



Original Research Article

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Co-Digestion of Biofuel Deoiled Cakes with Different Combinations of Cow Dung for Biogas Production and Nutrient Rich Manure

Priyanka Prasad^{1*}, Balakrishna Gowda¹, B.S. Nalini², G. Ashiwini²,
K.T. Prasanna¹ and K. Rajesh Kumar¹

¹Department of Forestry and Environmental Sciences, University of Agricultural Sciences,
Bengaluru- 560 065, Karnataka, India

²Department of Microbiology, University of Agricultural Sciences,
Bengaluru- 560 065, Karnataka, India

*Corresponding author

ABSTRACT

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Biogas is generated from the anaerobic-digestion of organic-wastes and is a rapidly emerging means for power generation. The seed-cake after oil extraction can serve as a good feedstock for the generation of biogas. This study presents summary of the results obtained in the anaerobic digestion of neem and pongamia deoiled-cakes with different proportions of cow-dung. The study revealed that the pH of cow-dung slurry at the beginning was 7.8; neem-cake, 5.0 and pongamia-cake, 5.8 suggesting significant differences in pH among the bio-gas substrates used. Two months after anaerobic-digestion, there was increase in pH with increase in the proportion of cow-dung and also increase in total-N and P in biogas spent slurry after anaerobic-digestion. There was no definite trend with reference to total-K. There was a decrease in total soluble solids (organic-matter), volatile solids, C/N-ratio and volatile fatty acids at the end of digestion. Significantly, very low C/N-ratio was recorded in treatments 4:1 cow-dung: neem-cake and 1:1 cow-dung: pongamia-cake (3.24) at the end of 60 days. The cumulative biogas for nine weeks was significant with cow-dung (15,755 cc) followed by 4:1 cow-dung: neem-cake. This suggests that oil-cakes can serve as good feed stocks for biogas production.

Introduction

Owing to the scarcity of coal and petroleum products, there is a need to search for alternative renewable energy from wide variety of sources such as solar energy, wind energy, Biofuel, biogas as distinct sources of renewable energy. The rising crude oil prices and increasing concerns for the environment has especially renewed focus on biodiesel from vegetable oils and animal fats as alternative to petroleum fuels. India has an enormous potential of tree borne non-edible

oil seeds with more than 300 tree species occurring in wild and along cultivated areas yielding oil in considerable quantities. In India, only non-edible oil can be used as a raw material for biodiesel production. These non-edible oil seed plants can be grown in non-fertile and waste lands. Non-edible oil seeds like jatropha, pongamia, mahua, and neem are found to be promising candidates to meet the alternative fuel needs of the country (Nina *et al.*, 2011).

Biogas technology offers a very attractive route to utilize certain categories of biomass for meeting substantial energy needs in rural as well as urban areas. The composition of biogas varies with the feed material fed to the biogas digester. Conventionally biogas is produced from regionally available raw materials such as cow dung, agricultural wastes and most of the recyclable organic wastes (Nagamani *et al.*, 2010). The primary constituent of biogas is methane (CH_4) and carbon dioxide (CO_2) and may have small amounts of hydrogen sulphide (H_2S), moisture and siloxanes. This gas is useful as fuel to substitute firewood, cow-dung, petrol, LPG, diesel, & electricity, depending on the nature of the task and local supply conditions and constraints. In fact, proper functioning of biogas system can provide multiple benefits to the users and the community can contribute towards resource conservation and environmental protection (Gouri *et al.*, 2013). It can effectively be used in a gas engine to convert the energy in gas into electricity and heat. It is mediated by a consortium of microorganisms, the performance depends on various factors such as pH, temperature, HRT (Hydraulic retention time), C/N ratio, etc. (Van der Berg and Kennedy, 1983). There have been a few attempts made in the past to increase biogas production by stimulating the microbial activity using various biological and chemical additives under different operating conditions. The biological additives include different plants, weeds, crop residues, microbial cultures *etc.* (Gunaseelan, 1987). This study is aimed at finding appropriate methods to optimally utilize the seed cake and its inherent energy before refining it as potential manure for crops.

Materials and Methods

The deoiled seed cakes of neem (*Azadirachta indica*) and pongamia (*Pongamia pinnata*) were collected from the Bio-fuel center.

Different proportions of seed cake and cow dung were mixed, dried in a hot air oven and analyzed for moisture content and total soluble solids. Based on this, different quantities of the substrates were weighed and the experimental units were setup for generation of biogas. The biogas units were constructed using polycarbonate carboys of five liter capacity fitted with a rubber cork with eight mm glass tube.

A rubber tubing of eight mm was connected to this glass tube for the collection of biogas into a one liter graduated measuring cylinder by the downward displacement of water using a beehive in a trough containing water. The mouth of the carboy fitted with rubber cork was sealed with wax to prevent any gas escaping from the carboy (Fig. 1). The bio-gas substrates namely, cow dung and deoiled cakes in different proportions were filled into the cans.

Water in required quantities were added to the carboys as per the different treatments and allowed for anaerobic digestion in the laboratory for 60 days. The amount of gas produced was noted down in the calibrated measuring cylinders. Then, the measuring cylinders were carefully removed from the trough in the same inverted position and lit with the help of match stick.

Characterization of substrates

Determination of moisture content

The substrates were dried in a hot air oven at 80°C till a constant weight was achieved and cooled in desiccators to record its final weight. The moisture content of the sample was calculated as follows.

$$\text{Moisture Content (\%)} = \frac{\text{Initial Wt.} - \text{Final Wt.} \times 100}{\text{Initial Wt.}}$$

Chemical characteristics of the substrates

The organic matter content of the substrates was determined by dry combustion method in a muffle furnace as per the procedure outlined by Jackson (1973). The physico-chemical characteristics analyzed were temperature, pH, total solid (TS), volatile solid (VS), volatile fatty acids (VFAs), total nitrogen, total phosphorus. The pH of the biogas substrates and their combinations was measured with a digital pH meter in 1:2.5 ratio of substrate to water(Jackson, 1973).The total nitrogen in samples was determined by semi micro Kjeldhal method as per the procedure outlined by Jackson (1973) using Gerhardt auto analyser. The total phosphorous content of the samples was determined by vanado-molybdo-phosphoric yellow colour method in nitric acid system as per the procedure given by Jackson (1973). The total potassium content was determined using flame photometer (Jackson, 1973) in the tri-acid digest. The total solids, volatile solids and volatile fatty acids were determined by adopting the procedures furnished by APHA, 1995.

The biogas produced was measured every day by the downward water displacement of water at atmospheric pressure using calibrated 1 liter measuring cylinders. The cumulative biogas production was determined by adding the daily biogas production.

Results and Discussion

The pH of cow dung slurry at the beginning was 7.8; neem cake slurry 5.0 and pongamia cake 5.8, suggesting that there were significant differences in pH among the biogas substrates. But, Bagudo *et al.*, (2008) did not observe much difference in pH of cow dung and other agricultural wastes. The differences observed in this study could be due to the varied nature of the substrates, in

that the oil seeds are highly proteinaceous while cow dung is more lingo-cellulolytic. Further, the increase in pH in combinations of different biogas substrates could be the result of added effect of cow dung in higher proportions to the deoiled cakes. The results also indicated that sixty days after anaerobic digestion, there was not much change in the pH of biogas slurry with different combinations of biogas substrates except with 4:1 cow dung and neem cake (T7) while in cow dung and pongamia combinations in 1:1 ratio, increase in pH was recorded. The general decrease in pH in spent slurries as compared to the fresh ones may be attributed to the formation of sulphides in the slurries due to the breakdown of biodegradable sulphur containing organic and inorganic compounds and also due to the formation of fatty acids by acetogenic methanogens during the process of digestion (Ahmad, 2000). Ndegwa *et al.*, (2000) suggested that the pH reduction may be attributed to mineralization of nitrogen and phosphorus into nitrites/nitrates and orthophosphates and bio-conversion of the organic material (Table 2).

Sufficient nutrients are also important for microbial growth. The macro nutrients such as carbon, hydrogen, nitrogen and oxygen are the main components of cells in the biomass, with others like sulphur, phosphorus, potassium, calcium, magnesium and iron which are required (McMahon *et al.*, 2001). The majority of nutrients can be inhibiting if they are present at high concentrations. The results in the present study have indicated that there were significant differences in N, P and K contents amongst the different treatments. The composition and nutritional availability of oil cakes widely vary based on the quality of the seed or nuts, method of oil extraction, storage parameters etc. (Sumithra *et al.*, 2007). Regarding combinations, there were significant differences among combinations of cow dung and each oil cake owing to the

fact that cow dung having less NPK, when mixed in higher proportions with the same quantity of deoiled cakes, show significant decreases in different treatments. In general, there was an increase in total-N content in the samples in bio-gas spent slurry after two months of anaerobic digestion. The highest N content was recorded in T3 (PC alone) (6.6%) followed by T8 (4:1 CD: PC) (6.1%), T9 (2:1 CD: PC) (5.6%) and least was in treatment T7 (4:1 CD: NC) (1.9%). Significant increase in N, P and K were recorded, that have been due to the mineralization of soluble organic compounds. Further, when organic material undergoes decomposition, carbon in the material gets lost as CO₂ resulting in mass reduction of substrate resulting in increase in K content in the bio-gas slurry (Table 1).

The potentialities of the three different residues as substrates for anaerobic digestion have been investigated in this study. The advantage of using deoiled cakes as a substrate for biogas production is their cheaper availability throughout the year. Moreover, with increasing emphasis on cost reduction industrial processes and value addition to agro-industrial residues, utilization of deoiled cakes as an energy source seems to be promising because of their higher energetic value. The production of bio-gas is a complex multi-step process involving degradation of substrates by various microorganisms under the influence of physico-chemical conditions within anaerobic environment. The process occurs in three stages; hydrolysis of the substrates, acedogenesis and methanogenesis (Garba, 1999, Dangoggo *et al.*, 2004). In either case, hydrolysis of the substrate is considered as the first step, suggesting that all the other stages depend on the rate and extent of hydrolysis of the substrate (Anderson, 1979). When the process parameters are considered, apart from pH, the total solids of the slurry are important and means, the part of the substrate remaining after the expulsion of

moisture. This is made up of both the volatile solids and ash contents of the substrates. Since, bio-gas is produced from the conversion of only the volatile solids (organic matter) of the substrates, it would be expected that the total solid content of the substrate to be higher than that of the spent slurry. The differences in the total solids between the substrate and its spent slurry should therefore be directly proportional to volume of bio-gas generated from the substrates. A general observation made in this study was a decrease in the total soluble solids (organic matter) over a period of sixty days in bio-gas slurry in all the treatments which could be attributed to the conversion of organic matter to bio-gas in the anaerobic digestion process. The total solids are very important as far as the process is concerned and according to Abdul karim *et al.*, (2010) at some point of time, any increase in percent total solids, further rise in the volume of the bio-gas gets seized. Balsam (2002) suggested that the optimum solid contents should be in the range of 7-9%. Baserja (1984) reported that the process was unstable below 7% (of manure) of total solids while a level of 10% caused an overloading of the digester. Further, Itodo and Awulu (1999) showed that slurries of higher TS concentrations were more acidic than that of lower TS concentrations. Different ratios of seed cakes were diluted and volatile solids of all the seed cake treatments and cow dung which was held showed a decrease in concentration after the retention period of sixty days (Table 3).

There was reduction in organic carbon in the biogas slurry during the process of anaerobic digestion. In general, there was a reduction in organic-C in all the treatments at the end of anaerobic digestion. This reduction in organic carbon in anaerobic process was therefore probably limited to the production of organic acids, H₂ and CO₂ by facultative bacteria (Hobson *et al.*, 1981).

Table.1 Nutrient concentration of biogas substrates and biogas spent slurry during anaerobic digestion

Sl. No.	Treatments	Nutrient Concentration (%)					
		Biogas Substrates			Biogas Spent Slurry		
		N	P	K	N	P	K
T1	Cow dung alone (control)	1.6 ^e	0.03	0.52 ^a	2.8 ^f	4.5 ^a	2.0 ^b
T2	NeemCake alone (NC)	3.9 ^a	0.06	0.14 ^h	2.1 ^h	0.3 ^f	1.0 ^e
T3	PongamiaCake alone (PC)	3.9 ^a	0.03	0.17 ^g	6.6 ^a	0.6 ^e	0.8 ^{ef}
T4	1:1 CD:NC	1.4 ^f	0.05	0.12 ⁱ	2.0 ^h	1.2 ^d	0.7 ^f
T5	2:1 CD:NC	1.8 ^d	0.09	0.43 ^d	2.9 ^f	2.3 ^c	1.2 ^d
T6	3:1 CD:NC	2.2 ^c	0.07	0.33 ^f	2.4 ^g	3.4 ^b	1.7 ^c
T7	4:1 CD:NC	2.6 ^b	0.05	0.39 ^e	1.9 ⁱ	4.5 ^a	2.2 ^a
T8	1:1 CD:PC	1.4 ^f	0.04	0.33 ^f	6.1 ^b	1.2 ^d	0.7 ^f
T9	2:1 CD:PC	1.8 ^d	0.03	0.47 ^b	5.6 ^c	2.4 ^c	1.2 ^d
T10	3:1 CD:PC	2.2 ^c	0.04	0.43 ^d	5.0 ^d	3.5 ^b	1.7 ^c
T11	4:1 CD:PC	2.6 ^b	0.03	0.45 ^c	4.1 ^e	4.6 ^a	2.2 ^a
S.Em		0.037	-	0.004	0.114	0.057	0.048
CD @ 5%		0.115	NS	0.013	0.354	0.177	0.148

Table.2 Changes in pH, Total soluble Solids and C/N ratio in biogas substrates and biogas spent slurry during anaerobic digestion

Sl. No.	Treatment	Biogas Substrates			Biogas Spent Slurry		
		pH	TSS (%)	C/N ratio	pH	TSS (%)	C/N ratio
T1	Cow dung (control)	7.8 ^a	62.2 ^e	21.70 ^c	8.0 ^a	51.8 ^c	10.75 ^b
T2	NeemCake alone (NC)	5.0 ^g	35.5 ^j	5.30 ^k	4.4 ^h	29.4 ^e	8.14 ^c
T3	PongamiaCake alone (PC)	5.8 ^{ef}	88.7 ^a	13.20 ^g	4.4 ^h	72.7 ^a	6.39 ^e
T4	1:1 CD : NC	5.5 ^f	24.4 ^k	6.71 ^j	5.2 ^g	22.5 ^f	6.55 ^e
T5	2:1 CD : NC	5.6 ^f	39.9 ^h	12.22 ^h	5.6 ^f	34.5 ^e	7.08 ^d
T6	3:1 CD : NC	5.7 ^f	55.5 ^f	17.60 ^e	5.8 ^e	51.8 ^c	12.54 ^a
T7	4:1 CD: NC	6.0 ^{de}	71.2 ^c	23.05 ^b	7.8 ^b	65.8 ^b	3.24 ^g
T8	1:1 CD: PC	5.9 ^{de}	37.9 ⁱ	8.72 ⁱ	6.9 ^c	34.1 ^e	3.24 ^g
T9	2:1 CD: PC	6.0 ^d	53.4 ^g	14.10 ^f	6.0 ^d	46.8 ^d	4.85 ^f
T10	3:1 CD: PC	6.2 ^c	68.9 ^d	19.60 ^d	6.1 ^d	66.4 ^b	7.71 ^d
T11	4:1CD: PC	6.5 ^b	84.4 ^b	25.03 ^a	6.1 ^d	75.4 ^a	10.67 ^b
S.Em		0.064	0.177	0.238	0.054	1.273	0.167
CD @5%		0.199	0.549	0.742	0.169	3.963	0.518

Table.3 Volatile fatty acids and titrable acidity in biogas spent slurry

Sl. No	Treatments	VFA (%)	Cumulative Biogas (cc)
T1	Cow dung (control)	05.2 ^k	15755
T2	NeemCake alone (NC)	26.2 ^b	595
T3	PongamiaCake alone (PC)	21.8 ^c	215
T4	1:1 CD : NC	07.8 ⁱ	587
T5	2:1 CD : NC	09.2 ^h	907
T6	3:1 CD : NC	10.5 ^g	993
T7	4:1 CD : NC	11.8 ^f	8418
T8	1:1 CD : PC	06.7 ^j	3783
T9	2:1 CD : PC	13.5 ^e	3690
T10	3:1 CD : PC	20.3 ^d	3090
T11	4:1CD : PC	27.1 ^a	2550
	S.Em	0.050	-
	CD @ 5%	0.156	-

The details of treatments

Treatments	Quantity of the substrates
T1 - Cow dung alone (Control)	1412g CD + 1588ml water
T2 - Neem cake(NC)alone	270g NC + 2730ml water
T3 - Pongamia cake (PC) alone	258gPC + 2742ml water
T4 - 1 CD :1 NC	225g CD + 225g NC + 2550ml water
T5 - 2 CD : 1 NC	370g CD + 185g NC + 2445ml water
T6 - 3 CD: 1 NC	495g CD + 165g NC + 2340ml water
T7 - 4 CD : 1 NC	610g CD + 153g NC + 2237ml water
T8 - 1 CD: 1 PC	228g CD + 227g PC + 2547ml water
T9 - 2 CD : 1 PC	364g CD + 182g PC + 2454ml water
T10- 3 CD: 1 PC	540g CD + 180g PC + 2280ml water
T11- 4 CD: 1 PC	567g CD + 142g PC + 2291ml water

There were 11 treatment combinations with two replications.

Fig.1 The deoiled seed cakes of neem (*Azadirachta indica*) and pongamia (*Pongamia pinnata*)



The carbon compounds are converted into CH₄ and other gases like CO₂. According to Richard (1996) and Wilkie (2005), the anaerobic bacteria do not or very slowly degrade lignin and some other hydrocarbons. In other words, higher lignin content will slow down biodegradability of wastes. The C: N ratio higher than 30 result in slow multiplication of microorganism due to N limitation and as a consequence, lower efficiency of substrate degradation is observed. On the other hand, if the substrate C: N ratio can result in successful digestion, however, when such low C: N ratios and nitrogen rich substrates are applied, a possible NH₄ inhibition must be considered. NH₄, although represents an ideal form of nitrogen for the growth of microorganisms, is toxic to mesophilic methanogenic organisms. Thermophilic methanogenic microorganisms are generally more sensitive to NH₄ concentration. However, NH₄ inhibition can very much depend on the type of substrates. In the present study, there was a decrease in C: N ratio at the end of anaerobic digestion of 60 days in all the treatments. Significantly, very low C/N ratio was recorded at the end of anaerobic digestion in treatments T7 with 4:1 CD: NC with and T8 with 1:1 CD: PC (3.24). The low yields in bio-gas in these treatments could be due to the toxicity of NH₄ in these treatments due to lower C: N ratios.

The anaerobic digestion is a complex process consisting of a series of microbial reaction catalysed by consortia of different bacteria (McInerney *et al.*, 1980). The inter dependence of bacteria is a key factor of the bio-gas process. Under conditions of unstable operation, intermediates such as volatile fatty acids (VFA) and alcohols accumulate (Gujer and Zehnder, 1983) at different rates depending on the substrate and the type of perturbation causing instability (Allioson, 1978). The most common disturbances causing imbalances are hydraulic or organic

over loading, the presence of inorganic or organic toxins or other disturbances in the process such as temperature and substrate changes (Switzenbaum *et al.*, 1990). Several parameters have been suggested as stress indicators. Some of the most commonly used indicators include measurements of gas production, gas composition, pH, destruction of volatile solids and VFA concentrations. In general, most of these indicators are suitable for detecting gradual changes. However, pH, reduction of volatile solids and gas composition are often too slow for the optimal detection of sudden changes (Algelidaki and Ahringeb, 1994). The pH changes are small in highly buffered systems, as often seen in reactors with high ammonia loads, even when the process is severely stressed (Algelidaki and Ahringeb, 1994). Some important features of a good process indicator are its ability to detect imbalances at an early stage and its ability to reflect the metabolic state of the system directly. It is also important that the relative change of the parameter following a perturbation is significant, compared to the background fluctuations and analysis uncertainty. For a long time it has been recognized that the VFA concentration is one of the most important parameters for the accurate control of anaerobic digestion (Hill and Bolte, 1989; McCarty and McKinney, 1961). Though, improvement of biogas production is expected based on the high oil content as in case of oil cakes, addition of cakes with other feed stocks, such as cow dung may result in the accumulation of free fatty acids. The accumulation of high free fatty acids can also cause problems in the production of biogas by indirectly causing toxic effects due to the resultant decrease in pH. Generally, hydrolytic and acedogenic microorganism grow about ten times faster than methanogens. This results in the accumulation of high free fatty acids before they get converted to methane by methanogens. In the present study, the

volatile fatty acids were significantly higher in T11 with 4:1 CD: PC (27.1%) and least was in T1 with cow dung alone (5.2%). Probably, this could be one of the factors why biogas production with only cow dung was high. The accumulation of volatile fatty acids (VFA) during the imbalance of the process reflects directly an uncoupling kinetics between acid producer and consumers (Hickey *et al.*, 1989). Therefore, concentration of VFA was suggested as one of the factors for the control and monitoring of anaerobic digester (Hill and Bolte, 1989). The toxic effects of high VFA concentrations on the anaerobic digestion process have been studied and reported by several authors (Ahring and Westermann, 1988; Gorris *et al.*, 1989). A drop in pH is generally considered to be the main cause of the toxicity (Hill 1982; Mosey and Fernandes, 1984). Several studies have showed that high concentrations of VFA in themselves have no adverse effect on biogas process (Gourdon and Vermande, 1987).

Biogas production technology is the only solution for sustainable development of rural lively hoods. In this regard, deoiled cakes, a by-product of biodiesel production has its own importance owing to its availability, mostly in rural areas. However, there is a need to standardize the cow dung to cake ratios as it has been revealed in the present study. Further, the spent slurry which is rich in plant nutrients can be used as valuable manure in agriculture. Therefore, efforts should be made to promote environmental friendly technologies to meet the ever growing energy demands of the rural population.

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