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Study on Biogas Production Potential of Leaves of *Justicia schimperiana* and Macro-Nutrients on the Slurry

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Abstract

Conversion of animal waste to biogas energy to replace traditional fuel and use of the slurry as a fertilizer is the current focus of the national biogas program of Ethiopia (NBPE). However, there are many plant species which could also be probed for their potential of biogas production. This paper presents the experimental results of the anaerobic digestion of *Justicia schimperiana* (JS) and cow dung each separately and with their various combinations at Addis Ababa University Environmental Science Laboratory. The biomass of JS and cow dung were characterized and then estimation of biogas production and methane content of each treatment, T1 (cow dung alone), T2 (1:1), T3 (2:1), T4 (3:1), T5 (JS alone), T6 and T7 (with digester effluent) was performed using indirect (water displacement) and absorption of CO_2 by 10% NaOH methods, respectively. Statistically significance difference (at 0.05 levels) on production of biogas among treatments was observed. It was found that T5 (JS alone) was highest in the amount of biogas production, but the highest in quality. Thus, T3 produced the optimum methane gas among treatments. Moreover, JS and its combinations with cow dung produced higher volume of biogas and contained more macro-nutrient in the slurry for plants than cow dung alone. Thus, JS appears to be a good material for biogas and bio-slurry production.

Keywords: Anaerobic digestion; Biogas; *Justicia schimperiana*; Nutrients; Slurry

Introduction

Justicia schimperiana (Hochst.ex A. Nees) T. Anders is a perennial herb or shrub with many branched stems, up to 0.8-3 m tall with slightly unpleasant smell. Leaves blade up to $15-24 \times 8-12$ cm, ovateelliptic, broadest near the base, base cuneate to attenuate entire along the margins, apex acuminate, pubescent along veins on both surfaces, or rarely pubescent all over both surfaces; lateral veins 5-9 pairs; petiole up to 10-40 mm long; pubescent with non-glandular hairs to nearly glabrous [1,2].

Justicia schimperiana (Hochst.ex A. Nees) T. Ander (JS) is distributed in high concentration in almost all regions of Ethiopia and other east African countries: Eritrea, Somali, Kenya and Tanzania [1,2]. Other studies revealed that this green plant is one of the most abundant, widespread and regenerative species in north western Ethiopia (352 seedling/ha) [3], northern Ethiopia [4], south western Ethiopia [5] and southern Ethiopia such as Bonga (10,000 seedling/ha), Boginda (4917 seedling/ha), Mankira [6], Wonango Wreda [7], and south eastern Ethiopia [8].

The Ethiopian government under NBPE in collaboration with the Netherland Development Organization (SNV Ethiopia), an international NGO, has embarked on an ambitious biogas program to construct biogas plants to address the rural energy crisis and indoor pollution caused by the burning of traditional biomass. The first phase program is being implemented in Amhara, SNNPRS, Oromia and Tigray regions [9]. However, the only targeted raw material being used for the biogas production is manure which has lower energy content than green shrubs since animals that produce it have already digested the substrate [10,11]. So, co-digestion using leaves of JS could be important for sustainable and uniform feeding of the digesters and therefore relatively higher biogas production and slurry use. It may also open the opportunity for those households who have less than four heads of cattle to be included in the program [12]. This study is therefore, intended to evaluate the biogas production potential of JS and its combination with cow dung through anaerobic digestion and to determine the macro-nutrient content of the slurry.

Materials and Methods

Procedure

The total solid (TS), volatile solid (VS), fixed solid (FS) and the C/N ratio of the feed stocks (JS and cow dung) had been determined before the anaerobic digestion process began, and the sample of the plant (JS) was then dried, purified, finely ground and the two substrates were mixed in different proportions. The process of anaerobic digestion for the generation of biogas was conducted in seven treatments (Table 1): T1 (cow dung alone), T2 (1:1), T3 (2:1), T4 (3:1), T5 (JS alone), T6 (2:1 plus inoculums) and T7 (3:1 plus inoculums) in the laboratory each with three replications. Tap water was added to each digester to improve the moisture content and enhance the process of digestion except for treatments T6 and T7 in which inoculums were added in a 1:1 ratio with water [13]. According to a recommendation made by Ituen et al. [14] and Rai [15], optimum biogas is produced when a total solid (TS) of 8% is obtained in the fermentation slurry. So the feed stocks were mixed with tap water to get 8% TS solution. Accordingly, the treatments, ratio of cow dung to JS, mass of fresh cow dung, dry JS, the amount of tap water that was added to get an 8% TS substrate and inoculums added are summarized in Table 2.

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Treatment	Pr	oportion (%) (CD : <i>J</i> S)	Fresh CD (g)	Dried JS (g)	Water added (g) (for 8% TS)	Inoculums (g)	Total mass (g)
T ₁ (control)	1:0	100:0	453.51	0	796.49	-	1250
T ₂	1:1	50:50	226.76	50	973.24	-	1250
T ₃	2:1	66.67:33.33	302.36	33.33	914.31	-	1250
T ₄	3:1	75:25	340.14	25	884.86	-	1250
T ₅	0:1	0:100	0	100	1150	-	1250
T ₆	2:1	66.67:33.33	302.36	33.33	457.15	457.15	1250
Τ,	3:1	75:25	340.14	25	442.43	442.43	1250

Table 1: Treatments and contents of each digester.

Biogas yield and its quality

The volume and methane content of the gas produced in the anaerobic reactors was measured by an indirect method. First, the volume of water displaced by the gas was measured by down ward displacement of water for each digester which corresponds to the amount of biogas produced. Subsequently, the methane content in the biogas was estimated by allowing the gas to pass through 10% NaOH solution as the CO₂ dissolves in it and form carbonate [16]. Thus, the amount of NaOH displaced is approximately equal to the amount of methane in the gas.

Determination of macro-nutrients of the slurry

Cow's rumen juice (as the source of microbial inoculum) was obtained and prepared as described by Eshete et al. [12]. The filtered rumen juice was transferred into a 2 L capacity gallon and supplemented with 20 g of glucose. This was done in order that the microbes trapped inside the juice would generate more energy from utilizing glucose as substrate to breakdown any complex organic polymer (such as cellulose) which may have been retained in the rumen juice after filtration. Following this, the rumen juice was injected with 1.8 ml of Na₂S.9H₂O (2% w/v) using a long needle attached to a 10 ml syringe and the gallon was screw capped with a specially designed cap which allowed us to evacuate biogas from the 2 L capacity gallon with time (Figure 1). Addition of hydrated sodium sulphide was done to reduce the rumen juice in order to promote the growth of strict anaerobic bacteria trapped inside the juice. Following this, the populations of aerobic and anaerobic bacteria were determined by cultural enumeration (See below) before and after subjecting the rumen juice to anaerobic digestion in the dark under ambient (laboratory) condition until biogas production was no longer observed (two month later).

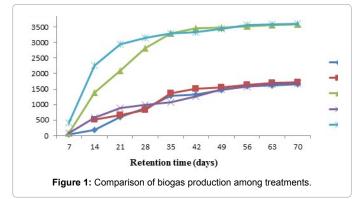
Results and Discussion

Characterization of feed stocks

The total solid and volatile solid content of both JS and cow dung were determined with three replications and their average values are summarized in Table 2. As it could be seen from the table, the total solid content of JS was 31%. Out of the total solid the volatile solid and ash (fixed solid) content of the substrate were 80.59 and 19.41%, respectively. This indicates that large fraction of JS is biodegradable and thus it can serve as an important feedstock for biogas production. For cow dung the total solid was 22.05% which is 2.05 more than 18-20% reported by Rai [15]. The total solid value of JS is slightly higher than Chat waste (29.35%) reported by Nigussie [17], and the volatile solid content value (80.59%) agrees with the value 75-80% reported by Steffen et al. [18]. The carbon to nitrogen ratio (C/N) of the feed stocks is another factor that affects the anaerobic digestion process. Methane yield and its production rates are highly influenced by the balance of carbon and nitrogen in the feeding material. The nitrogen content of

Parameters	Cow dung (%)	JS (%)	
Moisture content	77.95 ± 2.31	69 ± 1.83	
TS	22.05 ± 1.91	31 ± 1.24	
VS as percentage of TS	79.56 ± 2.33	80.59 ± 2.12	
Ash as percentage of TS	18.44 ± 1.25	19.41 ± 1.11	
OC	46.26 ± 1.64	46.85 ± 1.87	
TN	2.26 ± 0.22	2.13 ± 0.13	
C/N	20.47 ± 1.25	22.00 ± 1.15	





JS was 2.13%, which is by far higher than the expected value as most vegetable matter contains lower nitrogen (higher C/N ratio), but it still lower than the value 3.24% for Austrian winter peas and 4.10% for Hairy Vetch as reported by Harper and Henry (1924). The C/N of both JS and cow dung in this experiment was 22:1 and 20:1, respectively which agree with the value 20:1 to 30:1 reported by Dahlman and Forst [19]. This indicates that JS could serve as a substrate for biogas production even without mixing it with cow dung or other animal and human waste provided that it is available in the area. For the mixture treatments of these substrates, the possible ratio is still around 21:1. Thus, in both substrates the balance of carbon and nitrogen is good for the bacteria thus both could be used (their combination or each alone) for anaerobic digestion to produce biogas.

Characteristics of digesters

Temperature and pH are the main factors that affect bio-digestion. Consequently, the temperature of the room where digestion took place, and the pH of each digester were measured three times a day and within two days interval, respectively.

Temperature: Both the mean temperature and the temperature fluctuations adversely affect the performance of a biogas digester. The day time temperature of the room where digestion took place was measured.

It was found that the minimum and maximum day time

temperatures were 14 and 19.5°C, respectively. The mean daily temperature of the digestion room during the digestion period was 16-19°C. This means that there was a maximum fluctuation of 5.5°C. This fluctuation was minimized by thick covering of the digesters (about 10 cm radius) with sand which brought the digesters' temperature fluctuation to less than 1°C as recommended by NRCS [20]. Practically, the changes in temperature during biogas production can be minimized by constructing the digester in underground as done by the National Biogas Program of Ethiopia for household users.

In this experiment it can be deduced that it is possible to produce biogas in such temperature range (14-20°C), but it takes a longer hydraulic retention time (about 70 days in this case). Practically, the production in such temperature range can be compensated by using a digester having a larger volume rather than heating the reactor as it may need higher energy costs [13].

pH: pH is another factor that affects digestion of substrates in reactors. Thus, the pH of all the treatments was measured in two days interval regularly. The initial pH of each input mixture of treatments was 6.62 to 7.33. This is in agreement with a pH range of 6.25 to 7.5 which is conducive for methanogenic bacteria to function properly as indicated by Rai [15].

Amount and quality of biogas production

Biogas production and its methane content were measured for about ten weeks of digestion period, until gas production ceased. It was found that T5 produced the highest volume (410.67 ml) in the first week of digestion. During this period the other treatments produced below 100 ml except T2 which produced little as indicated in Figure 1.

A lag phase of about one week was observed at T2 which indicates that in the 1:1 ratio there should be sufficient period for acclimation in order to start up the digestion process. This supports the recommendation made by Rai [15], i.e., keeping the cow dung proportion above 50% is essential for immediate and better gas production in such a mixture. Though T5 (JS alone) produced the maximum in the first week of digestion, its average methane content especially in the first three days was very low (mean 39%) (Figure 2) which means that about 58% of the gas constituents in this period was CO₂. The gas therefore, cannot be used as an energy source directly during this period of digestion. The fact that no lag phase was observed at the beginning of the experiment, but low methane was produced suggests a higher hydrolytic-acidogenic than methanogenic activity in the reactors of this treatment [21]. In such cases two mechanisms are used to improve the quality of biogas. The first mechanism is absorbing (scrubbing) the CO₂ by basic substances: lime, sodium hydroxide or potassium hydroxide so that the percentage of methane could be maximized and the gas could burn easily [15]. The other one is removing the total gas produced in the first three days of the first week through the water drainage of the biogas plant installation and using the gas produced after these periods as currently practiced by the household biogas users in Ethiopia [22]. After the first week, the methane content of the treatment, T5, increased and remained in the range 55 to 69 which agrees with the literature value of 50 to 75 [23] and 55 to 80 [24]. Therefore, it could be important to use JS alone after one week of digestion in order to have optimum production of methane and low carbon dioxide.

As shown in Figure 2, the percentage composition of methane of treatments other than T5 was between 55 to 75 during the whole digestion period. This value agrees with the theoretical value of 50 to 75 as suggested by EEMBPM [23].

The positive correlation coefficients were; 0.958, 0.907, 0.941, 0.954, and 0.783 between retention time and the quality of gas of treatments T1, T2, T3, T4 and T5, respectively indicating that there is a statistically significant (p<0.01) linear relationship between these two variables. This means that, the quality of the gas increases as the digestion period increases, and remains above 60% after five weeks of digestion. The reason could be the existence of more and more methanogenic bacteria population for the conversion of acidic substances including CO₂ to CH₄ [25].

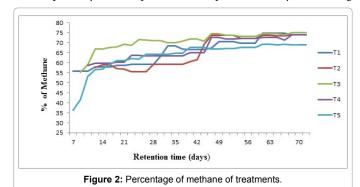
As it can be seen from Figure 3, all treatments of cow dung to JS combinations except those with digester effluent (T6 and T7) produced more volume of biogas than cow dung alone. But, its quality was better than T2 (1:1) and T5 (JS alone). T3 (2:1 ratio of cow dung to JS) is therefore, the best of all the treatments both in quality and quantity.

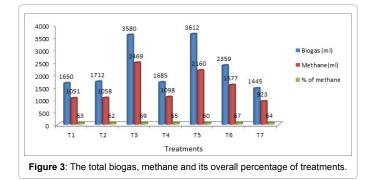
Least significant difference (LSD) method was employed and it was found that the biogas production of each treatment compared with the other treatments is significantly different at the 0.05 significance level.

Thus, the combination which produced relatively maximum biogas $(3580 \pm 8.5 \text{ ml})$ with maximum methane composition (69%) i.e., T3 (2:1 ratio of cow dung to JS) would be important in using it as a substrate for supplementing cow dung. T5 (JS alone) $(3612.33 \pm 11.37 \text{ ml})$ could be the second as its methane content is relatively lower (the quality of T3 was about ten percent more than T5 for the whole digestion period). So, using this combination by scrubbing the CO₂ or by removing the biogas produced within the three days of the first week may contribute much in providing a significant amount of biogas production.

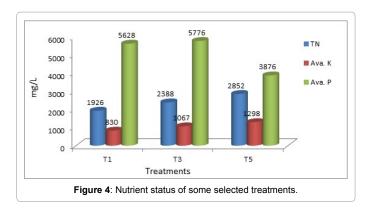
pH and nutrient values of the slurry

One advantage of anaerobic digestion is the use of the remnant (slurry) as organic fertilizer. As a result, the pH and the macronutrients for the slurry of treatments, T1 (cow dung alone), T3 (2:1) and T5 (JS alone) were determined and it was found that 6.38, 6.64 and 6.52, respectively were the pH results. The pH of the slurry of cow dung





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alone (T1), 6.38, is similar to 6.3 as reported by Fokhrul [26]. The pH of the slurry of T5 (JS alone) and T3 (2:1) were higher than T1 (cow dung alone), and the values in the three treatments were between the minimum and maximum accepted values of 6.0 and 8.5, respectively [26]. The values of the macro-nutrients are shown in Figure 4.

As it can be seen from Figure 4, the macro-nutrients of treatments of JS (T5) and with JS (T3) were higher than cow dung alone except that of the available phosphorus which was lowest for JS alone. T3 was highest in pH (near neutral), higher in TN and available K, and highest in available phosphorus. Thus, use of the 2:1 ratio of cow dung and JS could provide better fertility for land and alternative to chemical fertilizers [27-29].

Generally, for rural households especially those having less than four heads of cattle (a problem commonly observed in developing countries) JS can be used for anaerobic digestion as a supplementary feed stock with cow dung primarily to produce biogas and secondly to nutrient recovery for soil amendments.

Conclusion

Mixing cow dung with JS, especially in the 2:1 ratio will optimize gas yield, its quality and plant nutrient values. Thus, households with less than four heads of cattle could use JS as additional substrate to qualify for the national biogas programs. Furthermore, those who do not have cattle or agro-industries could use JS for biogas and slurry production.

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