

Experimental co-digestion of carbon rich source and earthworm bedding wastewater to improve Bio-methane potential using experimental design

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ABSTRACT

Corn cob and corn husk are a rich source of carbon content. The added high nutrients as carbon content to the anaerobic digestion of nitrogen-rich earthworm bedding wastewater could establish positive synergism for efficient methane production. The experimental results showed that the model had significant ($p < 0.05$) with R^2 value of 0.989. The maximum Bio-methane potential of 26.91% with methane content of 83% was obtained at the optimum conditions of 12% of TS of substrate and 50:50 of corn cob to corn husk ratio. Structure analysis by X-ray diffraction (XRD) showed that the cellulose structure of substrate was destroyed and changed. The destruction of the fiber structure on the surface of corn cob and corn husk after digestion was found to be clearly by SEM. The results confirmed that RSM technique with a central composite design were useful tools for optimizing the Bio-methane potential from co-digestion.

Keywords:

Earthworm bedding wastewater, Corn cob, Corn husk, Co- digestion, Response surface methodology.

1. INTRODUCTION

Energy is the most important factor in the development of countries. The increase in energy demand is growing rapidly for the industrial countries of the world and the limited amount of fossil fuels as a source of non-renewable energy. The main sources of the largest producer of pollution problems comes from the combustion process of fossil fuels like oil, spilling the different types of air pollution during transportation, burning of coal, natural gas, and gasoline to produce electricity resulting in the change in atmospheric condition and other changes. As a result, researchers began to search for new alternative energy. Renewable resources continue to draw attention all over the world. Energy production from lignocelluloses, as a biomass energy source is a feasible choice for the replacement of traditional fossil fuels because they are bio- renewable, sustainable, low cost, environmental friendly and clean energy for communities [1].

Currently, the operations of industrial agriculture produce many types of lignocellulosic wastes and their effect on the environment, including contamination of surrounding water bodies, ground water pollution and the atmosphere. Corn or maize is one of the most common crops grown in all regions of Thailand. In particular, sweet corn is one of the major exports products. So, each year there is a lot of lignocellulosic waste (corn cobs, stalks, leaves and husks as a solid waste) from processing corn production in the corn factory. The corn cob agricultural residue, which composes mainly hemicelluloses and cellulose content, can be used as a renewable raw material to produce alternative energy using a fermentation process. Among various lignocelluloses for cellulosic fermentation, corn cob residue shows advantages in its high cellulose content and no pretreatment requirement [2, 3]. Usefulness of these wastes has been studied by many researchers in order to reduce

environmental pollution and emissions [4], produce activated carbons [5], produce bio-char [6], produce bio-oil [7], produce ethanol [8] and produce biogas [9].

In recent years, interesting biogas technology has been implemented widely and rapidly. The technology of anaerobic digestion or fermentation is typically a process for biogas generation that is essentially produced from carbon by photosynthetic organisms, which capture light energy from the sun by the chlorophyll, using water, atmospheric CO₂ and soil nutrients. Residue from agricultural production and processing, manure or any other biodegradable feedstock from animal farms, sewage sludge, solid waste, wastewater from industrial and household sources all contain waste organic matter, which can be degraded and converted to biogas. The utilization of biogas typically is in a biogas engine, to produce electricity, transportation fuels and useful heat, at high efficiency (Fig. 1) [10].

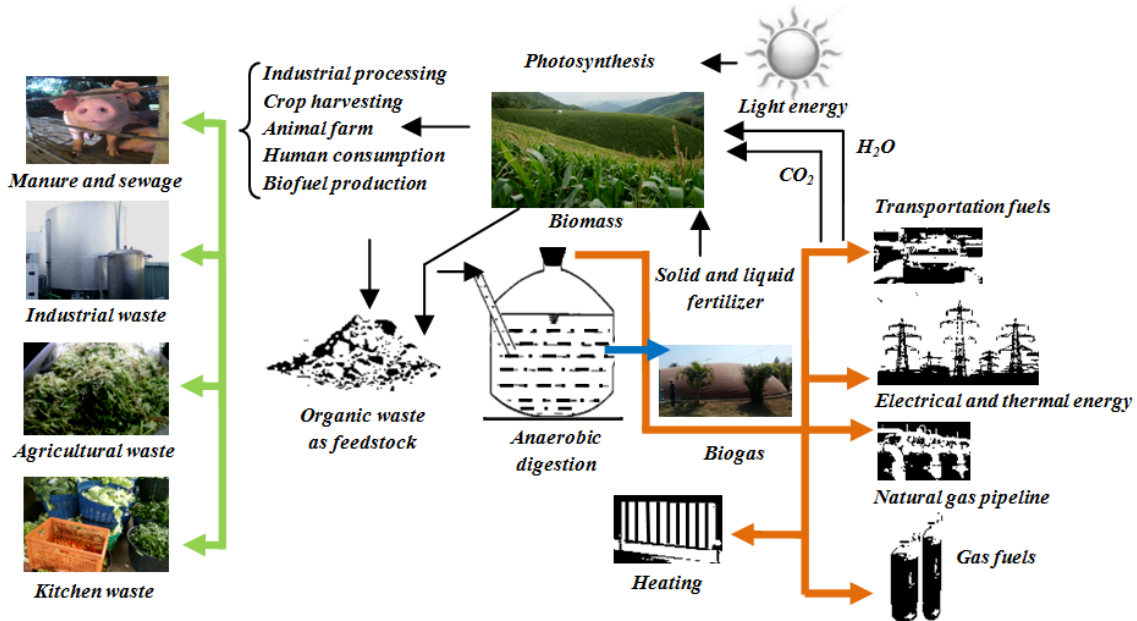


Fig. 1 A schematic flow diagram of global biogas cycle.

Anaerobic digestion for biogas production is a multi-step biological process. The important processes in anaerobic digestion are hydrolysis, fermentation, acetogenesis, and methanogenesis. The hydrolysis step is degradation of complex organic materials into smaller units by hydrolytic and fermentative bacteria. The hydrolysis of the substrates is utilized by fermentative bacteria in acetogenesis and acetogenesis process. Fermentation products such as acetate, hydrogen and carbon dioxide can be converted directly by methanogenic microorganisms into the main component of CH₄ and CO₂ [11]. Fig. 2 shows carbon flow diagram of the biogas process, with individual processes described as follows.

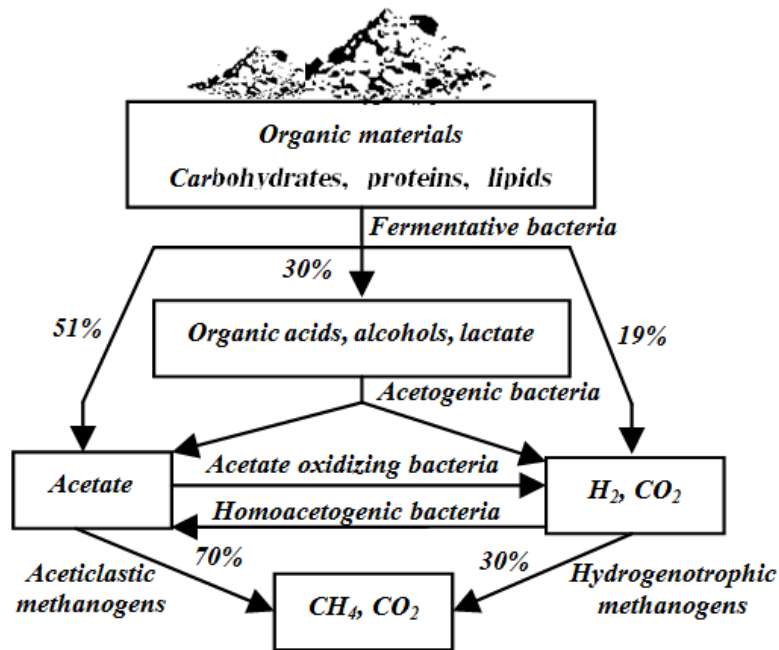


Fig. 2 Carbon flow diagram of the biogas process [11].

Generally, most of agricultural residues can be used to combine with the liquid manure for co-digestion performing for increase in CH_4 conversion. By the co-digestion, the increasing in content of organic substrate as shown in carbon to nitrogen ratio (C/N) has enhanced the biogas production. In the fermentation process, the C/N ratios deriving from swine manure is 10 ± 16 while combining with other agricultural residues can increase C/N ratio to 20 [12].

Presently, agricultural production releases large amounts of wastewater in daily farming. Especially, raising earthworms for profit is popular for rural farmers. The important part of raising earthworms is preparation of earthworm bedding. Bedding is the natural habitat material from any carbon-rich organic matter and nutrient-rich compost material. Animal manure is generally being used for earthworm composting bedding. In the process of the suitable bedding preparation, dry manure is soaked into the huge amount of water for 2 - 3 days to dilute more nutrients because the earthworm is very sensitive to pH and toxic materials. The large quantities of wastewater produced from this process are release into the river.

In this work, the main objective was to investigate the biogas production from co-digestion of wastewater from earthworm bedding preparation, sweet corncob milling process and corn husks to improve digestion performance of methane yield, in a Bio-methane potential (BMP) batch test using a statistical technique for designing experiments in order to optimize the multivariable system. The experimental design was to analyze the effect of the total solid of substrate (TS) and the ratio of sweet corncob milling process to corn husks on biogas production by DOE using the response surface methodology (RSM) technique in a batch reactor under anaerobic digestion.

2. MATERIALS AND METHODS

2.1 Substrates

The sweet corncob and corn husks used in this study were kindly provided from a corn canning company located in the north of Thailand. Sweet corncob and corn husks were milled to particles approximately 0.5 - 1.0 cm in size by a grinding machine before further use. During the experiment, substrates were directly used without any other pretreatment. Earthworm bedding wastewater samples

used in this study was collected from an earthworm farm in the north of Thailand where cow manure was used to prepare the earthworm bedding.

2.2 Inoculum

Inoculums were taken from the swine farm with 20 percentage working volume at a local swine farm located in north of Thailand, which were used in co-digestion process of earthworm bedding wastewater, sweet corn cob milling process and corn husks waste. The swine farm has been kept working for more than 5 years.

2.3 Batch laboratory anaerobic digestion tests

The anaerobic batch co-digestion tests in all experiments were carried out in duplicate. 1 L digester setup with 800 mL working volume was performed in brown glass bottles. Feed as the substrate was introduced into the reactor and immediately sealed using para-film and aluminum crimp caps with drilled holes to ensure anaerobic conditions where a slender plastic tube with a silicone tube connected to export gas into the gas collector for biogas volume by the water displacement method. The blank digester contained inoculums and distilled water to measure the background biogas production. The digesters were placed in a water bath at constant temperature of 35 ± 1 C°. The experiments were run for approximately 30 days and mixed once a day to avoid stratification. The biogas volume was recorded every day through the water displacement method and cumulative biogas volume was calculated. The biogas was packed in plastic back to measure the proportion of methane and other production by gas detector. Gas chromatography (GC) was used to measure and confirm the production of methane content in the gas.

2.4 Bio-methane potential (BMP) assay

The Bio-methane potential (BMP) assay can be used as an index of the anaerobic biodegradation potential to determine the methane yield of co-digestion substrate as follows (Eq. 1). BMP assay can be determined through stoichiometric conversation, CH₄ production is related to organic removal; 1 gCOD reduction equals 395 mLCH₄ (35 C°, 1 atm) [13]. However, methane production can vary by co-digestion substrate and due to multiply parameters in the digester.

$$\frac{BMP(mlCH_4)}{gCOD} = \frac{Biogasproduction(L) \times CH_4(\%)}{(COD_{in} - COD_{out})(mg / L)} \quad (1)$$

2.5 Experimental design and procedures

Response surface methodology (RSM) technique was used to find the optimum conditions to investigate the interactive effects of factors. Two factors of total solid of substrate (X_1) and the proportion of corn cob to corn husk (X_2) were varied for the experimental design in this study. A three-level, with central composite design (CCD) was used in the optimization to find out the individual and interactive effects of these two variables. The general form of second-order polynomial model is as follows (Eq. 2):

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \left(\sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij} x_i x_j \right)_{i < j} \quad (2)$$

Where y is the predicted response evaluated; x_i x_j are individual variables; β_0 is the offset term; β_i , β_{ii} , and β_{ij} are the linear coefficients, the quadratic coefficient and interaction coefficient, respectively; and k is the number of studied factors. The correlation coefficient, R^2 was used to evaluate the quality of the model equation. F-test and t-test were used to determine the statistical significant and the

significance of the regression coefficients and analysis of variance (ANOVA). According to the statistical graphics Design-Expert software design, 13 runs of experiments were performed with five replications of the center points where the axial point was determined to be 1.682. Subsequently, the response surface plot (3D) and contour plot (2D) of the response were displayed to give visual insight into the variable effects on Bio-methane potential values. The dimensionless ones (X_1, X_2) with the code values at levels: $-\alpha, -1, 0, +1, +\alpha$ were used for two independent variables.

2.6 Analytical Procedures

The parameters of initial substrate were analyzed such as pH, TS, VS, COD, ALK, moisture content and C/N ratio. Liquid samples in digesters were taken for pH value measurement by pH meter (pH620 BENCH-TOP). The laboratory batch test was measured following the AOAC standardized procedures [14]. The moisture content and TS was determined as the weight loss in a convection oven at 105 °C drying of a biomass sample. VS was measured as weight loss after burning in muffle furnace at 550 °C to drive off volatile solids in the sample. COD was analyzed using closed reflux by titration method. Carbon and nitrogen were analyzed to calculate the ratio of carbon to nitrogen in the substrate. Each analytical result was performed in three measurements and the average value was selected for determination. Scanning electron microscope (SEM) was used to observe surface morphology of sample before and after anaerobic digestion. The phase crystallite of sample was conducted with X-ray diffraction analysis using X-ray diffractometer with CuK_α radiation.

3. RESULTS AND DISCUSSION

3.1 Characteristics of the substrate

Analytical methods of the initial characteristics of the substrate and properties were investigated and listed in Table 1. Earthworm bedding wastewater was rich in total nitrogen and C/N ratio of 5.01 ± 0.12 with a high COD value of $36,560 \pm 10.42 \text{ mg L}^{-1}$ and 6.80 ± 0.10 of pH. The TS (%) in the current study was 8.16 ± 0.77 , which is higher than TS (%) of inoculums that had 4.86 ± 0.51 . C/N ratio of corncob and corn husk was 41.00 ± 0.54 and 32.21 ± 0.36 , respectively. The C/N ratio of corncob was higher than corn husk and the earthworm bedding wastewater. They were 1.2-fold and 8.2-fold, respectively. The C/N ratio was the critical parameter for biogas production. It showed the optimal nitrogen levels. The digestion of single raw material with low C/N ratio has been found to cause the retention of nitrogen conversion and increasing of ammonia resulting in the inhibition of microorganism activity for biogas production. The C/N ratio of 23-30 is often cited as optimal [15]. Therefore, corncob, corn husk and the earthworm bedding wastewater with different C/N ratio were used for co-digestion to adjust the C/N ratio of initial feedstock for digestion of methane gas. The corn husk and corncob were $51.35 \pm 2.51\%$ and $45.52 \pm 1.13\%$ of cellulose and hemicelluloses content on dry basis. The cellulose can be converted to biogas by biological process. However, the presence of lignin and hemicelluloses content causes difficulty in enzymatic hydrolysis due to its heterogeneity and crystallinity of structure [15]. The initial pH of inoculums and earthworm bedding wastewater was suitable for the methanogenic process in anaerobic digestion and initial alkalinity of substrate in the range 1,500 – 5,000 mg/L would retain the self-buffering capacity. [16]. The compositions of the substrate are shown in Table 1.

Table 1. Characteristics and chemical composition of the substrate used in the experiments (% dry matter, except C/N ratio).

Characteristics	Value			
	Inoculum	earthworm bedding wastewater	Corn cob	Corn husk
pH	7.30 ± 0.10	6.80 ± 0.10	NA	NA
TS (%)	4.86 ± 0.51	8.16 ± 0.77	93.12 ± 4.25	91.81 ± 5.14
VS (% of TS)	62.65 ± 2.28	70.42 ± 2.43	87.23 ± 3.43	88.27 ± 2.52
C/N ratio	12.13 ± 0.42	5.00 ± 0.12	41.00 ± 0.54	32.21 ± 0.36
COD (mg L ⁻¹)	8,560 ± 8.82	36,560 ± 10.42	NA	NA
ALK (mg CaCO ₃ L ⁻¹)	2,455 ± 11.57	2,120 ± 13.35	NA	NA
Cellulose (%dry basis)	NA	NA	45.52 ± 1.13	51.35 ± 2.51
Hemicellulose (%dry basis)	NA	NA	34.05 ± 2.53	27.26 ± 1.22
Lignin (%dry basis)	NA	NA	14.27 ± 0.50	13.55 ± 1.10

NA: no analysis

3.1 Optimization of Bio-methane potential

The experimental design of the variables in the coded and actual values for studying the effects of two independent variables was depicted in Table 2 with the experimental values of Bio-methane potential as the response. The quadratic model and evaluating the relationship between TS of substrate (X_1) and corncob to corn husk ratio (X_2) were used to predict values of Bio-methane potential by CCD. The response factor, which is Bio-methane potential, was calculated through Eq. (2). From the design of the experiment, the interaction plot compared all the influencing factors in a single point. The variable steepness of the curve indicated the most influence of the variable. The plot in Fig. 3 shows that factor X_1 (TS of substrate) is the most influential factor towards the response value which is the Bio-methane potential. The second most influential factor is X_2 (corncob to corn husk ratio). However, there was no interactive effect between these variables ($p > 0.05$). The comparison between the actual values calculated after experimentation and the predicted values calculated from the chosen model is shown in Fig. 4. The graph shows the predicted values versus the experiment values from anaerobic co-digestion. The points are a strong linear association, which indicates that the calculated values of the model are close to the experimental values. The production of Bio-methane potential depends on two factors that include TS of substrate and corncob to corn husk ratio. TS of substrate are found to be the most variable of influence as compared to corncob to corn husk ratio.

The three dimensions (3D) response surface and the two dimensions (2D) contour plots of TS of substrate and corncob to corn husk ratio on Bio-methane potential are shown in Fig. 5a-5b. The Bio-methane potential increases by increasing the TS of substrate up to 12% when corncob to corn husk ratio were kept at central point. However, the Bio-methane potential was produced at low levels at TS of substrates of 8 and 16%. The results are similar to that of previous reports [17]. The overloading in the digester resulted in the inhibition of digestion and reduction of the methane production process. The highest Bio-methane potential was approximately 26.91% with the maximum content of 83% of methane at 12% of TS of substrate when corncob to corn husk ratio was kept at 50:50.

Table 2 Experimental design for Bio-methane potential.

Run	Code values		Actual values		CH ₄ content (% v/v)	Bio-methane potential (%)
	X ₁	X ₂	X ₁	X ₂		
1	0	0	12.00	50.00	83.00	26.91*
2	-1	0	8.00	50.00	76.50	22.00
3	-α	+α	9.17	85.36	66.81	17.50
4	0	0	12.00	50.00	82.70	26.73*
5	+α	+α	15.21	85.36	42.32	12.70
6	0	+1	12.00	100.00	38.51	8.70
7	-α	-α	9.17	14.64	72.31	19.24
8	0	0	12.00	50.00	82.20	26.71*
9	0	0	12.00	50.00	82.00	26.72*
10	0	-1	12.00	0.00	48.41	14.70
11	0	0	12.00	50.00	82.50	26.71*
12	+1	0	16.00	50.00	51.70	15.40
13	+α	-α	15.21	14.64	50.00	14.91
blank	-	-	-	-	36.10	8.58

X₁ and X₂ were the code values of TS of substrate and corncob to corn husk ratio, respectively.

* Data center points

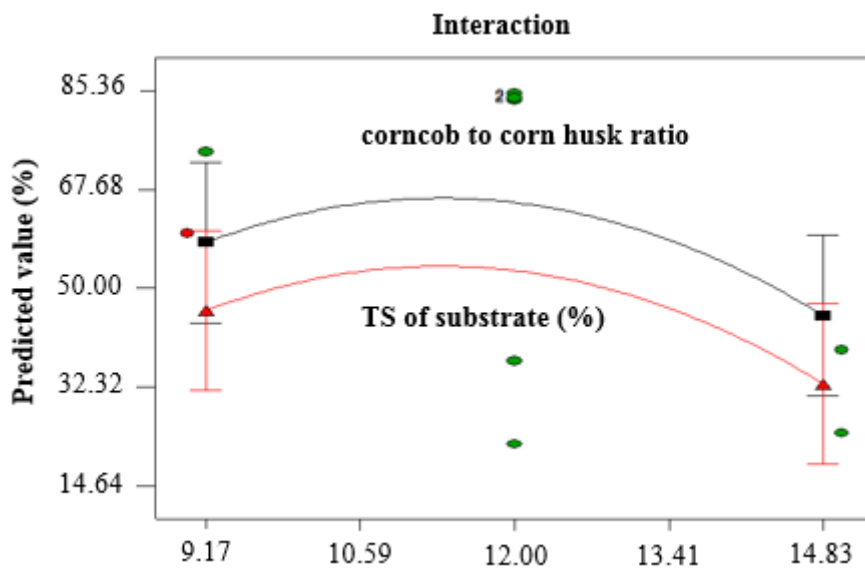


Fig. 3 The interaction plot of TS of substrate and corncob to corn husk ratio.

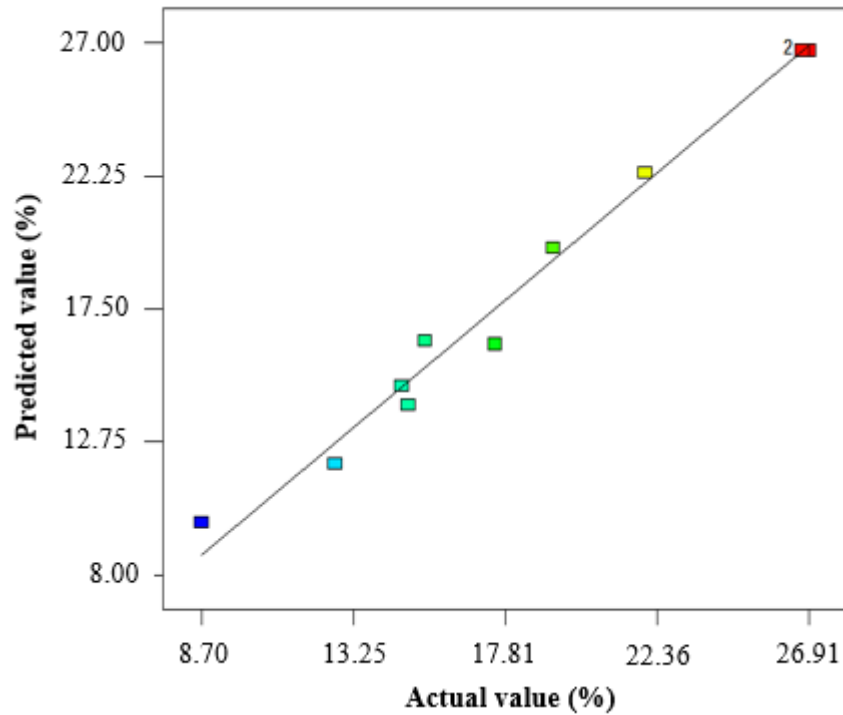


Fig. 4 Predicted vs. experimental Bio-methane potential values.

ANOVA was used to analyze the data obtained from the independent variables to confirm the significance and suitability of response variable. Table 3 shows the ANOVA analysis of quadratic polynomial model. P-value less than 0.05 ($p < 0.05$) for the variable indicates model terms are significant. The reliability of the model and variance of the response are represented by F-value. RSM suggested quadratic model with F-value of 180.13 that implied the significance of this model. In this case, X_1 , X_2 , X_1^2 , X_2^2 were significant model terms and the Lack of Fit F-value of 180.72 indicated that the model was significant.

Table 3. ANOVA analysis of quadratic polynomial model.

Source	Sum of squares	DF	Mean square	F-value	p-value
Model	489.69	7	122.42	180.13	< 0.0001 significant
X_1	37.33	1	37.33	54.93	< 0.0001
X_2	23.71	1	23.71	34.89	0.0004
X_1^2	98.63	1	98.63	145.13	< 0.0001
X_2^2	363.91	1	363.91	535.47	< 0.0001
Residual	5.44	8	0.68		
Lack of Fit	5.41	4	1.35	180.72	< 0.0001 significant
$R^2 = 0.989$	$R^2_{adj} = 0.984$		Adequate precision = 32.999		

The quadratic model equation in terms of coded and actual factors is the results of regression analysis. The statistical model in terms of actual factors by applying multiple regression the relationship between the Bio-methane potential (Y) and the coded values of independent factors and their respective interactions was described in Eq. (3).

$$Y = -42.6206 + 10.3105X_1 + 0.5286X_2 - 0.4609X_1^2 - 0.0057X_2^2 + 792X_3^2 \quad (3)$$

Where Y : is the response, X_1 (TS of substrate (%)) and X_2 (corn cob to corn husk ratio) are factors in DOE code. The value of R^2_{adj} for this model is 0.9835 which is in good agreement with R^2 value.

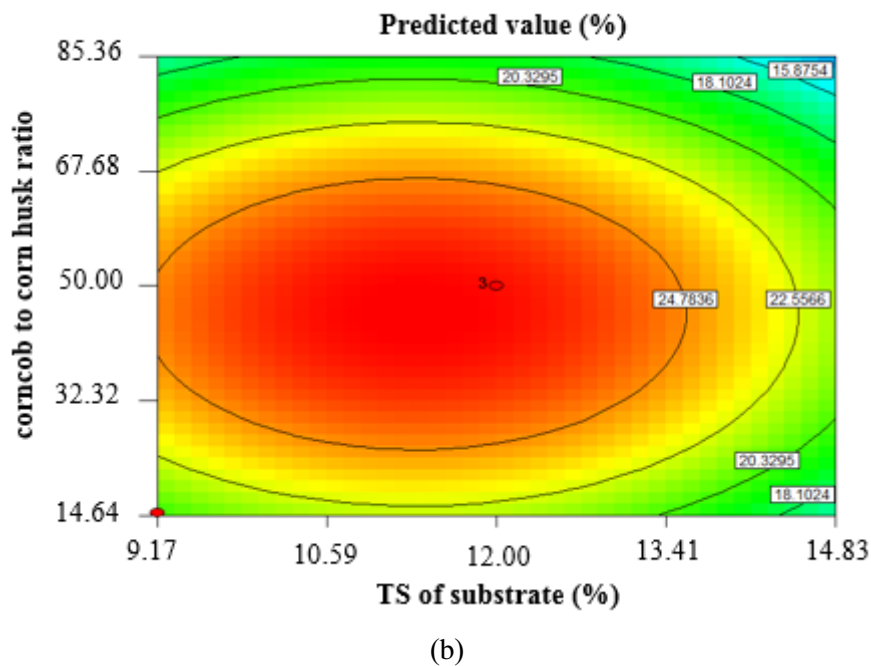
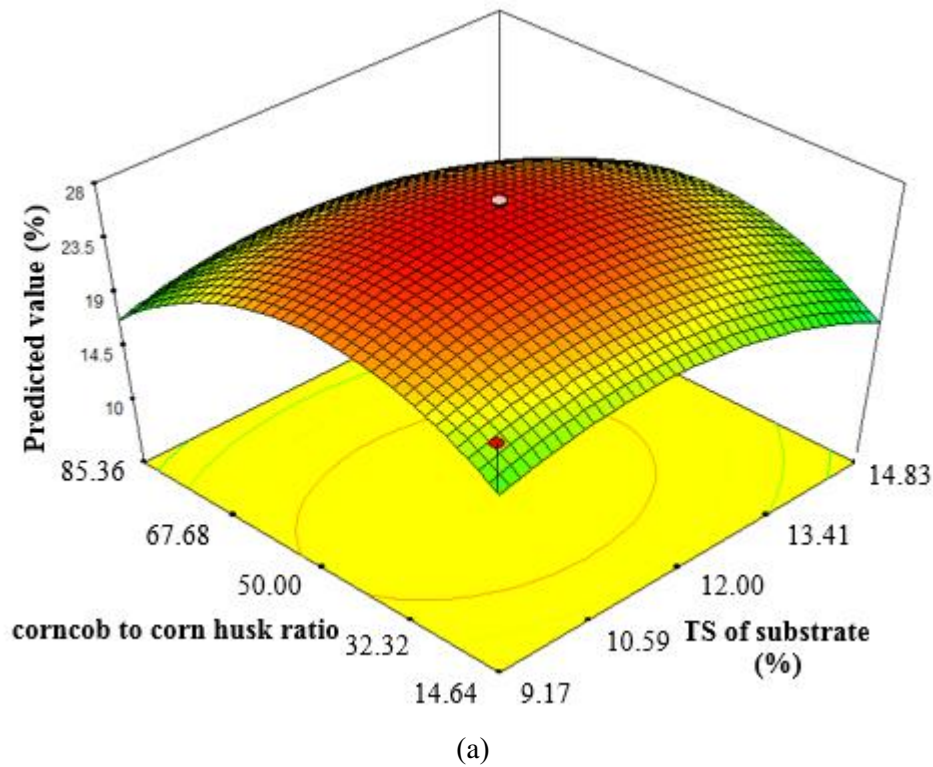


Fig. 5 Combined effect of TS of substrate and corncob and corn husk ratio: (a) the 3D plot and (b) the contour plot.

3.2 Morphological characteristic analysis of the substrate

The morphological structures of undigested and anaerobic digestion of corncob and corn husk are illustrated in Fig. 6. The morphological changes on surface after anaerobic digestion were observed by SEM. The magnification of the images is 100. The SEM micrograph of undigested corncob milling process and corn husk exhibited the cellular structure of the fiber which packed together (Fig. 6a - 6b). After anaerobic digestion, corncob and corn husk clearly showed the destruction of the fiber structure, network structure was destroyed and numbers of particles with different shapes and sizes of fiber surface

(Fig. 6c - 6d), which was expected for the digestion of the cellulose complex [18]. A detailed investigation was carried out to determine the effect of anaerobic digestion by anaerobic microorganisms in a digester that may cause destruction of macromolecular chains, leading to mutual displacement of separate structural elements and the displacement of structural elements of polymeric chains [19]. The presence of the small particles on the surface during the anaerobic digestion was digestion of the depolymerization of hemicelluloses [18].

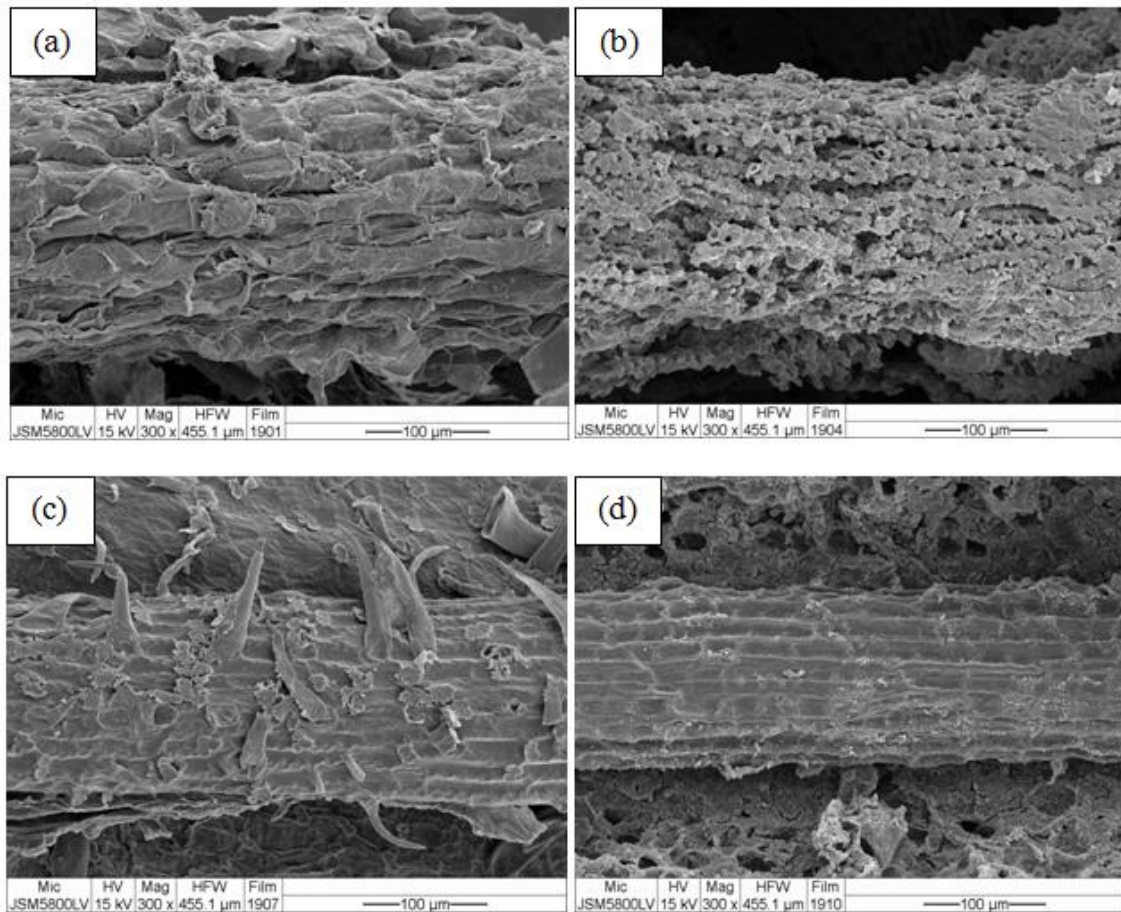


Fig. 6 SEM images of corncob before (a) and after (b) anaerobic digestion and corn husk before (c) and after (d) anaerobic digestion from Run6 and Run10, respectively.

3.3 Physical structure of the substrate

X-ray diffraction (XRD) patterns of the corncob milling process and corn husk before and after anaerobic digestion are shown in Fig. 7. The undigested corncob and corn husk appeared as a sharp high peak at 2-Theta closed to 22°, which is corresponding to the transverse arrangement of the lattice plane of cellulose I [0 0 2] [19]. The results show that the reflection [0 0 2] becomes wider and less intensive after anaerobic digestion, indicating that the cellulose structure was destroyed and changed due to a breakage of the original structure. This observation was further confirmed by XRD analysis.

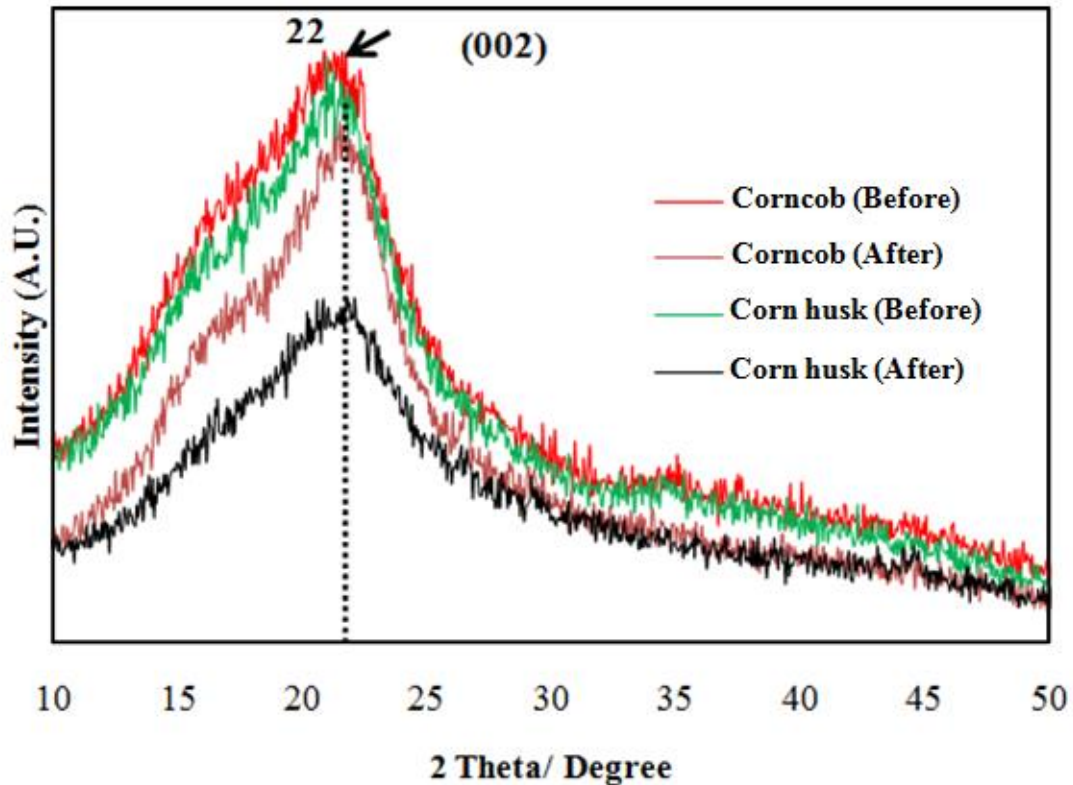


Fig. 7 X-ray diffraction patterns of corncob and corn husk before and after anaerobic digestion from Run6 and Run10, respectively.

4. Conclusions

The present study focuses on the optimization of different types of variables for the maximal Bio-methane potential from co-digestion of earthworm bedding wastewater, corncob and corn husk in batch tests. The response surface methodology using CCD was adopted for the parametric study on the Bio-methane potential in anaerobic digestion. The effects of two different types of variables namely, TS of substrate and corncob to corn husk ratio were studied. TS of the substrate showed the most influence of variables in the anaerobic digestion test for Bio-methane potential. The optimized conditions of the Bio-methane potential were TS of substrate of 12%, corncob to corn husk ratio of 50:50. The maximum Bio-methane potential was 26.91%. The model was found to have significant effects ($p < 0.05$) on efficient generation of Bio-methane potential in anaerobic digestion. The results showed that RSM was useful to optimize the prediction of Bio-methane potential in anaerobic digestion. The value of the R^2 for RSM was 0.989. The high value of R^2 confirmed that the model could be efficiently used for prediction of Bio-methane potential in this study successfully.

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