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Original Research Article

Effectiveness of bacterial inoculum as partial substitute for cow dung in the installation and stabilization of biogas plant

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ABSTRACT

The prominence of biogas plants for source centric waste management is increasing rapidly. Fresh cow dung is essential for installation of biogas plant. However, its availability is rare in urban areas. In order to help enhance the rate of installation of biogas plants in urban areas, inoculum might be useful. This study aims at developing a bacterial inoculum as a partial substitute for fresh cow dung that sustains the fermenting activity in the biogasdigester. Pure culture of cow dung-derived Bacilli sp. was made and their activity during the initial hydrolysis of organic matter in biogas plant was assessed. The active bacteria, Bacillus subtilis was identified by 16S r-DNA analysis. Dose-activity links of the inoculum under various feedstock combinations of cow dung and vegetable waste were studied based on 60-day biogas yield. Methane level of the 24-hour stock of biogas was sensed indirectly as heat value of combustion captured in 5 litres water. A feedstock combination of 30 kg fresh cow dung, 30 kg vegetable waste and 2.25-liter inoculum when used for installation of 1M3 biogas plant developed stable bacterial consortium in 15-day retention time and performed well on subsequent addition of vegetable waste. The inoculum was effective in the range 25 to 40 ml per kg cow dung-withdrawn. The study showed that cow dung-based biogas installation can be replaced by using one- fourth dung, an equal quantity of organic waste and the bacterial inoculum in the prescribeddose.

1. Introduction

Installation, stabilization and gas yield are the three prominent phases in a biogas plant. Bacterial fermentation of organic matter in the plant happens in four stages *viz.* solubilizing, volatile fatty acid-genesis, acetic acid-genesis and methane-genesis. The bacteria needed for this are drawn from animal dung whose quantity depends on the digester volume (Baba Shehu[1] *et a*l., 2012). About two week's retention is required for the growth, multiplication and stabilization of the bacterial consortium after installation of the biogas plant (Anna Schnurer[2] et al. 2009; Joanne M. Willey[3] *et al.*, 2007; Yeole[4] *et. a*l. 1992 and Gadre[5] *et al.*, 1990). Twenty species of fermentative bacterial species have been reported to play critical role in the production of biogas (Ramaswamy[6,7] *et al.,* 1991 & 1997and Girija[8] *et al.,* 2013). Among these, cow dung bacilli took a dominant role in the hydrolysis of organic matter in bio-digesters. Bacterial hydrolysis happens under aerobic, semi- aerobic and micro- aerobic environment that prevail in the digester in the initial days (Velmurugan[9] *et al.*, 2011). The bacteria *Bacillus subtilis* once believed as strict aerobe has now been recognized as facultative too (Michiko[10] *et a*l., 1997).

Notwithstanding the design of the biogas plant, the chemical and microbiological reactions that occur in the digester are largely dependent on the temperature, pH and concentration of the volatile organic matter in the digester fluid and are influenced by the substrate types and their feeding rate (Khanal[11] S.K. 2008, Francesco Fantozzi[12] *et al.*, 2011 and Berlian Sitorus[13] *et al.,* 2013). Substrate concentration in which total volatile matter is below 4% and above 10% inhibited anaerobic digestion (Zinder[14] *et al.,* 1984). Substrate loading rate of 3.8 kg fermentable organic

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matter per m^3 per day was prescribed for stable performance of biogas plants (Viswanath[15] *et al.*, 1992).

The objective of the study was to develop a cultured bacterial inoculum that would minimize the quantity of fresh cow dung needed for installing biogas plant with guaranteed efficiency.

2. Materials and Methods

2.1. Inoculum

Cow dung bacilli were isolated from fresh dung through serial dilution, spread plate technique and incubation at 37°C for 24 hrs. The colonies were subjected to series of sub culture until pure culture was obtained. Identification of bacteria was done through standard procedures such as microscopic examination and biochemical characterization (Figure 1). The species level identification was done through 16S r-D NA sequencing. The procedure involved were the extraction of bacterial genomic DNA, PCR amplification by Thermocycler, sequencing of the base pair in the DNA fragment and conformation of 16S r-DNA gene using automated sequencer (Frank Schwieger[16] *et a*l., 1998). The bacteria *Bacillus subtilis* was identified and selected as the inoculum for the purpose of this study. The facultative nature of the bacteria was confirmed by oil overlay test. Cellulolytic, lignolytic and amylase activities of the inoculum were ascertained by tests using CMC agar, META agar and starch agar respectively. Shelf life assessment by Total Viability Count Method revealed 126 days for the bacterial inoculum at 1x 10^6 cfu /ml concentration.

Fig. 1: *Bacil*lus on nutrient agar

2.2. Biogas plant

Prefabricated FRP model portable biogas plants having 600 litres digester- capacity and 400 litres floating gas-tank were used for the experiment (Figure 1). Fresh cow dung demand for the installation of this plant is 120 kg (moisture content about 80%). After the hydrolytic retention period, a loading rate in the range of 5 to 7.5 kg organic waste per day has been normally prescribed for this type of $1M^3$ plant.

The feedstock used in the experiment was prepared under uniform conditions to minimize error among experiments. The chemical characteristics of the feedstock are provided in Table 1. Yields as well as heat-value of biogas were chosen as indices for assessing the digester activity. Volume of gas accumulated in the floating drum in 24-hour interval was measured for 60 yield days in each experiment. Heat value of biogas was measured by the combustion of 24 - hour stock of the gas in a stove and capturing the heat in 5litres water. The highest temperature and the time taken to attain it were taken as indirect indications of methane content in the biogas. All measurements were done under identical conditions for the sake of error-free comparison among experiments.

Table 1: Chemical composition of cow dung and vegetable wastes

Parameters	Substrate (dry matter)	
	Cow dung	Vegetable waste
Moisture %	80.3	76.2
Ph	07.2	06.5
TVM %	15.0	19.9
TOC %	08.7	11.2
Nitrogen %	00.5	00.8
Phosphorus %	00.4	00.3
Potassium %	00.2	00.4
Dry matter %	19.7	23.8

TVM: Total Volatile Matter; TOC: Total Organic Carbon

Fig. 2: 1 M3 biogas plants

3. Experiments and Observation

The focus of the study was to develop a feedstock combination using minimum quantity of cow dung, veg waste and cultured inoculum that can together stabilize the bacterial consortium in a biogas digester ensuring

Table 2: Biogas yield of feedstock with and without inoculum

Feed stock (Kg)		Organic matter – Digester Volume Ratio (%)		Biogas yield : average of 60 yield days (Liters/day)			
				Volume of inoculum for withdrawing one kg cow dung			
Dung	Veg. waste	TOM*	TVM*	Nil	@ 40 ml	@ 25 ml	@ 30 ml
120	-	4.0	3.0	93	-	-	-
60	60	4.4	3.5	94	120	110	-
30	90	4.6	3.8	-	157	111	-
30	60	3.4	2.7	146	-	197	-
30	30	2.2	1.8	94	-	192	-
30	30+	2.2	1.8	105	-	140	-
60	30#	3.2	2.5	190	-	-	223

*TOM: Total Organic Matter; TVM: Total Volatile Matter (Dry matter) + Veg. waste @ 2.5 Kg/day was added from 2^{nd} -12^{th} day # Veg. waste @ 2.5 Kg/day was added from 26^{th} yield day own words

continuous production of gas. A thousand-liter capacity biogas plant installed using 120 kg cow dung and maintained for 60 days served as control experiment in batch-addition method. The dung conventionally used at this ratio ensured 4% TOM as well as enough bacterial diversity in the digester fluid. Any attempt to reduce the quantity of the dung must have compensated the consequent reduction of bacterial population as well as reduction in TOM content in the digester fluid. Dung at 60 kg and 90 kg were tried in the present study. In each case the efficacy of the cultured inoculum under test were validated at two dose levels; a lower dose of 25 ml and higher dose of 40 ml for withdrawal of one kg cow dung. Simultaneously, the most matching TOM dose among 90 kg, 60 kg and 30 kg vegetable wastes were searched in batch addition experiments. In all thirteen experiments of 60 -day duration was done using as many feedstock combinations with and without adding the inoculum. The data regarding feedstock, doses of inoculum and volume of biogas in 60 yield-day-averages are given in Table 2. A record of the temperature gain following the combustion heat of daily generated gas captured in 5liter water was also maintained.

4. Results and Discussion

Feedstock combination of 60 kg dung and 60 kg veg wastes with and without inoculum did not give desirable gas production. Feedstock holding 30 kg dung mixed with 90 kg and 60 kg veg wastes also failed to provide significant increment in gas production over the controls at both lower and higher dose levels of the inoculum. An average yield of 197 liters biogas was obtained in the 30-60 combination of dung and veg waste along with 2.25 liters inoculum. However, methane content in the gas generated was low because the rise in temperature obtained in the combustion experiment remained in the range 30^0 C-50^0 C during the yield days. This indicated the crowding effect caused by 60 kg veg waste. The burning rate of the gas was 10 liters per minute. Most promising result was obtained in the feedstock combination of 30 kg dung and 30 kg veg waste mixed with 2.25 liters of the inoculum. While the inoculum-dung ratio was 25 ml per kg dung-withdrawn, the matching ratio with veg waste was 75 ml per kg added. The average yield of biogas in this experiment was 192 liters which was 104% more than the control. It was better quality biogas in terms of methane content as the rise in temperature obtained in the combustion experiment remained in the range 50^0 C -72^0 C during the yield days. The burning rate of this gas was 10 liters per minute.

Effect of daily addition of veg waste was tested in 30 kg-30 kg- 2.5- liter combination by adding 2.5 kg veg waste from day 2 onwards. This method seemed to have disturbed the stabilization process of bacteria in the digester environment and hence the gas yield was low. Daily addition of veg waste from 26^{th} day onwards gave enhanced production of good quality gas. This showed that daily addition succeeding HRT increased the gas production possibly due to the availability of more substrate among stabilized bacterial consortium. Inoculum at the higher dose of 40 ml per kg dung-withdrawn generated more gas than the lower dose. The decrease in HRT observed in this experiment showed faster hydrolysis at the instance of the higher level of inoculum provided.

5. Conclusion

Biogas plant installation at present is entirely cow dung based in which daily addition of feedstock is preceded by a stabilization period of about 15 days. The study proposes use of one- fourth dung, an equal quantity of organic waste and inoculum in the dose range 25-40 ml per kg dung-withdrawal, as feedstock for installation. Daily addition of organic waste can be started after three weeks. This is applicable to biogas plants of all capacities. The long shelf life of 126 days makes storage and distribution of the inoculum easy.

6. Source of funding

None.

7. Conflict of interest

None.

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