RESEARCH PAPER

Experimental Investigation on Anaerobic Digestion of Cattle Dung and Aloe Vera Wastes

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ABSTRACT
Anaerobic digestion for mixture of aloe vera waste and cow dung in different ratio of 1:0, 1:4, 1:3, 1:2, 1:1, 3:1 and 0:1 was done in laboratory experiment. It was seen that digestion of treatment T3 offers a better nutrient balance for anaerobic microorganism growth resulting in obtaining the optimized biogas and methane yield compared to other treatment. The total biogas volume from treatment T3 was 42.86 L and the biogas volume at STP was 38.68 L. Total volume of dry biogas produced at STP was recorded as 34.77 L. The total specific biogas production during 40 days of retention period was observed as 247.15 L/kg dm and 335.23 L/kg VS respectively. The methane yield in treatment T3 was 24.58 L and total specific methane production at STP was recorded as 184.89 L/kg dm and 283.48 L/kg VS respectively. The CO2 yield in treatment T3 was 17.34 L and total specific CO2 production during 40 days of retention period were observed as 122.54 L/kg dm and 199.99 L/kg VS respectively.
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INTRODUCTION

The disposal of biodegradable waste without adequate treatment results in widespread environmental pollution (Trivedi *et al*., 2015). Among the various energy resources available biogas has become as a favorable fuel for the future with numerous advantages (Hafiz *et al*., 2016). Biogas technology is a clean low carbon technology and has potential for development of human being and tackling pollution (Bomboriya, 2012). This utilization of biogas technology can be used for domestic as well as industrial purpose and no skilled person requires for maintenance of biogas plant. Biogas contains around 55-65 per cent of methane, 30-40 per cent of carbon dioxide and small quantities of hydrogen, nitrogen, carbon monoxide, oxygen and hydrogen sulphide (Ahmed and Hussain, 2013, Bohn *et al*., 2007, Kapdi *et al*., 2005 and Ward *et al*., 2008). The calorific value of biogas is appreciably high around 4700 kcal or 20 MJ at around 55 per cent methane content. Biogas obtained by anaerobic digestion of animal waste (cattle dung), waste from rural based industries (agro / food processing), kitchen wastes, wastes from forestry and other loose and leafy organic matters can be used as energy source for cooking, lighting and other applications like refrigeration, electricity generation and transportation (Agrahari and Tiwari, 2013, Alvarez and Liden, 2008, and Bouallagui, *et al*., 2005).

Biogas technology or bio-methanation is complex microbacterial process which converted the organic wastes into clean renewable biogas and organic manure source in absence of oxygen. The process of bio-methanation involves an anaerobic decomposition decay of organic materials this bacterial degradation takes place in four stages in very specific conditions and is accompanied by the production of biogas which has high methane content namely hydrolysis, acidogenesis, acidogenesis and methanogenesis. In hydrolysis process organic polymers of the organic matter (fats, proteins, carbon hydrates) are split into monomers (fatty acids, sugar, amino acids). In acidogenesis process lower fatty acids like acetic acid, propionic acid and butanoic acid emerge from the monomers and in addition small amounts of lactic acids and alcohol are produced. The acidity of the substrate (pH value) increases in acidogenesis process. In acidogenesis process acid products are transformed into acetic acids, hydrogen and carbon dioxide and in the methanogenesis process bacterial synthesis using carbon dioxide and hydrogen produce methane (Zomalloa *et al*., 2011 and

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Saheli *et al*., 2014). Bio-methanation is an energy saving waste treatment has led to the development of a range of anaerobic reactor design. The digested slurry produced from biogas plant enriched with nitrogen, phosphorous, potassium and several micronutrients are an attractive option for organic farming. A good quality manures to improve soil fertility (Chandra *et al*., 2012 and Petersson *et al*., 2007).

In India, number of projects and applications has taken up by village level organization, institution, private entrepreneurs etc. to promote reliable decentralized power generation from biogas specifically in the small capacity range 3 kW to 250 kW. Under the national biogas and manure management programme, 1.11 lakh biogas plants were to be set up in 2015-16 and 42.7 per cent of the target had been achieved with 47,490 units installed. The state nodal agencies, khadi and village industries commission (KVIC) and biogas development and training center (BDTC) implemented biogas plant provides substantial subsidy for setting up plants for rural and semi-rural households to meet the fuel needs. In India model uses for biogas plant approved by ministry of new and renewable energy are available of 1 to 6 m^3 capacity for fixed dome and floating dome type plants. The ferro-cement and brick-masonry biogas plants, prefabricated deenbandhu biogas plants made of high density polyethelene and KVIC and deenbandhu plants made of fiber-glass reinforced plastic and reinforced cement concrete material also included in the approved plants (MNRE).

Advantages of biogas plant are the easily understood operation and the volume of stored gas is directly visible, gas pressure is constant which determined by the weight of the gas holder and construction is relatively easy, construction mistakes do not lead to major problems in operation and gas yield. Family size biogas plants in rural India are fed with 1:1 mixture of cattle dung and water as substrate. Besides inconvenience in mixing water and cattle dung the watery digested slurry discharged from the biogas plants rich in micro nutrients like nitrogen, phosphorus and potash which require a lot of time and space for drying before transportation to fields for use as manure (Deshpande *et al*., 2012, Chen *et al*., 2010, Comino, 2010 and Shyam *et al*., 2006). The rapid development of the aloe processing industry generates waste products that are aloe vera peel. Disposal of aloe vera waste after separation from gel without adequate treatment may result in environmental pollution. The bio-methanation studies helpful for safe disposal and getting energy from aloe vera waste. Aloe vera waste is rich in carbohydrates and other polysaccharides and has structural polymers as cellulose, pectin and hemicellulose, besides polymers associated with medicinal properties as the acemannan and aloe ride. This waste has been processed for the production of biogas (Quintal *et al*., 2010 and Adesuyi, *et al*., 2012). Aloe vera waste can be used as support and carbon sources in anaerobic digestion for the production of biogas. Co-digestion offers better nutrient balance and C/N ratio and also enhances the biodiversity in the digester and produces the synergistic effects (Zhang *et al*., 2016).

MATERIALS AND METHOD

The effectiveness of anaerobic co-digestion of aloe vera waste with cow dung for biogas production was investigated using a laboratory scale bioreactor working in batch. Aloe vera leaves rind is rich in carbohydrates and other polysaccharides thus this waste was processed for the production of biogas (Nagraju, 2011). The waste part of the aloe vera plant collected and mixed with cow dung in different proportions. After laboratory analysis, best proportion of mixture was decided considering onsite availability of waste feedstock and need of processing industry.

Experimental apparatus setup

The seven treatments in two replications were studied for laboratory experiment to minimize the error. The treatment T1 contained aloe vera waste alone and the subsequent treatments consisted of mixture of aloe vera waste and cow dung in 1:4, 1:3, 1:2, 1:1 and 3:1 for T2, T3, T4, T5, T6, and T7 respectively shown in Table 1. T9 with cow dung alone was taken as the control. The study period of the experiment was 40 days (Yadav *et al*., 2014).

Inoculum

The inoculum for the anaerobic digestion of aloe vera waste and cow dung was prepared by composting fresh cow dung under anaerobic condition for or presoaking period of five days in ambient temperature (Dhanya *et al*., 2009). For laboratory experiment, the digested slurry was added 15 per cent of total volumes to enhance the growth of micro-organisms in an anaerobic digestion process.

Laboratory experiment for bio-methanation

A 2 L capacity of conical flasks and graduated cylinders as single state digesters was carried out for laboratory study of the anaerobic digestion process. An inverted calibrated jar filled with water which was partly immersed in water bath used for collection of biogas. In this method, airtight water filled aspirator bottle having an outlet at the bottom is connected at the top to another glass reagent bottle containing organic waste material. As the gas is formed it increases the pressure on the water surface and correspondingly water was displaced through outlet tube till the pressure balance was maintained on the water surface and at the outlet of aspirator bottle. The water bottle was marked or graduated in milliliters. Amount of water displaced in the bottle is equal to the amount of gas formed (Anunputtikul and Rodtong, 2004).

Fig. 1: Laboratory experiment for bio-methanation

Table 1: Bio-methanation treatments considered for laboratory experimentation

Treatment composition	Quantity of aloe vera waste (g)	Quantity of cow dung (g)	Quantity of slurry (mL)	Amount of water (mL)	Total volume (mL)
T_1 Aloe vera waste alone	1000	-	150	140	1290
T_2 1:4 (20%:80%)	200	800	150	540	1690
T_3 1:3 (25%:75%)	250	750	150	515	1665
T_4 1:2 (33.3%:66.6)	335	665	150	472.5	1622.5
T_5 1:1 (50%:50%)	500	500	150	390	1540
T_6 3:1(75%:25)	750	250	150	265	1415
T_7 Cow dung alone	-	1000	150	640	1790

Theoretical calculation

Total solid content

The total solid content of the feed material was determined by using standard method (APHA, 1998). About 20-25 g sample was taken in the aluminum box and placed in the oven for 24 h at 104-105°C. The weight of the empty box and after filling the substrate sample and before placing it in the oven was recorded. The box and the lid were placed separately in the oven. After 24 h the box was taken out with covered lid and placed in a desiccator to cool down to ambient temperature. After some period weight of the box was measured. All the measurements of weight were made by the electronic balance. The percentage total solid content of the sample was calculated using the following empirical relation:

$$TS = \frac{M_d}{M_w} \times 100 \qquad \text{Eq…(1)}$$

Where,

M_w = Initial mass of the sample, g

M_d = Mass of the oven dried sample, g

Volatile solids content (VS) and carbon (%)

The volatile solids content (VS) and non volatile solids content of the substrate material was determined as per the standard method using standard method (APHA, 1998). Volatile solid percentage was determined by igniting a known mass of dried samples at 580°C ± 5ºC in a muffle furnace for 6 h and allowed the crucible to cool partially in the air and then transferred to desiccators for final cooling. The loss in mass was taken as volatile solid fraction (APHA, 1995). The volatile solids content and carbon

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content of the sample to be calculated using following equation:

$$VS = \frac{M_d - M_a}{M_d} \times 100 \qquad \text{Eq…(2)}$$

$$\text{carbon}\ \% = \frac{\text{Volatile solid}}{1.724} \qquad \text{Eq…(3)}$$

Where,

M_a = Mass of dry ash, g

M_d = Mass of the oven dried sample, g

The cumulative biogas, methane and carbon dioxide production over the study period was calculated by adding daily biogas, methane and carbon dioxide production respectively. Specific biogas production, specific methane production and total volatile solids removal efficiency (per cent) were determined by using standard formulae (Chandra *et al*., 2012).

Biogas production at STP and specific biogas production

Observed daily biogas production was corrected at standard temperature and pressure (STP) condition using following equation. STP refers to 0 °C (273 K) temperature and at one atmospheric pressure.

$$BV_0 = \frac{273BV}{273+T} \qquad \text{Eq…(4)}$$

Where,

BV_0= Volume of daily produced biogas at STP (at 0 °C), L or m^3

BV = Volume of daily produced biogas at temperature T, L or m^3

T = Observed biogas temperature, °C

The specific biogas production (per unit TS and VS) were calculated using following equation:

$$BV_{0\text{Specific dm}} = \frac{BV_0}{DMF \times TS} \qquad \text{Eq…(5)}$$

$$BV_{0\text{Specific VS}} = \frac{BV_0}{DMF \times VS} \qquad \text{Eq…(6)}$$

Where,

$BV_{0\text{Specific TS}}$ = Specific biogas production, m^3/kg dm

$BV_{0\text{Specific VS}}$ = Specific biogas production, m^3/kg VS

DMF = Daily mass of feed, kg

TS = Total solids content, decimal

VS = Volatile solids content, decimal

Methane and carbon dioxide production and Specific methane production

The daily production of methane and carbon dioxide in produced biogas were determined by:

$$CH_{4\text{Yield}} = \frac{CH_4\text{Conc.}}{100} \times BV_0 \qquad \text{Eq…(7)}$$

Where,

$CH_{4\text{Yield}}$ = Daily methane yield at STP, L or m^3

CH4 Conc. = Methane concentration in biogas, %

$$CO_{2\text{Yield}} = \frac{CO_2\text{Conc.}}{100} \times BV_0 \qquad \text{Eq…(8)}$$

Where,

$CO_{2\text{Yield}}$ = Carbon dioxide yield at STP, L or m^3

CO_2 Conc. = Carbon dioxide concentration in biogas, %

The specific methane production (per unit TS and VS) was determined using:

$$MV_{0\text{Specific TS}} = \frac{CH_{4\text{Yield}}}{DMF \times TS} \qquad \text{Eq…(9)}$$

$$MV_{0\text{Specific VS}} = \frac{CH_{4\text{Yield}}}{DMF \times VS} \qquad \text{Eq…(10)}$$

Where,

MV_0= Specific TS is the specific methane production, m^3/kg dm

MV_0 = Specific VS is the specific methane production, m^3/kg VS

Calculation of dry biogas volume at STP

The dry biogas volume were determined by multiplying biogas volume at STP to dry biogas factor (litre dry biogas at STP/ litre biogas) measured at T°C at given temperature.

The biogas volume at STP calculated may be artificially high. Since Charle's Law does not compensate for the change in relative volume of water vapor present in biogas. In order to obtain STP volume of dry biogas, a correction method was applied for water vapor mass and volume due to evaporation. The standard steam saturated volume values at respective temperatures which were to obtain water vapor density. The water vapor density for each temperature was then converted to fractional water vapor volume using the equation given below:

$$V = n \times \frac{RT}{P} \qquad \text{Eq…(11)}$$

Where,

V = Gas volume, L

n = Number moles of gas

R = Gas constant 0.08205 L atm/mol K

T = Absolute gas temperature, K

P = Gas pressure, Atmospheric

Value of term P is 1 at STP, the value of n was then determined from the equation shown below:

$$n = \text{Water vapor density}/\text{Molecular mass of water}$$

The non-water biogas volumes at respective temperatures were obtained by deducting the water vapor volume from unity. The dry biogas factor (DBF) at STP was calculated by multiplying non-water biogas volume to BV_0/BV values.

RESULTS AND DISCUSSION

The biogas produced during each treatment distinctive measure of gas production and composition which was influenced by various proportions of aloe vera waste and cow dung concentration substrate utilized amid 40 days of the test shown in table 2 and 3. The digested slurry having 15 per cent feeding substrate was added to each treatment to upgrade anaerobic process. For each treatment of laboratory experiments two replication having 10 per cent solidity of the mixture was prepared by adding tap water.

Table 2: Total biogas production

Treatment	Total biogas, L	Total biogas at STP, L	Total volume of dry biogas at STP	Total specific biogas, L/kg dm	Total specific biogas, L/kg VS
T_1	15.31	13.94	12.64	104	192.6
T_2	39.45	35.49	33.20	224.62	300.12
T_3	42.86	38.68	34.77	247.15	335.23
T_4	35.085	31.64	28.41	206.16	286.31
T_5	29.5	26.7	23.95	188.64	340.25
T_6	26.90	24.31	21.89	163.19	34.08
T_7	39.7	36	33.91	219.43	173.57

Table 3: Total Methane and CO_2 yield

Treatment	Methane yield, L	Total specific methane production at STP in L/kg dm	Total specific methane production at STP in L/kg VS	CO_2 yield, L	Total specific CO_2 yield production at STP in L/kg dm	Total specific CO_2 yield production at STP in L/kg VS
T_1	7.35	47.83	66.60	6.18	40.21	55.99
T_2	19.10	128.19	189.04	15.03	100.84	148.71
T_3	24.58	184.89	283.48	17.34	122.54	199.99
T_4	17.28	110.37	149.7	13.47	86.08	116.76
T_5	14.5	91.83	122.7	11.37	96.21	72
T_6	13.05	79.61	62.97	10.57	64.47	50.99
T_7	20.13	122.75	97.09	16.91	103.13	81.58

The treatment T_1 has feeding substrate aloe vera waste and cow dung in 1:0 proportion. The maximum biogas production was found during 11th to 34th day. The biogas generation started on 3rd day of treatment T_2 having aloe vera waste and cow dung in 1:4 proportion. The maximum biogas production was from 8th to 36th day. The treatment T_3 was having aloe vera waste and cow dung in 1:3 proportion and the biogas generation begun on the 2nd day. The maximum biogas production was from 6th to 34th day. The treatment T_4 having aloe vera waste and cow dung in 1:2 proportion started biogas generation on the 4th day. The maximum biogas production was from 10th to 32nd day. Treatment T_5 having aloe vera waste and cow dung in 1:1 proportion, biogas generation started on the 5th day. The maximum biogas production was from 10th to 30th day. Treatment T_6 having aloe vera waste and cow dung in 3:1 proportion biogas generation started on the 5th day. The maximum biogas production was from 10th to 30th day. Treatment T_7 having aloe vera waste and cow dung in 0:1 proportion biogas generation started on the 4th day. The maximum biogas production was from 11th to 30th day.

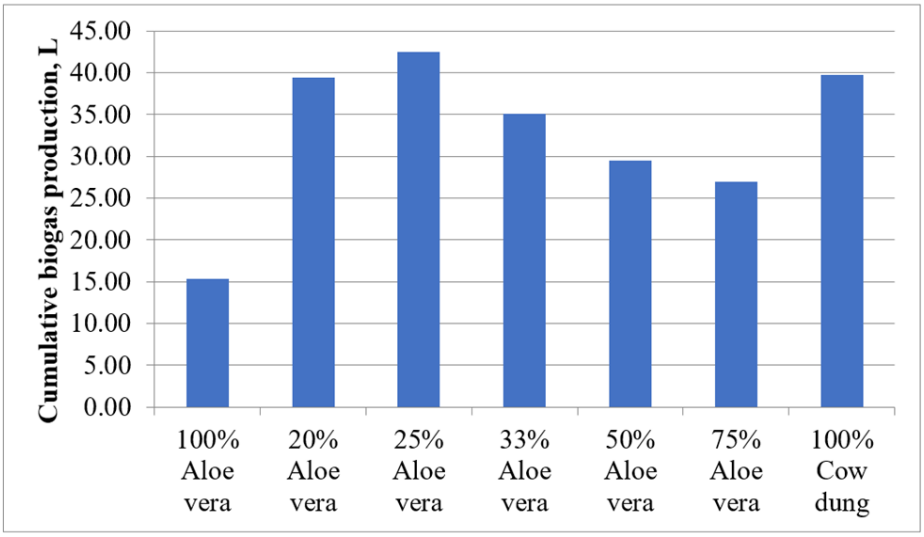

Figure 2: Cumulative biogas production from different treatments during experimentation of 40 day

It was observed that aloe vera as a single feedstock produce very less amount of gas in bio-methanation process. This might be due to alone aloe vera waste cannot easily be degraded in an anaerobic digestion process because of the presence of the high amount of lignocelluloses gave high C/N ratio which affects the digestion process of feedstock. The C/N ratio of aloe vera waste was of 30.6. The C/N ratio of treatment T_2, T_3, T_4, T_5, T_6 and T_7 are 18.2, 19.5, 25, 27.3, 29 and 24 per cent respectively. By increasing the percentage of aloe vera waste decreased the biogas production. It was seen that the biogas production increased up to T_3 treatment then decreased up to T_6. The daily biogas production for treatment T_1 was delayed by 3-5 day compared to other treatment possibly because of lower buffering capacity of aloe vera waste. In addition, all C/N ratios showed higher yield than aloe vera waste alone which indicated that the balanced nutrients in the mixed substance enhance the microbial activity in anaerobic digestion. The results of the cumulative methane yield in this study are consistent with the results of a previous study by Huang *et al*., 2016. Biogas production from treatment T_3 was found maximum compared to all other treatment. The increased biogas in treatment T_3 due to better nutrient balance and C/N ratio for anaerobic micro-organism growth and appropriate pH of 6.9 to avoid acidification and high buffering capacity for the rapid dropping of pH. Author Kumar *et al*., 2010 found that a C/N ratio range from 13.9 to 19.6 is acceptable for digestion of green and food waste. The Author Kumar *et al*., 2010 found that a C/N ratio range from 13.9 to 19.6 is acceptable for digestion of green and food waste. The researcher Li *et al*., 2015 observed that the anaerobic digestion efficiency increased initially with an increase in C/N from 17 to 23.4 following which it decreased with further increase in C/N. Biogas production from alone cow dung, treatment T_7 was higher than treatment T_4, T_5 and T_6. It was seen that digestion of treatment T_3 offers a better nutrient balance for anaerobic microorganism growth resulting in obtaining the optimized biogas and methane yield. The treatment T_2 and T_3 shows increasing trend of biogas but after treatment T_3 increased percentage of aloe vera decreases the production of biogas this might be due to increase the percentage of lignocelluse present in feeding subtract.

CONCLUSIONS

Disposal of aloe vera waste after separation from gel without adequate treatment may result in environmental pollution. The bio-methanation studies helpful for safe disposal and getting energy from aloe- industry waste. It was seen that digestion of treatment T_3 offers a better nutrient balance for anaerobic microorganism growth resulting in obtaining the optimized biogas and methane yield. The treatment T_2 and T_3 shows increasing trend of biogas but after treatment T_3 increased percentage of aloe vera decreases the production of biogas this might be due to increase the percentage of lignocelluse present in feeding subtract. The total biogas volume from treatment T_3 was 42.86 L and the biogas volume at STP was 38.68 L. Total volume of dry biogas produced at STP was recorded as 34.77 L. The total specific biogas production during 40 days of retention period was observed as 247.15 L/kg dm and 335.23 L/kg VS respectively. The methane yield in treatment T_3 was 24.58 L and total specific methane production at STP was recorded as 184.89 L/kg dm and 283.48 L/kg VS respectively. The CO_2 yield in treatment T_3 was 17.34 L and total specific CO_2 production during 40 days of retention period were observed as 122.54 L/kg dm and 199.99 L/kg VS respectively.

ABBREVIATION

BDTC- Biogas Development and Training Center

CH_4- Methane

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CO_2- Carbon dioxide

C/N ratio- Carbon-Nitrogen ratio

DBF- dry biogas factor

Dm-dry matter

kg-killo-gram

KVIC- Khadi and Village Industries Commission

L-Litre

STP-Standard temperature pressure

TS- Total solid

VS-Volatile solid

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