# Optimization of Methane Gas Production From Co-Digestion of Food Waste and Poultry Manure Using Artificial Neural Network and Response Surface Methodology

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# Abstract

Food waste with high carbohydrate content is considered as a suitable substrate for fermentation of methane gas. In this study, co-digestion of poultry manure (PM) and food waste (FW) was used. Response surface methodology (RSM) and artificial neural network (ANN) were applied to optimize parameters of co-digestion of PM and FW at different ratios, initial pH values and temperatures. A comparative analysis was done using RSM and ANN in a predictive model of the experimental data obtained in accordance with the central composite design. The combined effects of the independent variables (ratio, pH and temperature) as the most significant parameters of methane fermentation of PM and FW were investigated. Optimization using RSM and ANN showed a good fit between the experimental and the predicted data as elucidated by the coefficient of determination with  $R^2$  values of 0.991 and 0.998, respectively. Quadratic RSM predicted the maximum methane yield to be 537 mL CH<sub>4</sub>/g VS at the optimal conditions; ratio 80:20 (PM : FW); temperature 35 °C; and initial pH 7.11. The maximum predicted methane yield by the ANN model was 535.82 mL CH<sub>4</sub>/g VS at the following conditions; ratio of poultry manure to food waste 80:20; temperature 35 °C; and pH 7.00. The verification experiments successfully produced 538 mL CH<sub>4</sub>/g VS within 14 days of incubation. These experiments indicated that the developed model was successfully used to predict the fermentable methane production.

Keywords: food waste, poultry manure, response surface methodology, central composite design, artificial neural network

# 1. Introduction

Composting and directly applying poultry litter to the land as organic fertilizers are widely practised by farmers. The rise in environmental concerns associated with the production of energy with CO<sub>2</sub> mitigation policies has renewed interest in digestion technologies. Anaerobic digestion is a method for the treatment of organic wastes in the absence of oxygen. This process has advantages in waste stabilization and biogas recovery. It has also been proven as an efficient process in green technology for disposing crop residue, sewage sludge, food waste and animal manure (Wan et al., 2011; Li et al., 2009). Uncontrolled discharge of these wastes also has negative effects on the environment, society and also health, thus it is necessary to minimize the risks. Poultry manure (PM) has been considered as a very attractive animal waste for anaerobic digestion because it's high methane potential. Manure is a potential substrate due to its have high buffering capacity and also rich in a variety of nutrients where it was necessary for bacterial growth (Hartmann et al., 2002).

The production of methane will be higher when several wastes are combined in a single process and if a single waste only is used, a low methane yield will result due to its low biodegradability or the presence of inhibitory compounds such as potassium and lipids (Fernandez et al., 2005). Digestion of more substrates in the same reactor gives positive effects and the added nutrients could support microbial growth (Mata-Alvarez et al., 2000). Furthermore FW with its high concentration of carbohydrates, highly digestible, low cost and renewable (Pan et al., 2008) has the potential to be combined with PM. Among the factors that affect the methane fermentation are

pH, substrate and also temperatures. If the substrate does not fall into the suitable range of alkalinity, there is a tendency for potential toxicity and digester failure. Zinder (1994) reported that pH between 7-7.2 is suitable for methane fermentation. Mostly, the previous studies optimized the optimum conditions for biogas production by the conventional method by change one factor at a time. This is a method in which a single factor is varied while all the other factors are kept fixed at a specific condition and also it is time consuming, laborious and difficult of reaching the optimal conditions due to ignoring the interactions between variables.

To solve this problem, response surface methodology (RSM) and artificial neural network (ANN) were proposed for use to determine the influences of single factors and their interactive effects. RSM is a statistical method for designing experiments, evaluating the interactive effects of factors and searching optimal conditions and reducing the number of experiments. It has been extensively used in biological optimizations in recent years (Wang et al., 2005). In a recent study, a response surface model was developed for computing the H<sub>2</sub> yield for glucose degradation by a mixed batch anaerobic mesophilic culture under different experimental conditions (Ray et al., 2010). Approximately 85% of the theoretical H<sub>2</sub> yield (4.0 mol H2/mol glucose) was achieved in experiments conducted near the optimum factor setting identified by the D-optimality analysis (Debabrata et al., 2011). On the other hand, ANN is suitable for developing bioprocess models and the ANN models are exclusively data-based. The most widely utilized ANN architecture is multi-layered that approximates non-linear relationships existing between multiple input process variables and the corresponding dependent (output) variables (Nandi et al., 2001). ANNs were successfully used to model the results of biogas production and chemical oxygen demand (COD) removal with an upflow anaerobic sludge blanket reactor (UASB) (Mu & Yu, 2007) and an expanded granular sludge bed (EGSB) reactor (Guo et al., 2008). With RSM and ANN, the interactions of influencing parameters on methane gas can be evaluated. Therefore the main objective of this study was to optimize the effects of the different ratios of PM and FW, initial pH values of fermentation and different temperatures in the mesophilic range.

# 2. Materials and Methods

# 2.1 Raw Material

Poultry manure (PM) was collected from the chicken farm located at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang. Food waste (FW) was collected from the Cafeteria at Universiti Putra Malaysia (UPM). FW was separated into three categories; carbohydrate (rice), fiber (vegetable) and protein (meat and fish) at a ratio of 2:1:1. 500 g of FW was ground in 500 mL distilled water using a blender (Servco Servuces) and then stored at -20 °C prior to use.

# 2.2 Characterization of Waste

The collected PM and FW were analyzed for pH, total solids (TS), total suspended solids (TSS), total volatile solids (TVS), volatile suspended solids (VSS), chemical oxygen demand (COD) and moisture content in accordance with the standard methods (APHA, 2005).  $NH^+_4$ -N were measured using YSI equipment. The composition of the biogas produced everyday was determined by using a gas detector (Crowcon).

# 2.3 Operating Procedure

Batch digestion test was carried out in a column with a total capacity of 2.5 L and a working volume at 1.5 L. The PM and FW placed in the column at different ratios of PM and FW; 100:0, 80:20, 60:40 and 40:60 and incubated in a water bath at different temperatures (25, 30, 35, 40 and 45 °C). The pH values (6.5, 7.0, 7.5 and 8.0) were adjusted using 5M HCl and 5M NaOH. The composition of the gas was measured on a daily basis. The batch fermentation in the column was carried out for 14 days. Each column was flushed with nitrogen gas for 10 min with a flow rate of 2 L/min in order to remove the oxygen content.

# 2.4 Experimental Design and Optimization

The statistical software Design-Expert 6.0 (Stat Ease Inc. Minneapolis, USA) was used to determine the optimal combination of parameters equivalent to the highest fermentable methane produced. Design Expert 6.0.6 of central composite design (CCD) was used in the optimization of methane gas production from co-digestion of FW and PM. The pH ( $X_1$ ), temperature ( $X_2$ ) and ratio of PM to FW ( $X_3$ ) were chosen as shown in Table 1.

Variable	Parameters			Level		
		-2 (-α)	-1	0	1	2 (a )
X <sub>1</sub>	Initial pH	6.0	6.5	7.0	7.5	8.0
$X_2$	Temperature (°C)	25	30	35	40	45
X <sub>3</sub>	Poultry manure: Food waste	40:60	60:40	80:20	100:0	0:100

Table 1. Levels of factors and variables used for optimization

Each variable in the CCD was studied at five different levels assigned as -2, -1, 0, +1, and +2, respectively. Methane yield (mL CH<sub>4</sub>/g VS) were used as the output variables. According to the design, 20 runs of experiments were performed with six replications of the center points (Table 2) where the axial point was determined to be 1.682. For optimal point prediction, a second order polynomial model function was fitted to the experimental results. The regression model was calculated by analyzing the analysis of variance (ANOVA), *p*-and F-value. The adequancy of the model was expressed by the coefficient of determination,  $R^2$ . The model describes the interaction among the parameters influencing the response by varying them concurrently.

Table 2. Code and real values at initial ph, different temperature and different ratio of substrate

Run	Co	de va	lues	Re	al va	lues	Methane	Prodicted values
	$x_{I}$	$x_2$	<i>x</i> <sub>3</sub>	$X_1$	X <sub>2</sub>	X <sub>3</sub>	(mL CH <sub>4</sub> /VS)	Treuleeu values
1	0	-2	0	7.0	25	80	123	106.35
2	0	0	-2	7.0	35	40	152	132.39
3	0	0	0	7.0	35	80	550	535.13
4	2	0	0	8.0	35	80	511	503.35
5	0	0	0	7.0	35	80	544	535.13
6	1	-1	1	7.5	30	100	360	376.09
7	1	-1	-1	7.5	30	60	241	249.21
8	0	2	0	7.0	45	80	201	199.85
9	-1	1	-1	6.5	40	60	399	400.71
10	0	0	2	7.0	35	100	438	445.23
11	-1	1	1	6.5	40	100	293	302.59
12	0	0	0	7.0	35	80	521	535.13
13	-2	0	0	6.0	35	80	498	487.85
14	-1	1	1	6.5	30	100	340	337.84
15	0	0	0	7.0	35	80	538	535.13
16	1	1	1	7.5	40	100	354	342.84
17	0	0	0	7.0	35	80	551	535.13
18	0	0	0	7.0	35	80	530	535.13
19	-1	-1	-1	6.5	30	60	245	273.96
20	1	1	-1	7.5	40	60	358	377.96

ANN was applied to illustrate a nonlinear mapping between the input variables (pH, temperature and ratio) and the output variables (methane yield production). The topology of the neural network was designed so that the number of neurons in the input layer was fixed by the number of inputs, whereas the output layers were determined by the number of output variables as shown in Figure 1.



Figure 1. Interconnected group of nodes of artificial neural networks

The learning step is the critical part where the hidden layer of the network was determined by varying the number of nodes from 1-20. The sigmoidal function was used as a transfer function in both nodes of the hidden and output layers. The predictive values calculated by the RSM and the ANN models were plotted against the corresponding experimental values. The value of  $R^2$  was compared to visualize the modelling abilities towards the experimental data.

#### 3. Results and Discussion

The characteristics of PM used in this study based on TSS, VSS and pH were 90100 mg/L, 48500 mg/L and 8.25, respectively as indicated in Table 3. However, from the previous study (Magbanua et al., 2000) demonstrated that the characteristics of PM based on TSS and VSS were 14600 mg/L, 12500 mg/L, respectively.

Parameters	Unit	Poultry manure	Food waste
pH		8.25	5.25
Total Solid (TS)	mg/L	92400	95300
Total Suspended Solid (TSS)	mg/L	90100	74700
Total Volatile Solid (TVS)	mg/L	71400	68300
Volatile Suspended Solid (VSS)	mg/L	48500	46500
Chemical Oxygen Demand (COD)	mg/L	65900	34500
Ammonia-N	mg/L	895.30	294.90
Moisture content	%	55.10	60

Table 3. Characteristics of the poultry manure and food waste used

The characteristics of food waste based on TSS, VSS and pH were 74700 mg/L, 46500 mg/L and 5.25, respectively as indicated in Table 3. However, from the previous study (Elsayed et al., 2012) demonstrated that the characteristics of food waste such as TSS, VSS and pH were 48400 mg/L, 27900 mg/L and 4.6, respectively.

### 3.1 Response Surface Analysis Regression and Model Analytics

The design matrix of central composite design in predicted value and actual terms of specific methane production are shows in Table 2. The methane gas production was determined by dividing the methane yield by

the amount of volatile solid added in the column. The values obtained were subjected to response analysis to evaluate the relationship between initial pH of substrate  $(X_1)$ , different temperature at mesophilic condition  $(X_2)$  and different ratio of substrate  $(X_3)$ . By applying multiple regression analysis the results were fitted to a second order polynomial equation Thus the equation obtained based on mathematical regression models for methane production fitted in term of coded factors as follows:

 $Y_{SMP} = 535.13 + 3.88 X_1 + 23.38X_2 + 7.19X_3 - 9.88X_1^2 - 95.51X_2^2 - 97.09X_3^2 + 0.50X_1X_2 + 15.75X_1X_3 - 40.50X_2X_3$  (1) Analysis of variance (ANOVA) was used to determine the quadratic model was significance. A summary of the ANOVA is shown in Table 4.

Sources	Sum of square	df	Mean square	F- value	Prob > F
Model	3.810E+005	9	42334.48	122.70	< 0.0001
$\mathbf{X}_1$	240.25	1	240.25	0.70	0.4235
$X_2$	8742.25	1	8742.25	25.34	0.0005
$X_3$	543.93	1	543.93	1.58	0.2378
$X_1^2$	2525.84	1	2525.84	7.32	0.0221
$X_2^2$	2.359E+005	1	2.359E+005	683.76	< 0.0001
$X_{3}^{2}$	1.232E+005	1	1.232E+005	357.20	< 0.0001
$X_1X_2$	2.00	1	2.00	5.797E-003	0.9408
$X_1X_3$	1984.50	1	1984.50	5.75	0.0374
$X_2X_3$	13122.00	1	13122.00	38.03	0.0001
Residual	3450.26	10	345.03		
Lack of fit	2754.26	5	550.85	3.96	0.0787
C.V	4.80				
R <sup>2</sup>	0.9910				
Adjusted R <sup>2</sup>	0.9829				

Table 4. Analysis of variance (ANOVA) of the model

The Model F-value of 122.70 implies the model is significant. There is only 0.01% chance that a "Model F-Value" could occur due to noise. The *p*-Value represents the significance of the variables in which the smaller the *p*-Value, the higher the significance of each variable. The *p*-Value was less than 0.05 which indicated the model terms are significant. The low value of coefficient of variation (CV, 4.80) indicated a high degree of precision and a good deal of reliability of the experimental values (Wang et al., 2005). The fit of the model was also expressed by the coefficient of determination R<sup>2</sup>, which was found to be 0.9910. The regression analysis of the experimental design shown that the linear model terms (X<sub>2)</sub> quadratic model term (X<sub>1</sub><sup>2</sup>, X<sub>2</sub><sup>2</sup>, X<sub>3</sub><sup>2</sup>) and the interactive model term (X<sub>1</sub>, X<sub>2</sub>) are significant (P<0.05). However, the linear model term (X<sub>1</sub>, X<sub>3</sub>) and the interactive model term at (X<sub>1</sub>, X<sub>2</sub>) are insignificant (P>0.05).

#### 3.2 Response Surface Analysis and Interactions Among Factors

#### 3.2.1 Relationship Between pH and Different Temperature

One variable kept at its central level with three dimensional response surface plots were generated and the experimental range was varied with the others. As shown in Figure 2, methane production was higher when the temperature was at 35  $^{\circ}$ C, however when the temperature exceeded 35  $^{\circ}$ C the methane production decreased rapidly. The response surface of methane production illustrated that slight inhibition was observed when the temperature was lower than 30  $^{\circ}$ C.



Figure 2. Response surface and contour lines for the interactive effect of initial pH and different temperature at mesophilic condition on specific methane production

Bacteria have a limited range of temperatures for their activity. Usually, methanogens are very sensitive to temperature changes. In general, mesophilic anaerobic digestion is more widely used compared to thermophilic digestion because of the lower energy requirements and the higher stability of the process. Methane production reached the high value of 537 mL CH<sub>4</sub>/ g VS at the pH range 7-7.2 and the statistical difference was found to be insignificant (p > 0.01). This phenomenon could be attributed to the pH utilization of substrate between 6.5 - 7.5 and was limited for methane production.

# 3.2.2 Relationship Between the Effect of pH and Ratio of Substrate

Figure 3 illustrates the interactive effects of initial pH and different ratios of poultry manure and food waste on specific methane production (SMP). The ratios of different wastes significantly affected the SMP.



Figure 3. The interactive effect of the ratio PM and initial pH for the poultry manure and food waste of response surface and contour plots

Based on Figure 3, the methane production was reduced during single waste fermentation but increased to its peak at the ratio 80:20 of PM to FW. However, the production of methane yield declined when FW was added at more than 20 % into the PM during fermentation.

3.2.3 Relationship Between Effect of Ratio and Different Temperature

Methane production increased to its peak when the ratio of PM and temperature increased, and then declined with the increase of these two factors. However, the analysis of variance indicated that the ratio of PM, FW and different temperatures at mesophilic condition are significantly influenced on methane production (P < 0.05). The response of methane production indicated that the ratio of substrates and temperatures have interactive effects on each other.



Figure 4. Response surface and contour plots for the interactive effect of different ratio of PM and different temperatures at mesophilic condition

Based on Figure 4, the highest yield of methane (537 mL  $CH_4/g$  VS) was at the ratio of 80:20 of PM and FW at 35 °C. On the other hand, the lowest specific methane production (123 mL  $CH_4/g$  VS) was at the ratio of 60:40 of PM and FW and FW and the temperature was 30 °C. It was might be attributed to the fact that lower pH substrate could lead to acidic condition. When the pH dropped below 6.3, inhibition of methanogenesis would occur (Bitton, 1994; Van Haandel & Lettinga, 1994).

#### 3.3 Conditions for Optimum Response and Model Validation

The second order polynomial model was used in this study to determine the specific optimal conditions. Furthermore, the optimal conditions for maximizing SMP were calculated by setting the partial derivatives of Equation (1) to zero with respect to the corresponding variables (Wang et al., 2005). The optimal conditions were: initial pH 7.11, ratio of PM and FW being at 80:20 and temperature at 35 °C. The optimal conditions were: initial pH 7.11, ratio of PM and FW being at 80:20 and temperature at 35 °C. The maximum value for methane production was estimated as 537 mL CH<sub>4</sub>/g VS.

	Tama	Datio	]	RSM	A	NN
pН	remp (°C)	Kauo (PM·FW)	(mL (	CH <sub>4</sub> /g VS)	(mL C	CH <sub>4</sub> /g VS)
	( C)	(1 11.1 11) -	Actual	Predicted	Actual	Predicted
7.11	35	80:20	535.13	537	-	-
7	35	80:20	-	-	-	535.82

Table 5. The optimum parameters and methane production obtained by RSM and ANN

ANN is an efficient tool to control and simulate an anaerobic digestion process and the developed models could effectively predict the gas production and composition from the reactors (Holubar et al., 2002). Therefore Artificial Neuron Network (ANN) analysis was utilized to confirm the validity of the statistical experiment and to get a better understanding of methane fermentation from co-digestion of PM and FW.



Figure 5. Prediction of R<sup>2</sup> by using response surface methodology (RSM) and Artificial Neuron Network (ANN)

Figure 5 shows the curve of  $R^2$  from the RSM and ANN. The value of the  $R^2$  for RSM and ANN were 0.991 and 0.998, repectively. The high value of  $R^2$  showed that the model used was useful for the prediction of methane production from the co-digestion of PM and FW at different ratios and the obtained data fitted to estimated curve of  $R^2$  and also the high value of  $R^2$  also indicated that the model was useful for the prediction of methane production (Wang et al., 2005).

# 3.4 Production of Methane

The stability of parameters is important for the maximum methane yield of the anaerobic digestion process. Table 6 shows the comparison of the methane yield from this study with the previous study using the batch mode operation.

Feedstocks	Ratio	рН	Methane yield	References
			(mL CH <sub>4</sub> /g VS)	
Treatment chicken manure: Chicken manure	1:1	8-9	157	Fatma et al., 2010
Pig manure	1:0	7.4	226	Xie et al., 2009
Pig manure: Grass sillage	3:1	7.1-8	304.2	Xie et al., 2011
Poultry manure: Food waste	8:2	7.11	537	This study

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Based on the previous study on co-digestion of manure with other waste biomass, the methane yield was enhanced compared to the single fermentation. Our results showed high methane yield (537 mL  $CH_4/g$  VS) when compared to other reports (Table 6). Our findings showed a pH around 7.0-7.1 is most suitable for methane production.

# 4. Discussion

The characteristics of food waste (FW) used in this study in term of TSS, VSS and pH were higher compared to the previous study and this might be due to the different compositions of the organic fractions of municipal solid

wastes and also PM has more solid elements compared to the FW. Based on Figure 2 shows that temperature at 40°C the methane yield decreased which might be due to excess production of volatile fatty acids (VFA) and their inhibitory effects on the fermentation of organic wastes (Noike et al., 2002). Although microorganisms with potential importance to the anaerobic digestion process can exist under a wide range of temperature conditions, most studies of practical process applications have been performed under ambient (20-25 °C), mesophilic (26-40 °C), or thermophilic (40-55 °C) temperature conditions (Chynoweth & Isaacson, 1987). A study by De Baere (2000) found that most anaerobic treatment plants operated at mesophilic conditions, which was 62% as surveyed in 2000 and the choice may have been driven by the high sensitivity of bacteria to temperature fluctuations in the thermophilic range.

Previous studies (Bitton, 1994; Van Haandel & Lettinga, 1994) indicated the optimum pH at 7.0-7.2 and the rate of methane production decreased if the pH was lower than 6.3 or higher than 7.8 and if the pH dropped below 6.3, this might inhibit the methanogenesis process. The analysis of variance indicated that the interactive effects of initial pH and ratio of PM and FW were significant on SMP (P < 0.05) as shown in Figure 3. The optimum pH for methanogenic bacteria function at range between 6.7 and 7.4, but optimally at pH 7.0 - 7.2 (Zinder, 1994) and at the pH lower than 6.8 or higher than 7.8 will decrease the rate of methane gas production. pH acts as a factor for controlling methanogens activity in anaerobic fermentation. Active methanogens activity is in narrow pH range between 6.3-7.8 (Pan et al., 2008). An excessively alkaline pH can lead to the disintegration of microbial granules and subsequent failure of the digestion process (Sandberg et al., 1992). Anaerobic digestion of two or more substrates increased biogas or methane yield as it has been reported previously that high methane production of 603 mL CH4/g VS was obtained in the co-digestion of a mixture of 70% manure, 20% food waste and 10% sewage sludge at the OLR of 1.2 g-VS/L day (Marañón et al., 2012). Co-digestion of animal manure with agricultural residues also has been reported previously (Callaghan et al., 2002; Kaparaju & Rintala, 2005) and the co-digestion of poultry manure with the other waste such as food and agricultural waste that has the potential for methane production. Based on the Table 5, the predicted specific methane production of 537 mL  $CH_4/g$  VS by using RSM analysis was close to the actual value of 535.13 mL  $CH_4/g$  VS. The predicted methane yield was 535.82 mL CH<sub>4</sub>/g VS while 539 mL CH<sub>4</sub>/g VS was the actual methane yield by using ANN analysis. The predicted values of methane yield by using RSM and ANN analysis were shown to be almost similar to the actual values.

# 5. Conclusions

The factors of the ratio for poultry manure (PM) to food waste (FW) showed the most significant effect. Increasing the FW to PM ratio can significantly enhance the methane yield but the methane production decreased when the ratio of FW exceeded 20 percent since a higher ratio of FW to PM may lead to acidic conditions which decreased the methane yield. The ratio of FW to PM and pH showed significant interactive effects, which demonstrated that inhibition was caused by pH decrease. The optimal conditions were: initial pH 7.11, ratio of PM to FW at 80:20 and temperature at 35 °C. The maximum response value for methane production was estimated as 537 mL CH<sub>4</sub>/g VS. The model validation for experiment by ANN confirmed that the optimum methane yield was close to the estimated value by using RSM. The results thus showed that RSM was useful for the prediction of methane yield production from co-digestion of PM and FW fermentation process. The value of the R<sup>2</sup> for RSM and ANN were 0.991 and 0.998, respectively. These high value of R<sup>2</sup> demonstrated that both models could be efficiently used for the prediction of methane production from the co-digestion of PM and FW.

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