OPTIMIZATION OF FACTORS AFFECTING BIOGAS PRODUCTION FROM POME

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ABSTRACT: *This paper documents a study on biogas production from palm oil mill effluent (POME) by anaerobic digestion. The study aims to optimize biogas production from POME by employing the effect of optimal level of inputs and to develop an appropriate model to predict its processes. In this study, the continuous-stirred reactor was used to treat POME, where the inputs monitored, were Organic Loading rate, Hydraulic Retention Time and Sludge Retention Time. Waste fruit-based inoculum and NaOH were used to adjust Carbon to Nitrogen ratio and pH value. The novelty of this research was to harness the biogas production potential of hazardous POME in line with the waste to energy [WtE] concept. In order to come up with credible research design and analysis, DOE software was used. The findings in surface response diagrams of DOE manifested that the optimum inputs for maximum biogas production of 3.8L.d*$^{-1}$ *are; 5g L*$^{-1}$*d*$^{-1}$*for OLR, 28 for C/N and 6.5 days for HRT. The validation results of the developed model affirm that the overall error in model prediction is 1.51 percent with respect to actual outputs from the anaerobic reactor. Thus, this study justifies that further research on POME should be done at economic scale CSTR reactor using the optimum value of inputs for maximum productivity of biogas in order to contribute to achieving economic and environmental sustainability*.

Keywords: Hydraulic Retention, Sludge Retention, Biogas Production, Anaerobic Reactor, Production Optimization

1.0 INTRODUCTION

Biogas is an output of a biological process from anaerobic digestion of organic waste including biomass-enrich wastewater, manure, sewage sludge, municipal solid waste, and biodegradable feedstock [1–4]. The stages of decomposition of biosolids to biogas are hydrolysis, acidogenesis, acetogenesis, and methanogenesis [5, 6]. Raw biogas contains 50-65% biogas (CH_4), 30-45% carbon dioxide (CO_2), hydrogen sulphide (H_2S) and other impurities [7, 8].

With Malaysia being one of the largest palm oil producers in the world, which accounts for 17.73 million tons of crude palm oil (CPO) and 2.13 tons of palm kernel oil a year [8, 9]. Consequently, a huge amount of palm oil mill effluent (POME) produced to be known as a toxic and hazardous effluent for environment and health. The POME contains organic compounds (COD) and biomass-based volatile suspended solids (VSS) which both become the sources of biogas (CH_4) emission [9, 10]. It has been reported that the COD and VSS in POME are biogas potential elements [4]. In conventional POME treatment, the biogas is produced and emitted to atmosphere as GHG emission. It has also been reported that biogas emission is about 25-times higher global warming potential (GWP) compare to CO_2 [8, 11]. Despite, being POME a hazardous element to environment and health; it could be converted to biogas as a resource, which would contribute to achieving economic and environmental sustainability [9, 12]. The composition of POME-based biogas is listed in Table 1 [4, 13, 14], which reflects its biogas and methane potentials for capture and use for generating energy.

Table 1: Composition of Bio-gas [4, 13]

Element	Formulae	Composition (Vol. %)
Methane	CH_4	50 – 75
Carbon dioxide	CO_2	25 – 45
Water vapor	H_2O	2 – 7
Hydrogen Sulphide	H_2S	< 2.5
Other Gases	-	< 2

In this research, a two-stage anaerobic digester has used for optimizing biogas production by using waste fruit to inoculate the digestion process. Though several types of researches have been conducted for biogas production from POME; the optimization of factors that maximize productivity as well as inoculation of the digestion process with waste fruit had never been reported in published journals; ***in this regard, this research is Novel*****.**

1.1 The Chemistry of Biogas Production from POME

Thee digestion process begins with bacterial hydrolysis for breaking down insoluble long-chain polymers of fats, proteins, and carbohydrates into short-chain polymers. Then, acidogenic bacteria reduce the fatty acids, amino acids, and sugars into CO_2, H_2, NH_2, and organic acids. The acetogenic bacteria later convert these organic acids into acetic acid. Finally, methanogenic bacteria transform these products into gases which are mostly CH_4 and CO_2 [15], [16].

In order to have efficient biodegradation, the required nutrients shall be made available in the anaerobic reactor for microorganisms to build cells that produce biogas. The main chemical elements that would be utilised by microorganisms are carbon, oxygen, nitrogen, hydrogen, Generation of biogas requires an appropriate carbon-to-nitrogen ratio of at least 25:1 [17]. These stages are shown in Figure 1.

1.1.1 Biogas Production from POME

Hydrolysis Process - In this stage, the water of POME is broken down to form H^+ cations and OH^- anions. Water reacts with long-chain organic polymers including polysaccharides, fats, and proteins to form soluble shorter-chain polymers, such as Fatty Acids, Amino Acids and Sugars [19, 20]. The rate of breakdown depends on the composition of the substrate used in the anaerobic process [21–25]. Equation (1) shows the hydrolysis step of anaerobic digestion:

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + H_2 \quad \text{(Eq. 1)}$$

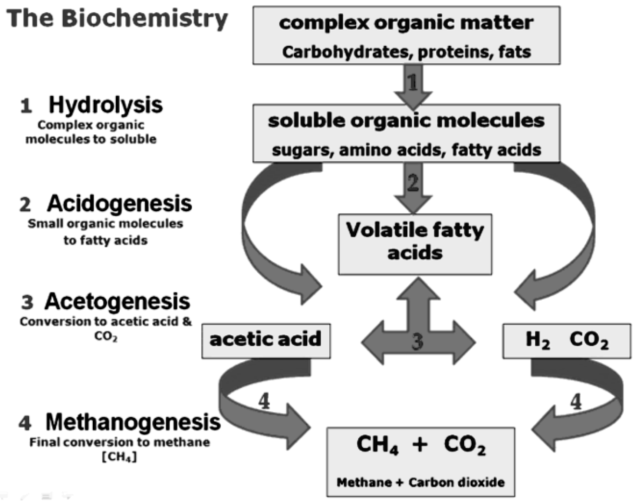


Fig. 1: Schematic Representation of Anaerobic Biodegradation [18]

Acidogenesis Process - A wide variety of different bacteria perform acidogenesis process by consuming available oxygen in an anaerobic digester. The fermentative bacteria produce an acidic environment in the digester to form organic products such as acetate, hydrogen, carbon dioxide, hydrogen sulphide, shorter volatile fatty acids, and carbonic acids [19]. Based on different populations of microorganisms, the acidogenesis process is divided into hydrogenation and dehydrogenation [26]. The hydrogenation involves with the hydrogen production [13]. The biogas is formed from acetate, H_2, and CO_2. However, the final products of this stage are sugars, long-chain fatty acids, and amino acids [21–25]. Equations (2), (3), and (4) present the acidogenesis step of anaerobic digestion:

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2H + 2CO_2 \quad \text{(Eq. 2)}$$

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \quad \text{(Eq. 3)}$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad \text{(Eq. 4)}$$

Acetogenesis Process - This is the pre-methanogenesis stage where Volatile Fatty Acid (VFA) and alcohols are produced by acetogenic bacteria [19]. VFA oxidize to form acetate, hydrogen, and carbon dioxide [27]; and hydrogen scavenging bacteria contributes to convert hydrogen to biogas [28]–[30]. Equations (5), (6), and (7) show the steps of acetogenesis in anaerobic digestion [21]–[25].

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad \text{(Eq. 5)}$$

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \text{(Eq. 6)}$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 3H_2 + H^+ \quad \text{(Eq. 7)}$$

Methanogenesis is the final step in anaerobic digestion. It utilizes the substrate formed during the previous stages such as acetic acid, carbon dioxide and hydrogen [26]. During the methanogenesis stage, biogas forms through two main routes. Firstly, the fermentation of acetic acid and acetophilic bacteria use acetic acid to produce biogas and carbon dioxide [31, 32]. Equations (8), (9), and (10) show the steps of methanogenesis [21–25]

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \text{(Eq. 8)}$$

$$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad \text{(Eq. 9)}$$

The secondary route uses hydrogen to reduce CO_2 to CH_4 by hydrogenophillic methanogens:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \text{(Eq. 10)}$$

Here, acetate, H_2, and CO_2 are key substrates for producing biogas. Based on stoichiometric relations, about 70% of biogas is produced from acetate, while the remaining 30% is produced from H_2 and CO_2.

1.2 Technology for Biogas Production from POME

Various technologies had been developed for producing biogas from POME. A few most successful and relevant models are cited here. Poh and Chong (2009) conducted a study on the performance of a UASB-HCPB reactor to evaluate the effects of Hydraulic Retention Time (HRT), organic loading rate (OLR) of volatile suspended solids (VSS) inputs on biogas production at thermophilic temperature (55°C). The results show that biogas production rate increased up to HRT 5 days at OLR range 4.28 g L^{-1} d^{-1}-9.19 g L^{-1} d^{-1}, and biogas production started to reduce after HRT 5 days with higher OLR than 13.75g L^{-1} d^{-1}. The experiment concluded that HRT, VSS, and OLR have a positive effect on biogas production; and the optimum level of these variables are important to optimize biogas gas production from POME [33].

Khemkhao et al. undertook a study entitled 'Simultaneous Treatment of Raw POME and Biodegradation of Palm Oil Fibre'. The performance of the reactor was tested with nine steps of OLR starting from 2.0 to 19.0 g $CODL^{-1}.D^{-1}$, where pH value was maintained at 7.0-8.0 to enhance methanogenesis. The findings demonstrated that biogas production increased with OLR, and declined when OLR reached up to 19.0g.$CODL^{-1}.D^{-1}$ [18]. The findings conclude that OLR has a positive effect on biogas production.

Krishnan *et al.* documented a study on POME entitled 'Effect of Organic Loading Rate on Hydrogen and Biogas Production in Two-stage Fermentation under thermophilic conditions'. The result demonstrated that at OLR of 12 kg COD per 1000 L of POME, the COD conversion efficiency to biogas was 85 percent. With this experiment, CH_4 content in biogas was 68 percent [34].

Choi *et al.* have conducted a study on POME using Combined High-rate Anaerobic Reactors. The experiment was conducted at a mesophilic temperature of 35-37°C, with adjusted pH 7.0. The maximum level of biogas production was achieved at 110 L d^{-1} at an OLR of 18.9 kg.d^{-1} per 1000 L of POME [35].

Malakahmad and Yee reported a study on energy production from POME by using an Anaerobic Baffled Reactor. The reactor was operated with a constant HRT of 4 days while processing the temperature and the pH was kept at 35°C and 7.2 respectively. The system achieved 82 percent COD removal efficiency at an OLR of 11.38 $gL^{-1}.d^{-1}$. The biogas yield improved from 0.05 $L.g^{-1}$ of COD to 0.25 $L.g^{-1}$ COD [36].

1.3 Factors Effect on Biogas Production

Biogas production from anaerobic digestion depends on COD decomposition and VSS degradation rate. It indicates that biogas potential depends on loading and decomposition rate of COD and VSS in an anaerobic reactor. The process performance of the anaerobic reactor depends on a few factors related to digestion process performance. The factors are HRT, SRT, OLR, pH, Carbon-to-Nitrogen Ratio (C/N), Toxicity and H_2S. The parameters influencing HRT is presented in the Equation (11) below:

$$HRT(t) = \frac{V}{Q} \qquad \text{(Eq. 11)}$$

Where, V = Volume of Reactor (m^3). Q = POME Influent flow rate (m^3hr^{-1}). HRT plays a vital role in decomposing VSS and COD to biogas. If HRT is less than the required time, biogas yield will be less than potential, and if HRT is higher than required time, reactor size needs to be larger which requires higher investment cost to build it [4,8,17].

Whereas, the parameters influencing SRT is presented in equation (12) below:

$$SRT(t) = \frac{X_i V_i}{Q_x X_x} \qquad \text{(Eq. 12)}$$

Where, X_i = Mixed liquor suspended solids (MLSS) in each reactor (mg/L). V_i = Individual reactor volume. Q_x = excess bio-solids removal rate (m^3/d). X_x = MLSS in the excess bio-solids flow (mg/L). The conversion efficiency from VSS and COD to biogas production significantly depends on sludge age [4, 8, 17].

The pH and Alkalinity Control - Process Alkalinity is a factor to maintain pH value in the reactor; in order to inhabit rapid pH drop, Alkalinity level is maintained between 2000mg/L to 3000 mg/L[4,8].

OLR Control - OLR is the number of volatile solids and COD loading rate into an anaerobic digester each day. OLR affects the performance efficiency of digestion to produce biogas. OLR is maintained between 1-11kg.$D^{-1}m^{-3}$ [37]

The Toxicity Control - Methanogens are sensitive to the toxicity of NH_3, H_2S, and VFAs, which depends on the pH of the substrate. In un-adapted cultures, a free level of above $NH_3$150mg/L POME can inhibit methanogen growth. The NH_3 is toxic at pH levels greater than 7.5 and lower than 6.0. H_2S and VFAs are also toxic at pH levels less than 6.0.

The H_2S Control - The concentration of H_2S shall be maintained within 200mg/L POME in order to continue methanogen bacteria growth [25].

The C/N Control - In order to have efficient biodegradation, nutrients level for microorganisms shall be maintained. Generation of biogas requires a range of carbon-to-nitrogen ratio from 20:1 to 40:1 [25].

1.4 Findings on Literature Review on Technology Used for Biogas Production from POME

Due to the high solids and oil content in POME, it is a great challenge to digest POME with UASB and EGSB reactors. In producing biogas from POME, pre-treatment facilities should be installed. Palm-oil mills typically use continuously-stirred tank reactors (CSTR), which are less expensive when compared to the other digesters, besides being relatively simple to operate and maintain. The process could be done under mesophilic or thermophilic temperatures, with either mechanical, hydraulic, or gas-injection mixing. Though biogas could be produced from POME with different anaerobic reactors, the optimization of biogas production depends on several controlling factors mostly OLR, HRT, SRT, and pH and C/N. Hence, the optimization of these factors would play a vital role in making the process economically and environmentally sustainable.

2.0 PROBLEM STATEMENT AND OBJECTIVES

Many advanced technologies have been developed to solve problems related to carbon emission from POME. Despite the implementation of such advanced technologies, a part of the problem exists in palm oil mill domain such as optimizing biogas production by optimizing the factors that affect biogas production process. However, it has been stated that palm oil mills are struggling to reduce carbon emission in order to mitigate its effects on environment and health. This prevailing predicament infers that a research gap exists in palm oil mill domain. Thus, this research has been formulated to optimize biogas production from POME by optimizing the factors that affect the anaerobic digestion process. Indeed, this research attempts to address the question of ***'how to optimize biogas production by optimizing factors that affect anaerobic digestion process?'.***

2.1 Objectives of Research

The broad objective of this study was to optimize biogas production from POME. To achieve this goal, the broad objective has been divided into the following specific objectives:

2.1.1 Optimization of Factors that affect on Biogas Production

The scope of work of this part of the study is related to the objective one stated in section 2.1.1. This section will 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 the research was to determine optimum level factors that contributed to optimizing biogas production from POME. To achieve this goal, data were analyzed by Design Expert (Version - 2018).

2.1.2 Model Development to Predict Optimum level of Biogas Production from POME

The scope of work of this part of the study is related to objective two stated in section 2.1.2. This section will answer the research question of "**What is the Model of output as a response (biogas) from POME that shows the result for producing Biogas?"**. The investigation under this section of research was to determine Model Building that contributed to the optimum amount of biogas from POME. To achieve this goal, data were analyzed by DOE (Version - 2018).

3.0 METHODOLOGY

The research methodology has four components: At the first part, the experimental setup at the laboratory. The second part is the POME collection from palm oil mill for conducting the experiment at the laboratory. The third part is a collection of required data from the experiment for analysis in order to achieve research goal. At the final stage, model estimate by using experimental data and report writing.

To achieve the stipulated research objectives, relevant equipment and machinery were selected and organized for this experiment, comprising feed tanks, feed pumps, CSTR type anaerobic reactor, flow switch (FS), and effluent collection tank as shown in Figure 2.

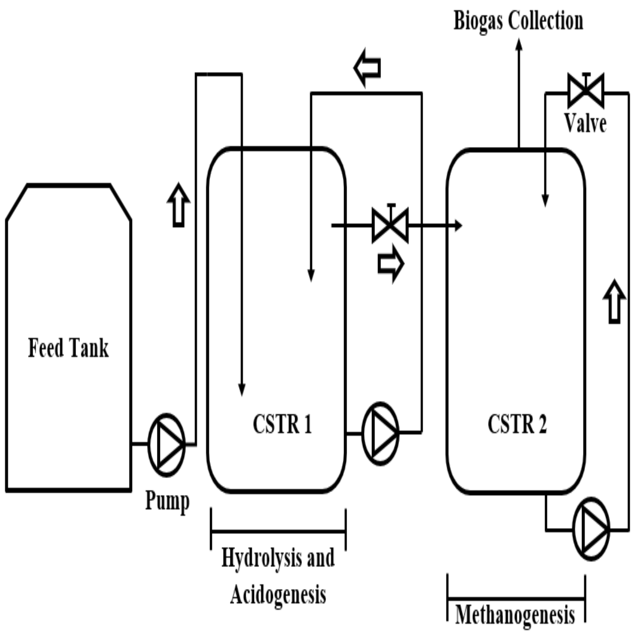


Fig. 2: Experimental Setup at Laboratory

3.1 Operating Procedure of CSTR

The batch digestion of biogas production from POME was performed by using two-stage CSTR; the capacity of each CSTR was 5L and 10% (v/v) of inoculum. The substrates were used in different doses as which listed in Table 3. The substrate and inoculum were mixed in the feedstock. The pH value was adjusted between 6-7.8 by using 5M NaOH [37]. The biogas production was measured on a daily basis. The batch fermentation in the reactor was carried out for 12 days for each run. The entire study was carried out at ambient temperature (33 ± 3 ºC) and then slowly circulate with a centrifugal pump for mixing the organic contents. At the beginning of the experiment, the headspaces of the reactor were flushed with POME in order to remove the oxygen content to ensure anaerobic condition. The volume of biogas was measured daily in every 24 hours of operations. All the experiments were carried out in triplicate and the results were expressed as means.

3.2 Characterization of Feedstock

The waste fruit was used to prepare inoculum and to maintain the C/N ratio from 20 to 40. The mesh size of the skin was converted to less than 1.0 mm and kept 30 days at atmospheric temperature before it was put into the feedstock. The weight of inoculum ware adjusted for each run of the experiment, as shown in Table 2.

Table 2: Characterization of Feedstock

Item(g/L)	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

3.3 Research Variables

The dependent variable of this study is biogas gas production. This variable depends on COD and VSS digestion efficiency in CSTR anaerobic reactor under the effects of HRT, SRT and C/N. pH, process temperature and OLR. Hence, these factors are the independent variables of this research.

3.4 Experimental Design and Optimization Method

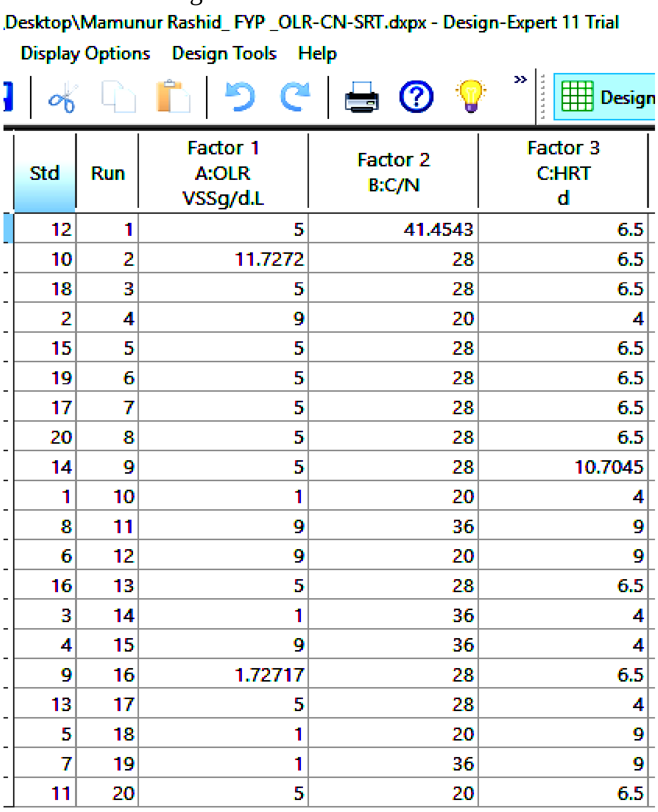
In this study, the statistical graphics Design of Expert software DOE Version–2018 was used, which include Central Composite Design (CCD) option. The CCD is used to estimate the level of inputs and to determine the optimum number of the experimental run to achieve the highest biogas production from the anaerobic digestion process at a minimum resource.

Response surface methodology (RSM) is a statistical technique used in this study to optimize inputs and outputs of CSTR. The level interactions among the factors related to biogas production from POME were estimated by DOE [38]. Productivity improvement in the production process is an important factor [39]. In order optimize biogas production; the process design and anaerobic reactor operations must meet required conditions of higher productivity[39]. Based on this fundamental, the levels of experiment were estimated by using CCD of DOE as tabulated in Table 3.

Table 3: Outputs of DOE on CCD for Experimental levels.

Variable	Code Variable Level				
	-α (1.6820	-1	0	+1	+α (1.6820
X_1(ORL)	-1.72	1	5	9	14.49
X_2(C/N)	-14.54	20	28	36	41.45
X_3(HRT)	2.29	4	6.5	9	10.7

As per the DOE and CCD method, the run 20 at 5 levels were selected. Each independent variable divided into five levels by CCD as stated -α, -1, 0, +1, and +α, respectively, which listed in Figure 3.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Std	Run	Factor 1 A:OLR VSSg/d.L	Factor 2 B:C/N	Factor 3 C:HRT d
12	1	5	41.4543	6.5
10	2	11.7272	28	6.5
18	3	5	28	6.5
2	4	9	20	4
15	5	5	28	6.5
19	6	5	28	6.5
17	7	5	28	6.5
20	8	5	28	6.5
14	9	5	28	10.7045
1	10	1	20	4
8	11	9	36	9
6	12	9	20	9
16	13	5	28	6.5
3	14	1	36	4
4	15	9	36	4
9	16	1.72717	28	6.5
13	17	5	28	4
5	18	1	20	9
7	19	1	36	9
11	20	5	20	6.5

Fig. 3: Experimental Runs with Combination

3.5 Model Development Method

To perform this research, DOE has been used to develop experimentation strategy using minimum resources by investigation all factors and their interactions [40]. The response from 3D surface was used to relate to the independent factors in a quadratic model consisting of linear, two factorials, and quadratic shown in Eq. (13).

$$Y = \beta_0 + \sum\beta_1X_1 + \sum\beta_2X_2 + \sum\beta_3X_3 + \sum\beta_{12}X_1X_2 + \sum\beta_{23}X_2X_3 + \sum\beta_{13}X_1X_3 + \sum\beta_{11}X_1^2 + \sum\beta_{22}X_2^2 + \sum\beta_{33}X_3^2 + \varepsilon \quad \text{(Eq. 13)}$$

In this polynomial equation, Y is the predicted response as biogas production. The organic loading rate X_1(ORL), C/N ratio (X_2), and HRT (X_3) were chosen as shown in Table 3. The β_0 is the offset term as the intercept of the model equation; β_1, β_2, and β_3 are the linear coefficients of independent variables, the quadratic coefficient and interaction coefficient. The regression model was estimated and ANOVA analysis was performed by DOE (Version 18) as well. The quality of the model was expressed by the value of correlation coefficient (β_1, β_2, and β_3) and R^2. Model term was evaluated by probability (P-value) with 95% confidence level. The model also described the interactions among the factors (X_1, X_2, and X_3) which influence the output variable by varying them concurrently.

3.6. Process Variable Control Method

The digestion process was designed to maintain the environment inside the anaerobic reactor to promote the smooth growth of methanogens bacteria for optimized biogas production. As methanogens are sensitive to toxicity of NH_3, H_2S, and VFAs; the toxicity level was maintained by controlling the pH of the substrate. The free NH_3 level of 150 mg/L in the digestion of POME was maintained by controlling pH between 7.5 and 6.0. The pH level beyond this limit also contributes to increasing toxicity level due to H_2S and VFAs. The concentration of H_2S was kept within 200 mg/l in order to maintain methanogen bacteria growth [17]. As methanogenic bacteria are also sensitive to higher oxygen concentration, it is necessary to control the resulting oxygen level inside the digester throughout the experiment.

In order to have efficient biodegradation, nutrients level for If we need to have efficient biodegradation, nutrients level for microorganisms must be regulated by adjusting the C/N ratio in the digestion process. In generating biogas, a range of carbon-to-nitrogen ratio from 20:1 to 40:1 was recommended [17]. Process Alkalinity is an important factor in maintaining pH value in the reactor. In order to ensure inhabitation of fast pH drop, alkalinity level was maintained between 2000 mg/L to 3000 mg/L.

3.7 Sample Collection Procedure

Fresh POME collected from Bau Palm Oil Mill, located nearby Kuching of Sarawak. A number of 25-liter high-density polyethylene (HDPE) containers were used to transport POME from mill to the operations research laboratory at Universiti Malaysia Sarawak.

3.8 The Properties of Intendant Process Variables

The biogas production process had divided into two parts. The first part was for Hydrolysis and Acetogenesis, which took place at CSTR1. The second part has taken place at CSTR2 to be known as methanogenesis . These processes are displayed in Figure 2 and the process variables are listed in Table 4.

Table 4: Stages of Experiment and Chemical Process

Stages of Process	Process Control Parameters
Fermentation Hydrolysis and Acidogenesis Process at CSTR 1 [19], [20]	pH = 4.0- 5.5 Temperature = 35^{o}C HRT = 5.0 days OLR = 1-11kg.$D^{-1}.m^{-3}$ C/N = 20-40
Anaerobic Reactor CSTR 2 The methanogenesis [28]–[30]	pH = 6.5-7.5 Temperature = 35^{o}C HRT = 5-10 days OLR = 1-11kg.$D^{-1}.m^{-3}$ SRT = 15-22 days C/N = 20-40

The SRT at digestion was 15-22 days as guide given by Verma et al.,(2002); and Zupančič and Grilc (2012) [42], [43]. The organic loading rate was 1.0kg.$m^{-3.}$ $D^{-1}$of POME [42, 43]. The sludge was recycled into the feed tank to increase the contact time in order to hasten the decomposition speed, this process was also used by Verma et al., (2002 [42]; and Poh and Chong (2009) [44]. The effluent and sludge were collected through a pipe as shown in Figure 3.

4.0 RESULTS AND DISCUSSION

The total process was carried out in two stages anaerobic digestion. At the first stage, it was performed at CSTR1, and HRT was from 2-5 days. At the second stage, the outputs of CSTR1 were fed to CSTR2 and HRT was maintained as per experiment design stated in Table 3. The total run and combination with variables were maintained per the CCD and DOE software outputs, which were listed in Table 4. The digester was prepared and start-up work was completed as per the experiment design mentioned in research methodology section 3.1.

4.1 Optimization of Factors that Affect on Biogas Production

The scope of work of this part of the study was related to the objective one stated in section 2.1.1. This section will answer the research question of "What are the optimum levels of inputs to POME digestion process that effect to produce Biogas?". Indeed, the investigation under this section of the research was to determine optimum level factors that contributed to optimizing biogas production from POME. To achieve this goal, data were analyzed by DOE (Version - 2018). The findings of data were analysed with respect to inputs listed in Figure 4 and Figure 5. The 3D surface response diagrams show that the optimum value of inputs for OLR was 5g. L^{-1}. $d^{-1;}$ for C/N was 28; and for HRT was 6.5 days, and the optimum biogas production was 3.8.d^{-1}. This finding indicates that if an anaerobic bioreactor setup and operate with these optimum inputs, the optimum level of biogas would be produced.

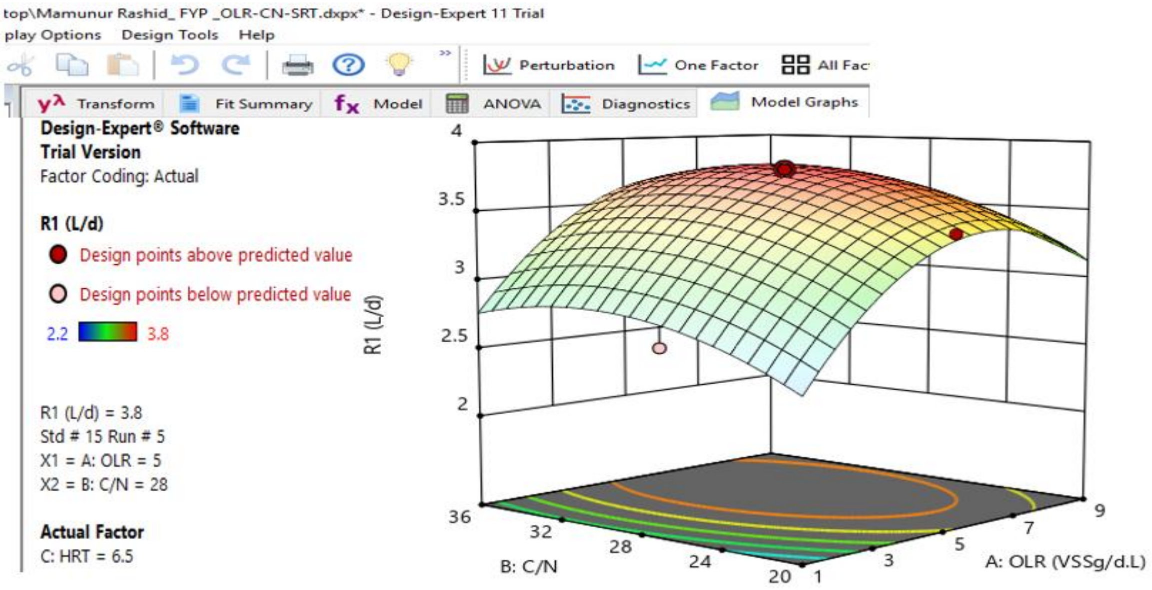

Fig. 4: Optimum Level of Biogas Production with Respect to Optimum Level of OLR and C/N as Inputs

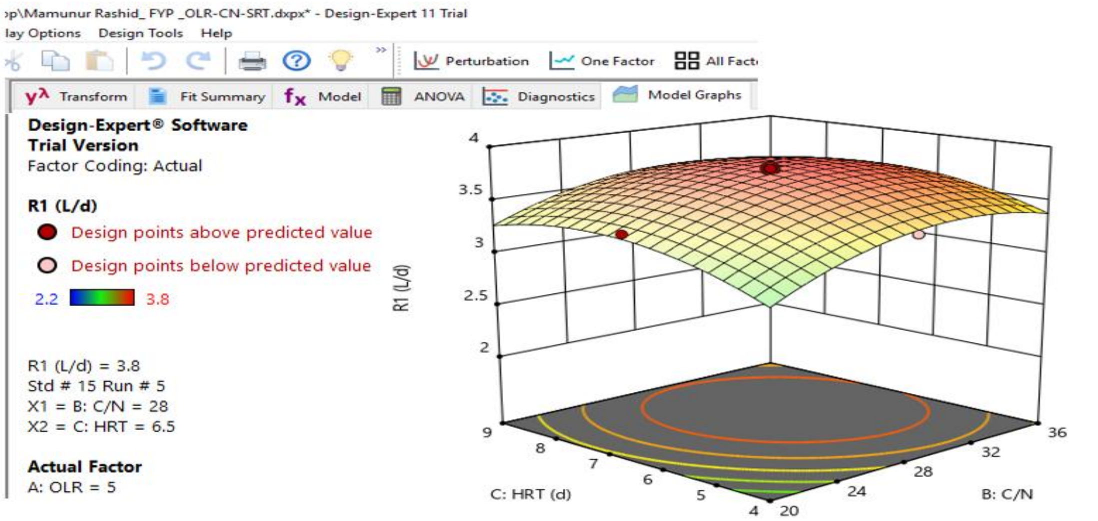

Fig. 5: Optimum Level of Biogas Production with Respect to Optimum Level of OLR and HRT as Inputs

4.1.1 Conclusion and Answer to the Research Question One

Bioreactors were run as per the combinations, shown in Figure 3 and Table 3. The summary of finding on optimum inputs and optimum outputs are shown in Figure 4 and Figure 5. Based on the findings, it can be deduced that if bioreactor run with POME as per the value stated in Figure 4 and Figure 5, it would contribute to producing the optimum level of biogas. In conclusion, it can be stated that if CSTR 1 and CSTR 2 were operate at the optimum value of factors such as 5g. L^{-1}. d^{-1} 5 for OLR, 28 for C/N and HRT 6.5, it would contribute to producing an optimum level of biogas. Thus, objective number one of the research is achieved and the research question one is replied.

4.2 Model Development to Predict Optimum level of Biogas Production from POME

The scope of work of this part of the study was related to objective two stated in section 2.1.2. 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 variables listed in Table 3 and Figure 3. Indeed, the DOE (Version- 2018) was used for data analysis and model development.

4.2.1 Data Analysis and Model Development

The results of the experiment were analyzed and findings were presented in 3D plot as in Figure 4 and Figure 5; basically, these are the outputs of DOE (Version-2018). The statistical quadratic model outputs of DOE (Version-2018). The statistical quartic model was developed by using information from Table 3 and Figure 3 and, which shown by Equation (14):

$$Q_{(Biogas)} = 3.74 + 0.3306X_1 + 0.1473X_2 + 0.0888X_3 - 0.0125X_1X_2 + 0.0122X_1X_3 - 0.0375X_2X3 - 0.5742(X_1)^2 - 0.2418(X_2)^2 - 0.2174(X_3)^2 \quad \text{Eq. (14)}$$

Where $Q_{(Biogas)}$: is the response, *X1* is OLR, X2 is C/N, and X3 is HRT are factors in DOE code. The model summary from ANOVA is listed in Table 5.

4.2.2 Model Summary

The model summary that was developed by the DOE is listed in Table 5.

Table 5: Model Summary

Factors	Coefficient	F-Value	p-Value
Intercept	3.74	8.86	0.0001*
X1(OLR)	0.33	16.60	0.0022*
X2(C/N)	0.14	3.53	0.08**
X3(HRT)	0.088	1.28	0.280**
X1.X2	0.0125	0.0193	0.8924**
X1.X3	0.0122	0.0193	0.8924**
X2.X3	0.0375	0.1734	0.6859**
$X1^2$	2.7	41.63	0.0001*
$X2^2$	0.5066	7.81	0.0190*
$X3^2$	0.4095	6.31	0.0308*
R^2=0 .8886, Coefficient of Variance (CV)=8.05			

The R-squared of 0.8886 revealed that the model could explain 88.86% of the variability in the Response. For a good statistical model, the R^2 should be in the range of 0.75–1.0 which indicates a good fit of the model [44]. The relatively high value of R^2 indicated that the quadratic equation could be used for getting a precision estimate. The coefficient of variation of 8.05% confirmed good precision and reliability outcome of this experiment.

4.2.3 Model Validation

The developed model was validated with actual outputs from anaerobic reactor and model estimate by variables listed in Table 3 and Figure 3. The distribution of model prediction and actual biogas production are presented in Figure 6.

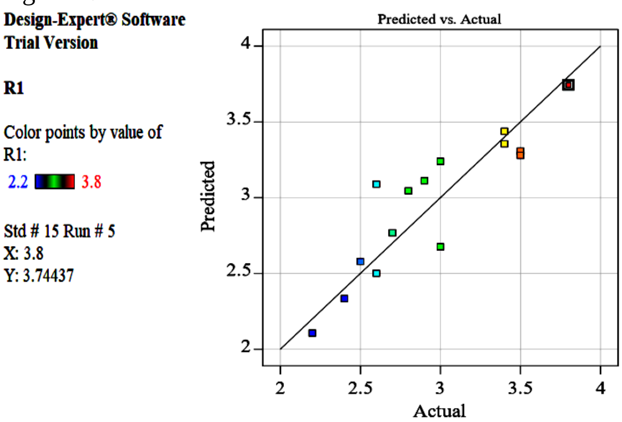

Fig. 6: Model Validation

Figure 6 demonstrates the model validation result of 20 experimental runs, which shows the average error in actual 3.8 (X:3.8, Figure 6) and in predicted 3.744 (Y:3.744, Figure 6). Indeed, the overall error in model prediction is 1.51 percent with respect to actual outputs from the anaerobic reactor. This finding demonstrated that biogas prediction model that presented in Equation (14) is useful in predicting outputs of an anaerobic reactor.

5.0 CONCLUSION AND DISCUSSION

The broad objective of this research was to determine the optimum level of three factors (OLR, C/N, and HRT) that contribute to producing the optimum level of biogas. To achieve this goal, CSTR type anaerobic reactor was run as per the combinations as shown in Figure 3. The inputs-outputs of experimental run presents by Figure 4 and Figure 5. The ANOVA was run to develop an empirical model, which shown by Equation (14). The model was validated by using data listed in Table 3 and Figure 3. The ANOVA outputs on actual and model prediction are presented in Figure 6.

The findings of surface response diagrams demonstrated that the optimum input for OLR is 5g. L^{-1}. d^{-1}; for C/N is 28; and for HRT is 6.5 days, and the optimum biogas production was 3.8.d^{-1}. These findings indicate that if an anaerobic bioreactor setup and operate with these optimum inputs, the optimum level of biogas would be produced. The validation results show that the overall error in model prediction is 1.51 percent with respect to actual outputs from the anaerobic reactor. These findings are very similar to the experiment conducted by Wong et al. (2013) [45] and Khemakhao et al. (2015) [46].

In conclusion, it can be stated that the model built to estimate biogas production from POME is quite fit to predict the output of the anaerobic reactor. The outcomes of this research with the results of validation demonstrated that the broad objective of this research has been achieved. Thus, this study recommends that further research should be carried out on POME treatment with economic-scale CSTR reactor by employing the optimum value of inputs for maximum production of biogas in order to contribute to achieving economic and environmental sustainability.

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