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Effect of Microbial Inoculum on Biogas Production from Cattle Dung under Anaerobic Batch Digestion

Shikha Mehta, Kamla Malik*, Nisha Verma and Ramesh Chander Anand

Department of Microbiology, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana-125004, India

**Corresponding author*

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A B S T R A C T

The compost, landfill waste, paddy soil and kitchen waste were used for development of microbial inoculum for the enhancement of biogas production from cattle dung under anaerobic batch conditions. Six digesters were set up containing, biogas slurry, compost, paddy soil, landfill waste and kitchen waste along with cattle dung in different concentration. The dehydrogenase activity, cellulase activity, N, P, K content, volatile solids, total solids, total volatile fatty acids, C/N ratio and biogas production was studied in samples at different time intervals. Enhanced biogas production from cattle dung supplemented with kitchen waste @ 10% was observed under anaerobic batch digestion. The maximum dehydrogenase and cellulase activities (1464.0 μg TPF g^{-1} sample per 24 hr and 296.2 μg glucose g^{-1} sample per 24 hr respectively) were found at 4^{th} week in digester no. 6. Total volatile fatty acids, total solids and volatile solids decreased after 8 weeks of digestion. The N, P and K content was increased whereas C/N ratio was found to decrease. Supplementation of kitchen waste with cattle dung through anaerobic digestion for biogas production is cost effective and environmental friendly technology. It also improves microbial growth which enhanced biogas production during digestion.

Introduction

Biogas is a clean and renewable energy source which can be a good substitution of conventional sources of energy (fossil fuels, oil) that are causing environmental as well as health problems (Yadvika *et al.*, 2004). Biogas is produced by anaerobic digestion of organic substrates. It is one of the oldest processes used for the treatment of industrial wastes and stabilization of sludge (Noyola *et al.*, 2006). The conversion of organic matters into biogas production can be divided into four stages *viz.* hydrolysis, acidogenesis, acetogenesis and methanogenesis; and in each individual stage, different groups of microorganisms such as hydrolyzing and fermentative bacteria, hydrogen producing acetogenic bacteria and methanogenic bacteria are involved (Abdeshahian *et al.*, 2016; Horvath *et al.*, 2016). The organic substrates such as food processing wastes like bakery waste, potato waste, kitchen waste, grease, oil or fats, municipal waste, plant material and animal manures *viz.* chicken, swine or cow manure can be digested to produce biogas. The biogas yield mainly depends on the composition and the biodegradability (under anaerobic conditions) of the waste. Biogas can be directly used as cooking fuel, in combined heat and power gas engines or upgraded to natural gas quality biomethane. The utilization of biogas as a fuel helps to replace fossil fuels (Wei *et al.,* 2011). Normally, anaerobic fermentation is a slow process. However, this slow process leads to a large volume of digester and hence high cost of the system. The biogas contains 50-75% CH_4, 34-45% CO_2 and CO, N_2, H_2, H_2S, O_2 in traces (Juanga *et al.*, 2007; Karellas, 2010).

Biogas has a very positive impact on the environment, since less CO_2 is formed during its combustion than used for photosynthesis by the plants from which it is produced (Otun *et al.,* 2015). Biogas production depends on several operating parameters such as temperature, pH, retention time, total solid content, carbon to nitrogen ratio (C/N ratio), mixing, chemical oxygen demand and volatile solid content which need proper monitoring and control to achieve maximum yield of biogas (Sidik *et al.,* 2013). In conventional biogas plants, a large hydraulic retention time (HRT) of 30-50 days is used. There is a need to improve and also increase the efficiency of biogas production and in turn, decrease the HRT. Several methods have been reported to increase the efficiency of biogas production through optimizing various parameters such as nutritional requirements of microbes, using different chemical and biological additives and also manipulating the feed proportions (Satyanarayan and Shivayogi, 2010; Wei *et al.,* 2010). Till date the development of microbial inoculum for enhancement of biogas production has not received much attention. Considering these aspects, the present investigation was undertaken to study the effects of microbial inoculum for enhancement of biogas production from cattle dung under anaerobic batch digestion.

Materials and Methods

Anaerobic batch digestion

Anaerobic digestion of fresh cattle dung and the developed microbial inoculum was carried out by batch system for eight weeks. Five kg of each sample (paddy soil, compost, landfill waste and kitchen waste) was added to 50 kg of biogas slurry and it was covered by a layer of cattle dung and kept for 30 days for enriching microbial inocula. Six digesters were set up for determining their effect on biogas production i.e. cattle dung + slurry @ 10%, cattle dung + slurry @ 20%, cattle dung + compost @ 10%, cattle dung + paddy soil @ 10%, cattle dung + landfill waste @ 10% and cattle dung + kitchen waste @ 10%.

Analysis of fresh substrate, influent and effluent

Cattle dung, compost, kitchen waste, landfill waste and paddy soil were analyzed for various parameters such as pH, total solids, volatile solids, organic carbon content, total volatile fatty acids, cellulose, hemicellulose and lignin contents (AOAC, 2000). The samples were also analyzed for dehydrogenase activity, cellulase activity, nitrogen, phosphorus and potassium content following the standard procedures (Casida *et al.,* 1964; Deng and Tabatabai, 1994; Bremner, 1965; John, 1970 and Antil *et al.,* 2002, respectively). The rate of biogas production was estimated by water displacement method.

Results and Discussion

To assess the effect of kitchen waste, compost, landfill waste and paddy soil on biogas production, various combinations of these inocula with cattle dung were made for the enrichment of microflora and digestion was carried out in batch type manner for eight weeks. The cattle dung, compost, kitchen waste, landfill waste and paddy soil were analyzed for various parameters (Table 1). The cattle dung, compost, kitchen waste, landfill waste and paddy soil had a pH of 7.8, 8.7, 8.1, 8.4 and 8.2 respectively. The highest total volatile fatty acid (TVFA), total solids and volatile solids (465 mg kg^{-1}, 13.58% and 89.3% respectively) was observed in kitchen waste as compared to other waste. The cattle dung, compost, kitchen waste, landfill waste and paddy soil had cellulose content of 29.4, 33.4, 27.5, 31.4, 27.6 %, hemicellulose content of 18.4, 21.3, 12.9, 11.7, 19.7% and lignin content of 11.0, 11.9, 8.4, 10.3 and 11.4%, respectively. The nitrogen content was highest in kitchen waste (1.37%). The potassium concentration was highest in compost (1.20%) and phosphorus content was highest in landfill waste (0.41%). The C/N ratios ranged from 29.8 to 35.9. After analysis of various parameters of these substrates then combination were made with cattle dung.

The influent slurry comprising of combinations of cattle dung with different inoculum was analyzed for various parameters (Table 2). The pH in various digesters varied in a range of 8.27 to 8.95. Total volatile fatty acid content (TVFA) was maximum (532.5 mg kg^{-1}) in cattle dung containing kitchen waste and minimum (375.0 mg kg^{-1}) in cattle dung containing paddy soil @ 10%. Cellulose and hemicellulose content ranged between 33.5 to 37.6 and 19.2 to 23.5%, respectively. Lignin content was maximum (12.9%) in cattle dung containing paddy soil. Total solid content was in the range of 14.11 to 15.75 %. Volatile solids ranged from 83.0 to 91.0 (% of TS).

Nitrogen, potassium and organic carbon content was maximum (1.42%, 1.29% and 52.7%) in digester-6 containing kitchen waste as supplement as compared to other digesters. The phosphorus content was highest in digester-5 (0.43%) containing landfill waste as supplement. The maximum C/N ratio was found in digester-4 (40.4) containing paddy soil as supplement. After completion of batch anaerobic digestion for eight weeks, decrease in TS and VS (% of TS) was observed in all the digesters (Table 3). The maximum degradation (28.8%) of total solids was observed in digester-6 (CD + kitchen waste @ 10%) and minimum degradation (13.7%) was in digester-4 (CD + paddy soil @ 10%). The same trend was observed in volatile solids degradation and maximum degradation (18.6%) was observed in digester-6 (Fig. 1). Zupančič *et al.,* (2008) reported 10% increase in volatile suspended solids degradation efficiency when house-hold organic waste was co-digested with municipal sludge. Degradation of volatile solids and total solids could be explained due to breakdown of substrates resulting in biogas production. A similar observation was reported by Shivaraj and Seenayya (1994) during methanogenesis of poultry litter waste slurry. The phosphorus and potassium content in effluent increased after eight weeks of digestion (Table 3). The biogas production was recorded for eight weeks in batch digestion. The temperature ranged from 14.7 to 25.9˚C during eight weeks. The maximum biogas production was observed in digester –6 (Cattle dung + kitchen waste @ 10%) with the range of 0.5 to 14.3 litres (Fig. 2). The biogas production increased up to 5th week of anaerobic digestion under batch digestion conditions and then deceased up to 8th week of incubation period probably due to depletion of substrates. The anaerobic batch digestion for production of biogas and co-digestion of three different types of biodegradable wastes (cow dung, fruit waste and food waste) was conducted using a 25 litres capacity plastic keg prototype biogas plant. The maximum biogas production (164.8%) was observed in co-digestion of cow dung and food waste, followed by co-digestion of the three wastes (91.0%) (Otun *et al.,* 2015).

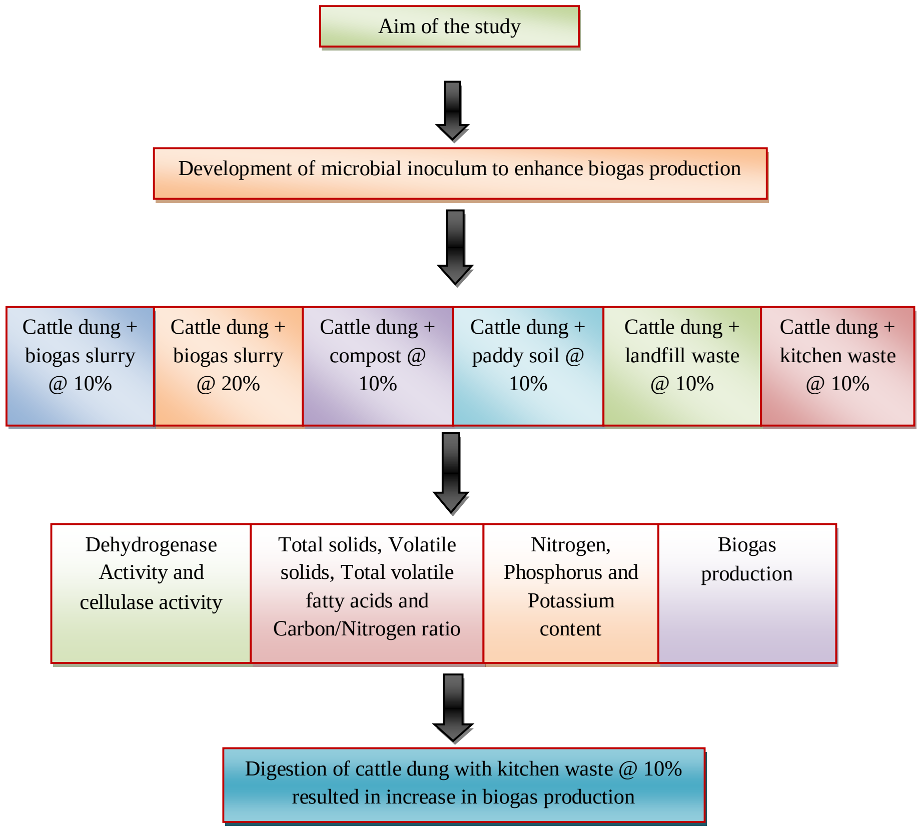

Fig.1 Degradation of total solids and volatile solids in different digesters

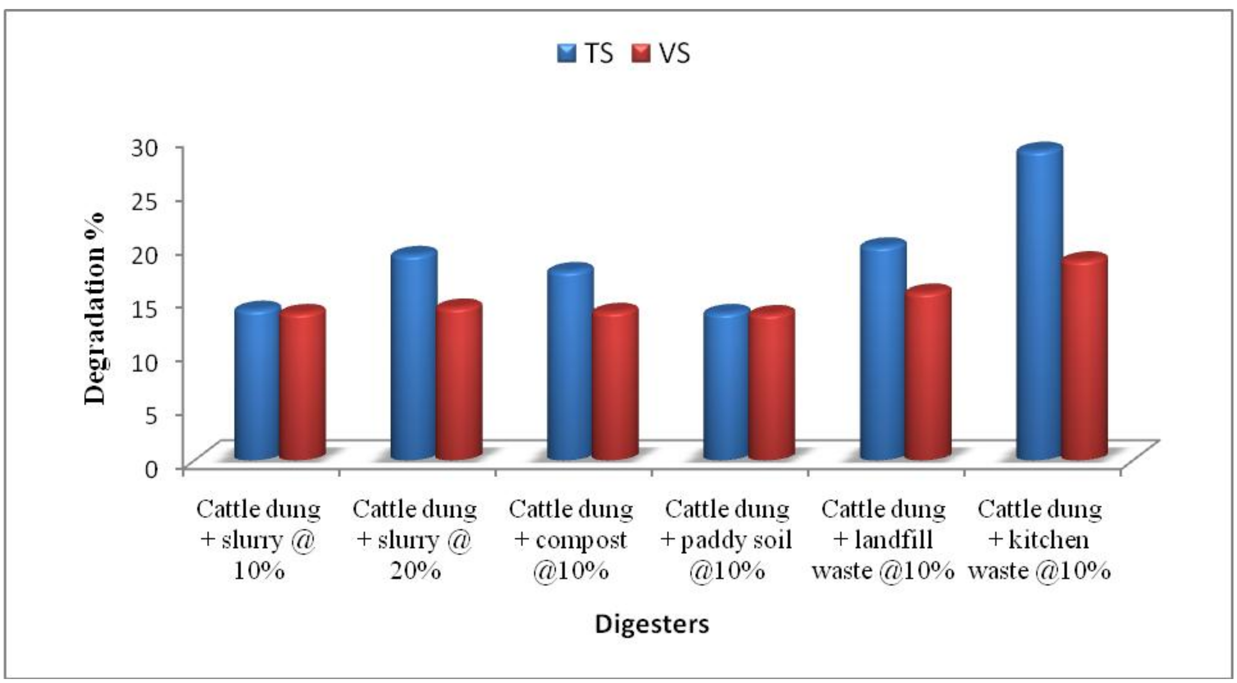

Fig.2 Weekly biogas production (litres) from cattle dung supplemented with different inocula during batch digestion

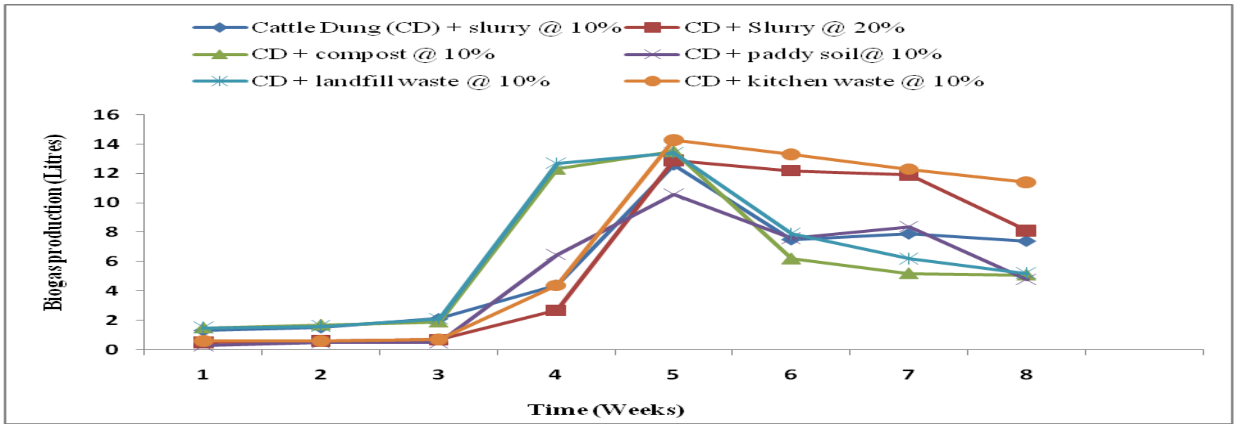

Table.1 Analysis of various parameters of the substrates used in anaerobic digestion

Parameter	Cattle dung	Compost	Kitchen waste	Landfill waste	Paddy soil
pH	7.8	8.7	8.1	8.4	8.2
TVFA (mg/kg)	395	410	465	435	315
Total solids %	12.72	12.43	13.58	13.29	13.17
Volatile solids (% of TS)	79.8	82.9	89.3	88.6	83.4
Cellulose (% of TS)	29.4	33.4	27.5	31.4	27.6
Hemicellulose (% of TS)	18.4	21.3	12.9	11.7	19.7
Lignin (% of TS)	11.0	11.9	8.4	10.3	11.4
Nitrogen (% of TS)	1.27	1.29	1.37	1.25	1.19
Phosphorus (% of TS)	0.40	0.39	0.36	0.41	0.32
Potassium (% of TS)	0.99	1.20	1.18	1.07	0.93
Organic carbon %	43.9	41.7	40.9	39.8	42.8
C/N Ratio	34.5	32.3	29.8	31.8	35.9

TVFA: Total volatile fatty acids, TS: Total solids, C/N: Carbon/ Nitrogen ratio

Table.2 Chemical composition and pH of influent for batch digestion

Parameter	Digesters					
	1	2	3	4	5	6
	Cattle Dung (CD) + slurry @ 10%	CD + Slurry @ 20%	CD + compost @ 10%	CD + paddy soil @ 10%	CD + landfill waste @ 10%	CD + kitchen waste @ 10%
pH	8.27	8.66	8.95	8.45	8.43	8.71
TVFA (mg/kg)	415.0	495.0	450.0	375.0	527.5	532.5
Total solids %	14.13	14.44	14.18	14.11	14.92	15.75
Volatile solids (% of TS)	87.0	84.0	83.0	84.5	89.5	91.0
Cellulose (% of TS)	34.6	35.6	35.1	33.5	36.4	37.6
Hemicellulose (% of TS)	23.5	21.3	21.4	20.2	22.5	19.2
Lignin (% of TS)	10.9	9.7	12.1	12.9	11.8	11.9
Nitrogen (% of TS)	1.34	1.36	1.35	1.21	1.39	1.42
Phosphorus (% of TS)	0.41	0.37	0.42	0.34	0.43	0.38
Potassium (% of TS)	1.17	1.22	1.26	1.13	1.20	1.29
Organic carbon %	50.4	48.7	48.1	49.0	51.9	52.7
C/N Ratio	37.6	35.8	35.6	40.4	37.3	37.1

TVFA: Total volatile fatty acids, TS: Total solids, C/N: Carbon/ Nitrogen ratio

Table.3 Chemical composition and pH of effluent after batch digestion

Parameter	Digesters					
	1	2	3	4	5	6
	Cattle Dung (CD) + slurry @ 10%	CD + Slurry @ 20%	CD + compost @ 10%	CD + paddy soil @ 10%	CD + landfill waste @ 10%	CD + kitchen waste @ 10%
pH	7.36	7.87	8.10	7.96	8.02	7.99
TVFA (mg/kg)	315.0	270.0	260.0	257.5	340.0	365.0
Total solids %	12.15	11.67	11.68	12.17	11.95	11.21
Volatile solids (% of TS)	75.0	72.0	71.5	73.0	75.5	74.0
Cellulose (% of TS)	30.2	30.4	32.0	32.5	34.7	33.9
Hemicellulose (% of TS)	19.2	20.1	18.5	17.4	18.9	17.0
Lignin (% of TS)	10.4	9.2	11.2	12.3	11.0	10.2
Nitrogen (% of TS)	1.41	1.38	1.39	1.26	1.44	1.49
Phosphorus (% of TS)	0.46	0.49	0.53	0.39	0.56	0.55
Potassium (% of TS)	1.23	1.31	1.33	1.16	1.29	1.34
Organic carbon %	43.5	41.7	41.4	42.3	43.8	42.9
C/N Ratio	30.8	30.2	29.7	33.5	30.4	28.7

TVFA: Total volatile fatty acids, TS: Total solids, C/N: Carbon/ Nitrogen ratio

Table.4 Dehydrogenase and cellulase activity during batch anaerobic digestion

Digester	Incubation period (weeks)									
	0 week		2nd week		4th week		6th week		8th week	
	D. A.	C. A.	D. A.	C. A.	D. A.	C. A.	D. A.	C. A.	D. A.	C. A.
Cattle dung + slurry @10%	1076.0	104.7	1193.0	154.2	1324.0	281.4	1224.0	186.4	1119.0	163.2
Cattle dung + slurry @20%	1159.0	113.4	1285.0	157.4	1393.0	279.9	1375.0	191.7	1315.0	176.4
Cattle dung + compost @10%	1050.0	100.4	1213.0	129.7	1570.0	207.6	1392.5	184.3	1220.0	171.9
Cattle dung + paddy soil @10%	934.0	97.7	1111.0	115.9	1237.5	195.4	1165.0	172.6	1051.0	167.2
Cattle dung + landfill waste @10%	1141.0	112.5	1233.0	134.3	1303.5	232.2	1278.5	189.9	1107.0	164.0
Cattle dung + kitchen waste @10%	1191.5	121.7	1351.0	159.4	1464.0	296.2	1441.0	207.1	1412.0	181.2

D. A. = Dehydrogenase activity (µg TPF g^{-1} sample per 24 hr), C. A. = Cellulase activity (µg glucose g^{-1} sample per 24 hr)

The biogas production from cattle dung, piggery faeces and poultry waste under different environmental conditions by anaerobic digestion was observed. A 6 kg of each of waste was mixed with four litres of water and loaded into three locally constructed digesters. The biogas produced was measured using water displacement method for every 5 days. It was found that piggery faeces gave the highest yield of biogas (1.07 l kg^{-1}), followed by cattle dung (0.71 l kg^{-1}), with poultry wastes the least

(0.42 *l* kg^{-1}) all under direct sunlight (Soom *et al.*, 2016). Microbial biomass in terms of dehydrogenase and cellulase activity was also determined at 15 days interval for eight weeks during batch digestion. The dehydrogenase and cellulase activity increased up to 4th week and subsequently decreased with increase in incubation period up to 8th week. The maximum dehydrogenase activity and cellulase activity was 1464.0 µg TPF g^{-1} sample per 24 hr and 296.2 µg glucose g^{-1} sample per 24 hr, respectively in cattle dung containing kitchen waste at 4^{th} week of incubation period (Table 4). This activity was maintained probably due to the adaption of microflora resulting in the increase of biogas production. Thus, cattle dung along with kitchen waste @ 5%, compost @ 5%, landfill waste @ 5% and paddy soil @ 5% or cattle dung along with biogas slurry @ 20% can be used for efficient production of biogas.

In conclusion, supplementation of compost, landfill waste, paddy soil and kitchen waste to cattle dung proved to be beneficial to achieve enhanced biogas production. The maximum biogas production (14.3 *l*) was observed on supplementation of cattle dung with kitchen waste @ 10% on dry weight basis after 5^{th} week of batch anaerobic digestion. As no increase in biogas production has been achieved after supplementation of paddy soil to cattle dung so use of paddy soil may be avoided.

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