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Physico-Chemical Properties of Before and After Anaerobic Digestion of *Jatropha* Seed Cake and Mixed With Pure Cow Dung

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Abstract

In this study, the biogas production potential of *Jatropha* seed cake (JSC) was assessed in the laboratory. The physico-chemical analysis data Total solids (TS%), Volatile solids (VS%), Organic carbon (OC%), Total nitrogen (N%), Total phosphorus (P%), Total potassium (K%), Moisture content (MC%) of the cakes confirmed that JSC is feasible of anaerobic digesters. By blending, JSC with cow dung in different ratios, the feedstock of the biogas digesters was prepared. From the nine treatments to analyze the changes in physico-chemical parameters of the fresh and digested slurry of moisture content is highest after digestion Treatment control-2, 15.8% and lowest before digestion (12.2%). But before digestion, Treatment control-2, TS is 98.2% and VS is 93.12% higher than after digestion. The organic nutrients content Treatment control-2 is C%, 27.37%, N% 2.67 and K % 1.98 of BD is higher than AD but P% 1.078% thus the phosphorous content had increased during the digestion process.

Keywords: *Jatropha* seed cake; Pure cow dung; Anaerobic digestion; Total solid; Volatile solid; Organic nutrient content.

Introduction

Jatropha curcas is a perennial shrub to small evergreen tree of up to 6m height, adapted to all kinds of soil and does not demand any special nutritive regime [1]. It has been introduced in Africa and Asia and is now cultivated worldwide. In India, it was believed to be introduced by the Portuguese settlers during 16th century. It is a multipurpose, deciduous, small tree reported to be cultivated in drier regions of central and western parts of India. Recently, it has also been introduced in the northern and southern states of India. The plant is widely distributed and fits easily into agriculture system in the form of hedges, windbreak and erosion barrier or as a source of fire wood [2].

In recent years there are growing concerns about the utility of *Jatropha curcas* as a source of biofuel plantations in order to generate alternate source of energy. *Jatropha* seed cake is used as a fertilizer but the literature and data regarding the efficacy of improving crop yield or conditioning soil is limited. *Jatropha curcas*, commonly known as physic nut, belong to the euphorbiaceous family. It is a hardy plant, thrives on degraded land and requires limited amount of nutrients and water. The seeds contain about 300-350 gkg^{-1} oil, which can be used as a fuel directly or, in its trans esterified form, as a substitute for diesel. Large scale planting of *jatropha* has taken place or is planned in India, china, Madagascar, Myanmar and many other developing countries, with the aim of using the oil as biodiesel [3] in these countries the oil is produced from whole seeds using a screw press. The seed has a hard, black outer shell and kernel range from 350 to 400 $gm.kg^{-1}$ and from 600 to 650 $gm.kg^{-1}$ respectively.

The seed cake left as a by-product after oil extraction by screw press can contain as much as 500-600 $gm.kg^{-1}$ indigestible shells. The generation of biogas from these cakes would be a best solution for its efficient utilization. Anaerobic digestion has been considered as waste-to-energy technology, and is widely used in the treatment of different organic wastes for example: organic fraction of municipal solid waste, sewage sludge, food waste, animal manure, etc. Anaerobic treatment comprises of decomposition of organic material in the absence of free oxygen and production of methane, carbon dioxide, ammonia and traces of other gases and organic acids of low molecular weight. Anaerobic digestion provides an alternative option for energy recovery and waste treatment.

Material and Methods

Statistics

The included Mean, Standard Error analysis was done using SPSS package and (Minitab 13 for windows software package) where used to analyze the Physico-chemical parameters VS, TS of feedstock and digestate, result of *jatropha* seed cake.

Experimental apparatus setup

Nine treatments in three replications were taken for the study. The treatment, T1 contained *Jatropha* seed cake (JSC) alone and the subsequent treatments consisted of cow dung (CD) and *Jatropha* seed cake in 1:1,1:2, 1:3, 1:4, 1:5, 2:1 and 3:1 for T2, T3, T4, T5, T6, T7 and T8 respectively. T9 with cow dung alone was taken as the control. The experiment was conducted during 84 days constant temperature around 35 ± 1°C for optimization of the biogas yield.

Hydraulic retention period

The optimum period for the economical gas production in batch fermentation depends largely on pattern of daily gas production and the pattern of changes in the calorific value of gas produced i.e. CH_4 content. The study period in the experiment was 84 days.

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Inoculum

The inoculum for the anaerobic digestion of non-edible oil seed cakes and cow dung will be prepared by composting the fresh cow dung under anaerobic condition for a period of five days in ambient temperature. The fresh cow dung contains active micro-organisms. A pinch of urea will be added in different treatments to supplement the nutrients so that it enhances the growth and multiplication of consortium of micro-organisms.

Anaerobic batch digestion assembly

Laboratory studies will be conducted by using 2L capacity conical flasks, and graduated cylinders, as batch digesters for carrying out the anaerobic digestion. The feed stocks will be diluted with the requisite amount of water so as to make 7-10% total solid concentration. The gas generated will be collected in inverted calibrated gas collection jar filled with water which will be partly immersed in water bath.

Measuring yield and quality of biogas

The gas production is measured by water displacement method whereas the quality, which is the percentage of methane from the biogas, is estimated by the displacement of sodium hydroxide, with a process held one next to the other. The gas volume produced in the anaerobic reactor was captured in a bottle filled with water, which was kept under pressure. When a gas bubble entered the bottle with water, the gas replaced the water, which was then forced out of the bottle into an empty bottle. The volume of water in the measuring cylinder thus resembled the gas production in the reactor. The displaced water is collected and then using a measuring cylinder its volume would be calculated. The displaced water indicates the total volume of biogas produced. Note that the gas coming out of the digester is stored in the displaced bottle. Adding back the displaced water to the displacement bottle would push out the biogas stored before; and passing it through 5% NaOH solution. The CO_2 from the biogas would be retained in the solution whereas the methane would displace its equivalent volume of NaOH. Collecting the displaced solution and measuring its volume using a measuring cylinder would give the volume of methane from the produced biogas [4]. Hence, it would be possible to estimate the percentage of methane in the biogas, using the following simple equation.

$$\text{CH4\%} = \frac{displaced\ NaOH}{displaced\ water} \times 100$$

Quantitative analysis of biogas yield

Cumulative yields of biogas (expressed in litres/kg of dry matter) from JSC and its admixtures with cow dung and the trends in biogas production are shown in Table 1.

The rate of biogas production from JSC was observed to be rapid as compared to cow dung during the second week of fermentation, the rate of production in the mixtures of cow dung and JSC increased substantially and biogas yield was faster than that from treatment with cow dung alone. JSC alone produced biogas of 76.5 L/kg dry matter (dm) within 14 days and there after its production attained steady pace. The trend pointed out a gradual increase to a maximum between 14 and 21 days, and then the gas production is maintained at a high level to the end of the experiment [5]. The total period taken for digestion was 84 days, beyond which the gas yield were more or less stopped as observed for all the treatments except CD:JSC in 2:1. But the treatment, CD: JSC in 2:1 ratio showed a steady increase in biogas generation throughout the study period and yielded the highest amount of biogas i.e. 500.15 L/kg dry matter followed by CD: JSC in 3:1, generating 420.7 L/kg dm. All the treatments except treatment with *Jatropha seed* cake alone have shown biogas potential higher than CD and produced 263, 256.34, 237.82, 273.54 and 276.94 L/kg dm of biogas by CD:JSC in 1:3,1:2,1:1,1:4 and 1:5 respectively. Compared to 184.34 L/kg from cow dung. This higher yield may be due to the synergistic action of micro-organisms from the co-digestion process.

Sample preparation and feed solution

Jatropha curcas seed was collected from Dehradun. The seed cake was stored in a plastic bag at constant temperature around 35 ± 1°C and was blended prior to experiment. After blending the seed cake were mixed with tap water at ratios of JSC alone, 1:1, 1:2, 1:3, 1:4, 1:5, and 2:1, 3:1, CD alone by weight to create slurry.

Estimation of Volatile Solids, Total Solids, Moisture Content, Organic Carbon, Total Nitrogen, Total Potassium, Total Phosphorus

Total solid (TS)

The total solid content of the *Jatropha* seed cake (JSC) and cow dung (CD) can be determined by the procedure given by APHA [6]. A 10 g of freshly collected sample has to be taken in a disc and placed inside a hot air oven at 105°C for one hour. Then it is taken out and cooled to room temperature in desiccators and weighed. The percent of total solids is computed using the formula.

$$\%\ \text{total solids} = \frac{C-A}{B-A} \times 100\ \%$$

Where,

A= Mass of empty clean and oven dried silica crucible, g

B= Mass of silica crucible + sample, g

C= Mass of silica crucible + sample after oven drying, g

Volatile solids (VS)

The volatile solid content of sample can be determined by adopting the following procedure furnished by APHA [6]. A knows mass of residue obtained from the determined of the total solids is placed in a silica crucible and ignited in a muffle furnace at 550 ± 500°C for 30 minutes. Then the crucible is taken out of the muffle furnace, partially cooled in air, kept in desiccators for few minutes and then weighed. Volatile solids is computed using the formula given below,

$$\%\ \text{VS} = \frac{B-C}{B-A} \times 100\ \%$$

Where,

A= Mass of empty clean and oven dried silica crucible, g

B= Mass of silica crucible + sample, g

C= Mass of silica crucible + sample after ignition, g

Moisture content

The moisture content will be determined using AOAC [7] method, about 15g of sample will be weighed in converted dish previously dried at 98-100°C, cooled in desiccator and weighed soon after reaching room temperature. Cover will be loosened and heated at 98-100°C to constant weight. At the end of drying, cover will be immediately tightened on dish, transferred to desiccator and weighed soon after reaching room temperature.

$$\text{Moisture\%} = \frac{\Delta SB - \Delta SA}{weight\ of\ sample} \times 100$$

Period (weeks)	TI JSC	T2 (1:1)	T3 (1:2)	T4 (1:3)	T5 (1:4)	T6 (1:5)	T7 (2:1)	T8 (3:1)	T9 CD
I	22.04	25.33	27.18	13.21	16.60	24.02	20.91	34.93	6.54
II	76.5	27.31	33.65	15.22	18.81	25.81	23.38	36.38	11.18
III	68.78	30.13	39.87	17.63	20.74	29.74	25.62	37.16	14.55
IV		33.65	38.65	18.26	24.62	34.03	26.05	38.32	18.48
V		31.39	31.23	20.25	27.11	37.96	28.14	38.48	21.59
VI		31.11	27.42	23.84	28.59	39.83	35.29	38.56	25.94
VII		30.34	28.06	33.43	32.79	41.74	38.12	38.63	28.32
VIII		28.56	30.28	36.46	34.54	43.81	45.32	38.78	28.52
IX				41.98	36.63		52.75	39.83	29.2
X				42.71	33.11		59.23	39.87	
XI							69.36	39.76	
XII							75.98		
TOTAL	**167.32**	**237.82**	**256.34**	**263**	**273.54**	**276.94**	**500.15**	**420.7**	**184.34**
Methane content (%)	**56.8**	**57.2**	**57.7**	**56.2**	**58.3**	**58.5**	**61**	**58.7**	**54**

Table 1: Periodic And Total Of Biogas And Methane Content (%) Obtained From Jatropha Seed Cake And Admixtures With Cow Dung (L/Kg At Dry Weight).

ΔSB = weight of dish and sample before drying.

ΔSA = weight of dish and sample after drying.

Carbon content

0.1g seed cake powder into a 500ml conical flask. Add 10ml of 1N potassium dichromate solution. Add 20 ml sulphuric acid and mix by gentle rotation for 1 minute taking care to avoid throwing seed cake powder up onto the sides of the flask. Let stand for 30minutes and dilute to 200 ml with distilled water. Add 10ml phosphoric acid, 0.2 g ammonium fluoride and 10 drops diphenylamine indicator. Titrate with 0.5 N ferrous ammonium sulphate solutions until the colour changes from dull green to a turbid blue. Add the titrating solution drop by drop until the end point is reached when the colour shifts to a brilliant green and prepare and titrate a blank in the same manner.

% Organic Matter=10[1(S/B)] X0.67

S=sample titration

B=blank titration

Nitrogen content

Nitrogen content will be determined using (Kjeldahl method, 1950) half a gram powdered sample will be digested with concentrated H_2SO_4 in presence of digestion mixture ($CuSO_4$ + K_2SO_4 + selenium powder in 20:10:1) till the digest give clear green colour. The digested sample will be further diluted carefully with distilled water to known volume. Then a known amount of aliquot will be transferred to distillation unit (Micro Kjeldahl apparatus) and liberated ammonia will get trapped in boric acid containing mixed indicator. Later, it will be titrated against standard H2SO4 and the amount of ammonia liberated indicator.

Potassium content

The potassium content of the samples will be estimated using the standard procedure as suggested by flame photometer method [8] which is explained below. For 10 ml of dried sample, 50 ml of 0.01 N ammonium acetate will be added. The sample gets leached with an additional 50 ml of ammonium acetate and made up the volume to 100 ml. The standard of potassium is fed to the flame photometer and the readings were noted down using K-filter. The procedure was repeated thrice and the average values are reported.

Phosphorus content

Plant and seed samples were digested with diacid mixture of HNO_3 and $HClO_4$ in the ratio of 9:4 and the extract was made to a definite volume. Total phosphorous was determined by vanado molybdate phosphoric acid yellow colour method at 730 nm [8].

Feedstock compositions of cow dung mixed with *jatropha* seed cake with different ratio were prepared as shown in Table 2.

Results and Discussion

In this study analysis of the different ratio of treatment composition of JSC and with CD showed that Table 3. The physico-chemical properties of the feedstock composition of the substrates before and after digestion in terms of moisture content, VS%, and TS% etc. Were analyzed and presented above Table 3.

Total solids (TS)

Different ratio of seed cake wastes were diluted and total solids of all treatments seed cake and cow dung which were held at for example one treatment TC-2, 98.2% showed a decrease after the retention period of 84 days which were found to be only 93.67%. Thus there was a 40% decrease in the TS% and thus indicated that the continuous feeding for digestion process is very small efficient in removing the total solids. Total solids are very important as far as the process is concerned. According to Abdulkarim et al. [9] at some point in the increase of the percent TS, no further rise in the volume of the biogas is obtained. Balsam [10] and Zennaki et al. found that the optimum solid content obtained for biogas production is in the range 7- 9%. Furthermore, Baserja [11] reported that the process was unstable below a total solids level of 7% (of manure) while a level of 10% caused an overloading of the digester. Itodo and Awulu [12] showed that slurries of higher TS concentrations were more acidic than that of lower TS concentrations.

Volatile solids (VS)

Volatile solids reduction occurred in the slurry during the digestion. The VS fed on an average basis into the digester were found to be all treatment for example one treatment like TC-2, 93.12%. The VS found after the digestion process was found to be 83.31%. Thus there was about 12% decrease in volatile solids. Volatile solids are the solids that are lost on ignition of the dry solids at 55°C centigrade. VS are responsible for biogas production. Since seed cake waste contains 93.12% of volatile solids thus it has a great potential of biogas production and can be used easily and potentially as a raw material for biogas production.

Label		Amount of seed cake(g)	Amount of cow dung(g)	Amount of water added(ml)	Total volume of slurry (ml)
Treatment composition	**Replication**				
TC-1 seed cake alone	R1	108.108	0	891.892	1000
	R2	108.108	0	891.892	1000
	R3	108.108	0	891.892	1000
TC-2 1:1	R1	54.05	225.733	720.22	1000
	R2	54.05	225.733	720.22	1000
	R3	54.05	225.733	720.22	1000
TC-3 1:2	R1	72.432	148.98	778.58	1000
	R2	72.432	148.98	778.58	1000
	R3	72.432	148.98	778.58	1000
TC-4 1:3	R1	81.081	112.86	806.06	1000
	R2	81.081	112.86	806.06	1000
	R3	81.081	112.86	806.06	1000
TC-5 1:4	R1	86.486	90.293	823.221	1000
	R2	86.486	90.293	823.221	1000
	R3	86.486	90.293	823.221	1000
TC-6 1:5	R1	90.05	74.94	835.006	1000
	R2	90.05	74.94	835.006	1000
	R3	90.05	74.94	835.006	1000
TC-7 2:1	R1	35.67	302.48	661.846	1000
	R2	35.67	302.48	661.846	1000
	R3	35.67	302.48	661.846	1000
TC-8 3:1	R1	81.081	112.86	806.059	1000
	R2	81.081	112.86	806.059	1000
	R3	81.081	112.86	806.059	1000
TC-9 Cow dung alone	R1	0	451.467	548.53	1000
	R2	0	451.467	548.53	1000
	R3	0	451.467	548.53	1000

Table 2: Feedstock composition of cow dung and jatropha seed cake and taken in 9 treatments with 3 replications.

Treatment Composition (TC)	Moisture content (%)		Total Solids (%)		Volatile Solids (%)	
	BD	AD	BD	AD	BD	AD
TC-1 JSC alone	4	7.5	92.5	88.21	93.77	82.13
TC-2 1:1	12.2	15.8	98.2	93.67	93.12	83.31
TC-3 1:2	11.7	14.7	97.5	92.99	92.88	82.11
TC-4 1:3	10.3	13.5	96.28	91.52	91.55	81.1
TC-5 1:4	8.8	11.3	94.56	90.21	90.22	79.22
TC-6 1:5	7.4	10.2	92.33	88.12	89.71	77.90
TC-7 2:1	5.1	8.6	90.18	84.01	88.95	76.66
TC-8 3:1	5.9	8.3	90.11	85.21	88.62	74.32
TC-9 CD alone	59.31	77.95	22.15	15.44	92.66	76.61

Table 3: Moisture content, Volatile solid and total solid content of treatment before and after anaerobic digestion.

Moisture Content (MC)

The moisture content of the digestate increased from all treatment for example one treatment TC-2, 12.2% to 15.8% thus there was an increase (Table 3). According to Sadaka and Engler [13], water content is one of very important parameter affecting anaerobic digestion of solid wastes. There are two main reasons i.e., (a) Water make possible the movement and growth of bacteria facilitating the dissolution and transport of nutrient and (b) water reduces the limitation of mass transfer of non-homogenous or particulate substrate. In general, the moisture content of the digestate increased with increase in the amount of VS and TS reduction. The moisture content to be maintained for the degradation depends upon the type of the waste. In the present study final moisture content was at 15.8%.

Organic carbon content (OC%)

Organic carbon content reduction occurred in the slurry during the digestion. The OC fed on an average basis into the digester were found to be all treatment for example one treatment like TC-2, 27.37%. The OC found after the digestion process was found to be 24.78%. Percent carbon content in the feedstock was found to 24.80 in cow dung slurry and 48.80 in the JSC waste slurry. The reduction of C by anaerobic processes was therefore probably limited to the production of organic acids, H_2 and CO_2 by facultative bacteria [14]. Thus continuous process of feeding is also efficient in removing carbon content. The carbon compounds are converted to the CH_4 and other gases like CO_2. According to Richard and Wilkie [15,16], anaerobic bacteria do not or very slowly degrade lignin and some other hydrocarbons. In other word, the higher lignin content will lower biodegradability of waste. As food waste was used, it contains enough

Treatment composition	OC%		P%		N%		K%	
	BD	AD	BD	AD	BD	AD	BD	AD
TC-1(JSC alone)	48.80	46.21	2.09	3.77	4.5	3.85	3.22	2.65
TC-2(1:1)	27.37	24.78	0.066	1.078	1.890	1.24	0.86	0.78
TC-3(1:2)	28.64	26.05	0.063	1.068	2.520	1.87	0.90	0.87
TC-4(1:3)	30.97	28.38	0.060	1.043	2.310	1.56	0.87	0.75
TC-5(1:4)	31.22	28.63	0.058	0.081	2.390	1.61	0.90	0.88
TC-6(1:5)	31.58	28.99	0.056	0.077	2.260	1.56	0.89	0.82
TC-7(2:1)	22.44	23.85	0.057	0.070	2.030	1.38	0.81	0.79
TC-8(3:1)	26.72	24.13	0.061	0.067	2.013	1.413	0.87	0.81
TC-9(CD alone)	24.80	22.21	0.064	0.075	1.92	1.61	1.12	0.78
SE ± (m)	1.329	1.328	0.108	0.200	0.134	0.129	0.125	0.099

Table 4: Organic nutrient content of various treatments before and after anaerobic digestion.

carbohydrates and less cellulose and lignin content, thus the removal of carbon content was fast.

Macronutrients content

In this study the amount of macronutrients content like K, N and P% which were present at the start of the digestion P is0.066%, K is 0.86% and N is 1.890% of the waste. The macronutrients content were again analysed at the end of the study period (84 days) and it were found to be P is 1.078%, K is 0.78% and N is 1.24%. Thus the macronutrients content like phosphorus content had increased, potassium content had decreased and nitrogen content had decreased during the digestion process. Increase and decrease in the quality of macronutrient were recorded in the slurry; (Table 4). Phosphorous, Nitrogen and Potassium a macronutrient is very important as far as plant growth is considered. It is present in every living cell. It is very important to enhance process stability and maintain a stable operation for anaerobic digestion of municipal solid waste [17].

Conclusion

The supplementation of JSC to cow dung up to 75 per cent can be advantageously used for the economic biogas generation from a total solid concentration of 8-10 per cent. This shows that the mixing of these two materials in different proportions enhanced the gas production by utilizing more of the complex substrates than when used alone. The effect may be due to the synergistic action of a variety of cellulolytic and hydrolytic bacterial species in the breakdown of raw material [18]. The decline in gas production in treatment with JSC alone may be due to high lignin content. It is worth mentioning that slurry made of 2:1cow dung and JSC was found the best among the various treatments in biogas generation. The admixtures of cow dung and JSC is a viable option for the energy production and for efficient management of *jatropha* seed cake which was otherwise creates environmental problems.

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