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RESPONSE SURFACE OPTIMISATION OF BIOGAS POTENTIAL IN CO-DIGESTION OF MISCANTHUS FUSCUS AND COW DUNG

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ABSTRACT

In this study, the effects of co-digestion operating conditions for the enhancement of biogas production from *Miscanthus Fuscus* mixed with cow dung, was investigated. The aforementioned organic wastes are good substrate resources for anaerobic co-digestion (AD) due to their high content of easily biodegradable materials. This source of effective and eco-friendly technology as AD is for generating energy from organic waste. Response surface methodology (RSM) based on the Box-Behnken (BBD) design was employed to evaluate and optimise four process variables: pH, temperature, and hydraulic retention time (HRT) and feedstock inoculum (F/I) ratio on the biogas production. This study signifies the interactions between the process conditions, and identifies the most significant variables of control in order to maximise the biogas production. A developed regression model established the relationship between the significant effect of the input variables and the response. The analysis of variance (ANOVA) showed a high coefficient of determination value (R^2= 0.9997) at 95% confidence level. The results show that the F/I ratio has a major impact on biogas production. The model developed predicted values which were well fitted ($P<0.005$) with the values obtained from the experimental data. Thus, the regression model confirmed findings. The RSM and BBD employed proved to be economical and a reliable tool for modelling, optimizing and studying the interactive effects of the four process factors (pH, temperature, HRT and F/I ratio) for the biogas production.

Keywords: Anaerobic digestion; Biogas; Cow Dung; Inoculum to feed ratio; Response surface methodology

1. INTRODUCTION

The world's petroleum reserves are rapidly depleting, as a result of fossil fuel combustion, the high threat of global warming and the release of toxic compounds and air pollutants into the environment (Troschinetz & Mihelcic, 2009). Energy consumption rates and waste generation are escalating with a depletion of fossil resources (Guenther, 2018). However, managing waste materials towards the sustainable production of biofuels and biogas can have economic merits and provide environmental protection (Sørensen et al., 2013; Tetteh et al., 2017). Currently, the growing problems associated with traditional energies, such as price and global warming depletion, have resulted in the promotion of renewable energy. In recent years, biogas has

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emerged as one of the alternative fuels and generated massive research interest for production. Reasons include; easy adaptability, clean fuel, and cheap and readily available materials (Khanal, 2008).

In many industrialized countries, the conversion of municipal organic waste to biogas has become popular in recent years as a sustainable technology producing green energy. Some of these widespread research technologies of renewable energy resources include municipal solid wastes, animal manures, fish waste, agricultural waste (El-Mashad & Zhang, 2010; Li et al., 2010) from a variety of feedstocks (Armah et al., 2017). However, the volume of livestock manure like cow dung is escalating, and inappropriate disposal can lead to adverse environmental and health problems such as eutrophication (Safari et al., 2018). In the same context,, *Miscanthus Fuscus* is a bamboo-like plant that grows rapidly up to 3 meters, generating a high yield of biomass with low ash content, suitable for use in electricity generation (Khanal, 2008; Sawatdeenarunat et al., 2016). It is a promising non-food crop yielding high-quality lignocellulose material which can be used in a number of ways, including energy and fiber production, thatching, and industrial application purposes (Sawatdeenarunat et al., 2016). *Miscanthus Fuscus* has been found suitable for biogas production and has a high methane yield potential per unit area (Al Mamun & Torii, 2015). According to Tetteh et al. (2017), using *Miscanthus Fuscus* together with cow dung is potentially a way of managing waste via biogas production, due to their high buffering capacity with the necessary nutrients for bacterial growth.

The synthesis of a renewable energy source, as an alternative to a non-renewable energy source, has been evaluated, where energy is produced from biogas through the anaerobic digestion (AD) process (El-Mashad & Zhang, 2010). Biogas consists mainly of 60-70% methane, 20-30% carbon dioxide, 0-3% nitrogen, 0-1% hydrogen, and 0-1% hydrogen sulphide (Rao et al., 2010; Appels et al., 2011). The AD process is a biological process involving bio-degradation of wastes or organic matter by microorganisms under poor or no oxygen conditions (Abbasi & Abbasi, 2010; Abbasi et al., 2012b; Hamawand, 2015). The AD process has been employed as effective technology to improve energy security and reduce environmental pollution (Appels et al., 2011). Several parameters within an AD process can have positive and negative effects on its physical environment and production efficiency. These parameters include temperature, hydraulic retention time (HRT), carbon to nitrogen ratio (C/N), solid to water content, pH, and the organic loading rate (OLR), thus making the process relatively slow (Abbasi et al., 2012a).

According to Ribeiro et al. (2017), a sharp change in the aforementioned parameters could adversely affect the biogas production process. Some studies have shown the use of a one-factor-ata-time (OFAT) approach in biogas production optimises the AD process to maximize the biogas production (Haris et al., 2018). This OFAT technique is a waste of time and uncertain to attain optimum conditions due to avoidance of the interactions between some of the parameters (Nasruddin et al., 2018). To resolve this problem, recent researchers have adapted response surface methodology (RSM), as an essential statistical tool for experimental design, generating models, analysing and evaluating single factors and interactive effects of the input variables on the response; and to obtain optimum conditions to predict the specified response (Appels et al., 2011; Kurniawan et al., 2014).

Response surface methodology is very important in experimental design, and has been applied successfully in many fields to minimize the number of experimental runs for optimization, improving and developing new processes. Some of these include sugar and oil refinery wastewater (Tetteh & Rathilal, 2018) and biogas production (Menon et al., 2017). Therefore, the RSM is an efficient tool to control and stimulate the complex influential factors of the AD process, to enhance the study of their interactive effects on biogas production (Kurniawan et al., 2014; Tetteh & Rathilal, 2017).

In order to establish the interactive effect of variables, the RSM design technique is used mostly for the experimental designs, analysing and modelling the experimental data to optimise the response (El-Gendy et al., 2013; Ani et al., 2018). The BBD design, one of the mostly commonly used RSM designs, is adapted for this study. Thus, BBD is arguably more efficient, compared to central composite design, due to its less number of runs (Protasov, 2018; Haris et al., 2018). However, it is not nearly as flexible compared to CCD, in terms of its ability to be conducted progressively (Kurniawan et al., 2014; Tetteh & Rathilal, 2017). This is because the BBD is efficient in estimating the coefficient of determination (R^2) for a specified model, experimental errors and good data fitness. The main objective of this study was to reveal how to maximise biogas production from *Miscanthus Fuscus* as a feedstock in a co-digester using cow dung as the inoculum. The BBD was employed for the experimental design, modelling and optimising the biogas AD process.

2. MATERIALS AND METHODS

Feedstock (F): *Miscanthus Fuscus* was harvested from local farmland at Adako Jachie in the Ashanti region of Ghana and was used as the main feedstock for the biogas production. Firstly, the feedstock was washed and dried to remove unwanted particles. It was then shredded and milled with a hammer mill (Fritsch Pulverisette 558, Germany) to obtain 10 mm sized particles.

Inoculum (I): The fresh cow dung used as inoculum was obtained from a cattle farm in the same municipality. To provide the necessary bacteria for the digestion process it was kept in sealed Schott bottles and stored at 4°C before further chemical analysis. Sodium hydroxide (NaOH) was used to adjust the pH of the anaerobic digestion process to cater for volatile acids.

A laboratory balance (Kern PCB 3500-2, United Kingdom) was used to weigh the masses, while a gravity convection oven (VWR DRY-line oven, Pennsylvania) was used for drying the feedstock and an inoculum and muffle furnace (Nabertherm, China) for ashing. The pH was measured with a pH meter (Thermo Scientific 'Orion' Star A121, United States of America).

2.1. Characterization

The America Public Health Association (APHA) standard methods were used to determine the chemical composition of the *Miscanthus Fuscus* and the cow dung as depicted in Table 1. The co-digestion of the cow dung (I) and *Miscanthus Fuscus* (F) were mixed at different mixing ratios such as 25:75, 50:50, 75:25 to enhance the biogas production.

Table 1 Experimental design for the feedstock and the inoculum

Biodigester ID	Inoculum (I)	Feedstock (F)
pH	7.4	7.2
Total solids, TS (%)	10.5	8.6
Volatile solids, VS (%)	86.5	92.6
COD mg/L	3960	3650
Organic carbon (%)	47.6	49.5

2.2. Experimental Setup and Procedure

The total solids and volatile solids of the feedstocks and inoculum were pre-determined and used to prepare the digestion samples in the 1000 ml Schott bottles (used as the biodigesters) with an effective volume of 800 ml. For each run, a headspace of 200 ml was left which was purged with N_2 to create the anaerobic environment (Tetteh et al., 2017). The biodigesters were closed air-tight with rubber caps and incubated in a circulating water bath. Since it is a batch system, it was made to run until anaerobic digestion was complete. Stirring was carried out periodically by cautiously shaking each biodigester to ensure uniformity. The composition of biogas was analysed from the BMP test using a gas chromatograph (SRI 8610 GC) equipped with a thermal conductivity detector, packed with 6' HayeSep-D/6' Molecular Sieve-13X. The anaerobic digestion system was designed to quantitatively determine the volume of biogas produced using a water displacement technique as depicted in the schematic diagram Figure 1.

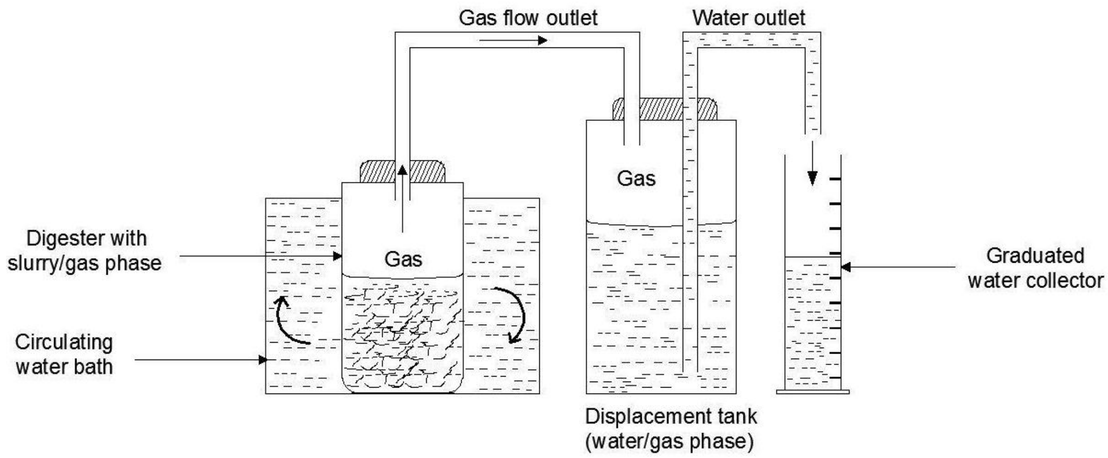

Figure 1 Schematic diagram of anaerobic co-digestion setup

2.3. Box Behnken Design

Data driven approach using RSM, 26 experiment runs were designed with BDD, using four factors such as pH (5 to7), HRT (20 to 40 days), temperature (20 to 30^{o}C) and F/I ratio (25 to 75%), and three levels (-1, 0, +1) as depicted in Table 2. The data obtained from the experiment were simulated using Design-Expert (10.03) software to produce a predictive model for the biogas. This was used to identify the interactional effects, optimize the process and maximize the biogas production. A quadratic polynomial model as represented in Equation 1, estimated the response surface, with adjusted and predicted R^2 values of 0.9993 and 0.9982, respectively.

Table 2 Box Behnken design matrix

Range	-1	1
pH	5	7
Temp (0'C)	20	30
HRT (days)	20	40
F/I (%)	25	75

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_{ii}^2 + \sum_{i<j}^{n} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where $X_i X_j$ represent the interaction terms, β_{ii} and β_{ij} represent the coefficients of the interaction factors respectively.

3. RESULTS AND DISCUSSION

The general application of the AD process includes treatment of waste water and generating clean fuel from renewable feedstocks. Thus, the biological degradation of the feedstocks by microbial community to produce the biogas can be used for electricity and heat generation. The performance of the AD depends on the raw materials and the microorganism adaptation to the environmental shocks or change in conditions. It is observed that co-digestion of the cow dung (I) and *Miscanthus Fuscus* (F) simultaneously increased in degradation and the bioconversion rate increased the biogas production,such that the grinding of the *Miscanthus Fuscus* into smaller sizes made the organic components more accessible to the microorganisms.

The fluctuation of the pH, might be due to the conversion of the volatile fatty acids into acids by the acidogenic bacteria presence. While pH above 6.5-7 led to alkalinity through the digestion of the organic compounds by methanogenic bacteria. This inhibited the microbial community and was favorable for the biogas production (Al Mamun & Torii, 2015).

The result selected to analyse the response was the actual biogas produced and that of the values for the independent variables as shown in Figure 2. It was found that the temperature of the AD process had a major effect on the biogas production, facilitating the degradation of the organic matter to produce the biogas. Thus, both the pH and temperature increased the metabolic rate of the microorganism. However, moving the pH towards the alkalinity range collapsed the biogas methanogenic process, which resulted in the low production of the biogas on the 3rd and 18th runs, as against the maximum biogas produced on the 17th run. It can be deduced that the significant increase in the biogas production rate, may be due to the biological activity. Thus, the bacterial produced hydrolysis enzyme increased because of the superior entree to the cellulosic material after the removal of the lignin structure (Tetteh et al., 2017). In addition, the substrate was not used effectively by the anaerobic system due to the lack of access to the cellulosic material. This may have been due to the number of bacteria for biodegradability, which was increased with the amount of the cow dung in the digester (Tetteh et al., 2017).

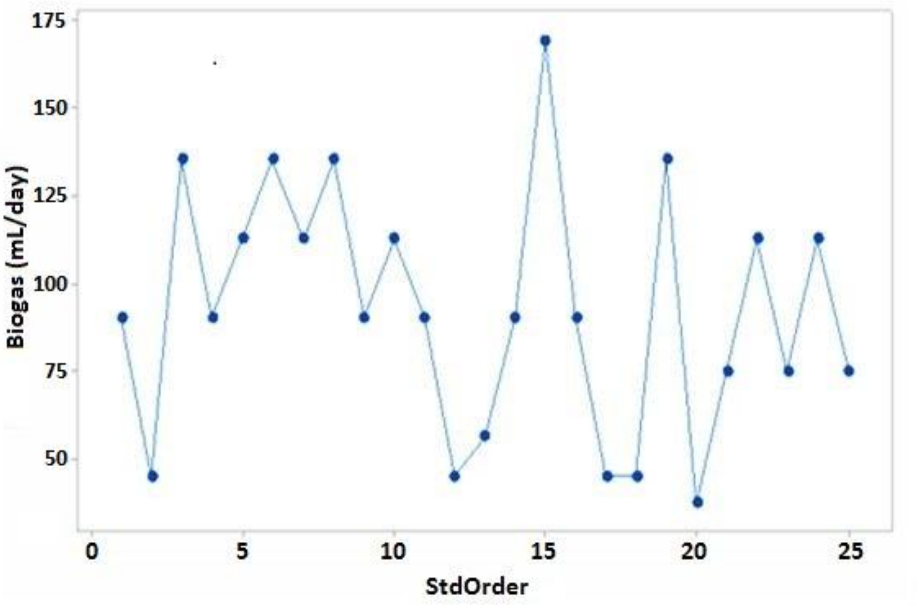

Figure 2 The standard runs for the biogas production, at pH (5-7), HRT (20-40 days), temperature (20-30ºC) and F/I (25-75 %)

From Figure 2, it was found that on the 17th run, the maximum biogas was produced at the operational conditions of HRT (20 days), temperature (30ºC) and F/I ratio (75%) with a favorable range for methanogenesis of pH (6). The 3rd and 18th runs showed almost the same amount of biogas volume (135 ml) produced with the same conditions of HRT (25 days) and

F/I ratio (75%), but with different pH and HRT values at 7 and 30 days for the 3rd run and 6 and 40 days for 18th run respectively. In addition, studies have shown that the source of the inoculum defines the content of macronutrients and trace elements, which could alter the AD process and therefore affect biogas production. Therefore, it is necessary to use inoculum with an active microbial community to enhance co-digesting of complex organic matter at a steady state in a large variety of the organic molecules (Tetteh et al., 2017).

3.1. Biogas Response Model

The exact expectation of the producible biogas amount is one of the most important aspects in the design of an anaerobic digester. The chemical composition of a feedstock determines the potential biogas yield, which is a function of the input variables. A second order regression model for the response surface was then developed for the biogas. This was expressed in the reduced form of the coded and actual values of the independent variables, indicating the influence of the input variables on the biogas yield as expressed in Equation 2. The positive and negative signs in front of each terms of the models exhibits synergetic and antagonistic effect on the response.

$$Y_{coded} = 90 + 45.63C - 18.75D - 9.37CD + 3.75D^2 \quad (2)$$

The Equation 2 in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1, likewise the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

$$Y_{actual} = 92.5 + 3.7C - 7.5D - 0.075CD + 0.15D^2 \quad (3)$$

The Equation 3 in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the centre of the design space.

3.2. Analysis of Variance (ANOVA)

Subsequently, the ANOVA multiple comparison test was employed, and it was found that the biogas model, and the corresponding terms, were all significant ($P<0.05$). The model validity was evaluated, and its statistical significance was controlled by the Fisher's exact test (F-value). The ANOVA values for the quadratic regression model obtained from the BBD employed in the optimisation of the biogas is given in Table 3.

In addition, an acceptable agreement of the adjusted and predicted determination coefficients values of 0.9993 and 0.9982, respectively were found. The value of the actual R^2 (0.9997) was closer to 1, which signified a high correlation existed between the actual values from the experiment and the predicted values (Table 3). The response model is highly statistically significant at 95% confidence level. The model F-value of 2484.19 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Likewise, the P-values less than 0.0500 indicate model terms are significant. In this case C, D, CD, D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant where model reduction was done to improve it. The standard deviation and adequate precision values obtained were 0.9232 and 183.61, respectively.

The actual and model predicted values for the biogas production is shown in Figure 3, corresponding to the change in operating conditions. Hence, the regression model explains that there is a relationship between the independent variables and the response, which explains the adequacy of the regression model (Safari et al., 2018).

Table 3 ANOVA results obtained to validate the model

Source	Sum of Squares	df	Mean Square	F-value	p-value	Comments
Model	29640.87	14	2117.2	2484.19	< 0.0001	significant
C-Inoculum concentration	24979.69	1	24979.69	29309.5	< 0.0001	
D-HRT	4218.75	1	4218.75	4950	< 0.0001	
CD	351.56	1	351.56	412.5	< 0.0001	
D^2	61.36	1	61.36	72	< 0.0001	
Residual	9.37	11	0.8523			
Lack of Fit	9.37	10	0.9375			
Cor. Total	29650.24	25				
Std. Dev.	0.9232					
R^2	0.9997					
Adjusted R^2	0.9993					
Predicted R^2	0.9982					
Adeq. Precision	183.611					

Thus, the experimental data points closer to the line of best fits indicated, the data was well fitted onto the model. Figure 4 shows the normal probability plots of the residuals for biogas production. The standardized residual plots indicate the normal distribution of the points follows a straight line, with only a few scattered. Although some scattering is expected, even with the normal data as shown in Figure 4, it can be presumed that the data is normally distributed (Protasov, 2018; Haris et al., 2018). Therefore, the normal probability plot indicated good validity and significance for the approximation of the regression model.

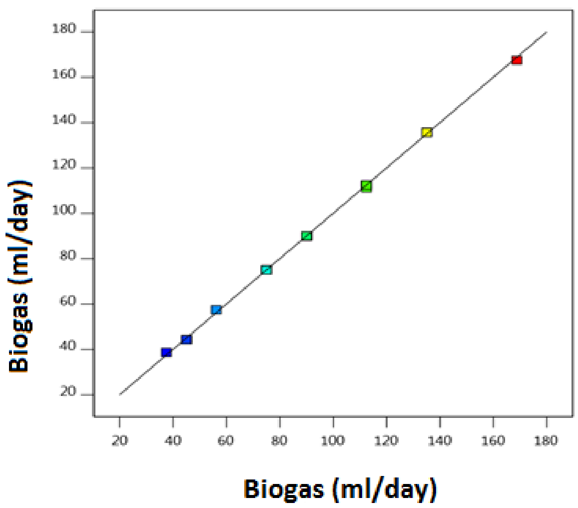

Figure 3 Experimental values versus the model predicted values

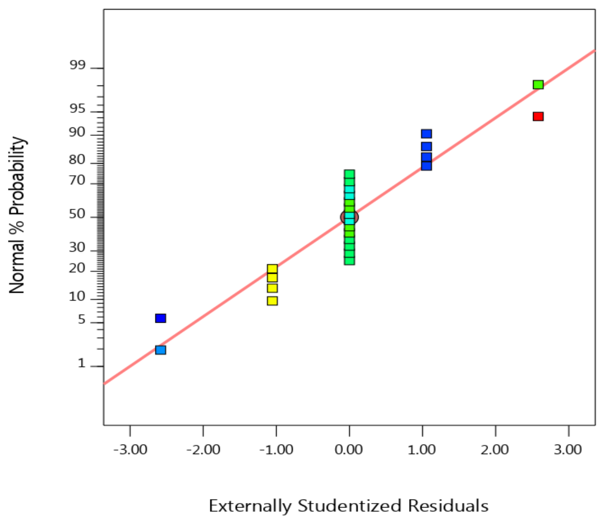

Figure 4 Normal probability plot

Figure 5 also shows residual versus predicted values for biogas yield. In this study, the experimental data points were scattered randomly within the constant range of residuals along the graph. This revealed that there was no obvious pattern and uncommon pattern. Increasing the temperature can significantly increase the biogas yield within mesophilic region (28-300C), and pH (5.9-6.1) and HRT (20 days). This shows the model is adequate, and there is no reason to suspect any violation of the independence or constant variance assumption in all the runs.

Both 3D plots in Figure 6 depicted that an increase F/I ratio from 25% to 75%, increased the biogas production. The three-dimensional (3D) plot (a) shows the response surface for investigating the interactive effect of the pH and F/I ratio, and the 3D plot (b) shows the temperature and F/I ratio to maximise the biogas production within the experimental ranges. It is observed that the F/I ratio was the main contributing factor (Safari et al., 2018). Therefore, in finding the solution of the operating conditions, and with the desired goal of the model to maximise the biogas production, the optimum values for the process variables.

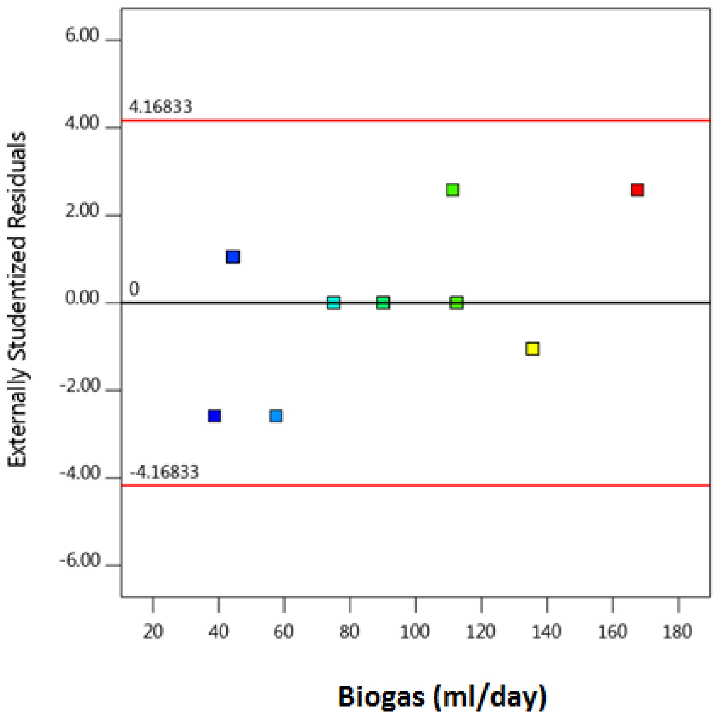

Figure 5 Diagnostic plots for residual versus predicted biogas yield

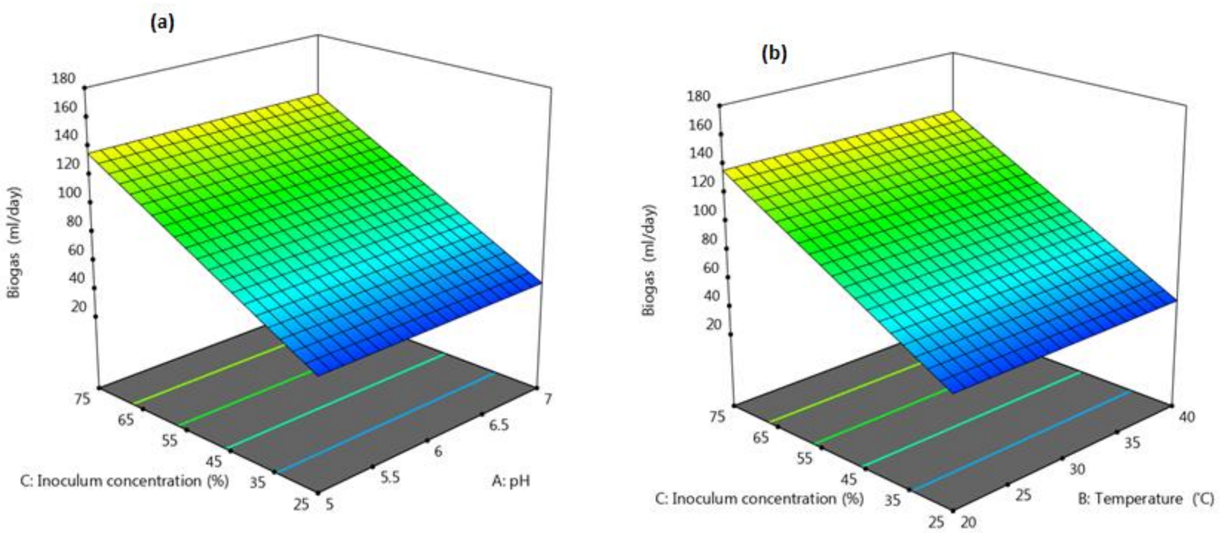

Figure 6 The 3D plot for the biogas response surface: (a) pH versus F/I ratio; (b) temperature versus F/I ratio

The desirability was optimised as a function based on the response goal as shown in Table 4, after verification through further experimental testing with the predicted values. The desirability function value was found to be 98% for the respective optimum conditions.

Table 4 The solution for the optimised process conditions to maximise the biogas production

Runs	pH	Temperature (ºC)	F/I ratio (%)	HRT(days)	Biogas produced (ml/day)	Desirability
1	6.0	30.0	75	20	167.5	98%
2	6.0	29.9	75	20	167.5	98%
3	6.1	29.7	75	20	167.5	98%
4	5.9	29.3	75	20	167.499	98%
5	6.1	29.4	75	20	167.499	98%
6	5.9	30.7	75	20	167.499	98%
7	6.1	29.3	75	20	167.499	98%
8	6.1	30.4	75	20	167.5	98%
9	5.9	28.9	75	20	167.498	98%
10	6.1	30.8	75	20	167.497	98%

4. CONCLUSION

The biogas production from *Miscanthus Fuscus* mixed with cow dung was investigated in a batch co-digester process. This could be of economic important in terms of protecting the environment and reducing greenhouse gases. The results showed that the ratio of *Miscanthus Fuscus* and cow dung (as F/I ratio of 3:1) had a significant impact on biogas production. The RSM and BBD employed proved to be economical and a reliable tool for modelling, optimizing and studying the interactive effects of the four process factors (pH, temperature, HRT and F/I ratio) for the biogas production. A highly significant (R^2 = 0.9997; $P < 0.0001$) regression quadratic model equation was obtained by analysing the experimental data obtained from the BBD matrix. The AD process conditions for effective control and biogas performance is therefore defined as a pH of 6, a temperature of 30ºC, HRT of 20 days and F/I ratio of 75%. Therefore, using a mixture of organic wastes such as cow dung (I) and *Miscanthus Fuscus* (F) for biogas production can be encouraged since it reduces waste generation and green energy, and is good for sustainable social economic developments and environmental cost.

5. ACKNOWLEDGEMENT

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