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Proximate composition of banana leaves and estimation of fungal degradation products of the leaves at mesophilic temperature

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Abstract
Banana Leaves were collected and treated for biogas and bioliquid production. The physical and chemical parameters of the leaves were determined before and after fermentation. The leaves were subjected to anaerobic degradation (fermetation) process at mesophilic temperature (33^oC) for 11 consecutive days, where biogas and bioliquid were generated. The amounts of gaseous and liquid degradation products generated in the process were collected and analysed. Various estimations were carried out using different equations/ relations. The work showed that the percentages of organic matter content of the leaves before and after fermentation outweighed the percentages of all other parameters of the leaves; the carbon to nitrogen ratio (C/N) of the leaves increased after fermentation; the weight of bioliquid extracted was greater than the weight of biogas collected; the biogas collected was composed of 68.860 % CH_4, 31.138 % CO_2 and 0.120 % H_2S; the meltenes content of the bioliquid was greater than its asphaltenes content and; the amounts of maltenes and asphaltanes obtained through extraction and estimation methods were almost equal.

Keywords: Banana leaves, physical and chemical parameters, mesophilic temperature, fungal degradation, degradation products

Introduction

The proportion and the relative composition of degradation products (biogas and bioliquid) generate from biodegradable substrates are mainly determine by the nature of the substrates and the prevailing physico–chemical degradation conditions (Yaro, 2011). In nature, there are many raw materials from which biogas and bioliquid can be extracted, which include: human and animal manures, leaves, twigs, grasses, agricultural and industrial wastes, and so on, whose organic content is greater than 2% (Ariane, 1985). However, the most important component of the substrates from which biogas and bioliquid are extracted is the biomass (Maduagwu, 2002).

Biomass in any material that is directly or indirectly derived from plant live excluding the moisture, which is renewable in time period of less than 100 years (Yaro, 2011). Typical biomass resources are energy crops, agricultural and municipal wastes. Animals wastes are also biomass materials because they are derived either directly or via the food chain from plants (Probstein and Hicks, 1982). Since anaerobic degradation of biomass into biogas and bioliquid is a microbial process, which involves the combined action of four different groups of bacteria in four different stages in the absent of air therefore, the better the living environment of the microbes involved, the faster the process as reported by Khandelwal and Mahdi (1986). The factors that enhance the anaerobic biodegradation includes: concentration, necessary nutrients, carbon to nitrogen ration (C/N), airtightness, suitable pH, optimum temperature, mixing / stirring, nature of the substrate and addition of nutrients (Ariane, 1985; Khandelwal and Mahdi, 1986; Yaro, 2011).

The technology of biogas and bioliquid production emerged as alternative route of generating renewable energy resources (gassous and liquid fuels), which are cheap and environmentally – friendly from renewable sources that could be used to replace petroleum products, which are comparatively very cost and scarce. The technology also helps in protecting environment from desertification, erosion and reduced biodiversity resulting from frequent felling of trees for domestic energy (especially in the rural area). This is because through bioconversion technology, renewable energy resources (biogas and bioliquid) can be generated with the qualities similar to those of gaseous petroleum products (natural and refinery gases) and liquid petroleum products (petrol, kerosene, gas oil and diesel), which could be used for domestic and industrial energy supply (Yaro, 2015).

In order to improve the technology of biogas and bioliquid production, as well as the quality of the degradation products (biogas and bioliqiud), several but different researches were conducted by different researchers using different substrates under different conditions for instance, Gumel and Yaro (2015) studied the possibility of generating biogas from Maize cob, as well as, the analysis of the combustible component of the gas using flame ionization detector (FID); Ekwanchi *etal* (2013), studied the production, isolation and adduction of praffins from banana leaves for domestic and industrial energy supply; Airehour (1994), investigated the effect of degradation temperature on the activity of the fermentative microbes and the quantity of fermentation products; effect of Na, Mg, Fe, Al, Sn and Cd salts as nutritive additives on the bioconversion of biomass (*Echonia crassipes*) was investigated by Singh *etal* (1992); effect of seeding on biogas production using pigeon droppings was investigated by Aliyu *etal* (1996); Garba *etal* (1996), studied the effect of slurry concentration on biogas production and; so on. This work reports studies on the proximate Composition of banana leaves, where the physical and chemical parameters of the leaves were analysed. The work also report studies on the percentage of the gaseous and liquid degradation products generated from the leaves at mesophilic temperature.

Material and Methods

Sample Collection and Preparation

The banana leaves were collected from a group of banana plants planted by Kano Agricultural and Rural Development Authority (KNARDA) in Bichi town, Kano State – Nigeria. The leaves were fresh and mature at the time of collection.

The banana leaves were sun-dried for 14 days, ground using wooden pestle and mortar and sieved with 100µm mesh.

Determination of Physical Parameters of the Sample

Moisture Content

The moisture content of the sample was determined at 105^0C for 4 hours in an oven. The percentage by mass of the moisture in the sample was evaluated from the weight of the moisture obtained and the weight of the sample taken for the analysis using the expression adopted by Yaro (2011).

Inorganic Matter (Ash) Content

The ash content of the sample was determined at 600^oC for 5 hours in a muffle furnance according to the method described by Yaro (2011) with few adjustments The percentage by mass of the ash in the sample was evaluated from the weight of the ash and the weight of the sample taken.

Organic Matter (Volatile Solids)

The volatile solids content of the sample was estimated from the percentages of the moisture and ash content of the sample as follows:

$$\%\text{volatile solids} = 100\% - [\%\text{moisture} + \%\text{ash}] \quad (1)$$

Determination of Chemical Parameters of the Sample

Organic Carbon Content

The organic carbon content of the sample was determined by Walkley – Black method as described by Yaro (2011) with few adjustments. The percentage by mass of the organic carbon in the sample was evaluated using the following expression as adopted by Yaro (2014):

$$\%\,OC = \frac{[CVk_2Cr_2O_7 - C^1\,(T_{FeSO4} - T_B)]\,0.003 \times F \times 100}{W} \quad (2)$$

Where % OC = percentage by mass of organic carbon is the sample

$CVk_2Cr_2O_7$ = molar concentration x volume of $K_2Cr_2O_7$ solution

C^1 = molar concentration of hydrated $FeSO_4$ solution

T_{FeSO4} = titre value of hydrated $FeSO_4$ solution with sample

T_B = Titre value of hydrate $FeSO_4$ for blank titration

F = Correlation factor =1.33 (i.e a recovery factor for what might be lost)

W = Weight of the sample taken = 0.5g

Total Nitrogen Content

The total nitrogen content of the sample was determined by micro – Kjeldahl method as describe by Yaro (2011) with few adjustment. The reactions that occurred during the process are illustrated by the equations below.

$$N_{2(g)} + 3H_2O_{(l)} + H_2SO_4 \rightarrow (NH_4)_2SO_{4(aq)} + 3/2\,O_{2(g)} \quad (3)$$

$$(NH_4)_2SO_{4(aq)} + 2NaOH_{(aq)} \rightarrow 2NH_{3(g)} + Na_2SO_{4(aq)} + 2H_2O_{(l)} \quad (4)$$

$$NH_{3(g)} + H_3BO_{3(aq)} \rightarrow NH_4^+ + H_2BO^-_{3(aq)} \quad (5)$$

$$NH^+_{4(aq)} + H_2BO^-_{3(aq)} + HCl_{(aq)} \rightarrow H_3BO_{3(aq)} + NH_4Cl_{(aq)} \quad (6)$$

The percentage by mass of the total nitrogen in the sample was evaluated using the expression below:

$$\%\,N = \frac{(T_{HCl} - T_B)\,(M_{HCl} \times 0.014)\,V_1 \times 100}{W \times V_2} \quad (7)$$

Where T_{HCl} = titre value of 0.01 M_{HCl} with sample

T_B = titre value for blank titration

M_{HCl} = molar concentration of standard HCl = 0.01M

0.014 = Weight by volume of nitrogen atom /dm^3 = 14g /$100cm^3$

V_1 = Volume of distilled water added to the digest = $60cm^3$

V_2 = Volume of digest sample (aliqout) pipette = $10cm^3$

W = Weight of sample taken = 0.5g

Production and Estimation of Degradation Products

The degradation products (biogas and bioliquid) were generated by Anaerobic fermentation (degradation) of ground banana leaves in the presence of yeast at 33^oC for 11 consecutive days according to the degradation method described by Garba (1998) with few adjustment in the quantity and nature of slurry, degradation time and size of the reactors. Triplicate sets were made and the averages of the degradation products (biogas and bioliquid) were evaluated. The collection and compositional analysis of the gaseous degradation product (biogas) were carried out in accordance with the methods adopted by Gumel and Yaro (2015). The extraction and concentration of the liquid degradation product (bioliqiud) were carried out according to the methods described by Ekwanchi etal (2013). The physical and chemical parameters of the digested substrate in the fermented slurry were also determined after fermentation.

Estimation of the Amount of Organic Matter Converted into Degradation products

The percentage by mass of the amount of organic matter content of the substrate that has been converted into biogas and bioliquid was evaluated as follows:

$$\% OMSC = \% OMSB - \% OMSA \quad (8)$$

Where OMSC = Organic matter of the sample converted into degradation products

OMSB = organic matter of the sample before degradation

OMSA = organic matter of the sample after degradation

Estimation of the Amount of Gaseous Degradation Product Generated in the Process.

The component of the gaseous degradation product (CH_4, CO2 and H_2S) were estimated using the general gas equation of state. The equation is

$$P_V = nRT \quad (9)$$

Where P = atmospheric pressure = 76ommHg = 1atm

V= volume of the component of biogas collected

n = number of moles of the component

R = Universal gas constant = 0.0821L atm. mol^{-1} k^{-1}

T = Fermentation temperature = 33^0C = 306k

The number of moles, n of each of the component was evaluated using the relation,

$$n = \left(\frac{PV}{RT}\right) \quad (10)$$

The weight (mass) of each component was estimated using the relation;

$$M = n \times Mm \quad (11)$$

Where M= mass of the component

Mm = molar mass of the component

The weight (mass) of the gaseous degradation product (biogas) was evaluated as the sum of the weights (masses) 0f the components of the biogas collected as follow:

$$W_{biogas} = WCH_4 + WCO_2 + WH_2S \quad (12)$$

Where W= weight

Since the biogas collected was from the organic matter content of the sample (banana leaves) therefore, the percentage by mass of the gaseous degradation product was evaluated as follows:

$$\% \text{ biogas} = \frac{W_{biogas} \times 100}{W_{omsc}} \quad (13)$$

Where W_{OMSC} = weight of the organic matter in the sample converted into gaseous degradation product.

The W_{OMSC} was obtained from the percentage by mass of the organic matter in the sample that has been converted into degradation product (%OMSC), and the weight of the sample (WS) used in the preparation of the slurry, as follow:

WS ⟶ 100%

Womsc ⟶ % OMSC

Where Vc = Volume of the component

Vbiogas = Volume of biogas collected

The percentage composition by volume of each the component ($\%V_C$) of the gaseous degradation product was evaluated as follows:

$$\% Vc = \left(\frac{V_C}{V_{biogas}}\right) \times 100 \quad (15)$$

Where Vc = volume of constituent (component)

V_{biogas} = volume of biogas collected

The percentage composition by weight of each the component of the gaseous degradation product was evaluated as follows:

$$\% Wc = \frac{Wc}{W_{biogas}} \times 100 \quad (16)$$

Where Wc = weight of component

Estimation of the Amount of Liquid Degradation Product Generated in the Process

Since the liquid degradation product generated in the process was also from the organic matter content of the leaves (banana leaves) just like the biogas, therefore, the weight of the bioliquid ($W_{bioliquid}$)was evaluated as the difference between WOMSC estimated using equation (14) and W_{biogas} obtained using equation (12) as follows:

$$W_{bioliquid} = WOMSC - W_{biongas} \quad (17)$$

The percentage by weight of the bioliquid generated was evaluated using equation (18) as follows:

$$\% bioliquid = \left(\frac{W_{bioliquid}}{WOMSC}\right) \times 100 \quad (18)$$

The quantity of bioliquid estimated was compared with the quantity of bioliquid extracted.

Results and Discussion

Results

The Results of all the analyses and estimations carried out in the research are presented in Tables 1-3 below. Table 1 gives the proximate composition of the substrate (banana leaves) before and after fermentation at 33^0C. Table 2 shows the cumulative volume of biogas collected, the weights and percentages the degradation products generated from the fermentation of 3.672g of the organic matter content of the substrate. Table 3 shows the cumulative volume, weights, and percentage of each of the components of the gaseous degradation product .Table 4 gives the quantity of bioliquid estimated and their respective compositions.

Table 1: Proximate Composition of Banana Leaves Before and After Fermentation at 33^0C

Parameters	Moisture (%)	ash (%)	Organic matter (%)	C (%)	N (%)	C/N
before fermentation	4.730	12.130	83.140	5.410	0.240	22.5:1
after fermentation	4.740	12.133	73.960	2.770	0.080	33:1

Table 2: Fungal Degradation Products of 3.672g of the Organic matter of leaves

Degradation products	biogas	bioliquid
Cumulative volume (dm^3)	0.835	N.D
Weight (g)	0.827	2.845
Percentage by weight	22.522	77.478

Key: N.D. = not determined

Table 3: Cumulative volumes and percentages of the components of gaseous degradation product

Component of Biogas	CH_4	CO_2	H_2S
Cumulative volume (dm^3)	0.575	0.260	0.001
Weight (g)	0.366	0.460	0.001
Percentage by Volume (%v)	68.860	31.138	0.120
Percentage by weight (%w)	44.256	55.623	0.121

Table 4: The Amounts of Bioliquid Obtained and their Respective Compositions

Component of biogas	extracted bioliquid	estimated bioliquid
quantity of the bioliquid (g)	3.022	2.845
maltenes content of the bioliquid (g)	1.609	1.515
asphaltenes content of the bioliquid (g)	1.413	1.330
% by weight of the maltenes (%)	53.243	53.251
% by weight of the asphaltenes (%)	46.757	46.749

Discussion

The results of proximate analysis of banana leaves before and after fermentation are shown in Table 1**.** From the result, it could be seen that the percentage of organic matter in the substrate (banana leaves) outweighed the percentage of all the other components. This signified high degradation potential of the leaves (Ariane, 1985). The result also showed that the moisture and ash contents of the substrate before and after fermentation were almost equal while the organic matter of the substrate has significantly decreased after fermentation. This may be connected to the fact that during microbial degradation (fermentation*)* of biodegradable substance, only the organic content undergo degradation *(*Airehrour, 1994*).* In addition to the decrease in the percentage of the organic matter content after fermentation, there was also a drastic decrease in the percentage of C and N after fermentation. The decrease in the percentage of carbon content, C(%) may be attributed to the utilization of the of the carbon content of the substrate during the formation of methane (CH_4) and also as source of energy for the microbes (Obinwanne,1999**)**. The decrease in the nitrogen content of the substrate, N(%) on the other hand may be connected to the utilization of the nitrogen by the microbes for their growth during fermentation for microbial growth during fermentation (Garba, 1998).The increase in the carbon to nitrogen ratio (C/N) increase from 22.5:1 to 33:1 after fermentation. may be associated with the exhaustion of the nitrogen content of the substrate by the microbes during fermentation (Chen and Inber, 1991). The low C/N of the substrate before fermentation (22.5:1) indicated high biogas and bioliquid production potential of the substrate because for a substrate to be good for biogas generation, its C/N should be maintained between 20:1 to 25:1 (Ariane, 1985).

Table 2: shows the fungal degradation products (biogas and bioliquid) generated from 3.672g of the organic matter content of the substrate. From the result, it could be seen that the quantity of the biogas generated was relatively lower than that of the bioliquid. The high bioliquid observed may be attributed to the fact that bioliquid is composed of heavier paraffinic, nephthanic and aromatic hydrocarbons and, some quantities of N,S and O while biogas is composed of only lighter gaseous paraffinic hydrocarbons (i.e. $C_1 – C_4$) per molecule (Yaro, 2011)**.** The high bioliquid observed may also be attributed to the dissolution of some biogas in the slurry during fermentation due to inadequate stirring (Khandelwal and Mahdi, 1986)

Table 3 shows the composition of the gaseous degradation product. From the result, it could be seen that the biogas collected was composed of CH_4, CO_2 and H_2S**.** The result also showed that the volumes of the components estimated were in the order: $CH_4 > CO_2 > H_2S$ while their weights were in the order**:** $CO_2 > CH_4 > H_2S$. The high percentage of CH_4 and relatively low percentage of CO_2 and H_2S in the biogas indicated the good fuel value and low environmental hazards of the biogas (Ekwenchi and Yaro, 2010).

The result of the amount of bioliquid and their respective composition obtained through extraction and estimation is shown in Table 4. The result showed that the amount of bioliquid collected through extraction (i.e. experimentally based data) was almost equal to the amount of bioliquid obtained through estimation (i.e**.** theoretically calculated data). This implied to accuracy of the experimentally based data collected and, agreement between the experimentally based data and the various relevant equations/relations applied for the analysis of the data. The result (Table 4) also showed that the amount of maltenes in the bioliquids was relatively higher than the amount of asphaltenes. This may be connected to the conversion of some asphaltenes (usually aromatic hydrocarbons) into maltenes (paraffins hydrocarbons) through dearomatization resulted from microbial activities during fermentation because of the ability of microbes to fermentation. Synthesize alkanes by dearomatization of aromatic hydrocarbons in the substrate during anaerobic fermentation. (Bird *etal*, 1974).

Conclusion

From the C/N of the substrate and the respective compositions of the degradation products, it can be concluded that through bioconversion technology under favorable anaerobic operational conditions of pH temperature and concentration, gaseous and liquids products that could used as alternatives renewable energy sources can be generated from banana leaves using low skill technology.

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