilic methanogenic process was using up-flow anaerobic sludge bed (UASB) reactor for methane production. It was operated at temperatures between 28–34 °C, pH of 7.5 and HRT of 20, 15, and 10 days. Parameters which were measured include pH, gas production and component, VFA distributions, COD, SS, oil concentration, total phosphorus, and total Kjeldahl nitrogen (TKN).

With operating conditions of ASBR at OLR of 60 g COD/L.d and HRT of 2 days, the maximum hydrogen production rate was recorded at 1.84 L H₂/L.d at an average of 1.8 L H₂/L.d. The average concentration of hydrogen in the biogas was 55%. The COD removal rate in ASBR was at 38%. The maximum methane production rate was recorded at 2.6 L

CH₄/L.d at HRT of 15 days and OLR of 6 g COD/L.d. In the UASB unit, the average concentration of methane and removal rate of COD were 73% and more than 90% respectively. The best result obtained from the ASBR was achieved at the pH of 5.5, temperature of 55 °C, HRT of 2 days, and OLR of 60 g COD/L.d giving a maximum hydrogen production rate at 1.84 L H₂/L.d. The optimum HRT in the UASB was 15 days in which maximum methane production rate of 2.6 L CH₄/L.d was obtained. The composition of biogas was 51% CH₄, 14% H₂, and 35% CO₂ in a 4.4 L biogas/L.d. The hydrogen and methane yield produced were 210 L H₂/kg COD and 315 L CH₄/kg COD respectively. The two-stage fermentation and methanogenic process were capable to recover energy up to 34% and 90% higher than single-stage methane production and single stage hydrogen production respectively.

2.10.8 Combined High-Rate Anaerobic Reactor

A study was conducted by Choi et al. (2013) on the performance of a high-rate anaerobic reactors to treat POME. The aim of this study was to evaluate the feasibility of combination systems for POME treatment and to investigate the performance of each reactor in terms of COD removal, OLR, SS, pH, biogas production, and methane yield. The high-rate anaerobic reactors were consisted of one hybrid reactor and two different filter reactors. The hybrid reactor was known as AHR. The filter reactors were known as ABF and anaerobic downflow filter (ADF). The secondary reactors were included to improve the removal efficiency of COD and ensured the stability of the anaerobic system. The baffle in ABF was made from acrylic resin. The ADF on the other hand was packed with clay ball media. The experiment was presented by Figure 2.9.

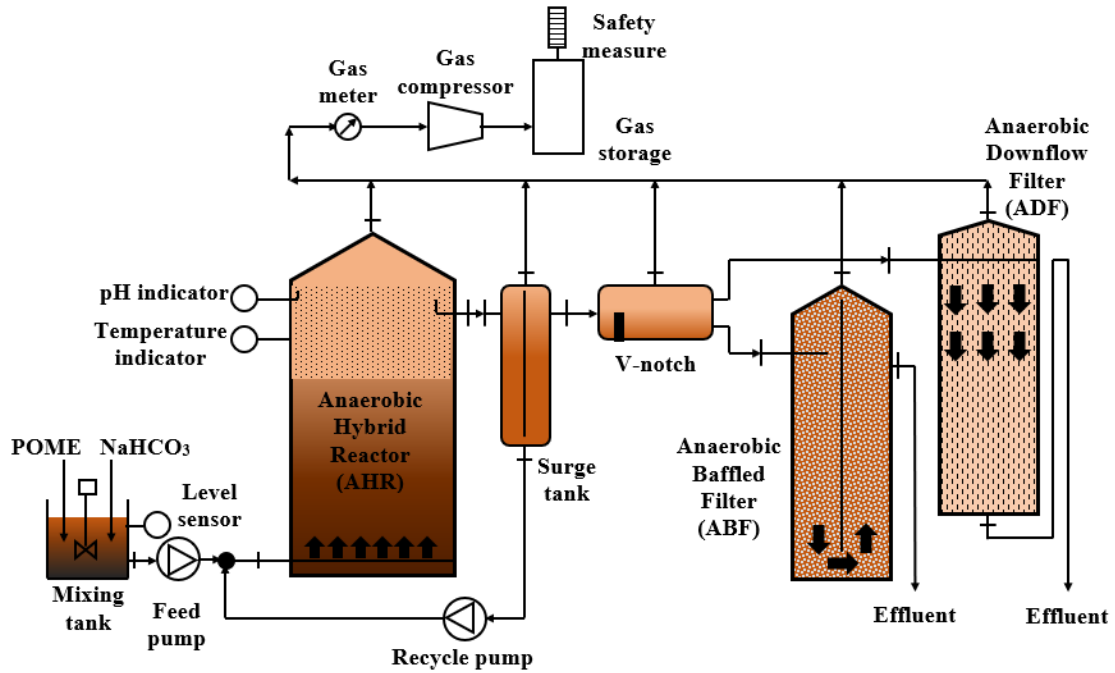


Figure 2.9: Schematic Diagram of AHR, ABF and ADF (Choi et al., 2013)

Raw POME underwent pre-treatment by a 3-phase screw decanter to remove excess SS and oil. The combined system was operated under a mesophilic temperature of 35–37 °C. pH was adjusted to around 7 during the initial stage. The overall HRT was in the range of 0.7 to 2.4 days. OLR was increased stepwise from 0.91 to 23 kg COD/m³/d. The parameters measured were COD values, SS, and volume of biogas.

Experimental findings demonstrated that when the OLR was 13 kg COD/m³.d in the AHR reactor, the maximum removal efficiency of COD was at 95.6%. Treated effluent from AHR was fed into ABF and ADF achieving COD removal efficiency of approximately 93.5%. As for biogas production, the maximum level of biogas production was achieved at 110 L/d at an OLR of 18.9 kg COD/m³.d. The average methane yield obtained was within the range of 0.171–0.269 L CH₄/gCOD_{removed}. Methanogens were probably not active at high OLR (19.9–21.9 kg COD/m³.d) causing methane yield to decrease. The combination system of AHR, ABF, and ADF was shown to perform well and stable under high OLR conditions.

2.10.9 Up-flow Anaerobic Sludge Blanket Reactor

A study was conducted by Ahmad et al. (2011) to examine the treatability of POME and other effects of CaO on methanogenesis process due to the granulation process in UASB reactors. The aim of this study was to determine the influent CaO-CKD concentration and the relationship between the CaO concentration in the feed and biomass accumulation. The specific granulation, methanogenic activity, density, and composition of granules were also studied. UASB reactors were used to conduct this experiment. The experimental setup is shown in Figure 2.10.

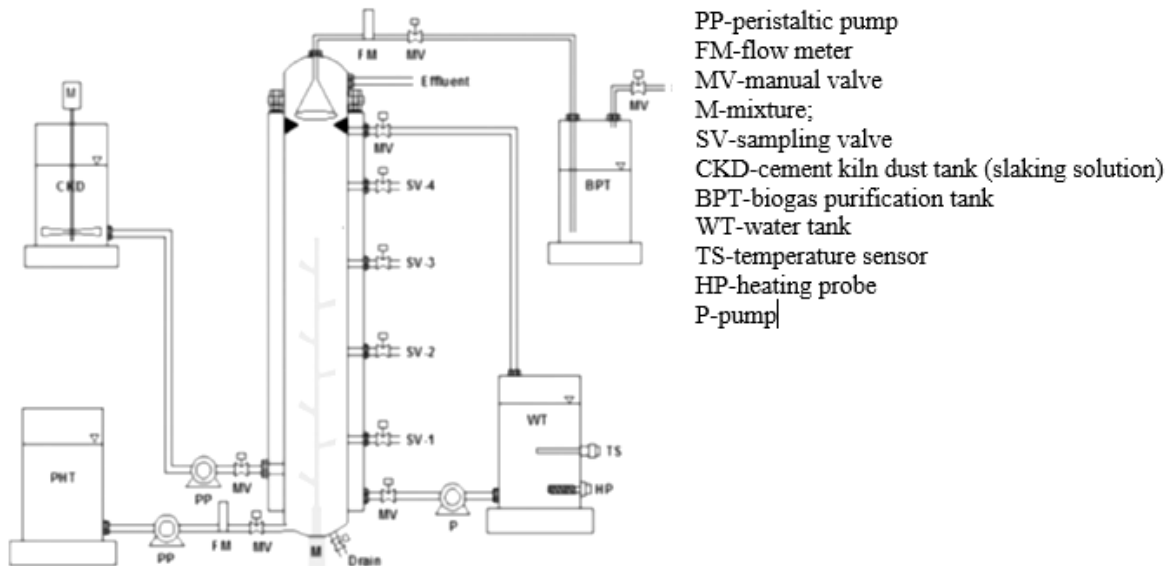


Figure 2.10: Experimental Setup of UASBR (Ahmad et al., 2011)

It was operated at temperature of 35 °C. The feed flow rate was set at 1.25 L/d and HRT was fixed at 4 days. Six reactors were used which were added with different concentration of CaO. A continuous feeding was performed with an initial OLR of 15 kg COD/m³.d. The reactor was monitored on the COD removal efficiency, VFA, effluent VSS, MLVSS concentration, and methane yields.

In the UASB reactor, CaO played important roles in the process of granulation. These included migrations of cells to the surface of other cells, increased precipitation, improved the adhesion of cells by microbial activities, and growth of cells to develop granules. Based on COD removal, the reactor achieved an average efficiency of 82.4% when it was fed with 10 g/L CaO at an OLR of 12.5 kg COD/m³.d at temperature of 35 °C. The average methane yield was measured at 0.91 L CH₄/g COD_{removed}. It was found that 88.6% of COD removed was converted into methane when UASB was operated at OLR of 12.5 kg COD/m³.d. When the concentration of calcium oxide increased, the specific activity of granules decreased resulting in a low bacterial specific activity.

2.10.10 Up-flow Anaerobic Sludge Blanket and Expanded Granular Sludge Bed

Reactors

A study was conducted by Fang et al. (2011) on the performance of UASB and EGSB on continuous treatment of POME and deoiled POME. The aim of this study was to compare both reactors in terms of methane production rate, methane yield, OLR, process stability, and COD removal efficiency. One 1.5 L UASB reactor and one 1.3 L EGSB reactor were used to conduct the experiment. Both reactors were operated at thermophilic temperature at 55 °C. Sodium carbonate (NaHCO₃) was added into the reactors to achieve pH value of more than 6. Both reactors were operated at HRT of 10 days and then reduced to 5 days. Different substrate concentrations were used to vary the OLR at 1.3 to 10.4 g VS/L.d. The parameters measured included biogas production, pH, and VFA.

When POME was used as a substrate, the methane potential achieved a maximum of 503 ml CH₄/g VS-added at 55 °C. On the other hand, deoiled POME achieved a maximum methane potential at 610 ml CH₄/g VS-added at 55 °C. For the treatment of POME, both

reactors were operated at maximum OLR of 5.8 g VS/L.d achieving removal rate of COD at an efficiency of 90% and methane yield of 438 ml CH₄/g VS.

For the treatment of deoiled POME, more than 90% of COD removal were produced at OLR of 2.6 g VS/L.d. The methane yield in UASB reactor for deoiled POME was 600 ml CH₄/g VS-added compared to EGSB reactor which was 555 ml CH₄/g VS-added. Based on this study, the UASB reactor was found to perform better than EGSB reactor in terms of stability, methane production, and low VFA concentration. Overloading of substrate could produce excess amount of acid which interrupted methanogenesis resulting in reduction of methane yield.

2.10.11 Up-flow Anaerobic Sludge Blanket Reactor

A study was conducted by Khemkhao et al. (2012) on the microbial diversity and performance of UASB for treating POME. The aim of this study was to evaluate the effects of OLR and operating temperature on the microbial density and performance of UASB treating POME. Two UASB reactors were used to conduct this experiment. The first reactor (U1) was operated at mesophilic temperature of 37 °C. The second reactor (U2) was operated from mesophilic at 37 °C to thermophilic at 57 °C with increments of 5 °C. Raw POME was filtered and pre-treated before it was used as substrate. Sodium hydroxide (NaOH) was added into the sample to obtain pH value of 7. Both reactors were operated at HRT of 2.4 days with up-flow velocity of 0.3 m/h. The OLR of both reactors were increased at 2.23, 3.95, 5.76, 7.77, and 9.47 g COD/L.d. Parameters measured included COD, VSS, and biogas composition.

The study revealed that both reactors achieved COD removal of more than 80% as OLR was increased. However, when the OLR reached 9.47 g COD/L.d, only U2 was able to achieve COD removal efficiency of more than 80%. As for biogas production, the volume of biogas for U1 and U2 were 10 L and 13 L respectively. U2 produced methane content of 76% which was higher compared to U1 in which methane content was 75%. Based on the findings, thermophilic anaerobic digestion was the preferable operating condition to treat POME as compared to mesophilic anaerobic digestion. Besides that, the desired microbial community for treating POME can be achieved in a thermophilic reactor and high OLR.

2.10.12 Up-flow Anaerobic Sludge Blanket Reactor

A study was conducted by Ahmad and Ghufuran (2014) to evaluate the effect of cement kiln dust (CKD) as a substrate to neutralize and granulate POME in a laboratory-scale UASB reactor fed with CaO-CKD. The aims of this study were to evaluate the characteristics of granule at different OLR and HRT as well as bio-kinetics of granule and mass fraction of methanogens (f) with different types of substrates. A UASB reactor was used to conduct this experiment operating at temperature of 35 °C. The substrate was fed at a flow rate of 0.53 L/d and at HRT of 3.35–34.5 days. A 5-rpm stirrer was installed in the reactor to prevent CaO-CKD from settling. The reactor was continuously fed with an initial OLR of 4.5 kg COD/m³.d and varied from 1.5 to 46 kg COD/m³.d. pH value of the reactor was at 7.5. Parameters such as BOD, COD, gas volume, VFA, pH, TS, total Kjeldahl nitrogen, and oil and grease were measured.

The research findings stated that the removal efficiencies of COD and BOD achieved values of 97% and 79% respectively as HRT was increased from 12.5 to 24.5 days. The values of granulation and growth yield were found to be highest at HRT of 24.5 days. VSS

and MLVSS concentration exhibited the same trend, which decreased as HRT was increased to 34.5 days. As for methane production, it was increasing up to 2.5 L/d at HRT of 24.5 days under COD concentration of 46 g/L. The production rate of methane was at 0.85 L/g COD_{removed} at HRT of 24.5 days. Based on this study, CKD was chosen as a substitute for lime to increase the pH of POME. Methanogens were bacteria which can grow and produce maximum amount of methane in a neutral environment.

2.10.13 Thermophilic Continuous Stirred Tank Reactor

A study was conducted by Irvan et al. (2012) on the effect of a thermophilic CSTR on the treatment of POME. The aim of this study was to evaluate the methane emission in a thermophilic anaerobic digestion of POME. The pH of POME was adjusted with NaHCO₃ before it was fed. A premixed chemical solution consisted of FeCl₂, NiCl₂·6H₂O, and CoCl₂·6H₂O was also added as a nutrition for methane-producing bacteria. The reactor was operated at HRT of 4, 6, and 8 days. The temperature of the reactor was maintained at 55 °C. Parameters measured included amount of gas generated, pH, amount of feeding and discharging of substrate, TS, M-alkalinity of substrate, and VS of POME.

The study revealed that the highest amount of biogas was produced at HRT of 4 days which was 16.14 L/d. For the decomposition rate of VS, HRT of 8 days gave the highest at 63.5% while the lowest was at HRT of 4 days at 53.5%. The low decomposition rate of VS could be a result of instability inside the reactor in which high amount of COD and low nitrogen found in POME. As for CH₄ concentration, HRT of 4 days gave the highest amount at 67% compared to 66% and 63% produced at HRT of 6 and 8 days respectively. The acceptable HRT for CSTR was at HRT of 8 days as high amount of VS and COD decomposition rate were produced. The methane content recorded for HRT of 8 days was

64%. A balance between high and low HRT was needed to achieve acceptable results in methane production. Other operating conditions including pH, SRT, and OLR were among the factors that could affect methane production as well.

2.10.14 Membrane Anaerobic System

A study was conducted by Abdurahman et al. (2011) on the performance of membrane anaerobic system (MAS) as an alternative and cost-effective method to treat POME for reducing its hazardous elements. The aims of this study were to evaluate the performance of MAS in treating POME to produce methane and to determine the kinetic parameters of the process based on Monod, Contois, and Chen and Hashimoto models. This system was mainly consisted of a cross flow ultra-filtration membrane (CUF) and an anaerobic reactor. The membrane was used to separate biomass solids from the solid suspension to be recycled. The experimental setup was presented by Figure 2.11.

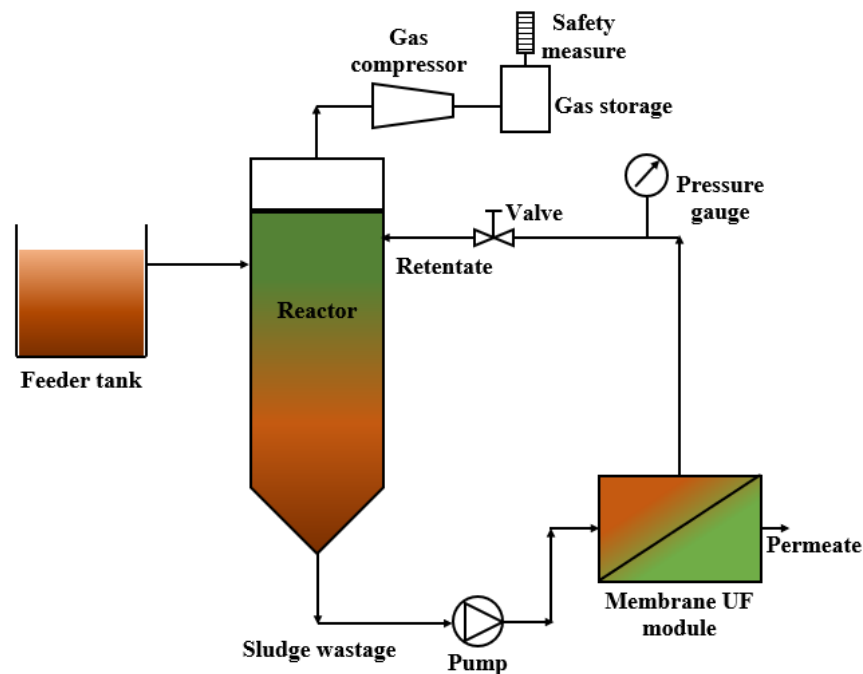


Figure 2.11: Experimental Setup of MAS (Abdurahman et al., 2011)

The reactor was operated at OLR ranging from 2 to 13 kg COD/m³.d, SRT from 800 to 11.6 days, and HRT from 400.6 to 5.7 days. Parameters measured included COD, TSS, pH, VSS, substrate utilization rate (SUR), and specific substrate utilization rate (SSUR). Six steady-states were achieved in this operation. The pH of the system was maintained within the optimal condition for anaerobic reactors which was between 6.7–7.8.

The findings revealed that at OLR of 13 kg COD/m³.d, the MAS obtained COD removal efficiency of 94.8% which produced effluent COD of 2,279 mg/L. The COD removal efficiency was in the range of 94.8–96.5% as HRT was increased from 5.7 to 400.6 days. The treated POME was found to be much clearer in terms of colour compared to raw POME. As for biogas production, the rate increased from 0.27 L/g.d to 0.83 L/g.d as OLR was increased from 2 to 13 kg COD/m³.d. However, the methane content decreased at increasing OLR and the values ranged from 68.7–74.2%. To ensure that anaerobic reactors to perform successfully, factors such as pH, mixing, operating temperature, nutrient availability, and organic loading rate must be operated effectively.

2.10.15 Carrier Anaerobic Baffled Reactor

A study was conducted by Malakahmad and Yee (2014) on the performance of carrier anaerobic baffled reactor (CABR) on harvesting biogas at short HRT. The aim of this study was to investigate the biogas production rate from POME during start-up of a laboratory scale CABR without effluent recycling and under the effects of OLR, alkalinity supplementation, and short HRT. The CABR system contained a series of up-flow packed-bed attached growth reactors. The substrate was forced to flow under and over the vertical packed-bed baffles. This increased the contact time between substrate and active biomass at short HRT. Effluent produced was free from biological solids. The benefits of using CABR

are able to sustain high loading of COD, require small reactor volumes, and simple operating conditions. The experiment is pictured in Figure 2.12.

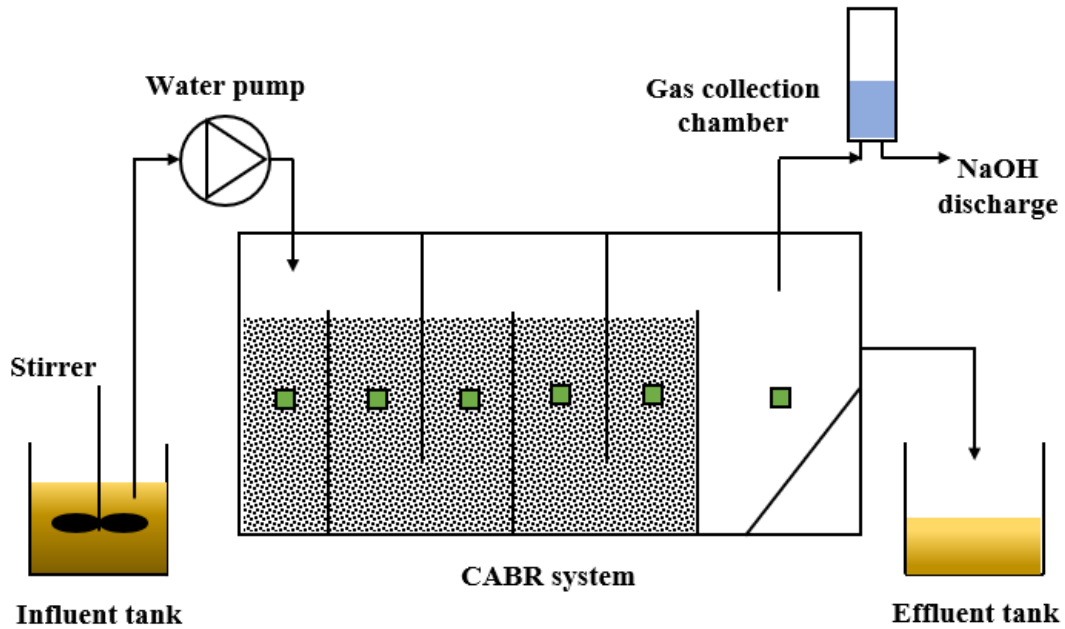


Figure 2.12: Schematic Diagram of CABR (Malakahmad & Yee, 2014)

The CABR was operated at initial OLR of 0.46 g COD/L.d and a constant HRT of 4 days. The operating temperature was maintained around 35 °C and pH about 7.2. The parameters measured in this study included HRT, OLR, solid loading rate (SLR), food-to-microorganisms (F/M) ratio, influent flow rate, volume of reactor, biomass concentration (MLVSS), COD, and methane production rate.

The findings reported that the COD removal efficiency was steady throughout the experiment. The removal efficiency increased as OLR was increased. The CABR system achieved COD removal efficiency of 82% with an OLR of 11.38 g COD/L.d. The methane yield was improved from 0.05 to 0.25 L CH₄/g COD as the OLR was increased. The amount of methane contained in the biogas was measured to be 54–75%. As for VFA concentration,

it was increased at increasing OLR resulting in the decrease of pH in the system. At high OLR, it illd encourage the growth of acidogens resulting in the decrease of pH and methane content. The pH illd increase as methanogens beian to convert hydrogen and acetic acid into methane gas and carbon dioxide resulting in the increasd of methane content.

2.10.16 Semi-Continuous Stirred Tank Reactor

A study was conducted by Alrawi et al. (2011) on the efficiency of co-digestion of POME with rumen fluid (RF) using a semi-continuous stirred tank reactors (SCSTR). The aim of this study was to treat POME anaerobically by co-digestion with RF and compare it with mono-digestion of POME by using SCSTR under mesophilic conditions around 37 °C. The experimental setup was presented by Figure 2.13.

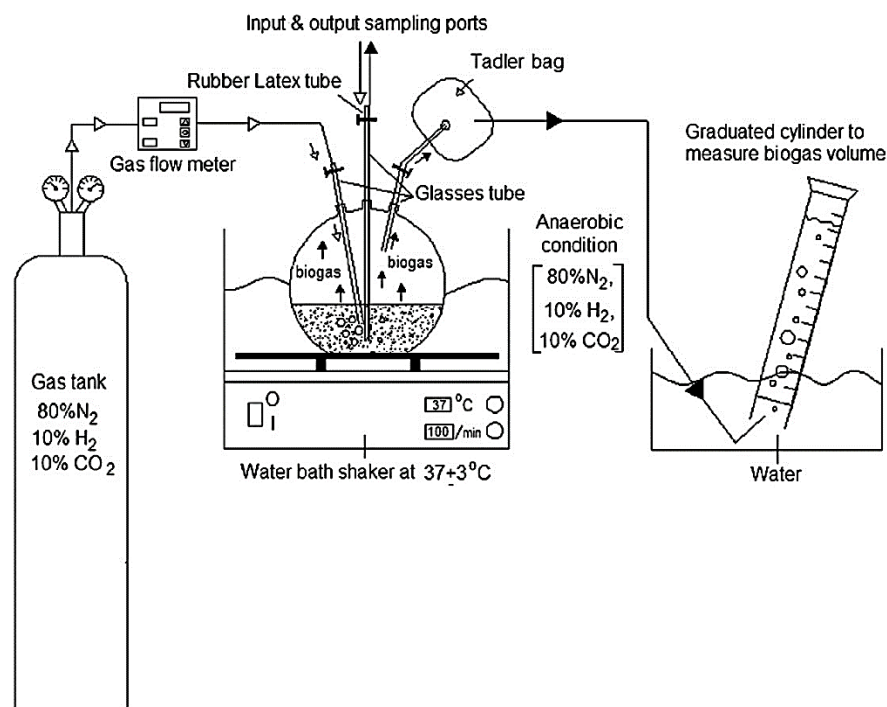


Figure 2.13 Schematic Diagram of SCSTR (Alrawi et al., 2011)

The experiment was conducted at HRT of 20, 10, and 6 days and OLR of 1.65, 2.85, and 4.06 g COD/L.d. 3 different mixtures known as mixture A, B, and C were prepared for

this experiment. Mixture A consisted of 5% RF and 95% POME. Mixture B consisted of 10% RF and 90% POME. Mixture C consisted of 100% POME. The main parameters which were measured included pH, COD, MLVSS concentration, and rate of CH₄ production.

The result demonstrated that the rate of CH₄ production increased as COD removal efficiency increased. Digester with mixture B produced the highest COD removal efficiency of 96.48% at OLR of 1.65 g COD/L.d and HRT of 20 days. However, the highest CH₄ production rate was 61.85% at OLR of 4.06 g COD/L.d and HRT of 6 days. The pH of digesters with mixture A, B, and C were relatively stable at different HRT and OLR. The pH measured was in the range of 7.05–7.55 providing a suitable environment for methanogenesis. Based on this study, co-digestion had shown to be more effective compared to mono-digestion. Co-digestion of POME with other co-substrate could prove to be more effective in COD removal efficiency as well as improving the production rate of methane.

2.10.17 Ultrasonic Membrane Anaerobic System

A study was conducted by Nour and Nour (2017) to evaluate the performance of ultrasonic membrane anaerobic system (UMAS) on the treatment of POME. The aim of this study was to evaluate the performance and kinetics of UMAS based on Monod, Contois, and Chen and Hashimoto models. A new designed laboratory scale UMAS reactor was used for this experiment. The reactor was consisted of a crossflow ultrafiltration membrane and an anaerobic reactor. The experimental setup was presented by Figure 2.14.

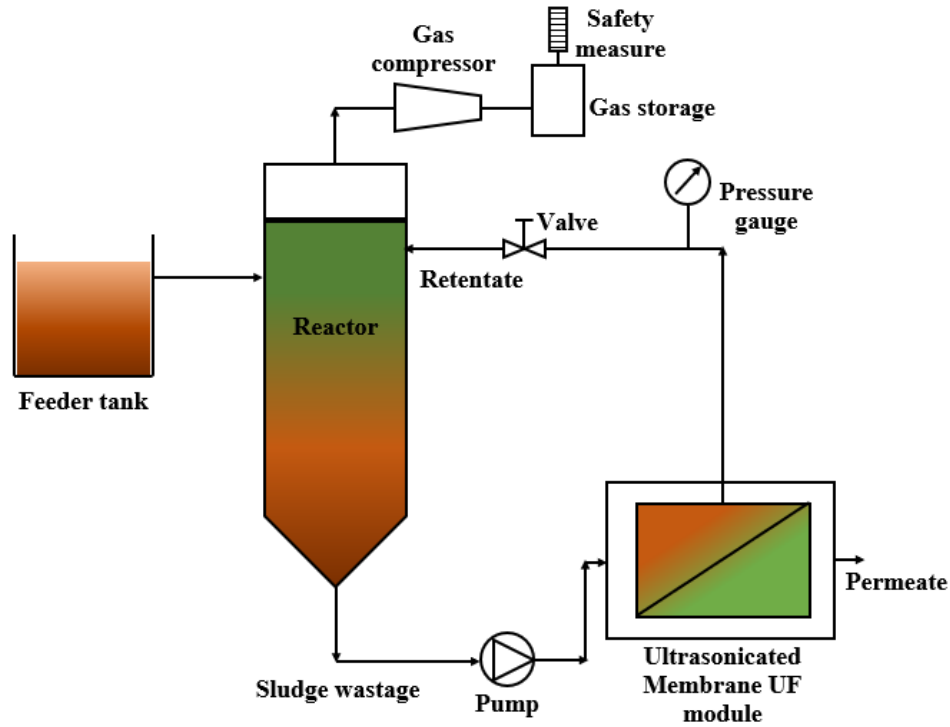


Figure 2.14: Experimental Setup of UMAS (Nour & Nour, 2017)

The UMAS was operated at different COD concentration ranging from 70,400–90,200 mg/L. As for HRT and OLR, the UMAS was operated from 14.7–500.8 days and from 1.5–9.0 kg COD/m³.d respectively. The pH of raw POME was adjusted by adding NaOH to obtain a value around 7.0. Parameters such as COD, BOD, pH, VSS, TSS, and methane gas production were measured. The sixth steady state which was fed with the highest COD concentration at 90,200 mg/L at OLR of 15 kg COD/m³.d produced a COD removal efficiency of 92.8%. the first steady state which was fed with the lowest COD concentration at 70,400 mg/L at OLR of 1.0 kg COD/m³.d produced a COD removal efficiency of 98.3%.

The findings demonstrated that COD removal efficiency decreased from 98.3 to 92.8% as HRT was decreased from 500.8 to 8.6 days. However, the UMAS reactor still achieved COD removal efficiency of more than 90% which proved that it performed as good

as other anaerobic system. The increased in biogas production from 0.48 to 0.81 L/g.d was measured as OLR increased from 1.0 to 15 kg COD/m³.d. The methane content dropped from 81 to 64.6% as OLR was increasing while HRT was decreasing. The newly designed UMAS performed rather well to remove COD around the range of 92.8–98.3%. It was also able to recover 64.6–81% of methane from the treated POME.

2.10.18 Two-Stage Anaerobic Digestion Process

A study was conducted by Trisakti et al. (2016) on the performance of a two-stage anaerobic digestion process in which substrate agitation was performed in the digester. The aims of this study were to evaluate the effects of increasing of OLR or reduction of HRT and agitation rate on methanogenesis stage during anaerobic digestion of POME to biogas. The experiment was conducted using a CSTR in which a two-stage process of anaerobic digestion was performed. The experiment was shown in Figure 2.15.

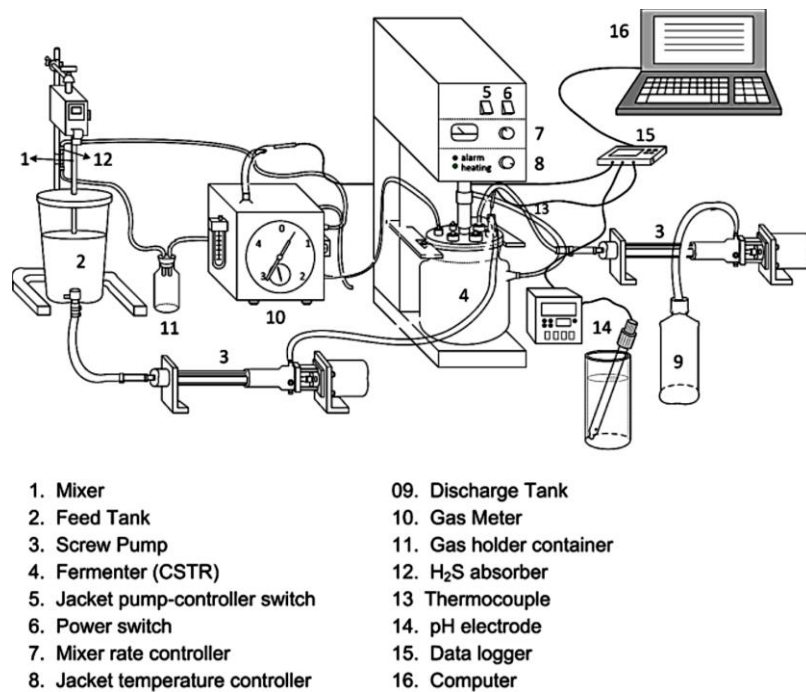


Figure 2.15: Experimental Setup of CSTR (Trisakti et al., 2016)

The first experiment was conducted with varying HRT of 100, 40, 6, and 4 days at mixing rate of 100 rpm. It was operated at room temperature and pH was adjusted to around 7. The second experiment was conducted with varying agitation rate at 50, 100, 150, and 200 rpm by maintaining HRT of 4 days. It was operated at temperature of 55 °C and pH was also adjusted to around 7. Parameters which were measured included TS, VS, TSS, VSS, COD, and production rate and composition of biogas.

From the experiment, the findings revealed that the number of microorganisms which was represented as VSS, increased as the rate of agitation was increased. Agitation rate of 200 rpm produced the highest VSS value of $26,404.33 \pm 944.94$ mg/L. As for microbial activity, the highest COD removal and VS decomposition were achieved at 100 rpm giving values of 81% and $67.44 \pm 3.59\%$ respectively. Agitation rate of 100 rpm also produced the highest biogas production at 58.87 ± 6.27 L/kg VS. Therefore, the best results were obtained at agitation rate of 100 rpm. The purpose of mixing or agitation is to increase the contact time between substrate and microorganisms, maintained pH value, prevented sedimentation of denser solids, and aided in release of biogas bubbles.

2.11 Process Optimization of POME Digestion

The anaerobic digestion process offered many benefits to recover resources from POME mainly in the form of biogas, water, and organic fertilizers. Anaerobic digestion was widely used in industries especially in wastewater treatment process either to recover biogas or produce cleaner effluent. The optimization of the anaerobic process was still needed because this process was influenced by various factors such as microbial communities, retention time, amount of food for bacteria, temperature, and pH. Appels et al. (2011) stated that the process of anaerobic digestion could be optimized if the following fields were

identified: (1) the understanding of roles of microorganisms in digestion process and (2) the importance of mathematical model on production optimization.

2.11.1 Roles of Microorganisms in Digestion Process

Fundamental knowledge on the stages of anaerobic digestion was crucial to understand the mechanism of anaerobic digestion process. Different stages of anaerobic digestion involved different types of bacteria. Somehow, the basic understanding of the digestion system was lacking due to the complexity of the microbial activity. Studies conducted on the composition of microbial community would indeed add extra knowledge on the advancement and optimization of anaerobic system (Rittmann et al., 2008). As an example, a study was conducted by Chouari et al (2005) on a combination of 16S ribosomal RNA gene clone library sequencing and dot blot hybridization to analyse the structures of microbes during digestion of waste-activated sludge. Another study conducted by Cirne et al. (2007) on a two-stage batch digestion of sugar beets and grass/clover was able to determine the microbial groups which are responsible during the hydrolysis stage. A study on methanogens was conducted by Morris et al. (2014) using quantitative polymerase chain reaction (PCR) found that digesters with high methane production rate had higher numbers of methanogen-specific gene *mcrA* per nanogram DNA and *mcrA* transcripts per nanogram RNA.

2.11.2 Mathematical Model on Production Optimization

The technique to achieve optimization in the production of biogas could be adapted from Operations Research (Ba et al., 2016; Trigeorgis & Tsekrekos, 2018). In optimization, the decision maker could formulate strategies to develop a feasible solution. In each optimization problem, the objective was usually to minimize or maximize a function over a

set of variables under certain constraints. In linear programming, the optimization problem could be solved by the mathematical models stated by Equation 2.16 to Equation 2.19:

Maximize objective function Z , where X is a variable and C is a constraint.

$$Z = C_1X_1 + \cdots + C_nX_n \quad \text{Eq. 2.16}$$

Subject to constraints b_1 and b_m .

$$a_1X_1 + \cdots + a_nX_n < b_1 \quad \text{Eq. 2.17}$$

$$a_mX_1 + \cdots + a_mX_n < b_m \quad \text{Eq. 2.18}$$

$$X_1 > 0, \cdots, X_n > 0 \quad \text{Eq. 2.19}$$

The typical input-output production optimization was presented in Figure 2.16.

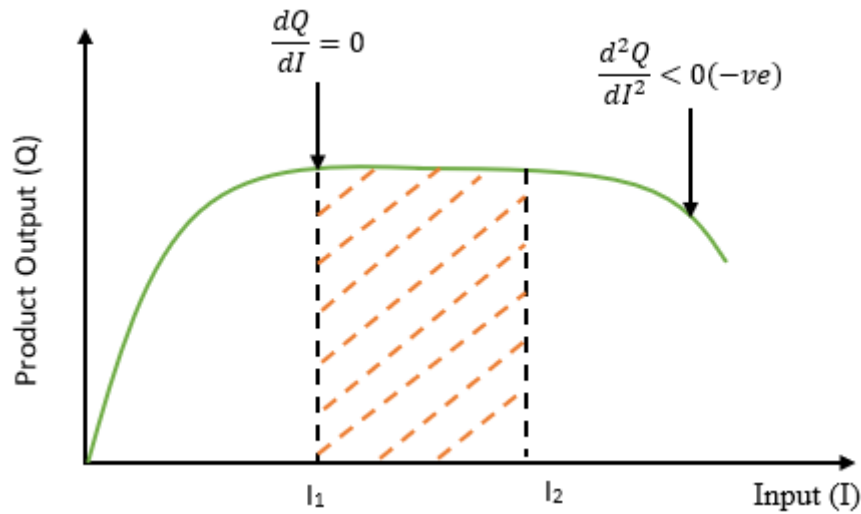


Figure 2.16: Input-Output Optimization (Burdett, 2016)

Based on Figure 2.16, the maximum output was produced at input I_1 and the point in which $\frac{dQ}{dI} = 0$ was known as the optimum point. Inputs greater than I_1 would not increase the amount of output. The shaded region under the curve was known as non-value-added inputs. These non-value-added inputs would contribute to increase production cost and

reduced the effectiveness of the output production. In the aspect of COD reduction, which was the objective of the current research, the input-output optimization model could be applied to optimize factors with regards to COD reduction.

2.11.3 Central Composite Design on Production Optimization

CCD was the most commonly used response surface designed experiment (Dima et al., 2020). CCD consisted of a factorial or fractional factorial design with center points, augmented with a group of axial points (also called star points) to estimate the curvature. CCD could be used to predict first- and second-order terms efficiently. By adding center and axial points to a previously done factorial design, a response variable with curvature could be modelled. CCD was especially useful in sequential experiments because it could build on previous factorial experiments by adding axial and center points. CCD had the desired properties of orthogonal blocks and rotatability (Talha et al., 2018).

- Orthogonal blocks: Often, CCD was done in more than one block. CCD could create orthogonal blocks, allowing model terms and block effects be estimated independently and minimizing the variation in the regression coefficients.
- Rotatability: Rotatable designs provided constant prediction variance at all points that were equidistant from the design center.

2.11.4 Fundamental of CCD

The CCD could be used as a statistical method based on the multivariate nonlinear model to optimize process variable and determine the regression model with operating conditions from the appropriate experiments (McVoitte & Clark, 2019). It was suitable to study the relationships of various parameters that affected the process. Generally, the optimization process consisted of three important steps:

- Performing the statistically designed experiments,
- Estimating the coefficients in a mathematical model, and
- Predicting the response and checking the adequacy of the model.

The regression model in Equation 2.20 could be used to analyze the effect of parameter interactions.

$$Y = \beta_0 + \beta_i x_i + \beta_{ij} x_i x_j + \beta_{ii} x_{ii}^2 + \epsilon \quad \text{Eq. 2.20}$$

Where Y is the response variable; β_0 is the intercept; β_i , β_{ij} and β_{ii} are coefficients of the linear effect, double interactions; x_i , x_j are the independent variables or factors and ϵ is error (Arunkumar et al., 2014). Statistical experimental designs were powerful tools to search for the key factors rapidly from a multivariable system. Plackett-Burman design was one such method that had been frequently used for screening multiple factors at a time (Vi et al., 2017). This experimental design was particularly useful for initial screening as it was used for the estimation of only the main effects. The significant factors obtained from the screening experiments could be further optimized by employing response surface methodology that enabled the study of interaction effects among different variables. RSM was a statistical experimental technique applied under appropriate experimental design to resolve multi-variable equations.

2.11.5 Benefit of Using CCD in Experiment Design

The CCD, D-optimal, and Box-Behnken were found to be widely used optimization techniques for methane production and COD reduction from POME because of the advantage of optimizing multifactor problems with optimum number of experimental runs (Dima et al., 2020). Besides, these methods had limitation of increased number of experiments if several factors were selected for process optimization. With multiple factors,

these techniques were not appropriate because it increased the cost of chemicals, time, and physical efforts. Therefore, a simplified design strategy was required that could be used to overcome these problems. CCD suggested more experiments than other methods which gave a more appropriate result which was more precise (Talha et al., 2018). An example of methane production optimization, CCD suggested up to 20 experimental runs if three factors were required to be studied. At optimized conditions, methane production would be able to achieve 79 volume per day for CCD. Interactive study of operating parameters by 3D contour plots produced by CCD also exhibited pH, C/N, and VSS as the most significant factors. However, quantification of contribution was not possible with CCD.

It could be concluded that CCD was a robust statistical tool for experimental design and process optimization (Kainthola et al., 2020). Data analysis and optimization of operating parameters were possible with fewest numbers of experiments, less computational experience, and graphs obtained which were easy to read and understand. The optimized values obtained for CCD was in good agreement to be used in engineering applications. Therefore, it can be concluded that it was very essential for Engineering Design.

2.11.6 Response Surface Methodology on Production Optimization

Response surface methodology (RSM) was a collection of mathematical and statistical techniques useful for developing, optimizing, and improving products and industrial processes. It was highly useful when many factors affected production, yield, or percent removal of a process. RSM initiated from the DOE was used to determine the significant factors affecting an experiment. It aimed at reducing the number of experimental runs while maximizing output through the data generated. The data could be used to develop practical models that correlated the response to the experimental factors. The model

facilitated the search for a better process response that was validated through experiments. The model repeated till an optimal process was identified or the limit of experimental resources was reached (Danmaliki et al., 2017).

The RSM was the most popular multivariate statistic technique, which had been used in the optimization of food processes (Baş & Boyacı, 2007). RSM was a collection of statistical and mathematical methods established on the fit of a polynomial model to the data that must depict the behaviour of a data set with the purpose of making statistical predictions. The approach was useful for optimizing, designing, developing, and improving processes where a response or responses were affected by several variables (Ghorbannezhad et al., 2016; Kaushik et al., 2006). The objectives of RSM included the determination of variable settings for which the mean response was optimized and the estimation of the response surface near this good location. RSM could be used to analyze and model their adsorption behaviour. RSM could also predict the suitability of output parameters.

In RSM method, the least square multiple regression methodology was usually applied to investigate the relationship between the independent and dependent variables. A multiple regression equation could be used to fit the second-order polynomial equation based on the experimental data as follows which was presented by Equation 2.21.

$$Y = \beta_0 + \beta_i x_i + \beta_{ij} x_i x_j + \beta_{ii} x_{ii}^2 + \epsilon \quad \text{Eq. 2.21}$$

Where y represents the predicted response, β_0 is the model intercept, and β_i , β_{ii} , and β_{ij} are the regression coefficients for the linear, quadratic, and interactive effects of the model, respectively. x_i and x_j are the factors and k is the number of factors (Ghorbannezhad et al., 2016). The model used for optimizing food processes in the RSM methodology was

not always a second order. The regression coefficients that had a statistically significant effect on response should be considered in the equation. Therefore, if quadratic terms were not statistically significant, those coefficients are removed, and equation will be linear (Yolmeh & Jafari, 2017).

2.12 Research Gap Analysis and Chapter Summary

This study has attempted to review the literature published on the operational conditions that affects COD reduction from POME. Major areas considered in the literature review framework revealed the effects of pH, C/N, OLR, HRT, and SRT on COD reduction at optimum operating conditions. Emphasis was given to conduct this study by using a two-stage fermentation, an anaerobic reactor and inoculum. Table 2.3 listed the summary of research findings on POME treatment.

Table 2.3: Summary of Research Findings for POME Treatment

Type of anaerobic digester used	Controlling parameters of experiment	Result of experiment	References
UMAS	HRT = 8.6 d OLR = 15 kg COD/m ³ /d	COD removal: 92.8% HRT of 8.6 days and methane content of 64.6%	Nour and Nour (2017)
CSTR	HRT = 4 d, At 55 °C and 100 rpm	COD removal: 81% HRT of 4 days and methane content of 76.64%	Trisakti et al. (2017)
UASB-CSTR	1 st stage: HRT = 9 h, at 55 °C OLR = 75 g COD/L/d 2 nd stage: HRT = 12 d, at 55 °C OLR = 12 g COD/L/d	COD removal: 40.0% HRT of 12-9 hours and methane content of 5% COD removal: 85.0% HRT of 12 days and methane content of 68%	Krishnan et al. (2016)
Modified CSTR	HRT = 3.3 d at 55 °C OLR = 19 g COD/L/d	COD removal: 82.0% HRT of 3.3 days and methane content of 68%	Khemkhao et al. (2015)
ASBR-UASB	1 st stage: HRT = 2 d, at 55 °C OLR = 60 g COD/L/d 2 nd stage: HRT = 15 d, at 37 °C OLR = 6 g COD/L/d	COD removal: 38.0% HRT of 2 days and methane content is unavailable COD removal: 95.0% HRT of 15 days and methane content of 51%	Mamimin et al. (2015)
EGSB	HRT = 9.8 d at 35 °C OLR = 6.45 kg COD/m ³ /d	COD removal: 94.89% HRT of 9.8 days and methane content of 65-70%	Wang et al. (2015)
UASB	HRT = 24.5 d, at 35 °C OLR = 46 kg COD/m ³ /d	COD removal: 97% HRT of 24.5 days and methane content of 91%	Ahmad and Ghufraan (2014)
AHR-ABF	Total HRT = 6.2 d, at 37 °C OLR = 15 kg COD/L/d	COD removal: 90.0–93.0% HRT of 6.2 days and methane content of approximately 68%	Jeong et al. (2014)

Table 2.3 continued

AHR-ABF	Total HRT = 6.2 d, at 55 °C OLR = 15 kg COD/L/d	COD removal: 93.0–95.0% HRT of 6.2 days and methane content of approximately 65%	Jeong et al. (2014)
CABR	HRT = 4 d, at 35 °C OLR = 11.38 g COD/L/d	COD removal: 82% HRT of 4 days and methane content of 54-75%	Malakahmad and Yee (2014)
UASB-HCPB	HRT = 2 d at 55 °C OLR = 27.65 g COD/L/d	COD removal: 91.8% HRT of 2 days and methane content of 57.4%	Poh and Chong (2014)
SCAR	HRT = 12 d at 35 °C OLR = 6.91 g COD/L/d	COD removal: 66.09% HRT of 12 days and methane content of 48.5%	Wong et al. (2014)
AHR followed by ABF and ADF	HRT = 0.7–2.4 d, at 35–37 °C OLR = 19–23 g COD/L/d	COD removal: 93.5% HRT of 0.7-2.4 days and methane content of 65%.	Choi et al. (2013)
CSTR	HRT = 8 d, at 55 °C	COD removal: 77% HRT of 8 days and methane content of 64%	Irvan et al. (2012)
UASB	HRT = 2.4 d, at 55–57 °C OLR = 9.5 g COD/L/d	COD removal: 81% HRT of 2.4 days and methane content of 76%	Khemkhao et al. (2012)
MAS	HRT = 5.7 d OLR = 13 kg COD/m ³ /d	COD removal: 94.8% HRT of 5.7 days and methane content of 68.7%	Abdurahman et al. (2011)
UASB	HRT = 4 d, at 35–38 °C OLR = 12.5 g COD/L/d	COD removal: 82% HRT of 4 days and methane content of 48%	Ahmad et al. (2011)
SCSTR	HRT = 6 d, at 37 °C OLR = 4.06 g COD/L/d	COD removal: 85% HRT of 20-6 days and methane content of 61.8%	Alrawi et al. (2011)
EGSB	HRT = 5 d, at 55–57 °C OLR = 9.7 g COD/L/d	COD removal: 92% HRT of 5 days and methane content of 60%	Fang et al. (2011)
EGSB	HRT = 5 d, at 55–57 °C OLR = 19.4 g COD/L/d	COD removal: 92% HRT of 5 days and methane content of 73%	Fang et al. (2011)

Table 2.3 continued

UASB	HRT = 5 d, at 55–57 °C OLR = 9.7 g COD/L/d	COD removal: 93% HRT of 5 days and methane content of 61%	Fang et al. (2011)
UASB	HRT = 5 d, at 55–57 °C OLR = 19.4 g COD/L/d	COD removal: 94% HRT of 5 days and methane content of 70%	Fang et al. (2011)

The gap between previous studies and this proposed research is reported in this section. The identified gap is determining the scope of study and to establish novelty. The main operational conditions considered in COD reduction are pH, C/N, OLR, HRT, and SRT. Based on Table 2.3, COD reduction methods are listed were reported, but the difference between the published research and the current one is the use of indigenous inoculum from banana peel which was not reported earlier. This inoculum would be used to adjust C/N (from 20 to 30) to achieve optimum level of COD reduction from POME. The research with inoculum with banana peel had not been published in papers. With this background, the research gap is on the use of banana peel waste to adjust C/N to achieve optimum level of COD reduction.

This study was undertaken to fill up the gap exists in the POME treatment process. The findings of literature review, publications and research gap analysis were the rationale of undertaking the current research “Optimization of factors affecting chemical oxygen demand reduction from palm oil mill effluent using anaerobic digestion”.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Overview of Research Methodology

This chapter describes the development of research methodology and materials for achieving the research objectives. This is applied research in engineering field focuses on COD reduction optimization from POME. The broad aim of this research is divided into three specific objectives and the research methodology is designed accordingly for achieving objectives. The experiment involved feedstock preparation with inoculum, anaerobic digestion, sample collection and laboratory analysis of both influent and effluent quality.

The research design for achieving goal is presented in Section 3.2. The anaerobic reactor start-up and feedstock preparation are stated in Section 3.3 and Section 3.4. Sample collection and analysis are explained in Section 3.5. Research variables are presented in Section 3.6.

3.2 Research Design for Achieving Research Objectives

A two-stage fermentation for reducing the COD in POME was a preferable method to separate acidogens and methanogens as conducted by Mamimin et al. (2015). The feed tank was used to prepare feedstock for POME before it is transferred into reactor 1. Reactor 1 was used for hydrolysis and acidogenesis, which is the first stage. Reactor 2 was used for acetogenesis and methanogenesis which is the second stage. Daily, the fermented POME in the reactor was recirculated by circulating pumps (P_2 and P_3 from Figure 3.1) and effluent recycling to mix the organic contents. POME samples were collected from output water tank (T_2 from Figure 3.1) to measure its COD.

The capacity of the reactor was 350 L which contains POME and inoculum. The POME and inoculum were mixed in the feed tank. The experiment was conducted at outdoor ambient temperature about 30–35 °C (mesophilic temperature) as conducted by Choi et al. (2013). A study was conducted by Jeong et al. (2014) showed that the anaerobic reactors performed similarly in both mesophilic and thermophilic condition and the organic content is highly biodegradable in mesophilic condition. The pH of the system was adjusted to 5–6 and 7–8 for the first stage and second stage respectively as done by Mamimin et al. (2015) by adding sodium hydroxide (NaOH). The pH range was measured using a pH meter. The pH of 5–6 was favourable for acidogens to grow but not methanogens (Ozgur & Peksel, 2013). The pH of 7–8 was to minimize the effects on biogas production as methanogens are sensitive to pH (Abdurahman et al., 2011). The HRT was measured as the residence time (hydraulic retention time) of POME inside the reactor. The sludge kept in a storage tank to obtain maturity, so the maturity time was measured as SRT. The experiments were carried out in triplicate to validate the outcomes of the experiment and the outcomes were used in 2 sigma variation.

Five significant factors were selected from literature reviews which may affect COD reduction. These factors include pH, C/N, OLR, HRT, and SRT. The inputs (pH, C/N, OLR, HRT, and SRT) were transferred into Design Expert software to obtain 5 level inputs based on CCD. A total of 50 runs with a combination of five independent variables were produced from DOE using Design Expert. ANOVA was performed to obtain the significant level of factors in COD reduction. The significant test was conducted at 95% confidence level. Factors with p-value of less than 0.05 were known as significant factors that contributed to COD reduction. For this case, it is related to specific objective one.

3.2.1 Research Design for Achieving Specific Objective Two

The experimental setup to achieve specific objective 2 was shown in Figure 3.1. The explanation on the methodology used was stated in Section 3.2. The data from the experiment was analysed to determine the effect of each factor on COD reduction.

3.2.2 Research Design for Achieving Specific Objective Three

The experimental setup to achieve specific objective 3 was shown in Figure 3.1. The explanation on the methodology used was stated in Section 3.2. The data from the experiment was analysed to determine the optimum values of each input using Design Expert software.

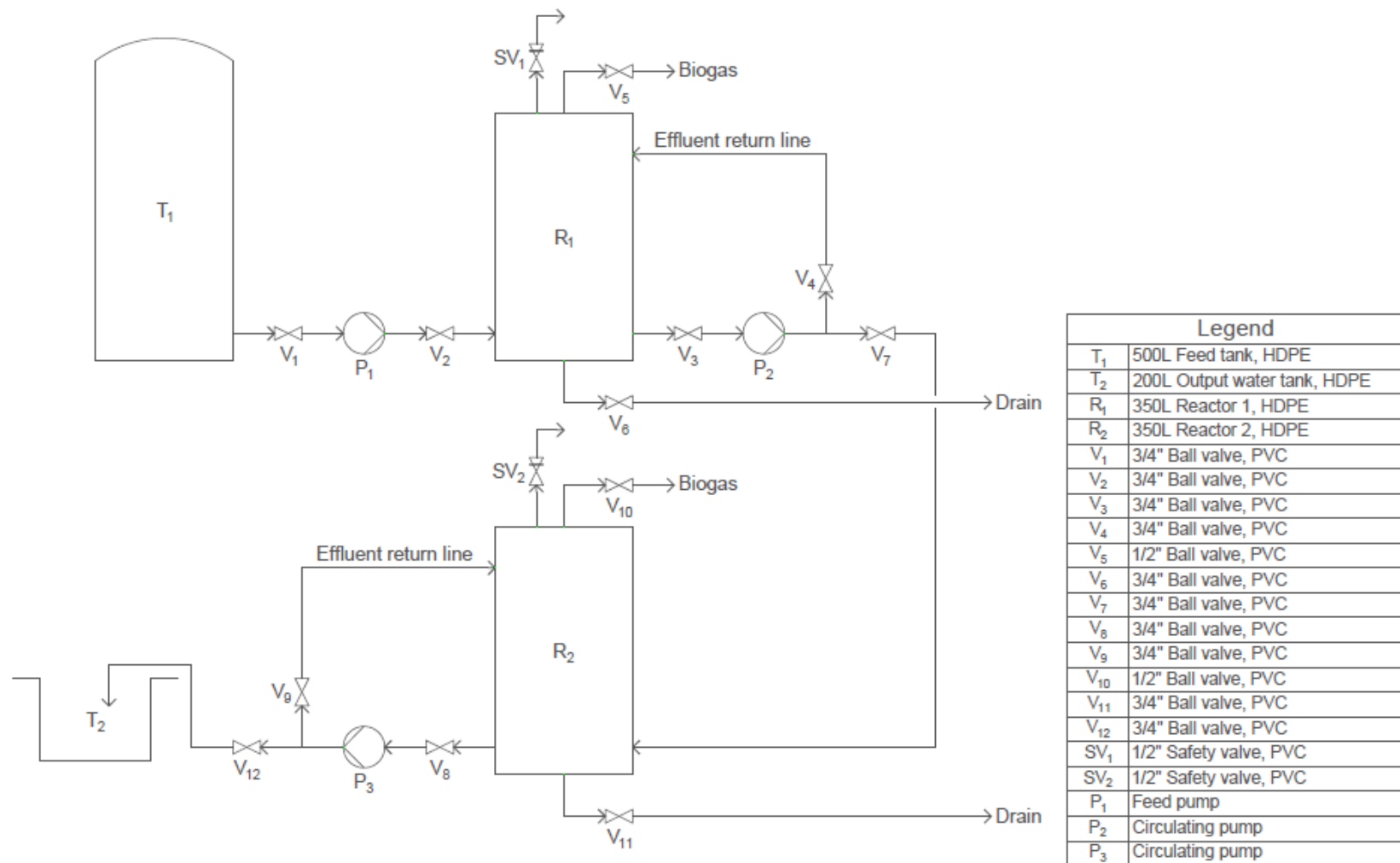


Figure 3.1: Lab-scale Reactor

3.3 Anaerobic Reactor Start-up

The start-up process was required to achieve smooth anaerobic reactor operation. Start-up process allows microorganisms found in the POME to grow and proliferate in order to aid in digestion of the organic matter (Idris et al., 2018). Acclimatization is essential to allow microorganisms to adapt to the current environmental conditions, allowing it to maintain their performance (Choong et al., 2018). A steady state was achieved when the performance of the bioreactor remained constant. The performance of the anaerobic reactor was in POME process and evaluated based on the COD of POME.

3.4 Feedstock Preparation

The substrate was a mixture of POME and inoculum. Waste banana peel was used to prepare the inoculum. The mesh size of the skin was converted to less than 1.0 mm and was kept 30 days at atmospheric temperature before it is added into the feedstock. In order to adjust the C/N, the weight of inoculum was adjusted before it is mixed with POME. Table 3.1 shows the characterization of the feedstock and method of preparation for inoculum is attached in Appendix C1.

Table 3.1: Characterization of Feedstock (Shahidul et al., 2018)

Properties	Value		
	POME	Inoculum	Substrate*
COD (g/L)	96	0.0	75
VSS (g/L)	30	80	35
pH	4.5	5.5	7.5
TS (g/L)	75	11	50
C/N	7	23.1–35	11–40

* The properties of substrate are due to mixing of POME and inoculum.

**pH of substrate was adjusted to 7.5 using sodium hydroxide (NaOH).

3.5 Sample Collection and Analysis

Fresh POME for this experiment was collected from the sludge pit of anaerobic pond at Bau Palm Oil Mill (BAPOM) using 25-litre high density polyethylene (HDPE) containers, before being transported to Operations Research Laboratory of Universiti Malaysia Sarawak.

3.5.1 Chemical Oxygen Demand Analysis

The reduction in COD was determined by collecting POME samples in the feed tank (initial COD) and output water tank (final COD). The COD tests were carried out according to DOE 2011, Reference Method.

3.5.2 Volatile Suspended Solids Analysis

A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105 °C. The total suspended solids were measured by weighing the amount of residue remaining on the filter. The residue was ignited to constant weight at 550 °C. The weight loss on ignition is the volatile suspended solids.

3.5.3 pH Analysis

A pH meter (Extech PH100) was used to measure the pH of a sample. Calibration was conducted by rinsing the measuring probe with distilled water before it was submerged in buffer solution of pH 7 and rinsed again with distilled water. The steps were repeated by using buffer solution of pH 4 and pH 10.

3.5.4 Total Solids Analysis

Water from a well-mixed sample in a dish is evaporated and the sample is dried to constant weight in an oven at 103 to 105 °C. Total solids are measured by weighing the amount of solids present in the dish.

3.5.5 Carbon-to-Nitrogen Analysis

The C/N ratio of inoculum was 35 and C/N ratio for POME was 7. An example for 100 L of POME and the goal of C/N is 26, the amount of inoculum was determined by using the ‘Goal Seek’ function in Excel. Figure 3.2 (a) shows the table used to calculate volume of POME and inoculum based on their carbon and nitrogen contents. Figure 3.2 (b) shows the ‘Goal Seek’ function to determine the volume required.

	C%	N%
Inoculum	35	1
POME	7	1
	Total kg C	Total kg N
Inoculum	73.88882	2.111109
POME	7	1
Total	80.88882	3.111109
	POME	Inoculum
Volume (L)	100	211.1109
		311.1109
C/N	25.99999	

Figure 3.2 (a): Carbon and Nitrogen Contents of POME and Inoculum

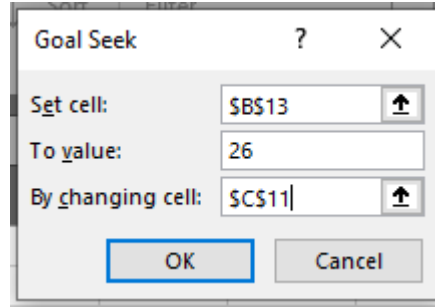


Figure 3.2(b): 'Goal Seek' Function

3.6 Research Variables

In this study, there are two variables which are dependent variable and independent variable. The dependent variable is the output of this research which is the COD reduction. The independent variable is the input of this research which consist of pH, C/N, OLR, HRT, and SRT.

3.6.1 Optimization of Inputs on COD Reduction

In this study, Design Expert (Version-2018) software was used which include the CCD option. In CCD, the minimum number of factors it can accommodate is two, such as numerical or continuous factors. The range of input was $-\alpha$, -1 , 0 , $+1$, and $+\alpha$ (Adeleke et al., 2019). From $-\alpha$ to $+\alpha$ was estimated using the CCD software. The number of experimental obtained at each number of factors is given by the formula stated in Equation 3.1.

$$N = 2^n + (2 \times n) + nc \quad \text{Eq. 3.1}$$

Where, N is the number of runs, n is the number of factors, nc is the number of center points the designer desire. In an experiment where the researcher wants to look at the effect of temperature and pressure on methanol yield aiming to reduce COD. For example, if we have 3 factors then the example calculation would be demonstrated as follows:

$N = 2^3 + 2 \times 3 + nc$ (which can be set between 2 to 6). Therefore, $N = 8 + 6 + 6$ (if the number of center point is assumed to be 6). $N = 20$ runs (max runs for 3 factors). To get value for the axial point we need to first get the value for α and the formula is given by Equation 3.2.

$$\alpha = 2^{\frac{k}{4}} \quad \text{Eq. 3.2}$$

Where, k is the number of factors. Equation 3.3 displays the calculation example used in this study. Therefore,

$$\alpha = 2^{\frac{k}{4}} = 2^{\frac{5}{4}} = 2.3784 \quad \text{Eq. 3.3}$$

To get the value of axial point, we apply the following equation. Axial point = $X \pm \alpha$ (Range/2), where X is the value of central point and range is between the upper and lower level divided by 2. From Equation 3.3 example,

$$\text{Axial point} = 6.5 \pm 2.3784 [(7-6)/2]$$

$$= 6.5 \pm 2.3784 (1/2)$$

$$= 6.5 \pm 1.1892$$

$$\text{For the upper axial point } (+\alpha) = 6.5 + 1.1892 = 7.6892$$

$$\text{For the lower axial point } (-\alpha) = 6.5 - 1.1892 = 5.3108$$

The outputs on the experiment range and level of the independent variables are shown in Table 3.2 and Appendix C2.

Table 3.2: Experimental Range and Levels of Independent Variables

Range and Level					
Variables	-α (2.3784)	Low (-1)	Central (0)	High (1)	+α (2.3784)
X ₁ (pH)	5.3108	6	6.5	7	7.6892
X ₂ (C/N)	11.7295	20	26	32	40.2705
X ₃ (OLR)	-6.9597	2	8.5	15	23.959
X ₄ (HRT)	-0.1352	4	7	10	14.1352
X ₅ (SRT)	5.8648	10	13	16	20.1352

The range of pH in the digestion process that marked by X₁(pH) has five values 5.310, 6, 6.5, 7 and 7.689, which indicates the experiment is conducted with five pH. This range of pH has been used by Bolzonella (2011), Carballa et al. (2011), Franco et al. (2007), and Sánchez et al. (2005) in the POME digestion process in anaerobic condition.

The range of C/N that marked by X₂(C/N) has five values 11.729, 20, 26, 32, and 40.270, which indicates the experiment is conducted with five C/N. This range of C/N has been used by Shahidul et al. (2018) and Shanmugam and Horan (2009) in the POME digestion process in anaerobic condition.

The range of OLR to digestion process that marked by X₃(OLR) has five values - 6.959, 2, 8.5, 15, and 23.959 which indicates that the experiment is conducted with five OLR. This range of OLR has been used by Jeong et al. (2014), Khemkhao et al. (2015), and Shahidul et al. (2018) in the POME digestion process in anaerobic condition.

The range of HRT that marked by X₄(HRT) has five values -0.135, 4, 7, 10 and 14.135 which indicates the experiment is conducted with five HRT. This range of HRT has been used by Malakahmad and Yee (2014) and Shahidul et al. (2018) in the POME digestion process in anaerobic condition.

The range of SRT that marked by $X_5(\text{SRT})$ has five values 5.864, 10, 13, 16 and 20.135 which indicates the experiment is conducted with five SRT. This range of SRT has been used by Lau et al. (2009) and Mohd Idris et al. (2014) in the POME digestion process in anaerobic condition.

3.6.2 Experimental Runs and Order

Experimental runs and order were formulated using DOE using Design Expert software. The outputs from DOE produced a total of 50 runs which are a combination of five independent variables listed in Table 3.2. The experimental runs are shown in Table 3.3.

Table 3.3: Experimental Runs

Std	Run	Factor 1 A:pH	Factor 2 B:C/N	Factor 3 C:OLR	Factor 4 D:HRT	Factor 5 E:SRT
22	1	7	20	15	4	16
4	2	7	32	2	4	10
36	3	6.5	40.2705	8.5	7	13
28	4	7	32	2	10	16
11	5	6	32	2	10	10
29	6	6	20	15	10	16
6	7	7	20	15	4	10
37	8	6.5	26	6.95969	7	13
8	9	7	32	15	4	10
7	10	6	32	15	4	10
43	11	6.5	26	8.5	7	13
19	12	6	32	2	4	16
24	13	7	32	15	4	16
17	14	6	20	2	4	16
34	15	7.68921	26	8.5	7	13
18	16	7	20	2	4	16
49	17	6.5	26	8.5	7	13
42	18	6.5	26	8.5	7	20.1352
35	19	6.5	11.7295	8.5	7	13
1	20	6	20	2	4	10
33	21	5.31079	26	8.5	7	13
44	22	6.5	26	8.5	7	13
48	23	6.5	26	8.5	7	13

Table 3.3 continued

10	24	7	20	2	10	10
38	25	6.5	26	23.9597	7	13
9	26	6	20	2	10	10
30	27	7	20	15	10	16
16	28	7	32	15	10	10
13	29	6	20	15	10	10
14	30	7	20	15	10	10
39	31	6.5	26	8.5	7	13
23	32	6	32	15	4	16
2	33	7	20	2	4	10
46	34	6.5	26	8.5	7	13
5	35	6	20	15	4	10
3	36	6	32	2	4	10
12	37	7	32	2	10	10
32	38	7	32	15	10	16
21	39	6	20	15	4	16
40	40	6.5	26	8.5	14.1352	13
20	41	7	32	2	4	16
25	42	6	20	2	10	16
45	43	6.5	26	8.5	7	13
15	44	6	32	15	10	10
26	45	7	20	2	10	16
41	46	6.5	26	8.5	7	5.86476
50	47	6.5	26	8.5	7	13
31	48	6	32	15	10	16
27	49	6	32	2	10	16
47	50	6.5	26	8.5	7	13

3.6.3 Design of Experiment for Production Optimization

The DOE is a set of techniques that revolve around the study of the influence of different variables on the outcome of a controlled experiment. Generally, it involves identifying the independent variables or factors that affect a product or process, and then studying their effects on a dependent variable or response (Alghoul, 2018). DOE is used for the input (pH, C/N, OLR, HRT, SRT) and output (Q_{COD}) design research. Variables and ranges understanding are the key enabler of assuring input (pH, C/N, OLR, HRT, SRT) result in the final (Q_{COD}) design. Knowledge is achieved by establishing models correlating the

inputs with the outputs of the process. The mathematical relationships of the Critical Process Parameters (CPPs) and Material Attributes (CMAs) with the Critical Quality Attributes (CQAs) define the design space. Consequently, process understanding is well assured and rationally leads to a final output meeting the Maximum Target Desired Profile (MTDP). Fractional factorial 7-level designs are used in this analysis (Das & Dewanjee, 2018; Peng & Lin, 2019).

- Selection of process variables (pH, C/N, OLR, HRT, SRT) and response (Q_{COD}): this choice is justified based on the DOE objective and should assure that the whole design is executable and appropriate for satisfying its scope. This means that factor types either numeric or categorical and their levels, i.e., their values within the design, are selected accordingly.
- Take the value of 5 levels ($-\alpha$, -1 , 0 , $+1$, $+\alpha$) factor: this result can justify the range between each level.
- Selection of an experimental design (Response surface method, central composite design): based on DOE objectives, screening, characterization or optimization of process and formulation, several types of designs can be chosen, e.g., factorial or mixture. Decisions on randomization, blocking, and replications should be also addressed.
- Checking that the data of COD reduction (Q_{COD}) with the experimental: DOEs are exceptionally effective in identifying outliers, arising from potential poorly executed trials.
- Analyzing the results: ANOVA and graphical tools are very helpful in identifying main effects of the significant factors and interactions thereof. The results are

evaluated not only on a statistical basis but also versus the established theories and practically lead to process knowledge.

- Use and interpretation of the results: at this step, the evaluation of the DOE outcome can support scientifically sound decision making on following actions, such as executing confirmatory runs, augmenting designs, or further proceeding with scale up and technology transfer activities. It is evident that the success of these seven steps heavily depends on the establishment of an appropriate environment that can support the seamless execution of the DOE.
- Check the correlation and intercept in between input variables (pH, C/N, OLR, HRT, SRT).
- Report, graph collecting based on run, predicted and actual values.
- Get the 3D graph with optimum values for specific variables (pH, C/N, OLR, HRT, SRT) for highest COD reduction output (Q_{COD}).
- Visualize the central composite design (CCD) for optimum level of COD reduction (Q_{COD}).

CHAPTER 4

RESEARCH FINDINGS AND DISCUSSION

4.1 Introduction

This chapter presents the outcome of research conducted on COD reduction from POME. Section 4.2 of this chapter describes the data collection, data analysis and findings relating to objective one. Section 4.3 presents the data collection, data analysis and findings relating to objective two. Finally, Section 4.4 gives the outline of data collection, data analysis and findings relating to objective three.

The independent variables of this research are pH, C/N, OLR, HRT, and SRT and the dependent variable is COD reduction.

4.2 Determination of Significant Factors affecting COD Reduction from POME

The reduction in COD was analysed with respect to the five variables by using Design Expert software. The normal probability plot is shown in Figure 4.1.

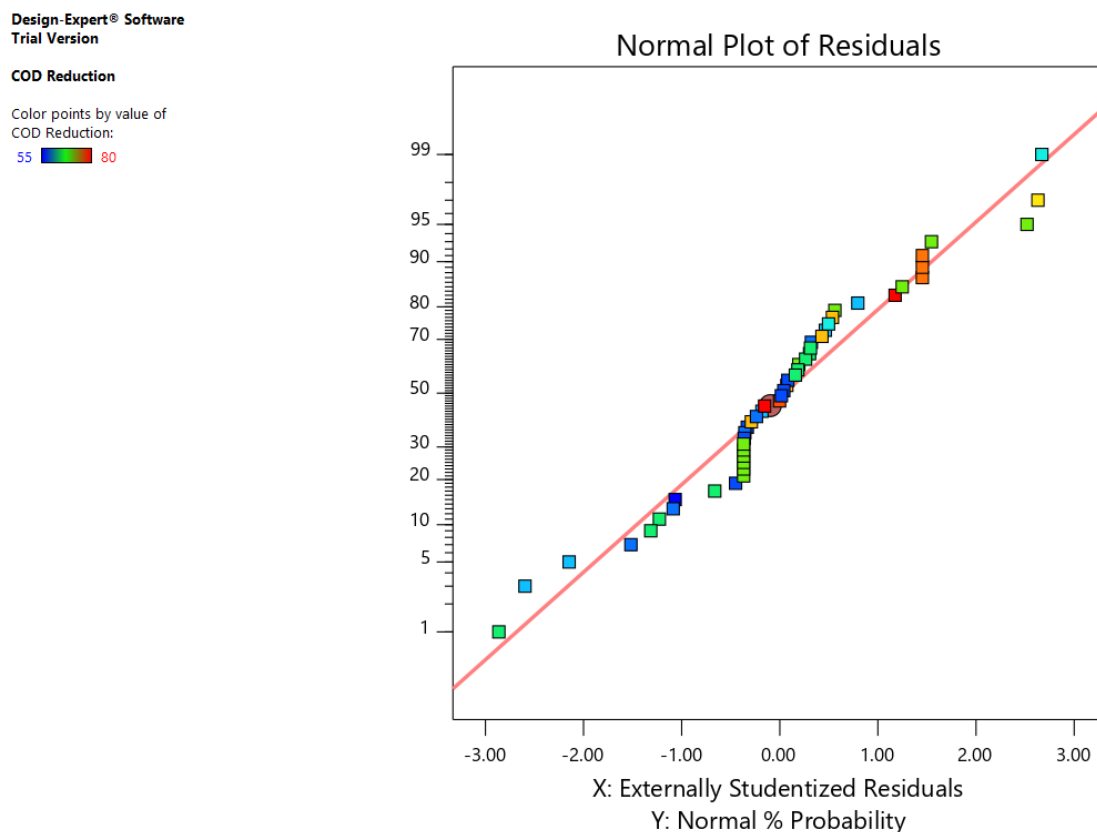


Figure 4.1: Data Distribution of COD Outputs

Figure 4.1 demonstrates that the outputs of COD reduction are normally distributed; also indicates that the range of outputs is within -2.00 standard deviation to +2.00 standard deviation with a small dispersion. This information indicates the outputs are within the acceptable limit of 95% probability. The studentized residuals represent a measure of the strength of the difference between observed and expected values where outliers could be identified and deleted if the residuals (data response – fitted value) is three times greater than estimated standard deviation ($ESR > 3$). Experimental data collected was analysed by Design Expert software. The statistical parameters were analyzed by whose outputs were summarized in Table 4.1.

Table 4.1 shows that the p-value of pH with respect to COD reduction is 0.0001 ($p < 0.05$), which is significant at 95% confidence level. This result indicates that pH has a

significant effect on COD from POME. The p-value of C/N with respect to COD reduction is 0.0227 ($p < 0.05$), which is significant at 95% confidence level thereby indicating that C/N has a significant effect on COD reduction from POME. As for OLR, the p-value with respect to COD reduction is 0.0001 ($p < 0.05$), which is significant at 95% confidence level. This finding demonstrates that OLR has a significant effect on COD reduction from POME. The p-value of HRT with respect to COD reduction is 0.1290 ($p > 0.05$). This finding shows that HRT is not significant to COD reduction from POME. The p-value of SRT with respect to COD reduction is 0.6362 ($p > 0.05$). This finding shows that SRT is not significant to COD reduction from POME. The summary of the correlation for COD reduction with level of significance for each of those factors under study is shown in Appendix C3.

Table 4.1: Correlation Model of Factors in COD Reduction from POME

Factors (input variables)	p-value of Factors	Significance Level
pH	0.0001	As $p < 0.05$; Significant
C/N	0.0227	As $p < 0.05$; Significant
OLR	0.0001	As $p < 0.05$; Significant
HRT	0.1290	As $p > 0.05$; Not significant
SRT	0.6362	As $p > 0.05$; Not significant
Coefficient of determination, $R^2 = 0.8134$ Adjusted $R^2 = 0.6846$ Adequate precision = 8.935 Coefficient of Variation (CV) = 6.19%		

4.2.1 Critical Discussion on Model Developed

In reference to Appendix C3 to C6, the regression model of input-output data is built with R^2 and p-value. The R^2 value is 0.8134, which indicates that the conversion of inputs to COD reduction is about 81.34%. This finding demonstrates that the performance of POME digestion process is acceptable. The R^2 value represents the coefficient of determination of variables involved, normally ranging from zero to one but usually

expressed in percentage. Cohen (1969) categorised his effective sizes (d values) of 0.2, 0.5 and 0.8 as small, medium and large respectively. With the correlation coefficient between inputs and output of $R = 0.90 > 0.5$, three of the input variables (pH, C/N, OLR) are found to be strongly positive (Rumsey, 2016) with significant impacts on output where their p-values ($p < 0.05$) at 95% confidence level, as depicted in Table 4.1. Although the impact of HRT and SRT are statistically insignificant ($p > 0.05$) to COD reduction (output), they are still considered important factors which are responsible for providing optimal conditions for microorganisms to function effectively.

The CV value is 6.19%, reflecting that the performance of POME digestion process in the anaerobic reactor is good. As a statistical measure of the dispersion of data points around the mean, the lower the value of CV, the more precise the estimate around the mean. On the other hand, we can consider that adequate precision value in Table 4.1 as a measure of signal to noise level, representing the ratio of mean to standard deviation which reflects the severity of noise and other interferences. Here, our experimental result indicates that we have got adequate signal of 8.935. A ratio greater than 4 is desirable for the model to be used to navigate the design space. Lower noise level would give higher signal to noise ratio (SNR), which implies better statistical robustness.

4.2.2 Scenario Analysis on Significant Factors affecting COD Reduction

The objective one has three elements which are the independent variables as pH, C/N, and OLR. The results of the experiment show that pH, C/N, and OLR are significantly important ($p < 0.05$) in reducing COD from POME. Other factors such as HRT and SRT are important factors as well although they are not significant ($p > 0.05$). pH is an essential chemical factor in the solubility and dispersion of biological organic contaminants, which

controls the performance of the anaerobic digestion process. pH has also been linked to the microbial activities in anaerobic digestion (Andersson & Nilsson, 2001; Nayono, 2010). At pH level below 6.6, the methanogenic activities decrease due to the slowed growth rate of methanogens (Angelidaki et al., 2003). Some studies reported that methanogenic processes are fully stopped at pH levels below 4 and above 9.5 (Beccari et al., 1996; Gerardi, 2003). This is also supported by Shahidul et al. (2018) by stating the fact that at pH less than 5.5 and pH more than 8, the solution of POME becomes toxic which kill methanogens and ultimately affect COD reduction. A similar study was conducted by Wong et al. (2013) reported that the performance of anaerobic digestion increases as pH increases up to 7.5 in POME processing. It has been also reported that at acidogenesis process, the optimal pH value is ranging between 5.5 and 6.5 (Kim et al., 2003).

In conclusion, a two-stage anaerobic digestion is a preferred method to conduct research separately; one part is hydrolysis and acidification and the other part is acetogenesis and methanogenesis processes (Van Kessel & Russell, 1996; Jiunn-Jyi et al., 1997; Kim et al., 2003; Bolzonella, 2011; Yossan et al., 2012). The study recommends to maintain the pH value in the anaerobic reactor between 6.5–7.5.

The C/N is an important manipulating variable in optimizing the digestion process of POME (Okonkwo et al., 2016). It was reported that higher carbon content contributes to increasing carbon dioxide formation and lower pH, while the high value of C/N (>40) will enhance the production of ammonia gas that could increase pH (Dioha et al., 2013). Thus, two ways, POME would be toxic for methanogenic process. Adjusting the C/N could be done by co-digestion, which means to introduce a co-substrate into the system. The co-digestion process offers the anaerobic digestion system some benefits (Esposito et al., 2012).

Co-digestion also increases microbial population variation associated with the biochemical reaction process. El-Mashad and Zhang (2010) reported that co-digestion process affects COD reduction effectively.

In conclusion, Tanimu et al. (2014) in their study revealed that the anaerobic system demonstrated better pH stability as the C/N increased from 17 to 30. The optimal C/N for methanogenesis in an anaerobic system is about 20 as mentioned by Kossmann et al. (2011). It can be concluded that the recommended C/N range for anaerobic digestion is between 20–35 (Kayhanian, 1999; Habiba et al., 2009).

The OLR is a measurement of a biological system's conversion capacity. Increasing OLR in an anaerobic system can lead to decrease COD reduction efficiency and process instability (Sánchez et al., 2005). The addition of large volumes of organic material can alter the environment inside the anaerobic reactor and overwhelms the methanogens. This is due to the increasing of microbial activity during hydrolysis and acidogenesis, resulting in the rise of VFA production. The accumulation of acid will cause the pH inside the reactor to drop. As a result, the efficiency of methanogenic bacteria in converting VFA to methane is reduced (Mao et al., 2015).

Rincón et al. (2008) discovered that anaerobic reactor performed effectively in OLR ranged from 1.5 to 9.2 kg-COD/m³.d; any further increase of OLR resulted in process instability. Krishnan et al. (2016) disclosed that at higher OLR (8.3 to 15.8 kg-COD/m³.d), the performance of COD reduction improved. It can be concluded that the recommended OLR range for anaerobic digestion is between 1.5–15.8 kg-COD/m³.d (Krishnan et al., 2016; Rincón et al., 2008).

4.2.3 Conclusion and Answer to Research Question for Objective One

Research objective one is described in Section 1.5(a) which was to determine the factors that significantly affect COD reduction from POME. The results of the experiment show that pH, C/N, and OLR produced significance levels of $p < 0.05$ which are significant to COD reduction. HRT and SRT also contributed to COD reduction but in terms of significance level, they are not significant to COD reduction. It may be due to the ineffectiveness of reactor performance. This experiment shows that pH, C/N, and OLR are the significant factors that contribute to COD reduction. Therefore, research objective one is achieved.

4.3 Determination of Impact of Manipulating Variables on COD Reduction

This section revealed the impact of one factor on COD reduction while other four factors remained constant. However, the contribution of other factors on COD reduction were still important.

4.3.1 Impact of pH on COD Reduction

The range of pH were 5.310, 6, 6.5, 7, and 7.689, which indicated the experiment was conducted with five pH. At pH 5.310, the COD reduction obtained was 62%. At pH 6, the COD reduction obtained was in the range of 55–70%. At pH 6.5, the range of COD reduction obtained was between 62–77%. As for pH 7, the contribution of this factor was able to reduce 57–80% COD. At pH 7.689, the COD reduction was able to achieve up to 80%.

In general, the methanogenesis process prefers a neutral pH. The efficiency of COD reduction relies on the performance of anaerobic bacteria especially methanogens. The

higher the amount of organic matter converted into biogas, the higher the efficiency of COD reduction. Methanogens improve biogas production at pH range of 6.5–7.5 and the productivity increases at pH of 6.8–7.6 (Stronach et al., 1986; Nayono, 2010). However, the performance of an anaerobic digestion decreases at pH below than 6.5 and higher than 7.6 (Nayono, 2010). Liu et al. (2008) stated that the optimal pH value depends on the substrate and digestion system. A study conducted by Wong et al. (2013) reported that the concentration of methane increases as pH increases in the treatment of POME in a SCABR. Methanogenesis and acidogenesis have different optimal pH levels. As for acidogenesis, the optimal pH value is ranging between 5.5 and 6.5 (Kim et al., 2003). A two-stage anaerobic digestion was the preferred method to separate acidogenesis and methanogenesis due to the differences in optimal pH range.

4.3.2 Impact of C/N on COD Reduction

The range of C/N were 11.729, 20, 26, 32, and 40.270, which indicated the experiment was conducted with five C/N. At C/N 11.729, the COD reduction obtained was 70%. At C/N 20, the range of COD reduction was between 55–75%. At C/N 26, the range of COD reduction was between 62–80%. As for C/N 32, the COD reduction was in the range of 57–80%. At C/N 40.270, the COD reduction was able to achieve up to 75%.

During anaerobic digestion, Yadvika et al. (2004) reported that microorganisms utilize carbon 25 to 30 times faster than nitrogen. Several authors agree on an optimal C/N of between 20 and 35 (Kayhanian, 1999; Habiba et al., 2009) for anaerobic digestion. According to Khalid et al. (2011) the optimal C/N for anaerobic degradation of organic waste was 20 to 35. Nurul Adela et al. (2014) reported that the C/N of the POME is in the range of 10.08–11.44 which is significantly lower than required C/N value of 20 to 35.

Therefore, co-digestion was necessary to increase the C/N during POME digestion. The addition of a co-substrate would help to reduce harmful compounds in the mixture and improve the pH and moisture content of the system (Esposito et al., 2012). The presence of two different substrates would increase the variation of microbial population in the biochemical reaction process resulting in better COD removal efficiencies and volatile solids reduction (El-Mashad & Zhang, 2010; Sidik et al., 2013; Nurliyana et al., 2015).

4.3.3 Impact of OLR on COD Reduction

The range of OLR were 2, 6.959, 8.5, 15, and 23.959, which indicated the experiment was conducted with five OLR. At OLR 2, the COD reduction obtained was in the range of 55–65%. At OLR 6.959, the COD reduction obtained was 65%. At OLR 8.5, the range of COD reduction was between 62–80%. As for OLR 15, the COD reduction range was from 58–80%. At OLR 23.959, the COD reduction was able to achieve up to 62%.

Different researchers had selected different ranges of OLR for the optimization of an anaerobic digestion system. Rincón et al. (2008) conducted a study using OLR in the range of 1.5 to 9.2 and they were able to obtain COD reduction efficiencies in the range of 77–97%. OLR could affect the pH and alkalinity of a system. A study conducted by Krishnan et al. (2016) observed that as the OLR increased from 4 to 12 kg-COD/m³.d, pH and alkalinity in the continuous stirred-tank reactor (CSTR) unit increased until it reached a maximum level at OLR of 12 kg-COD/m³.d. As the OLR was further increased to 20 kg-COD/m³.d, the system's pH and alkalinity decreased sharply. Chaisri et al. (2007) conducted a study by increasing OLR (2.50 to 10.0 kg-COD/m³.d) in an up-flow anaerobic sludge bed (UASB) reactor and they found that the COD reduction efficiency declined from 96.3% to 87.4%. Similarly, they increased OLR (1.10 to 10.0) in up-flow anaerobic filter (UFAF) reactor and

found that the COD reduction efficiency also decreased from 91.6% to 66.3%. This showed that OLR had an effect in COD reduction. The addition of large volume of organic material (increase OLR) would interrupt the environment of the system and affect the fermentation process (Abdelgadir et al., 2014). High OLR would increase the microbial activities during hydrolysis and acidogenesis which would lead to an increase in VFA production. The increasing presence of VFA would increase the pH in the system and as a consequence, methanogenic microorganisms were unable to perform effectively at low pH conditions (Mao et al., 2015).

4.3.4 Impact of HRT on COD Reduction

The range of HRT were 4, 7, 10 and 14.135, which indicated the experiment was conducted with four HRT. At HRT 4, the COD reduction obtained was in the range of 55–75%. At HRT of 7, the COD reduction obtained was in the range of 62–80%. At HRT 10, the range of COD reduction was between 57–80%. As for HRT 14.1335, the COD reduction obtained was 74%.

It has been reported that for efficient digester operation, selection of HRT value is important; the range of HRT could vary from 5–10 days (Krzysztof Ziemiński, 2012; Nour et al., 2010). Another study was conducted by Siddique et al. (2016) found that the COD reduction efficiency was increasing steadily from 68.2% to 98.5% as HRT increased from 3 to 9 days. Likewise, Wong et al. (2014) reported that the highest COD reduction efficiency was recorded as 66.09% at HRT of 12 days and was lowest at 47.30% for HRT of 4 days. A well-adapted microbial population in anaerobic condition could lead to an increase in the rate of digestion of organic matters (Alrawi et al., 2011b). HRT was one of the anaerobic conditions which provided an effect on COD reduction of wastewater. A longer HRT was

found to achieve better COD removal efficiencies and was suitable for digestion of POME. A short HRT might reduce the contact time between organic matter and bacteria resulting in the insufficient reproduction of methane-forming bacteria (Maroun & Fadel, 2007; Poh & Chong, 2009). From these studies, it could be concluded that the COD reduction efficiency increased as HRT was increasing.

4.3.5 Impact of SRT on COD Reduction

The range of SRT were 5.864, 10, 13, 16, and 20.135 which indicated the experiment was conducted with five SRT. At SRT 5.864, the COD reduction obtained was 65%. At SRT of 10, the COD reduction obtained was in the range of 55–78%. At SRT 13, the range of COD reduction was between 62–80%. As for SRT 16, the COD reduction obtained was 57–80%. At SRT 20.135, the COD reduction was able to achieve up to 70%.

Generally, increasing the SRT improved the removal efficiency of COD (Dereli et al., 2014). The effect of increasing SRT by effluent recycling improved the removal efficiency of COD in fermentation process (Busu et al., 2010; Gnanapragasam et al., 2010; Lee et al., 2010). During the fermentation of POME, COD removal efficiency achieved 85% when effluent recycling was used. Meanwhile, the COD removal efficiency obtained 76% without effluent recycling (Irvan et al., 2017). Effluent recycling was necessary for suspended growth process where it would increase contact between sludge and biomass; hence allowed microorganisms to further broke down the organic materials to reduce COD of effluent (Ali, 2012). Mahmoud et al. (2003) stated that methanogenesis began between 5 and 15 days of SRT at a temperature of 25 °C. A high SRT performed the function of allowing microbial community to be acclimatized to the environment of the components available in these wastewaters (Dvořák et al., 2016). As SRT was increased, microorganisms

began to compete for nutrients resulting in the formation of antibiotics to prevent the growth of other microorganisms. As a result, certain microbial population would not be able to survive and died. Dead cells of microorganism then dissolved into the bioreactor causing the concentration of soluble microbial particles to increase resulting in increasing supernatant COD (Huang et al., 2001).

4.3.6 Scenario Analysis on Impact of Manipulating Variables on COD Reduction

The COD removal performance was determined by the change of COD over COD_{in} . The average COD_{in} for POME was about 80,000 mg/L. The average COD_{out} was about 25,000 mg/L. The average efficiency of COD reduction was about 69%.

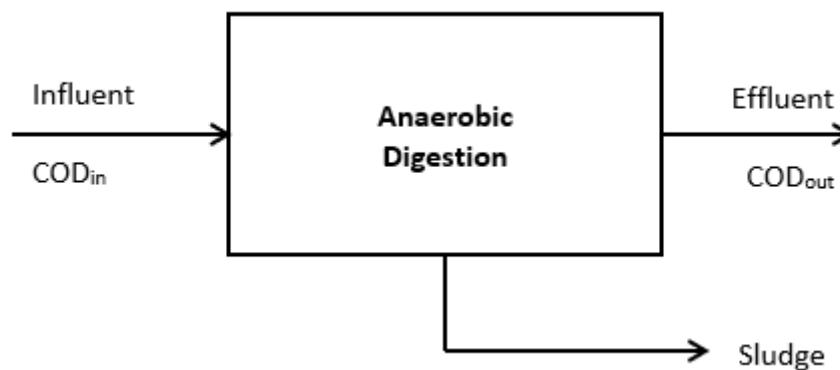


Figure 4.2: COD Reduction Process

The highest and lowest value of COD reduction were 80% and 57% respectively. The highest COD reduction efficiency reported by Wong et al. (2014) was 66.09% at HRT of 12 days. The current study was able to achieve 80% COD reduction, 13.91% more at a lower HRT of 7 days. In this study, the application of co-digestion by introducing an inoculum to increase the C/N of the feedstock was a factor to COD reduction. The presence of an inoculum increased the population of microorganisms inside the system for better COD removal (El-Mashad & Zhang, 2010; Esposito et al., 2012).

A similar study was conducted by Khemkhao et al. (2015) stated that they achieved 82% COD reduction efficiency at 19.0 g COD/L.d and HRT of 3.3 days. The COD reduction efficiency they achieved was slightly higher than the current study might be due to the effect of temperature. The current study was conducted at mesophilic temperature while Khemkhao et al. (2015) conducted their study at thermophilic temperature. A study investigated by Fang et al. (2011) on an expanded granular sludge bed (EGSB) reactor operated at a thermophilic condition produced higher COD reduction efficiency and biogas production. The performance of a CSTR in terms of process stability, COD reduction efficiency, and biogas production were better in thermophilic conditions compared to those under mesophilic conditions (Choorit & Wisarnwan, 2007).

A study conducted by Mamimin et al. (2015) using a two-stage thermophilic fermentation and mesophilic methanogen process managed to achieve COD reduction rate of more than 90% in the UASB unit. Although it is higher than the current study (80%), the two-stage process was a preferable method in COD reduction. This was because a two-stage process could improve the digestion efficiency and negative effects of inhibitors in the feedstock (Li & Yu, 2011). A two-stage process improved the net energy balance, process stability, OLR, and the methanogenic process. Thus, these could lead to higher rate of methane production, COD reduction efficiency, and volatile solids (VS) reduction. It can be concluded that, the two-stage process was a preferable method for reducing COD from POME.

4.3.7 Conclusion and Answer to Research Question for Objective Two

Research objective two is described in Section 1.5(b) which was to determine the impact of manipulating variables (pH, C/N, OLR, HRT, SRT) on COD reduction from

POME. The scope of study consists of five elements which were pH, C/N, OLR, HRT and SRT. The contribution of these five factors were able to achieve up to 80% COD reduction whereas, the lowest value of COD reduction contributed by these five factors was 57%. The impact of each variable on COD reduction was determined to achieve the research objective.

4.4. Determination of Optimum Value of Factors that Contribute to COD Reduction from POME

The scope of study under this section was related to objective three stated in Section 1.5(c). The study was conducted to determine the optimum value of factors (pH, C/N, OLR, HRT, SRT) and COD reduction by using regression analysis of experimental data. The Design Expert software was used to perform regression analysis and generate the response surface diagrams in Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6, and Figure 4.7.

4.4.1 Relationship between Independent Variables and COD Reduction

4.4.1 (a) Effect of pH and C/N on COD Reduction

The response surface diagram demonstrated the combined effects of two factors (pH and C/N) on COD reduction output while keeping the other three independent variables constant at their centre point. This scenario is presented in Figure 4.3.

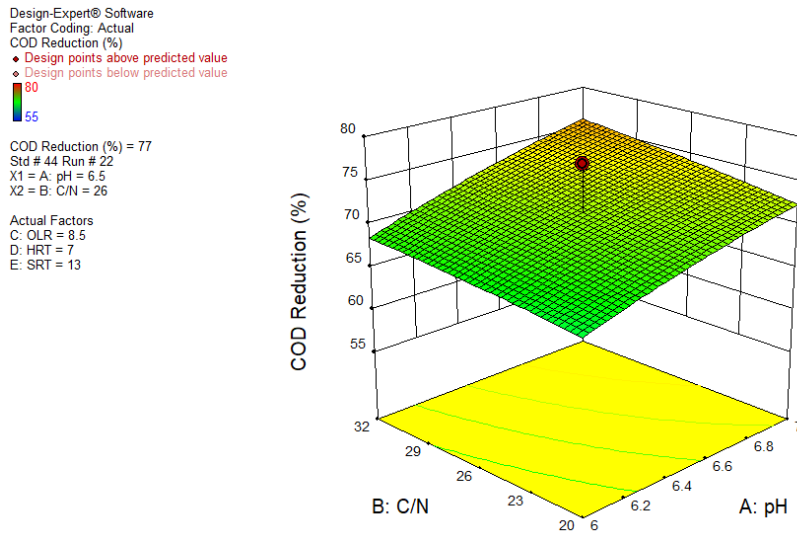


Figure 4.3: Response Surface Diagram of pH, C/N and COD Reduction

It can be deduced from Figure 4.3, the optimum COD reduction due to effect of pH and C/N is 77%. The 77% COD reduction is obtained at pH of 6.5 and C/N of 26.

4.4.1 (b) Effect of C/N and OLR on COD Reduction

The response surface diagram demonstrated the combined effects of two factors (C/N and OLR) on COD reduction output while keeping the other three independent variables constant at their centre point. This scenario is presented in Figure 4.4

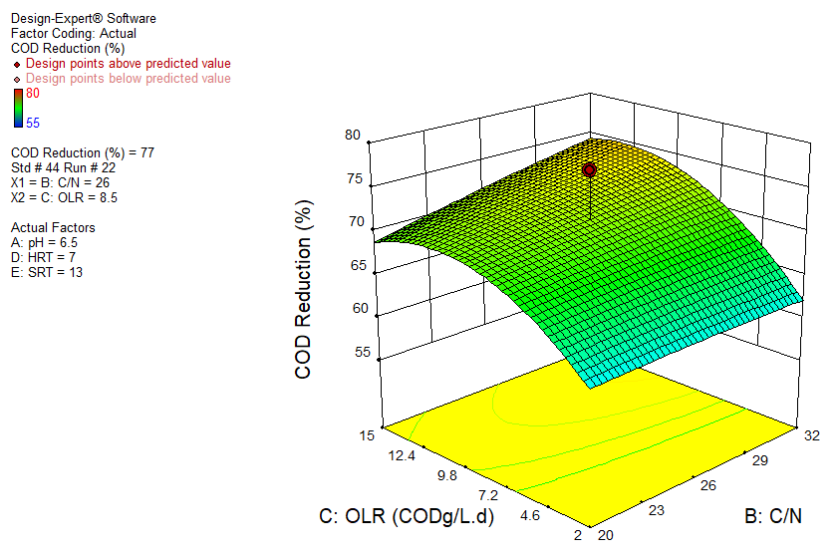


Figure 4.4: Response Surface Diagram of C/N, OLR and COD Reduction

It can be deduced from Figure 4.4, the optimum COD reduction due to effect of C/N and OLR is 77%. The 77% COD reduction is obtained at C/N of 26 and OLR of 8.5.

4.4.1 (c) Effect of OLR and HRT on COD Reduction

The response surface diagram demonstrated the combined effects of two factors (OLR and HRT) on COD reduction output while keeping the other three independent variables constant at their centre point. This scenario is presented in Figure 4.5.

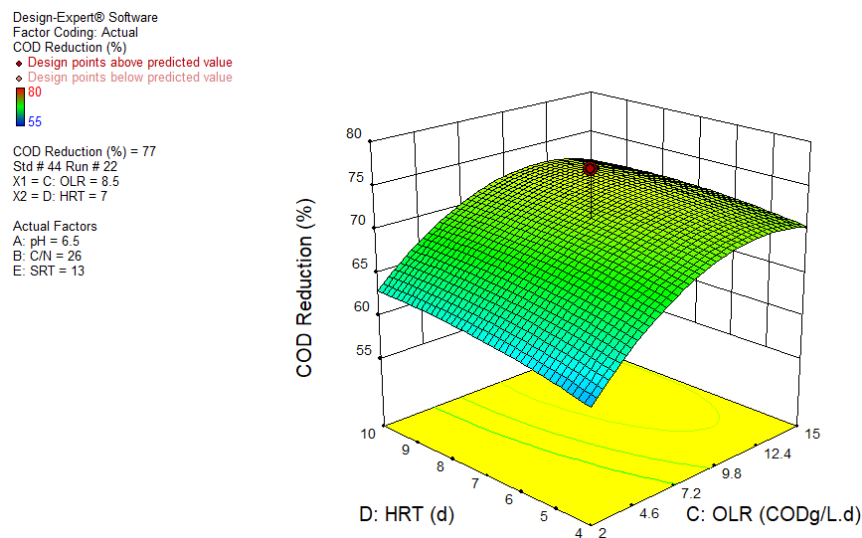


Figure 4.5: Response Surface Diagram of OLR, HRT and COD Reduction

It can be deduced from Figure 4.5, the optimum COD reduction due to effect of OLR and HRT is 77%. The 77% COD reduction is obtained at OLR of 8.5 and HRT of 7.

4.4.1 (d) Effect of HRT and SRT on COD Reduction

The response surface diagram demonstrated the combined effects of two factors (HRT and SRT) on COD reduction output while keeping the other three independent variables constant at their centre point. This scenario is presented in Figure 4.6.

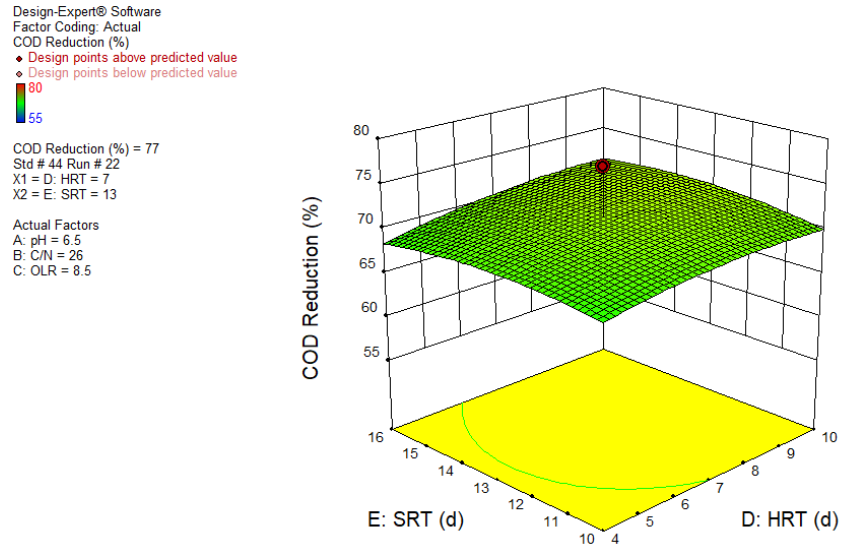


Figure 4.6: Response Surface Diagram of HRT, SRT and COD Reduction

It can be deduced from Figure 4.6, the optimum COD reduction due to effect of HRT and SRT is 77%. The 77% COD reduction is obtained at HRT of 7 and SRT of 13.

4.4.1 (e) Effect of SRT and pH on COD Reduction

The response surface diagram demonstrated the combined effects of two factors (SRT and pH) on COD reduction output while keeping the other three independent variables constant at their centre point. This scenario is presented in Figure 4.7.

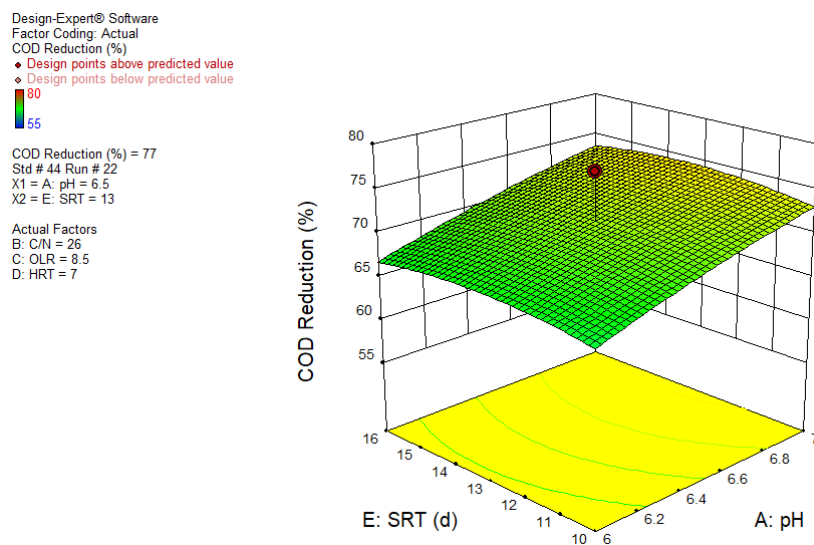


Figure 4.7: Response Surface Diagram of SRT, pH and COD Reduction

It can be deduced from Figure 4.7, the optimum COD reduction due to effect of SRT and pH is 77%. The 77% COD reduction is obtained at SRT of 13 and pH of 6.5. Figure 4.3 to Figure 4.7 establish the relationship among inputs and output. The individual relationship between input and output described in Section 4.4.1 (f).

4.4.1 (f) Confirmation Optimization of COD Reduction with respect to Input

The optimum value was determined by Design Expert software after experimental data was collected and analysed. pH of 6.5 was the optimum value to achieve 77% COD reduction. pH lower than 6.5 would reduce the growth rate of methanogens, resulting in lower COD reduction (Malakahmad & Yee, 2014). A pH which is too high would be unfavourable to methanogens because they are pH-sensitive (Wong et al., 2013). This would reduce the performance of COD reduction as well. Merlin et al. (2014) stated that majority of enzymes (>3000) preferred neutral pH with fairly narrow pH range to remain active.

Similarly, the effect of C/N, OLR, HRT and SRT on COD reduction can be explained as follows. A lower C/N reflected high value of nitrogen to produce ammonia gas, causing pH to increase and interrupt microbial growth (Dobre et al., 2014). A higher C/N (high carbon content) resulted in the formation of carbon dioxide and decreased pH when carbonic acid reacted with water (Okonkwo et al., 2018). Lower OLR could result in undersupply of organic materials for microorganisms to digest (Krishnan et al., 2017). Meanwhile, higher OLR affects the process stability in the system resulting in rapid build-up of VFA which would decrease the pH value (Mao et al., 2015). A shorter HRT would lead to a build-up of VFA because it was unconverted by microorganisms (Mao et al., 2015), while a longer HRT would increase the contact time between microorganism and organic material resulting in better removal efficiency (Siddique et al., 2016). A shorter SRT would provide insufficient

time for microorganisms to reproduce and restore their population in the system (Abdelgadir et al., 2014), while a longer SRT would allow microorganisms to adapt to the environment and reproduce in order to break down the organic material (Dvořák et al., 2016). The optimum value of all the factors including independent (pH, C/N, OLR, HRT, and SRT) and dependent variables (COD reduction) are tabulated in Table 4.2.

Table 4.2: Optimum Values of Inputs and Output

Figure No.	Input Factors	Optimum Value	Optimum Output of COD Reduction (%)
4.3, 4.4, 4.5, 4.6, 4.7	pH	6.5	77
	C/N	26	
	OLR	8.5	
	HRT	7	
	SRT	13	

4.4.2 POME Digestion Process Modelling

The COD of POME is the objective function of the reaction. The POME with the required inoculum prepared from banana peel (fruit waste) was fed into the anaerobic digester for reducing COD. The COD removal performance depends on OLR process, operating environment (COD and biomass loading rate) in anaerobic reactor and the efficiency of the digestion process. The environment in anaerobic reactor depends on pH, HRT and SRT. C/N also contributes to supply nutrient for microbial growth, with carbon as food for microorganisms while nitrogen acting as enzyme for microbial activities. The COD of POME would be reduced at the end of the biochemical process of anaerobic digestion. The conceptual model for input-output POME digestion process for the reduction of COD is presented in Figure 4.8 for mathematical analysis (Miller & Blair, 2009; Liang et al., 2010; Zulfiqar, 2012):

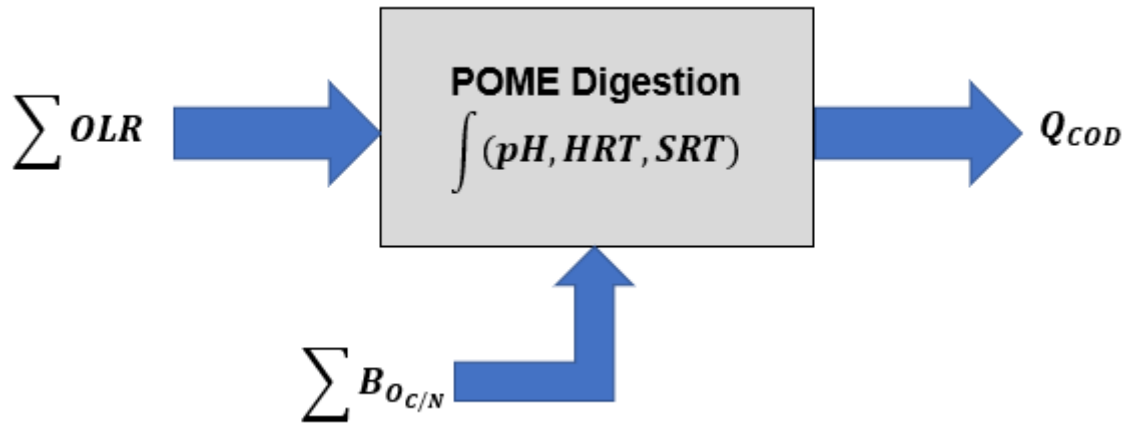


Figure 4.8: Conceptual Model of POME Digestion

Where:

C/N = Adjusted biomass with high carbon content that contributes to provide elemental carbon (C) and nitrogen (N) to speed up the required bacteria growth for the digestion.

HRT = Hydraulic retention time, contributor to accelerate POME digestion for COD reduction.

SRT = Sludge retention time as a contributor to accelerate POME digestion for COD reduction.

K = Process control factor of input conversion to output as COD reduction ($k < 1$), where the range of K is 0.7 to 0.9 [$0.7 < K < 0.9$] (Seborg et al., 2004).

The process capability K of the reactions depends on many factors and among these are pH, HRT and SRT. The process capability factor K is the key parameter to determine the designed process, which could be presented as a function of these factors as follows:

$$K = \int (pH, HRT, SRT) \quad \text{Eq. 4.1}$$

It infers that the conceptual model depicted in Figure 4.8 is a non-linear process which can be expressed mathematically by Equation 4.2.

$$Q_{COD} = A_o(OLR)^\alpha \cdot \left(B_{O_C} \frac{C}{N}\right)^\beta K \quad \text{Eq. 4.2}$$

Subsequently, this equation can be converted to linear form (straight line) by taking logarithm in both sides as shown in Equation 4.3.

$$\log(Q_{COD}) = \log A_o + \alpha \log(OLR) + \beta \log\left(B_{O_C} \frac{C}{N}\right) + \log K + \log \varepsilon \quad \text{Eq. 4.3}$$

Where, A_o is the intercept of the line at y-axis. The coefficient of OLR ' α ' is the contribution to COD reduction from POME. The coefficient of B_o related to C/N ' β ' is the contribution to COD reduction from POME. Whereas the regression term error, ε was zero, with k being the process capability factor for all inputs with respect to COD reduction in the system.

4.4.3 Model Estimate

The coefficient of input-output model (Equation 4.3) estimate represents the expected change in response per unit change in inputs when all the remaining factors (inputs) are held constant. The intercept in an orthogonal design is the overall average response of all the experimental runs.

It is standard practice to use the coefficient p-values to decide whether to include variables which are significant ($p < 0.05$) in the final model. Hence, the interactions and quadratic terms were not considered in the model where their coefficients are not significant

($p > 0.05$) or negative values (antagonistic effects) as reflected in Table 4.3 and Appendix C5. The experimental data were analysed by Design Expert software to obtain coefficient of the model. The data analysis is presented in Appendix C5. From Appendix C5, the coefficients of the model are listed in Table 4.3. Hence, only the main effects were considered to form the final empirical model.

Table 4.3: Coefficient Estimate of the Linear Regression Model

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept (A_0)	71.45	1	1.29	68.81	74.10	
A-pH	3.41	1	0.62	2.14	4.69	1.00
B-C/N (β)	1.50	1	0.62	0.22	2.77	1.00
C-OLR (α)	4.84	1	0.71	3.39	6.29	1.13
D-HRT	1.11	1	0.71	-0.34	2.57	1.13
E-SRT	0.30	1	0.62	0.98	1.57	1.00

With reference to the model analysis presented, the respective values of coefficients of the empirical model (Equation 4.3). The coefficients are listed in Table 4.3.

Coefficient of OLR (α) = 4.84, coefficient of C/N (β) = 1.50 and the intercept A_0 = 71.45. R^2 in the model presented in Table 4.1 is the value of K, which is 0.8134. (K = 0.8134, which indicates the conversion of input to output is 81.34%)

By using these coefficients in Equation 4.3, the desired statistical model could be rewritten as linear form shown in Equation 4.4.

$$\log(Q_{cod}) = \log 71.45 + 4.84 \log(OLR) + 1.50 \log(B_{OC}) + \log 0.8134 + \log \varepsilon \quad \text{Eq. 4.4}$$

The nonlinear form of that model could be derived from Equation 4.2, to be represented by Equation 4.5.

$$Q_{COD} = 71.45(OLR)^{4.84} \cdot (B_{OC})^{\frac{1.50}{N}} 0.8134 \quad \text{Eq. 4.5}$$

4.4.4 Model Validation with Input Factors

The developed model in Equation 4.5 has also been validated by using values (Appendix C6) from different experimental runs. The graph in Figure 4.9 presents the “x-axis” as the actual experimental outputs (actual data response) of 50 runs; and “y-axis” as the predicted outputs.

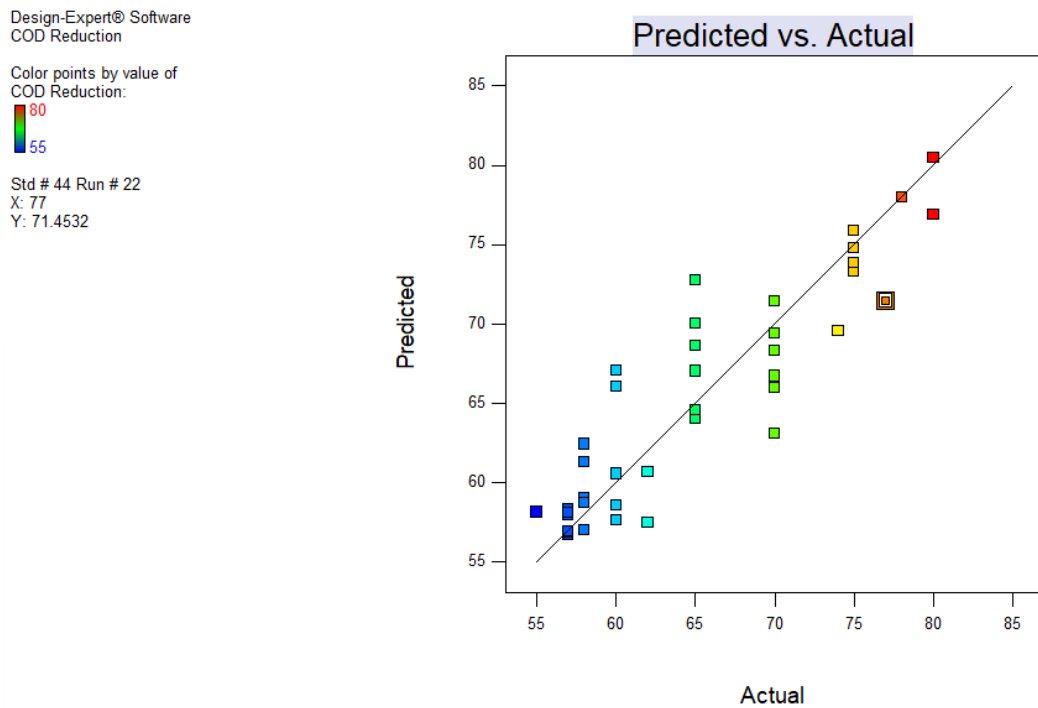


Figure 4.9: Distribution of Actual and Predicted Data on COD Reduction

The experimental values with optimum inputs for both actual and prediction model outputs (COD reduction) are depicted in Table 4.4 and listed in Appendix C6.

Table 4.4: Model Validation with Inputs Factors

Optimum Input Values	Run	Actual	Predicted	Standard Error (%)	Standard Deviation in Error
pH = 6.5 C/N = 26 OLR = 8.5 HRT = 7 SRT = 13	4	60.00	66.05	9.16	3.79
	10	60.00	67.08	10.55	
	18	70.00	65.99	6.07	
	29	60.00	60.56	0.92	
	35	70.00	63.09	10.95	
	40	74.00	69.56	6.38	
	48	65.00	67.03	3.03	
	Average Error between Actual and Model Prediction			6.72	

Based on the findings in Table 4.4, the average standard error between actual and model prediction is 6.72%, with standard deviation of error 3.79. A small value of standard deviation error indicates good model fitting by giving close value between predicted and actual values for the response (Behera et al., 2018). It validates that model developed is quite fit to predict COD reduction within 10% of error limit.

4.4.5 Conclusion and Answer to Research Question for Objective Three

Research objective three was described in Section 1.5(c) which was to determine the optimum value of factors that contribute to COD reduction from POME. The optimum inputs and output are given in Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6, and Figure 4.7. Based on the 3D plots, the optimum value for pH is 6.5; C/N is 26; OLR is 8.5 kg/d.m³; HRT is 7 days, and SRT is 13 days. The optimum output of COD reduction is 77%. This indicates that an anaerobic reactor operated at these conditions (pH is 6.5; C/N is 26; OLR is 8.5 kg/d.m³; HRT is 7 days, and SRT is 13 days), the optimum COD reduction can achieve up to 77%.

The validation results show that the actual value and predicted error is within 6.72% with a standard deviation 3.79. Based on the findings, it can be stated that the COD reduction prediction is quite fit to predict COD reduction within the error limit. Therefore, research objective three is achieved.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Chapter Overview

This chapter covers the conclusion and recommendations of this study. Section 5.2 presents the conclusion of this study. Section 5.3 presents the recommendations for future study related to POME.

5.2 Conclusion of Study

The research objective one is to determine the significant factors that affect COD reduction from POME. Experimental results reveal that contributing factors such as pH, C/N and OLR are significant at 95% confidence level ($p < 0.05$). HRT and SRT also contribute to COD reduction although their significance level is more than 0.05. It might be due to the inefficiency of reactor performance. Therefore, the research objective one is achieved and answer to research question one is replied.

The research objective two is to determine the impact of manipulating variables (pH, C/N, OLR, HRT and SRT) on COD reduction from POME. The contribution of these five factors to COD reduction is 80% whereas, the lowest value is 57%. The impact of each variable on COD reduction is presented in Section 4.3 of Chapter 4. Therefore, the research objective two is achieved and answer to research question two is replied.

The research objective three is to determine the optimum value of factors that contribute to optimize COD reduction. The optimum values of this process variables are pH 6.5; C/N 26; OLR 8.5 kg/d.m³; HRT 7 days, and SRT 13 days. The optimum output of COD reduction is 77%. An empirical model is developed with ANOVA (Design Expert software),

which is presented in Equation 4.12. The developed model is validated by using values from different experimental runs as presented in Appendix C6. The validation results give an average error of 6.72% with standard deviation error of 3.79%. It could be stipulated that the model built to estimate COD reduction from POME is fit to predict the output of anaerobic reactor as error is within 10% limit with respect to predicted values. Thus, the research objective three is achieved and answer to research question three is replied.

In this study, banana peels are chopped to smaller size and used as inoculum. This inoculum is used to prepare the substrate to increase the C/N. The findings demonstrate that with this substrate, the COD reduction achieved 77% and thus, the null hypothesis is accepted. The experiment concludes that the problem stated in problem statement has been addressed. Additionally, also proved that factors such as pH, C/N, OLR, HRT and SRT have played vital roles in reducing COD. This study has also answered the research questions raised as stated in Section 1.3 of Chapter 1 and addressed the research gap that exists in POME domain. This study also proves that anaerobic digestion system with banana peel is an efficient process to reduce COD and enhance the optimization process. Thus, the findings of this study add new knowledge in treating POME.

5.3 Limitations of Study

This experiment experienced a few limitations. The limitations are:

- The distance between the Operations Research Lab and Bau Palm Oil Mill is quite far that affects the POME quality during transportation.
- There are many insignificant factors that are not considered in this experiment that might affect the performance of the anaerobic system.

- Only COD test was conducted to measure the performance of the system. BOD and other tests were not conducted.

5.4 Recommendations for Future Study on POME

Some recommendations are suggested for future studies to overcome these limitations and improve the current study. The following recommendations include:

- Experiment setup could be nearby the mill to maintain the quality of POME collected.
- Further experiment can be conducted with up-flow velocity, digestion temperature, VSS concentration, and MLSS concentration to investigate the COD reduction. By including these factors, they can contribute to better outcomes such as higher COD reduction rate.
- Other substrates that are available naturally could be used to reduce COD reduction from POME by anaerobic reactor namely kitchen waste, sugarcane bagasse, decanter cake and so on. A study on these types of substrates should be undertaken to determine which substrate can improve the performance of POME treatment in terms of biogas production, COD reduction, and BOD reduction.

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APPENDICES

Appendix A: List of Publications

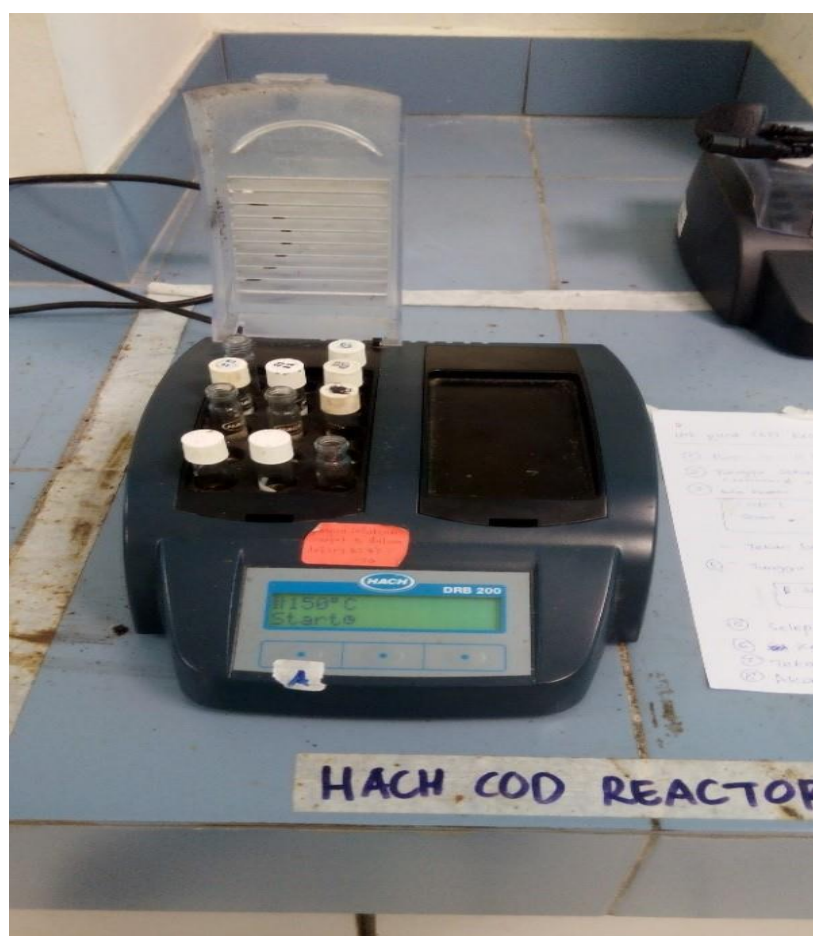
1. Shahidul, M., Baini, R., Tanjong, S. J., Said, M. A. M., & Joy, E. J. (2018). Effects of hydraulic retention time and solid retention time of POME on COD removal efficiency. *International Journal of Automotive and Mechanical Engineering*, 15(2), 5347–5355.
2. Eugene JJ, Shahidul MI, & Mamunur R. (2019). Optimizing organic materials reduction from hazardous industrial bio-effluent by the aid of C/N enriched inoculum: An approach to use traditional anaerobic reactor to achieve environmental sustainability. *Science International (Lahore)*, 31(4), 649–654.

Appendix B: Experimental Setup

Appendix B1: 350 Litre Lab-Scale Reactor



Appendix B2: COD Reactor



Appendix B3: Biomass Measuring Scale



Appendix B4: Flow Meter



Appendix B5: pH Meter



Appendix C1: Method of Preparation for Inoculum

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Appendix C2: Outputs of Experimental Range and Levels from CCD

Central Composite Design

Each numeric factor is set to 5 levels: plus and minus alpha (axial points), plus and minus 1 (factor combination of the categorical factor levels).

Numeric factors: 5 (2 to 50)

☒ Horizontal

Categoric factors: 0 (0 to 10)

☐ Vertical

	Name	Units	Low	High	-alpha	+alpha
A [Numeric]	pH		6	7	5.31079	7.68921
B [Numeric]	C/N		20	32	11.7295	40.2705
C [Numeric]	OLR	CODg/L.d	2	15	-6.95969	23.9597
D [Numeric]	HRT	d	4	10	-0.135243	14.1352
E [Numeric]	SRT	d	10	16	5.86476	20.1352

☒ Enter factor ranges in terms of +/- 1 levels

☐ Enter factor ranges in terms of alphas

Type: Full

Blocks: 1

Points

Non-center points 42

Center points 8

alpha = 2.37841

Options...

50 Runs

Appendix C3: Model Summary from ANOVA

Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2122.37	20	106.12	6.32	< 0.0001	significant
A-pH	504.42	1	504.42	30.04	< 0.0001	
B-C/N	97.22	1	97.22	5.79	0.0227	
C-OLR	778.70	1	778.70	46.37	< 0.0001	
D-HRT	41.01	1	41.01	2.44	0.1290	
E-SRT	3.84	1	3.84	0.23	0.6362	
AB	3.78	1	3.78	0.23	0.6387	
AC	75.03	1	75.03	4.47	0.0433	
AD	69.03	1	69.03	4.11	0.0519	
AE	0.031	1	0.031	1.861E-003	0.9659	
BC	52.53	1	52.53	3.13	0.0875	
BD	0.031	1	0.031	1.861E-003	0.9659	
BE	7.03	1	7.03	0.42	0.5227	
CD	11.28	1	11.28	0.67	0.4191	
CE	0.78	1	0.78	0.047	0.8307	
DE	3.78	1	3.78	0.23	0.6387	
A ²	12.74	1	12.74	0.76	0.3909	
B ²	2.46	1	2.46	0.15	0.7049	
C ²	629.40	1	629.40	37.48	< 0.0001	
D ²	20.13	1	20.13	1.20	0.2826	
E ²	67.93	1	67.93	4.05	0.0537	
Residual	487.01	29	16.79			

Appendix C3 continued

Residual	487.01	29	16.79		
Lack of Fit	389.01	21	18.52	1.51	0.2811 not significant
Pure Error	98.00	8	12.25		
Cor Total	2609.38	49			
The Model F-value of 6.32 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AC, C² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 1.51 implies the Lack of Fit is not significant relative to the pure error. There is a 28.11% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.					
Std. Dev.	4.10		R-Squared	0.8134	
Mean	66.18		Adj R-Squared	0.6846	
C.V. %	6.19		Pred R-Square	0.1143	
PRESS	2311.05		Adeq Precisor	8.933	
-2 Log Likelihood	255.71		BIC	337.86	
			AICc	330.71	

Appendix C4: Experimental Data on COD Reduction

Select	Std	Run ▼	Factor 1 A:pH	Factor 2 B:C/N	Factor 3 C:OLR CODg/L.d	Factor 4 D:HRT d	Factor 5 E:SRT d	Response 1 COD Reducti... %
	22	1	7	20	15	4	16	65
	4	2	7	32	2	4	10	60
	36	3	6.5	40.2705	8.5	7	13	75
	28	4	7	32	2	10	16	60
	11	5	6	32	2	10	10	57
	29	6	6	20	15	10	16	58
	6	7	7	20	15	4	10	70
	37	8	6.5	26	6.95969	7	13	65
	8	9	7	32	15	4	10	75
	7	10	6	32	15	4	10	60
	43	11	6.5	26	8.5	7	13	70
	19	12	6	32	2	4	16	60
	24	13	7	32	15	4	16	75
	17	14	6	20	2	4	16	57
	34	15	7.68921	26	8.5	7	13	80
	18	16	7	20	2	4	16	57
	49	17	6.5	26	8.5	7	13	77
	42	18	6.5	26	8.5	7	20.1352	70
	35	19	6.5	11.7295	8.5	7	13	70
	1	20	6	20	2	4	10	55
	33	21	5.31079	26	8.5	7	13	62
	44	22	6.5	26	8.5	7	13	77
	48	23	6.5	26	8.5	7	13	77
	10	24	7	20	2	10	10	65
	38	25	6.5	26	23.9597	7	13	62

Appendix C4 continued

9	26	6	20	2	10	10	57
30	27	7	20	15	10	16	75
16	28	7	32	15	10	10	78
13	29	6	20	15	10	10	60
14	30	7	20	15	10	10	65
39	31	6.5	26	8.5	7	13	70
23	32	6	32	15	4	16	70
2	33	7	20	2	4	10	57
46	34	6.5	26	8.5	7	13	70
5	35	6	20	15	4	10	70
3	36	6	32	2	4	10	58
12	37	7	32	2	10	10	65
32	38	7	32	15	10	16	80
21	39	6	20	15	4	16	58
40	40	6.5	26	8.5	14.1352	13	74
20	41	7	32	2	4	16	58
25	42	6	20	2	10	16	57
45	43	6.5	26	8.5	7	13	70
15	44	6	32	15	10	10	65
26	45	7	20	2	10	16	65
41	46	6.5	26	8.5	7	5.86476	65
50	47	6.5	26	8.5	7	13	70
31	48	6	32	15	10	16	65
27	49	6	32	2	10	16	58
47	50	6.5	26	8.5	7	13	70

Appendix C5: Output of DOE on Coefficient Estimate and Standard Error

Factor	Coefficient	df	Standard	95% CI		VIF
	Estimate		Error	Low	High	
Intercept	71.45	1	1.29	68.81	74.10	
A-pH	3.41	1	0.62	2.14	4.69	1.00
B-C/N	1.50	1	0.62	0.22	2.77	1.00
C-OLR	4.84	1	0.71	3.39	6.29	1.13
D-HRT	1.11	1	0.71	-0.34	2.57	1.13
E-SRT	0.30	1	0.62	-0.98	1.57	1.00
AB	0.34	1	0.72	-1.14	1.83	1.00
AC	1.53	1	0.72	0.050	3.01	1.00
AD	1.47	1	0.72	-0.013	2.95	1.00
AE	-0.031	1	0.72	-1.51	1.45	1.00
BC	1.28	1	0.72	-0.20	2.76	1.00
BD	-0.031	1	0.72	-1.51	1.45	1.00
BE	0.47	1	0.72	-1.01	1.95	1.00
CD	-0.59	1	0.72	-2.08	0.89	1.00
CE	0.16	1	0.72	-1.33	1.64	1.00
DE	0.34	1	0.72	-1.14	1.83	1.00
A ²	-0.47	1	0.54	-1.58	0.64	1.03
B ²	-0.21	1	0.54	-1.32	0.90	1.03
C ²	-4.50	1	0.74	-6.00	-3.00	1.14
D ²	-0.80	1	0.73	-2.30	0.70	1.14
E ²	-1.09	1	0.54	-2.20	0.018	1.03

Appendix C6: ANOVA on Actual and Model Prediction

Run Order	Actual Value	Predicted Value	Residual	Leverage	Studentized Residual	Studentized Residual	Cook's Distance
1	65.00	68.63	-3.63	0.469	-1.216	-1.227	0.062
2	60.00	58.60	1.40	0.475	0.471	0.464	0.010
3	75.00	73.84	1.16	0.580	0.436	0.429	0.012
4	60.00	66.05	-6.05	0.469	-2.025	-2.147	0.172
5	57.00	56.75	0.25	0.469	0.083	0.082	0.000
6	58.00	61.28	-3.28	0.454	-1.083	-1.086	0.046
7	70.00	69.41	0.59	0.469	0.197	0.194	0.002
8	65.00	70.05	-5.05	0.098	-1.299	-1.315	0.009
9	75.00	74.78	0.22	0.469	0.073	0.071	0.000
10	60.00	67.08	-7.08	0.469	-2.372	-2.596	0.237
11	70.00	71.45	-1.45	0.099	-0.374	-0.368	0.001
12	60.00	57.62	2.38	0.475	0.801	0.796	0.028
13	75.00	75.88	-0.88	0.469	-0.294	-0.290	0.004
14	57.00	56.88	0.12	0.475	0.041	0.041	0.000
15	80.00	76.90	3.10	0.580	1.168	1.176	0.090
16	57.00	56.95	0.048	0.475	0.016	0.016	0.000
17	77.00	71.45	5.55	0.099	1.426	1.453	0.011
18	70.00	65.99	4.01	0.580	1.510	1.546	0.150
19	70.00	66.72	3.28	0.580	1.236	1.248	0.100
20	55.00	58.16	-3.16	0.475	-1.063	-1.066	0.049
21	62.00	60.66	1.34	0.580	0.503	0.497	0.017
22	77.00	71.45	5.55	0.099	1.426	1.453	0.011
23	77.00	71.45	5.55	0.099	1.426	1.453	0.011
24	65.00	64.08	0.92	0.469	0.308	0.303	0.004
25	62.00	57.51	4.49	0.796	2.426	2.670	1.093 ¹
26	57.00	58.00	-1.00	0.469	-0.336	-0.331	0.005
27	75.00	73.35	1.65	0.454	0.543	0.537	0.012
28	78.00	78.01	-6.045E-003	0.454	-0.002	-0.002	0.000
29	60.00	60.56	-0.56	0.454	-0.185	-0.182	0.001
30	65.00	72.76	-7.76	0.454	-2.562	-2.863	0.260
31	70.00	71.45	-1.45	0.099	-0.374	-0.368	0.001
32	70.00	68.30	1.70	0.469	0.568	0.561	0.014
33	57.00	58.36	-1.36	0.475	-0.457	-0.451	0.009

Appendix C6 continued

34	70.00	71.45	-1.45	0.099	-0.374	-0.368	0.001
35	70.00	63.09	6.91	0.469	2.315	2.520	0.226
36	58.00	57.03	0.97	0.475	0.327	0.322	0.005
37	65.00	64.20	0.80	0.469	0.268	0.263	0.003
38	80.00	80.48	-0.48	0.454	-0.157	-0.155	0.001
39	58.00	62.43	-4.43	0.469	-1.484	-1.517	0.093
40	74.00	69.56	4.44	0.796	2.397	2.631	1.065 ¹
41	58.00	59.07	-1.07	0.475	-0.362	-0.356	0.006
42	57.00	58.10	-1.10	0.469	-0.368	-0.363	0.006
43	70.00	71.45	-1.45	0.099	-0.374	-0.368	0.001
44	65.00	64.43	0.57	0.454	0.188	0.185	0.001
45	65.00	64.05	0.95	0.469	0.318	0.313	0.004
46	65.00	64.57	0.43	0.580	0.161	0.159	0.002
47	70.00	71.45	-1.45	0.099	-0.374	-0.368	0.001
48	65.00	67.03	-2.03	0.454	-0.669	-0.663	0.018
49	58.00	58.72	-0.72	0.469	-0.241	-0.238	0.002
50	70.00	71.45	-1.45	0.099	-0.374	-0.368	0.001

Appendix C7: Test Report on COD of POME



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MS ISO/IEC 17025
TESTING
SAMM NO. 057

TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer : Eugene Anak Jackson
1384, Lorong D7
Taman Satria Jaya, BDC
Jalan Stutong
93350, Kuching

Lab No. : CK/CL105/114097/18
Type (No.) of Sample : Effluent (4)
Date Received : 14th August 2018
Date of Report : 24th August 2018
Service Order : -

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)
114097-1	POME Date : 11/08/18	50,900
114097-2	POME Date : 12/08/18	22,500
114097-3	POME Date : 13/08/18	39,100
114097-4	POME Date : 14/08/18	18,400

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Appendix C8: Test Report on COD of POME



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Tel: +06-82-548366 Fax: +06-82-548388 / 548399
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TEST REPORT
* NOT FOR ADVERTISEMENT PURPOSES *

Customer : Eugene Anak Jackson
1384, Lorong D7
Taman Satria Jaya, BDC
Jalan Stutong
93350, Kuching

Lab No. : CK/CL105/114219/18
Type (No.) of Sample : Effluent (3)
Date Received : 17th August 2018
Date of Report : 3rd September 2018
Service Order : -

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)
114219-1	POME Date : 15/08/18	21,600
114219-2	POME Date : 16/08/18	15,200
114219-3	POME Date : 17/08/18	18,500



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Appendix C9: Test Report on COD of POME



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TEST REPORT

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Customer : Eugene Anak Jackson
1384, Lorong D7
Taman Satria Jaya, BDC
Jalan Stutong
93350, Kuching

Lab No. : CK/CL105/113492/18
Type (No.) of Sample : Effluent (3)
Date Received : 16th July 2018
Date of Report : 20th July 2018
Service Order : -

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)
113492-1	POME Date : 13/07/18	93,600
113492-2	POME Date : 14/07/18	38,600
113492-3	POME Date : 15/07/18	35,300

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Appendix C10: Test Report on COD of POME

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	Email: laboratory@chemsain.com
	
	

TEST REPORT

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Customer :	Eugene Anak Jackson	Lab No. :	CK/CL105/113521/18
	1384, Lorong D7	Type (No.) of Sample :	Effluent (2)
	Taman Satria Jaya, BDC	Date Received :	17 th July 2018
	Jalan Stutong	Date of Report :	31 st July 2018
	93350, Kuching	Service Order :	-

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)
113521-1	POME Date : 16/07/18	71,700
113521-2	POME Date : 17/07/18	70,200

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Appendix C11: Test Report on COD of POME



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TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer : Eugene Anak Jackson 1384, Lorong D7 Taman Satria Jaya, BDC Jalan Stutong 93350, Kuching	Lab No. : CK/CL105/113566/18 Type (No.) of Sample : Effluent (3) Date Received : 19 th July 2018 Date of Report : 9 th August 2018 Service Order : -
--	--

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)	*Mixed Liquor Volatile Suspended Solids (MLVSS), mg/L (Method: APHA 2540 E, 2005)
113566-1	Sample A Date : 18/07/18	73,500	1,560
113566-2	Sample B Date : 19/07/18	62,000	-

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Appendix C12: Test Report on COD of POME

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	Email: laboratory@chemsain.com	

TEST REPORT

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Customer :	Eugene Anak Jackson	Lab No. :	CK/CL105/113137/18
	1384, Lorong D7	Type (No.) of Sample :	Effluent (3)
	Taman Satria Jaya, BDC	Date Received :	2 nd July 2018
	Jalan Stutong	Date of Report :	10 th July 2018
	93350, Kuching	Service Order :	-

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)
113137-1	POME Date : 29/06/18	76,100
113137-2	POME Date : 30/06/18	53,700
113137-3	POME Date : 01/07/18	50,700


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Appendix C13: Test Report on COD of POME



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TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer : Eugene Anak Jackson
 1384, Lorong D7
 Taman Satria Jaya, BDC
 Jalan Stutong
 93350, Kuching

Lab No. : CK/CL105/113235/18
 Type (No.) of Sample : Effluent (3)
 Date Received : 5th July 2018
 Date of Report : 18th July 2018
 Service Order : -

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)	*Mixed Liquor Volatile Suspended Solids (MLVSS), mg/L (Method: APHA 2540 E, 2005)
113235-1	Sample A Date : 02/07/18	53,700	24,100
113235-2	Sample B Date : 03/07/18	67,800	-
113235-3	Sample C Date : 04/07/18	88,700	-

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Appendix C14: Test Report on COD of POME

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TEST REPORT

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Customer : Eugene Anak Jackson 1384, Lorong D7 Taman Satria Jaya, BDC Jalan Stutong 93350, Kuching	Lab No. : CK/CL105/113286/18 Type (No.) of Sample : Effluent (1) Date Received : 6 th July 2018 Date of Report : 20 th July 2018 Service Order : -
--	--

Parameter	POME Date : 05/07/18	Method
Chemical Oxygen Demand, mg/L	38,100	DOE 2011, Reference Method

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Appendix C15: Test Report on COD of POME



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TEST REPORT

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Customer : Eugene Anak Jackson 1384, Lorong D7 Taman Satria Jaya, BDC Jalan Stutong 93350, Kuching	Lab No. : CK/CL105/112325/18 Type (No.) of Sample : Effluent (5) Date Received : 15 th May 2018 Date of Report : 28 th May 2018 Service Order : -
--	---

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)
112325-1	POME Date : 27/04/18	97,300
112325-2	POME Date : 28/04/18	88,800
112325-3	POME Date : 29/04/18	85,100
112325-4	POME Date : 30/04/18	90,300
112325-5	POME Date : 01/05/18	84,400

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Appendix C16: Test Report on COD of POME



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Tel: +06-82-548366 **Fax:** +06-82-548388 / 548399
Email: laboratory@chemsain.com




TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer : Eugene Anak Jackson
 1384, Lorong D7
 Taman Satria Jaya, BDC
 Jalan Stutong
 93350, Kuching

Lab No. : CK/CL105/112054/18
 Type (No.) of Sample : Effluent (7)
 Date Received : 30th April 2018
 Date of Report : 11th May 2018
 Service Order : -

Lab No.	Identification of Sample (s)	Chemical Oxygen Demand, mg/L (Method: DOE 2011, Reference Method)
112054-1	POME Date : 08/04/18	98,000
112054-2	POME Date : 09/04/18	95,100
112054-3	POME Date : 10/04/18	93,600
112054-4	POME Date : 11/04/18	92,500
112054-5	POME Date : 12/04/18	85,700
112054-6	POME Date : 14/04/18	91,000
112054-7	POME Date : 15/04/18	90,200

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Appendix C17: Test Report on COD of POME

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	Tel: +06-82-548366 Fax: +06-82-548388 / 548399	
	Email: laboratory@chemsain.com	

TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer :	Eugene Anak Jackson	Lab No.	: CK/CL105/111328/18
	1384, Lorong D7	Type (No.) of Sample	: Effluent (1)
	Taman Satria Jaya, BDC	Date Received	: 21 st March 2018
	Jalan Stutong	Date of Report	: 29 th March 2018
	93350, Kuching	Service Order	: -

Parameter	POM Effluent Date : 08/03/18 Time : 2.00 pm	Method
Chemical Oxygen Demand, mg/L	82,900	DOE 2011, Reference Method


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B. Sc. (Hons), M. Env.Sc.,
MMIC (2747/5242/08/08)
SENIOR CHEMIST



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Appendix C18: Test Report on COD of POME

	CHEMSAIN KONSULTANT SDN BHD (130904-U)	 
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TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer :	Eugene Anak Jackson 1384, Lorong D7 Taman Satria Jaya, BDC Jalan Stutong 93350, Kuching	Lab No. :	CK/CL105/111354/18
		Type (No.) of Sample :	Effluent (1)
		Date Received :	22 nd March 2018
		Date of Report :	3 rd April 2018
		Service Order :	-

Parameter	Set A Date : 22/03/18	Method
Chemical Oxygen Demand, mg/L	91,400	DOE 2011, Reference Method


TANG JOCK KJIE
B. Sc. (Hons), M.Env.Sc.,
MMIC (2747/5242/08/08)
SENIOR CHEMIST



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Appendix C19: Test Report on COD of POME

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	172, Rock Road, 93200 Kuching Sarawak.	
	Tel: +06-82-548366 Fax: +06-82-548388 / 548399	
	Email: laboratory@chemsain.com	

TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer :	Eugene Anak Jackson	Lab No. :	CK/CL105/111355/18
	1384, Lorong D7	Type (No.) of Sample :	Water (1)
	Taman Satria Jaya, BDC	Date Received :	22 nd March 2018
	Jalan Stutong	Date of Report :	4 th April 2018
	93350, Kuching	Service Order :	-

Parameter	Set B Date : 22/03/18	Method
Biochemical Oxygen Demand, mg/L	24,200	DOE 2011, Reference Method
Chemical Oxygen Demand, mg/L	105,000	DOE 2011, Reference Method

Date of commencement of BOD₅ analysis : 22nd March 2018


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B. Sc. (Hons), M. Env. Sc.
MMIC (2747/5242/08/08)
SENIOR CHEMIST



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Appendix C20: Test Report on COD of POME



CHEMSAIN KONSULTANT SDN BHD (130904-U)
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TEST REPORT

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Customer : Eugene Anak Jackson
1384, Lorong D7
Taman Satria Jaya, BDC
Jalan Stutong
93350, Kuching

Lab No. : CK/CL105/111412/18
Type (No.) of Sample : Effluent (2)
Date Received : 26th March 2018
Date of Report : 3rd April 2018
Service Order : -

Lab No.	111412-1	111412-2	Method
Parameter	POME SET A Date : 26/03/18	POME SET B Date : 26/03/18	
Chemical Oxygen Demand, mg/L	82,500	95,800	DOE 2011, Reference Method



TANG JOCK KJE
B. Sc. (Hons), M. Env. Sc.,
MMIC (2747/5242/08/08)
SENIOR CHEMIST



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3) The result(s) relates to the sample(s) tested.

Appendix C21: Test Report on COD of POME



CHEMSAIN KONSULTANT SDN BHD (130904-U)
 172, Rock Road, 93200 Kuching Sarawak.
 Tel: +06-82-548366 Fax: +06-82-548388 / 548399
 Email: laboratory@chemsain.com




TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer : Eugene Anak Jackson
 1384, Lorong D7
 Taman Satria Jaya, BDC
 Jalan Stutong
 93350, Kuching

Lab No. : CK/CL105/110967/18
 Type (No.) of Sample : Effluent (4)
 Date Received : 5th March 2018
 Date of Report : 15th March 2018
 Service Order : -

Lab No.	110967-1	110967-2	<i>Method</i>
Parameter	POME 1 Date : 05/03/18 Time : 8.00 am	POME 2 Date : 05/03/18 Time : 10.00 am	
Chemical Oxygen Demand, mg/L	76,400	70,500	DOE 2011, Reference Method

Lab No.	110967-3	110967-4	<i>Method</i>
Parameter	POME 3 Date : 05/03/18 Time : 12.00 pm	POME 4 Date : 05/03/18 Time : 2.00 pm	
Chemical Oxygen Demand, mg/L	78,700	73,100	DOE 2011, Reference Method



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Appendix C22: Test Report on COD of POME

	CHEMSAIN KONSULTANT SDN BHD (130904-U)	 
	172, Rock Road, 93200 Kuching Sarawak. Tel: +06-82-548366 Fax: +06-82-548388 / 548399 Email: laboratory@chemsain.com	

TEST REPORT
* NOT FOR ADVERTISEMENT PURPOSES *

Customer :	Eugene Anak Jackson 1384, Lorong D7 Taman Satria Jaya, BDC Jalan Stutong 93350, Kuching	Lab No. :	CK/CL105/111041/18
		Type (No.) of Sample :	Effluent (1)
		Date Received :	8 th March 2018
		Date of Report :	15 th March 2018
		Service Order :	-

Parameter	POM Effluent Date : 07/03/18 Time : 2.30 pm	Method
Chemical Oxygen Demand, mg/L	80,500	DOE 2011, Reference Method



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Appendix C23: Test Report on COD of POME

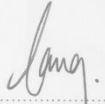
	CHEMSAIN KONSULTANT SDN BHD (130904-U)	
	172, Rock Road, 93200 Kuching Sarawak.	
	Tel: +06-82-548366 Fax: +06-82-548388 / 548399	
	Email: laboratory@chemsain.com	
		

TEST REPORT

* NOT FOR ADVERTISEMENT PURPOSES *

Customer :	Eugene Anak Jackson	Lab No. :	CK/CL105/111065/18
	1384, Lorong D7	Type (No.) of Sample :	Effluent (1)
	Taman Satria Jaya, BDC	Date Received :	8 th March 2018
	Jalan Stutong	Date of Report :	15 th March 2018
	93350, Kuching	Service Order :	-

Parameter	POM Effluent Date : 08/03/18 Time : 2.00 pm	Method
Chemical Oxygen Demand, mg/L	91,700	DOE 2011, Reference Method


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