

OPTIMIZATION OF BIOGAS PRODUCTION FROM POULTRY MANURE WASTEWATER IN 250 ML FLASKS

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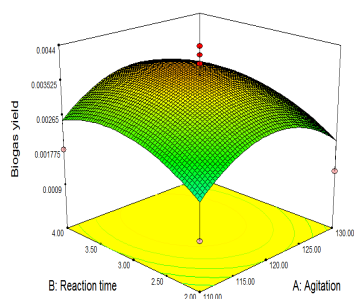
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Graphical abstract



Abstract

A research was conducted on anaerobic digestion from poultry manure wastewater to produce biogas. This research was considered as a triumph to the concept of waste-to-wealth. The poultry manure collected was characterized and pre-treated to remove excessive ammonia-N which caused inhibition to the biogas production. Central Composite Design (CCD) with five replicates at centre points was used to investigate the simultaneous effect of the variables: agitation (110-130 rpm) and reaction time (2-4 days) on the biogas production. Then, the experiment was designed and analyzed using Design Expert V7.0 software by applying response surface methodology (RSM) concept. The biogas production performance was evaluated on the basis of biogas yield from initial Chemical Oxygen Demand (COD) and was found ranged from 0.49 to 4.37 mL/g COD. Quadratic model was well fitted ($R^2 > 0.80$) with a confidence level higher than 95%. The optimum biogas production condition was at agitation: 120 rpm and reaction time: 3.3 days. Under this condition, 4.45 mL/g COD of biogas yield was obtained. This counted for 5.82% error from predicted values.

Keywords: Optimization, biogas production, poultry manure wastewater, Central Composite Design (CCD), anaerobic digestion

Abstrak

Penyelidikan telah dijalankan pada pencernaan anaerobik dari najis ayam untuk menghasilkan biogas. Penggunaannya untuk menghasilkan biogas akan dianggap satu kejayaan kepada konsep menukar sisa kepada hasil berharga. Najis ayam yang diperolehi telah melalui proses pencirian dan pra-rawatan untuk mengurangkan ammonia-N yang berlebihan yang akan menyebabkan perencatan dalam penghasilan biogas. Reka Bentuk Komposit Pusat (CCD) dengan lima replikasi di tempat pusat telah digunakan untuk mengkaji kesan serentak pembolehubah: pengadukan (110-130 rpm) dan masa tindak balas (2-4 hari) terhadap penghasilan biogas. Eksperimen telah direka dan dianalisis oleh perisian Design Expert versi 7.0 menggunakan metodologi permukaan tindak balas (RSM). Prestasi penghasilan biogas telah dinilai berdasarkan hasil biogas daripada Keperluan Oksigen Kimia (COD) awal yang didapati dalam julat antara 0.49 hingga 4.37 mL/g COD. Model kuadratik dapat mewakili data eksperimen dengan baik ($R^2 > 0.80$) dengan tahap keyakinan yang lebih tinggi daripada 95%. Penghasilan biogas optimum adalah menggunakan pengadukan: 120 rpm dan masa tindak balas: 3.3 hari. Di bawah keadaan ini, 4.45 mL/g COD hasil biogas diperolehi. Ini diambil kira untuk ralat 5.82% daripada model yang diramalkan.

Kata kunci: Pengoptimuman, penghasilan biogas, najis ayam, Reka Bentuk Komposit Pusat (CCD), pencernaan anaerobik

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1.0 INTRODUCTION

The daily production of laying hen manure can be approximated with 138 g/day (25% dry substance) and 90 g/day (40% dry substance) of a broiler [1]. A proper disposal management of poultry manure still offers a valuable treat to industry in order to sustain the in house sanitation and meet the latest environmental regulations [2]. To overcome this problem, anaerobic digestion (AD) was suggested for the poultry manure treatment. AD is advantageous with its positive energy balance and it would result in smaller quantities of biomass sludge generation compares to aerobic treatment. For these reason, the method is increasingly used [3]. However, in order to understand the process dynamic and reactors configurations of AD, several studies were carried out so that the technology could be applied effectively at the poultry farm [4-5].

According to Bezerra *et al.* [6], central composite design (CCD) is the most utilized design of optimization for the development of analytical procedures compared to the others as their low efficiency of the latter especially for a numbers of variables. The relationships between predicted and experimental results were illustrated and analyzed by CCD. In this software, the goodness of fit was determined by coefficient of determination, R-squared, while the statistical significance of the regression model was checked by the Fisher statistical test (F-test) in analysis of variance (ANOVA) [7]. Effects with a confidence level higher than 95 % (p-value less than 0.05) were preferable to represent the reliability of a result [8]. Mohd Salleh *et al.* [9] carried out the optimization process by comparing CCD and full factorial design (FFD). The R-squared obtained were 0.998 and 0.96 for CCD and FFD respectively. This implies that CCD has the higher accuracy compared to others such as FFD.

2.0 MATERIALS AND METHODS

2.1 Collection of Samples

There were two types of samples involved in this research work. The first sample was raw poultry manure as substrate and second one was poultry soil as source of inoculums. Raw poultry manure was collected from a moderate size poultry farm located at Gambang, Kuantan, Pahang (Malaysia). The poultry soil on the other hand, was collected just besides the poultry barn where the raw poultry manure was taken. Samples were collected in bulk plastic container and then stored in an industrial type refrigerator at 4°C.

2.2 Preparation of Poultry Manure Wastewater

In order to maintain the moisture consistency, the poultry manure sample collected was mixed thoroughly with distilled water at a feed ratio of 1:1 for 5 to 10 minutes to produce poultry manure wastewater (PMW). After that, it was kept in freezer at 4°C to minimize odour and substrate decomposition before experiment [10]. Demirci and Demirer [11] reported that nutrients content in the manure can be sufficient for anaerobic microbial growth if sufficient amount of water is present. According Fernandez *et al.* [12], significant quantities of biogas will not be produced if the substrate not diluted. The PMW was then characterized and kept at 4°C until further used in order to prevent prior fermentation of the organic substrate.

2.3 Characterization and Pre-treatment of Substrates

The PMW used as model substrate was tested for its biochemical characteristics as presented in Table 1. After characterization, PMW was gone through pre-treatment processes to remove excessive ammonia-N which might cause inhibition on biogas production.

The type of soil collected for pre-treatment purpose, namely peat soil (PS) was kept frozen just prior to use. Upon pre-treatment, PS was mixed thoroughly for 5 to 10 minutes with distilled water to produce soil water (SW). Previous screening study suggested that the best pre-treatment condition using PS to distilled water of 1:6 ratio to produce SW, and SW to PMW at 1:4 ratios without agitation for 5 hours reaction time [13].

The pre-treatment experiment was conducted at laboratory scale study by using 250 ml conical flask under aerobic condition. The flask was filled with PMW first, and then the SW was added to pre-treat PMW. Ammonia-N and COD concentration were determined by using HACH Spectrophotometer DR/2800 following Method 8155 and Method 8000, respectively with similar dilution factor of 10.

2.4 Preparation of Inoculums

Soil used in soil mixed culture (SMC) preparation was different from PS used for pre-treatment part. The soil used in SMC was poultry soil collected besides the poultry barn. The poultry soil was mixed thoroughly with distilled water at a ratio of 1:6 for 5 to 10 minutes to produce SMC. Treated PMW was acclimatized with SMC anaerobically at substrate inoculums ratio (SIR) of 0.25 in 5 litres plastic digester. The hydraulic retention time (HRT) for acclimatization was 30 days under ambient temperature. This produced inoculums to be used in the AD process. The inoculation of the fresh material with either digested material or the liquid fraction from the reactor was used by most reactors to minimize washout of microorganisms [14].

Table 1 Test method for characterization of poultry manure wastewater

No.	Parameter	Unit	Test method
1	pH	-	Standard Methods APHA, 1998
2	Suspended solid (SS)	mg/L	Standard Methods APHA, 1998
3	Biological oxygen demand (BOD)	mg/L	Standard Methods APHA, 1998
4	Chemical oxygen demand (COD)	ppm	HACH Spectrophotometer Method 8000
5	Ammoniacal nitrogen (AN)	mg/L	HACH Spectrophotometer Method 8155
6	Nitrate	mg/L	HACH Spectrophotometer Method 8171
7	Nitrite	mg/L	HACH Spectrophotometer Method 8153
8	Phosphorus	mg/L	HACH Spectrophotometer Method 10127

2.5 Optimization of Biogas Production from PMW

The design of experiment (DOE) for optimization in this study was response surface methodology (RSM), which generated by CCD in Design Expert Software Version 7.1.6 (Stat-Ease Inc., Minneapolis, MN, USA). The software applies important statistical and

mathematical methods to find the best model to describe the response data. A three dimensional surface graph for the responses will be modelled out where the optimization point can be easily obtained from [15]. The experimental design by CCD was summarized in Table 2.

Table 2 Independent variables involved in CCD

Factor	Symbol	Unit	Level				
			(-α)	(-1)	(0)	(+1)	(+α)
Agitation	A	rpm	100	110	120	130	140
Reaction time	B	days	1	2	3	4	5

* α=2

2.6 Experimental Set Up and Analysis

Laboratory scale experimental set up was prepared at Environmental Laboratory of Civil and Earth Resources Engineering, University Malaysia Pahang. Upon experimental start-up, substrates were poured before inoculums into 250 ml of conical flasks. The SIR was 0.25. This fact was supported in which high instantaneous substrate inoculums ratio favoured the metabolic activity and microbial growth to produce biogas from poultry manure wastewater [16]. Then, the flasks were closed with rubber stopper with gas line piping and sealed with parafilm to avoid contamination.

Mixing took place by using the New Brunswick Scientifics Shaker for agitation purpose followed the set up as shown in Table 2. The frequency and intensity of agitation were controlled by agitation adjustment knob with timer. The collection of biogas from the reactor was carried out with water displacement set up and reading of biogas volume was taken daily until end of reaction time. Biogas production performance can be evaluated on the basis of biogas yield from initial Chemical Oxygen Demand (COD) of the substrate as follows:

$$\text{Biogas yield (Y)} = \left(\frac{\text{biogas production volume}}{\text{initial concentration of COD}} \right) (L/g \text{ COD}) \quad (1)$$

The analysis of initial COD of the substrates was by using HACH Spectrophotometer namely DR 5000 following Method 10212.

2.7 Validation of the Model

After obtaining the optimal condition suggested by CCD for optimum biogas production, validation of the model was tested. Comparison was made between experimental and predicted values in order to justify the validity of the model.

3.0 RESULTS AND DISCUSSION

3.1 Characterization of Substrates

The characteristics of PMW and treated PMW were listed in Table 3. The characteristics of poultry manure wastewater studied by Yetilmezsoy and Sakar [17] were almost similar compared to this study. In that study, the pH, COD, suspended solid and phosphorus

concentration, were 7.30, 21,100 mg/L, 446 mg/L, respectively.

These pHs were in good range as the anaerobic microorganisms for biogas production were less sensitive and can function in a wider range of pH between 4.0 to 8.5 [18]. When pH was below 4.0 or above 8.5, AD was inhibited. When pH was below 4.0, the activity of the methanogens was completely suppressed. Only when pH value strictly regulated in the range of 4.0 to 8.5, methanogens can grow healthily and played a role of biocatalyst. If pH was out of optimized range, the amount of soluble organic matter and other sulphur-contained organic compounds increased greatly in the AD. These then led to growth inhibition of methanogenic bacteria which yielded biogas [19].

In this study, the initial COD concentration for PMW of 35,600 mg/L was about 7 times higher than treated PMW at 4985 mg/L. Cakir and Stenstrom [20] reported that wastewater having wide range of COD concentration of 2000 to 20,000 mg/L. Biological and chemical oxygen demand (BOD and COD), are water quality analyses commonly used to indicate the amount of organic matter present in wastewater. BOD and COD are biodegradable and could degrade readily in soil [21].

An important characteristic was suspended solids (SS) content, which affected the mixing, process dynamics and digester feeding method. The SS value for PMW and treated PMW were both above 750 mg/L. The exact value could not be obtained due to equipment limitation. However, both of the values were in a good range for biogas production [17]. Yetilmezsoy and Sakar [17] conducted a study on

treatment of PMW with SS value of 5020 mg/L and 1130 mg/L for PMW and treated PMW respectively. Anaerobic digester must be operated in suitable range (>750mg/L) of SS to ensure stabilization in the process and increase of biogas production [22].

The ammoniacal nitrogen (AN) concentration of PMW reduced after treatment process from 1490 mg/L to 440 mg/L. The treatment used soil water were able to decrease the AN content to avoid inhibition. The AN content reduced after the PMW treatment using soil water. It was estimated that microorganisms with more than 100 million in population and several thousands of species lived in 1 g of soil [23]. This may due to some reaction between the soil water and PMW because soil reduced ultimate sludge quantity, destroyed most of pathogens present in the sludge, and eliminated unpleasant smell problems. For more understanding regarding to this matter, further mechanism study required. In this research, the focus was on biogas production while treatment was study to help improving biogas production only. If AN inhibition occurs, Bujoczek *et al.*, [24] reported that nearly no biogas production, even after 120 days of reaction time. Based on Sung and Liu [25], AN concentration below 200 mg/L were beneficial to anaerobic process. However, AN inhibition can start at AN content up to 1000 mg/L. A few previous studies dealt with higher initial AN concentration compared to this study, such as at 1500 mg/L [26] and also 2250-3000 mg/L [27]. A few more studies, have demonstrated that acclimatization at high AN concentration was effective to raise AN tolerance for biogas production [11, 28].

Table 3 Characteristics of PMW and treated PMW

No	Parameter	Unit	PMW	Treated PMW
1	pH	-	8.1	7.5
2	BOD	mg/L	18300	2300
3	COD	mg/L	35600	4985
4	Suspended solids	mg/L	> 750	> 750
5	Ammoniacal nitrogen	mg/L	1490	440
6	Nitrate	mg/L	2270	1210
7	Nitrite	mg/L	58	20
8	Phosphorus	mg/L	710	140

3.2 Optimization Studies with CCD

In this design of experiment, CCD was implemented for the optimization of biogas production. The two factors involved in this study were agitation speed and reaction time. By using CCD, a total of 13 runs were generated with different set up condition. The response was biogas yield (L/g COD) in term of

Response 1 (R1). The result data shown in Table 4 was obtained from the laboratory experimental run. With CCD, the goodness of fit was able to be determined by coefficient of determination, R-squared while the statistical significance of the regression model was checked by the Fisher statistical test (F-test) in analysis of variance (ANOVA) [7]. Effects with a confidence

level higher than 95 % (p-value less than 0.05) were preferable to represent the reliability of a result [8].

Table 4 Experimental results for optimization

Run	Agitation (rpm)	Reaction time (day)	Biogas yield (L/g COD)
1	120	3	0.00361
2	130	4	0.00204
3	130	2	0.00125
4	120	5	0.00250
5	120	1	0.00133
6	110	2	0.00096
7	140	3	0.00064
8	120	3	0.00437
9	120	3	0.00395
10	110	4	0.00180
11	120	3	0.00395
12	120	3	0.00416
13	100	3	0.00049

3.3 Statistical Analysis

From ANOVA result summarized in Table 5, the model F-value of 7.86 in F-test implied the significant of the model. There was only a 0.86 % probability that a model F-value this large could occur due to noise.

The Sum of Squares for the Model source was 2.125×10^{-5} , which represented the summation of Regression Sum of Squares for the quadratic regression model. Each regression source had corresponding degrees of freedom (DF) of one and hence contributed a total DF of 5 for the model source. The Mean Squares of the Model was 4.259×10^{-6} , which was the division of Sum of Squares by the corresponding DF.

The Lack of Fit, F-value of 14.39 indicated the significant relative to the pure error. There was only a 1.31 % chance that it could occur due to noise. This means that there was some significant effect that has been neglected and that effect was a function of the factors which already existed in the model. A little change in the parameters might affect the fit of model. It was advisable to add more factors such as temperature and S/l ratio in order to make the lack of fit to become desirably insignificant. Apart from that, it was recommended to widen the range of the parameters so that outliers can be included.

This model had a satisfactory R-Squared value of 0.8489 which implied the model was adequate for the design space navigation. The adequate precision measured the signal to noise ratio which compared the predicted values range at points of design to the average prediction error. A ratio greater than 4 was desirable for an adequate model. In this particular case, the ratio of 7.327 indicated adequate signal discrimination.

Table 5 Result for ANOVA

Source	Sum of Squares	DF	Mean Square	F-value	p-value
Model	2.125×10^{-5}	5	4.259×10^{-6}	7.86	0.0086
A	5.741×10^{-8}	1	5.741×10^{-8}	0.11	0.7541
B	1.313×10^{-6}	1	1.313×10^{-6}	2.43	0.1632
AB	6.250×10^{-10}	1	6.250×10^{-10}	1.155×10^{-3}	0.9738
A²	1.774×10^{-5}	1	1.774×10^{-5}	32.8	0.0007
B²	6.741×10^{-6}	1	6.741×10^{-6}	12.46	0.0096
Lack of fit	3.470×10^{-6}	3	1.157×10^{-6}	14.39	0.00131

3.4 Residuals Analysis and Diagnostic Plots

Residual analysis is necessary to ensure that the assumptions for the ANOVA are met. From the least squares fit, the residuals (e_i) played a crucial role in judging the adequacy of the model and were defined by Equation (2). The difference between the actual individual values was indicated as y_i while the predicted value from the model was indicated as \hat{y}_i .

$$e_i = y_i - \hat{y}_i \quad \text{where } i = 1, 2, 3, \dots, n \quad (2)$$

Diagnostic plots generated from CCD using Design Expert V7.0 were reviewed in residuals analysis to determine the feasibility of the model. The normality assumption may be checked by a normal probability

plot of the residuals. The experimenter handbook by Kraber *et al.* [29] stated that a good normal probability plot should shows a linear straight line whereas an S shape indicating a bad normal plot. The handbook also mentioned that good residuals versus predicted response plot should be random scatter whereas a bad plot of the kind will shows a megaphone shape. If the variance of the response depends on the mean level of y , then this plot will often exhibit a funnel-shaped pattern. This is also suggestive of the need for transformation of the response variable y . A review on the normal probability plot for biogas yield as illustrated in Figure 1 revealed that the residuals generally fall on a straight line implying that the errors are distributed normally. On the other hand, the residuals versus

predicted response as shown in Figure 2 revealed that they were random scattered without obvious pattern and unusual structure. This general impression implied that the model proposed was adequate and there was no reason to suspect any violation of the independence or constant variance assumption.

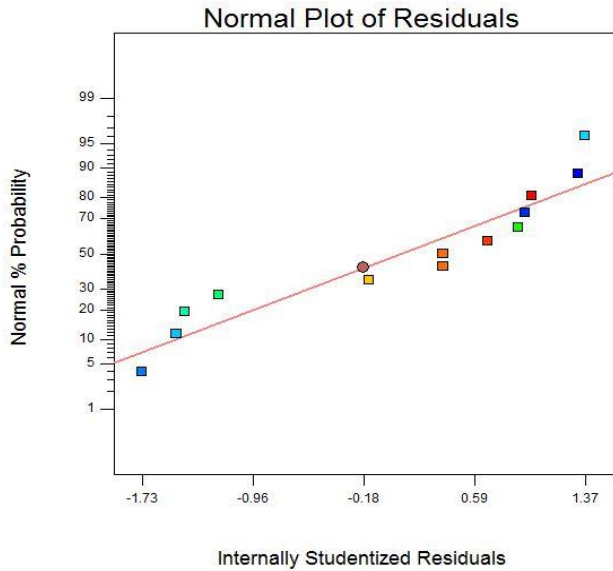


Figure 1 Normal probability plot of residuals for biogas yield data

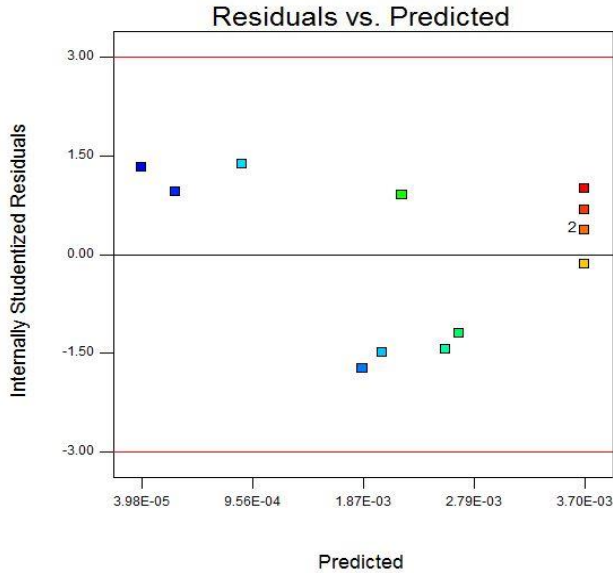


Figure 1 Residuals versus predicted response plot for biogas yield data

3.5 Main Effect Contribution

The contour plot graph of the effects of agitation and reaction time on the biogas yield was illustrated as in Figure 3. The units for the response biogas yield, agitation and reaction time were L/g COD biogas, rpm and days, respectively. Figure 3 clearly showed

that the agitation of 120 rpm and reaction time of 3 days yield highest biogas production. The yield of biogas decreased when agitation and reaction time were out of this condition. From the contour plot, the elliptical profile proved an extraordinary interaction between agitation and reaction time. It can be explained that as agitation increased, the yield of biogas was increased. This also happened to another parameter as the proportional relationship between reaction time and biogas yield. Nevertheless, once the agitation and reaction time were greater than the centre point value, the reverse trend was observed. From Figure 4, the three-dimensional surface graph generated in a dome shape in which maximum points was obtained at standard Run 8 which yield 0.00437 L/g COD biogas.

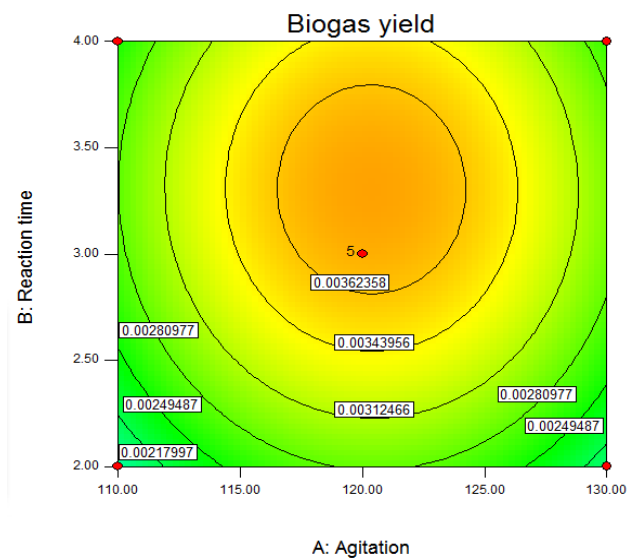


Figure 2 Contour plot graph of optimization

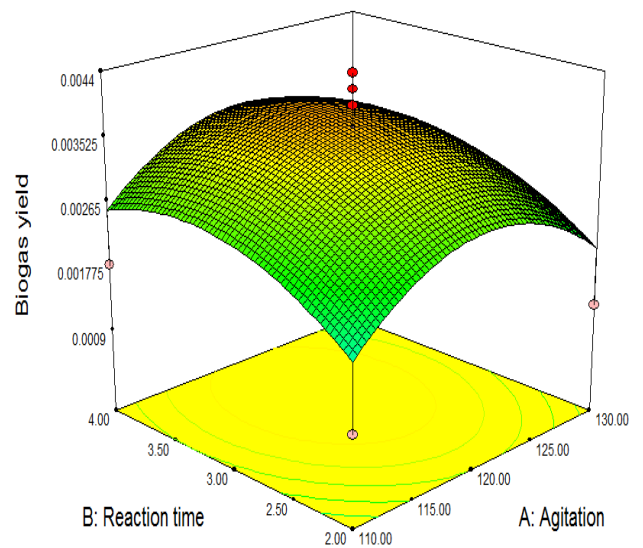


Figure 3 Model graph of optimization

This result in optimal conditions was at agitation speed of 120 rpm and reaction time of 3 days. The final equation was defined as follows:

$$\text{Biogas yield (L/g COD)} = (-0.1304) + (2.1258 \times 10^{-3} \times A) + (3.7547 \times 10^{-3} \times B) - (1.3874 \times 10^{-6} \times AB) - (8.8112 \times 10^{-6} \times A^2) - (5.4293 \times 10^{-4} \times B^2) \quad (3)$$

In order to get a better understanding of the results, the response function for RSM data was assessed graphically by the use of perturbation plot. The perturbation plot helps to compare the effect of all the factors at a particular point in the RSM design space. It displays the effect of changing one factor from the reference point while holding the other factor constant. As can be seen from Figure 5, both agitation (A) and reaction time (B) affected the biogas yield in an almost similar trend of curvature. This indicates that both agitation and reaction time factors showed significant quadratic effects that contributed to the biogas yield.

For factor A, the biogas yield increased up to a certain point, which was at coded unit of 0.000, and dropped when the agitation speed increasing. Tailing of biogas yield peak reduced due to higher agitation than the 0 coded units which might cause substrate disruption. In this study, the effect of agitation to the optimization of biogas production was crucial because agitation provides auxiliary mixing which enhances the efficiency of substrate conversion in digester by provides intimate contact between poultry manure wastewater and its inoculums [30]. Mass and heat transfer also can be fostered by agitation which can improve efficiency of mixing [31]. Besides, it avoids both the scum layers formation on the surface and the sedimentation of sludge on the bottom of the digester [32]. In addition, there will be occurrence of natural mixing in the anaerobic digester due to gas bubbles rise and the currents of thermal convection when the sludge is added with inoculums which generate reaction once combined [33]. Inadequate mixing will results in foam production due to overloaded [34]. Nevertheless, the structure of microbial substrate will be disrupted by vigorous continuous mixing [35].

On the other hand, for factor B, the biogas yield showed an upward trend when the reaction time increased. However, the tailing of growing trend started to slow down after the coded unit range of 0.000 to 0.500. Reaction time can be considered as another vital factor in the determination of optimum condition for biogas production. This due to the fact that an optimum hydraulic retention time (HRT) was crucial to the treatment of liquid poultry manure. AD of poultry waste was preferably to operate at shorter HRT so as to meet the requirement of economics and environmental beneficial extent. This was because under short HRT, the decomposition of organic matter can be achieve efficiently without accumulating excessive residual and other intermediate products such as volatile fatty acids [36].

HRT depends on other factors, such as feed stock and operational temperature [37]. Based on Sakar et al. [37], the HRT of AD of poultry manure studied were between 13.2 h and 91 days under mesophilic conditions which maintained between 25 and 35°C. Therefore, the optimization result from this research study need to be further validated.

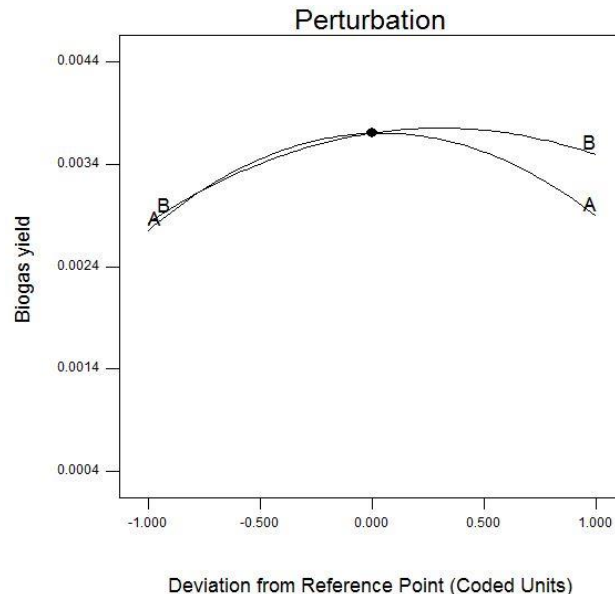


Figure 4 RSM Perturbation plot for biogas yield

3.6 Interaction of Factors

The interactive effect of agitation and reaction time on biogas production from poultry manure wastewater was plotted as in Figure 6. The non-parallel lines displayed in the interaction plot indicated that there was an interaction effect between agitation (A) and reaction time (B) on biogas production. According to Bakeman [38], the less parallel the lines are, the most likely there is to be a significant interaction. In Figure 6 the lines were not parallel and there was no cross-over interaction, but an interaction would be expected. The biogas yield response grew curvilinear when the agitation increased at a fixed level of reaction time factor.

At lower coded time factor (B-) which was 2 days of reaction time, agitation had a significant effect on biogas production. This was because during limited reaction time period, the agitation became the crucial factor in biogas production. In such short reaction time, the capability of biogas yield from AD of poultry manure wastewater was relatively lower compared to longer reaction time. Higher agitation at 120 rpm can supply adequate mixing which hence enhances the efficiency of substrate conversion in anaerobic digester by provides intimate contact between poultry manure wastewater and its inoculum. However, too high agitation (over 120 rpm) will cause cell disruption to

microbial methanogens. This will directly lead to reduction of biogas production.

Similarly, at higher coded time factor (B+) which was 4 days of reaction time, agitation also showed a significant effect on biogas yield. In such case, the biogas yield response also affected in the same manner by the agitation as in lower coded time factor. In this longer reaction time, the biogas production was slightly increase because the poultry manure wastewater substrates were given longer duration of intact with the inoculum. This longer duration of reaction time supplied with high agitation of 120 rpm may definitely promise a higher yield of biogas from poultry manure wastewater as compared to short reaction time. However, too long period of reaction was tried to be prevented due to economical factor and the extent to the beneficial of environment.

The Least Significant Difference (LSD) bars acted as the visual aids in assisting to interpret effect on interaction plots. As shown in Figure 6, the overlapping of the LSD bars for 2 indicated that both lower coded time factor (2 days) and higher coded time factor (4 days) covered the same range of biogas yield. In the other words, it defined that the difference in those means was not large enough to be declared significant using a t-test. The overlaps

between pairs of LSD bars indicated that the associated means differ was not lie on 95 % confidence levels.

3.7 Validation of Experiment

The suitability of the model equation for the prediction of the optimum response values was validated using the optimal conditions suggested by CCD.

showed the biogas yield according to the suggested agitation and reaction time based on predicted and experimental values. The error deviations lower than 30 % can be accepted in the validation run. From the result obtained, the experimental values were closed to the predicted values and it confirmed the validity and adequacy of the predicted models. Under condition with 120 rpm and reaction time of 3 days, the percentage error for experimental values was 8.50 % from the predicted value. On the other hand, the percentage error for experimental values was 5.82% from the predicted value under suggested optimal condition of 120 rpm and 3.3 days. The result of analysis proved that the response model was adequate for reflecting the expected optimization and the model of equation (2) was satisfactory and accurate.

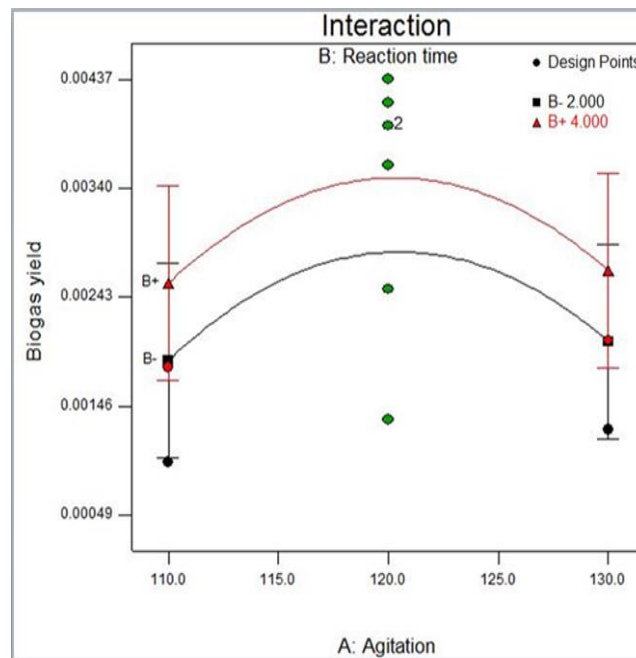


Figure 6 Interaction plot of agitation and reaction time on biogas yield

Table 6 Predicted and experimental values of the optimization parameter.

No	Agitation (rpm)	Reaction time (days)	Biogas yield (L/g COD)	Predicted biogas yield (L/g COD)
1	120	3	0.00425	0.00370
2	120	3	0.00283	0.00370
3	120	3	0.00308	0.00370
4	120	3.3	0.00391	0.00375
5	120	3.3	0.00354	0.00375
6	120	3.3	0.00445	0.00375

3.8 Comparison with Other Researcher

Table 7 showed the comparison of biogas yield with other researchers. The biogas yield from poultry manure wastewater in this study was 0.00445 L/g COD. In daily biogas production basis, AD process of poultry manure wastewater in this study yielded 1.48×10^{-3} L/g COD day⁻¹. Kafle and Kim [39] utilized swine manure as substrate to undergo AD for biogas production had yielded 0.95×10^{-3} L/g COD day⁻¹. Under their same study by replaced substrate with apple waste, the biogas yield was slightly lower which were only 0.75×10^{-3} L/g COD day⁻¹. The experimental studies of Syaichurrozi *et al.* [40] and Vlassis *et al.* [41] produced 2.21×10^{-3} L biogas/g COD vinasse day⁻¹ and 1.11×10^{-3} L biogas/g COD glycerol day⁻¹, respectively.

The operating temperature for all researchers including this study was set in mesophilic range between 25 - 38 °C. The HRT for AD of poultry manure wastewater recorded the lowest value of 3.3 days only. The reactor used in this study was with 250 ml and operated in batch mode. The result obtained proved that poultry manure wastewater was a potentially substrate for biogas production. It recorded the highest biogas yield compared to other substrate of swine manure, apple waste and glycerol, except for vinasse.

In experiments of Kafle and Kim [39], the AD under batch mode operation took place in 1.2 L glass

bottles (liquid volume of 0.8 L). The substrate of swine manure took 22 days for highest biogas yield while the substrate of apple waste took 146 days to achieve highest biogas production. The low biogas yield from AD of swine manure might due to its high ammonia content which was a major limitation that has plagued anaerobic digesters for many years [42-44]. Similarly, fruit and vegetable waste such as apple waste also had major limitations to its usefulness in AD because of its characters that rapidly acidifies, stressing and activity inhibition by methanogens [45-46].

Syaichurrozi *et al.* [40] who employed vinasse as substrate for AD yielded the highest amount of biogas within the comparison among researchers listed in Table 7. The HRT for his batch AD experiment was 60 days used 5 L polyethylene digesters. It produced higher biogas than in this study because vinasse contained sufficient nitrogen sources which were needed by bacteria to build cell structure [47]. However, too high amount of nitrogen sources might inhibit bacteria activity.

Vlassis *et al.* [41] conducted AD experiments with substrate of glycerol under continuous stirred tank reactor mode of operation. Within a HRT of 378 days, the AD yielded biogas of 1.11×10^{-3} L /g COD day⁻¹, this was only slightly lower than that obtained in this study.

Table 7 Comparison of biogas yield with other researchers

Study	Substrate	HRT	Temperature	Biogas yield (10 ⁻³)
This study	Poultry manure wastewater	3.3 days	25.0 °C	1.48 L/g COD day ⁻¹
Kafle and Kim (2013)	Swine manure	22 days	36.5 °C	0.95 L/g COD day ⁻¹
Syaichurrozi <i>et al.</i> (2013)	Vinasse	60 days	25.0 °C	2.21 L/g COD day ⁻¹
Kafle and Kim (2013)	Apple waste	146 days	36-38 °C	0.75 L/g COD day ⁻¹
Vlassis <i>et al.</i> (2013)	Glycerol	378 days	35.0 °C	1.11 L/g COD day ⁻¹

4.0 CONCLUSION

CCD was used to determine the optimum condition for the production of biogas from poultry manure wastewater. The ANOVA showed that the effect of agitation and reaction time for biogas yield was significant. Quadratic model was used in predicting all the responses. The optimal conditions determined were agitation of 120 rpm and 3.3 days of reaction time. Under this condition, 44.5×10^{-4} L/g COD of biogas yield was obtained. This counted for 5.82 % error from predicted models. Therefore, it was suggested the models obtained can be used to optimize the biogas production from poultry manure wastewater.

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