

PRODUCTION OF BIOGAS BY MICROBIAL DIGESTION OF BANANA LEAVES AND ADDUCTION OF PARAFFINS FROM SATURATES USING UREA THIOUREA SOLUTIONS

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ABSTRACT

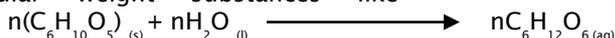
A slurry prepared from banana leaves was subjected to microbial digestion in the absence of air at 33°C for 2 weeks. The biogas and bioliquid generated were separately collected. The biogas was quantitatively and qualitatively evaluated and the bioliquid was separated into maltenes (soluble component) and asphaltenes (insoluble component) by precipitation. The saturates content of the maltenes was obtained by column chromatographic separation using n-hexane. The paraffins content of the saturates (n-alkanes, iso-alkanes and cycloalkanes) were adducted using urea and thiourea solutions. For the adduction of n-alkanes, a saturated solution of urea in acetone was used. The iso-alkanes and cycloalkanes were adducted using thiourea in methanol in the ratio of 1:1(v/v). The work showed that: banana leaves can be used to generate biogas of good fuel value through microbial digestion; urea and thiourea can be used as molecular sieves for separating paraffins of different molecular structures; the biogas generated was composed of 68.78% CH₄, 31.10% CO₂ and 0.12% H₂S and; the amounts of n-alkanes, iso-alkanes and cycloalkanes adducted from 38.00mg saturates of banana leaves were 22.64mg, 10.36mg and 2.57mg, respectively.

KEYWORDS: Banana leaves; biogas; bioliquid; microbial digestion; saturates, adduction; paraffins; urea; thiourea.

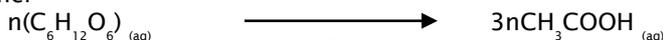
INTRODUCTION

Microbial digestion of biodegradable substances into biogas and bioliquid is a process, which involves the combined action of four groups of bacterial in four different stages as reported by Ariane (1985) and Khandelwal and Mahdi (1986). The stages are illustrated below:

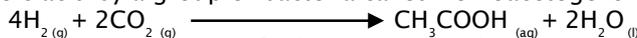
The First Stage is the degradation of higher molecular weight substances like



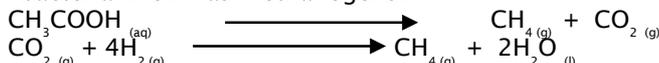
The Second Stage is the conversion of the product of first stage into organic acid and by-products like CO₂, H₂O, H₂, NH₃ etc by a group of bacteria known as acetogens, which are collectively called "acid former"



The third stage is the conversion of hydrogen and simple carbon compounds produced in the second stage into ethanoic acid by a group of bacteria called homoacetogens.



The fourth stage is the conversion of ethanoic acid and some other compounds like CO₂ and H₂ into CH₄ by a group of bacteria known as methanogens.



The advantages of biogas technology include solving fuel problem by generating renewable energy resources (biogas) from organic waste; promoting agricultural production by greatly augmenting the quantity and quality of organic fertilizer (biofertilizer) obtained as digested slurry, because it has been reported by khandelwal and Mahdi (1986) that digested slurry contained more nitrogen (N), phosphorus oxide (P₂O₄) and potash (K₂O) than undigested farm yard manure and; improving health and sanitation because huge amount of biodegradable wastes are converted into useful products and the digested slurry formed is free

cellulose, starch, fast, proteins etc present in organic materials into low molecular weight compounds like fructose, glucose etc. that are able to pass through bacterial membrane by a group of hydrolytic bacteria. Thus polymers are transformed into monomers by enzymatic hydrolysis.

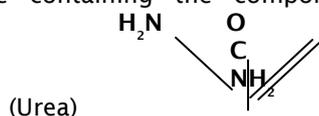
from foul smell and, the transfer of fungal and pathogens is drastically reduced as reported by Ahmed (2000).

Biogas (paraffins content) is a flammable, colourless gas obtained by anaerobic digestion of biodegradable substrates. It contains 50-70% CH₄, 30-40% CO₂ and trace of H₂, N₂ and H₂S as reported by Maishanu and Hussain (1990). On the other hand, Bioliquid is the extract of digested slurry of biodegradable substrate, which is relatively heavier than biogas and generally composed of maltenens and asphaltenes as reported by Ekwenchi and Yaro (2012).

The soluble component of bioliquid known as maltenes or desphaltned oils is obtained from lignocellulosic component of a biomass as a result of microbial activities. Maltenes are composed of large amount of saturates, few aromatics and very small quantities of resins and organic polar as reported by Ekwenchi and Yaro (2012).

Saturates are a fraction of maltenes, which are chiefly composed of paraffins of different molecular structures. They are stable, nonpolar and generally unreactive towards common reagents under normal conditions as reported by Bamkole and Ogunkoya (1977). Paraffins are saturated hydrocarbons (n-alkanes, iso- alkanes and cyclo-alkanes), which are formed by the combination of carbon and hydrogen atoms in the simplest possible way, and illustrate this chain-forming property in a striking manner. The carbon atoms can be linked together in a straight chain known as n-alkanes or in a branched known as iso-alkanes. The end carbon atoms of an open aliphatic chain can sometimes join together to form a closed system or ring such compounds are known as cyclic hydrocarbons or cyclo-alkanes as reported by Ababio (1985).

Adduction as applied to the separation of components of a mixture could be described as the process by which a component (adduct) of a mixture is separated using appropriate adducting substances (molecular sieves). Adduction of component involves the dissolution of the substance containing the components to be



It should be noted that the difference in the crystalline structures of urea and thiourea as shown above, enables thiourea to adduct paraffins that can not be adducted by urea. The research is aimed at generating biogas and bioliquid from banana leaves by microbial digestion, evaluating the quantity and quality of the biogas generated and assessing the potentials of urea and thiourea as molecular sieves for separating paraffins of different molecular structures. The amounts of n-alkanes, iso-alkanes and cycloalkanes that can be adducted from 38.00mg saturates of banana leaves using urea and thiourea solutions are also going to be determined.

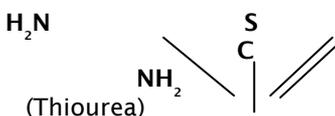
MATERIALS AND METHODS

Collection and Preparation of Sample

The sample (fresh banana leaves) was collected from a group of banana plants planted by Kano Agricultural and Rural Development Authority (KNARDA) in Bichi town, Kano State - Nigeria. The sample was oven dried at 105°C for three hours, ground using pestle and mortar and

adducted (adducts) using suitable solvent; adducting the adduct using appropriate molecular sieve; extracting the adduct using appropriate extracting solvent and; drying the extract containing the adduct using appropriate technique as reported by Mailabari (1983). During the process of adduction, components that are appreciably smaller than the pores of the molecular sieve crystals diffuse into the interior of the molecular sieve crystals and strongly adsorbs within the interstitial spaces of the crystalline structure of the molecular sieve while component that are relatively larger than the pores of the molecular sieve crystal passes through between the inter grains-space of the crystalline structure of the molecular sieve as reported by Bayawa (2000).

Molecular sieves are the natural or synthetic crystalline materials characterized by high degree of porosity and having all pores of the same size (Bayawa, 2000). Examples of the substances used as molecular sieves includes urea and thiourea amongst many others. Urea is a white, crystalline diamide derivatives of trioxocarbonate IV acid (H_2CO_3), which is obtained by evaporating an aqueous solution of ammonium cyanate (NH_4CNO) as reported by Murray (1977). In contrast, thiourea is an organic compound of carbon, nitrogen, sulphur and hydrogen with the formula CSN_2H_4 or $(NH_2)_2CS$, which is similar to urea, except that the oxygen atom in the urea is replaced by sulphur atom. The structures of urea and thiourea are as follows:



sieved to a mesh size of less than $250 \times 10^{-6}m$ using mesh of different porosity.

Digester or Reactor

A 500cm³ Buchner filter flask (BFF) was used as a reactor. A polyvinyl chloride (PVC) tube of 50cm length and 0.8cm internal diameter was attached to the outlet of the BFF using araldite, with one end of the PVC free (i.e unattached). The unattached end was used to convey the gaseous degradation product (biogas) formed in the BFF to gas jar. All the reactors used in this work were constructed as such.

Production and Collection of Biogas

The Slurry used in the reactor was prepared by dissolving 40.0g of the sample (banana leaves) in 250cm³ distilled water containing 1.8g yeast. The reactor was made air-tight and placed in water-bath maintained at 33°C and labeled A. The digestion process was allowed to continue for 2 weeks, with the reactor being shaken periodically to ensure even distribution of microbes and substrate and prevent scum formation in the reactor.

The biogas produced was collected by the downward displacement of brine (28 % NaCl) in an inverted 1dm³ cylinder. The experimental set up was made in triplicate and the average volume of the biogas collected was evaluated.

In order to evaluate the composition of the biogas, 2 parallel set ups were made each in

triplicate and labeled B and C according to the method adopted by Gumel and Yaro (2012). The average volumes of CO₂ and H₂S absorbed in sets B and C, were respectively evaluated. The average volume of CH₄ in set A was also evaluated using the average volumes of CO₂ and H₂S obtained in sets B and C, respectively.

Compositional Evaluation of Biogas

The composition of the biogas collected was evaluated as follows:

$$\begin{aligned} \text{CO}_2 &= \text{A} - \text{B} \\ \text{H}_2\text{S} &= \text{A} - \text{C} \\ \text{CH}_4 &= \text{A} - (\text{CO}_2 + \text{H}_2\text{S}) \end{aligned}$$

The percentages composition of the biogas were evaluated as follows:

$$\% \text{CO}_2 = \frac{\text{A} - \text{B}}{\text{A}} \times 100$$

$$\% \text{H}_2\text{S} = \frac{\text{A} - \text{C}}{\text{A}} \times 100$$

$$\% \text{CH}_4 = 100 \% - (\% \text{CO}_2 + \% \text{H}_2\text{S})$$

Where A = Volume of biogas (CH₄ + CO₂ + H₂S) in set A

B = Volume of CH₄ + H₂S in set B

C = Volume of CH₄ + CO₂ in set C

Extraction of Bioliquid from Digested Slurry

The bioliquid was extracted from the digested slurry by soxhlet extraction using absolute methanol (as extracting solvent) at 60°C for 72 hours and subsequently recovered from the extracting solvent by evaporation using rotary evaporator and finally concentrated to a constant weight by heating at 37°C using heating-mantle in a fume cupboard.

Isolation of Maltenes by Precipitation

For the isolation of maltenes from the bioliquid, 1.0g of the concentrated bioliquid was dissolved in a mixture of methanol and hexane in the ratio 1:40 (v/v) and kept in refrigerator for 24hrs. the maltenes being the soluble component of the bioliquid was carefully removed from the asphaltenes (insoluble component) by decantation according to the decantation method adopted by Ekwenchi and Yaro (2012).

Separation of Saturates from Maltenes

The saturates were collected from the maltenes by column chromatographic separation using n-hexane as eluent. The column used was a glass column of 40cm length and 0.85cm internal diameter with a bed volume of 22.72cm³ as evaluated. The column was first packed with silica gel of particle size 0.63 - 0.74nm (activated at 100°C over night in an oven) followed by alumina of particle size 3.74cm in the ratio 1:2 (silica gel to alumina). The sample (Maltenes) to packing materials ratio was 1:70 for silica gel and 1:140 for alumina, where 5.13g of silica gel and 10.13g alumina were used as packing materials. The packed column was first washed with 30cm³ n-hexane before loading the sample after which 72.5mg of the sample was loaded in the column as concentrated solution of n-hexane and eluted with 30cm³ n-hexane. The fraction collected was dried in fume cupboard as saturates.

Urea Adduction of n-alkanes from Saturates.

In order to adduct n-alkanes content of the saturates, 38.0mg of the saturates collected above was dissolved in n-hexane and a saturated solution of urea in acetone was subsequently added and, white crystals were formed, which contained the suspected n-alkanes. When no more crystals were formed with addition of saturated urea solution, the addition was stopped and the crystals formed were filtered off, washed with n-hexane and dissolved in distilled water. The dissolved crystals were extracted several times with n-hexane using separating funnel. The n-hexane-extract obtained, which contained the n-alkanes was air-dried in a beaker to a constant weight and weighed as n-alkanes. The percentage of the n-alkanes adducted from the saturates was evaluated as follows;

$$\% \text{ n-alkanes} = \frac{\text{weight of n-alkanes adducted}}{\text{weight of the saturates used}} \times 100$$

Thiourea Adduction of iso-alkanes and Cycloalkanes

The urea non adducts (i.e, iso-alkanes and cycloalkanes) left in the aqueous solution were adducted using thiourea solution in methanol in the ratio 1:1 (v/v).

The aqueous solution containing the iso alkanes and cycloalkanes was mixed with the thiourea in methanol and kept at room temperature for 4 days, where crystals formed. The crystals formed were filtered off and washed with benzene and subsequently dissolved in distilled water. The dissolved crystals were extracted several times with benzene. The benzene-extract obtained was air-dried in a beaker to a constant weight and weighed as cycloalkanes. The filtrate on the other hand, was also air-dried in a clean and dry beaker and weighed as iso-alkanes.

The percentages of both the cycloalkanes and iso-alkanes adducted from the saturates were each evaluated as follows:-

$$\% x = \frac{\text{Weight of } x \text{ adducted}}{\text{Weight of the saturated used}} \times 100$$

Where x = cycloalkanes or iso-alkanes as the case may be.

3.0 RESULTS

The results of all the analyses carried out in this work are presented in Tables 1, 2 and 3 below: Table 1 shows the daily average volumes of gaseous degradation products generated in sets A, B and C, each from 40g banana leaves over a period of 14 days. Table 2 gives the total volumes and percentages of CH₄, CO₂ and H₂S in the biogas. Table 3 shows the amounts and percentages of n-alkanes, iso-alkanes and cycloalkanes adducted from 38mg saturates of banana leaves.

The results of all the analyses carried out this work are shown in the tables below:

Table 1: Compositional Analysis of Biogas Generated at 33°C (cm³).

Time (Day)	Set A (Direct collection of biogas)	Set B (Absorption of CO ₂)	Set C (Absorption of H ₂ S)
01	308.00	193.00	306.00
02	128.00	98.00	130.00
03	102.00	62.00	120.00
04	122.00	51.00	100.00
05	85.00	85.00	84.00
06	48.00	49.00	47.00
07	30.00	0.00	18.00
08	12.00	24.50	17.00
09	0.00	0.00	0.00
10	0.00	13.00	0.00
11	0.00	0.00	12.00
12	0.00	0.00	0.00
13	0.00	0.00	0.00
14	0.00	0.00	0.00
Total	835.00	575.50	834.00

* KEY: Set A = CH₄ + CO₂ + H₂S; Set B = CH₄ + H₂S and; Set C = CH₄ + CO₂

Table 2: Total Volume and Percentages of CH₄, CO₂ and H₂S in the Biogas Produced.

Composition	Volume (cm ³)	Percentage (%)
CH ₄	574.50	68.78
CO ₂	259.50	31.10
H ₂ S	1.0	0.12

Table 3: Amounts and Percentages of N-alkanes, Iso-alkanes and Cycloalkanes Adducted from 38.00mg Saturates of Banana leaves using urea and thiourea

Component Adducted	Amount (mg)	Percentage (%)	Molecular Sieve
n-alkanes	22.64	59.58	urea
iso-alkanes	10.36	27.26	thiourea
cyclo-alkanes	2.57	6.76	thiourea
Total	35.57	93.60%	urea and thiourea

DISCUSSION

Table 1 shows the daily and the total average volumes of biogas generated from 40.0g banana leaves at 33°C in the absence of air for both the direct technique (Set A) and absorption techniques (Set B and C). From the result, it could be seen that appreciable quantities of gaseous degradation products were generated and collected from banana leaves. This proved the potential productivity of banana leaves (an Agricultural waste) for biogas generation, which could be used as an alternative energy resource as reported by Garba and Ojunkwu (1998).

Evaluation of the total volumes of the gaseous degradation products (CH₄, CO₂, and

H₂S) in the biogas collected revealed that the biogas generated in this work is composed of 68.78% CH₄, 31.10% CO₂ and 0.12% H₂S as presented in Table 2. From the result shown in the table (Table 2), it could be said that banana leaves can be used to generate biogas of good fuel value (i.e, high calorific value) and low environmental hazards due to high CH₄ and relatively low CO₂ and H₂S contents. This is based on the fact that, for gaseous fuels (Biogas inclusive) to be of good fuel value, and low environmental hazards, percentage of the combustible component (CH₄) must exceed 60% with CO₂ content below 40% as reported by Ekwenchi and Yaro (2010).

From the result shown in Table 3, it could be seen that the amount of n-alkanes, iso- alkanes and cycloalkanes adducted from 38.00mg saturates of banana leaves using urea and thiourea solutions were 22.64mg, 10.36mg and 2.50mg, respectively. The result (Tables 3) also shows that urea and thiourea are good molecular sieve for separating paraffins of different molecular structures, because 93.60% of the paraffins in the saturates were adducted. This is in accordance with the findings of Mailabari (1983) and Bayawa (2000).

The large quantity of n-alkanes and the relatively low quantities of iso-alkanes and cyclo-alkanes observed may be associated with the conversion of some iso-alkanes and cyclo-alkanes in the substrate by microorganisms into n-alkanes during degradation as reported by Khandelwal and Mahdi (1986). The relatively large quantity of n-alkanes observed may also be associated with the dearomatization of some aromatic compounds in the substrate. This is based on the fact that some micro-organisms have the ability to synthesize alkanes by dearomatization of aromatic compounds into straight chain alkanes as reported by Ekwenchi *et al* (1990). On the other hand, the low - iso-alkanes adducted as compared to n-alkanes maybe attributed to the degradation of complex compounds in the substrate by micro-organisms into simple organic compounds, which eventually be converted into -alkanes (usually CH₄) as reported by Ariane (1985) and Airehrour (1994). The very small amount of cycloalkanes observed

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may be connected to the fact that during the degradation of organic substrates (of any kind), H₂ and other by-products are formed as reported by Ariane (1985); Khandelwal and Mahdi (1986) and, the H₂ formed reacts with the cycloalkanes content of the substrate and eventually reduces the cycloalkanes to n-alkanes through hydrogenation as reported by Murray (1977).

CONCLUSION

From the respective volumes of CH₄, CO₂ and H₂S (574.50cm³, 259.50cm³ and 1.0cm³), the percentage composition of the biogas (i.e. 68.78% CH₄, 31.10% CO₂ and 0.12% H₂S), the amount of saturates (38.00mg) collected from 40g banana leaves and the respective quantities of n-alkanes, iso-alkanes and cycloalkanes (i.e 22.64mg, 10.36 mg, and 2.57mg) adducted from the saturates using urea and thiourea solutions, it can be concluded that, banana leaves have the potential productivity for generating biogas of good fuel value and, urea and thiourea are good molecular sieves for separating paraffins of different molecular structures.

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