



Faculty of Engineering

Optimization of Biogas Production from POME mixed with Indigenous Inoculum using Two-Stage Anaerobic Reactor Operating at Mesophilic Temperature, Coupled with Nanomembrane Separator

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Optimization of Biogas Production from POME mixed with Indigenous
Inoculum using Two-Stage Anaerobic Reactor Operating at Mesophilic
Temperature, Coupled with Nanomembrane Separator

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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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With unwavering commitment to life-long learning, it is inspiring to note the words of Napoleon Hill who stipulated that ‘Anticipation is sweeter than realization as man must be motivated by the pursuit of some yet unattained object, that which is at hand does not satisfy. The only enduring satisfaction is what the person keeps alive in his heart, the hope of better future.’ Due to constant challenge from industrial and economic development towards Industrial Revolution 4.0 on mankind and environment, I feel compelled to undertake this research study with this esteemed university, under the supervision of Professor Dr. M. Shahidul Islam, supported by two other co-supervisors Professor Ir. Dr Andrew Ragai Henry Rigit and Dr Magdalene Andrew Munot from Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, UNIMAS, Sarawak. I would like to register my greatest appreciation and sincere gratitude to all the people involved, especially my main Supervisor Professor Dr M. Shahidul Islam for his steely patience and unflinching resolve in helping me to complete this thesis over the last four years. Special mentions are also accorded to our Research Fellow (Eugene) as well as the academic staff of Engineering faculty and Centre for Graduate Studies (CGS) for their unsparing assistance and services throughout my enrolment with UNIMAS, Sarawak. Lastly, I wish to thank SALCRA and its staff for allowing us to visit and collect samples from BAPOM for the experiments. Special thanks to sponsors, supporters, friends, and my family members who had rendered their continuous support in terms of money, time, and space to enable timely completion of this important research which could contribute to both economic and environmental sustainability of the palm oil industry in Malaysia.

MALCOLM MUSSEN LAMOH

ABSTRACT

The broad objective of this research is to optimize biogas production from Palm Oil Mill Effluent (POME) by reducing Chemical Oxygen Demand, Biological Oxygen Demand and sludge levels in high-strength POME before disposal in order to assuage its negative impacts on environment, economy and health. The COD and VSS of POME would be converted by microorganisms to reusable resources such as biogas, potable water and organic sludge for achieving social, economic and environmental sustainability. The novelty of this research is to develop a model by using Central Composite Design (CCD) combined with Design of Experiment (DOE) to optimize the number of experiments in a double stage fermentation process. Based on the findings of literature review, five important process factors such as HRT, C/N, SRT, pH, OLR were identified with their limits of input to digestion process. The limit of each factor was analysed by CCD and DOE to determine steps of experiment and number of experimental runs for finding the significant factors and their respective optimum values relating to optimum level of biogas production from POME. Two-stage fermentation was used to separate and accelerate acidogenesis and methanogenesis in five sets of 5-litre CSTR reactors for POME digestion to produce biogas. Fifty (50) experimental runs as per CCD and DOE software outputs were needed to determine optimum level of inputs in developing the model for optimal biogas production. The experiment revealed that the factors significantly ($p < 0.05$) affect biogas production are HRT, SRT, pH and OLR; but C/N has also contributed to produce biogas though its contribution was not significant ($p > 0.05$). The experiment also unlocked the information related to optimum value of factors that contributed to optimize biogas production. Based on the 3D plots of Response Surface Methodology (RSM), the optimum value for OLR is 5 kg/m³/d, pH is 6.6–7.1, C/N is 30.5, HRT is 6.5 days and SRT is 13.5 days with optimum output of biogas production at 3.0 L/d.

This finding has been validated using similar experiments with two 350 L CSTR reactors whose results demonstrated that an empirical model built to estimate biogas production from POME appeared quite fit to predict output of anaerobic reactor within the 5% error limit.

Keywords: Optimization, biogas production, palm oil mill effluent, anaerobic reactor, mesophilic temperature

***Pengoptimuman Pengeluaran Biogas dari Efluen Kilang Minyak Sawit (POME)
Menggunakan Reaktor Anaerobik yang Beroperasi di Suhu Mesofilik dan diginding
dengan Pemisah Nanomembran***

ABSTRAK

Objektif utama penyelidikan ini adalah untuk mengoptimumkan pengeluaran biogas dari POME dengan mengurangkan COD, BOD, dan tahap enapcemar dalam kekuatan tinggi POME sebelum dilupusan untuk mengurangkan kesan negatif ke atas alam sekitar, ekonomi, dan kesihatan. COD dan VSS dari POME akan ditukar oleh mikroorganisma kepada sumber-sumber yang boleh digunakan semula seperti biogas, air bersih, dan enapcemar organik untuk mencapai kelestarian sosial, ekonomi, dan alam sekitar. Keaslian penyelidikan ini adalah untuk membangun model dengan menggunakan Central Composite Design (CCD) digabungkan dengan reka bentuk eksperimen (DOE) untuk mengoptimumkan jumlah eksperimen dalam proses penapaian dua peringkat. Berdasarkan hasil kajian literatur, lima faktor proses penting seperti HRT, C/N, SRT, pH, dan OLR telah dikenal pasti dengan hadnya dalam proses penghadaman. Had faktor-faktor ini telah dianalisa menggunakan CCD dan DOE untuk menentukan langkah eksperimen dan jumlah eksperimen untuk mencari faktor yang penting dan nilai optimum yang berkaitan dengan tahap optimum pengeluaran biogas dari POME. Penapaian dua peringkat telah digunakan untuk memisahkan dan mempercepatkan proses acidogenesis dan methanogenesis dalam lima set 5-liter CSTR reaktor untuk pencernaan POME untuk menghasilkan biogas. Lima puluh (50) jumlah eksperimen diperlukan untuk menentukan tahap optimum input dalam membangun model untuk pengeluaran biogas yang optimum. Eksperimen ini menunjukkan bahawa faktor ketara ($p < 0.05$) yang mempunyai sumbangan yang besar bagi pengeluaran biogas adalah HRT, SRT, pH, dan OLR; dan C/N juga telah menyumbang kepada menghasilkan biogas tetapi sumbangannya tidak ketara ($p > 0.05$). Eksperimen ini

juga menjelaskan maklumat yang berkaitan dengan nilai optimum faktor-faktor yang menyumbang kepada pengeluaran biogas yang optimum. Berdasarkan plot 3D Response Surface Methodology (RSM), nilai optimum untuk OLR ialah 5 kg/m³/d, pH ialah 6.6–7.1, C/N ialah 30.5, HRT ialah 6.5 hari, dan SRT ialah 13.5 hari dengan output optimum pengeluaran biogas pada 3.0 L/d. Penemuan ini telah disahkan menggunakan eksperimen yang sama dengan dua reaktor CSTR 350 L yang menunjukkan bahawa model empirikal dibina untuk menganggarkan pengeluaran biogas daripada POME kelihatan agak memuaskan untuk meramal output reaktor anaerobik dalam had ralat 5%.

Kata kunci: *Pengoptimuman, pengeluaran biogas, efluen kilang minyak sawit, reaktor anaerobik, suhu mesofilik*

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LIST OF ABBREVIATIONS

BOD	Biochemical Oxygen Demand
C/N	Carbon-to-Nitrogen Ratio
CCD	Central Composite Design
COD	Chemical Oxygen Demand
CV	Coefficient of Variation
DOE	Design of Experiment
F/M	Food-to-Microorganisms Ratio
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

CHAPTER 1

INTRODUCTION

1.1 Study Background

Being predominantly an agriculture-based economy, the palm oil industry has grown into one of the major industries in Malaysia, making it the second biggest suppliers of palm oil in the world after Indonesia. For the corresponding year of 2015 and 2016, the total volumes of production of CPO recorded by Malaysian Palm Oil Board (MPOB) were about 19.961 and 17.318 million tons respectively, as reflected in Table 1.1.

Table 1.1: Crude Oil Palm Production in Malaysia (CPO) for 2015 and 2016 (MPOB, 2015, 2016)

Data No.	Months	Production for 2015 (‘000 tons)	Production for 2016 (‘000 tons)
1	Jan	1,161	1,130
2	Feb	1,122	1,043
3	Mar	1,495	1,219
4	Apr	1,693	1,301
5	May	1,811	1,365
6	Jun	1,764	1,533
7	Jul	1,816	1,585
8	Aug	2,051	1,702
9	Sep	1,959	1,715
10	Oct	2,038	1,678
11	Nov	1,654	1,575
12	Dec	1,399	1,473
	Total Production	19,961	17,318

Malaysia registered a substantial increase of 4.6% in the total planted area under oil palm covering 5.64 million hectares in 2015 compared with 5.39 million hectares in the previous year 2014. Currently, Sabah and Sarawak constitute the largest planted areas at 27% and 26% respectively, while the remaining 47% of the total planted areas are scattered in Peninsular Malaysia (MPOB, 2015, 2016). Consequently, Malaysia produces significant

portion in the exports of oils and fats to the world market (Madaki & Seng, 2013), amounting to 12% and 27% for the respective products.

Undoubtedly, the palm oil industry provides a significant source of livelihood for rural dwellers in government land development schemes and private small holdings, as well as more employment opportunities for technical and general posts in the agricultural estates. Conversely, the palm oil industry creates some adverse impacts on environment in terms of soil, water and air pollution in our lithosphere, hydrosphere and atmosphere respectively.

Over the years, the palm oil mill effluent or POME has notoriously become the most significant pollutant from palm oil mills. Being a by-product of crude palm oil (CPO), POME is a rich biodegradable effluent, usually discharged to water bodies without adequate treatment. The oily mixture can be decomposed completely with the presence of oxygen (known as BOD) where raw POME can possess about 100 times higher BOD than domestic sewage (Madaki & Seng, 2013).

In terms of content of POME, water constitutes 95–96%, total solids are 4–5% with suspended solids at 2–4%, while oil and grease at 0.6–0.7% only (Langerak, 2018). As an estimate, the production of one tonne of crude palm oil (CPO) from the mill would generate about 2.5–3.5 m³ of palm oil mill effluent known as POME. The major characteristics of this hazardous effluent are its high content of organic materials (COD and BOD) and other nutrients which make it toxic with low pH as reflected in Table 1.2 (MPOB, 2012; Shahidul et al., 2018).

When untreated POME discharges into the water bodies, the biodegradable elements in POME tend to consume dissolved oxygen from water at a faster rate compared to the rate of

oxygen that can be dissolved in the receiving water. Consequently, fish and other marine organisms would not be able to tolerate the depletion of oxygen in the water. When polluted water is used in agriculture activities, crops absorb the harmful pollutants which contribute to change in cell structure and nutritional content of crops and then toxic elements of POME would get into food chain. Subsequently, human health will be severely affected which could result in kidney failure and other fatal disease. Rural dwellers rely on river water to perform domestic chores, be it cooking, drinking or bathing; use of such polluted water could damage their skins, liver and kidneys in the long haul.

Table 1.2: Properties (Characteristics) of POME (MPOB, 2012; Shahidul et al., 2018)

Parameter	Characteristics of Pome		
	Units	Mean	Range
Chemical Oxygen Demand or COD	mg/L	51000	15000 to 100000
Biochemical Oxygen Demand or BOD	mg/L	25000	10250 to 43750
pH	-	4.2	3.4 to 5.2
Total Solids	mg/L	40000	11500 to 79000
Suspended Solids	mg/L	18000	5000 to 54000
Volatile Solids	mg/L	34000	9000 to 72000
Oil and Grease	mg/L	6000	130 to 18000
Ammoniacal Nitrogen	mg/L	35	4 to 80
Total Nitrogen	mg/L	750	180 to 1400

Biodegradation of POME would generate biogas, comprising mainly methane and carbon dioxide as listed in Table 1.3. If these obnoxious gases are not captured or contained and released to the atmosphere, it would result in the formation and emission of greenhouse gases (GHGs) which cause global warming.

Table 1.3 manifests that significant percentage of GHGs would be generated from POME which could have adverse effects on environment in general; and among these, effects on public health, economy and global warming are significant.

Table 1.3: Composition of Biogas from POME (Langerak, 2018; Shahidul et al., 2018)

Elements of Biogas	Volumetric content (%)
Methane (CH ₄)	50–75
Carbon dioxide (CO ₂)	25–45
Water vapour (H ₂ O)	2–7
Oxygen (O ₂)	<2
Nitrogen (N ₂)	<2
Hydrogen Sulphide (H ₂ S)	<2
Ammonia (NH ₃)	<1
Hydrogen (H ₂)	<1

On health issues, the air containing higher density CH₄, CO₂ and hydrogen would be harmful to the local communities, making them more susceptible to respiratory diseases when subjected to constant exposure to the polluted air. Animals and plants may not survive in such contaminated environment and would eventually perish. On the economic aspect, 1.0 m³ of POME is estimated to produce around 28 m³ of biogas (Wang et al., 2015) which contains about 64.5% methane, giving it a high economic value for energy generation. With regards to environment, global warming potential of those gases becomes a contagious issue. The formation of a GHG layer in the atmosphere would trap thermal energy above the Earth's surface, which reflects its global warming potential (GWP). In this aspect, POME is renounced to have considerably higher GWP compared to other industrial waste (Shahidul et al., 2018).

In order to address the issue of the GHGs emission, a few researches have been conducted with aim to capture CH₄ emitted by POME; where most of these studies were confined to evaluation of anaerobic digester performance in biogas production as well as methane potential of POME. But research efforts on biogas production optimization from POME using central compost design (CCD) together with design of experiment (DOE) have not been substantial. However, background of this research is to optimize the biogas production

from POME with involvement of biochemistry and biochemical process, which could contribute to achieve economic and environmental sustainability in the industry.

1.2 Problem Statement

Several methods of anaerobic digestion are used for the reduction of COD and biomass contents in POME for achieving economic and environmental sustainability. At this juncture, some new methods and technologies have been developed for more efficient POME treatment and disposal. However, most palm oil mills are still struggling to comply with more stringent requirements by Department of Environment (DOE) Malaysia in terms of limits of COD content in effluent and methane emission to the air. The current practice of using the conventional ponding system and open digesting tanks for reducing COD and biomass decomposing are environmentally disastrous with the emission of biogas, especially methane into the atmosphere. Moreover, these conventional methods would require longer processing time and bigger space or land area (Choi et al., 2013; Loh et al., 2013; Wu et al., 2009).

Thus, the current problems associated with POME treatment which converts both COD and biomass to biogas must be resolved as it would present a constant threat to environment, economy and public health. This research aims to address these problems by recovering these waste resources and converting them to useful outputs such as biogas for energy generation in order to get economic and environmentally sustainable solution.

In order to change the negative perceptions on the status of POME as waste to resource, more advanced technologies which are environmentally friendly need to be developed and applied in the palm oil mills. Treating POME not only focuses on the capturing of methane;

even clean water and organic fertilizers can be recovered. Then, problems associated with biogas emission could be resolved by promoting pollution control measures through cleaner production technology encompassing the international 5R (reduction, replacement, reuse, recovery, and recycling) policy on waste. Indeed, POME treatment could provide the potential for recovery of its valuable resources including biogas.

1.3 Research Questions and Hypotheses

Based on the above statement, what are the significant factors that influence optimal biogas production from POME? What are the optimum values of factors that contribute to optimize biogas production from POME? What empirical model is fit for the prediction of optimal biogas production from POME under anaerobic digestion?

Hence, this research is tailored for getting the relevant answers to these questions above. The most critical decision involves the selection of research variables (factors) and design of experiments to achieve the above objectives by addressing the following issues:

Does each of the selected factors have significant effect on biogas production?

Does optimal value of significant factors contribute to optimise biogas production?

Would the developed model be fit for prediction of optimal biogas production?

The research hypothesis is sub-divided into three components to provide answers to the research questions.

1.3.1 Hypothesis One

To test individual significance of inputs (t-test) at 95% confidence interval, whether each input as independent variable has effect on the dependent variable (output):

- H_0 : Each of the independent variables (pH, C/N, OLR, HRT, SRT) has no correlation or statistically significant relationship with dependent variable (output) at $p\text{-value} \leq 0.05$ confidence interval.
- H_A : Each factor (independent variable) has correlation with dependent variable at $p\text{-value} \geq 0.05$ confidence interval.

1.3.2 Hypothesis Two

The overall significance (f-test) on the combinations of independent variables (inputs) to jointly affect the dependent variable (output) must be statistically significant in order to reject the null hypothesis that all parameters or coefficients are not statistically different from zeros. Basically, it involves testing outputs of the digestion process whether total output is statistically significant at 95% confidence level. If the group of variables are jointly significant at a given probability of confidence interval at 95%, the model terms with non-zero coefficients should be included to get better fit than intercept-only model.

H_0 : The model estimate $Y(Q)$ as outputs of the anaerobic digestion is not statistically significant at $p\text{-value} > 0.05$, with all parameters or regression coefficients are not significantly different from zeros.

H_A: The model estimate Y(Q) as outputs of the anaerobic digestion is statistically significant at $p\text{-value} \leq 0.05$, where the model is statistically significant with at least one parameter not equal to zero.

1.3.3 Hypothesis Three

- In order to test the developed model is fit to predict the optimal level of biogas production from POME, f-test for equality of means (Lack of fit test) at the stipulated confidence interval ($p \geq 0.05$) could be applied. Any significant difference between model error and pure error indicates the Lack of Fit in the model, where p-value is small ($p < 0.05$) and f-statistic is greater than the critical f_c value from F-table.
- H_O: Model error means is roughly equal to the hypothesized or pure error.
- H_A: Model error means is significantly greater than pure error with large F-statistic value.

1.4 Objective of Research

The broad objective of this research focuses on the capture of biogas from POME for energy generation in order to achieve social, economic, and, environmental sustainability; though, POME had been regarded as a hazardous waste effluent causing adverse effects on health, environment and economy. However, the category of this research is to develop model in line with waste to energy (WtE) concept, which could directly benefit the palm oil industry itself. Eventually, this study would definitely give additional knowledge on the conversion of biomass into recoverable energy, through further improvements in the process design for POME treatment.

In this study, the broad objective is further broken down into the three specific objectives as follows:

- a) To determine the significant factors (parameters) which influence biogas production from POME under anaerobic digestion.
- b) To determine the optimum value of each factor that could contribute to optimal production of biogas from POME.
- c) To develop an empirical model for predicting optimal production of biogas from POME.

1.5 Scope of Study Required for Achieving Research Goals

This research is limited to the following scope of work in order to achieve the research goal. The experiment is to be conducted by using anaerobic reactor, operated at mesophilic temperature (about 30–35°C). The limits of variables and experimental runs would be determined by CCD and DoE to minimise the steps of experiment and experimental runs for finding the significant factors and their respective optimum value relating to optimum level of biogas production from POME. In order to attain our research goals, the study is designed to cover three areas of research work as follows.

The objective one of this research is to determine the significant factors affecting biogas production from POME. The study would begin by gathering information through literature review on the latest study of treating POME. The experiment involves setting up of two-stage fermentation and an anaerobic reactor for treating POME. To achieve research objective one, Minitab Version 18.1 and Design Expert Version 2018 software are used for

data analysis to estimate the p-value at 95% significant level of the factors that could contribute to optimize biogas production from POME. By using the data collected for specific objective one, the optimum values of factors that contribute to optimize biogas production from POME for specific objective two can then be determined using outputs from Design Expert software with Response Surface methodology (RSM).

As with sequential experiments, the previous two results can be used to achieve the specific objective three of this study, which is to develop the empirical model to predict the optimum level of biogas produced by POME. Outputs from Minitab Version 18.1 and Design Expert Version 2018 software would be used in data analysis for developing statistical predictive model.

1.6 Research Methodology to Achieve Research Goals

With the stipulated objective one of this research, the experiment would be conducted to determine the significant factors affecting biogas production from POME. A 5 L reactor with working volume of 3 L will be set up for treating POME in an anaerobic condition. A two-stage fermentation process will be adopted. Biogas will be collected and measured using the water displacement method. Five factors namely pH, C/N, OLR, HRT, and SRT will be transferred into Minitab Version 18.1 and Design Expert Version 2018 software to obtain 5 level inputs based on Central Composite Design (CCD). A total of 50 runs with a combination of six different variables are produced from DOE using Design Expert. ANOVA will be performed to obtain the significant level of factors in biogas production. The significance test will be conducted at 95% confidence level. P-value of inputs less than 0.05 will categorise them as the significant factors that contribute to biogas production.

With the stipulated objective two of this study, the experiment would be carried out to determine the optimum values of factors that contribute to optimize biogas production from POME. A 5 L reactor with working volume of 3 L would be set up for treating POME in an anaerobic condition by adopting a two-stage fermentation process. Biogas would be collected and measured using the water displacement method. The results from the first specific objective will be used to achieve the second specific objective. Using Design Expert Version 2018 software, the optimum values of these factors would be determined. Graphical representation in 3D plots would be presented.

With the stipulated objective three of this study, the experiment would be conducted to develop the empirical model to predict the optimum level of biogas produced by POME. The data from the first and second specific objectives would be used to achieve the third specific objective. A multiple linear regression model with all the inputs will be developed using Design Expert software and experimental data of biogas production from POME. A model validation will be performed using Design Expert and data analysis will be presented in a graph.

A similar setup as the first part of the experiment will be used for data validation, by setting all the operating parameters (inputs) at their optimal levels. However, bigger reactor of 350 L with working volume of 200 L for two-stage fermentation process will be used for validation.

1.6.1 Research Framework for Achieving Research Goals

The research framework of this research is elucidated by Figure 1.1.

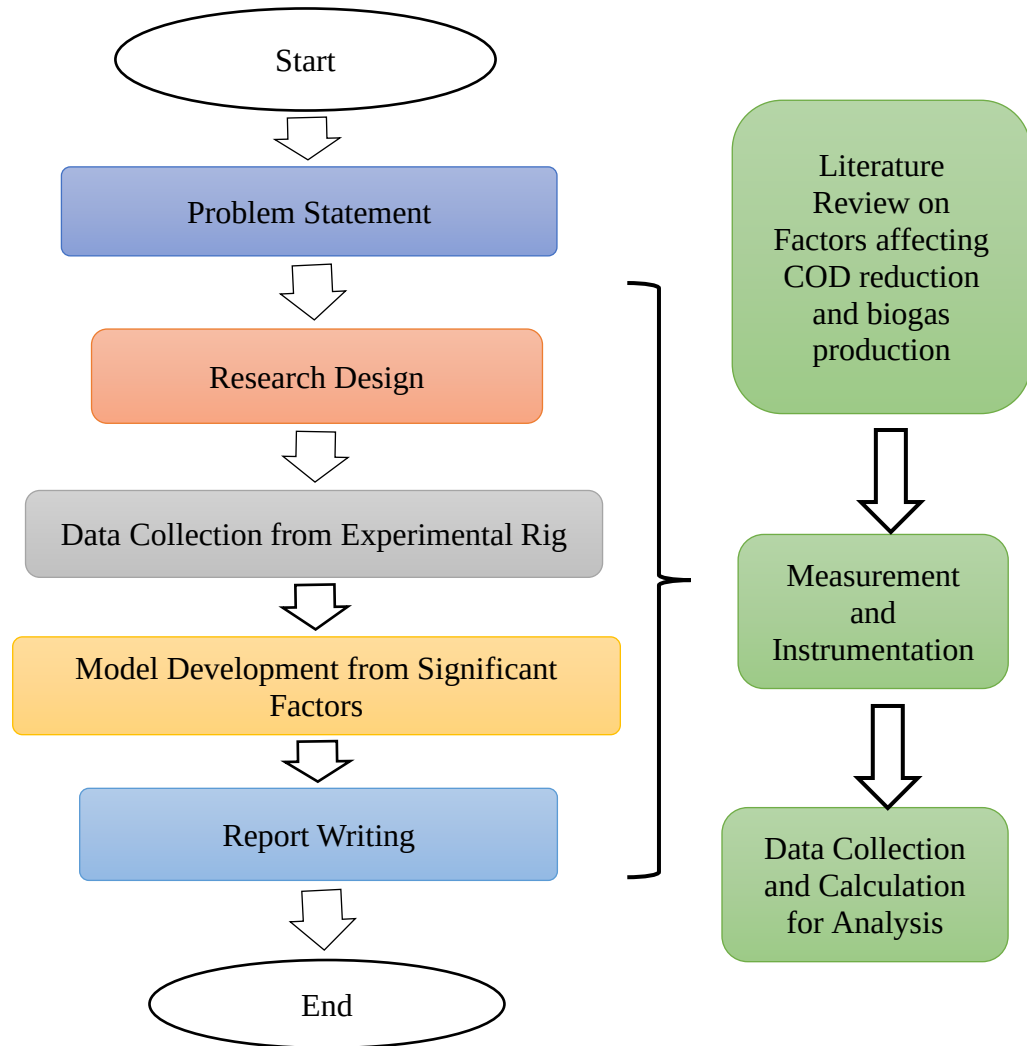


Figure 1.1: Framework of Research

1.6.2 Structure of Thesis

The structure for this report is presented in Figure 1.2.

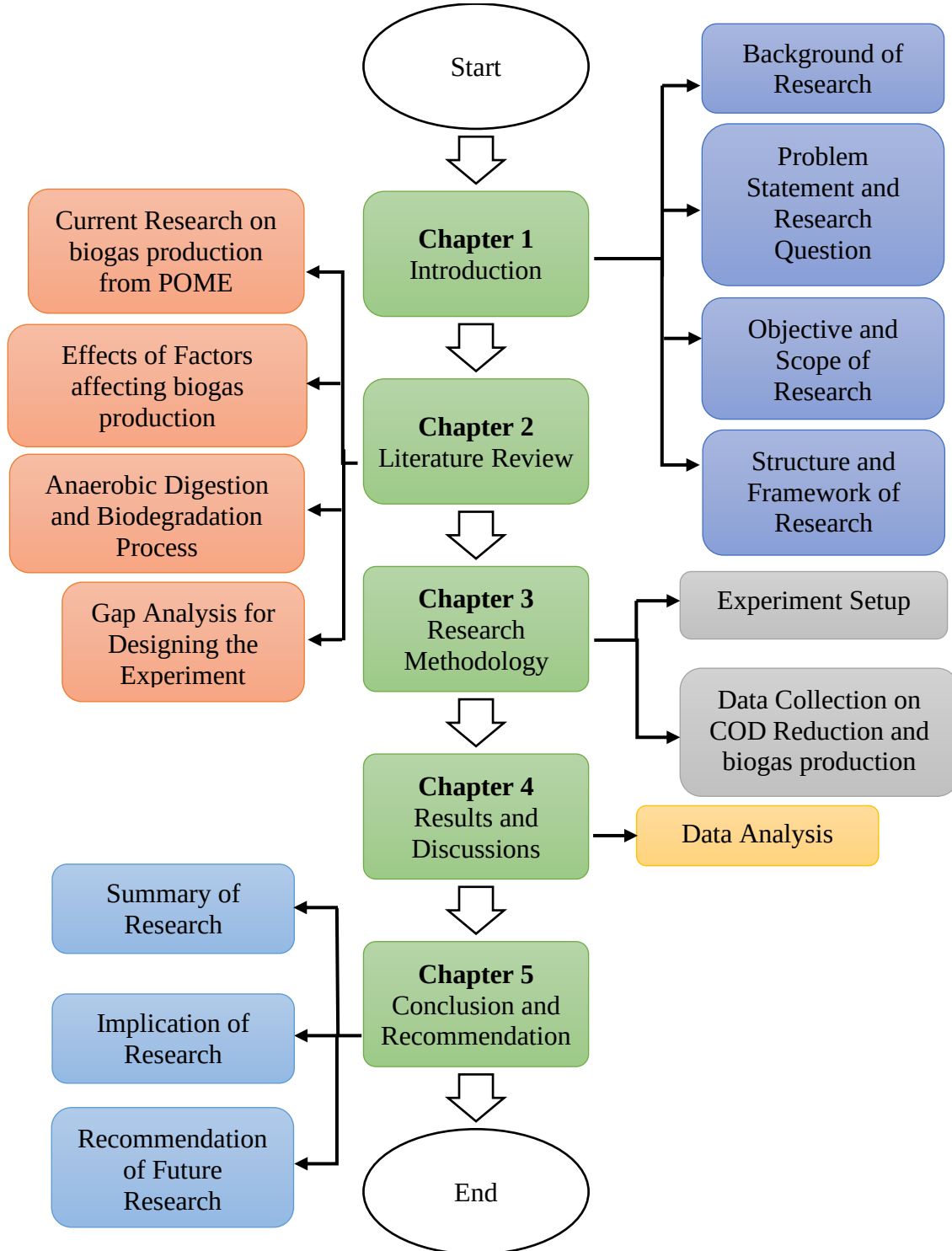


Figure 1.2: Structure of Report

1.7 Organisation of the Thesis

In order to realise the objectives of this study, the report is presented in five chapters. Chapter 1 provides a general introduction about the research covering background of study, problem statement, research question, objective of research, scope of study, framework of research, and structure of report. Chapter 2 includes literature review on current research on COD reduction and biogas production from POME, factors affecting COD reduction, chemical oxygen demand (COD), biochemical oxygen demand (BOD), and 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. The study focuses on COD reduction and the corresponding biogas production from POME. Chapter 4 comprises of data analysis, results and scenario analysis of research findings with other previous work. Here, collected data are calculated and analysed with the aid of tables and graphs to present the findings. The software Minitab Version 18.1 and Design Expert Version 2018 software are being used for running the experiments on POME digestion. Chapter 5 deliberates on outcomes of research, implications of research findings, novelty of research, recommendations of future research, limitations and conclusion of study. At the end of the report, Appendices are included to present supporting data and background of study. References, list of publications and other relevant documents are also attached herewith for further review.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Literature Review

This chapter presents the review of literature related to the latest study on biogas production optimization from POME with the aid of anaerobic digestion. The literature will also cover our strategic focus and objectives of literature review, methods applied for reviewing literature review, POME generation process, impacts of POME, operations of bioreactor, stages of anaerobic biodegradation for POME, mechanics of optimization for producing biogas from POME, and establishment of research gap analysis in the fields. The last section summarises the relevant findings of our literature review.

What transpired from the outcomes of many research studies on treatment of POME, culminates into a bold contention that POME should no longer be regarded as a waste product. POME is highly beneficial to the palm oil industry as it contains water and biomass. In this study, POME is treated in an anaerobic condition to convert biomass to biogas (mainly methane and carbon dioxide). This research aimed to evaluate the operating factors affecting biogas production from POME. Hence, this study would contribute to add new knowledge into the advancement of converting biomass into recoverable energy as biogas with appropriate optimization techniques. In this regard, the outcome of this study would make it beneficial for the palm oil millers to undertake the recovery of usable energy from POME for increasing productivity.

This study attempts to review the literature on the operational conditions affecting the biogas production from POME. Major areas considered in the literature review framework are to

find out the effect of HRT, pH, SRT and C/N on biogas production at optimum operating conditions as well as to reveal the biogas production optimization techniques. Emphasis would be given to conduct this study using a two-stage fermentation and anaerobic reactor for more efficient POME digestion at mesophilic temperature.

Standard literature review method had been used to study relevant published paper. Preliminarily about 300 journals on POME process which were published between year 2000 to 2017 were studied. Subsequently, about 100 papers were short-listed for review as they are relevant to the objectives of this research. In addition, other articles; books, daily newspapers, and newsletters were also reviewed in order to get an update on the latest research on POME treatment process particularly in producing biogas (biomethane).

The researcher then narrowed down to the search articles in peer reviewed journals and conferences. The paper 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: HRT, pH, SRT, C/N, biogas production, reduction of COD and BOD, and their combinations as independent and dependent variables in the treatment process of POME.

2.2 Sources and Procedures of Palm Oil Mill Effluent (POME) Generation

The three major operations that contribute to the generation of POME in different proportions (Rupani & Singh, 2010) are: 36% from the sterilization of fresh fruit bunch (FFB), 60% from clarification of the extracted crude palm oil (CPO), and only 4% from hydrocyclone separation of cracked mixture of kernel and shell generated in the mills. The extraction of palm oil comprises numerous process operations as elucidated in Figure 2.1.

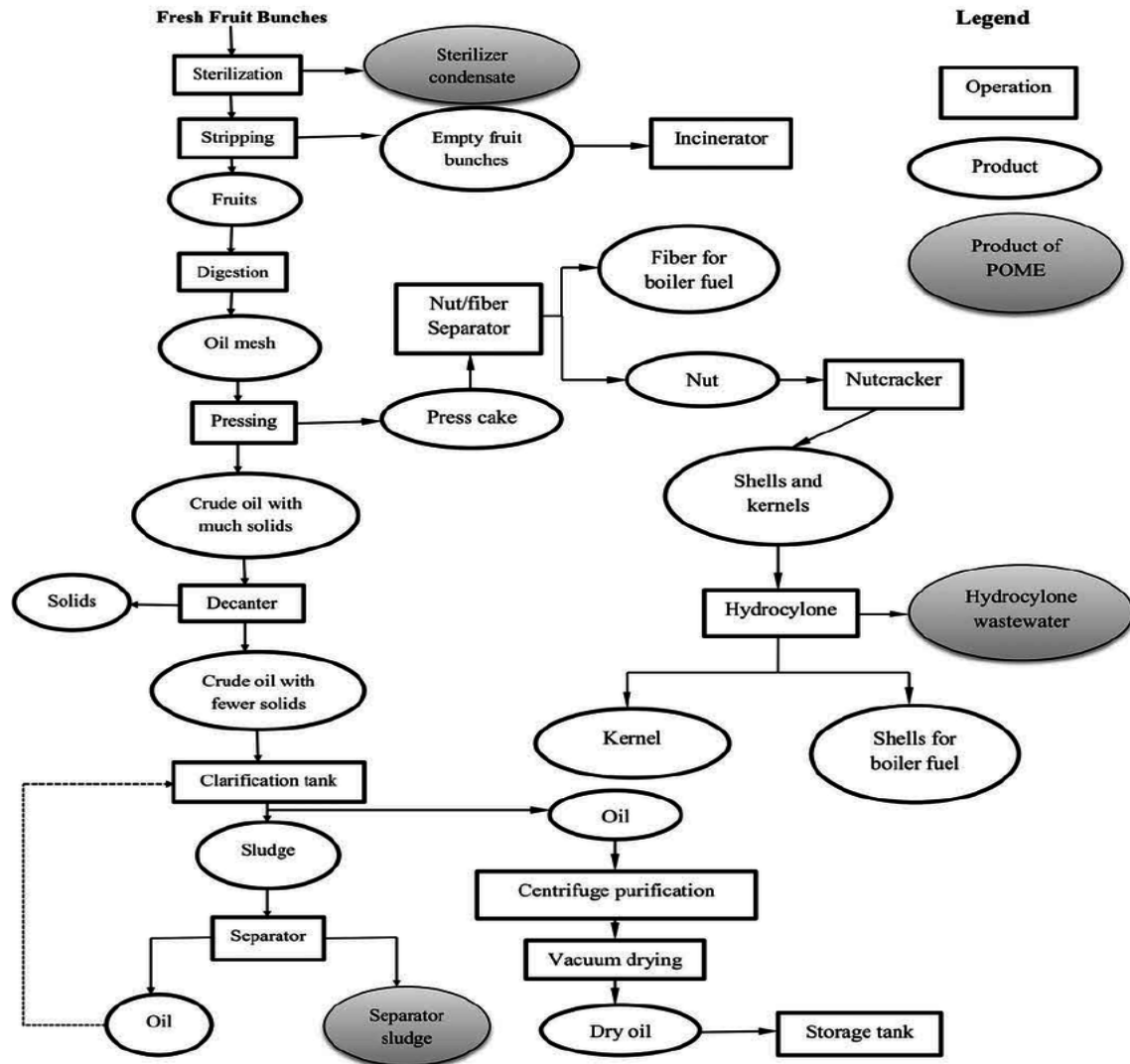


Figure 2.1: Process Operations and Processes in Palm Oil Extraction (Wu et al., 2010)

2.2.1 Steam Sterilization

Sterilization of FFBs is done as the first stage in extraction of crude palm oil (CPO) from palm oil fruits. The aim of sterilization is to loosen the individual fruits which are attached to the stalks or bunch to ensure the success of the upcoming processes (Subramaniam et al., 2010). Besides, FFB sterilization deactivates the biological factors that are responsible for the breakdown of oil and the rise of FFA (Vijaya et al., 2013). Sterilization can be conducted at 100°C or greater because lipase enzyme becomes inactive at 55°C (Matthäus, 2012).

Sterilization conducted at the higher pressure reduced the number of unstripped fruits from bunches thus increasing oil recovery compared to that conducted at atmospheric pressure. Steam can penetrate better to the inner part of the bunch at high pressure. The possibility of hydrolysis is enhanced with the presence of heat and water which causes the oil-bearing cell to rupture. However, insufficiently cooked or excessively cooked FFB has its disadvantages. An insufficiently cooked FFB may prevent some of the fruits from being stripped. An excessively cooked FFB may soften the fruits which can cause ripping and oil loss. Both Rupani and Singh (2010) and Ujang et al. (2010) used autoclave for FFB sterilization by applying steam at 120–140°C for 75-90 minutes, where high temperature is sufficient to interrupt the oil-containing cells in the mesocarp to release out the palm oil. The other common operating conditions for sterilization of FFBs are 131°C and a pressure of 40 psi (Noerhidajat et al., 2016). Some palm oil mills use a continuous sterilizer to carry out the operation at atmospheric pressure and steam temperature of 100°C (Noerhidajat et al., 2016). In other case, FFB is steamed at 45-50 pound-force per square inch gauge (psig) for 90 minutes at the sterilization station. Since this sterilization process is intended to soften the mesocarp and dehydrate the fruits, high pressure of 40 lb/in² at temperature of 140°C with triple cycle may be applied for about 90 minutes. Sterilization of FFB is also meant to prevent the rapid formation of free fatty acids during the pulping process and to remove any attached fruit. FFB are sterilized at 140°C and at 300 kPa for 50 minutes (Hosseini & Abdul Wahid, 2013). In similar process, an operating pressure of 3 kg/cm² and temperature of 140°C for 80–90 minutes has been employed by Sarah and Taib (2013). Those prevalent operating conditions for FFB sterilization process are summarised in Table 2.1.

Table 2.1: Parameters for Steam Sterilization

Parameters	References
Sterilizer chamber Time: 90 minutes	Vijaya et al. (2013)
Temperature: 131°C Pressure: 40 psi or 275.79 kPa	Noerhidajat et al. (2016)
Continuous sterilizer Temperature: 100°C Pressure: 101.325 kPa	Noerhidajat et al. (2016)
Pressure: 45–50 psig or 310.264–344.738 kPa Time: 90 minutes	Man & Baharum (2011)
Autoclave Temperature: 140°C Time: 75–90 minutes	Rupani & Singh (2010)
Autoclave Temperature: 120–140°C Pressure: 3–3.5 bar or 300–350 kPa Time: 75 minutes	Ujang et al. (2010)
Temperature: 140°C Pressure: 300 kPa Time: 50 minutes	Hosseini & Abdul Wahid (2013)
Sterilization chamber Live steam Time: 90 minutes	Subramaniam et al. (2010)
Temperature: 140°C Pressure: 3 kg/cm ² or 294.2 kPa Time: 80–90 minutes	Sarah & Taib (2013)

Any of these sterilization processes employed would affect the Deterioration of Bleachability Index (DOBI) of CPO extracted, which determines the ease of refining crude palm oil as reflected by its oxidative status. DOBI is an indicator of ‘fitness for use’ or ‘good merchantable quality’ which may constitute quality specifications on free fatty acid (FFA), together with moisture and impurities (M and I) by CPO traders. A good DOBI value necessitates proper air-release just before sterilization, shorter sterilizing time and lower process temperature otherwise these parameters may affect the stripping and digestion process later (Jusoh et al., 2013).

2.2.2 Stripping of Fresh Fruit Bunches

Fresh fruit bunches (FFBs) are dropped through the stripper in order to separate the fruits from the bunches. The detached fruits fall through the spaces between the bars of the stripper. Bucket conveyors are used to gather and transport the fruits to the next station (Rupani & Singh, 2010). The flow process of stripping FFB is shown in Figure 2.2.

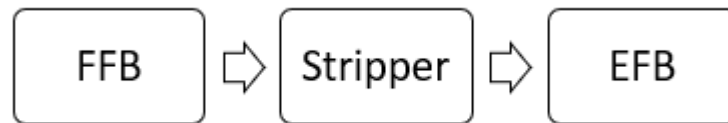


Figure 2.2: Process Flow of Stripping FFB

After sterilized fruits are been detached and separated from the fruit bunches by the thresher, they would eventually become empty fruit bunch (EFB). The EFBs are used as fuel for boiler and can also be used as organic fertilizers (Man & Baharum, 2011). In the stripper, fruitlets on the sterilized FFBs are separated from the stalks or bunches, and eventually known as EFB (Vijaya et al., 2013). Threshing is the removal of palm oil fruits form the fruit bunches. In a manual process of threshing, an axe is used to cut the fruit-laden spikelets before the fruits are separated (Ujang et al., 2010). Sterilized FFB are sent to a stripper to separate the fruitlets from the stalks or bunches which could then be called EFB. These EFBs are normally sent back to the plantations to be used as mulches and substitute fertilizer (Subramaniam et al., 2010). In other case, sterilized fruits are separated from the bunch stalks by using a rotary drum thresher (Faisal & Mahidin, 2013).

2.2.3 Digestion of Fresh Fruit Bunches

This stage of operation involves the mashing up of fruits under steam heated condition. The presence of heat would break the mesocarp oil-bearing cells as the fruits are mashed. The

digester is a vertical cylindrical vessel fitted with rotating arms. In order to improve the flow of oils during digestion, hot water is added (Rupani & Singh, 2010). The vessel is heated to a temperature between 80 and 90°C to allow removal of the mesocarp from the nuts (Hosseini & Abdul Wahid, 2013).

At certain point, oil-containing cells would burst and oil flows readily during pressing operation (Owolarafe & Faborode, 2008). Both mass transfer and heat transfer occurred during digestion. Water (moisture content and steam), solid (fibre, sludge) and oil are the major elements that can be found within the digester vessel. It has been reported that mainly water, oil and sludge are produced during digestion (Noerhidajat et al., 2016). The total mass of the fruit which includes the mass of the fruit, oil released, and sludge increases with the duration of the digestion process. However, the conversion of fibre to sludge does not affect water absorption (Noerhidajat et al., 2016). Two types of sludge are formed during the digestion process, which are classified based on their densities as light-density and heavy-density sludge. The light sludge is usually located between the water and oil while the heavy density sludge accumulates below the water (Noerhidajat et al., 2016).

One of the key factors in the digestion process is the condition of digester blades whose sharpness is vital for cutting and breaking the mesocarp fibre of the fruit. In order for the effects of shear, friction and pressure to be distributed evenly to the fruits, they should be mixed thoroughly (Noerhidajat et al., 2016).

After further softening and rupturing of oil-bearing cells to release the oil, the fruitlets are sent to the digester to be converted into homogeneous oil mash by mechanical stirring process (Subramaniam et al., 2010; Vijaya et al., 2013). This process releases palm oil in the

fruit through cracks on the oil-bearing cells. The digester comprises a steam-heated cylindrical vessel with central rotating shaft that is filled with several beater arms. The fruit is pounded by the rotary beater arms at high temperature which reduces the oil viscosity. This destroys the outer covering (exocarp) of fruit and completes the disruption of the oil-bearing cells. In order to maximize the effect of the agitation, the digester must be filled up to the maximum (Ujang et al., 2010). Besides crushing the fruit, the digester also separates the flesh from the seeds which would enhance the pressing process (Faisal & Mahidin, 2013).

2.2.4 Pressing of Digested Fresh Fruit Bunches

Homogeneous oil mash from the digester will pass through a screw press to obtain the crude palm oil (CPO) which still contains a mixture of palm oil (35–45%), water (45–55%) and fibrous materials in different portions. By passing the fibre and nuts through a depericarper, they will be separated (Rupani & Singh, 2010). In the screw press, maximum oil can be extracted from fruitlets and press cake is ready for kernel recovery (Man & Baharum, 2011). Most of the extracted CPO is obtained from the pressing of homogeneous oil mash (Faisal & Mahidin, 2013; Subramaniam et al., 2010; Vijaya et al., 2013). By passing the palm fruits through the pressing machine, the oil is extracted from fibre and seeds (Ujang et al., 2010). Then, fibre and nuts are removed from the mixture of oil, water and press cake.

2.2.5 Decanter of Pressed Palm Oil

After pressing, decanter is used to remove solids as much as possible from the extracted CPO which still contains a mixture of oil, water and fruit. Decanter works according to the basic principles of separating solid elements from the liquid elements. The segregation of pressed palm oil, sludge from settling tanks or empty fruit bunch liquor into dry solids (cake),

mill effluent (water) and recovered oil fractions takes place in a horizontal cylindrical bowl equipped with a screw conveyor. The palm oil product is fed into the bowl via an inlet feed zone. Centrifugal force causes instant sedimentation of the dry solids on the wall of the bowl, and the liquid phases – heavy phase (water) and light phase (oil) – separate in layers. Usually heavy phase is discharged under high pressure and the light phase is discharged by gravity. The resulting mixture of oil, water and fruit then pass through a vibrating screen in order to remove most of the solid wastes (Man & Baharum, 2011; Vijaya et al., 2013). The CPO extracted consists of water and vegetable impurities in the form of dissolved materials or indissoluble solids suspended in water. Other means of water removal in CPO can be done by passing it through a settling and centrifuging process (Hosseini & Abdul Wahid, 2013). The structural drawing of a decanter is shown in Figure 2.3.

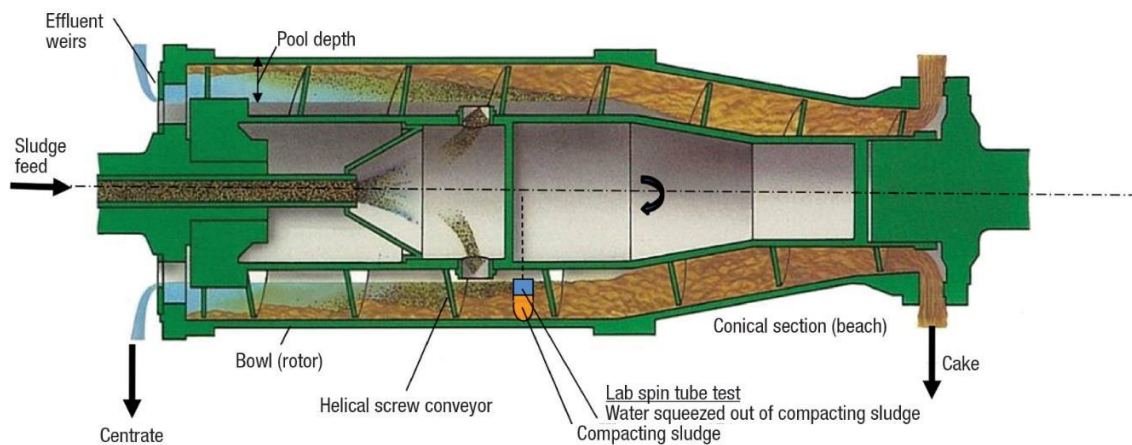


Figure 2.3: Structure of Decanter

2.2.6 Hydrocyclone of Influent

The palm kernels produced by the nutcracker need to be treated before sending them for storage in the kernel dispatch silo or processing in the kernel crushing plant to produce crude palm kernel oil (CPKO) (Man & Baharum, 2011). Kernels are separated from the empty

shells of the cracked nuts using centrifugal force and water in the hydrocyclone mechanism (Hosseini & Abdul Wahid, 2013). The hydrocyclone components are consisted of the upper and lower cone. The lower cone is connected to the apex or spigot which constitutes the discharge point of the cyclone, known as the underflow. When the slurry is directed into the cyclone at certain velocity through the feed entry point which is called the inlet or the vortex finder, the velocity and entry point would help to create a spiral path around the outside of the body, the upper and lower cone. The centrifugal force generated by this spin would force the heavy particles outwards towards the wall of the cyclone. It is the apex that is responsible for creating the spin required for the centrifugal sizing of the slurry. The hydrocyclone uses that centrifugal force and water to separate the kernel from the shell shown in Figure 2.4. The vortex finder is a point of separation between particles of different weights, where light particles exit through the overflow and the heavy material will be discharged through the underflow.

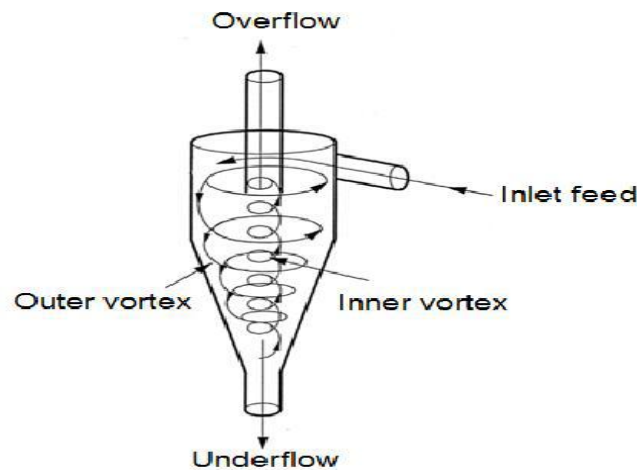


Figure 2.4: Schematic Diagram of Hydrocyclone (Hosseini et al., 2015)

2.3 Impacts of Palm Oil Mill Effluent

2.3.1 Impact of POME on Surface Water Quality

Palm oil mill effluent (POME) can affect water quality when large amounts of wastewater are discharged into the waterways during milling process. There are three major operations responsible for the generation of POME. POME is mainly generated from sterilization of fresh fruit bunches (FFBs), clarification of the extracted crude palm oil (CPO), hydrocyclone separation of cracked mixture of kernel and shell hydrocyclone, which contribute 36%, 60% and 4% of POME respectively (Rupani & Singh, 2010). About 5–7.5 tonnes of water are required for producing one tonne of CPO (Ahmad et al., 2003, 2005; Wu et al., 2009). Out of that quantity of water used, about 50–79% would end up as POME (Awotoye et al., 2011; Chavalparit et al., 2006; Ohimain and Izah, 2013; Okwute & Nnennaya, 2007; Singh et al., 2010). It follows that about 0.9, 1.5 and 0.1 m³ of POME are produced from these sources (clarification wastewater, sterilizer condensate and hydrocyclone wastewater) for each tonne of CPO. Small-scale mill operators usually discharge POME into the surrounding with little or no treatment. Discharging of POME into the waterways causes the water to turn brown, smelly and slimy. POME is a potential breeding ground for mosquitos especially during rainy seasons. With a higher concentration of both organic and inorganic compounds, POME discharge can increase these quality parameters including temperature, pH, total alkalinity, total solid, total dissolved solid, total suspended solid, magnesium, calcium, sodium, potassium, chloride, sulphate, nitrate, phosphate, zinc, iron, manganese, dissolved oxygen and biochemical oxygen demand in the receiving river (Awotoye et al., 2011), rendering the water unhealthy for domestic purposes.

2.3.2 Impact of POME on Soil

The discharge of POME into the soil can affect its pH value which is one of the main factors that determine the nutrients availability in plants. Since POME is acidic in nature, it will certainly increase the acidity of the soil. POME affects the soil through leaching of heavy metals, thereby changing its physicochemical properties. POME increases the acidity of soil, organic carbon, total nitrogen, phosphate, sulphate, phosphorus, sodium, potassium, calcium, magnesium, aluminium and hydrogen (Eze et al., 2013; Okwute & Nnennaya, 2007). The presence of phosphorus, sodium and potassium can enhance the growth of plants. However, high contents of nitrogen, phosphorus and potassium can affect the crop yield if the pH of soil is abnormal. The presence of phosphorus in POME soil will increase its pH level and other nutrient determinant available in the soil (Eze et al., 2013).

Due to the alteration of soil physicochemical properties and other vital nutrients as a result of POME discharge, soil texture and particulate size can be affected. Besides that, it can increase the bulk density of soil (Awotoye et al., 2011). The textural class of the soil depends on the organic matter content in which POME is discharged into. Due to the presence of unrecovered oil and debris from mill processing, the POME on soil retains water. As a result of POME, the appearance of soil and some properties including vegetation, colour, odour and constitution are changed. The soil may lose its vegetation cover and damp with humus. This causes clogging and water logging of the soil pores which could result to death of vegetation on contacts, with the environment being bare (Eze et al., 2013). Apart from that, pollution of the land and ecosystem leads to loss of land and resources to local organisms such as the soil micro flora and some biodiversity (Sridhar & Adeoluwa, 2009).

2.3.3 Impact of POME on Greenhouse Gases

Greenhouse gases (GHGs) create a layer in the atmosphere which contains thermal energy between the Earth's surface and atmosphere. When thermal energy tries to escape into the atmosphere, GHGs layer reflects the energy back to Earth's surface. This causes the temperature of the Earth's surface to rise, which leads to global warming. During anaerobic digestion of POME in palm oil mills, greenhouse gases such as carbon dioxide (CO₂) and methane (CH₄) are released into the atmosphere. The release of these greenhouse gases is the main offender of global warming which contributes to climate change (IPCC, 2014). The anaerobic treatment of POME in palm oil industry has been known as an important source of methane. The most common anaerobic treatment systems for POME are open digesting tank and lagoon treatment system. Oxygen is naturally available during the treatment process in the system. However, the amount of oxygen is unable to cater for all aerobic decomposition of organic matter contained in the wastewater. As a result, the decomposition of organic matter turns anaerobic, resulting in the generation of biogas, which would be released into the atmosphere. During anaerobic digestion of POME, methane is 25 times more powerful than carbon dioxide in trapping heat (Madaki & Seng, 2013). Therefore, methane is the chief contributor to global warming.

2.3.4 Impact of POME on Air

The anaerobic digestion of POME generates biogas in the form of CO₂ and CH₄. Harris et al. (2017) reported that methane in the atmosphere can create ground-level ozone. Ozone is not only harmful to human health, but also contributes to global warming. Ground-level or "bad" ozone is a colourless and highly irritating gas which is not emitted directly into the air. It is known as a secondary pollutant and it is formed by chemical reactions between

nitrogen oxides (NO_x) and volatile organic compounds (VOC) with the presence of sunlight and stagnant air. Ground-level ozone is also the main component of photochemical smog. Photochemical smog is a major contributor to air pollution. Smog refers to a yellowish or blackish fog which constitutes the mixture of pollutants in the atmosphere such as fine particles and ground-level ozone. Smog can contribute to reduction in visibility especially in urban areas and large population centres (Dislich et al., 2017). The implications of photochemical smog to plants and animals are serious. Smog can hinder the growth of plants. It will lead to the damage of crops, trees, and vegetation. Continuous exposure of crops and vegetables to smog would disrupt their ability to fight infections, making them more susceptible to diseases. Besides that, smog makes it difficult for animals to adapt themselves to the toxic condition. Animal species that could not survive in such environment will perish or become susceptible to illness (Dislich et al., 2017).

2.3.5 Impact of POME on Human Health

POME alone can contribute to different types of impacts on water quality, soil, greenhouse gases, air, agricultural products and fish production. Ultimately, these impacts are associated with human health. Unhygienic water, especially contaminated water contains a number of viruses and harmful bacteria which are damaging to human health. Drinking contaminated water results in diarrhoea, bacterial dysentery, cholera, typhoid and many other water-related diseases. Furthermore, poor water quality poses health hazard when it is used for bathing. When this contaminated water comes into contact to human skin, their skin may get itchy and cancerous. Continuous consumption of unclean water can damage the human organs such as liver, lung, bladder and kidney (Dislich et al., 2017; Harris et al., 2017).

Increase in Earth's temperature due to greenhouse gases pose major health risks especially to elderly people and those who work outside such as farmers and construction workers. They can suffer from exhaustion due to heat and heat stroke. Incidence and spread of diseases such as malaria and cholera would also increase as temperature and rainfall increase. Mosquitos that transmit the malaria virus can survive in hot and humid weather. Long-term exposure to polluted air can affect the lungs of humans which may develop into cardiovascular and respiratory diseases. Agricultural products and fish which are contaminated by POME discharge to soil and waterways could result in accumulation of harmful chemicals in humans who continue to consume those foods. Health problems associated with exposure to these chemicals include cancer, liver disease and developmental disorder (Dislich et al., 2017; Harris et al., 2017).

2.3.6 Impact of POME on Economy

Discharging of untreated POME into the water can reduce the quality of water. It can affect the economy as it can be expensive to treat and mitigate such contamination. POME also releases methane into the atmosphere during anaerobic digestion. Utilizing the potential of POME to generate energy in the form of biogas can contribute to world economic and sustainable growth (Madaki & Seng, 2013). Resource is known as a source or supply from which benefit is obtained. POME has the potential to be a resource rather than becoming a waste or by-product. Proper treatment of POME can achieve this feat by adopting an international trend of promoting pollution prevention through cleaner production, which is based on the 5R policy (Wu et al., 2009). The implementation of 5R policy encompasses the reduction, replacement, reuse, recovery and recycling of useful resources in POME using appropriate technologies to reduce environmental damage while simultaneously changing the status of POME from waste to resource.

Conventional method of treating POME such as ponding system in palm oil mills in Malaysia has been used for a very long time. Considering its low-cost and simple operating system, more than 85% of palm oil mills adopt fully the use of ponding system (Rupani & Singh, 2010). The open ponding system does not capture methane which escapes directly into the atmosphere. The emission of methane into the air can contribute towards climate change making the process not environmentally friendly. The capture of biogas in palm oil mills can be an effective and affordable way to recover and reuse methane to generate heat and electricity. While the initial cost to implement this technology is high, the benefits of capturing methane and using it as a source of energy can outweigh the cost. This makes the technology appealing to palm oil mills operators from an economic point of view (Shahidul & Malcolm, 2017).

On the other hand, as ambient temperature increases due to global warming, more power would be required for air-conditioning and ventilation, resulting in higher operating costs of running facilities and individual homes at thermal comfort zone.

2.3.7 Impact of POME on Society

Direct release of this effluent (POME) from palm oil mills into the waterways can cause water pollution which can affect downstream plants and animals as well as human beings. Farmers who use the water from rivers for irrigation purposes are hugely affected. Farmers will struggle to produce good harvest from their crops due to polluted water. Their farm productivity would suffer in terms of both quantity and quality of agricultural products due to crops becoming more susceptible to insect infestations. Ultimately, the livelihoods of affected families and communities would be jeopardised when their main source of income is under threat (Byrd & Demates, 2014).

Rising temperature of the Earth's surface is the effect of greenhouse gases. More prevalent occurrences of severe storms and hurricanes should be expected as more water vapours are sucked up from the earth's surface at higher temperature, causing serious threats or damages to crops and housings, exacerbated by the substantial rise of water or sea level due to global warming (Levermann et al., 2013), adversely affecting cities or resettlements at low-laying areas. More power would be required for air-conditioning and ventilation of residences, office facilities and public amenities, making it more expensive to operate and maintain them around thermal comfort zones (Mishra & Ramgopal, 2013) both for humans and animals.

2.3.8 Impact of POME on Environment

POME has the potential to cause contamination to the air, water and soil. The release of methane and carbon dioxide into the atmosphere from untreated POME results in the formation of GHG. GHGs can contribute to the increase in temperature of the Earth's surface. Rising temperature will affect the biotic communities on the Earth. Though some plants can adapt and utilize the increase in CO₂ and high temperature, other plants may fail to adapt themselves to survive in the new environment. As these plants begin to decrease in numbers, herbivorous insect species will get extinct due to loss of food and habitats. Discharging of POME into the river can lead to water pollution. Naturally, POME is acidic. When POME is released into the river untreated, it will reduce the pH of the water, being acidic in nature. This renders the water not suitable as habitat for aquatic organisms. POME causes the water to turn brown, smelly and slimy. The population of fish will be adversely affected by POME discharge. Some species of fish will die due to the acidic condition of water and some will migrate to other sources of freshwater which are much cleaner. Besides that, unhygienic or contaminated water can expose consumers to many types of water-borne diseases such as diarrhoea, cholera, typhoid and dysentery.

The discharge of POME into the soil can result in soil or land pollution. POME affects the soil by leaching of heavy metals and alters the physicochemical properties of the soil. Crops will not be able to tolerate such conditions of soil which could lead and death of crops. Furthermore, the contaminated land or soil will lead to loss of land and resources to local inhabitants such as the soil micro flora and other biodiversity.

2.4 Anaerobic Digestion for Biogas Production

Anaerobic digestion is the most suitable treatment method for effluents which contain high concentration of organic carbon such as POME. It is the most beneficial stabilization technique as it is cost effective and environmentally friendly through reduction of sludge quantity and its ability to recover and recapture energy in the form of biogas/methane (CH_4). This biological process involved depends on anaerobic microbes to digest the organic matter (pollutants) and thereby, reduces the biochemical oxygen demand (BOD) of the effluent. The complex polymers of POME are degraded by activities of hydrolytic, acetogenic, acidogenic, methanogenic microorganisms. The end products of anaerobic digestion are biogas which consist of 65% CH_4 , 35% carbon dioxide (CO_2) and some trace amounts of hydrogen sulphide.

At the mill, POME is collected in a pond or sludge pit and is retained for one or two days. The residual oil found in POME would be allowed to float and removed before it is pumped to a treatment plant for further processing. It is crucial to effectively remove any residual oil from POME before it enters the treatment plant, otherwise its presence may affect the efficiency of the preceding treatment process. Bio-methanation or methanogenesis is the formation of methane gas by microbes that decompose biodegradable matter in an anoxic environment. There are several factors that are involved in the decomposition of palm oil

biomass to CH₄. The factors that influence the overall methane yield include organic loading rate (OLR), hydraulic retention time (HRT) and solid retention time (SRT), pH value and C/N of substrate.

2.4.1 Organic Loading Rate in POME Digestion

In the palm oil industry, anaerobic sludge blanket processes such as up-flow anaerobic sludge blanket (UASB), up-flow staged sludge bed (USSB), expanded granular sludge bed (EGSB), internal circulation (IC), and inverse anaerobic fluidized bed (IAFB) reactors can bear very high loading rates, up to 40 kg-COD/m³/d (Chen et al., 2010). An increase in OLR can 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; hence the efficiency of the treatment will be affected. OLR is the mass of organic material that is fed daily to the treatment system per unit volume of reactor. By changing the influent concentration and flow rate, values of OLR can be varied. Under the conditions of changing HRT and flow rate, OLR can be expressed in the following form:

$$OLR = \frac{Q \times COD}{V} \quad \text{Eq. 2.1}$$

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 can be simplified into:

$$OLR = \frac{COD}{HRT} \quad \text{Eq. 2.2}$$

Table 2.2 depicts the various OLRs used by previous researchers in treating POME over those years.

Table 2.2: OLRs used by Previous Researchers

OLR (kg-COD/m³/d)	References
1.5–9.2	Rincón et al. (2008)
10	Karnchanawong & Phajee (2009)
5.0	Sulaiman et al. (2009)
1.4	Babae & Shayegan (2011)
4.53	Chotwattanasak & Puetpaiboon (2011)
7.5	Nurul et al. (2011)
12.5	Chen et al. (2014)
1.68–27.90	Osman & Hamad (2015)
6.45	Wang et al. (2015)
7.76	Moharram et al. (2016)

2.4.2 Hydraulic Retention Time

Hydraulic retention time (HRT) is a measure of the average duration of time that a compound remains in a storage unit. In a palm oil mill, HRT indicates the average time the palm oil mill effluent (POME) resides in reactor during biogas production. The HRT can be measured with the following equation:

$$HRT[t] = \frac{\text{Volume of aeration tank [m}^3\text{]}}{\text{Influent flow rate } \left[\frac{\text{m}^3}{\text{h}}\right]} = \frac{V}{Q} \quad \text{Eq. 2.3}$$

HRT is known as hydraulic retention time (t), usually stated in hours or days. V is the volume of reactor or aeration tank (m³) and Q is the flow rate of influent (m³/h).

In most bioreactors, the production of biogas decreases as the HRT increases. The properties of HRT could affect the production of other biogas such as bio-hydrogen. The HRT is closely related to the organic loading rate (OLR) and substrate concentration; thus, a good balance has to be achieved for good digester operation (Abdurahman, 2011).

Table 2.3 displays the various HRTs used by previous researchers, obtained from the literature review.

Table 2.3: HRTs used by Previous Researchers

HRT	References
1–4 days	Rajaletchumy (2010)
6, 10, 20 days	Alrawi et al. (2011)
5 days	Fang et al. (2011)
0.7–2.4 days	Choi et al. (2013)
2–12 days	Wong et al. (2013)
4 and 6 days	Malakahmad et al. (2014)
8–24 days	Wong et al. (2014)
4 days	Trisakti et al. (2015)
10 days	Wang et al. (2015)
3 days	Ahmad et al. (2016)
6–15 days	Choong et al. (2018)

2.4.3 Sludge Retention Time

SRT is a key parameter which can affect biochemical and physical properties of sludge. It is a key factor in determining the eventual amount of hydrolysis and methanogenesis in a UASB system at an operating temperature. SRT in the reactor is usually defined as the ratio of the sludge mass to the discharge rate of the sludge (d):

$$SRT = \frac{\text{Sludge mass in the digester}}{\text{Sludge discharge rate}} = \frac{X_i V_i}{Q_x X_x} \quad \text{Eq. 2.4}$$

Where X_i is the mixed liquor suspended solids (MLSS) in each reactor (mg/L), V_i is the individual reactor volume (m^3), 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 starts at SRT between 5 and 15 days. At 15°C, it starts at SRT between 30 and 50 days. The maximum methanogenesis found at 25°C amounted to 51% and 25% at 15°C. Maximum hydrolysis occurs at 75 days SRT and amounted to 50% at 25°C and 24% at 15°C (Halalsheh et al., 2005). The SRT and temperature have important effects on the hydrolysis of proteins, carbohydrates, and lipids. The most substantial portion

of the digestion of proteins, carbohydrates, and lipids occurs within the first 10 and 15 days at process temperatures of 35°C and 25°C, respectively (Mahmoud et al., 2004). Table 2.4 shows the SRTs used by previous researchers, derived from literature review.

Table 2.4: SRTs used by Previous Researchers

SRT	References
15–20 days	Seadi et al. (2008)
11–30 days	Lau et al. (2009)
15 and 30 days	Tian & Su (2012)
20–40 days	Chen et al. (2012)
10 days	Montañés et al. (2013)
4, 15 and 30 days	Mohd Idris et al. (2014)
6–8 days	Salim et al. (2014)
20 days	Yi et al. (2014)
10–20 days	Choong et al. (2018)

2.4.4 Chemical Oxygen Demand and Biochemical Oxygen Demand

Organic matters are usually quantified as BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) while inorganic matters are mainly quantified as sulphate, chloride, ammonium, heavy metals and others (Lee & Nikraz, 2014).

COD is the amount of oxygen required by the total amount of organic compounds and inorganic matter to be oxidized by chemicals (oxidizing agents) in the wastewater. BOD is the amount of dissolved oxygen needed by the microorganisms to oxidize the organic compounds by biological-oxidation effect in a certain condition (Pujar et al., 2014). BOD value is taken for a given water sample at certain temperature (20°C) over a specified time period (5 days). Both COD and BOD are measured according to the APHA Standard Methods where COD is always higher than BOD for any biomass or wastewater.

COD test uses a strong chemical oxidant in an acid solution (potassium dichromate in a 50% sulphuric acid solution) and heat to oxidize both organic and inorganic substances in wastewater sample to obtain CO_2 and H_2O . 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 will change colour from orange to green based on the amount of oxidation and it can be read by using a laboratory calorimeter. The test data is available in few hours giving it a major advantage over the 5-day BOD test, as it provides faster water quality assessment and process control.

The BOD test is based on the principle that if enough oxygen is available, aerobic biological decomposition by microorganisms will continue until all waste is consumed. The test would be known as BOD_5 if it measures the amount of dissolved oxygen at the beginning to the end of a five-day period, while the sample is kept in a dark and incubated condition. 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 provide historical data only. Therefore, the BOD test is not recommended for rapid water quality assessment or optimal process control.

Since BOD is mainly a biochemical parameter, the BOD:COD ratio represents a good indicator of the proportion of biochemically degradable organic matter to total organic matter. Relatively, substrate COD strength has significant effect on the ultimate amount of biogas yield, as well as the methane content (Ghani & Idris, 2009). COD gives a precise estimation of the organic (degradable) material content of a given sample (Curry & Pillay, 2012). Therefore, higher values of COD are favourable for anaerobic digestion (Tewelde et al., 2012).

2.4.5 Carbon-to-Nitrogen

The carbon-to-nitrogen (C/N) is known as the ratio of elemental carbon to the elemental nitrogen present in the material. Different materials have different C/N. The overall C/N can be altered when two different materials are combined giving a new C/N. A high C/N will leave carbon still available while nitrogen has been consumed causing the bacteria to starve and eventually die. Conversely, when nitrogen content is too much, digestion would stop if all available carbon has been consumed. The correct ratio of carbon to nitrogen will prevent the loss of methane content (Okonkwo et al., 2018). The C/N was calculated based on the chemical composition (Shanmugam & Horan, 2009). The C/N can be determined by dividing the total organic carbon content by the total nitrogen content, according to the following equation:

$$\frac{C}{N} = \frac{W1 \times C1 + W2 \times C2}{W1 \times N1 + W2 \times N2} \quad \text{Eq. 2.5}$$

where, W1 and W2 are the volatile solid (VS) weight 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.

Several authors agree on an optimal C/N of between 20 and 35 (Habiba et al., 2009; Kayhanian, 1999) for anaerobic digestion. In anaerobic digestion, the methanogenic bacteria require 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 and 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 to 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 can affect the pH value of a slurry. The increase in carbon content will give rise to more carbon dioxide formation and lower pH value, while high value of nitrogen will enhance production of ammonia gas that could increase the pH to the detriment of the microorganisms (Dioha et al., 2013). Table 2.5 shows the C/N of co-substrate used by previous researchers for co-digestion.

Table 2.5: C/N of Co-substrate used by Previous Researchers

Co-substrate	C/N	References
Night soil	10/1	Dioha et al. (2013)
Horse excreta	13/1	Dioha et al. (2013)
Pig excreta	15/1	Dioha et al. (2013)
Food waste	16/1	Brown & Li (2013)
Chicken excreta	22/1	Dioha et al. (2013)
Cow dung	24/1	Brown & Li (2013)
Giant King Grass (Napier Pak Chong I)	35/1	Brown & Li (2013)
Rice husks	47/1	Dioha et al. (2013)
Sugar cane bagasse	53/1	Dioha et al. (2013)
Municipal solid waste	20–30/1	Wang et al. (2015)
Sewage sludge	8.16/1	Zahan et al. (2016)
Fruit wastes	25–49/1	Moujannı et al. (2018)

2.4.6 Up-flow Velocity

The up-flow velocity is regulated by installing a valve for maintaining a pre-set up-flow velocity and a gravity transmission system. Wastewater flows directly into the effluent tank under gravity, which results in rather high flowrate. However, this high flowrate can be overcome by adding equalization tank between the influent tank and reactor, which is equipped with mixer to produce consistency in the wastewater (Syafudin et al., 2015). The term up-flow velocity represents the ratio between the influent flow rate and the cross-sectional area of the reactor, as expressed by Equation 2.6:

$$W_{up} = \frac{F_{in}}{A} \quad \text{Eq. 2.6}$$

Or alternately it can be obtained by dividing the height of the reactor by the hydraulic retention time:

$$W_{up} = \frac{h}{HRT} \quad \text{Eq. 2.7}$$

The up-flow water velocity in a UASB reactor usually ranges from 0.1–1.4 m/h (Korsak 2008, Kalyuzhnyi et al. 2006). Effluent recirculation was set to maintain the required up-flow velocity (Oda & Pandya, 2016). Up-flow velocity ranging from 5–15 m/h are applied by effluent recycling and results in sludge bed expansion throughout the reactor's height. The high total biomass allowed the improved system, called expanded granular sludge bed (EGSB) system, to accommodate higher organic loading rate (OLR) (Karnchanawong & Phajee, 2009). The sludge bed and the blanket are formed mostly of particles or granules suspended in flowing water. Therefore, the up-flow velocity in the sludge bed is somewhat higher compared to up-flow velocity in the upper part of the reactor. This is called the Venturi effect, created by the presence of particles which constricts the space for water to flow, causing its velocity to increase.

Table 2.6 shows the different up-flow velocities in the reactors used by previous researchers.

Table 2.6: Up-flow Velocity used by Previous Researchers

Up-flow Velocity (m/h)	References
0.5–2.0	Francese et al. (1998)
0.9–1.5	Kavitha & Murugesan (2007)
0.1–1.4	Korsak (2008)
4	Karnchanawong & Phajee (2009)
1.38	Karnchanawong & Phajee (2009)
0.5	Dhir & Ram (2012)
0.13	Wang et al. (2015)
0.18–0.24	Moharram et al. (2016)
0.35	Moharram et al. (2016)
0.56	Moharram et al. (2016)

2.5 Anaerobic Biodegradation to Recover Resources from POME

The processes of hydrolysis, acidogenesis, acetogenesis and methanogenesis are simplified in a schematic representation in Figure 2.5.

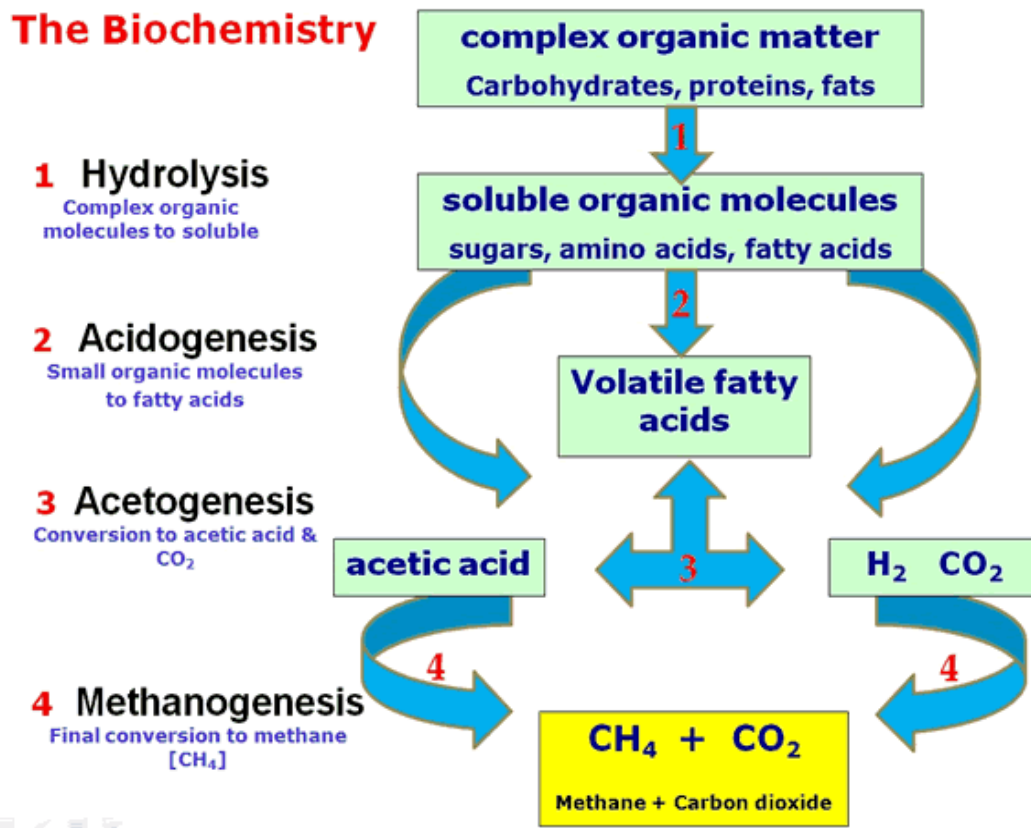


Figure 2.5: Schematic Representation of Anaerobic Biogas Production (Monge et al., 2013)

2.5.1 Hydrolysis for POME Digestion

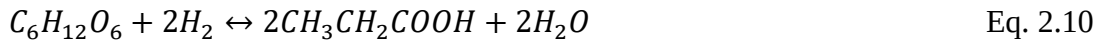
In general, hydrolysis is a chemical reaction in which water is broken down to form H⁺ cations and OH⁻ anions. It is the first step in anaerobic digestion process. This process involves enzyme which facilitates the conversion of organic polymers such as carbohydrates, proteins, lipids, and nucleic acids into soluble monomers and dimers such as simple sugars, amino acids and fatty acids (Adekunle & Okolie, 2015). This is carried out by several hydrolytic enzymes such as cellulases, cellobiase, xylanase, amylase, lipase and protease secreted by hydrolytic microbes (Weiland, 2010). Hydrolysis is important because microorganisms are unable to absorb large organic polymers to be used as an energy source. Different types of organic polymers require different enzymes. Therefore, the rate of breakdown depends on the composition of the substrate. Usually, the decomposition rate of cellulose and hemicellulose occur at a slower rate compared to decomposition rate of proteins (Schunurer & Jarvis, 2009). The majority of the monomers which are still large in size must be further broken down in the next step called acidogenesis, in order to produce methane. Equation 2.8 shows the step of hydrolysis during anaerobic digestion (Biarnes, 2013; Haandel & Lubbe, 2012; Ostrem, 2004; Verma, 2002; Zupančič & Grilc, 2012).



2.5.2 Acidogenesis for POME Digestion

Acidogenesis or acidification phase is the second step in anaerobic digestion process. In acidogenesis, microorganisms such as facultative and obligatory bacteria further break down the hydrolysed products into simpler organic products. The fermentative bacteria produce an acidic environment in the digestive tank to form organic products such as ammonia, acetate, hydrogen, carbon dioxide, hydrogen sulphide, shorter volatile fatty acids, carbonic

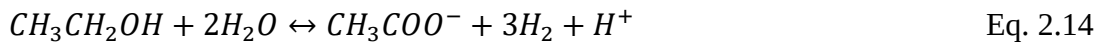
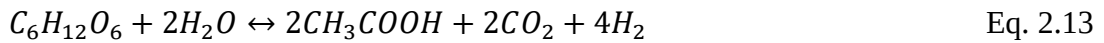
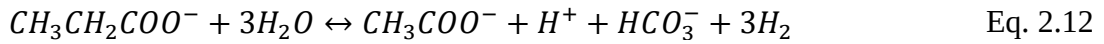
acids and alcohols (Adekunle & Okolie, 2015). Due to the interaction of different populations of microorganisms, the acidogenesis process is divided into hydrogenation and dehydrogenation (Krzysztof, 2012). Hydrogenation involves the addition of hydrogen while dehydrogenation is the removal of hydrogen (Sanfilippo & Rylander, 2000). Acetate, H₂ and CO₂ will soon become the precursors of biomethane formation. While some of the final products are still too large, they must undergo the next process which is acetogenesis. Equation 2.9, Equation 2.10, and Equation 2.11 show the steps of acidogenesis during anaerobic digestion (Biarnes, 2013; Haandel & Lubbe, 2012; Ostrem, 2004; Verma, 2002; Zupančič & Grilc, 2012).



2.5.3 Acetogenesis for POME Digestion

Acetogenesis is the formation of acetate, a derivative of acetic acid and the products during this phase are consumed by microorganisms. It is the third step in anaerobic digestion process. Acetogens convert products generated during acidogenesis into VFA and alcohols because these products cannot be converted directly into methane (Adekunle & Okolie, 2015). VFA will be oxidized to form acetate, hydrogen, and carbon dioxide. Meanwhile, VFA containing longer carbon chains that one unit undergoes oxidation to form acetate and hydrogen (Seadi et al., 2008). During anaerobic oxidation, the production of H₂ was caused by the role of protons as the final electron acceptor. The oxidation process depends on the partial pressure of hydrogen and will only occur if the partial pressure is low. The formation of hydrogen during acetogenesis is regarded as a waste product, which has toxic effects on

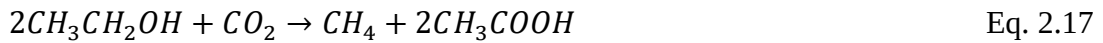
the microorganisms carrying out this step. Hence, it hinders the work of acetogenic bacteria. However, hydrogen scavenging bacteria or methanogens can utilize the hydrogen produced and convert them to methane (Seadi et al., 2008). This explains the importance of a cooperation between acetogens and methanogens in which transfer of hydrogen occurs (Chandra et al., 2012; Deublein & Steinhauser, 2010; Nurliyana et al., 2015). Equation 2.12, Equation 2.13 and Equation 2.14 show the steps of acetogenesis during anaerobic digestion (Biarnes, 2013; Haandel & Lubbe, 2012; Ostrem, 2004; Verma, 2002; Zupančič & Grilc, 2012).



2.5.4 Methanogenesis for POME Digestion

Methanogenesis is the final step in anaerobic digestion. It utilizes the substrates which are formed during the previous stages such as acetic acid, carbon dioxide and hydrogen (Krzysztof, 2012). Methane is produced by two groups of methanogens that are classified as acetotrophic methanogens and hydrogenotrophic methanogens. Acetotrophic methanogens convert acetate into methane and carbon dioxide. Meanwhile, hydrogenotrophic methanogens utilize hydrogen as electron donor and carbon dioxide as electron acceptor to produce methane (Demirel & Scherer, 2008). The formation of methane and carbon dioxide are performed by methanogens at strict anaerobic environments (Aslanzadeh, 2014). These methane-formers are susceptible to even small amount of oxygen present in the system. According to Seadi et al. (2008), methanogenesis is a vital step because the rate of biochemical reaction in this stage is 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 corresponds to the removal efficiency of COD or BOD during the treatment process of anaerobic wastewater. Equation 2.15, Equation 2.16, and Equation 2.17 show the steps of methanogenesis during anaerobic digestion (Biarnes, 2013; Haandel & Lubbe, 2012; Ostrem, 2004; Verma, 2002; Zupančič & Grilc, 2012).



2.6 Effect of Various Operating Parameters on Performance of Anaerobic Digestion

Lot of research work has been done on the use of different types of anaerobic reactors for treating POME with the applications of principles of biotechnology and microbiology (Abdelgadir et al., 2014) because of their high efficiency, low energy use and green energy generation in the form of biogas. The process parameters of anaerobic digestion such as temperature, pH, hydraulic retention time (HRT), organic loading rate (OLR) and sludge retention time (SRT) had been reviewed to take into account the optimal conditions for living, growth and proliferations of anaerobic bacteria. Though temperature plays a significant role in improving or accelerating the conversion of organic matter into gaseous state by enhancing mixing and energising microbes to act on the substrate, the need to apply external heat to maintain operating temperature at thermophilic range would offset the cost of energy recovery from the system. Hence, most treatment systems are designed to operate

at mesophilic range (El-Mashad et al., 2004; Ge et al., 2011), which is around the average ambient temperature in the tropics.

Due to constraints of resources (time, money, equipment), only five input factors (OLR, pH, C/N, HRT and SRT) were considered for further review on similar research works.

2.6.1 Up-flow Velocity, pH, OLR on Performance of Anaerobic Digestion

Up-flow velocity is an operating condition which can contribute to the performance of anaerobic digestion. The up-flow velocity in an anaerobic reactor produces two different outcomes on the removal efficiency of organic matter. High up-flow velocity results in proper mixing between substrate and biomass in the reactor, which enhances their duration of contact. On the contrary, high up-flow velocity can reduce the biological removal efficiency when it is greater than the settling velocity of particles, resulting in the untimely separation of biomass from substrate (Mahmoud et al., 2003). Chu et al. (2005) have conducted a study by applying different up-flow velocities in a membrane-coupled EGSB reactor and the removal efficiency of COD was high at higher up-flow velocities.

A pH (potential of hydrogen) value determines if the solution is acidic or alkaline. During anaerobic digestion, pH is an essential parameter which determines the performance and stability of the system for biogas production. Methanogens are microorganisms which are sensitive to the change in pH and the production of biogas increases at pH range of 6.5–7.8; best at pH range of 6.8–7.6 (Nayono, 2010). At pH levels below 6.6, the methanogenic activities decrease 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 result in the reduction of biogas production rate. 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 ranges between 5.5 and 6.5 (Kim et al., 2003). Due to different optimal pH values in both processes, a two-stage anaerobic digestion is the preferred method to separate the hydrolysis/acidification (first stage) and acetogenesis/methanogenesis (second stage) processes.

The biogas production in an anaerobic reactor increases with increasing OLR. However, there are consequences in increasing OLR as the stability and efficiency of digestion process may be affected. The addition of large volume of organic material may alter the environment inside the reactor and interrupts the fermentation process during the early stages. An extremely high OLR leads 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 will decrease. As a result, the efficiency of methanogenic archaea to convert high amount of VFA to methane will become lesser (Mao et al., 2015). Besides that, high OLR may result in sludge bed flotation, extreme foaming, build-up of undigested substrate, and decreasing treatment effectiveness (Abdelgadir et al., 2014).

2.6.2 Effect of HRT on Biogas Production

HRT is an operational condition which has an effect on microbial activities, metabolic stages of digestion, product of intermediates, and end-products during anaerobic digestion process (Choong et al., 2018). In anaerobic digestion, the effect of low HRT leads to the accumulation of VFA. A short HRT may result in system failure because it is affected by the growth rate of microbial population, insufficient hydrolysis, process imbalance, and organic overloading (Mao et al., 2015; Nurul Adela et al., 2014; Romero Aguilar et al.,

2013). Although a low HRT produces higher biogas production rate, the decomposition rate of organic matter is low. Meanwhile, the effect of higher than optimal HRT results in the ineffectiveness of digestion process. An optimal HRT is required to prevent methanogens being washed out from the reactor. In summary, a low OLR and a long HRT provide the best strategy for achieving constant and maximal methane yields (Mao et al., 2015). While treating petrochemical wastewater and activated manure, Siddique et al. (2016) found that the proportion of methane in biogas was highest ranging from 71.89–73.89% at HRT of 9 days, as compared to other HRTs at 12, 6, and 3 days.

2.6.3 Effect of HRT on COD and BOD Reduction

COD represents the total organic matter under anaerobic conditions. During anaerobic digestion, COD is digested and converted into harmless end products which include methane, carbon dioxide, and water. An increase in methane production reflects an increase in COD removal. A well-adapted microbial population in anaerobic condition leads to an increase in the rate of digestion of organic matters (Alrawi et al., 2011). HRT is one of the anaerobic conditions which provide an effect on COD reduction of wastewater. The removal efficiency of co-digested 10% rumen fluid and 90% anaerobically digested sludge-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 is suitable for digestion of POME. A short HRT may reduce the contact time between organic matter and bacteria resulting in the insufficient reproduction of methane-forming microbes (Maroun & El 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 microbial population might have adapted to the new environment of petrochemical wastewater.

However, as the HRT increased to 12 days, there was no improvement in COD removal efficiency.

High strength wastewater is usually categorized by its high BOD value. BOD is expressed as the organic matter which is decomposed by microorganisms during anaerobic digestion process (Merino-Solís et al., 2015). Organic matter is being utilized during anaerobic digestion, resulting in a significant decrease in BOD values. A study conducted by Ali (2012) on the treatment of dairy wastewater revealed that the removal efficiency of BOD increases with HRT. The BOD removal efficiency was about 90% at HRT of 72 hours. Meanwhile, HRT of 18 hours produced BOD removal efficiency of 20%. A study on the effects of HRT on treatability of textile and domestic wastewater (Nawaz & Khan, 2013) obtained BOD removal efficiency of 73% at HRTs of 12 and 8 hours. There was no increment in BOD removal efficiency when HRT was increased because BOD was readily broken down by bacteria and removed at 8 hours.

2.6.4 Effect of SRT on Biogas Production

The performance of an anaerobic reactor depends on the SRT. SRT is the key factor in an anaerobic system which determines the amount of hydrolysis and methanogenesis (Abdelgadir et al., 2014). Besides that, SRT plays a role in controlling the stability of a digester. A condition in which SRT is low provides insufficient amount of time for microorganisms to reproduce and restore the microbial population that are lost in the effluent. Washout occurs when the growth rate of microorganisms is lower than the rate of microbial loss. Critical SRT is identified as the SRT when washout starts to happen (Ali, 2012; Demirel et al., 2005; Metcalf & Eddy, 2003). The long duration of SRT allows adequate methanogenesis to occur in an anaerobic system. Biogas is formed through the

conversion of VS and it is affected by SRT. SRT can be increased by sludge recycling which results in greater residence time of sludge in the digester (Burke, 2001). The effect of SRT on VS decomposition rate with sludge recycling conducted by Irvan et al. (2017) showed an increase in VS decomposition rate as SRT was increased. The highest value of VS decomposition was at SRT of 21 days by achieving 84% while the lowest value obtained at SRT of 8 days was 58%. Irvan et al. (2017) proved that the conversion of VS into biogas is a function of SRT. Another study conducted by Bolzonella et al. (2005) registered a decrease in specific gas production by 25% as SRT was increased from 10 to 20 days. The amount of biogas produced at SRT of 12 days is higher by three times compared to SRT of 35 days. Foaming, accumulation of VFA and increased alkalinity occurred at SRT of 9 days during the digestion of dewatered sewage sludge resulting in process instability (Nges & Liu, 2010).

2.6.5 Effect of SRT on COD and BOD Reduction

Generally, increasing the SRT improves the removal efficiency of COD (Dereli et al., 2014). Industrial wastewaters such pharmaceutical wastewaters, saline wastewaters or both in combination are difficult to decompose (De Vrieze et al., 2014; Ng et al., 2014; Shi et al., 2014). A high SRT would allow 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 is carried out. Meanwhile, the COD removal efficiency obtained 76% without sludge recycling (Irvan et al., 2017). Previous researchers have also conducted studies on COD removal efficiencies and they found that the removal efficiencies was higher with sludge recycling than 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. 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 and hence, supernatant COD increased as well (Huang et al., 2001).

Effluent recycling involves separating biomass and returning it into the reactor. It is necessary for the suspended growth process (Ali, 2012) in order to maintain the sludge inside the reactor to increase the contact between biomass and sludge. This prolonged contact allows bacteria to further break down the biomass and thus, reduces the BOD and COD contents of effluent. Wastewaters which consist of high concentration of substrate or solid require longer SRT to decompose. A system treating sewage sludge which contains high solids has a greater production rate of methane compared to a system with low solids at the same SRT in mesophilic conditions (Duan et al., 2012). The SRT shall be prolonged if another complex substrate is added into the reactor. Besides that, the high concentration of biomass can be maintained inside the reactor by increasing SRT which will reduce the HRT (Demirel et al., 2005). In warm climates, most wastewater treatment plants which used the traditional activated sludge process operating at a short HRT were able to achieve 85 to 95% BOD removal efficiency (Leu et al., 2012).

2.6.6 Effect of C/N on Biogas Production

In anaerobic digestion, C/N determines the availability of nutrients in the system in which microorganisms require both carbon and nitrogen. The ratio of carbon to nitrogen impacts

the performance and efficiency of an anaerobic system. Substrate with extremely high C/N would deprive microorganisms with nitrogen to maintain cell biomass leading to low production rate of biogas. Meanwhile, substrate with extremely low C/N may result in increase to the amount of total ammonia nitrogen (TAN) and VFA in the system. TAN and VFA are harmful to methanogens by undermining their efficiency to consume carbon sources (Mao et al., 2015; Tanimu et al., 2014). During anaerobic digestion, microorganisms consume carbon 25 to 30 times faster than nitrogen. Hence, C/N ranging between 20 to 35 is known to be the optimum ratio for anaerobic digestion (Puñal et al., 2000; Yen & Brune, 2007; Zhang et al., 2013).

Co-digestion is a process of mixing two different feedstocks. A feedstock with high biodegradable carbon can be mixed with a co-substrate with low nitrogen or vice versa in order to achieve a desired C/N (Tanimu et al., 2014). Co-digestion offers more benefits compared to digestion carried out with a single substrate. These include a more stable collection of nutrients, higher loading rate, lower concentration of harmful ammonia and VFA, and improved biogas yields. A study conducted by Kim et al. (2013) on co-digestion of POME and EFB with C/N 57–59:1 produced specific methane production of 50.8 mL-CH₄/g-VSS/d. In another case, Wu et al. (2010) conducted a study on the co-digestion of swine manure with corn stalks at C/N of 20. The authors obtained an increase in cumulative biogas production up to 11 times and the cumulative net methane volume increased 16 times when compared to mono-digestion of swine manure.

It would be useful to use other fruit wastes such as banana or pineapple for co-digestion with POME, which are known to possess biomethane potentials under anaerobic conditions. Though several tons of banana peel (which accounts for 30–40% of the total fruit weight)

were produced daily in small-medium food processing industry, marketplaces, household garbage and restaurants in Malaysia (Mohammed & Chong, 2014), banana peels have not been fully utilised for production of useful by-products, mainly discarded as waste. Apart from being used as amendment material to improve composting of EFB (Zahrim et al., 2015), several attempts had been made to produce biomethane directly from banana peels (Bardiya et al., 1996; Clarke et al., 2008; Pisutpaisal et al., 2014) which could be captured for energy generation. With very high carbon content (Hapsoh et al., 2015; Nathoa et al., 2014), banana peel could be used as inoculum in POME digestion where the C/N ratio of the resulting substrate can be increased to the optimal range with the appropriate dosing rate.

2.6.7 Effect of C/N on COD and BOD Reduction

The introduction of a co-substrate into a digestion system to increase the C/N is known as co-digestion. The process of co-digestion provides few benefits to the anaerobic digestion system (Esposito et al., 2012). The presence of a co-substrate may reduce the concentration of harmful compound available in the system. By adding a co-substrate, the pH and moisture content of a system can be improved. Besides that, co-digestion provides the required buffer capacity of a mixture. Co-digestion also improves the biodegradability of an organic matter present in an anaerobic system. Co-digesting two different substrates increases the variation of microbial population involved in the biochemical reaction process. An increase in COD removal efficiency and reduction of volatile solids correspond to the increase in biogas production (El-Mashad & Zhang, 2010). This is due to the effective acclimatization of methanogens in the anaerobic system which produces an efficient removal of COD, BOD and volatile solids.

A study was conducted by Sidik et al. (2013) on the co-digestion of POME and cow manure. The highest removal efficiency of COD achieved by the author was 67% when digester was fed with 70% POME and 30% cow manure (CM). The lowest removal efficiency of COD was 46.3% when digester was only fed with cow manure. The digestion of 70% POME and 30% CM produced a C/N in the range of 25 to 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 63–78% 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. The BOD removal efficiency achieved 92–97% at changing C/N from 25 to 45. The BOD reduction is slightly lower in co-digestion due to the small addition of oxygen demand from EFB. 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).

Where C/N ratio for POME only ranges from 10.1–11.4 (Nurul Adela et al., 2014), it is necessary to increase the resulting substrate upon co-digestion with EFB with C/N values ranging from 45–70 (Salètes et al., 2004) in order to optimize biogas production at optimal range of C/N ratio for anaerobic process, as specified earlier.

2.7 Findings on Current Technology of Anaerobic System

This section presents the current technology that has been used by previous researchers to conduct studies on biogas production and reduction of COD and BOD from POME.

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

Poh and Chong (2014) tested the performance of UASB-HCPB reactor to evaluate the effects of hydraulic retention time (HRT), organic loading rate (OLR) and mixed liquor volatile suspended solid (MLVSS) concentration of POME. A packed bed was used to stop the biomass from moving and reduce sludge washout, as elucidated in Figure 2.6.

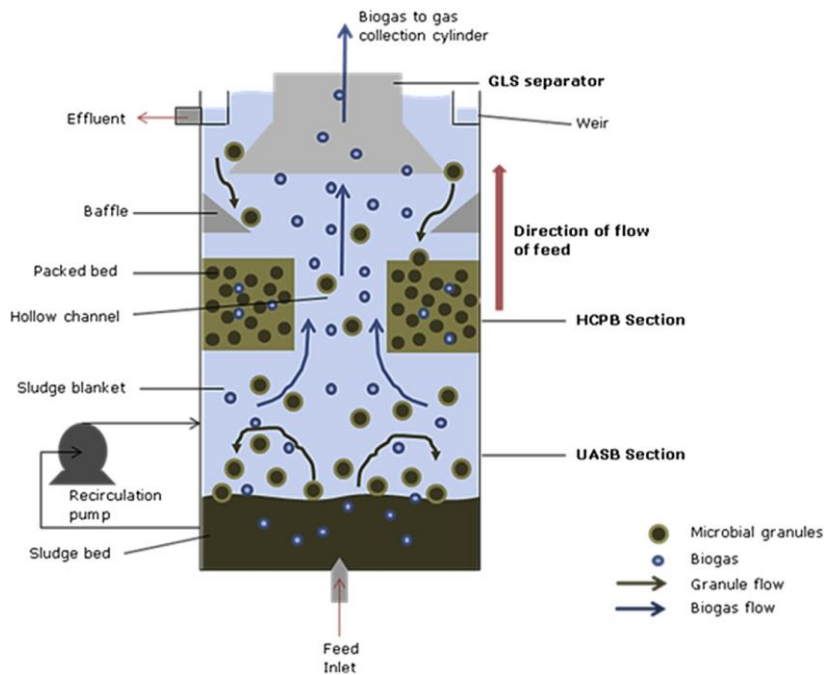


Figure 2.6: Design of UASB-HCPB Reactor (Poh & Chong, 2014)

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 reactor was evaluated at different HRTs while feed and MLVSS concentration are maintained at a constant value under thermophilic condition of 55°C.

Subsequently, the effect of OLR was determined by varying the feed COD concentration while keeping HRT and MLVSS concentration constant. Finally, the effect of MLVSS concentration was determined by changing its concentration through the addition or removal of the inoculum while maintaining HRT at 5 days.

The relevant parameters such as Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Suspended Solid (TSS), alkalinity, methane concentration and Volatile Fatty Acid (VFA) were then obtained from 13 experimental runs. Those parameters fluctuated throughout the experiment where runs 1–9 showed an increasing trend in overall COD and 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-COD/L/d) and reduced until run 9 which could be due to high corresponding OLR (13.75–27.65 g-COD/L/d). Runs 10–13 were conducted to study the effects of MLVSS concentration. HRT and OLR were 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-COD/L/d, HRT of 5 days and MLVSS concentration of 14.98 g/L was the best performance of the UASB-HCPB. Nonetheless, the imbalance of acidogen and methanogen population in the reactor which was related to HRT and OLR, would affect the performance of anaerobic digestion system. Besides, an increase in MLVSS concentration will lead to lower production of methane. HRT, OLR and MLVSS concentration are important operating conditions which will determine the performance of an anaerobic digestion system.

2.7.2 High-Rate Continuous Stirred Tank Reactor for Biodegradation of Palm Oil Fibre and Residual of POME using Raw POME as Substrate

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, operated at thermophilic temperature. Two types of CSTR were used in this study, which comprises the typical CSTR and a modified CSTR with a deflector installed at its upper section, as shown in Figure 2.7. The deflector was installed to promote the retaining of suspended solids inside the reactor.

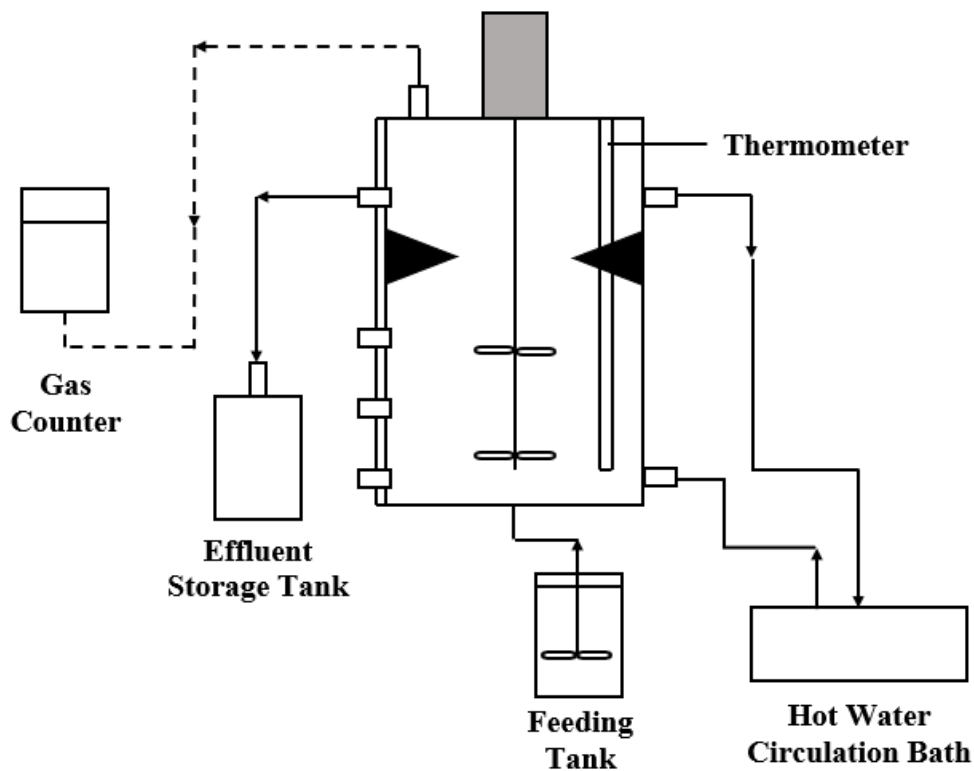


Figure 2.7: Diagram of a CSTR (Khemkhao et al., 2015)

Raw POME was used as the substrate for this study where neither physical nor chemical pre-treatment was done to remove the fibres and fat, oil and grease (FOG). The determination of

the reactor performance was evaluated for nine different steps of OLR starting from 2.0 to 19.0 g-COD/L/d while pH in the reactor was maintained at 7.0–8.0 to suit methanogenesis.

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 can 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 might affect the methane content although the OLR was increased.

2.7.3 Expanded Granular Sludge Bed Reactor

Wang et al. (2015) investigated the treatment efficiency of POME and stability of an expanded granular sludge bed (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, as shown in Figure 2.8 below.

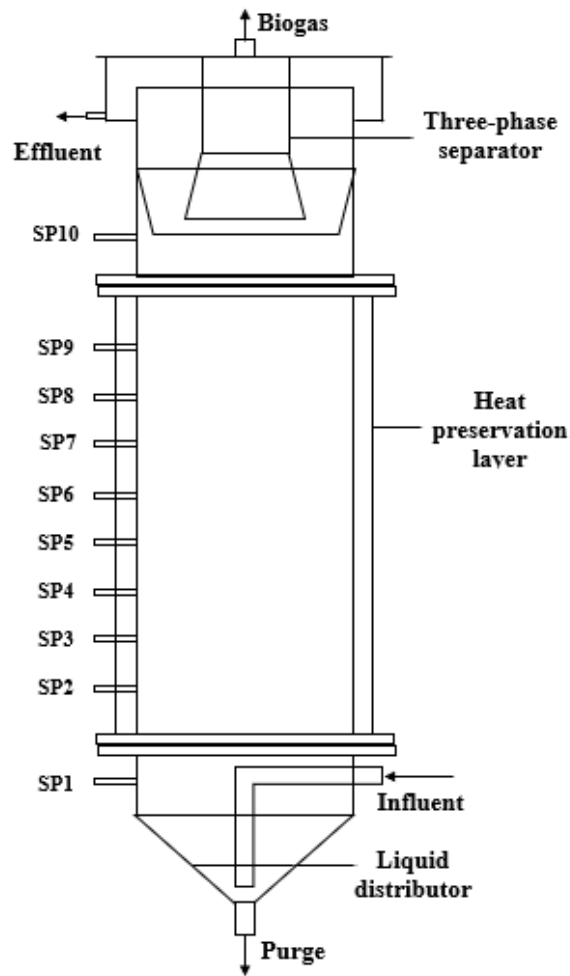


Figure 2.8: Schematic Diagram of EGSB Reactor (Wang et al., 2015)

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 suspended solids (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 in order to shorten the start-up period.

The anaerobic EGSB reactor was able to reduce the COD of POME from 71179 mg/L to 12341 mg/L. The removal of suspended solids (SS) by using DAF further reduced the COD to an average of 3587 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 process stability of an anaerobic reactor can be achieved by sufficient mixing, biogas recirculation and recycling of effluent. However, excessive mixing as well as accumulation of VFA might have caused pH value to drop inside the reactor which ultimately affects methanogenesis, resulting in lower production of methane.

2.7.4 Suspended Closed Anaerobic Reactor for Methanogenic Degradation

Wong et al. (2014) used this SCAR reactor to evaluate the effectiveness of suspended growth anaerobic degradation process in terms of pH, COD reduction and biogas production, as shown in Figure 2.9 below. Parameters which were analysed include pH, COD, soluble COD, microbial growth, VFA, total alkalinity and biogas composition.

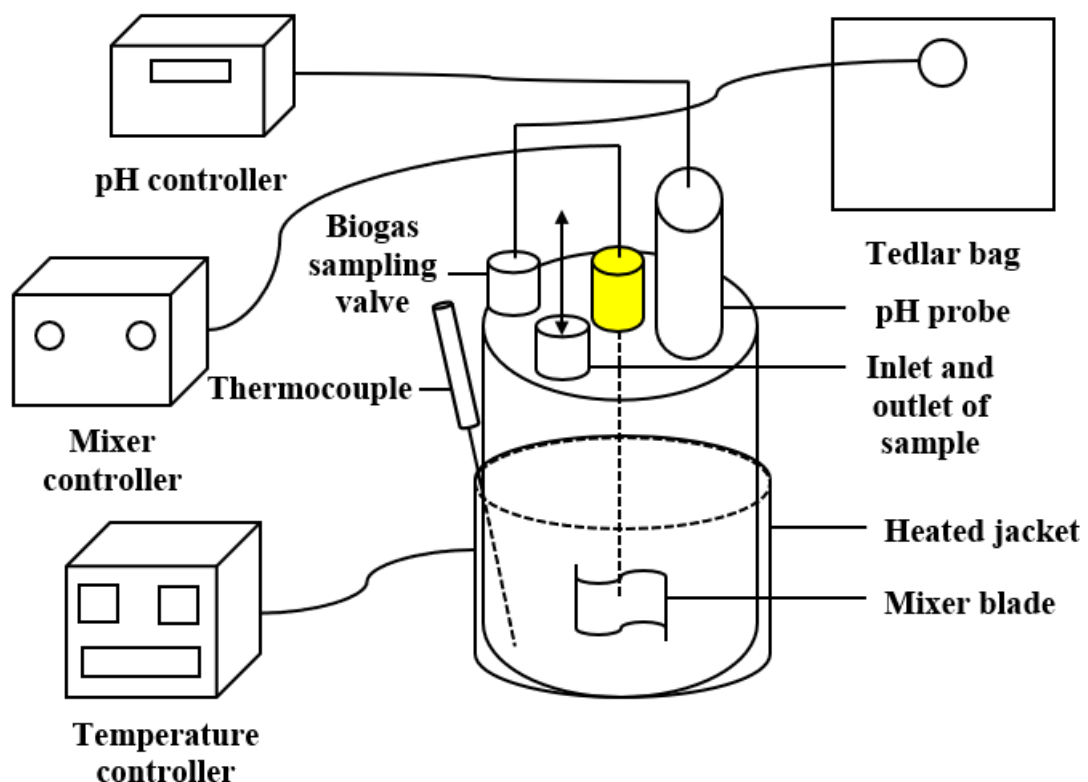


Figure 2.9: Schematic Diagram of SCAR (Wong et al., 2014)

In this study, SCAR reactor was operated with varying HRT of 12, 10, 8, 6 and 4 days by using different feed flow rates of 375 mL, 450 mL, 560 mL, 750 mL and 1125 mL respectively. The feeds were then subjected to 16 hours of continuous stirring before giving another 6 hours break for the 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 1097 mL-biogas/d to 3977 mL-biogas/d as HRT increased. 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 can affect the growth of methanogens which are responsible for the formation of methane. The rate of methane production can be increased with low OLR and longer HRT. pH not less than 7 and HRT more than 10 days are operating conditions which are recommended to achieve a good performance in anaerobic degradation of POME.

2.7.5 Anaerobic Hybrid Reactor and Anaerobic Baffled Reactor under both Mesophilic (37°C) and Thermophilic (55°C) Temperatures

Jeong et al. (2014) compared and evaluated the performance of this combined anaerobic system for POME treatment, which comprised anaerobic hybrid reactor (AHR) as the primary digester and anaerobic baffled reactor (ABF) as the secondary digester as depicted in Figure 2.10. At the lower part of the reactor, POME was treated by microorganisms from

the sludge bed. Subsequently, POME was treated by a biofilm formed on the filter media the upper of the reactor. Meanwhile, the ABF is a combination of anaerobic baffle reactor and anaerobic filter (AF).

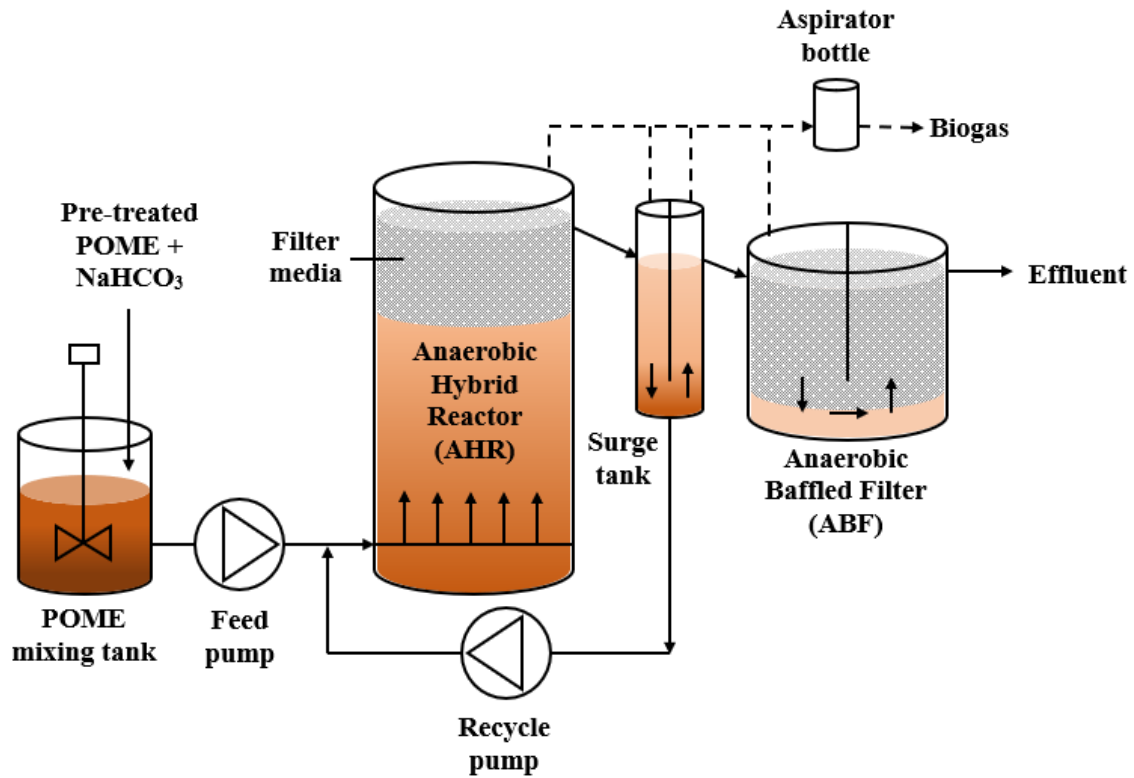


Figure 2.10: Schematic Diagram of AHR-ABF (Jeong et al., 2014)

A pre-treatment on raw POME was done by a screw decanter to remove the SS and residual oil. The feed flow rate was fixed to 0.73 and 0.77 L/d as OLR was gradually increased 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 combined system of AHR and ABF produced removal rates of COD at 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 L/d to 13.5 L/d and 1 L/d to 20.0 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. Methane production in thermophilic reactor was higher in COD removal and biogas production compared to the mesophilic reactor as high temperature tends to speed up the metabolic processes.

2.7.6 Anaerobic Sludge Blanket-Continuous Stirrer Tank Reactor

Krishnan et al. (2016) evaluated the effects of various OLR on H_2 and CH_4 production using this two-stage UASB-CSTR reactor operated at thermophilic conditions ($55^\circ C$), as shown in Figure 2.11.

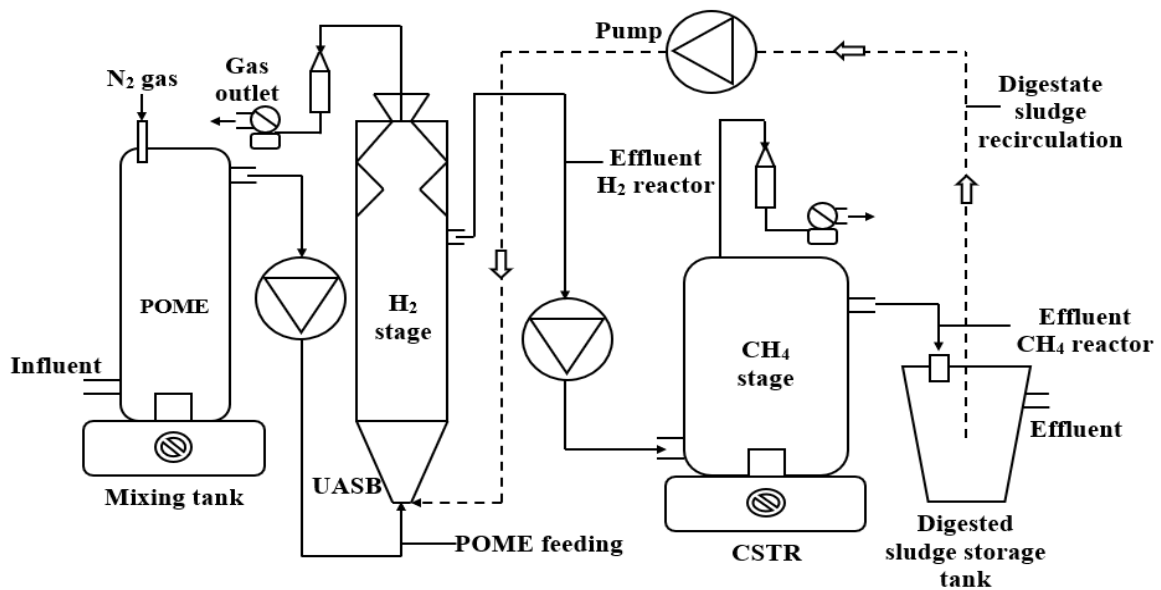


Figure 2.11: Schematic Design of UASB-CSTR (Krishnan et al., 2016)

The first stage using the UASB reactor focused on the production of H_2 while CSTR was used in the next stage to focus on the production of CH_4 . The raw POME was sieved to

remove coarse particles before feeding it into the UASB reactor with the addition of NaOH solution to achieve pH value of 5.5.

The HRT was kept constant at 6 hours. The mixing of feedstock in CSTR was maintained at 120 rpm. The parameters measured include hydrogen and methane production, VFA composition and MLVSS and effluent VSS composition.

The UASB unit recorded maximum value for gas production and COD removal at 4.5 L/d and 40% respectively, when OLR was at 75 kg-COD/m³/d, but decreased substantially at OLR 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. Fittingly, the two-stage fermentation process was designed to separate the microbial activities of acid formers and methane formers as acidogens and methanogens have different growth rate at different temperature range. It is known that acidogens grow rapidly while methanogens have a slower growth rate.

2.7.7 Two-Stage Thermophilic Fermentation and Mesophilic Methanogenic Process

Mamimin et al. (2015) studied the effects on process stability and microbial community in two-stage thermophilic fermentation and mesophilic methanogenic process for hydrogen and methane production from POME, as shown in Figure 2.12.

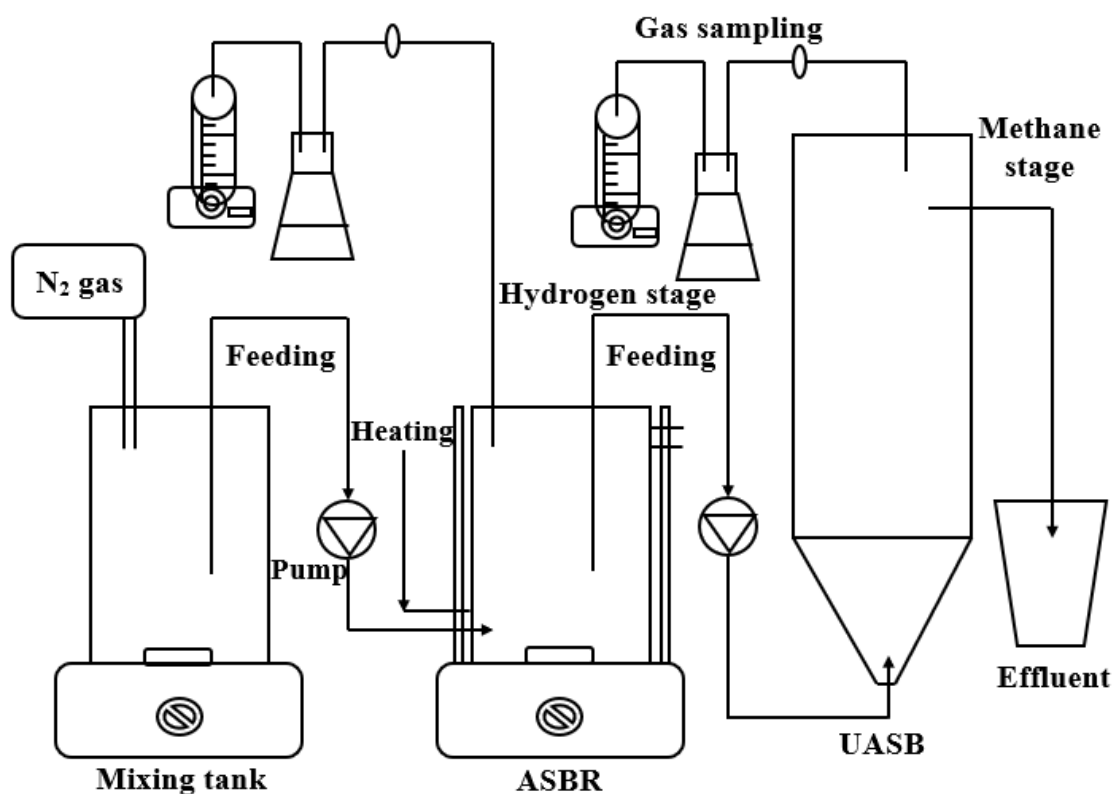


Figure 2.12: Schematic Diagram of Two-stage Hydrogen and Methane Process (Mamimin et al., 2015)

At thermophilic temperature of 55°C, this anaerobic sequencing batch reactor (ASBR) was tested for hydrogen production while setting pH value at 5.5 and HRT of 2 days. Subsequently, the up-flow anaerobic sludge bed (UASB) reactor was tested at mesophilic temperatures (28–34°C) for methane production, keeping pH at 7.5 and different HRT of 20, 15 and 10 days. Parameters 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 COD removal rate were recorded at 73% and more than 90% respectively. The best results obtained from the ASBR was under 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. It was observed that 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.

2.7.8 Combined High-Rate Anaerobic Reactor under Mesophilic Temperature (35–37°C)

Choi et al. (2013) evaluated the performance and feasibility of combined system of high-rate anaerobic reactors for POME treatment, 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, as displayed in Figure 2.13. The hybrid reactor was known as anaerobic hybrid reactor (AHR). The filter reactors were known as anaerobic baffled filter reactor (ABF) and anaerobic down-flow filter reactor (ADF). The secondary reactors were included to improve the removal efficiency of COD and ensure 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.

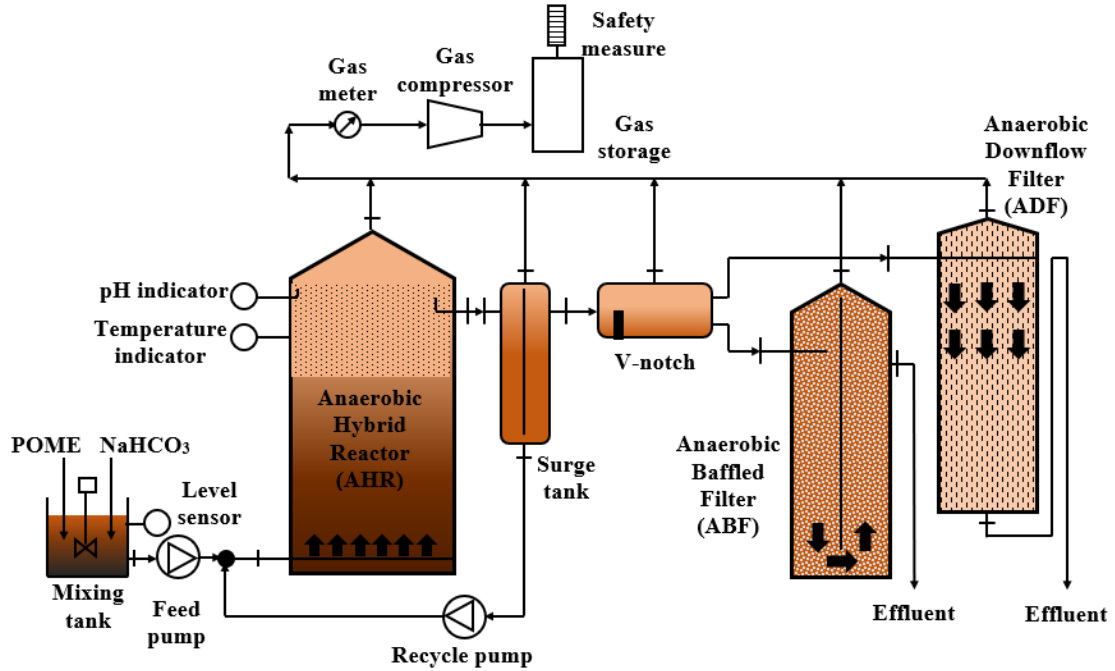


Figure 2.13: Schematic Diagram of AHR, ABF and ADF (Choi et al., 2013)

Raw POME underwent pre-treatment by a 3-phase screw decanter to remove excess SS and oil. pH value was adjusted to around 7 during the initial stage, and the overall HRT was varied from 0.7 to 2.4 days, as OLR was increased step by step from 0.91 to 23 kg-COD/m³/d. The parameters measured were COD values, SS and volume of biogas. The maximum removal efficiency of COD at 95.6% was achieved in the AHR reactor, when the OLR was at 13 kg-COD/m³/d.

Subsequently, COD removal efficiency of approximately 93.5% could be attained when treated effluent from AHR was fed into ABF and ADF reactors for further 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 and removed was within the range of 0.171 to 0.269 L-CH₄/g-COD. 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.

2.7.9 Up-flow Anaerobic Sludge Blanket Reactor with COA-CKD

Ahmad et al. (2014) examined the treatability of POME and effects of CaO on 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, specific granulation, methanogenic activity, density and composition of granules. UASB reactors were used to conduct this experiment, as shown in Figure 2.14.

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. 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.

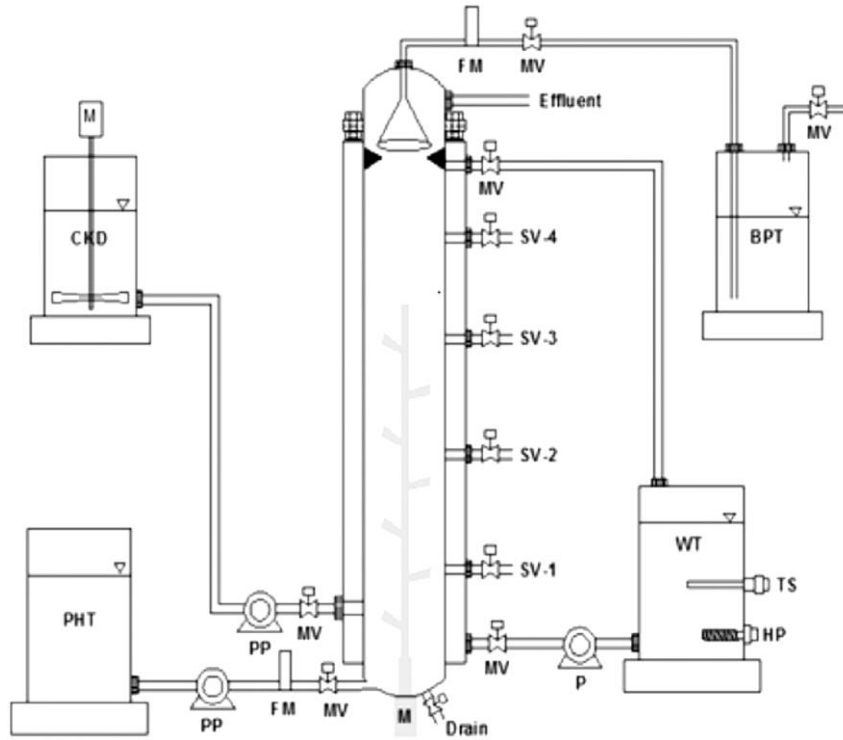


Figure 2.14: Experimental Set-up of UASBR (Ahmad et al., 2014)

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.

Based on COD removal, the reactor achieved an average efficiency of 82.4% when it was fed with 10 g/L CaO at OLR of 12.5 kg-COD/m³/d. The average methane yield was measured at 0.91 L-CH₄/g-COD, where 88.6% of COD removed was converted into methane in the UASB reactor. It was observed that the specific activity of granules decreased when the concentration of calcium oxide increased, due to low bacterial specific activity.

2.7.10 Up-flow Anaerobic Sludge Blanket and Expanded Granular Sludge Bed Reactors for Raw and De-oiled POME Treatments

Fang et al. (2011) compared the performance of 1.5 L EGSB reactor and 1.3 L EGSB reactor for both POME and de-oiled POME, in terms of methane production rate, methane yield, organic loading rate (OLR), process stability and COD removal efficiency. Both reactors were operated at thermophilic temperature at 55°C, with HRT initially set at 10 days and then reduced to 5 days. NaHCO₃ was added into the reactors to achieve pH value of more than 6. Different substrate concentrations were used to vary the OLR from 1.3 to 10.4 g-VS/L/d. The parameters measured include biogas production, pH and volatile fatty acids (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, de-oiled 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 COD removal efficiency of 90% and methane yield of 438 mL-CH₄/g-VS. For the treatment of de-oiled POME, more than 90% of COD removal efficiency was achieved 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 gave 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 can produce excess amount of acid which interrupts methanogenesis resulting in reduction of methane yield.

2.7.11 Up-flow Anaerobic Sludge Blanket Reactors for both Mesophilic and Thermophilic Digestion of POME

Khemkhao et al. (2012) evaluated the effects of organic loading rate (OLR) and operating temperature on the microbial diversity and performance of two different UASB reactors. The first reactor (U1) was operated at mesophilic temperature of 37°C. The second reactor (U2) was operated under increasing temperature with 5°C increments, ranging from mesophilic (37°C) to thermophilic (57°C) level. 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/hr. 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 include COD, VSS and biogas composition.

Both reactors achieved COD removal efficiency 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 this study, it was found that thermophilic anaerobic digestion was the preferable operating condition to treat POME as compared to mesophilic anaerobic digestion. Moreover, the desired microbial community for treating POME can be achieved in a thermophilic reactor with higher OLR for optimal methane production.

2.7.12 Up-flow Anaerobic Sludge Blanket Reactor

Ahmad and Ghufuran (2014) conducted a study to analyse bio-kinetics of cement kiln dust (CKD) as a substrate to neutralize and granulate POME in a laboratory-scale up-flow

anaerobic sludge blanket (UASB) reactor, as a function of hydraulic retention time. 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 substrate. 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 HRTs 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, while keeping pH value of the reactor at 7.5. Parameters such as BOD, COD, gas volume, VFA, pH, TS, total Kjeldahl nitrogen and oil and grease were measured.

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 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 are microorganisms (archaea) which can grow and produce maximum amount of methane in a neutral environment.

2.7.13 Thermophilic Continuous Stirred Tank Reactor for Methane Emission from POME under Thermophilic Temperature (55°C)

Irvan et al. (2012) evaluated the performance of this CSTR reactor with pH value being adjusted with the addition of NaHCO₃ before feeding. A premixed chemical solution consisted of FeCl₂, NiCl₂·6H₂O and CoCl₂·6H₂O was added to provide nutrition for methane-

producing bacteria. By varying HRT at 4, 6 and 8 days, data was collected on the amount of gas generated, pH, amount of feeding and discharging of substrate, TS, M-alkalinity of substrate and VS of POME.

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 might 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% as compared with 66% and 63% recorded at HRT of 6 and 8 days respectively. The acceptable HRT for CSTR was at 8 days where it produced high amount of VS and COD decomposition rate. The methane content recorded for HRT of 8 days was 64%. A balance between high and low HRT is needed to achieve acceptable results in methane production. Other operating conditions including pH, SRT and OLR are among the factors that can affect methane production as well.

2.7.14 Membrane Anaerobic System for Bio-methanation from POME

Abdurahman et al. (2011) tested the performance of membrane anaerobic system (MAS) as an alternative and cost-effective method to treat POME. This study was done 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, as shown in Figure 2.15. The membrane was used to separate biomass solids from the solid suspension in order to be recycled.

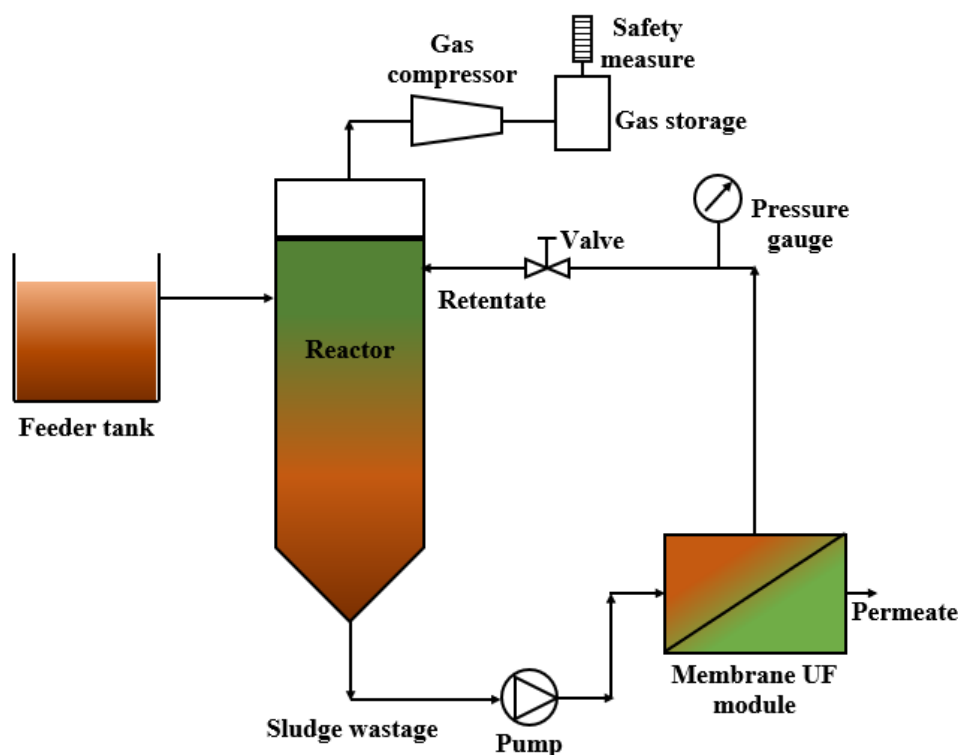


Figure 2.15: Experimental Set-up of MAS (Abdurahman et al., 2011)

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 include chemical oxygen demand (COD), total suspended solids (TSS), pH, volatile suspended solids (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 6.7 to 7.8.

At OLR of 13 kg-COD/m³/d, the MAS obtained COD removal efficiency of 94.8% which produced effluent COD of 2279 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-COD.d to 0.83 L/g-COD.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% to 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.

2.7.15 Carrier Anaerobic Baffled Reactor with Polymeric Media

Malakahmad and Yee (2014) investigated the biogas production rate from POME using laboratory-scale CABR reactor without effluent recycling in order to evaluate the effects of organic loading rate (OLR), alkalinity supplementation and short HRT. The CABR system contained a series of up-flow packed-bed attached growth reactors, as shown in Figure 2.16.

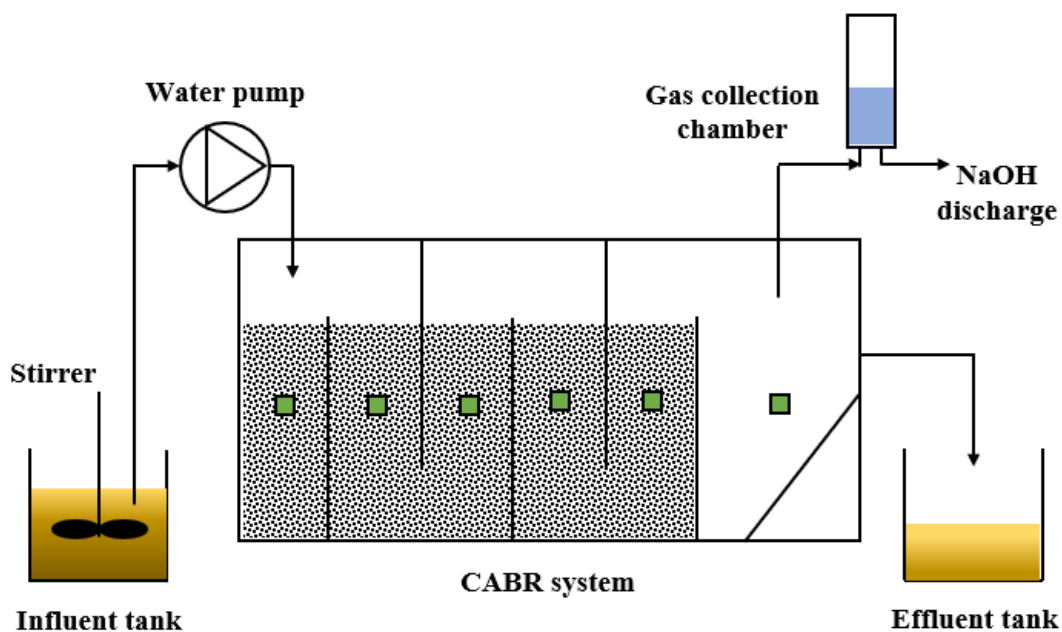


Figure 2.16: Schematic Diagram of CABR

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 its ability to

sustain high loading of COD, which requires small reactor volumes and simple operating conditions. 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 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 L-CH₄/g-COD 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 increased with increasing OLR resulting in the decrease of pH in the system. At high OLR, it will encourage the growth of acidogens resulting in the decrease of pH and methane content. However, pH value will increase as methanogens begin to convert hydrogen and acetic acid into methane gas and carbon dioxide resulting in the increase of methane content.

2.7.16 Semi-Continuous Stirred Tank Reactor

Alrawi et al. (2011) evaluated the efficiency of co-digestion of POME with rumen fluid (RF) as a co-substrate using this SCSTR reactor as shown in Figure 2.17 and compared it with mono-digestion of POME, under mesophilic condition (37°C).

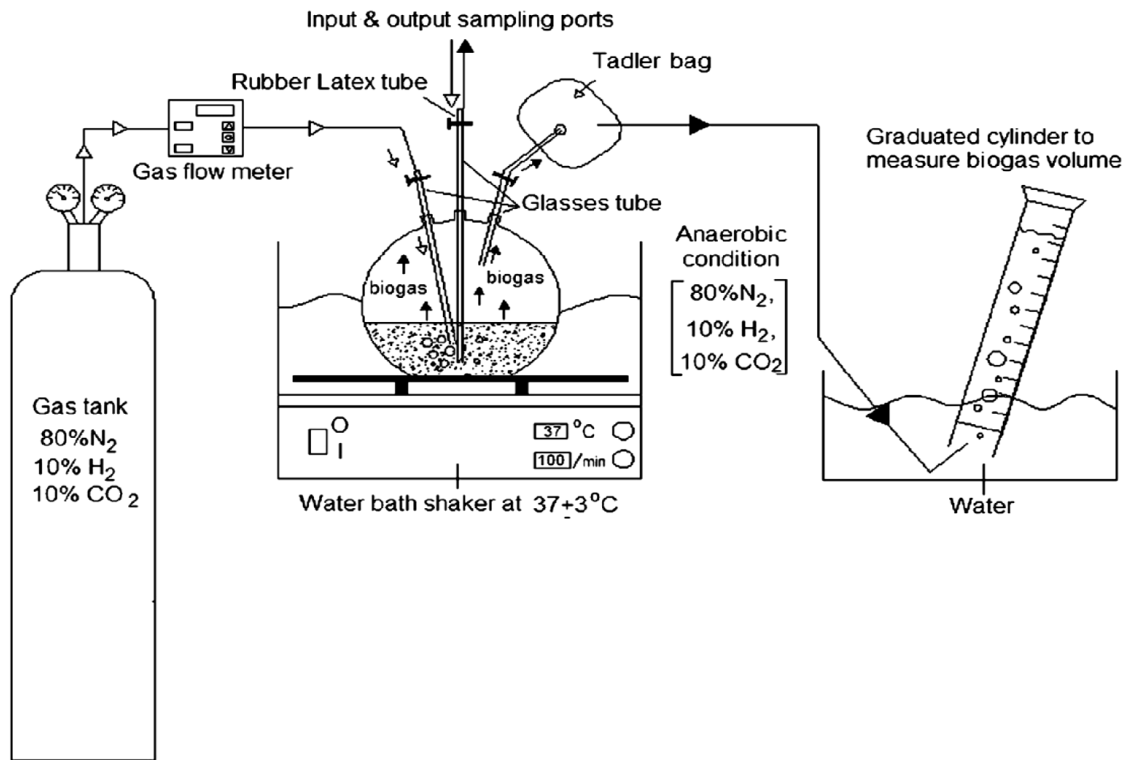


Figure 2.17: Schematic Diagram of SCSTR (Alrawi et al., 2011)

The experiment was conducted at hydraulic retention time (HRT) of 20, 10 and 6 days and organic loading rate (OLR) of 1.65, 2.85 and 4.06 g-COD/L/d respectively. Three 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 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 was relatively stable at different HRT and OLR. The pH measured was in the range of 7.05

to 7.55 providing a suitable environment for methanogenesis. Based on this study, co-digestion has shown to be more effective compared to mono-digestion. Co-digestion of POME with other co-substrate may prove to be more effective in COD removal efficiency as well as improving the production rate of methane.

2.7.17 Ultrasonic Membrane Anaerobic System

Nour and Nour (2017) evaluated the performance and kinetics of this UMAS reactor for biogas production from POME based on Monod, Contois, and Chen and Hashimoto models. This newly designed laboratory scale UMAS reactor comprises a cross-flow ultrafiltration membrane and an anaerobic reactor as shown in Figure 2.18.

The UMAS was operated at different COD concentration of 70,400 to 90,200 mg/L. As for hydraulic retention time (HRT) and organic loading rate (OLR), the UMAS was operated at 500.8 to 14.7 days and 1.5 to 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.

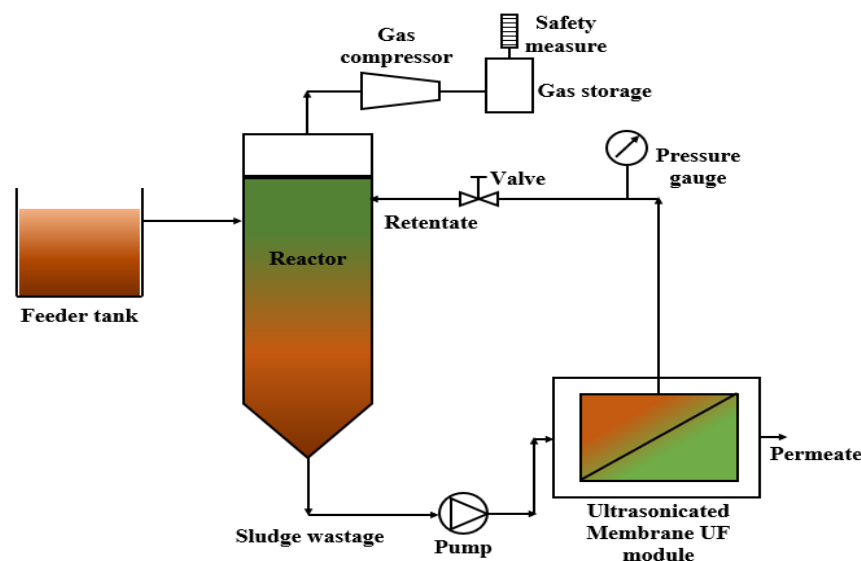


Figure 2.18: Experimental Set-up of UMAS (Nour & Nour, 2017)

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 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 increase in biogas production from 0.48 L/g-COD.d to 0.81 L/g-COD.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 increased and HRT was decreased accordingly. It was noted that 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.

2.7.18 Two-Stage Anaerobic Digestion Process of POME into Biogas

Trisakti et al. (2017) investigated the effect of substrate agitation on methanogenesis with increasing organic loading rate (OLR) or reduction of hydraulic retention time (HRT). The experiment was conducted using a continuous stirred tank reactor (CSTR) in which a two-stage process of anaerobic digestion was performed, as shown in Figure 2.19.

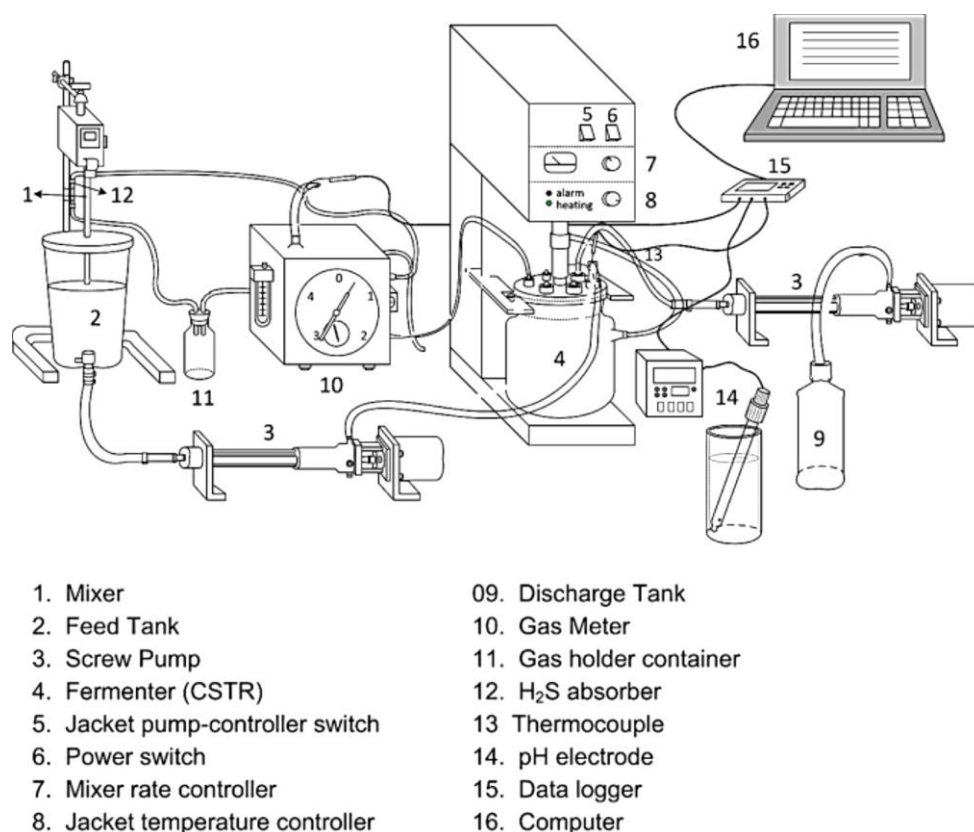


Figure 2.19: Experimental Set-up of CSTR (Trisakti et al., 2017)

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 total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), and production rate and composition of biogas.

From the experiment, the number of microorganisms which constitutes VSS, increased as the rate of agitation was increased. Agitation rate of 200 rpm produced the highest VSS value of 26,404.33±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±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, maintaining pH value, prevents sedimentation of denser solids and aids in release of biogas bubbles.

2.8 Models to Estimate Methane Potential

This section deliberates on the different models used to estimate methane potential of POME. Many models have been published in various journals, where they were used in designing anaerobic reactors for capturing methane produced from POME digestion. The most common models are presented as follows:

2.8.1 Gompertz Model Equation

Gompertz curve or Gompertz function is a mathematical model of time series, developed by Benjamin Gompertz (1779-1865). This model has been used to estimate methane of MSW and POME (Nopharatana et al., 2007), which can be presented mathematically as:

$$M_p = P_m - e^{\left[\frac{R_m}{P_m(X_0 - X)\varepsilon}\right]} + 1 \quad \text{Eq. 2.18}$$

Where M_p is methane yield (mL), P_m is methane potentials (mL), R_m is methane production rate (mL/d), X is lag-phase time (d) and ε is exponential value as constant (2.718). This particular model is useful for methane emission from leachate of municipal solid waste (MSW) as well as palm oil mill effluent (POME).

2.8.2 Modified Gompertz Model Equation

The application of this modified Gompertz model enables the evaluation of the kinetics of biogas production from microbial activities (Matheri et al., 2016; Yusuf et al., 2011). Its modified equation was used to model cumulative biogas production, as expressed by the following equation:

$$Y(t) = Ae^{\left[\frac{\mu e}{A(\lambda-1)}\right]} + 1 \quad \text{Eq. 2.19}$$

Where: $Y(t)$ = Cumulative biogas production (mL). A = Biogas production potential (mL). μ = Maximum biogas production rate (d^{-1}). λ = Lag phase period. t = Cumulative time for biogas production (d). e = Mathematical constant (2.718).

2.8.3 Vedrenne Model

Michel Vedrenne from Technical University of Madrid (UPM) developed this model to estimate methane production from bio-fluid (Vedrenne et al., 2007):

$$Q(CH)_4 = B_o \times M_o \times MCF \times S_g \quad \text{Eq. 2.20}$$

Where: B_o = Methane potential per kg of biodegradable matter. M_o = Biodegradable matter. S_g = Portion of faeces fed into the anaerobic system. MCF = Methane conversion factor, ranging from 0.4 to 0.77.

2.8.4 CDM Model

Donoso-Bravo et al. (2011) developed this popular CDM model in the palm oil mill domain for estimating methane potential. The model is presented as:

$$Q(CH)_4 = [S_y \times COD \times FCM \times COD_f \times F]1.33 \quad \text{Eq. 2.21}$$

Where: S_y = Volume of wastes feeding to bio-digester. The COD = Fraction of biodegradable organic matter. FCM = Methane conversion factor. COD_f = Fraction of COD associated with biogas production. F = Fraction of methane in biogas, 1.33(16/12) is the conversion factor of carbon to methane.

2.8.5 COD Equivalent Model

This model has been used to estimate methane potentials from POME (Taylor et al., 2014). The model is:

$$Q_{MP} = Q \times COD \times Efficiency \times MCF \times B_o \times UF \times GWPCH_4 \quad \text{Eq. 2.22}$$

Where: Q = Volume of POME. COD = Chemical oxygen demand of POME. MCF = Methane correction factor for POME. Efficiency = COD removal efficiency. B_o = Methane production capacity of POME (methane yield). UF = Model correction factor to account for model uncertainties. $GWP(CH_4)$ = Global warming potential of methane.

2.8.6 COD Proportional Model

The coefficient of biogas yields can be calculated from the following equation (Rashidi et al., 2012):

$$CH_4(m^3/d) = (COD_{methane})(0.00035) \quad \text{Eq. 2.23}$$

$$COD_{methane} \left(\frac{g}{d} \right) = COD_{in} - COD_{VSS} - COD_{eff} \quad \text{Eq. 2.24}$$

$$COD_{in} \left(\frac{g}{d} \right) = [COD_{in} (g/m^3)](Q) \quad \text{Eq. 2.25}$$

$$COD_{eff} \left(\frac{g}{d} \right) = \left(1 - \frac{RE}{100} \right) Q \quad \text{Eq. 2.26}$$

$$COD_{VSS} \left(\frac{g}{d} \right) = \left(1.42 \frac{gCOD}{gVSS} \right) \times \left(0.04 \frac{gVSS}{gCOD} \right) \left(1 - \frac{RE}{100} \right) (COD_{in}) \quad \text{Eq. 2.27}$$

Where, $COD_{in}(g/d)$ = COD concentration in influent. $COD_{eff}(g/d)$ = COD concentration in effluent. $COD_{vss}(g/d)$ = Volatile suspended solids in POME. $COD_{methane}(g/d)$ = COD associated with methane yield. $CH_4(m^3/d)$ = Methane yield per day. $Q(m^3/d)$ = Inlet flow rate of POME. RE = COD removal efficiency.

2.8.7 The Modified COD Equivalent Model

With this modified COD Equivalent model, the methane potential from POME could be estimated using the following equations:

$$CH_4[m^3(gCOD_{MP})^{-1}] = (COD_{MP})(0.00035) \quad \text{Eq. 2.28}$$

$$COD_{MP}(g) = COD_{in} - COD_{VSS} - COD_{eff} \quad \text{Eq. 2.29}$$

$$COD_{VSS}(g) = \left(1.42 \frac{gCOD}{gVSS}\right) \times \left(0.04 \frac{gVSS}{gCOD}\right) \left(1 - \frac{RE}{100}\right) (COD_{in}) \quad \text{Eq. 2.30}$$

$$COD_{eff}(g) = \left(1 - \frac{RE}{100}\right) Q \quad \text{Eq. 2.31}$$

$$COD_{in}(g) = [COD_{in} (g/m^3)](Q) \quad \text{Eq. 2.32}$$

Where: COD_{MP} = Methane potential of 1.0 m³ POME. $COD_{vss}(g)$ = VSS equivalent. $COD_{eff}(g)$ = COD removal efficiency. $COD_{in}(g)$ = Organic materials density in POME at inlet to reactor.

The seven models listed have been commonly used in various projects in producing methane from bio-fluid. After comparing the appropriateness of those models listed earlier, the models described in Subsection 2.8.6 and Subsection 2.8.7 had appeared relevant in estimating methane (biogas) generated from POME as required for our research purpose.

2.9 Mechanics of Optimization in POME Digestion

The anaerobic digestion process offers many benefits in the recovery of resources from POME mainly in the form of biogas, water and organic fertilizers. Hence, this technology has been widely used especially in wastewater treatment process either to recover biogas or produce cleaner effluent. However, the optimization to the current technology is still needed because anaerobic digestion process is influenced by various factors such as microbial communities, retention time, temperature, pH value and the amount of food available for microorganisms. Appels et al. (2011) stipulated that the process of anaerobic digestion can be optimized if the following fields are identified: (1) the understanding of roles of microorganisms in digestion process and (2) the importance of mathematical model on production optimization.

Various mathematical models are being adopted in studies related to anaerobic digestion to enhance the performance of the anaerobic digester. Currently, more attention is given to the development of accurate models in anaerobic digestion. Tomei et al. (2009) have reviewed different types of mathematical models on the anaerobic digestion process which ranges from simple, steady-state models to very complicated models. The International Water Association (IWA) Task Group developed the IWA Anaerobic Digestion Model No. 1 (ADM1) which is regarded as widely applicable or baseline for modelling of anaerobic digestion. It has the ability to predict the major activities during the conversion of complex organic substrates into CH_4 and CO_2 (Batstone et al., 2002). A schematic representation of the metabolic pathways is displayed in Figure 2.20.

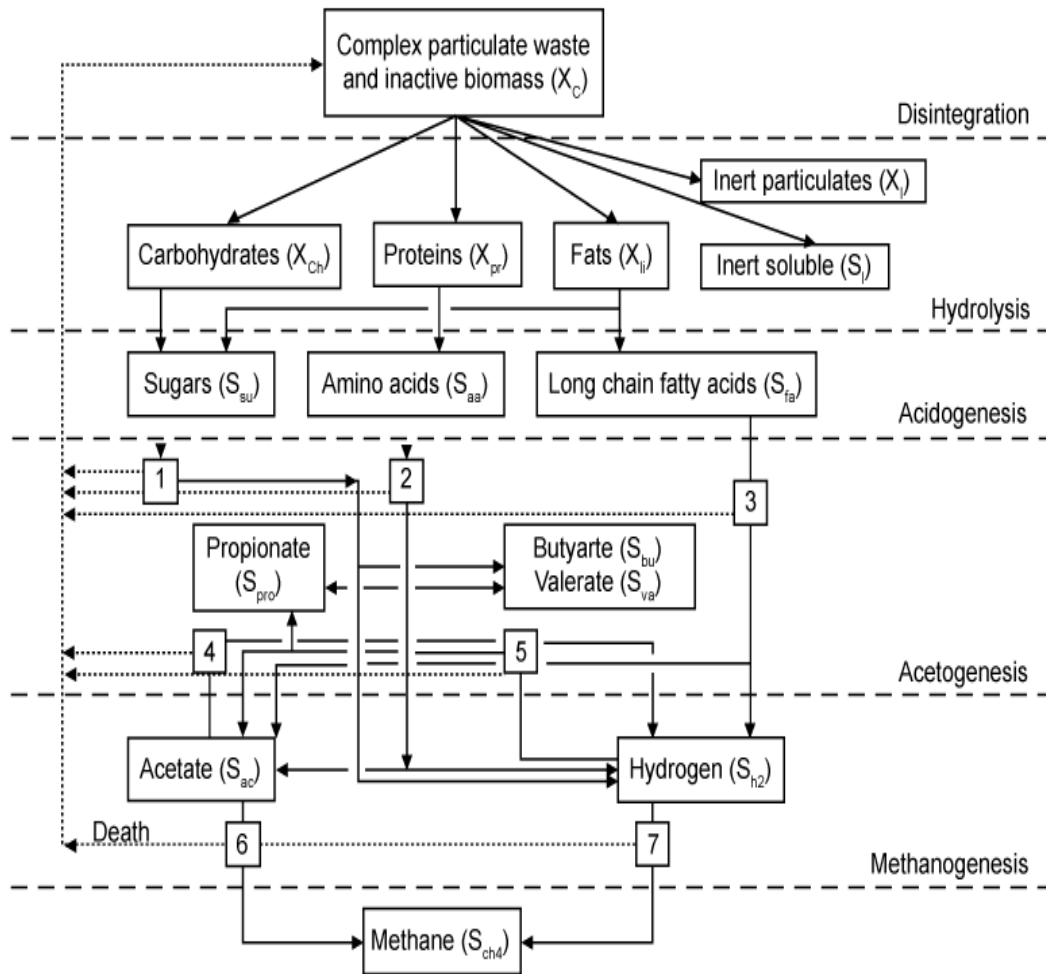


Figure 2.20: Metabolic Pathways for ADM1 Model X37 in POME Digestion. The pathways include (1) acidogenesis from sugars; (2) acidogenesis from amino acids; (3) acetogenesis from LCFA; (4) acetogenesis from amino acids; (5) acetogenesis from butyrate and valerate; (6) acetoclastic methanogenesis; (7) hydrogenotrophic methanogenesis.

Fundamental knowledge on the stages of anaerobic digestion is crucial in order to understand the mechanism of anaerobic digestion process. Different stages of anaerobic digestion involve different types of microorganisms. Somehow, the basic understanding of the digestion system is lacking due to the complexity of the microbial activity. Studies conducted on the composition of microbial community will 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 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.

The technique to achieve optimization in the production of biogas can be adapted from Operations Research. In optimization, the decision maker is able to formulate strategies in order to develop a feasible solution. In a given optimization problem, the objective is usually to minimize or maximize a function over a set of variables under certain constraints. In linear programming, the optimization problem can be solved by the following mathematical models:

Maximizing objective function,

$$Z = C_1X_1 + \dots + C_nX_n \quad \text{Eq. 2.33}$$

Subject to constraints,

$$a_1X_1 + \dots + a_nX_n < b_1 \quad \text{Eq. 2.34}$$

$$a_mX_1 + \dots + a_mX_n < b_m \quad \text{Eq. 2.35}$$

$$X_1 > 0, \dots, X_n > 0 \quad \text{Eq. 2.36}$$

The typical input-output production optimization is presented in Figure 2.21.

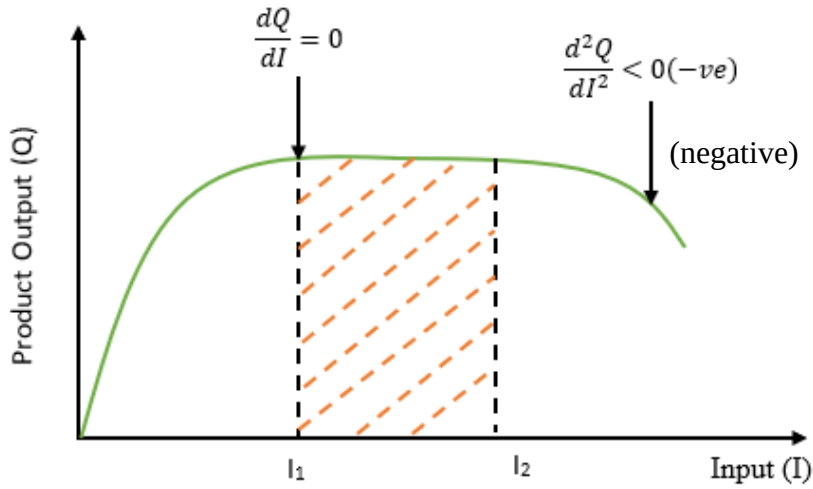


Figure 2.21: Input-Output Optimization (Burdett, 2016)

Based on Figure 2.21, the maximum output is produced at input I_1 and the point in which $\frac{dQ}{dI} = 0$ is known as the optimum point. Otherwise, inputs greater than I_1 will not increase the amount of output. The shaded regions under the curve are known as non-value-added inputs. These non-value-added inputs (between I_1 and I_2) may contribute to increase production cost and reduce the effectiveness of the output production.

With regards to biogas production, which is the objective of the current research, the input-output optimization model can be applied for optimizing biogas production (output) by changing OLR, pH, HRT, SRT, and C/N in order to get their optimal values (inputs). The illustrations of input-output optimization model for biogas production are presented in Figure 2.22, Figure 2.23 and Figure 2.24.

In the model to evaluate the effects of HRT on biogas production, the values of HRT are varied from 1 to 5 days while other variables such as SRT and C/N are kept constant.

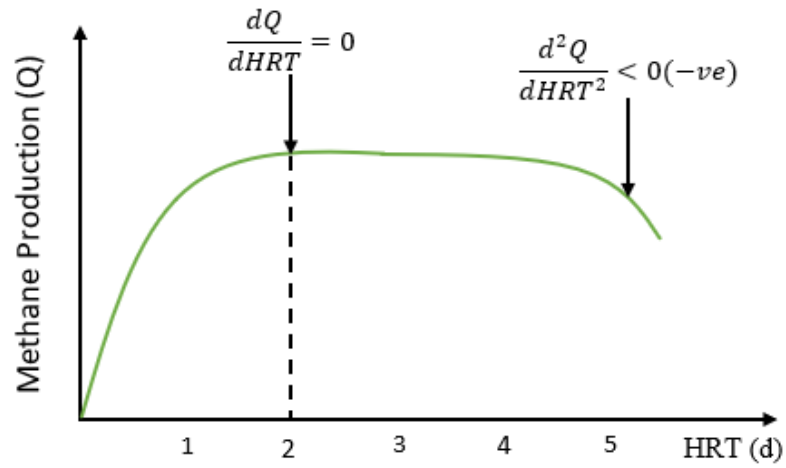


Figure 2.22: Input-Output Optimization Model of HRT on Methane Production

From Figure 2.22, the optimization point $\left[\frac{dQ}{d(HRT)} = 0 \right]$ can be obtained when the production of methane is highest at HRT of 2 days. As the HRT is increasing until 5 days, the production of methane will start to decline. Hence, the optimum HRT from this model is at 2 days.

In the model to determine the effects of SRT on methane production, the values of SRT are varied from 5 to 25 days while other variables such as HRT and C/N are kept constant.

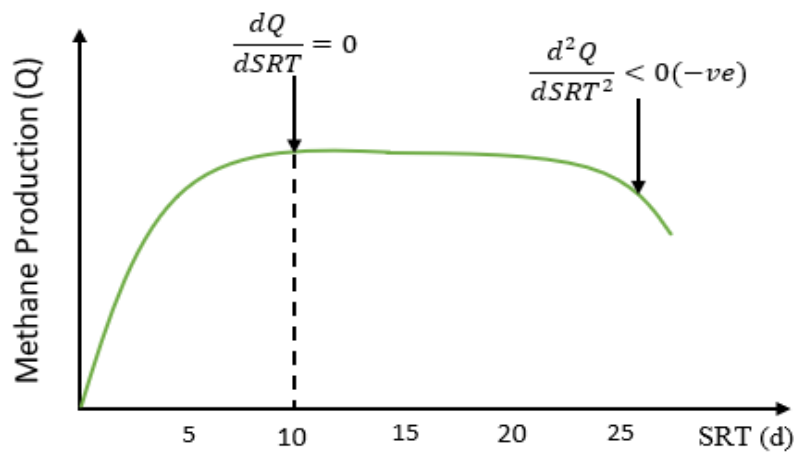


Figure 2.23: Input-Output Optimization Model of SRT on Methane Production

From Figure 2.23, the optimization point $\left[\frac{dQ}{d(SRT)} = 0\right]$ can be obtained when the production of methane is highest at SRT of 10 days. As the SRT is increasing up to 25 days, the production of methane will decrease. Therefore, the optimum SRT from this model is at 10 days.

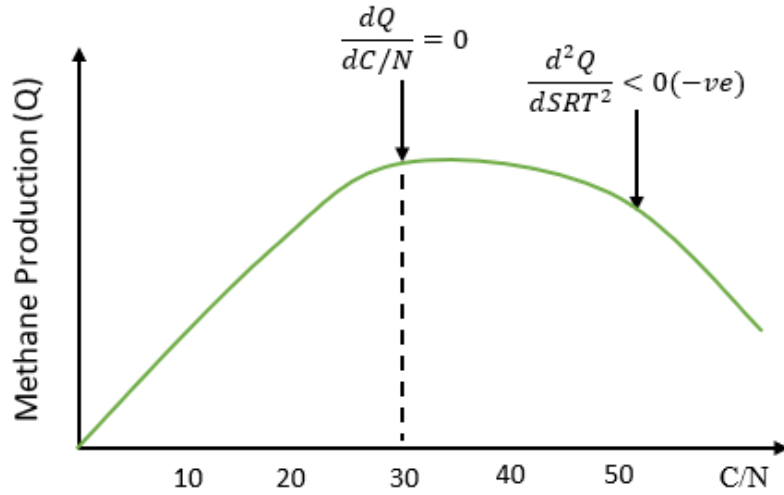


Figure 2.24: Input-Output Optimization Model of C/N on Methane Production

In the model to measure the effects of C/N on methane production, the values of C/N are varied from 10 to 50 by adding co-substrates while other variables such as HRT and SRT are kept constant. From Figure 2.24, the optimization point $\left[\frac{dQ}{d(C/N)} = 0\right]$ is obtained at C/N of 30, giving the highest production of methane. The production of methane is reducing as C/N goes beyond 30. Hence, the optimum C/N from this model is at 30.

2.9.1 Central Composite Design on Production Optimization

A central composite design is the most commonly used in response surface designed experiment. Central composite designs are a factorial or fractional factorial design with center points, augmented with a group of axial points (also called star points) which could be used to estimate the curvature. A central composite design can also be applied to

efficiently estimate first- and second-order terms. It can model a response variable with curvature by adding center and axial points to a previously done factorial design. Central composite designs are especially useful in sequential experiments because you can often build on previous factorial experiments by adding axial and center points. Central composite design has the desired properties of orthogonal blocks and rotatability, which may be elucidated as follows.

Orthogonal blocks: Often, central composite designs are done in more than one block. Central composite designs can create orthogonal blocks, enabling model terms and block effects be estimated independently while minimizing the variation in the regression coefficients.

Rotatability: Rotatable designs provide constant prediction variance at all points that are equidistant from the design center.

Design of Experiments (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. In this report, we will give an example on how to use Central Composite Designs in a DOE design folio in Weibull+ to determine the settings of factors that would result in the optimum value of the response.

2.9.2 Fundamental of CCD

CCD has been the widely used statistical method based on the multivariate nonlinear model for the optimization of process variables of biodegradation as well as for determining the regression model equations and operating conditions from the appropriate experiments. It is

also useful in studying the interactions of the various parameters affecting the process. The CCD can be used for fitting a second-order model which requires only a minimum number of experiments for modelling.

Basically, the optimization process involves three major steps:

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

In order to analyze the effect of parameter interactions, the following equation may be applied:

$$Y = \beta_0 + \beta_i x_i + \beta_{ij} x_i x_j + \beta_{ii} x_{ii}^2 + \epsilon \quad \text{Eq. 2.37}$$

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 the error (Arunkumar et al., 2014).

In RSM method, the least square multiple regression methodology is usually applied to investigate the relationship between the independent and dependent variables. A multiple regression equation can be used to fit the second-order polynomial equation based on the experimental data as in Equation 2.37 (Ghorbannezhad et al., 2016). The regression coefficients that have a statistically significant effect on response should be considered in the equation. Therefore, if quadratic terms are not statistically significant, those coefficients are removed, and equation will be linear (Yolmeh & Jafari, 2017).

Statistical experimental designs are powerful tools for searching the key factors rapidly from a multivariable system. Plackett-Burman design is one such method that has been frequently used for screening multiple factors one at a time. This experimental design is particularly useful for initial screening as it is 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 enables the study of interaction effects among different variables. For example, optimization of media components for the production of laccase by response surface methodology has been reported in the case of different fungal strains. Hence, Response Surface Methodology is a statistical experimental technique applied under appropriate experimental design to resolve multi-variable equations.

2.9.3 Detail Steps of CCD

Central Composite Design is a response surface design which apart from the 3 level factors has axial or star points. The axial or star point usually denoted as (α) increases the number of levels to 5 levels thereby giving flexibility to the experimental design. It allows the experimental designer to know what effect the factors had on response as the experimental designer goes beyond or below the chosen levels of factors. In Central Composite Design, the minimum number of factors it can accommodate is two such as numerical or continuous factors. The number of experimental runs obtained at each number of factors is given by the formula;

$$N = 2^n + (2 \times n) + nc \quad \text{Eq. 2.38}$$

Where, N is the number of runs; n is the number of factors; nc is the number of center points the designer desires. In an experiment where we want to look at the effect of temperature and pressure on methanol yield based on 3 factors then;

$N = 2^3 + 2 \times 3 + n_c$ (which can be set between 2 to 6).

Therefore, $N = 8 + 6 + 6$ (if the number of center points 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 Alpha (the distance of each axial point or star point from the center of a central composite design);

$$\alpha = 2^{\frac{k}{4}} \quad \text{Eq. 2.39}$$

Where, k is the number of factors. In the example above where the value of k is two.

Therefore,

$$\alpha = 2^{\frac{k}{4}} = 2^{\frac{2}{4}} = 2^{\frac{1}{2}} = \sqrt{2} = 1.414 \quad \text{Eq. 2.40}$$

To get value for the axial point, we apply this equation:

Axial point = mean of both the upper or lower level $\pm \alpha$ (Range between the upper and lower level divided by 2)

Axial point = $X \pm \alpha$ (Range/2)

Where, X is the value of central point.

For the above example,

$$\begin{aligned} \text{Axial point} &= 75 \pm 1.414 ((90-60)/2) = 75 \pm 1.414 (30/2) \\ &= 75 \pm 1.414 (15) = 75 \pm 21.21 \end{aligned}$$

For the upper axial point $(+\alpha) = 75+21.21 = 96.21$

For the lower axial point $(-\alpha) = 75-21.21 = 53.79$

2.9.4 Benefit of Using CCD in Biogas Process Design

By observing the work of optimization techniques for methane production rate from POME, we can determine the benefit of using CCD in engineering design. Central composite design (CCD), D-optimal, and Box-Behnken are found to be widely used optimization techniques for methane production from POME, because of the advantage of optimizing multifactor problems with optimum number of experimental runs as shown in Figure 2.25 and Figure 2.26. However, these methods have limitation of increased number of experiments if several factors were selected for process optimization. For multiple factors, these techniques would not be appropriate due to increase in the cost of chemicals, time, and physical efforts required. It is noted that Box Behnken Design can handle only three level factors unlike CCD which can have up to five factors. Therefore, a simplified design strategy is required to overcome these problems.

↓	C1	C2	C3	C4	C5	C6	C7	C8
	StdOrder	RunOrder	PtType	Blocks	OLR	PH	HRT	CH4 (Total)
1	13	1	0	1	11	6.9	7	1488
2	5	2	2	1	2	6.9	4	1050
3	14	3	0	1	11	6.9	7	1500
4	2	4	2	1	20	6.0	7	1350
5	6	5	2	1	20	6.9	4	1300
6	10	6	2	1	11	7.8	4	1400
7	9	7	2	1	11	6.0	4	1380
8	3	8	2	1	2	7.8	7	1075
9	4	9	2	1	20	7.8	7	1320
10	8	10	2	1	20	6.9	10	1320
11	15	11	0	1	11	6.9	7	1488
12	7	12	2	1	2	6.9	10	1080
13	1	13	2	1	2	6.0	7	1000
14	11	14	2	1	11	6.0	10	1450
15	12	15	2	1	11	7.8	10	1460

Figure 2.25: CCD Output with Three Input Factors (OLR, pH, and HRT)

Box-Behnken Design

Design Summary

Factors:	3	Replicates:	1
Base runs:	15	Total runs:	15
Base blocks:	1	Total blocks:	1

Center points: 3

Design Table (randomized)

Run	Blk	A	B	C
1	1	0	0	0
2	1	-1	0	-1
3	1	0	0	0
4	1	1	-1	0
5	1	1	0	-1
6	1	0	1	-1
7	1	0	-1	-1
8	1	-1	1	0
9	1	1	1	0
10	1	1	0	1
11	1	0	0	0
12	1	-1	0	1
13	1	-1	-1	0
14	1	0	-1	1
15	1	0	1	1

Figure 2.26: Box-Behnken Design for CH₄ versus OLR, pH, and HRT

While Box Behnken does not have embedded factorial design, CCD is more useful for sequential experimentation by retaining information from correctly planned factorial experiments done previously. Although CCD suggested more experiments than other methods, it could give a more appropriate result with better precision. For example, CCD suggests up to 20 experiments if three factors are required to be studied. At optimized conditions, methane production would achieve 79 volumetric units per day with CCD. Interactive study of operating parameters by 3D contour plots produced by CCD also exhibits pH, VSS, and CH₄ as the most significant factors (variables). However, quantification of contribution is not possible with CCD.

In any case, it can be concluded that CCD is a robust statistical tool for experimental design and process optimization. Data analysis and optimization of operating parameters are possible with fewest numbers of experiments, less computational experience, and graphs obtained are easy to read and understand. The optimized values obtained for CCD was in good agreement to be used in engineering applications, making it very essential for Engineering Design.

2.9.5 Response Surface Methodology on Biogas Production Optimization

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for developing, optimizing, and improving products and industrial processes. It has become the most popular multivariate statistical technique used in the optimization of food processes (Baş & Boyacı, 2007). It is highly useful when many factors affect production, yield, or percentage removal of a process. RSM initiated from the design of experiment (DOE) is used to determine the significant factors affecting an experiment. It aims at reducing the number of experimental runs while maximizing output through the data generated. The data can be used to develop practical models that correlate the response to the experimental factors. The model facilitates the search for a better process response that is validated through experiments. The model iterates till an optimal process is identified or the limit of experimental resources is reached (Danmaliki et al., 2017). RSM is 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 is useful for optimizing, designing, developing, and improving processes where a response or responses are affected by several variables (Ghorbannezhad et al., 2016; Kaushik et al., 2006). The objectives of response surface methodology include

the determination of variable settings for which the mean response is optimized and the estimation of the response surface near this good location.

2.9.6 The Use of Design of Experiment on Biogas Production Optimization

Based on literature review, Design of Experiments (DoE) is most suited to be used for the input (OLR, pH, C/N, HRT, SRT) and output (CH₄) design research. Understanding on variables and experimental ranges are the key enabler in assuring input (OLR, pH, C/N, HRT, SRT) result in the final (CH₄) design. Knowledge is achieved by establishing models for 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 5-level designs could be used for our analysis, as follows:

- Selection of process variables (factors OLR, pH, C/N, HRT, SRT) and response (CH₄): 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, α) factor: this result can justify the range between each level.
- Selection of an experimental design (RSM, CCD): 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 the data of methane (biogas) with the experimental design: DoE 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 based on the established theories which 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.
- Checking the correlation and intercept in between input variables (OLR, pH, C/N, HRT, and SRT).
- Reporting with graph collection based on run, predicted and actual values.
- Getting the 3D graphs with optimum values for specific variables (OLR, pH, C/N, HRT, and SRT) for highest output methane (biogas).
- Visualize the central composite design (CCD) for optimum level of methane (biogas) production.

2.10 Findings of Literature Review and Gap Analysis

This section was built to justify the current research on ‘Optimization of Biogas Production from POME mixed with Indigenous Inoculum using Two-Stage Anaerobic Reactor Operating at Mesophilic Temperature, Coupled with Nanomembrane Separator’. Extensive literature review had been conducted to study the performance of anaerobic digestion of POME treatment in terms of biogas production and reduction of COD and BOD from POME. About 18 papers published between year 2011 to 2017 were reviewed to get the latest findings on this issue. The gap between previous studies and this research would be reported to identify the scope of study and to establish its novelty. The main operational conditions considered in biogas production are OLR, HRT, SRT and C/N. The references of performance of anaerobic digestion and major findings would be summarized in Table 2.7.

Despite the known advantages of using more advanced anaerobic reactors against the convectional ponding system, industrial players are still reluctant to implement the route to energy recovery from POME considering the financial and technical constraints (Qamaruz-Zaman et al., 2016). With few researches done on the recovery of usable resources from POME by using fermentation and an anaerobic reactor, the findings listed in Table 2.7 also reveal that research on production optimization is nearly nil and factor of optimization is also not published extensively. In view of this, the research gap is on the optimization of methane (biogas) production from POME by using fermentation and an anaerobic reactor. However, this study is undertaken to fill up the gap. The findings of literature review, publications and research gap analysis are the rationale of undertaking the current research on ‘Optimization of Biogas Production from POME mixed with Indigenous Inoculum using Two-Stage Anaerobic Reactor Operating at Mesophilic Temperature, Coupled with

Nanomembrane Separator'. The use of CSTR reactors is preferred due to its simple design and cheaper construction with better controllability of operation and ease of maintenance (Ahmed et al., 2015). Except for mixing (stirring) and recycling of substrate by pumps, the energy requirement for operation of this treatment system is relatively low when operated at mesophilic temperature, thereby reduces operation cost.

The use of fruit waste in the form of banana peel as inoculum for co-digestion with POME would give added novelty to this research by improving the performance efficiency of two-stage anaerobic reactor to convert COD and VSS in POME to biogas for capture. In the past, few attempts had been made to produce biomethane directly from indigenous fruit wastes such as banana and pineapple (Bardiya et al., 1996; Clarke et al., 2008; Divyabharathi et al., 2017; Gunaseelan, 2004). If left to degrade in the open, fruit waste such as banana peel with high carbon (McDowell & Clark-McDowell, 2016) and mineral contents would contribute substantially to biogas emission (Ilori et al., 2007; Nathoa et al., 2014; Pisutpaisal et al., 2014) which is detrimental to the environment. In line with the waste-to-energy (WtE) concept, it would be beneficial to collect and utilise such plentiful fruit wastes for production of green energy in the form of biogas, instead of being regarded as waste materials.

Table 2.7: Performances of Anaerobic Digestion for POME Treatment
(a) Relation between HRT and Biogas Production

Anaerobic digester	Operating conditions	Biogas production	COD removal (%)	References	Relation between HRT and biogas production
UASB-HCPB	HRT = 2 d at 55°C OLR = 27.65 g-COD/L/d	36.76 L/d	91.8	Poh & Chong (2014)	At HRT 2 days, methane content at 57.4%
Modified CSTR	HRT = 3.3 d at 55°C OLR = 19 g-COD/L/d	6.2 L/d	82.0	Khemkhao et al. (2015)	At HRT 3.3 days, methane content at 68%
EGSB	HRT = 9.8 d at 35°C OLR = 6.45 kg-COD/m ³ /d	27.65 m ³ /m ³ -POME	94.89	Wang et al. (2015)	At HRT 9.8 days, methane content at 65–70%
SCAR	HRT = 12 d at 35°C OLR = 6.91 g-COD/L/d	1.097 L/d	66.09	Wong et al. (2014)	At HRT 12 days, methane content at 48.5%
AHR-ABF	Total HRT = 6.2 d, at 37°C OLR = 15 kg-COD/L/d	13.5 L/d	90.0-93.0	Jeong et al. (2014)	HRT 6.2 days, methane content at 68% (approx.)
CSTR	HRT = 4 d, At 55°C and 100 rpm	58.87±6.27 L/kg-VS	81	Trisakti et al. (2017)	HRT of 4 days and methane content of 76.64%

Table 2.7(a) continued

UASB-CSTR	1 st stage: HRT = 9.0 h, at 55°C OLR = 75 g-COD/L/d	2.1 L/d	40.0	Krishnan et al. (2016)	HRT of 12-9 hours and methane content of 5%
	2 nd stage: HRT = 12 d, at 55°C OLR = 12 g-COD/L/d	13.0 L/d	85.0		HRT of 12 days and methane content of 68%
ASBR-UASB	1 st stage: HRT = 2.0 d, at 55°C OLR = 60 g-COD/L/d	1.84 L/d	38.0	Mamimin et al. (2015)	HRT of 2 days and methane content is unavailable
	2 nd stage: HRT = 15 d, at 37°C OLR = 6 g-COD/L/d	2.4 L/d	95.0		HRT of 15 days and methane content of 51%
AHR followed by ABF and ADF	HRT = 0.7–2.4 d, at 35–37°C OLR = 19–23 g-COD/L/d	2.8 L/d	93.5	Choi et al. (2013)	HRT of 0.7–2.4 days and methane content of 65%.
UASB	HRT = 4 d, at 35–38°C OLR = 12.5 g-COD/L/d	3.2 L/d	82	Ahmad et al. (2011)	HRT of 4 days and methane content of 48%
EGSB	HRT = 5 d, at 55–57°C OLR = 9.7 g-COD/L/d	4.2 L/d	92	Fang et al. (2011)	HRT of 5 days and methane content of 60%
EGSB	HRT = 5 d, at 55–57°C OLR = 19.4 g-COD/L/d	1.98 L/d	92	Fang et al. (2011)	HRT of 5 days and methane content of 73%

Table 2.7(a) continued

UASB	HRT = 5 d, at 55–57°C OLR = 9.7 g-COD/L/d	4.2 L/d	93	Fang et al. (2011)	HRT of 5 days and methane content of 61%
UASB	HRT = 2.4 d, at 55–57°C OLR = 9.5 g-COD/L/d	3.0 L/d	81	Khemkhao et al. (2012)	HRT of 2.4 days and methane content of 76%
UASB	HRT = 24.5 d, at 35°C OLR = 46 kg-COD/m ³ /d	2.5 L/d	97	Ahmad & Ghufraan (2014)	HRT of 24.5 days and methane content of 91%
CSTR	HRT = 8 d, at 55°C	9.8 L/d	77	Irvan et al. (2012)	HRT of 8 days and methane content of 64%
MAS	HRT = 5.7 d OLR = 13 kg-COD/m ³ /d	395 L/d	94.8	Abdurahman et al. (2011)	HRT of 5.7 days and methane content of 68.7%
CABR	HRT = 4 d, at 35°C OLR = 11.38 g-COD/L/d	2.3 L-CH ₄ /L.d	82	Malakahmad & Yee (2014)	HRT of 4 days, methane content a 54–75%
SCSTR	HRT = 6 d, at 37°C OLR = 4.06 g-COD/L/d	-	85	Alrawi et al. (2011)	HRT of 20-6 days and methane content of 61.8%
UMAS	HRT = 8.6 d OLR = 15 kg-COD/m ³ /d	540 L/d	92.8	Nour & Nour (2017)	HRT of 8.6 days and methane content of 64.6%

Table 2.7: Performances of Anaerobic Digestion for POME Treatment
(b) Relation between SRT and Biogas Production from POME

Technologies/System	Description	Performance	SRT (days)	References
Membrane Anaerobic System	The system consists of cross flow ultra-filtration membrane (CUF) and an anaerobic reactor. The membrane is used to separate biomass solids from the solid suspension in order to be recycled.	COD removal 94.8%; Methane yield 0.58 L/g-COD/d	800–11.6	Abdurahman et al. (2011)
Continuous Stirred Tank Reactor	A complete mixing system in which digested slurry is settled in an external clarifier and microbe-rich sludge is recycled back into digester.	Biogas production 7–9 L/d; VS decomposition 84%; COD removal 85%	8–22	Irvan et al. (2017)
Membrane Bioreactor	Combination of membrane separation and activated sludge process. The flat sheet membranes are arranged parallel with defined spaces between every single plate.	Effluent COD below 30 mg/L; Supernatant COD 5.0 mg/L; COD removal more than 90%	30	Chen et al. (2012)
Semi-Continuous Laboratory-Scale Stirred Tank Reactor	Mesophilic co-digestion of sewage sludge and vegetable waste (sugar beet pellets).	Biogas productivity at average 0.68 m ³ -CH ₄ /m ³ /d; COD removal 84.23%	10	Montañés et al. (2013)
Bench-scale Membrane Bioreactor	Hollow fibre membrane module was submerged in the central compartment of a 40L tank. Synthetic wastewater was used in this study.	COD removal 95%; BOD removal 93%; TSS removal 99%	30	Mohd Idris et al. (2014)

Table 2.7: Performances of Anaerobic Digestion for POME Treatment
(c) Relation between Carbon-to-Nitrogen (C/N) Ratio and Biogas Production

Digestion System	Description	Performance	C/N	References
Digesters closed with rubber septa and screw cap	POME sample was fixed at 100 mL while amount of EFB was adjusted to obtain the selected C/N. Co-digestion was conducted at facultative conditions.	Methane production and methane yield 2.03 L and 0.5932 mL-CH ₄ /g-VS respectively at C/N of 45. BOD 97% and COD 58%, the highest from control sample.	25–55	Nurliyana et al. (2015)
Intermittent-continuous stirred tank reactor	EFB was added to POME at various EFB to POME ratios on COD basis	EFB:POME ratio of 0.25–0.31 produced 50.8 mL-CH ₄ /g-VSS.d methane production.	57–59	Kim et al. (2013)
Flask with thermometer and gas delivery glass tubing fitted in holed rubber corks.	Cow excreta was placed inside 250 mL flat bottom conical flask at temperature range of 33–42°C. Biogas production was measure.	Cow excreta produced the highest biogas yields of 0.70 m ³ /kg of volatile solids compared to other organic wastes.	24	Dioha et al. (2013)
Glass fermenter apparatus with one digester vessel and one water displacement gas collection reservoir	Co-digestion of natural water, cow dung, rice straw, and water hyacinth at various ratio for efficient biogas production.	Ratio of 2:1:1:1 produced maximum methane concentration of 61.47% and highest amount of biogas at 1650 mL/d.	31	Keanoi et al. (2013)

2.11 Summary of Literature Review

This study has attempted to review the literature on the operational conditions affecting the biogas production optimization in order to achieve research goal. Basically, HRT relates to capital investment; higher HRT translates into high cost for construction of tank to hold POME. In this regard, optimization of inputs such as HRT means higher productivity at lower capital investment, while optimizing the biogas output. Major areas considered in the literature review framework are to reveal the effect of pH, HRT, SRT and C/N on biogas production at optimum operating conditions. Emphasis is given to conduct this study by using two-stage anaerobic reactor, coupled with nanomembrane separator. The main objective of this research is the optimization of biogas production from POME mixed with indigenous inoculum under anaerobic digestion at mesophilic temperature.

It can be stipulated that with the basis of the literature review, the research gap is identified. The gap analysis presented in Section 2.10 postulates that this is an important research that has not been conducted before and this research is essential for acquiring new knowledge that could contribute to achieve higher productivity for those mills as well as to enhance environmental sustainability in the palm oil industry.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Overview of Research Methodology

This chapter describes the development of research methodology for achieving the research objectives. This is an applied research in engineering field that focuses on optimizing the biogas production from palm oil mill effluent (POME). The study on POME process includes anaerobic digestion, anaerobic biodegradation with inoculum, and factors affecting biogas production in anaerobic reactor, coupled with nanomembrane. It started with overview of research methodology in Section 3.1. materials and methods are explicated in Section 3.2 which comprised research variables in Subsection 3.2.1 and experimental runs with specific order numbers in Subsection 3.2.2, Data collection methodology in Subsection 3.3.3 and experimental set-up in Subsection 3.2.4. Sample collection procedure of POME for conducting those experiments is elucidated in Section 3.3, whereas feedstock preparation is covered in Section 3.4. Model validation methodology is elucidated in Section 3.5, followed by the hypothesis tests in Section 3.6. The chapter is concluded with a summary that is presented in Section 3.7.

As mentioned earlier, the research goal of this study was to optimize biogas production from POME by using anaerobic reactor, coupled with nanomembrane separator. To achieve this goal, this study was divided into three specific objectives which are reflected in Figure 3.1.

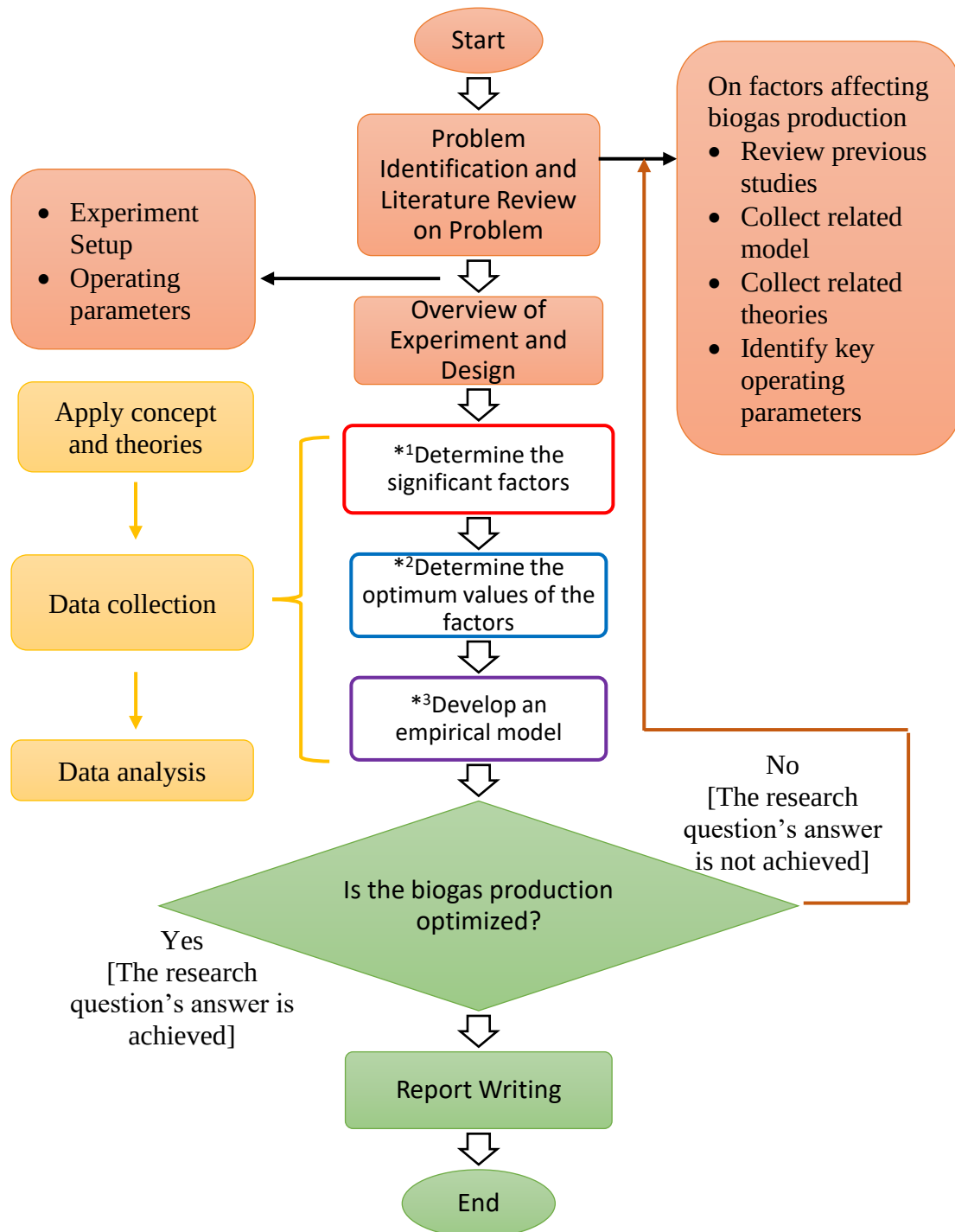


Figure 3.1: Research Methodology

*¹ Determine the significant factors that affect biogas production.

*² Determine the optimum values of the factors affecting biogas production.

*³ Develop an empirical model to predict the optimum value of biogas production.

Figure 3.1 illustrates the steps involved in the current research:

- Step 1 is the statement on problem mentioned in Section 1.2. To define problem, literature is reviewed on factors affecting biogas production from POME.
- Research methodology step 2 is done to achieve specific objective 1 which is to determine the significant factors affecting biogas production from POME. The concepts of anaerobic digestion and theories on biogas production are gathered from published scientific journals and are applied to conduct the experiment. Data are collected for analysis using Minitab Version 18.1 and Design Expert Version 2018 software to achieve research goal.
- Research methodology step 3 is taken to achieve specific objective 2 which is to determine the optimum values of factors that contribute to biogas production from POME. The concepts of anaerobic digestion and theories on biogas production are gathered from published scientific journals and are applied to conduct the experiment. Data are collected for analysis using Minitab Version 18.1 and Design Expert Version 2018 software to achieve research goal.
- Research methodology step 4 is employed to achieve specific objective 3 which is to develop an empirical model to predict optimum level of biogas production from POME. The concepts of anaerobic digestion and theories on biogas production are gathered from published scientific journals and are applied to conduct the experiment. Data are collected for analysis using Minitab Version 18.1 and Design Expert Version 2018 software to achieve research goal.

3.2 Materials & Methods

3.2.1 Research Variables

In this study, there were two variables which were dependent variable and independent variable. Dependent variable was the output of this research which is the biogas production. The independent variable was the input of this research which consists of pH, C/N, OLR, HRT, and SRT, as selected based on our literature review and known to be have significant effects on anaerobic digestion of POME.

This study was designed to identify input factors, which were significant at 95% confidence level ($p < 0.05$) in producing biogas from POME. The inputs factors with limits were obtained from literature reviews discussed in Section 2.7 to Section 2.12. With due considerations on both financial and time constraints in our research, the five factors selected were pH, C/N, OLR, HRT, and SRT. The inputs (pH, C/N, OLR, HRT, and SRT) were transferred into Minitab Version 18.1 and Design Expert Version 2018 software with Central Composite Design (CCD) option to obtain 5 level inputs required to achieve optimal biogas production from the anaerobic digestion process at a minimum resource. The outputs of the analysis on the experimental range and levels of the independent variables are shown in Table 3.1. The CCD outputs are attached in the Appendix C2.

Table 3.1: Experimental Range and Levels of Independent Variables

Range and Level					
Variables	$-\alpha$ (2.3784)	Low (-1)	Central (0)	High (1)	$+\alpha$ (2.3784)
X ₁ (OLR)	0.2431	3	5	7	9.7568
X ₂ (pH)	5.1729	6	6.6	7.2	8.027
X ₃ (C/N)	19.7971	26	30.5	35	41.2029
X ₄ (HRT)	0.5539	4	6.5	9	12.446
X ₅ (SRT)	5.1755	10	13.5	17	21.8244

From Table 3.1, the range of organic loading rate to digestion process marked by X_1 (OLR) has five values such as 0.2431, 3, 5, 7, and 9.7568. These values were used to conduct experiments with OLR. This range of OLR had also been used by Jeong et al. (2014), Khemkhao et al. (2015), and Shahidul et al. (2018) in the POME digestion process.

The range of pH in the digestion process, denoted by X_2 (pH) has five values of 5.1729, 6, 6.6, 7.2, and 8.02. These values of input would be used to conduct the experiments with pH. This range of pH was used by Bolzonella (2011) and Sánchez et al. (2005) for the POME digestion process in anaerobic condition.

The range of carbon to nitrogen ratio denoted by X_3 (C/N) has five values of 19.7971, 26, 30.5, 35, and 41.2029. These values of input would be used to conduct the experiments with C/N. This range of C/N had been used by Shahidul et al. (2018) and Shanmugam and Horan (2009) for the POME digestion process in anaerobic condition.

The range of hydraulic retention time denoted by X_4 (HRT) has five values of 0.5539, 4, 6.5, 9, and 12.446, which indicates that the experiment is to be conducted with five different HRT. This range of HRT has been used by Malakahmad and Yee (2014) and Shahidul et al. (2018) for the POME digestion process in anaerobic condition.

The range of sludge retention time denoted by X_5 (SRT) has five values of 5.1755, 10, 13.5, 17, and 21.8244. These values of input would be used to conduct the experiments with SRT. This range of SRT had been used by Lau et al. (2009) and Mohd Idris et al. (2014) for the POME digestion process in anaerobic condition.

3.2.2 Experimental Runs and Order for Biogas Production

The optimal number and order of 50 experimental runs with a combination of five independent variables comprising 32 factorial points, 10 axial points and 8 replicates at centre points were produced from DOE using Design Expert software, whereas the total outputs from the software with six different variables (five independents plus one dependent variable) are presented in Appendix C3. A full factorial design was employed for resolving the main effects of inputs with minimal number of experimental runs. The order of experimental runs was randomised to diminish the error and effects of uncontrolled factors on anaerobic digestion of POME. It was necessary to replicate the experiment at centre point with 8 replicates, in order to provide independent estimate of the experimental error (Box & Hunter, 1957). The outputs from software are presented in Figure 3.2(a) and Figure 3.2(b).

Std	Run	Factor 1 A:OLR VSSg/L.d	Factor 2 B:pH	Factor 3 C:C/N	Factor 4 D:HRT d	Factor 5 E:SRT d
2	1	7	6	26	4	10
44	2	5	6.6	30.5	6.5	13.5
7	3	3	7.2	35	4	10
18	4	7	6	26	4	17
36	5	5	8.02705	30.5	6.5	13.5
35	6	5	5.17295	30.5	6.5	13.5
15	7	3	7.2	35	9	10
46	8	5	6.6	30.5	6.5	13.5
41	9	5	6.6	30.5	6.5	5.17555
47	10	5	6.6	30.5	6.5	13.5
19	11	3	7.2	26	4	17
22	12	7	6	35	4	17
38	13	5	6.6	41.2029	6.5	13.5
32	14	7	7.2	35	9	17
1	15	3	6	26	4	10
25	16	3	6	26	9	17
39	17	5	6.6	30.5	0.553964	13.5
5	18	3	6	35	4	10
26	19	7	6	26	9	17
42	20	5	6.6	30.5	6.5	21.8244
17	21	3	6	26	4	17
3	22	3	7.2	26	4	10
23	23	3	7.2	35	4	17
31	24	3	7.2	35	9	17
43	25	5	6.6	30.5	6.5	13.5
4	26	7	7.2	26	4	10
37	27	5	6.6	19.7971	6.5	13.5
45	28	5	6.6	30.5	6.5	13.5

Figure 3.2(a): Experimental Runs of CCD

Std	Run	Factor 1 A:OLR VSSg/L.d	Factor 2 B:pH	Factor 3 C:C/N	Factor 4 D:HRT d	Factor 5 E:SRT d
23	23	5	7.2	35	4	17
31	24	3	7.2	35	9	17
43	25	5	6.6	30.5	6.5	13.5
4	26	7	7.2	26	4	10
37	27	5	6.6	19.7971	6.5	13.5
45	28	5	6.6	30.5	6.5	13.5
33	29	0.243172	6.6	30.5	6.5	13.5
50	30	5	6.6	30.5	6.5	13.5
11	31	3	7.2	26	9	10
34	32	9.75683	6.6	30.5	6.5	13.5
24	33	7	7.2	35	4	17
30	34	7	6	35	9	17
21	35	3	6	35	4	17
49	36	5	6.6	30.5	6.5	13.5
27	37	3	7.2	26	9	17
29	38	3	6	35	9	17
13	39	3	6	35	9	10
16	40	7	7.2	35	9	10
40	41	5	6.6	30.5	12.446	13.5
28	42	7	7.2	26	9	17
20	43	7	7.2	26	4	17
12	44	7	7.2	26	9	10
48	45	5	6.6	30.5	6.5	13.5
14	46	7	6	35	9	10
9	47	3	6	26	9	10
10	48	7	6	26	9	10
8	49	7	7.2	35	4	10
6	50	7	6	35	4	10

Figure 3.2(b): Experimental Runs of CCD

In order to address specific objective one, ANOVA test were performed using Minitab 18.1 software to establish the statistical significance of factors in biogas production from POME at 95% confidence level. P-value of less than 0.05 would categorise them as the significant input factors to biogas production. The predicted output (biogas production) from the reactors would be analysed by the Design Expert software to check on distribution (scatter) characteristics of data involved.

To achieve the specific objective two, the Response Surface Methodology was used to determine the optimal value of each variables for optimising the biogas output from 3D plots and contour optimization diagram generated by the statistical ‘Design Expert 2018’ software.

The results from the first specific objective were used to setup experiments for achieving the second specific objective. The Minitab Version 18.1 and Design Expert Version 2018 software were used to determine the optimum values of these factors. Response surface methodology (RSM) was used to evaluate the value of response for every possible outcomes of the experiment related to the input factors and output. The outcomes of these experiments were presented by graphical representation in 3D plots. Consequently, the optimization feature in the software would be able to generate the optimum operating parameters (inputs) and output from the digestion process of POME.

As with sequential experiments, results from the first two specific objectives could be used to undertake the development of mathematical model for specific objective three. Coefficient estimates of those significant factors (independent variables) from regression analysis from DOE software would be used to form the model terms in the prediction model. Lastly, model validation would be done to compare actual and predicted biogas production from POME and to check its adequacy for that purpose.

3.2.3 Data Collection Methodology

This study was performed in a batch process. The capacity of each reactor was 5 L with working volume of 3 L which contained substrate and 10% (v/v) inoculum. The substrate and inoculum were mixed in the feedstock as per the software outputs. The operating

temperature were maintained at mesophilic condition of 32–35°C as conducted by Choi et al. (2013). A study conducted by Jeong et al. (2014) showed that the anaerobic reactors performed similarly in both mesophilic and thermophilic condition and the organic content was highly biodegradable in mesophilic condition. The pH of the system was maintained at 5–6 for the first stage to enable acidogens to grow but not methanogens (Ozgur & Peksel, 2013). The pH range of 7–8 was maintained in the second stage to maximize the effects on biogas production as methanogens are sensitive to lower pH (Abdurahman et al., 2011). The batch fermentation process in the first stage was carried out up to 12 days based on the different settings of input combinations, before the substrate could be transferred to the second tank reactor for methanogenesis. As the volume of substrate involved was small (3 L), adequate mixing of substrate and microbes was achieved by shaking the tank reactor at periodic intervals throughout the experiment. The experiments were carried out in triplicate and the results are expressed in means, taking average value of outputs from three runs at each setting of input variables.

As with sequential experiments, the data from the first and second specific objective were used to achieve the third specific objective. A multiple linear regression model with all the inputs would be developed using Design Expert software and experimental data of biogas production. A model validation was performed using Design Expert and data analysis would be presented in a graph.

3.2.4 Experimental Setup

In the first part of data collection, five sets of reactors used concurrently to reduce time taken to conduct the whole experiment as per Figure 3.3 where the feedstock could be prepared earlier to get the required C/N ratio of the resulting substrate by mixing POME with the

correct amount of inoculum. The indigenous inoculum was made from waste banana peel after being kept for 30 days before it could be added into the digestion process (Choi et al., 2013). The C/N in the substrate was adjusted by varying the inoculum dosing rate which ranged from 11.8 to 40.3. Figure 3.4 shows the schematic diagram of the experimental setup for data collection where the setting of the remaining factors such OLR, pH, HRT and SRT can be varied individually at the different reactors based on the input combinations listed in Figure 3.2 and Table 3.1. Biogas generated from each reactor can be collected independently by water displacement method as shown in Figure 3.3.



Figure 3.3: Experimental Set-up of 5L Reactor

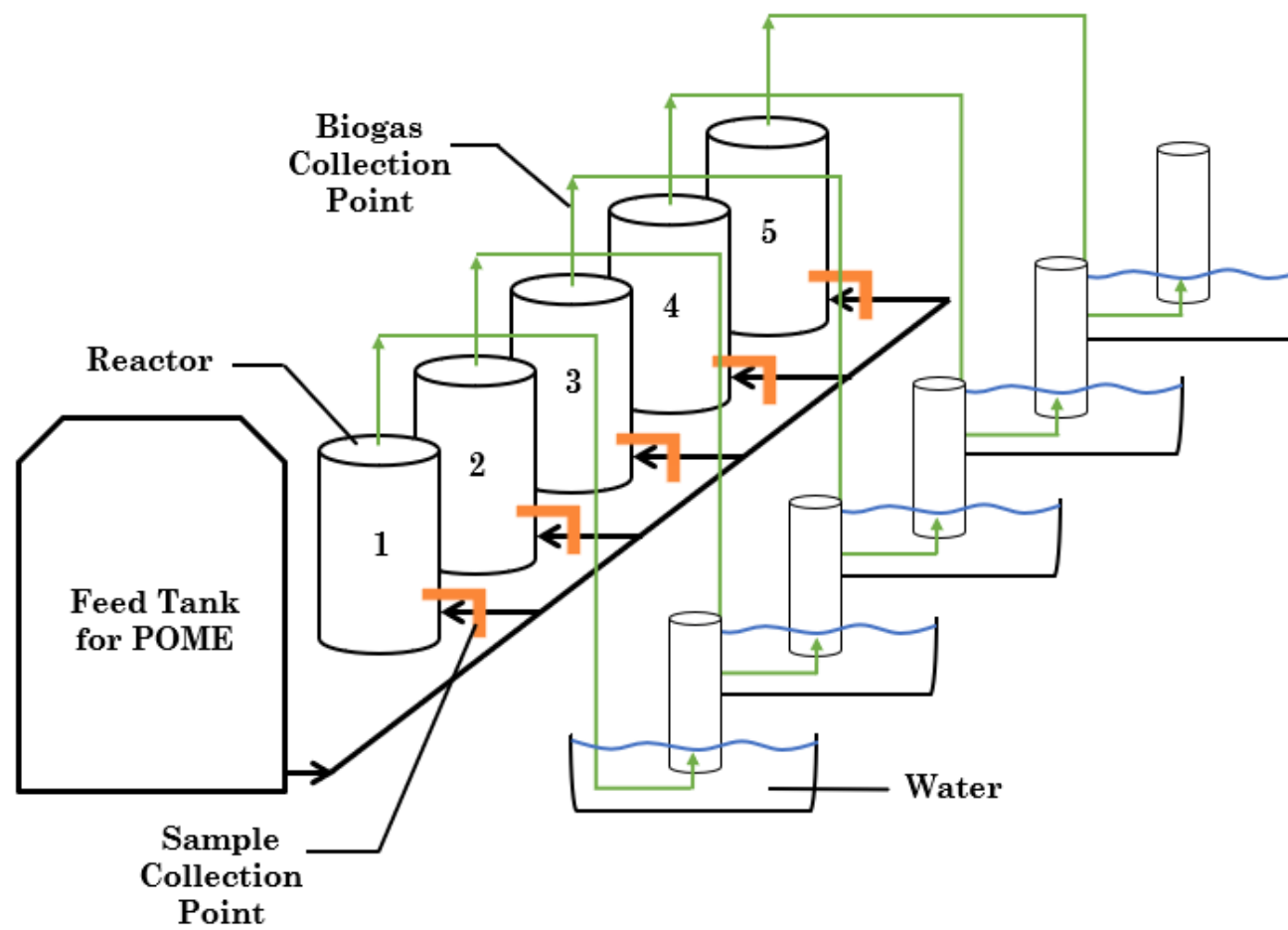


Figure 3.4: Schematic Diagram of Experiment Setup for Data Collection



Figure 3.5: Biogas Collection Method

3.3 Sample Collection Procedure of POME

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. After 3 days of fermentation in the feed tank with appropriate feedstock characterization inputs, that batch of POME was transferred into the reactor for methanogenesis as per design. On daily basis, this fermented POME in reactor would be recirculated slowly to mix the organic contents for better digestion.

3.4 Feedstock Preparation

The substrate is a mixture of POME and inoculum. Banana skin was used to prepare the inoculum. The mesh size of the skin was converted to less than 1.0 mm and was kept (microbial pre-treatment) for 30 days at atmospheric temperature before it was added into the feedstock. In order to maintain the C/N from 20 to 40, appropriate weight (dosage) of

inoculum would be mixed with POME for each set of samples for different experimental runs. Table 3.2 shows the characterization of the feedstock and method of preparation for inoculum is attached in Appendix C1.

Table 3.2: Characterization of Feedstock

Item	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	83	30

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

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

The high carbon content of banana peel (Divyabharathi et al., 2017; Nathoa et al., 2014) made it possible to achieve C/N ratio of the resulting substrate at optimal range of $20 < C/N < 35$ (Khalid et al., 2011) for efficient anaerobic digestion. Fittingly, Bardiya et al. (1996), Ilori et al. (2007), and Pisutpaisal et al. (2014) had attempted to produce biomethane to generate energy directly from banana peel with the natural microbial consortia available in such fruit waste.

3.5 Model Validation Methodology

As for data validation, 350 L reactors were used as shown in Figure 3.6 where the experiment was conducted at the optimal setting of inputs combination as generated by the software. Figure 3.7 displays the schematic diagram of the experimental setup for two-stage anaerobic reactors for data collection.

A similar setup as the first part of the experiment was used. The only difference was that the capacity of the reactor is 350 L with working volume of 200 L. The CSTR 1 was used for hydrolysis and acidogenesis which was the first stage. The CSTR 2 was meant for acetogenesis and methanogenesis which was the second stage.



Figure 3.6: Continuous Stirred-Tank Reactors

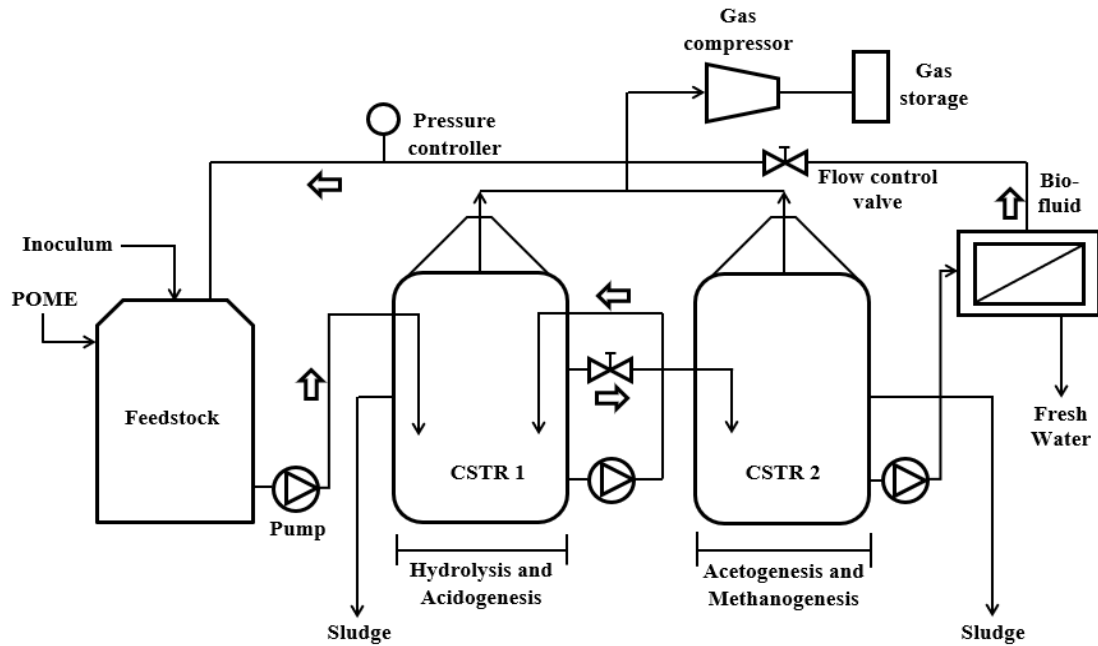


Figure 3.7: The Schematic Diagram of Experimental Setup for Model Validation

The first CSTR used for hydrolysis and acidogenesis was operated at pH of around 5.0 in order to break down long chain organic materials into short chains (Shahidul et al., 2018). Mamimin et al. (2015) and Kim et al. (2013) had also used this method for operating anaerobic reactor to break down long chain organic materials of POME into short chains. At this stage, the HRT was at least three days and CSTR was operated at 35°C. In the second CSTR, the acetogenesis and the methanogenesis processes were performed at pH from 6.5–7.5. The range of pH values of the substrate was adjusted by adding sodium hydroxide (NaOH), where measurements were taken using the pH meter in Appendix B2(g).

Pumps were installed at the outlet of CSTR 1 and 2 to allow recirculation to occur. Recirculation of fermented POME can improve the efficiency of digestion (Irvan et al., 2017). The effluent would later be fed to membrane system to separate undigested organic materials and water as shown in Figure 3.6. The concentrated organic material-enriched biofluid would be recycled back through CSTR 1 and CSTR 2. The biogas was collected

from both reactors and membrane which would be used to evaluate the effects of C/N, SRT, HRT, OLR and nano membrane on biogas production from POME.

3.6 Method of Hypothesis Test

Three hypotheses were established in relation to the specific objectives as discussed in Section 1.3 of Chapter 1. Based on the outcomes of the experiments, it could be concluded whether the null hypotheses would be accepted or rejected. The results of the hypothesis test would be discussed in Chapter 4.

3.7 Chapter Summary

This chapter presents the development of research methodology to achieve the research goal. The research goal is to optimize biogas production from POME. This research methodology was designed to achieve three specific objectives. A two-stage fermentation for biogas production was used to separate acidogenesis and methanogenesis which required different optimal conditions for efficient digestion of POME (Irvan et al., 2017). The first specific objective was to determine the significant factors affecting biogas production from POME. Five factors were selected from literature reviews, which are known to affect biogas production. These factors include pH, C/N, OLR, HRT, and SRT. The inputs (pH, C/N, OLR, HRT, and SRT) were transferred into Minitab Version 18.1 and Design Expert Version 2018 software to obtain 5 level inputs based on Central Composite Design (CCD). A total of 50 runs with a combination of six different variables were produced from DOE using Design Expert (five independent variables or inputs plus one dependent variable or output) as displayed in Appendix C3. ANOVA was performed to obtain the significant level of factors in producing biogas. The significance tests were conducted at 95% confidence level.

P-values of inputs less than 0.05 would categorise them as the significant factors that contributed to produce biogas from POME.

The second specific objective was to determine the optimum values of factors that contribute to biogas production from POME. The results from the first specific objective would be used to achieve the second specific objective. Using Minitab Version 18.1 and Design Expert Version 2018 software, the optimum values of these inputs were to be determined. Graphical representation in 3D plots would be presented. The third specific objective was to develop an empirical model to predict the optimum level of biogas production from POME. The data from the first and second specific objective would be used to achieve the third specific objective. A multiple linear regression model with all the inputs would be developed using Design Expert software and experimental data of biogas production. A model validation would be performed using Design Expert and data analysis would be presented in a graph. However, the outcomes from this chapter would be presented and used in Chapter 4 and Chapter 5 to derive the answers to the research questions.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Overview on Research Findings

This chapter depicts the results and research findings relating to (main part of title) objectives stated in Section 1.3. This was an applied research in engineering field that concentrates on optimizing biogas production from palm oil mill effluent through anaerobic digestion. With a closed digestion system, the biogas produced from hazardous palm oil mill effluent (POME) could be captured in order to contribute to achieving economic and environmental sustainability. Indeed, the factors that affected the biogas production from POME were organic loading rate (OLR), pH, carbon-to-nitrogen (C/N), hydraulic retention time (HRT), sludge retention time (SRT) and environment in digestion process (Shahidul et al., 2018); and accordingly, the independent variables were all of these input factors and the dependent variable was biogas production.

The aim of this research had been divided into three specific objectives. The research objective one was to determine the significant factors affecting biogas production from POME. The research objective two was to determine the optimum values of factors for maximizing biogas production from POME. The research objective three was to develop an empirical model for predicting the optimum level of biogas production from POME.

4.2 Anaerobic Bioreactor Start-up

Being an important part of this research for reaching research goal, the start-up process must ensure smooth operations of anaerobic bioreactor. Start-up process allowed microorganisms found in the POME to grow and proliferate in order to participate in digestion of the biogas

potential materials. Acclimatization was essential to allow microorganisms to adapt to the current environmental conditions, enabling them to maintain their performance. A steady state would be achieved when the performance of the bioreactor remained almost constant. The performance of the anaerobic bioreactor in POME digestion process and biogas production would be evaluated based on the reduction of chemical oxygen demand (COD) as well as volatile suspended solid (VSS) degradation.

4.3 Determination of Factors that have Significant Effect on Biogas Production

The scope of work of this part of study is related to research objective one stated in Section 1.4 (a). This section would answer the research question of “**What are the significant factors (inputs) to POME digestion process that make it economically feasible for biogas capture?**”.

A total of five batch reactors of 5-litter capacity each were used concurrently to conduct 50 experimental runs in triplicates, which would reduce the total time taken to complete the whole experiment. The inputs to digestion system, different combinations of independent variables are listed in Figure 3.2(a) and Figure 3.2(b), which comprised 32 factorial points, 10 axial points plus 8 replicates at centre point as displayed in Appendix C2. Replications of centre points in those experimental runs would give very good ‘power of prediction’ at the middle (centre) of our experimental region. The outputs (biogas) of 50 runs with respect to inputs of five variables were presented in Appendix C3.

The distribution of biogas production from reactors were analysed by Design Expert software, which is displayed in Figure 4.1(a) and Figure 4.1(b). It is important to check on the residual plots to ensure an unbiased estimate by the developed model.

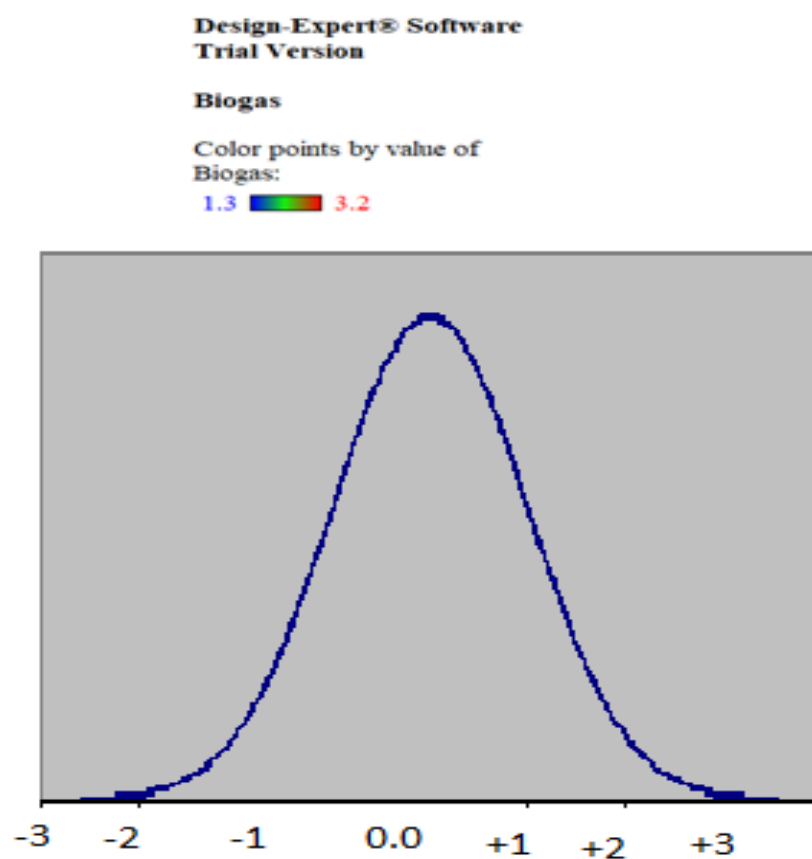


Figure 4.1(a): Data Distribution of Biogas Outputs from Batch-type Reactors

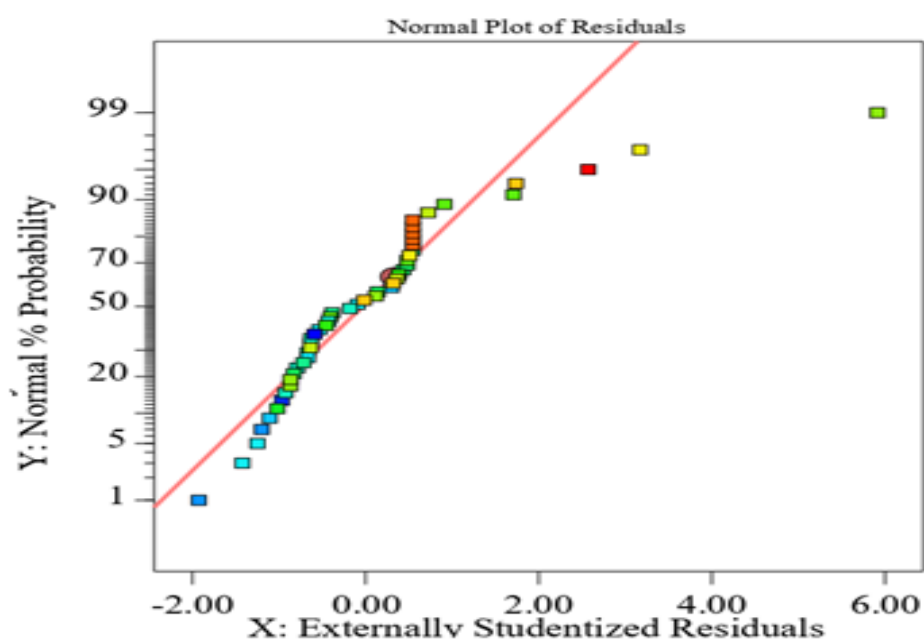


Figure 4.1(b): Data Distribution of Biogas Outputs from Batch-type Reactors

Figure 4.1(a) and Figure 4.1(b) demonstrate that the outputs of reactors are normally distributed, where the range of outputs is within (-2 standard deviation) to (+2 standard deviation), displaying a small dispersion. This information indicates that outputs of reactors are within the acceptable limit of 95% probability. The studentized residuals in Figure 4.1(b) 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 ($ESR > 3$).

4.3.1 Experimental Data Analysis and Findings on Objective One

Experimental data was analysed by Minitab Version 18.1 and Design Expert Version 2018 software. The Statistical parameters were estimated by using ANOVA from Minitab 18.1 whose outputs are presented in Figure 4.2 and summarised in Table 4.1.

Table 4.1: Factors that contribute significantly to biogas production from POME

Factors (Input Variables)	p-value of factors	Significance level of factors	The effect size as contribution and biogas production statistics
OLR	$p_{OLR} = 0.0197$	As $P_{OLR} < 0.05$; significant	$R^2 = 0.925$ Adjusted $R^2 = 0.3624$ Adequate precision = 5.5858 Coefficient of Variation (CV) = 16.67%
pH	$p_{pH} = 0.0495$	As $P_{pH} < 0.05$; significant	
C/N	$p_{C/N} = 0.7027$	As $P_{C/N} > 0.05$; Not significant	
HRT	$p_{HRT} = 0.0466$	As $P_{HRT} < 0.05$; significant	
SRT	$p_{SRT} = 0.0398$	As $P_{SRT} < 0.05$; significant	

4.3.1(a) The Output Model and Significance Level

Table 4.1 manifests that at point A, OLR, the p-value with respect to biogas production for OLR is 0.0197 ($p < 0.05$), which is significant at 95% confidence level.

This result indicates that OLR has a significant effect on biogas production from POME. The value of OLR would influence the quantity of biodegradable compounds which constitute COD and VSS contents that could be converted to biogas during anaerobic digestion process of POME. The output of DOE is listed in Appendix C4(a).

At point B, pH, the p-value with respect to biogas production for pH is 0.0495 ($p < 0.05$), which makes it significant at 95% confidence level. Hence pH has a significant effect on biogas production from POME by creating favourable environment for microorganisms to grow and proliferate, feeding on the available biodegradable compounds in POME.

At point C, C/N, the p-value with respect to biogas production for C/N is 0.7027 ($p > 0.05$). This finding demonstrates that C/N has effect but not significant to biogas production from POME at 95% confidence level. The right balance between the availability of elemental carbon and nitrogen is crucial to enable smooth digestion process of POME, as microorganisms would consume carbon about 30 times faster than nitrogen (Yadvika et al., 2004). It has been discovered that anaerobic digestion of POME would improve with addition of co-substrate or inoculum where its C/N ratio can be adjusted to the optimal value.

At point D, HRT, the p-value with respect to biogas production for HRT is 0.0466 ($p < 0.05$), which is significant at 95% confidence level. This finding shows that HRT has a significant effect on biogas production from POME, by providing ample to time for microbes to feed on the biodegradable compounds in the substrate. Hence, recycling of substrate daily is necessary to prolong the HRT.

At point E, SRT, the p-value with respect to biogas production for SRT is 0.0398 ($p < 0.05$), which is significant at 95% confidence level. It reflects that SRT has a significant effect on biogas production from POME, by giving adequate contacts between microorganisms and sludge to complete the digestion process. Obviously, longer SRT would allow the conversion of volatile solids (VS) to form biogas up to certain extent. As its population grows, these microorganisms begin to compete for the available nutrients, thereby producing antibiotics to prevent the growth of other microbes which could lead to death.

The summary of the correlation for biogas production with level of significance for each of those factors under study is listed in Figure 4.2, Appendix C4(a), and Appendix C4(b).

ANOVA for Quadratic model							
Response 1: Biogas							
	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	6.73	20	0.3364	2.39	0.0158	Significant
	A-OLR	0.8568	1	0.8568	6.09	0.0197	Significant
	B-pH	0.5910	1	0.5910	4.20	0.0495	Significant
	C-C/N	0.0209	1	0.0209	0.1486	0.7027	Insignificant
	D-HRT	0.6075	1	0.6075	4.32	0.0466	Significant
	E-SRT	0.6518	1	0.6518	4.64	0.0398	Significant
± Fit Statistics ± Model Comparison Statistics							
	Std. Dev.	0.3750			R²	0.925	
	Mean	2.25			Adjusted R²	0.3624	
	C.V. %	16.67			Predicted R²	-0.5964	

Figure 4.2: Biogas Outputs Model of Batch-type Reactors

The effect size R^2 is 0.925 which infers that the conversion of inputs to biogas production is about 92.5%, reflecting a good performance of the POME digestion process in the anaerobic reactor. The R^2 value also 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 $R = 0.96 > 0.5$, four of the input variables (OLR, pH, HRT, SRT) are found to be strongly positive (Rumsey, 2016) with significant impacts on output where their p-values ($p < 0.05$) at 95% confidence interval, as depicted in Table 4.1. Though the impact of C/N seems insignificant ($p > 0.05$) to biogas production (output), its value can be adjusted by adding co-substrate or inoculum that could provide adequate carbon as food for microbes and nitrogen for enzyme required for all microbial activities.

The Coefficient of Variation (CV) is 16.67%, expressed as the percentage ratio of standard deviation over mean, reflecting that performance of the POME digestion process in the anaerobic reactor is moderate and not high. 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 5.5858. 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.

4.3.2 Discussion on Answer to Research Question One

The results for all the experimental runs with the combinations of inputs are shown in Figure 3.2(a) and Figure 3.2(b) are presented in Appendix C2 and Appendix C3. Whereas the summary on significance level of input factors is presented in Table 4.1. Results presented

in Table 4.1 demonstrate that the OLR, pH, HRT and SRT input factors (independent variables) have significant ($p<0.05$) effect on biogas production from POME. However, C/N did contribute to biogas production but not significantly at 95% confidence level. Its insignificant effect on biogas production may be due to ineffectiveness of anaerobic digester operations.

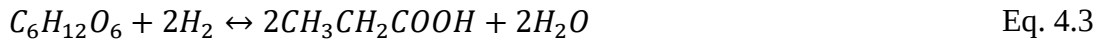
The science of significant ($p<0.05$) effect of inputs OLR, pH, HRT and SRT on biogas production from POME inferred that the 95% potentials of these inputs were used to convert COD and biomass of POME to biogas. It concurred that the effectiveness of four steps of POME digestion process in anaerobic reactors such as hydrolysis, acidogenesis, acetogenesis and methanogenesis displayed in Figure 2.5 of Section 2.5 were about 95% by the influence of input variables especially OLR, pH, HRT and SRT as follows:

At hydrolysis process, the water of POME reacted at a rate of 95% with long-chain organic polymers including polysaccharides, fats, and proteins to form soluble shorter-chain polymers, such as fatty acids, amino acids, and sugars (Begum et al., 2013; Papageorgiou et al., 2009); and input factors such as OLR, pH, HRT and SRT had played a vital role in this process. Succinctly, this chemical reaction can be expressed by Equation 4.1:

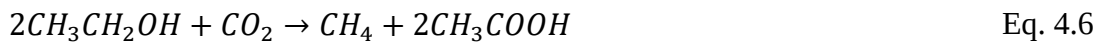


At the acidogenesis process, the outputs of hydrolysis process would be converted to volatile fatty acids (acetate, propionate, butyrate, lactate), alcohols as well as by-products of ammonia, hydrogen and carbon dioxide, with a conversion performance of 95%. Subsequently in acetogenesis, about 95% of those acids produced by acidogenesis would be converted to acetate, H_2 , and CO_2 , in which OLR, pH, HRT and SRT have played a vital role

in the process. These bio-chemical reactions involved the various steps of methanogenesis (Ahmad et al., 2003; Azri et al., 2017; Chin et al., 2013; Irvan et al., 2012; Sarwani et al., 2013), which can be expressed by the following Equation 4.2, Equation 4.3, and Equation 4.4:



At acetogenesis and methanogenesis stages, the biogas was produced by using 95% of the outputs of the acidogenesis process. The final reaction to produce biogas was achieved through two main pathways. After the fermentation of acetic acid, acetoclastic methanogens would use acetic acid to produce biogas and carbon dioxide (Donoso-Bravo et al., 2011; Taylor et al., 2014), as expressed in Equation 4.5 and Equation 4.6.



Another route involved the reduction of carbon dioxide to methane, using hydrogen or formic acid as sources of electrons with the presence of hydrogenotrophic methanogens, as expressed by Equation 4.7:



In these processes, factors such as OLR, pH, HRT and SRT were significantly effective in converting potentials of POME to biogas. In conclusion, it can be stipulated that these factors had significantly contributed to produce biogas from POME. Thus, research objective one

was achieved and the research question one was answered, where null hypothesis one is rejected (H_0 : Input variable has no effect on output).

4.3.3 Scenario Analysis on Significant Factors affecting Biogas Production from POME

The results of the experiment showed that OLR, pH, HRT and SRT produced significance levels of $p < 0.05$ which were significant to biogas production from POME.

The OLR is a measure of conversion capacity of a biological system. Increasing OLR in an anaerobic system can lead to decrease in 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 which could overwhelm the methanogens. This is due to the increase of microbial activities during hydrolysis and acidogenesis, resulting in the rise of volatile fatty acid (VFA) production. The accumulation of acid will cause the pH inside the reactor to drop. As a result, the efficiency of methanogenic archaea to convert high amount of VFA to methane will definitely be reduced (Mao et al., 2015).

Potential of hydrogen (pH) is an essential chemical factor in the solubility and dispersion of biological organic contaminants which control the performance of the anaerobic digestion process. It was noted that 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). A study 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). Due to different optimal pH values in both processes, a two-stage anaerobic digestion is a preferred method to separate the hydrolysis and acidification from acetogenesis and methanogenesis processes (Jiunn-Jyi et al., 1997; Kim et al., 2003; Van Kessel & Russell, 1996; Yossan et al., 2012).

C/N is an important manipulating variable in optimizing the digestion performance of POME (Okonkwo et al., 2018). It was reported that higher carbon content would contribute to increasing carbon dioxide formation and lower pH, while the high value of nitrogen would enhance the production of ammonia gas that could increase pH (Dioha et al., 2013). Increasing the C/N can be done with co-digestion by introducing a co-substrate into the system. The co-digestion process offers the anaerobic digestion system some benefits (Esposito et al., 2012). Apart from reducing the concentration of harmful compounds found in the system, co-digestion also increases microbial population variation associated with the biochemical reaction process. El-Mashad & Zhang (2010) reported that the increase in COD reduction efficiency in co-digestion is because methanogens are effectively acclimatized in the anaerobic system.

4.4 Optimization of Factors Affecting Biogas Production

The scope of work of this part of study is related to research objective two stated in Section 1.4 (b). This section would answer the research question of “**What are the optimum levels of inputs to POME digestion process that affect biogas production?**”.

The investigation under this section of research was to determine optimum level of factors of experimental runs in the experimental setup shown in Figure 3.5 that contributed to

optimize biogas production from POME. To achieve this goal, data were analysed by Minitab Version 18.1 and Design Expert Version 2018. The findings of data analysis with outputs (biogas) is presented in Appendix C3. Results listed in Table 4.1 demonstrated that the OLR, pH, C/N, HRT and SRT inputs had contributed to biogas production from POME. The experiment range and levels of the independent variables are shown in Table 3.2 in Section 3.2 earlier.

4.4.1 Data Analysis relating to Research Objective Two

The Statistical Design Expert 2018 software had been used to study the regression analysis of experimental data and to generate the response surface diagrams in Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6.

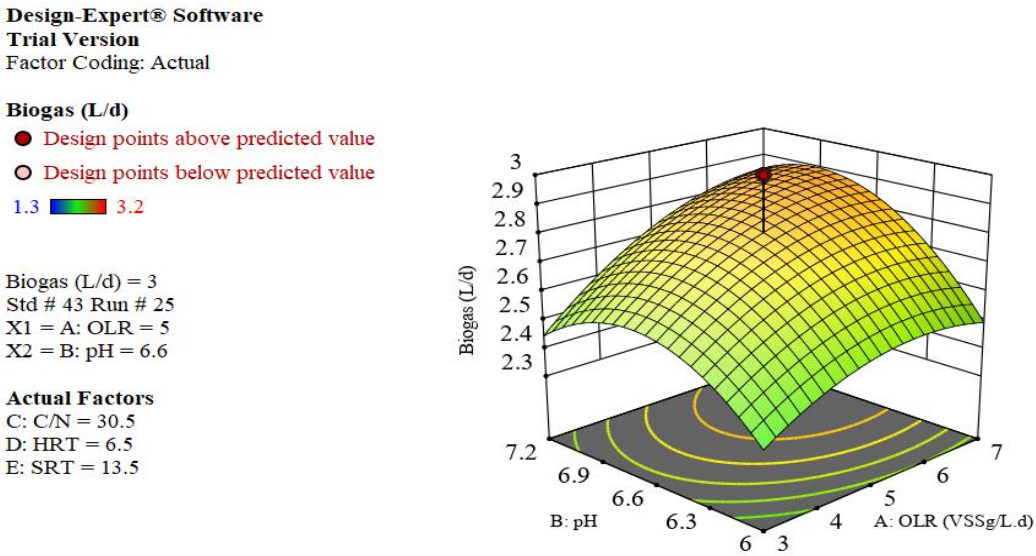


Figure 4.3: Surface Response Optimization of OLR, pH, and Biogas Production

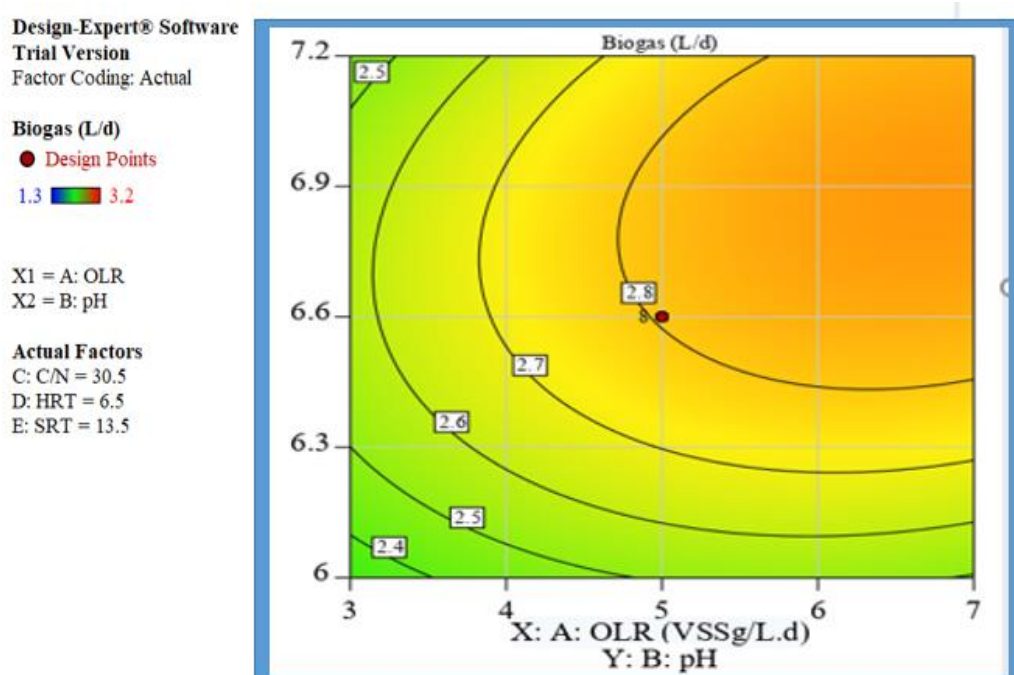


Figure 4.4: Contour Optimization on OLR, pH, and Biogas Production

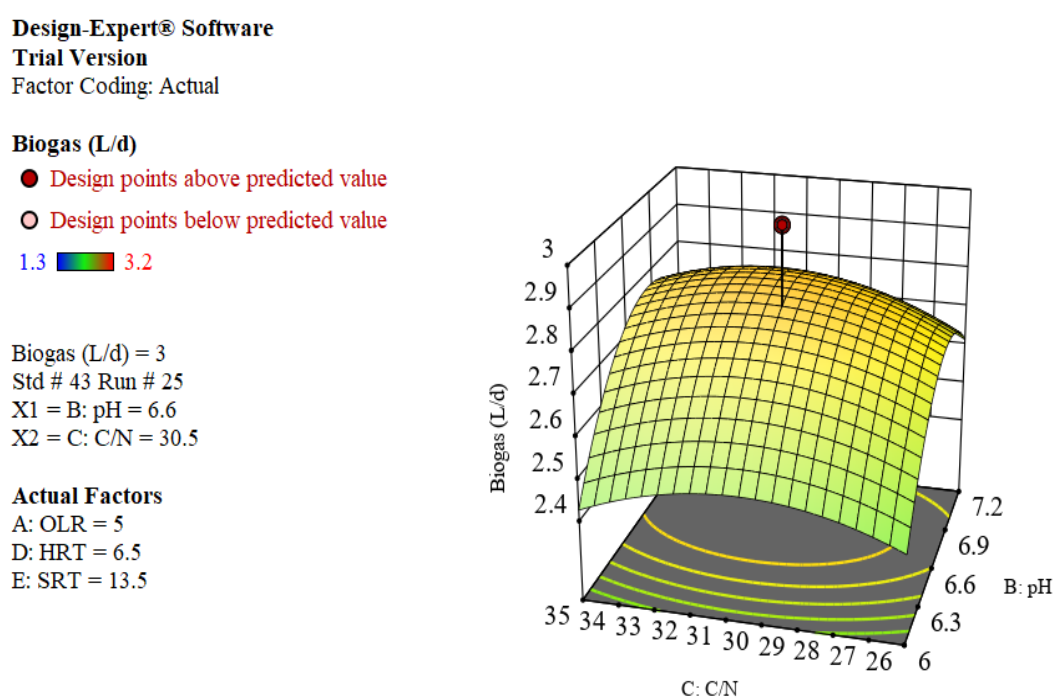


Figure 4.5: Surface Response Optimization C/N, pH and Biogas Production

4.4.1 (a) Biogas Production Optimization with respect to OLR and pH

The DoE software had produced 3D plots on inputs-outputs of anaerobic bioreactors, which are depicted in Figure 4.3 and Figure 4.5. It could be deduced from Figure 4.3 and Figure 4.5 that the optimum biogas output of 3.0 L/d could be obtained with the optimum level of OLR at 5 kg/m³/d. Chotwattanasak and Puetaiboon (2011) recorded the best performance with CSTR reactor under mesophilic temperature of 35°C with OLR of 4.52 kg/m³.d to produce maximum biogas production of 20 m³/m³ of POME at HRT 7 days, with corresponding COD reduction efficiency of 64%. Whereas in different experiment, Krishnan et al. (2016) recorded increase of pH value in CSTR reactor as OLR was increased from 6 to 12 kg/m³/d, before it decreased sharply to turn acidic with low alkalinity as OLR exceeded that range.

4.4.1 (b) The optimization of Biogas Production with respect to pH and C/N

The DoE software had produced 3D plots on inputs-outputs of anaerobic bioreactors, with respect to factor C: C/N and B: pH, which are presented in Figure 4.6. The contour optimisation plot in Figure 4.5 gives the range of inputs that affect output (biogas production). It could be deduced from Figure 4.5 that the optimum inputs required for the system to achieve optimal biogas output of 3.0 L/d were at pH values ranging 6.6–7.1 and C/N at 30.5. Alrawi et al. (2011) recorded pH range of 7.05–7.55 to give suitable environment for methanogenesis in SCSTR reactor for co-digestion of POME with rumen fluid (RF). The optimal C/N obtained in this study concurs with findings by several researchers who postulated that methanogenic bacteria require optimal range of C/N between 20 and 35 (Habiba et al., 2009; Kayhanian, 1999; Khalid et al., 2011).

4.4.1 (c) Biogas Production Optimization with respect to SRT and HRT

The DoE software had generated 3D plots as inputs-outputs of the bioreactors with respect to factors E:SRT and D:HRT, which are depicted in Figure 4.7.

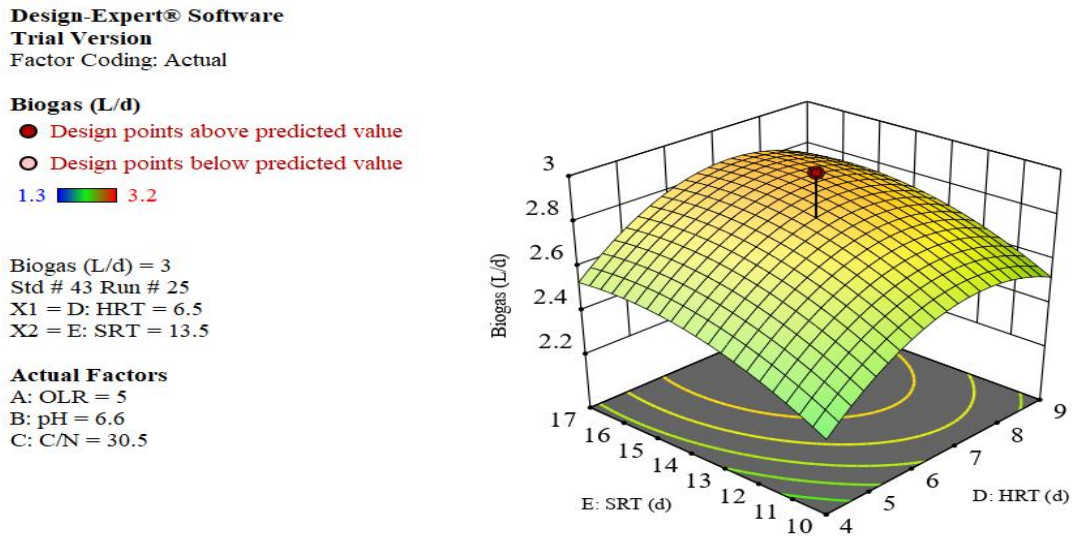


Figure 4.6: Surface Response Optimization SRT, HRT and Biogas Production

The Figure 4.6 demonstrates the combined effects of SRT and HRT on the biogas output while keeping the other independent variables constant at their centre points. Hence, it can be deduced from the 3D plot that the optimal levels of inputs required to produce optimum biogas output of 3.0 L/d from POME digestion are at HRT 6.5 days and SRT 13.5 days. Choorit and Wisarnwan (2007) recorded the best performance of CSTR at HRT 7 days with OLR at 12.25 kg-COD/m³/d, operating under mesophilic temperature of 37°C, producing 3.75 L/d of biogas with 71.1% COD removal efficiency. Mahmoud et al. (2004) used range of SRT from 10 to 15 days where most substantial portion of proteins, carbohydrates and lipids were digested at process temperature between 25–35°C.

4.4.2 Research Findings on Objective Two with Discussions

These optimum values of all variables including independent and dependent variables are presented in Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6, which could be summarised as in Table 4.2.

Table 4.2: Optimum Value of Factors from and Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6

Figure No	Input Factors	Optimum Value	Optimum Outputs as Biogas (L/d)
4.3,4.4, 4.5, 4.6	OLR (kg/m ³ /d)	5	3.0
	pH	6.6–7.1	
	C/N	30.5	
	HRT (d)	6.5	
	SRT (d)	13.5	

In this study, the optimum value of OLR is 5 kg/m³/d in order to get the optimum output of biogas from POME digestion process. For OLR of more than 5 kg/m³/d, the stability of the anaerobic digestion process would be affected (Calzada et al., 1984). Furthermore, large addition of organic material into the system could interrupt the fermentation process during the early stages of digestion, thereby affecting the efficiency of POME digestion process to produce biogas. When there is a rapid increase in the rate of hydrolysis and acidogenesis, VFA concentration would build up rapidly which decreases the pH during the digestion process and methanogenesis process will be affected (Mao et al., 2015). Hence, biogas production is reduced. Conversely, a low OLR can result in inefficient performance of POME digestion to produce biogas (Calzada et al., 1984) due to an undersupply of organic matter for microorganisms to digest. When the substrate is insufficient (OLR<5 kg/m³/d), hydrolysis and acidogenesis processes will be affected (Krishnan et al., 2017; Zinatizadeh et

al., 2006). Consequently, when the production of VFA is decreased, biogas production could also be reduced.

In this study, the optimum value of pH ranges from 6.6–7.1 as displayed in Figure 4.5. Methanogens are sensitive to pH and it has been reported that the biogas production increases if pH is more than 6.5 (Nayono, 2010). Microorganisms in acetogenesis and methanogenesis processes perform better when pH is at about 6.5 which is almost a neutral environment (Wong et al., 2013). On the contrary, methanogenic activities decrease if pH is lower than 6.5, due to the slowed growth rate of methanogens (Angelidaki et al., 2003), resulting in the reduction of biogas production rate. However, hydrolytic and acidogenic bacteria thrive well in pH lower than 6.5, favouring an acidic environment (Kim et al., 2003). As methanogens begin to convert hydrogen and acetic acid into methane and carbon dioxide, pH would increase resulting in the increase of methane content (Malakahmad & Yee, 2014).

In this study, the optimum value of C/N is 30. A C/N more than 30 has higher carbon content that contributes to increasing carbon dioxide formation and lower pH as carbonic acid reacts with water readily, increasing acidity (Dioha et al., 2013; Dobre et al., 2014). A high amount of carbon will leave carbon in excess while nitrogen has been consumed causing the bacteria to starve and eventually die (Dioha et al., 2013; Okonkwo et al., 2018; Shanmugam & Horan, 2009). Several authors agreed that C/N of more than 30 and less than 50 is the ideal C/N for biogas production from POME (Habiba et al., 2009; Kayhanian, 1999; Yen & Brune, 2007). The methanogens require optimum C/N of more than 30 reflecting a moderate N concentration for sufficient metabolism (Okonkwo et al., 2018) where nitrogen would be used to form enzyme for microbial activities. Conversely, C/N less than 30 has high value of nitrogen that would enhance the production of ammonia gas, thereby increasing pH

through protein degradation or by its presence in the charging flux, making it detrimental for the growth of microorganisms (Dobre et al., 2014). Excess nitrogen could cause digestion to stop when all available carbon had been consumed (Dioha et al., 2013; Okonkwo et al., 2018; Shanmugam & Horan, 2009). Then, it will affect the digestion process of POME and reduces the biogas production rate.

In this study, the optimum value of HRT is 6.5 days. If HRT is more than 6.5, the contact time between substrate and microorganisms will increase, resulting in increased production of methane-forming bacteria (Maroun & El Fadel, 2007; Poh & Chong, 2009). Longer HRT would give the microbial population sufficient time to adapt to the new environment (Siddique et al., 2016). A well-adapted microbial population in anaerobic condition leads to an increase in the digestion rate of organic matters (Alrawi et al., 2011). This will eventually improve the production of biogas. On the contrary, the effects of HRT lower than 6.5 could lead to the accumulation of VFA. A short HRT may result in system failure due to reduction in the growth rate of microbial population, insufficient hydrolysis, process imbalance, and organic overloading (Mao et al., 2015; Nurul Adela et al., 2014; Romero Aguilar et al., 2013). Although a low HRT produces higher biogas production rate, the decomposition rate of organic matter is low.

In this study, the optimum value of SRT is 13.5 days where SRT significantly affects biogas formation through the conversion of volatile solid (VS). When SRT is more than 13.5, the long duration of SRT allows adequate methanogenesis to occur in an anaerobic system. This enables microbial community to be acclimatized to the environment of the available components during the digestion process of POME (Dvořák et al., 2016). However, when SRT is further increased, microorganisms begin to compete for nutrients resulting in the

formation of antibiotics that prevent the growth of other microorganisms. As a result, certain microbial population will not be able to survive and die (Huang et al., 2001). Ultimately, biogas production during the digestion process will be affected. Conversely, SRT lower than 13.5 would not give sufficient amount of time for microorganisms to reproduce and restore the microbial population that are lost in the effluent (Abdelgadir et al., 2014). When the growth rate of microorganisms is lower than the rate of microbial loss, washout will occur (Ali, 2012; Demirel et al., 2005; Metcalf & Eddy, 2003). During washout, the number of microorganisms in the digestion system will be reduced, taking them longer time to break down the biomass to produce biogas (Ali, 2012). The digestion process will be affected and hence, reduces the production rate of biogas.

4.4.3 Discussions on Answer to Research Question Two

Bioreactor was run as per data displayed in Figure 3.2(a) and Figure 3.2(b) in Chapter 3. The findings from the software are displayed in Figure 4.3, Figure 4.4, Figure 4.5, and Figure 4.6. The summary of findings on optimum inputs and optimum output are presented in Table 4.2.

Based on the analysis of findings it can be concluded that if bioreactor operates at the combinations of independent variables listed in Table 4.2, optimum level of biogas production from POME could be achieved. In conclusion, the optimum level of biogas production from POME was obtained with C/N at 30.5; pH at 6.6–7.1; OLR at 5.0 kg/m³/d; HRT at 6.5 days, and SRT at 13.5 days. Thus, objective number two of the research was achieved and the research question two is answered, with the null hypothesis two being rejected (H_0 : All parameters are not significantly different from zeros).

4.4.4 Scenario Analysis on Optimum Values of Factors that Contribute to Produce Biogas from POME

The optimum value for pH is 6.6–7.1; for C/N is 30.5; for OLR is 5.0 kg/m³/d; for HRT is 6.5 days, and SRT is 13.5 days. The optimum output of COD reduction is 80%, resulting in biogas production from POME of 3.0 L/d. In general, the methanogenesis process prefers a neutral pH value. 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 (Nayono, 2010; Stronach et al., 1986). However, the performance of an anaerobic digestion decreases at pH below 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. Therefore, the optimum pH value (6.6–7.1) in this study is within the acceptable range of 6.5–7.5, as discovered by Stronach et al. (1986) and Nayono (2010).

Different researchers have 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 1.5–9.2 kg-COD/m³/d) and they were able to obtain COD reduction efficiencies in the range of 77–97%. OLR can 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 is further increased to 20 kg-COD/m³/d, the system's pH and alkalinity decreased sharply. Ronnachai et al. (2007) conducted a study by increasing OLR from 2.50 to 10.0 kg-COD/m³/d in an up-flow anaerobic sludge bed (UASB) reactor and discovered 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 demonstrates that OLR has an effect in COD reduction and biogas production from POME. Therefore, the optimum value of OLR of 5.0 kg/m³/d is acceptable in this study.

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, 2012; Nour et al., 2010). Another study 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 at 66.09% with maximum biogas production of 3.977 L/d at HRT of 12 days. Hence, the optimum value of HRT at 6.5 days is acceptable in this study.

Generally, increasing the SRT would improve the removal efficiency of COD (Dereli et al., 2014). This is further reinforced by the effect of increasing SRT through sludge recycling which improved the removal efficiency of COD in fermentation process up to 85%. Meanwhile, the COD removal efficiency could still be obtained at 76% without sludge recycling (Irvan et al., 2017). Mahmoud et al. (2003) stated that methanogenesis commences between 5 and 15 days of SRT at a temperature of 25°C. Therefore, the optimum value of SRT at 13.5 days is an acceptable value in this study.

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 (Habiba et al., 2009; Kayhanian, 1999) for anaerobic digestion.

According to Khalid et al. (2011) 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 is in the range of 10.08–11.44 which is significantly lower than required C/N value of 20–35. Hence, co-digestion is necessary to increase the C/N during POME treatment process. Thus, the optimum C/N (30.5) in this study is acceptable.

4.5 Model Development to Predict Optimum Level of Biogas Production from POME

The scope of work of this part of study is related to objective three stated in Section 1.4 (c). This section will answer the research question of “**What is the empirical model that could be used to predict optimum level of biogas production from POME?**”.

The scope of study under this section was to develop a multiple linear regression model with all variables listed in Table 4.1 by using experimental data and ANOVA program. The Minitab 18.1 and Design Expert 2018 software were used for data analysis and model development.

4.5.1 POME Digestion Process Modelling and Process Control

The COD and VSS of POME are the potential sources of biogas production. The POME with the required inoculum prepared from banana peel (fruit waste) was fed into the anaerobic digester for producing biogas. The conceptual model for input-output POME digestion process is presented in Figure 4.7 for mathematical analysis as follows:

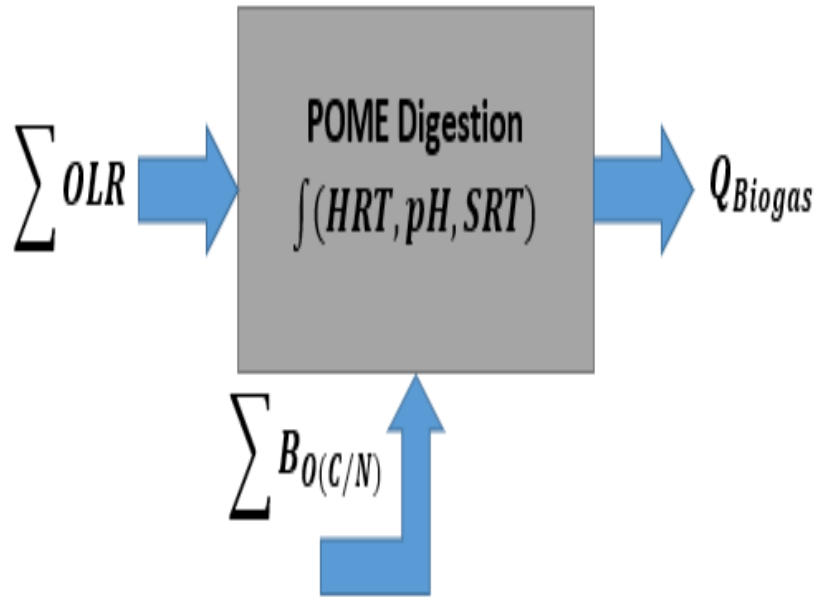


Figure 4.7: POME Digestion for Biogas Production

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 biogas. SRT = Sludge retention time as a contributor to accelerate POME digestion for biogas. K = Process control factor of input conversion to output as biogas ($k < 1$), where the range of K is 0.7 to 0.9 [$0.7 < K < 0.9$] (Seborg et al., 2004).

The biogas yield depends on OLR process, operating environment (COD and biomass loading rate) in anaerobic reactor and the efficiency of the digestion process. Environment in anaerobic reactor depends on HRT, SRT and pH; C/N also contributes to supply nutrient for microbial growth, with nitrogen acting as enzyme for microbial activities. The COD and biomass of POME would be converted to biogas in four steps of bio-chemical reactions, as elucidated by Figure 2.5 in Section 2.5 of Chapter 2.

The process capability K of those reactions depends on many factors and among these pH, HRT, SRT and C/N have significant role. 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:

$$K = \int \left(pH, HRT, SRT, \frac{C}{N} \right) \quad \text{Eq. 4.8}$$

It infers that the process conceptual model depicted in Figure 4.8 is a non-linear process which can be expressed mathematically by Equation 4.9.

$$Q_{biogas} = A_o(OLR)^\alpha \cdot \left(\frac{B_{OC}}{N} \right)^\beta K \quad \text{Eq. 4.9}$$

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

$$\log(Q_{biogas}) = \log A_o + \alpha \log(OLR) + \beta \log\left(\frac{B_{OC}}{N}\right) + \log K + \log \varepsilon \quad \text{Eq. 4.10}$$

Where, A_o is the intercept of the line at y-axis. The coefficient of OLR ' α ' is the contribution to biogas production from POME. The coefficient of $B_{OC/N}$ related to C/N ' β ' is the contribution to biogas production from POME. Whereas the regression term error, ε was considered to be zero, with k being the process capability factor for all inputs to produce biogas in the digester.

4.5.2 Data Analysis and Model Development

The data from the experiment were analysed and outputs are presented in 3D plots as displayed in Figure 4.3, Figure 4.5 and Figure 4.6, which are the outputs of the Minitab Version 18.1 and the Design Expert Version 2018. The outputs of ANOVA are presented in

the following Table 4.3, while the DOE output on relevant coefficient estimate and standard error is presented in Table 4.4.

Table 4.3: Model of Biogas Production from POME

ANOVA for Quadratic model

Response 1: Biogas

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	6.73	20	0.3364	2.39	0.0158	significant
	A-OLR	0.8568	1	0.8568	6.09	0.0197	
	B-pH	0.5910	1	0.5910	4.20	0.0495	
	C-C/N	0.0209	1	0.0209	0.1486	0.7027	
	D-HRT	0.6075	1	0.6075	4.32	0.0466	
	E-SRT	0.6518	1	0.6518	4.64	0.0398	
	AB	0.1250	1	0.1250	0.8891	0.3535	
	AC	0.0200	1	0.0200	0.1423	0.7088	
	AD	0.0013	1	0.0013	0.0089	0.9255	
	AE	0.0313	1	0.0313	0.2223	0.6408	
	BC	0.0113	1	0.0113	0.0800	0.7793	
	BD	0.0200	1	0.0200	0.1423	0.7088	
	BE	0.0450	1	0.0450	0.3201	0.5759	
	CD	0.0200	1	0.0200	0.1423	0.7088	
	CE	0.0200	1	0.0200	0.1423	0.7088	
	DE	0.0013	1	0.0013	0.0089	0.9255	
	A ²	0.4799	1	0.4799	3.41	0.0749	
	B ²	1.83	1	1.83	12.99	0.0012	
	C ²	0.3930	1	0.3930	2.79	0.1053	
	D ²	2.01	1	2.01	14.29	0.0007	
	E ²	0.4799	1	0.4799	3.41	0.0749	

Table 4.4: The Coefficient Estimate of the Linear Regression Model

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.81	1	0.1316	2.54	3.07	
A-OLR (X_1)	0.1406	1	0.057	0.0241	0.2572	1
B-pH (X_2)	0.1168	1	0.057	0.0003	0.2333	1
C-C/N (X_3)	0.022	1	0.057	-0.0946	0.1385	1
D-HRT (X_4)	0.1184	1	0.057	0.0019	0.235	1
E-SRT (X_5)	0.1227	1	0.057	0.0062	0.2392	1

The coefficient estimate represents the expected change in response (y) per unit change in input (X_n) 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 or negative values (antagonistic effects) as reflected in Table 4.3 and Appendix C5. Hence, only the main effects were considered to form the final empirical model.

With reference to the model analysis presented, the respective values of coefficients of the empirical model from Table 4.4 above are:

$\alpha = 0.1406$, $\beta = 0.022$, $A_0 = 2.81$. K is the process efficiency 0.925 (regarded as R^2 in Figure 4.3).

By using these coefficients in Equation 4.10, the desired statistical model could be rewritten as linear form shown in Equation 4.11.

$$\log(Q_{biogas}) = \log 2.81 + 0.1406 \log(OLR) + 0.022 \log\left(\frac{B_{OC}}{N}\right) + \log 0.925 + \log \epsilon \quad \text{Eq. 4.11}$$

The nonlinear form of that model could be derived from Equation 4.9, to be represented by Equation 4.12.

$$Q_{biogas} = 2.81(OLR)^{0.1406} \cdot \left(\frac{B_{OC}}{N}\right)^{0.022} 0.925 \quad \text{Eq. 4.12}$$

4.5.2(a) Developed Model Analysis

With reference to Appendix C5 and the prediction model Equation 4.9 above, the developed model comprises four parts. The first part is the intercept of the straight line at y-axis with a value of 2.81. The second part is the contribution of OLR (X1 at Table 4.4) to biogas production. It could be deduced from Table 4.4 and Figure 4.2 that the coefficient of OLR to biogas production is 0.1406 with significant level (p-value at $0.0197 < 0.05$) of contribution to biogas production with an effective process capability (K) 0.925 (at Appendix C4(b) regarded as R^2). This scenario could be represented by Figure 4.8.

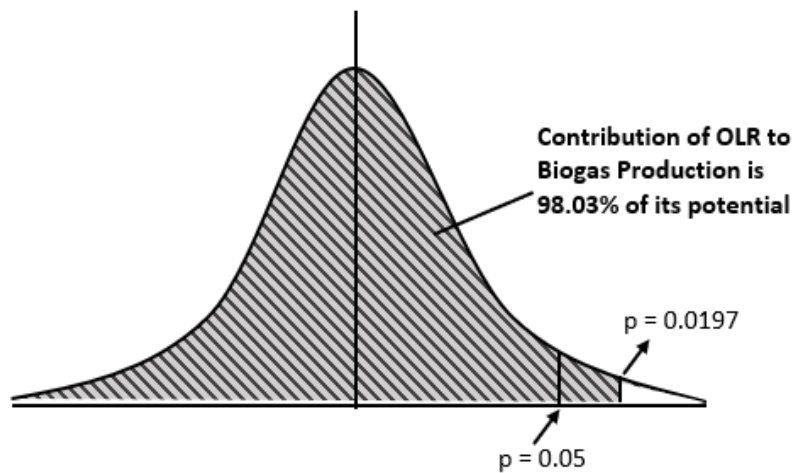


Figure 4.8: Contribution of OLR to Biogas Production

The shaded area in this Figure 4.8 represents the contribution of OLR to biogas at 98.03% ($p = 0.0197$) which is more than 95% ($p < 0.05$). This finding affirms that the contribution of OLR to biogas production was significant at 95% confidence level.

The third part is the contribution of $B_{OC/N}$ (related to C/N) to biogas production. The coefficient of $B_{OC/N}$ (X3 at Table 4.4) to biogas production is 0.0220 with p-value at 0.7027 ($p > 0.05$) of contribution with a process capability (k) 0.925 (regarded as R^2) as illustrated in Figure 4.9. In Equation 4.8 where K is a function of all the input factors under study, it represents a common effect size index R^2 for all those factors, encompassing the proportion of variance in one output variable explained by the other input variable. It can also be regarded as the coefficient of determination R^2 which normally range from zero to one (as $K < 1$), usually expressed in percentage (Ferguson, 2009).

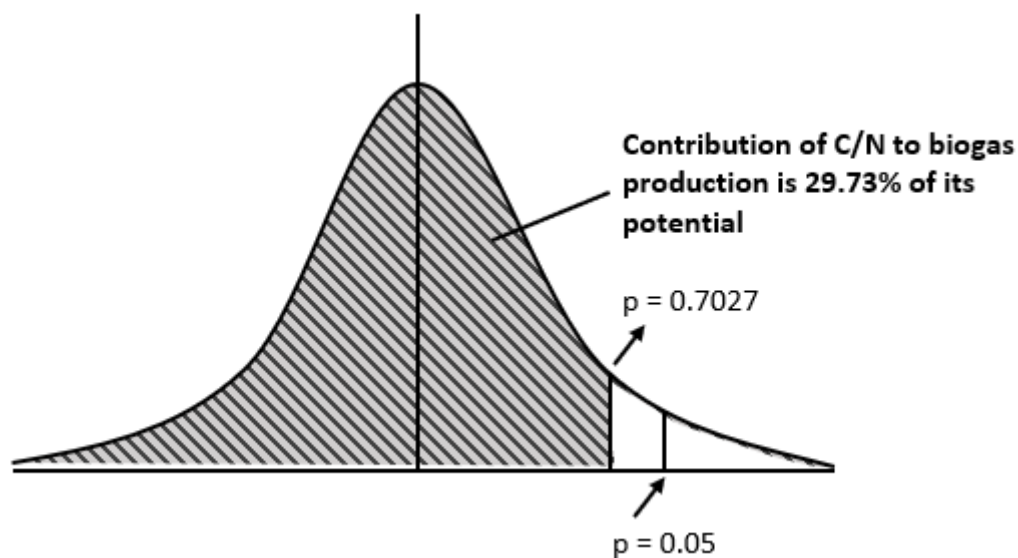


Figure 4.9: Contribution of C/N to Biogas Production

The fourth part is the process capability K which in statistics is regarded as effect size R^2 . Then, the process capability or effect size of the digestion process is about 92.5%, which

demonstrated that about 92.5% of input potentials are converted into biogas during the digestion process.

The biogas process control factors are pH, HRT and SRT as shown in Figure 4.8; and the function of these factors is to create digestion environment in anaerobic reactor; the contribution of each of these factors can be depicted similar to Figure 4.8. The coefficient of pH (at point B-pH) to biogas production is 0.1168 with significant level (p-value at $0.0495 < 0.05$) of contribution to biogas production. The coefficient of HRT (at point D-HRT) to biogas production is 0.1184 with significant level (p-value at $0.0466 < 0.05$) of contribution to biogas production. The coefficient of SRT (at point E-SRT) to biogas production is 0.1227 with significant level (p-value at $0.0398 < 0.05$) of contribution to biogas production. This information demonstrates that the process control factors had played a significant role in digestion process in anaerobic reactor.

4.5.2(b) Output Model and Multi-Collinearity Properties Among Independent Variables

In statistics, multi-collinearity is a phenomenon in which one predictor variable in a multiple regression model can be linearly predicted for the other variable with a substantial degree of accuracy. Hence, the coefficient estimates of those factors of multiple regressions can give erratic response to small changes in the model or the data set.

In this study, the level of multi-collinearity properties in the POME digestion process could be assessed from Table 4.3 and Appendix C5. Multi-collinearity properties among independent variables presented by Variance Inflating Factor (VIF) bespeak how the variables are related to each other, by quantifying its severity in the ordinary least squared regression analysis. It determines whether inputs factors are correlated or uncorrelated. The

VIF of OLR, C/N, pH, HRT and SRT is 1.0, indicating there is no collinearity among the independent variables (Ringle et al., 2015). The VIF of interactions among OLR, C/N, pH, HRT and SRT is 1.05 ($VIF < 5$), inferring that the multi-collinearity among the variables during the reaction is acceptable. Otherwise, if collinearity exists among independent variables, it tends to inflate the variance of at least one estimated regression coefficient, making it difficult to fit model and interpret the results.

4.5.3 Model Validation with 50 Experimental Outputs

The conceptual model is displayed in Figure 4.7 and prediction (equation) model is presented by Equation 4.9. The model was estimated by using inputs data shown in Table 3.2, Figure 3.2(a), and Figure 3.2(b). The actual data on biogas production listed in Appendix C3 would be used to validate the developed model. The distribution of predicted values of model and actual biogas production are presented in Figure 4.10.

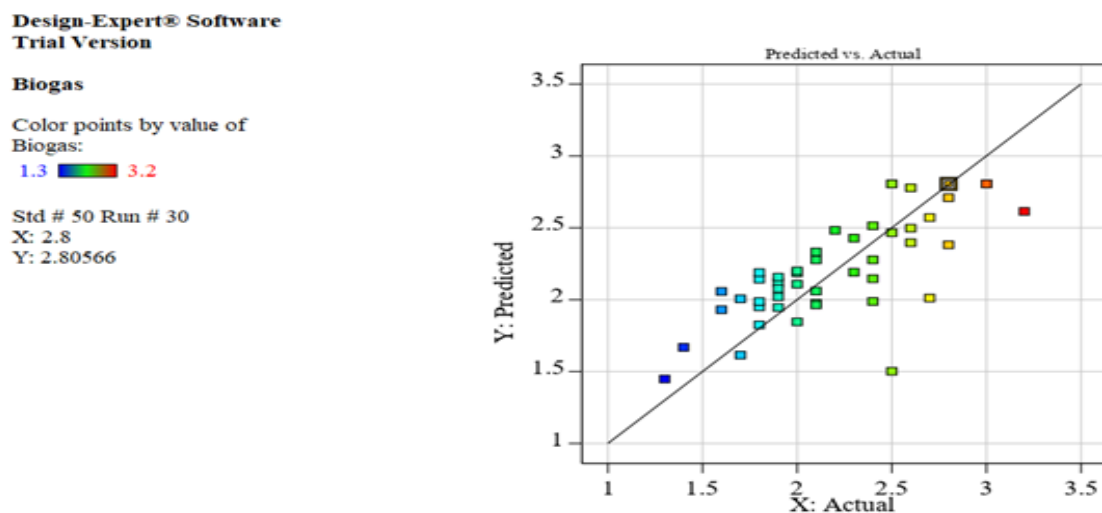


Figure 4.10: Distribution of Data on Actual Biogas and Prediction Model

The graph in Figure 4.10 presents the “x-axis” as the actual experimental outputs (actual data response) of 50 runs; and “y-axis” as the model estimate in Equation 4.12. It can be

deduced from Figure 4.10 and Appendix C6 that the optimal biogas output of 50 runs is 3.0 L/d [x: 3.0], whereas the value of model prediction (fitted value on the regression line) is 2.81 L/d [y: 2.8]. This finding demonstrates that average error in the prediction model with 50 runs is about 6.6%. It indicates that model developed for estimating biogas prediction is quite fit to predict biogas production within 10% of error.

4.5.4 Model Validation with Optimum Inputs

The developed model in Equation 4.12 has also been validated by using optimum values of inputs listed in Table 4.2. The experimental values with optimum inputs for both actual and prediction model outputs (biogas) are depicted in Table 4.5 and listed in Appendix C6.

Table 4.5: Model Validation with Optimum Inputs Factors

Optimum Input Values	Run	Actual	Predicted	Standard Error (%)	Standard Deviation in Error
OLR = 5 pH = 6.6–7.1 C/N = 30.5 HRT = 6.5 SRT = 13.5	4	3.1	2.95	5.17	1.60
	10	2.85	2.82	0.70	
	18	3.0	2.94	2.0	
	29	2.85	2.81	1.4	
	35	2.91	2.88	1.0	
	40	2.79	2.81	0.7	
	48	2.88	2.90	0.69	
	Average Error Between Actual and Model Prediction			1.66	

Table 4.5 demonstrates that the average standard error between actual and model prediction is 1.66%, with standard deviation of error 1.6. Such small value of standard deviation error indicates good model by giving close value between predicted and actual values for the response (Behera et al., 2018). It validates that model developed to estimate biogas prediction is quite fit to predict biogas production within 5% of error limit.

Based on DOE outputs on ANOVA in Figure 4.2, the developed model was highly significant with p-value of 0.0158 ($p < 0.05$), inferring that the model terms of independent variables were significant at 95% confidence level, as reflected by their small p-values. Hence, we reject the null hypothesis two based on F-test for overall significance where the model with proper terms having non-zero coefficients fits the experimental data better than intercept-only model (H_0 : All parameters or coefficients are not significantly different from zeros).

The lack of Fit test with ANOVA in Appendix C4(a) generated F-value of $3.29 < F_c$ (3.40 from F-table), with p-value of 0.0559 ($p > 0.05$) which is not significant at 95% level to reject the null hypothesis three (H_0 : For equality of means, model error or residual is roughly equal to the hypothesized or pure error). We cannot reject the null hypothesis if the f-statistic value is smaller than the critical f_c value from the F-table at the given degrees of freedom ($df_1 = 1$, $df_2 = 22$), unless the p-value is small ($p < 0.05$) which implies the 'Lack of Fit' is significant. Therefore, lack of fit exists only if there is significant difference between the two means. Hence in this study, there was not enough evidence at 95% significant level to conclude there is lack of fit in the developed (linear regression) model.

4.5.5 Conclusion and Answer to Research Question Three

Bioreactor was run as per the combinations of variables shown in Appendix C3; and the inputs-outputs on experimental run are presented in Figure 4.3, Figure 4.4, Figure 4.5, and Figure 4.6. The ANOVA was run to develop an empirical model, which is represented by Equation 4.12. The model was validated by using 5 variables listed in Table 3.2, Figure 3.2(a), and Figure 3.2(b). The ANOVA outputs on actual and prediction model are presented in Figure 4.10 and Table 4.5. The validation results show that the actual value and predicted

error is within 1.66% with a standard deviation 1.6. Based on the findings, it can be stated that the model built to estimate biogas production from POME is deemed fit to predict output of anaerobic reactor within the error limit. In conclusion, it could be stated that objective number three of the research is achieved and the research question three is answered, with the null hypothesis three based on F-test on equality of means being accepted.

4.5.6 Scenario Analysis on Empirical Model to Predict the Optimum Level of Biogas from POME

The empirical model to predict the optimum level of biogas production from POME is presented is Equation 4.9 of Section 4.5.1 which appeared as:

$$Q_{biogas} = A_o(OLR)^\alpha \cdot \left(\frac{B_{OC}}{N}\right)^\beta K \quad \text{Eq. 4.13}$$

As for the experimental results in Table 4.2, the highest and lowest value of biogas production were 3.10 L/d and 2.79 L/d respectively, which involved corresponding COD reductions of 80% and 57% respectively (Readings of COD values taken with optimal settings of input only). The highest COD reduction efficiency reported by Wong et al. (2014) with suspended closed anaerobic reactor (SCAR) was 66.1% at HRT of 12 days. The current study was able to achieve 13.9% more at a lower HRT of 6.5 days. In this study, the application of co-digestion was done by introducing an inoculum to increase the C/N of the feedstock as a factor to COD reduction and biogas production from POME. The presence of an inoculum increases the population of microorganisms (natural consortia of microbes in waste banana peel) inside the system for better COD removal from the substrate (El-Mashad & Zhang, 2010; Esposito et al., 2012).

This two-stage process is a more efficient method in reducing COD level up to more 90% removal efficiency by segregating the Stage 1 (hydrolysis and acidogenesis) and Stage 2 (acetogenesis and methanogenesis) which would assuage the negative effects of inhibitors in the feedstock. A two-stage process improves the net energy balance, process stability and methanogenic process which would lead to higher rate of methane production, COD reduction efficiency and volatile solids (VS) reduction, making it a preferable method for POME treatment.

4.5.7 Effects of Cross-flow Membrane Separator on Biogas Production from POME

As in Figure 4.11, the waste biomass left undigested by the anaerobic reactor would be passed through nanomembrane (NFM) with pore size of 2.47 nm and the resulting biofluid be recycled back into the reactors before disposal. Keeping the cross-flow velocity at 0.9 m/s with pressure drop up to 15 bars (Shahidul et al., 2015), the hydraulic permeability of each membrane is influenced by its pore size. Concentrations of effluent in both permeate and concentrate were determined by anion exchange chromatography in accordance with the method used by Van Riel and Olieman (1991).

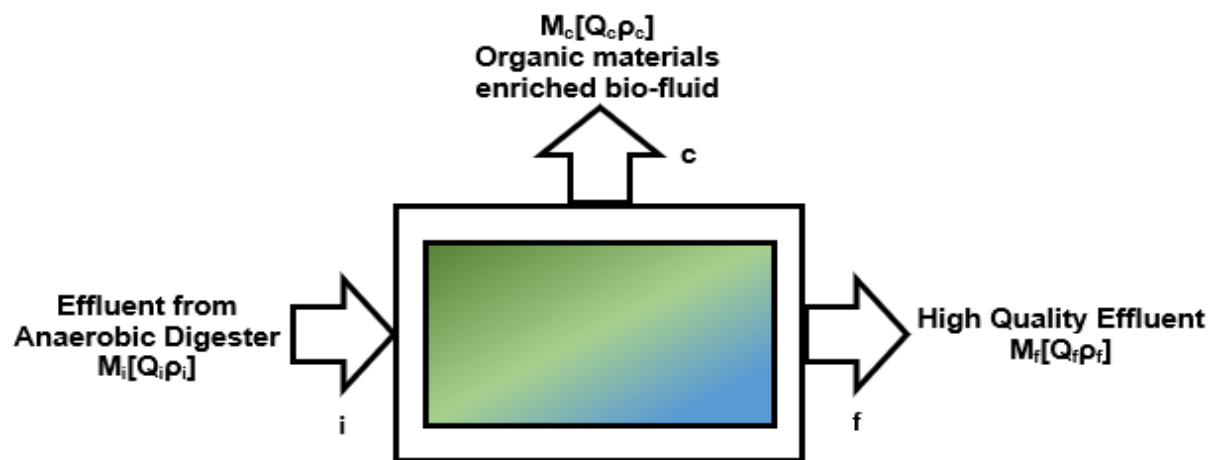


Figure 4.11: Schematic Diagram of Nanomembrane Separation (Shahidul et al., 2019)

Such subsequent treatment with nanomembrane managed to increase utilization rate of substrate up to 92% which improved overall performance of the system by increasing COD removal efficiency up to 95% as displayed in Table 4.6. There was an improvement on substrate utilization rate of about 30% (from 62.26 to 92.5%) when the substrate was recycled back after passing through the nanomembrane separator.

Results from Li and Yu (2011), Chen et al. (2012) and Mohd Idris et al. (2014) required SRT of 30 days for membrane bioreactor to achieve COD removal efficiency of more than 90% as stipulated in Table 2.7(b). In this study, the optimal SRT could be attained not more than 14 days with this system. Lastly, the average total biogas output from POME digestion was at 3.9 L.kg-COD/m³/d, registering 30% increase as compared to 3.0 L.kg-COD/m³/d obtained without membrane.

Table 4.6: Effects of Nanomembrane on Biogas Production and Effluent Quality

Estimated Parameters	Outputs with CSTR and without membrane	Outputs with CSTR and with membrane					
		L1	L2	L3	L4	Average	P-Value
Utilization of Substrate, R ² (%)	62.26	89	91.3	93.4	96.3	92.5	0.0345
Biogas production (L/d)	3	3.3	3.6	4.2	4.5	3.9	0.045
COD Reduction (%)	80	89.5	94.5	97.5	98.5	95	0.033
Effluent quality (mg/L)	20,000	10,500	5,500	2,500	1,500	5,000	0.001

4.6 Chapter Summary

This chapter presents the results of this study to achieve the research objectives stated in Section 1.4. To achieve research objective one, factors that significantly affect biogas

production from POME were determined. The results of the experiment show that pH, HRT, SRT and OLR have significant level of 95% confidence level ($p < 0.05$) contribution to produce biogas as shown by their p-values in Figure 4.2 in Section 4.3.3. C/N also contributed to biogas production, though its contribution was not significant at 95% confidence level. Thus, research objective one is achieved.

The second part of this chapter is related to research objective two in determining the optimum levels of inputs. The experiment was run as per the combinations shown in Figure 3.2(a) and Figure 3.2(b). Based on the 3D plots presented in Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6, the optimum value for OLR is 5 kg/m³/d; pH is 6.6–7.1; C/N is 30.5; HRT is 6.5 days, and SRT is 13.5 days. Thus, research objective two is achieved.

If the settings of those significant factors (input variables) are varied at different values from their optimal level, the effects on biogas production would not be optimized as elucidated in Table 4.7.

Table 4.7: Effects of Different Values of Factors (Inputs) on Biogas Production

Input Variable	Effects on Biogas Production (Output)
Organic Loading Rate (OLR) in kg/m ³ /d	High OLR (OLR>5) affects process stability (Calzada et al., 1984), with excess organic materials interrupting the fermentation process (hydrolysis and acidogenesis), resulting in rapid build-up of VFA concentration which decreases the pH, adversely affecting methanogenesis (Mao et al., 2015). Low OLR (OLR<5) causes inefficient performance of POME digestion (Calzada et al., 1984) with undersupply of organic matter for microorganisms to digest. Insufficient substrate at low OLR reduces production of VFA during hydrolysis and acidogenesis (Krishnan et al., 2017; Zinatizadeh et al., 2006).
Potential of Hydrogen (pH)	If pH>6.6, biogas production increases (Nayono, 2010) as methanogens are sensitive to lower pH while they prefer a neutral environment (Wong et al., 2013). Majority of enzymes (>3000 types) prefer optimal pH around 7.0 (neutral) with fairly narrow pH range to remain active (Merlin Christy et al., 2014). If pH<6.6, hydrolytic and acidogenic bacteria thrive well in lower pH, favouring acidic environment (Kim et al., 2003). As methanogens convert hydrogen and acetic acid into methane and carbon dioxide, pH would increase, resulting in increase of methane content (Malakahmad & Yee, 2014). However, the growth rate of methanogens reduced (Angelidaki et al., 2003) at pH<6.6, resulting in the reduction of biogas production rate.
Carbon to Nitrogen Ratio (C/N)	If C/N>30.5, higher carbon content of substrate causes carbon dioxide formation and lower pH as carbonic acid reacts with water readily, increasing acidity (Dioha et al., 2013; Dobre et al., 2014). Excess carbon left when all available nitrogen is consumed; bacteria would starve to death (Dioha et al., 2013; Okonkwo et al., 2018). Habiba et al. (2009) and Kayhanian (1999) postulated 20>C/N>35 as the ideal ratio for biogas production required moderate N concentration for efficient metabolism (Okonkwo et al., 2018) where nitrogen would be used to form enzyme for microbial activities. Low C/N<30.5 reflects high value of nitrogen to produce of ammonia gas, thereby increasing pH through protein degradation, making it detrimental for microbial growth (Dioha et al., 2013; Dobre et al., 2014). Excess nitrogen could cause digestion to stop when all available carbon had been consumed (Dioha et al., 2013; Okonkwo et al., 2018; Shanmugam & Horan, 2009), thereby reducing the biogas production.

Table 4.7 continued

Hydraulic Retention Time (HRT) in days	Longer HRT ($HRT > 6.5$) increases the contact time between substrate and microorganisms, enhancing production of <i>methane-forming archaea</i> (Maroun & El Fadel, 2007; Poh & Chong, 2009). Giving ample time for the microbial population to adapt to the new environment (Siddique et al., 2016) would increase digestion rate of organic matters (Alrawi et al., 2011) to produce more biogas. Shorter HRT ($HRT < 6.5$) leads to the accumulation of VFA by leaving it unconverted by microbes, ending up in system failure with reduction in growth rate of microbial population, insufficient hydrolysis, process imbalance, and organic overloading (Mao et al., 2015; Nurul Adela et al., 2014; Romero Aguilar et al., 2013).
Sludge Retention Time (SRT) in days	Longer duration ($SRT > 13.5$) allows adequate methanogenesis to occur in an anaerobic system, by getting acclimatized to the environment (Dvořák et al., 2016). If SRT increases further, microorganisms begin to compete for nutrients by producing antibiotics to prevent the growth of other microorganisms, rendering certain microbial population to die (Huang et al., 2001). Hence, the remaining microbes might not be able to couple up with the available nutrients in the substrate. Lower SRT ($SRT < 13.5$) will not give sufficient time for microbes to reproduce and restore their population in that batch (Abdelgadir et al., 2014). When growth rate of microbes is lower than the rate of microbial loss, washout will occur (Ali, 2012; Demirel et al., 2005; Metcalf & Eddy, 2003). During washout, the number of microorganisms left in the digestion system reduced, taking them longer time to break down the biomass to produce biogas (Ali, 2012).

The final part of this chapter addresses research objective three. To achieve research objective three, an empirical model needs to be developed for predicting biogas production from POME. After assessing the various available models described in Section 2.14, an empirical model was developed using ANOVA which is presented in Equation 4.12. The model was validated in two ways. Firstly, predicted values and actual values of 50 runs using 5 variables are listed in Table 3.2. Secondly, predicted values and actual values of 50 runs with the optimum levels of inputs are listed in Table 4.2. The results from ANOVA on biogas outputs for both actual and prediction model are presented in Appendix C6. It could be

stipulated that the model built to estimate biogas production from POME is fit to predict the output of anaerobic reactor as the average error between actual value and predicted value is 1.66% which is less than 5% error limit. The small value of standard deviation error of 1.60 indicates good model that gives close (near) value between predicted and actual values for the response (Behera et al., 2018). Moreover, the lack of fit F-test with the outputs of ANOVA in Appendix C4(a) proves that there was not enough evidence at 95% significance level ($p = 0.0559 > 0.05$) to conclude there was lack of fit in the developed (linear regression) model. Thus, research objective three is achieved.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Chapter Overview

This chapter covers the conclusion and recommendations. Section 5.2 deliberates on the outcomes of research. Section 5.3 stipulates the novelty of research and its contribution to knowledge system. Implications of research outcomes are elucidated in Section 5.4. In Section 5.5, limitations of study would be mentioned. Section 5.6 discusses the conclusion of study. Finally, recommendations for future study on biogas production from POME are presented in Section 5.7.

5.2 Outcomes of Research

A total of 50 runs of experiment were conducted in order to achieve the research goals. The findings of this study were only partial, but the knowledge gained on POME treatment could be used to address its significant impacts on society, environment, and economy. The findings would be elaborated in detail in Section 5.2.1, Section 5.2.2 and Section 5.2.3.

The outcomes of this study translated into the achievement of research goals. To achieve the research goals, this study was divided into three parts. First, the significant factors that affect biogas production from POME were determined. Then, the optimum values of factors that contribute to optimize biogas production were determined. Finally, an empirical model for predicting the optimum level of biogas production from POME was developed. The research outcomes of three research components could then be summarised figuratively in Figure 5.1.

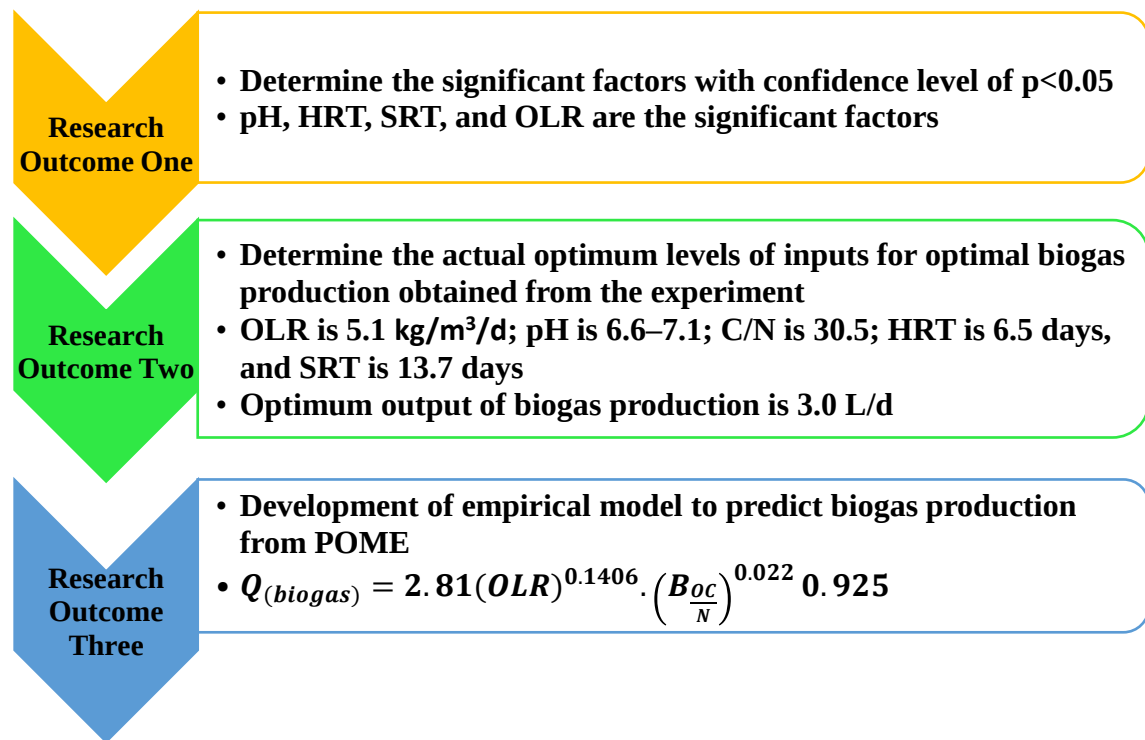


Figure 5.1: Research Outcomes of Study

POME is better known as a resource rather than a waste. Clean water can be recovered from POME as it is consisted of 95% water. Biogas (CH_4) can be captured using a closed digester tank and to be used for boiling or heating process. These resources if recovered would benefit the palm oil mills by reducing production and operation costs of running such processing facility. The implementation of environmentally sound technology will be economically sustainable to the palm oil mill operators while reducing their reliance on the usage of fossil fuels. Biogas with calorific value of 20 MJ/Nm^3 (Myles, 1987) can be used as alternative fuel or converted to electricity for internal use or for sale to the electrical grid supply for additional revenue.

With proper treatment of POME, the water and air quality of the surroundings will consequently improve. Then, animals and plants living nearby will not be affected by the

harmful effects of water and air pollution. Being a greenhouse gas (GHG), capturing of the released methane from POME during anaerobic digestion in palm oil mills, could help to mitigate global warming and drastic climatic changes that threaten our own livelihood.

POME contains extremely high amount of degradable organic matter, with the presence of some amount of unrecovered palm oil, classified under FOG (fat, oil and grease). This high strength effluent could cause pollution of waterways by depleting dissolved oxygen in the water when it starts to discompose and degrade aerobically. Hence, removal of high concentration of COD and BOD in POME before disposal to the environment will protect the habitat of aquatic organisms and help to sustain the ecosystem with rich biodiversity.

Soil becomes a habitat for large number of microorganisms and other living organisms, producing enzymes which ensure the continuation of element cycles in nature. Being an environmental pollutant with high composition of total solids, suspended organic solids and dissolved organic matter, untreated POME would alter the environmental parameters such as BOD, DO, COD, C/N, moisture content, and general soil quality. When such pollutants are reduced, the physicochemical properties of the soil return to normal which could enhance the growth and development of crop plants and animals that feed on them.

5.2.1 Research Outcome Number One

The research objective one was to determine the significant factors that affect biogas production. Results from experiments affirmed that contributing factors such as pH, HRT, SRT, and OLR gave significance levels of 95% confidence level ($p < 0.05$) for significant COD reduction and biogas production from POME. The contribution of C/N was not significant in the experiment as reflected by its small regression coefficient in Table 4.1,

might be due to the inefficiency of reactor performance. Therefore, the research objective one was achieved and answer to research question one was replied.

5.2.2 Research Outcome Number Two

The research objective two was to determine the optimum value of factors that contribute to optimize biogas production. The optimum inputs and output are presented in Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6. Based on the 3D plots, the optimum value for OLR was 5 kg/m³/d; pH was 6.6–7.1; C/N was 30.5; HRT was 6.5 days, and SRT was 13.5 days. The optimum output of biogas production was 3.0 L/d. Therefore, the research objective two was achieved and answer to research question two was replied.

5.2.3 Research Outcome Number Three

The research objective three was to develop an empirical model to predict biogas production from POME. An empirical model was developed with ANOVA and Regression Analysis using Minitab and Design Expert software, which is presented in Equation 4.12. The model was validated in two ways. Firstly, predicted values and actual values of 50 runs using 5 variables are listed in Table 3.2. Secondly, predicted values and actual values of 50 runs with the optimum levels of inputs are listed in Table 4.2. The results from Design Expert software on biogas outputs for both actual and prediction model are presented in Appendix C6. The validation results gave an average error within 1.66% and standard deviation error less than 1.6%. It could be stipulated that the model built to estimate biogas production from POME was fit to predict the output of anaerobic reactor as error was within 5% error limit between actual value and predicted value. Thus, the research objective three was achieved and answer of research question three was replied.

5.3 Novelty of the Research and Contribution to Knowledge System

In this study, an indigenous substrate which is naturally available was used to adjust the C/N for different settings of the experiments. The substrate used as an inoculum was made from the skin of fruit waste (banana peel) which would normally be discarded upon consumption of banana, with high biogas potential if left to degrade it the open. With its high carbon content ($C/N = 83$), the dosing of this inoculum in co-digestion with POME managed to increase biogas production substantially. Hence, this study could improve the current knowledge of treating POME in an anaerobic digestion system more efficiently with the developed model by optimizing the production of its resources or generation potentials for recovery and reuse. In this regard, the current research work is novel.

5.4 Implications of Research Outcomes

The results of this study may contribute to understanding the need to improve and develop better processes for POME treatment. As the palm oil industry in our country is developing, much attention should be given to improve the processes of POME treatment. Applications of more advanced processing technologies would contribute to better pollution controls with greater potentials for waste resources such as POME to become a new source of renewable energy.

5.4.1 Implication of Research Outcome to the Environment

Conventional treatment of POME using the open ponding system being the cheapest method, is very common in most palm oil mills (50% of 420 mills in Malaysia). Discharging of untreated POME into the water bodies can lead to water pollution as it reduces the quality of water. Conversely, it is possible to achieve high COD reduction for biogas production from POME by using an anaerobic system, the remaining COD level in the effluent could

be reduced to the extent that water can be recovered and reused by palm oil mills. Discharging of POME into the river can be evaded, thereby reduces water pollution at that vicinity. When biomass from POME is disposed irresponsibly into landfills or otherwise left to rot in the open, it would cause smell pollution as methane gas with unpleasant odour is released into the atmosphere. But a closed anaerobic digestion tank has the potential to capture biogas from POME. The biogas can be collected to be used as an alternative fuel source for heating in the milling process as well as electric generation using gas engine (turbine) on site. This would help to reduce the air pollution and mitigate the risk of climate change.

5.4.2 Implication of Research Outcome to the Economy

The potential to recover resources from POME is high. Biomass from POME can be converted into bio-fertilizers. Thus, it can reduce the usage of chemical fertilizer in plantation and concurrently saves cost. A cleaner water can be recovered from POME to be reused by the palm oil mills. Hence, palm oil millers would be able to reduce the water consumption for the milling process. Usage of an anaerobic system can harvest biogas from POME. This biogas can be used for heating of boilers since methane gas is highly combustible, with high calorific value (36 MJ/kg). Although the initial cost to implement this technology is high, the benefits of capturing methane and using it as a source of alternative fuel can outweigh the cost. A palm oil mill of 60 ton-FFB/hr capacity roughly generates 800 m³/d of POME which could give potential power of 2.1 MW to be used on site or sold for profit to the existing electricity power grid (Tong & Jaafar, 2006). This would make the technology appealing to palm oil mills operators from an economic point of view.

5.4.3 Implication of Research Outcome to Industry

The current study can contribute to better understanding of factors that affect COD reduction and biogas production where these factors could then be optimized in order to achieve high COD reduction efficiency and optimum biogas production in POME. This study could serve as a guideline for individuals involved in treating POME in an anaerobic system. It is also useful to palm oil millers to determine the best practices or methods to manage and utilize POME as a resource to achieve economic and environmental sustainability. With the right knowledge and technology, the palm oil industry can benefit from the potentials of POME and overcome the negative perception towards this industry.

5.4.4 Implication of Research Outcome to Society

As science, technology, and innovation are improving over time, with the right implementation and knowledge, the society will begin to acknowledge the importance of treating POME before it is discharged into the water and air. Water pollution has become prevalent in rural areas which creates encumbrances in undertaking daily domestic chores. Palm oil mills that can treat POME into clean water would reduce the negative effects of water pollution on the rural society. Besides, methane gas can also be captured and utilised to generate electricity in rural areas. By using the waste-to-energy approach to mitigate such pollutions from this industry, creation and preservation of cleaner environment with rich biodiversity would enhance the quality of life and overall wellbeing of all communities in the society concerned.

5.4.5 Implication of Research Outcome to the Policy Issue

Improving the water quality that is polluted by discharging of untreated POME can be realized using locally developed or foreign technologies. Many researches have been done

on the potential of POME similar with the current study, by trying to find the most preferable method to reduce its COD level before disposal. The Government can implement these strategies for utilizing the full potential of POME as one of the sources of renewable energy in Malaysia. Biogas, clean water and organic fertilizers can be recovered from POME. The government can provide incentives to new mills and existing mills which provide facilities to recover biogas, clean water and organic fertilizers. This will encourage palm oil millers to venture into green technologies which promote economic and environmental sustainability. As one of signatories to 2015 Paris Climate Accord with 0.67% global GHG emission contribution (United Nations, 2015), Malaysia should put more efforts to help mitigate global warming phenomena within the prescribed target of less than 2°C increase in global temperature above the pre-industrial level. Otherwise the expected sea-level rise due to global warming (Levermann et al., 2013) would adversely endanger those communities and cities located at low-laying areas of Borneo Island and Peninsula Malaysia. Hence, the existing National Biomass Strategy 2020 (Agensi Inovasi Malaysia, 2011) for renewable energy should be widely implemented throughout the whole spectrum of industries in Malaysia that contributes to global warming phenomena.

5.5 Limitations of Study

This research had several limitations that influenced the scope and outcomes of those experiments. Time was a limiting factor in this study. There were many factors that could contribute to the performance of the anaerobic system. However, only five factors were selected. Resources (financial, physical and manpower) were also limited. Only biogas collection was conducted to measure the performance of the system. COD and BOD and other tests were also not conducted throughout the course of experiments.

The quality of those samples collected from oxidation ponds at Bau Salcra Mill located 50 km away could have been severely affected over time since its discharge from the mill, exacerbated by loading and unloading of POME from the container upon reaching the laboratory at UNIMAS. Hence, leakage and direct escape of biogas during the whole digestion process for each batch could not be totally avoided.

Some discrepancies in readings or measurements of different parameters (inputs and output) could occur as they were taken at different ambient conditions, mostly done manually. Moreover, fluctuations in temperature could have substantial effects on those readings where it could be hot during the day and colder at night, ranging from 37 to 24°C at various seasons throughout the year. Where thousands of enzymes (>3000 types) are at work for digestion and energy production or respiration, they require a range of temperature to remain active, with certain temperature to work optimally. However, mesophilic bacteria can endure around 3°C temperature fluctuations without significant influence on biogas production (Deublein & Steinhauser, 2008; Kundu et al., 2014).

5.6 Conclusion of Study

The study on COD reduction and biogas production from POME will add new knowledge to the current technology for POME treatment. Besides that, this research is conducted to understand the potentials of POME in anaerobic digestion in which microorganisms convert organic compounds into biogas without oxygen while concurrently remove contaminating compounds from the effluent. Most palm oil mills are still using the conventional ponding system to treat POME and the research question was designed accordingly. The research on optimization of biogas production from POME was done to answer the research question.

The first research objective of this study was to determine the significant factors affecting biogas production from POME. Results presented in Section 4.3 demonstrate that the OLR, pH, HRT, and SRT input factors (independent variables) had significant level of 95% confidence intervals ($p < 0.05$) effect on biogas production from POME. However, C/N did contribute to biogas production but not significantly at 95% confidence level. Its insignificant effect on biogas production might be due to ineffectiveness of anaerobic digester operations. Thus, the research question one was replied and objective one was achieved.

The second research objective of this study was to determine the optimum value for each factor with respect to biogas production from POME. The actual optimum value obtained from the experiment for OLR was 5.1 kg/m³/d; pH 6.6–7.1; C/N was 30.5; HRT was 6.5 days, and SRT was 13.7 days. The optimum output of biogas production was 3.0 L/d. Thus, the research question two was replied and objective two was achieved.

The third research objective was to develop an empirical model to predict the optimum level of biogas production from POME. By using experimental data and ANOVA, a multiple linear regression model was developed with all the variables, as represented by Equation 4.10. Data analysis and model development was done using Minitab Version 18.1 and Design Expert Version 2018 software. The model was validated by using 5 variables listed in Table 3.2, Figure 3.2(a) and Figure 3.2(b). The ANOVA outputs on actual and prediction model are presented in Figure 4.10 and Table 4.4. The validation results show that the actual value and predicted error is within 1.66% with a standard deviation 1.6. The model built to estimate biogas production from POME is fit to predict output of anaerobic reactor within

the error limit. Thus, the research question three was answered and objective three was achieved.

The application of an indigenous substrate such as banana peel to adjust the C/N would augur well for achieving higher COD reduction for biogas production from POME treatment. This application of closed digestion system of POME with efficient anaerobic reactor would be beneficial to palm oil mills by reducing its adverse effects on the environment, economy and society. This research concludes that future study on other factors that can contribute to COD reduction and biogas production such as up-flow velocity and temperature would be important to gain new knowledge in treating POME. Sources of COD need to be further identified in order to improve the current COD reduction and biogas production rate from POME. These studies will improve the current method of treating POME and a better COD reduction rate with higher biogas production can be achieved.

5.7 Recommendations for Future Study on Biogas Production from POME

The current study was conducted to develop an empirical model which focuses on six variables namely pH, carbon-to-nitrogen (C/N), organic loading rate (OLR), hydraulic retention time (HRT), sludge retention time (SRT) and biogas production from POME. Due to limited time and resources constrain, certain areas that affect the performance of anaerobic system of treating POME are not conducted in this study. Future studies in anaerobic treatment of POME in the following areas are suggested:

- From the literature reviews, there are other factors that can contribute to biogas production from POME namely up-flow velocity, digestion temperature, VSS concentration, and MLSS concentration. By including these factors, they can

contribute to give better outcomes such as higher biogas production and greater COD removal efficiency.

- In this study, the performance of the anaerobic system is analysed based on COD and VSS reduction. Other methods should be studied to measure the performance of the anaerobic system mainly biochemical oxygen demand (BOD), biogas production rate, methane content, and carbon dioxide content.
- POME has the potential to be a type of resource in which clean water, methane gas, and organic sludge can be recovered for use in achieving both economic and environmental sustainability in the palm oil industry. Other more efficient methods for recovering these potentials from POME should be developed for this purpose.

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APPENDICES

Appendix A: List of Publications

1. Islam, M. S., Shahnur, B., Lamoh, M. M., Hashmi, M. S., & Islam, M. S. (2018). The Role of Engineering in Mitigating Global Climate Change Effects: Review of the Aspects of Carbon Emissions from Fossil Fuel-Based Power Plants and Manufacturing Industries. *Reference Module in Materials Science and Materials Engineering*. <https://doi.org/10.1016/B978-0-12-803581-8.11274-3>
2. Shahidul, M. I., & Malcolm, M. L. (2017). *Advanced Eco-farming Technology to Achieve Sustainable Agriculture Growth in Sarawak*. UNIMAS.
3. Shahidul, M. I., Malcolm, M. L., & Eugene, J. J. (2018). Methane Production Potential of POME: A Review On Waste-To-Energy [WtE] Model. *Science International (Lahore)*, 30(5), 717–728.
4. Shahidul, M. I., Malcolm, M. L., Eugene, J. J., & Mamunur, R. (2018). Optimization of factors affecting biogas production from POME. *Science International (Lahore)*, 30(6), 851–859. Retrieved from <https://ir.unimas.my/22601/>
5. Shahidul, M.I., Malcolm, M.L., Eugene, J.J., & Sharafatul, M.I. (2019): A noval study of biogas production from palm oil mill effluent with two-stage anaerobic digester and nanomembrane, *Science International (Lahore)*, 31(6), 811-819, ISSN 1013-5316; CODEN: SINTE 8
6. Shahidul, M. I., Malcolm, M. L., S.J. Hashmi, M., & Alhaji, M. (2018). Waste Resources Recycling in Achieving Economic and Environmental Sustainability: Review on Wood Waste Industry. *Reference Module in Materials Science and Materials Engineering*. <https://doi.org/10.1016/B978-0-12-803581-8.11275-5>

Appendix B: Experimental Set-up

Appendix B1: 5 Litre Lab-Scale Reactor



Appendix B2(a): 350L CSTR Reactor



Appendix B2(b): Bioreactor



Appendix B2(c): Gas Flow Meter



Appendix B2(d): COD Reactor



Appendix B2(e): Biomass Measuring Scale



Appendix B2(f): Flow Meter



Appendix B2(g): pH Meter



Appendix B2(h): Air Compressor



Appendix C1: Method of Preparation for Inoculum

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Appendix C2: Output of CCD on Experiment Range and Level of Independent Variables

iew Display Options Design Tools Help



Central Composite Design

Each numeric factor is set to 5 levels: plus and minus alpha (axial points), plus and minus 1 (factorial points) and the center point. If categoric factors are added, the central composite design will be duplicated for every combination of the categorical factor levels.

Numeric factors: (2 to 50) ☒ Horizontal ☒ Enter factor ranges in terms of ± 1 levels

Categoric factors: (0 to 10) ☐ Vertical ☐ Enter factor ranges in terms of alphas

	Name	Units	Low	High	-alpha	+alpha
A [Numeric]	OLR	VSSg/L.d	3	7	0.243172	9.75683
B [Numeric]	pH		6	7.2	5.17295	8.02705
C [Numeric]	C/N		26	35	19.7971	41.2029
D [Numeric]	HRT	d	4	9	0.553964	12.446
E [Numeric]	SRT	d	10	17	5.17555	21.8244

Type: Blocks:

Points

Non-center points: 42

Center points: 8

alpha = 2.37841 50 Runs

Appendix C3: Biogas Outputs of 50 Runs with respect to Inputs of Five Variables

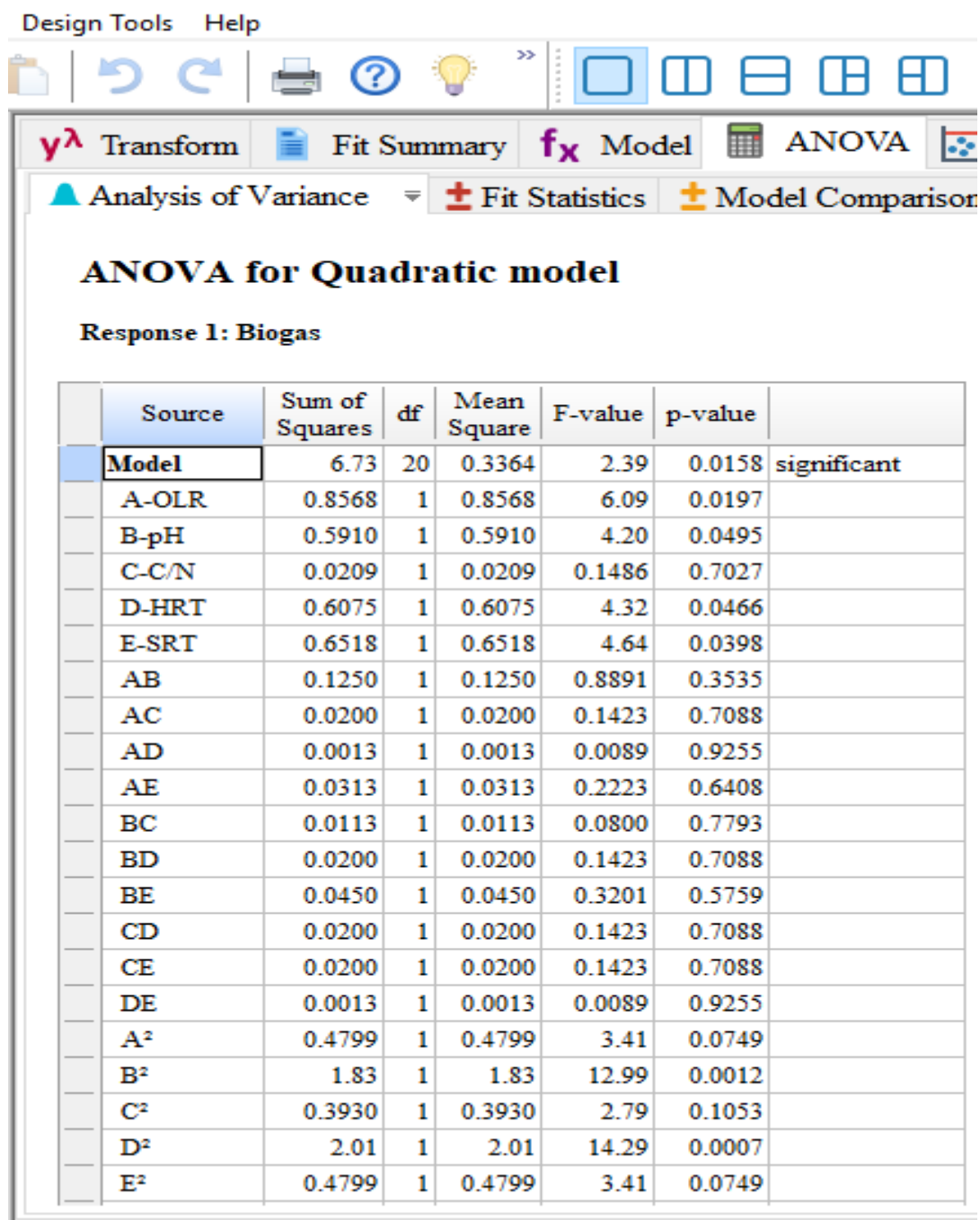
Design Tools Help

<

Appendix C3 continued

Design Tools		Help					
Design Layout		Column Info		Pop-Out View			
Std	Run	Factor 1 A:OLR VSSg/L.d	Factor 2 B:pH	Factor 3 C:C/N	Factor 4 DHRT d	Factor 5 E:SRT d	Response 1 Biogas L/d
33	29	0.243172	6.6	30.5	6.5	13.5	1.9
50	30	5	6.6	30.5	6.5	13.5	2.8
11	31	3	7.2	26	9	10	2.1
34	32	9.75683	6.6	30.5	6.5	13.5	3.2
24	33	7	7.2	35	4	17	2.6
30	34	7	6	35	9	17	1.8
21	35	3	6	35	4	17	1.9
49	36	5	6.6	30.5	6.5	13.5	2.5
27	37	3	7.2	26	9	17	2.6
29	38	3	6	35	9	17	1.9
13	39	3	6	35	9	10	1.8
16	40	7	7.2	35	9	10	2.4
40	41	5	6.6	30.5	12.446	13.5	2.7
28	42	7	7.2	26	9	17	2.6
20	43	7	7.2	26	4	17	2.5
12	44	7	7.2	26	9	10	2.2
48	45	5	6.6	30.5	6.5	13.5	3
14	46	7	6	35	9	10	1.8
9	47	3	6	26	9	10	1.6
10	48	7	6	26	9	10	2
8	49	7	7.2	35	4	10	2.1
6	50	7	6	35	4	10	1.7

Appendix C4(a): Output of DOE on ANOVA



Appendix C4(a) continued

dit View Display Options Design Tools Help							
yλ Transform Fit Summary fx Model ANOVA Diagnostics Model Graphs							
Analysis of Variance Fit Statistics Model Comparison Statistics Coefficients Coded Eq							
	DE	0.0013	1	0.0013	0.0089	0.9255	
	A ²	0.4799	1	0.4799	3.41	0.0749	
	B ²	1.83	1	1.83	12.99	0.0012	
	C ²	0.3930	1	0.3930	2.79	0.1053	
	D ²	2.01	1	2.01	14.29	0.0007	
	E ²	0.4799	1	0.4799	3.41	0.0749	
	Residual	4.08	29	0.1406			
	Lack of Fit	3.72	22	0.1690	3.29	0.0557	not significant
	Pure Error	0.3600	7	0.0514			
	Cor Total	10.80	49				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 2.39 implies the model is significant. There is only a 1.58% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, D, E, B², D² 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.

Appendix C4(b): Output of DOE on Fit Statistics

Design Tools Help

File Edit View Tools Help

Transform Fit Summary Model ANOVA

Analysis of Variance Fit Statistics Model Comparison

Fit Statistics

Std. Dev.	0.3750	R ²	0.6226
Mean	2.25	Adjusted R ²	0.3624
C.V. %	16.67	Predicted R ²	-0.5964
		Adeq Precision	5.5858

A negative **Predicted R²** implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 5.586 indicates an adequate signal. This model can be used to navigate the design space.

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

Design Tools Help

Transform
 Fit Summary
 Model
 ANOVA
 Diagnose

Analysis of Variance
 Fit Statistics
 Model Comparison Statistics

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.81	1	0.1316	2.54	3.07	
A-OLR	0.1406	1	0.0570	0.0241	0.2572	1.0000
B-pH	0.1168	1	0.0570	0.0003	0.2333	1.0000
C-C/N	0.0220	1	0.0570	-0.0946	0.1385	1.0000
D-HRT	0.1184	1	0.0570	0.0019	0.2350	1.0000
E-SRT	0.1227	1	0.0570	0.0062	0.2392	1.0000
AB	0.0625	1	0.0663	-0.0731	0.1981	1.0000
AC	-0.0250	1	0.0663	-0.1606	0.1106	1.0000
AD	-0.0062	1	0.0663	-0.1418	0.1293	1.0000
AE	-0.0312	1	0.0663	-0.1668	0.1043	1.0000
BC	0.0188	1	0.0663	-0.1168	0.1543	1.0000
BD	0.0250	1	0.0663	-0.1106	0.1606	1.0000
BE	0.0375	1	0.0663	-0.0981	0.1731	1.0000
CD	-0.0250	1	0.0663	-0.1606	0.1106	1.0000
CE	-0.0250	1	0.0663	-0.1606	0.1106	1.0000
DE	-0.0063	1	0.0663	-0.1418	0.1293	1.0000
A ²	-0.0929	1	0.0503	-0.1958	0.0099	1.05
B ²	-0.1813	1	0.0503	-0.2842	-0.0784	1.05
C ²	-0.0841	1	0.0503	-0.1870	0.0188	1.05
D ²	-0.1902	1	0.0503	-0.2930	-0.0873	1.05
E ²	-0.0929	1	0.0503	-0.1958	0.0099	1.05

Appendix C6: ANOVA on Biogas Outputs for both Actual and Prediction Model

Run Order	Actual Value	Predicted Value	Standard Error	Standard Deviation	Cook's Distance
1	1.8	1.95	-0.0015	0.00288922	0.011
2	3	2.81	0.0019	0.00288124	0.002
3	2	1.85	0.0015	0.00289813	0.012
4	1.9	2.12	-0.0022	0.00292021	0.025
5	1.6	2.06	-0.0046	0.00293438	0.206
6	2.5	1.5	0.01	0.00288494	0.977
7	2	2.11	-0.0011	0.00249638	0.006
8	3	2.81	0.0019	0.00252078	0.002
9	2.4	1.99	0.0041	0.00253135	0.166
10	2.5	2.81	-0.0031	0.00247238	0.005
11	2.1	2.06	0.0004	0.00246127	0.001
12	1.9	2.08	-0.0018	0.00249083	0.016
13	2.8	2.38	0.0042	0.00250963	0.171
14	2.8	2.71	0.0009	0.00243761	0.004
15	1.4	1.67	-0.0027	0.0024633	0.036
16	2	2.2	-0.002	0.00246424	0.02
17	1.3	1.45	-0.0015	0.00248133	0.022
18	1.8	1.83	-0.0003	0.00250793	0
19	2.1	2.33	-0.0023	0.00254667	0.027
20	2.7	2.57	0.0013	0.00255733	0.016
21	2.1	1.96	0.0014	0.00258706	0.009
22	1.7	1.61	0.0009	0.0026156	0.004
23	2.3	2.19	0.0011	0.00265393	0.006
24	2.3	2.43	-0.0013	0.00269047	0.008
25	3	2.81	0.0019	0.00273376	0.002
26	2.4	2.15	0.0025	0.00275436	0.033
27	2.4	2.28	0.0012	0.00275022	0.015
28	3	2.81	0.0019	0.00278844	0.002
29	1.9	1.95	-0.0005	0.00280377	0.002
30	2.8	2.81	-0.0001	0.0028697	0
31	2.1	1.98	0.0012	0.0029385	0.008
32	3.2	2.61	0.0059	0.00298481	0.336
33	2.6	2.5	0.001	0.00261918	0.005
34	1.8	2.19	-0.0039	0.00264661	0.076
35	1.9	2.02	-0.0012	0.00263517	0.007
36	2.5	2.81	-0.0031	0.00272104	0.005
37	2.6	2.4	0.002	0.0027552	0.021
38	1.9	2.16	-0.0026	0.00274043	0.033
39	1.8	1.99	-0.0019	0.00281397	0.018
40	2.4	2.51	-0.0011	0.00292309	0.007
41	2.7	2.01	0.0069	0.00306459	0.465
42	2.6	2.78	-0.0018	0.00177548	0.016

Appendix C6 continued

43	2.5	2.47	0.0003	0.00188157	0.001
44	2.2	2.48	-0.0028	0.00186689	0.04
45	3	2.81	0.0019	0.00196882	0.002
46	1.8	2.14	-0.0034	0.00130239	0.06
47	1.6	1.93	-0.0033	0.00131336	0.055
48	2	2.19	-0.0019	0.0012727	0.018
49	2.1	2.28	-0.0018	0.00155041	0.016
50	1.7	2.01	-0.0031	0.00218355	0.047
		Average=	-1.2E-05	0.00251108	