



Liquid State Anaerobic Co-digestion of Cattle Manure and Wheat Straw at Various Mix Ratios for Optimal Biogas Production

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ABSTRACT

Anaerobic codigestion study was performed on cattle manure (CM) and wheat straw (WS) at 5% total solids in mesophilic temperature. CM and WS were mixed on the basis of total solids in six ratios, namely, 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. The highest biogas production of 170.89 mL/g- $v_{s_{added}}$ was observed in the reactor fed with 100% CM (100:0). A considerable amount of biogas was measured up to 60:40 mix ratio (CM:WS). With the increase in proportion of WS in the mix, biogas production was decreased. Reactor stability in terms of pH and volatile fatty acids were also decreased with increase in the proportion of WS. The last three mix ratios showed low biogas production due to the less methanogenic activity caused by the low pH. Three kinetic models, namely modified gompertz model, growth and monod were employed to predict cumulative biogas production. Among these, modified gompertz was found the most suitable model.

Keywords: Anaerobic codigestion, Biogas, Agriculture residue, Cattle manure, Wheat straw, Kinetic study.

INTRODUCTION

India is an agriculture-based country, where 58% of the population lives under agriculture sector¹. The major activities of the agriculture sector are crop production and dairy farming. These activities resulting in by-production of biodegradable materials as agriculture residue and cattle manure. In some parts of India (particularly in Haryana, Punjab, Uttar Pradesh) burning of agriculture residue to eliminate the excess waste^{2,3}. It is causing air pollution in the neighbouring regions along with loss of valuable resource. Similarly, the

cattle manure produced from dairy farms is either stored in a manure pile or burned when it gets dry. Such improper management results in emission of greenhouse gases such as carbon dioxide and methane along with other pollutants⁴. This study aimed to produce valuable resources (biogas and natural fertilizer) from agriculture residue and cattle manure through anaerobic digestion.

Anaerobic digestion is a complex process of digesting organic material using microorganisms in absence of oxygen and produces the valuable products as biogas and



sludge^{5,6}. During anaerobic digestion, organic material passes through four stages of digestion process. The first stage is hydrolysis, where the polysaccharides, cellulose, hemicellulose, starch etc, get converted into carbohydrates, proteins, and lipids. Acidogenesis is the second stage, where the hydrolysed products transform into long-chain fatty acids, amino acids, and alcohols. In the third stage, acetogenesis, long-chain fatty acids are disintegrated into smaller molecules such as acetic acid and other small chain volatile fatty acids along with the hydrogen. Methanogenesis is the final stage of anaerobic digestion process, in which methanogenic bacteria convert the acetic acid, small chain volatile fatty acids and hydrogen into biogas^{7,8}. On the basis of total solids (TS) content of the substrate anaerobic digestion can be classified into three groups. They are liquid state (0% < TS < 10%), semi solid state (10% < TS < 15%) and solid state (TS > 15%) anaerobic digestion^{9,10}.

Anaerobic digestion of agriculture residues (for example wheat straw) alone may cause hindrance to the process due to the presence of relatively less biodegradable lignocellulosic and other materials¹¹. Literature indicates that Co-digestion of agriculture residue with cattle manure may be suitable and economical options at least in rural areas of developing countries. Co-digestion of wheat straw (WS) along with cattle manure (CM) may enhance the biodegradability of lignocellulosic component of agriculture residue^{12,13}.

In view of the above, the present study aimed to examine the fundamental obstacles that occur in the codigestion of WS and CM. An anaerobic batch experiment was performed at 5% total solids

content in mesophilic temperature. Three kinetic models, the modified Gompertz model, growth model, and Monod models were applied to predict the biogas production, lag phase, and hydrolysis rate.

MATERIALS AND METHODS

Substrate and Inoculum

Wheat straw (WS) as agriculture residue and cattle manure (CM) were collected from the local dairy farms nearby the National Institute of Technology Patna. Well mixed sun dried WS was grinded using kitchen grinder, passed through 1.18 mm sieve and store in airtight container for further use. The collected CM was properly mixed and passed through 1.18 mm sieve before storage in an airtight container at 4°C.

Experimental procedure

Six black colored glass bottles of 1 L volume were used as batch anaerobic digester. Each reactor was filled with 600 g of CM and WS mixture (100:0, 80:20, 60:40, 40:60, 20:80, and 100:0), on total solids (TS) basis as shown in the Table 1. Required amount of distilled water was added to each reactor to achieve 5% TS in the system. The reactor with 100% WS (100:0) was also supplied with 2% CM to ensure the presence of anaerobic bacteria. All the reactors were placed in a temperature-controlled water bath at mesophilic temperature (35±0.5°C) for a digestion period of 50 days. Biogas was collected using water displacement method. Volume of displaced water was noted down on daily basis as it is equal to the biogas production from each reactor. Contents of the reactors were manually mixed for a duration of one minute per day. The reactors were indicated as Rx, where x stands for reactor mix ratio, as shown in Table 1.

Table 1: Mix ratios of cattle manure (CM) and wheat straw (WS) and their weights

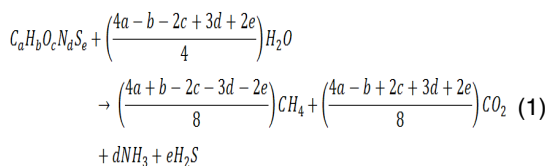
Reactor	%TS ratios of CM and WS	TS required in grams (CM: WS)	Weight of CM (gm)	Weight of WS (gm)	CM+WS (gm)	Water to be added in mL
R ₁	100: nil	30: NIL	190.48	Nil	190.48	409.52
R ₂	80: 20	24: 6	152.38	6.60	158.98	441.02
R ₃	60: 40	18: 12	114.29	13.19	127.48	472.52
R ₄	40: 60	12: 18	76.19	19.79	95.98	504.02
R ₅	20: 80	6: 24	38.10	26.39	64.49	535.52
R ₆	Nil: 100	Nil: 30	Nil	32.99	32.99	567.01

Analytical methods for the characterization of CM and WS

All the chemical tests were performed using the analytical grade chemicals. Moisture content (MC), total solids (TS), volatile solids (VS), pH, alkalinity, volatile fatty acids (VFA) were determined according to the testing procedures described in standard methods for water and wastewater treatment technologies¹⁴. Moisture content (MC), total solids (TS), and volatile solids (VS) were measuring using raw samples. For measuring pH, alkalinity, and volatile fatty acids (VFA), 5 g sample was mixed in 100 mL distilled water and filtered through the 11 μm filter paper. The filtrate was analyzed to determine the pH, alkalinity, and volatile fatty acids (VFA). pH was measured using a digital pH meter. Alkalinity was determined by the titration method. VFA were measured using the distillation method followed by titration.

Determination of theoretical biogas production

Modified Buswell-Boruff equation (Equation 1) is employed to calculate the theoretical biogas production (Equation 1)¹⁵.



Following the assumptions were made while employing the modified Buswell and Boruff equation;

- The experiment was performed at a constant temperature and biogas was collected at room temperature.
- All the biomass is completely digested.
- The biogas is composed of methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3), and hydrogen sulfide (H_2S).
- Number of moles of the biogas produced is equal to the sum of number of moles of CH_4 , CO_2 , NH_3 , and H_2S .

The quantity of biogas was calculated using above equation is in terms of mole. Thus, amount of biogas was measured in terms of mole is converted employing ideal gas equation (Equation 2).

$$V = \frac{nRT}{p} \quad (2)$$

Where V is the volume of gas (L), n is the number of moles, R is the universal gas constant (0.082057 atm. L/mole. K), T is equal to 300.15 K (273.15+27°C), P is equal to 1 atm, and n denotes the number of moles of gas. n can be obtained from the modified Buswell-Boruff equation. Therefore, volume of biogas production or theoretical biogas production will be;

$$\text{Theoretical biogas production (TBP)} = \frac{\left[\left(\frac{4a+b-2c-3d-2e}{8} \right) + \left(\frac{4a-b+2c+3d+2e}{8} \right) + d+e \right] \times R \times T}{P \times (12a+1b+16c+14d)} \quad (3)$$

Extent of anaerobic digestion can be estimated by the amount of material degraded through the anaerobic digestion and termed as biodegradability (BD)¹⁶. It is the ratio of experimental biogas production (EBP) and TBP, which is;

$$BD = \frac{EBP}{TBP} \times 100\% \quad (4)$$

Kinetic modelling of daily biogas production

The biogas data obtained from the anaerobic digestion of CM and WS were fitted in three kinetic models, namely modified gompertz, first order exponential growth and monod models.

Modified Gompertz model (Equation 5) was used to perform the nonlinear kinetic fitting¹⁷.

$$B = B_m e^{\left(-e^{\left(\frac{\mu \times e}{B_m} (\alpha - t) + 1 \right)} \right)} \quad (5)$$

Where B is the biogas production (mL/g- VS_{added}) with respect to time t in days, B_m is the maximum biogas potential (mL/g- VS_{added}), μ is the rate of biogas production (mL/g- VS_{added} /day), α stands for the lag phase in days and e stands for the Euler's constant, which is equal to 2.71828.

First-order exponential growth equation (Equation 6) and monod (Equation 7) models were also used to predict the biogas production along with the rate of hydrolysis constant^{18,19}.

$$Y = Y_m (1 - e^{-Kt}) \quad (6)$$

$$B = \frac{F \times K^1 \times t}{1 + K^1 \times t} \quad (7)$$

Where Y and B is the biogas production (mL/g VS_{added}), Y_m and F represents the maximum biogas yield (mL/g VS_{added}), K and K^1 stands for the rate of hydrolysis (d^{-1}).

RESULTS AND DISCUSSIONS

Characterization of substrates (CM and WS)

Characterization of substrates is an essential step to perform anaerobic digestion. It defines the stability of the digestion process and biogas production. Characteristics of CM and WS are presented in Table 2. The pH of the CM and WS were measured 6.33 and 7.04 respectively. The TS and VS contents in CM was 19.95% and 12.25%, whereas WS contains 90.95% and 83.6% TS and VS respectively. These values are in close range with the values reported in literature^{20,21}. The ratio of VS to TS for CW was 0.64 whereas for WS was 0.92. Thus, the reactor with more WS implies the more organic loading. The CHNS analysis were taken from the literature. The C/N ratios of CM and WS were 15.12 and 47.78, respectively. The reactor with 100% CM (R1) has a C/N ratio of 15.12, and the reactor with 100% WS is 47.78. Thus, the C/N ratio increases with increase in WS content in the mix. For the anaerobic digestion the suggested value of C/N ratio is 20 to 30²². Only the reactors R₂ and R₃ have C/N ratio in the range of 20 to 30.

Table 2: Initial physicochemical characteristics of cattle manure (CM) and wheat straw (WS)

Parameter	Cattle manure (CM)	Wheat straw (WS)
MC%	80.05	9.05
TS % of wet weight	19.95	90.95
VS% of wet weight	12.85	83.60
pH	6.33	7.04
Alkalinity (g/kg)	0.41	0.65
VFA (g/kg)	9.28	-
C(%)	38.71	41.57
H(%)	5.12	5.85
N(%)	2.56	0.87
S(%)	0.31	0.17
O(%)	42.38	44.45

Note: CHNSO values of CM were extracted from²³⁻²⁷, and for the WS, CHNSO values were extracted from^{20,21,28-30}.

Biogas production

Reactors containing mix of CM and WS in ratio 100:0, 80:20, 60:40, 40:60, 20:80, and 100:0 while maintaining 5% TS are designated as R₁, R₂, R₃, R₄, R₅, and R₆, respectively. Daily and cumulative biogas production from the reactors are presented in Fig. 1 and Fig. 2 respectively. The highest daily biogas production 283 mL was observed in R₁ on 13th day. The reactors R₂ and R₃ have produced the highest daily biogas 220 mL and 138 mL on

32nd and 30th days. The remaining reactors, R₄, R₅, and R₆ have not produced considerable amount of biogas. As the CM content decreases or WS content increases, the highest daily biogas production also decreases. The time duration from the start of reactor to the highest daily biogas production was also decreased (Table 3). This may be due to the fact that increasing proportion of WS increases cellulose, hemicellulose, and lignin content in the mix. Literature indicated that these materials are hard to digest anaerobically¹². Further, decreasing content of CM in the reactor R₄, R₅ and R₆ may also be responsible the less anaerobic activity.

Cumulative biogas production from R₁, R₂, R₃, R₄, R₅, and R₆ was 170.38, 108.86, 61.96, 9.52, 6.85, and 11.87 mL/g-VS_{added}, respectively Table 3. The highest cumulative biogas production of 170.38 mL/g-VS_{added} was reckoned from R₁, and the lowest cumulative biogas production was not noticeable. Such low biogas productions were observed in a study on solid-state anaerobic digestion of WS, where WS was Co-digested with dog food and canteen food waste³¹. A study on CM and WS Co-digestion using anaerobic CM sludge measured an increase in biogas production up to 100:30 ratio of CM and WS³². The current study also measured a sharp dip in the biogas production after R₃ which contains 60:40 mix ratio of CM and WS. The reactors, R₄, R₅, and R₆, have low or zero CM content and resulted in negligible biogas production. Most of the studies have applied the pretreatment techniques along with the anaerobic bacteria in the form of inoculum for the better anaerobic digestion of WS and obtained optimum biogas production³³⁻³⁶. Therefore, the reactors which contain more than 50% CM have produced a considerable amount of biogas.

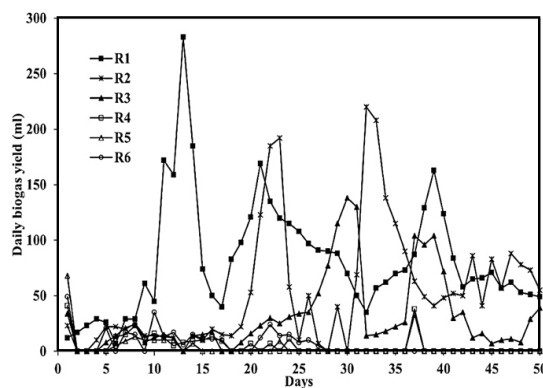


Fig. 1. Daily biogas production from the reactors

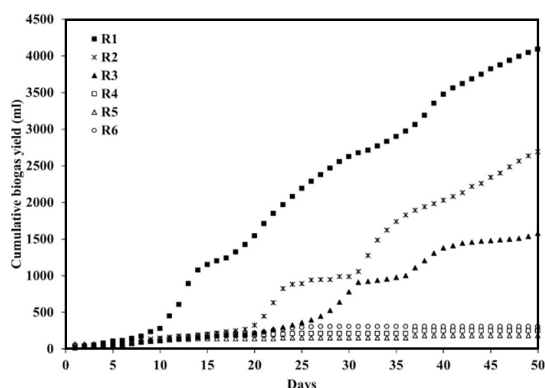


Fig. 2. Cumulative biogas production from the reactors

Volatile solids reduction

Biogas production is directly proportional to the extent of volatile solids (VS) reduction³⁷. The relation between biogas production (mL) and VS reduction (%) is presented in Fig. 3. The highest VS reduction and biogas production of 10.73% in reactor R₁, which is also corresponds to the production of highest cumulative biogas, 4092 mL. The amount of VS reduction and cumulative biogas production from all six reactors are linearly correlated with a higher value of coefficient of determination (R^2) of 0.94, which is also statistically supported by the one-way ANOVA ($p < 0.05$).

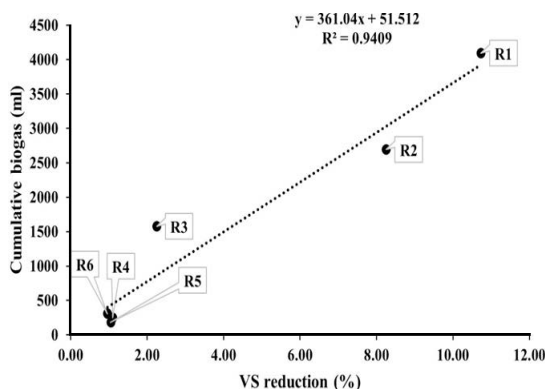


Fig. 3. Relation between volatile solids (VS) reduction and cumulative biogas production

Biodegradability

Biodegradability may also be considered as the ratio of experimental cumulative biogas production to theoretical biogas production¹⁶. Theoretical biogas production was calculated using modified Buswell equation. Similar to the VS reduction biodegradability is also decreased with increase in WS content in the reactor. Fig. 4 indicates, cumulative biogas production ($\text{mL/g-VS}_{\text{added}}$) and biodegradability and they are linearly correlated till R₅. The highest cumulative biogas production and the highest biodegradability of 170.38 $\text{mL/g-VS}_{\text{added}}$ and 15.37% respectively were observed in R₁, which contains 100% CM. While the lowest biogas production and the lowest biodegradability of 6.85 $\text{mL/g-VS}_{\text{added}}$ and 0.64, respectively were observed in R₅, which contains 20% CM and 80% WS. From the modified Buswell equation, the theoretical biogas for all the reactors lies between a close range, 1070 to 1150 $\text{mL/g-VS}_{\text{added}}$ Table 3. Whereas in the case of experimental observation, the biogas production varied from 170.38 to 6.85 $\text{mL/g-VS}_{\text{added}}$. This may be due to the recalcitrant part of WS (cellulose, hemicellulose, and lignin) and lack of anaerobic bacteria restricted the biogas production^{12,34}.

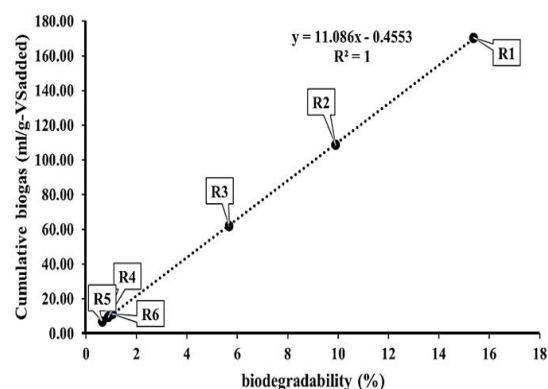


Fig. 4. Linear relation between biodegradability (%) and cumulative biogas production ($\text{mL/g-VS}_{\text{added}}$)

Table 3: Experimental and theoretical biogas production and biodegradability of the reactors

Reactor	Highest daily biogas production (mL)	Time to reach the highest daily biogas production (days)	EBP (mL)	EBP ($\text{mL/g-VS}_{\text{added}}$)	TBP ($\text{mL/g-VS}_{\text{added}}$)	BD [CBP/TBP×100 %]
R ₁	283	13	4092	170.38	1108.91	15.37
R ₂	220	32	2692	108.86	1099.48	9.90
R ₃	138	30	1576	61.96	1091.18	5.68
R ₄	-	-	249	9.52	1083.80	0.88
R ₅	-	-	184	6.85	1077.21	0.64
R ₆	-	-	308	11.17	1071.28	1.04

Note: EBP-cumulative biogas production, TBP-theoretical biogas production, and BD-biodegradability

pH, VFA, and alkalinity

pH, volatile fatty acids (VFA), and alkalinity are useful in monitoring the anaerobic digestion process. These parameters may vary during the process of anaerobic digestion³⁸. VFA are the intermediate products of the anaerobic digestion process. pH and VFA are associated with each other and varies inversely. pH, VFA, and alkalinity of all the digestion mixtures were measured at the initial and final stages of the digestion period and the same are shown in Fig. 5. The optimum pH range for anaerobic digestion is 6.5 to 7.5³⁸. The initial pH of the reactors was observed in the range of 6 to 7.5. The final pH of R₁ and R₃ was close to 5.5 and they have produced a biogas of 49 mL and 39 mL respectively, on the final day of digestion period. The reactors R₄, R₅ and R₆ having a final pH approximately 4.5, stopped producing biogas by the end of digestion period. It may be due to the more VFA production and low buffering capacity of the mix due to less amount of CM³⁹. The lignocellulosic components in the WS may got hydrolyzed by the end of the digestion period and produced relatively high VFA. The alkalinity of these three reactors was also slightly lowered compared to R₁, R₂, and R₃. Thus, the reactors which contain relatively more CM (R₁, R₂, and R₃) have shown relatively more buffering capacity and thus able to produce a considerable amount of biogas.

Kinetic study

Cumulative biogas (mL/g-VS_{added}) data obtained from the experimentation was fitted using three kinetic models, namely modified gompertz model, first order-exponential growth model and monod model to investigate the appropriate model which can better describe the data. The modified Gompertz model is able to quantify the maximum biogas production, biogas production rate, and

lag phase. While the remaining two models are useful in assessing the hydrolysis rate constant. Parameters obtained from the models while fitting the cumulative biogas are presented in Table 4. Results indicated that, modified Gompertz model was able to describe the experimental data very well particularly for R₁, R₂ and R₃ reactors. However, first order and Monod models were highly over predicted against the experimental cumulative biogas. The coefficient of determination, R₂ values of all the applied models were decreased with increase in WS content from R₁ to R₆ Table 4. R₂ values of the modified Gompertz model were in the accepted range (R₂>0.9) for R₁, R₂ and R₃ reactors. The rate of biogas production, μ of R₁, R₂, and R₃, were 4.88, 3.42, and 2.23 mL/g-VS_{added}/day, respectively. The lag time, α of R₁, R₂, and R₃ were 7.08, 15.90, and 17.06 days respectively. It indicates that, as the WS portion increases from R₁ to R₃, the μ values were decreased, and α values were increased. It may be due to the presence of hard recalcitrant materials of WS. A similar α value of 12.69 days was observed in the codigestion of untreated WS with animal manure from biogas plant as inoculum⁴⁰.

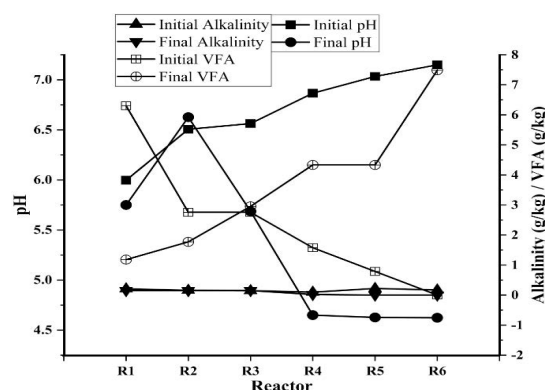


Fig. 5. pH, Alkalinity, and Volatile fatty acids (VFA) variation among the reactors

Table 4: Estimated kinetic parameters of modified Gompertz model, growth model, and monod model

Parameter	R1	R2	R3	R4	R5	R6
Experimental biogas potential (mL/g VS _{added})	170.38	108.86	61.96	9.52	6.85	11.17
B _m	189.84	151.52	81.95	55.45	51.29	62.75
μ	4.88	3.42	2.23	0.24	0.13	0.27
α	7.08	15.90	17.06	3.73	0.00	0.00
R ² (modified gompertz model)	0.99	0.99	0.99	0.79	0.82	0.75
Y _m	81279.60	588978.57	47836.42	11170.78	50010.57	70421.39
K	4.27E-05	3.13E-06	2.31E-05	2.21E-05	3.59E-06	4.31E-06
R ² (growth model)	0.99	0.94	0.92	0.85	0.85	0.81
F	616135.09	139528.48	421667.02	32600.68	359201.81	359328.05
K ¹	6.11E-06	1.32E-05	2.62E-06	7.59E-06	5.00E-07	8.45E-07
R ² (monod-model)	0.99	0.94	0.92	0.85	0.85	0.81

CONCLUSION

Batch experiments were performed on six different mixtures of cattle manure (CM) and wheat straw (WS) at mesophilic temperature. Results showed a decrease in biogas production with an increase in WS content. Reactor stability in terms of pH and stable VFA was observed better in R₁, R₂, and R₃ reactors. These reactors were fed with 100:0, 80:20, and 60:40 mixture of CM and WS. Low biogas production and high VFA was observed in R₄, R₅, and R₆ reactors, in which ratio of CM and WS were 40:60, 20:80, and 0:100 respectively. Of the three kinetic models employed in the study, the modified Gompertz model was found the best to describe the experimental data

of cumulative biogas production and shown optimum fitness up to R₃ reactor (i.e., for the 60:40 ratio of CM and WS). Therefore, the optimum mix ratio range of cattle manure and wheat straw for anaerobic digestion might be 20:80 and 60:40.

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Conflict of interest

There is no conflict of interest.

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