

The Potential of Mango (*Mangifera indica*) seeds as Substrate for Bioethanol Production

Bashar Badamasi Lailaba^{1*}, Abdulsalam Abdulrahman Ayodeji², Adejumo Mutiu³

Department of Science Laboratory Technology, Kebbi State Polytechnic, Dakingari^{1,2,3}

* Corresponding Author

Abstract: The problem of global warming is world widely known. One of the causes of this situation is the increase in the emission of greenhouse gasses from the utilization of fossil fuel. One of the possible solutions to this environmental degradation is the production of biofuel from lignocellulosic biomass. Bioethanol is a widely liquid biofuel which is sustainable and environmentally friendliness. Mango seeds, a renewable and abundant resources can serve as an alternative lignocellulosic biomass for the production of bioethanol which solved the problem of energy required and environmental degradation to more sustainable source of energy. This study was carried out to produce bioethanol from mango (*Mangifera indica*) seeds. The seeds were sundried, grounded into fine powdered and pretreated using combination of different concentrations of organic and inorganic dilute acids hydrolysis. Physico-chemical analysis of the raw sample and pretreated sample was analyzed. Reducing sugar was determined after hydrolysis using UV Spectrophotometer at 540nm with p H of 4.5. The results of the physic-chemical analysis revealed high carbohydrate, moisture and crude fiber content with significant ash content. Also, 40.97%, 24.30% of cellulose and hemicelluloses content respectively. The results of the pretreated sample shows decrease in lignin and hemicelluloses content and increase in cellulose content. The high reducing sugar was noticed with 20.97 in 15%/10% of formic acid/hydrochloric acid pretreatment. The highest yield of bioethanol was observed in 15%/10% with 39% bioethanol yield. The results indicate the suitability of mango seeds as a good potential resource for quality bioethanol.

Keywords: Lignocellulosic biomass, Renewable energy, Mango seeds, Fossil fuel., Bioethanol

I. INTRODUCTION

The concern on energy security, effects of dangerous gas emission, has necessitated to look for an alternative source of energy. Moreso, in millions of years, fossil fuel will be exhausted. Hence, given rise to high cost of production. This petroleum crisis has led to interest in alternative power from biomass. Energy from renewable substrate has advantages over fossil fuel which is environmentally friendly, productive, and affordable (Devi *et al.*, 2020). Liquid fuel from lignocellulosic biomass demands the availability of raw materials. The introduction of biofuel as a substitute to fossil fuel whose emission has resulted to destruction of ozone layer (Abo *et al.*, 2019). The alternative energy has become highly interested in the last few decades with the increasing awareness of exhausting primary energy resources, and intensifying research in solar energy and biofuels.

However, the use of first-generation biomass has been greatly reduced for a more sustainable resource. Non-edible feedstocks have been identified for its sustainability (Devi *et al.*, 2022) and reduce dependency on gasoline. However, bioethanol production has solved the problems of energy demand and environmental problem (Liu *et al.*, 2020). Lignocellulosic biomass has received a wide attention due to its abundance, viability, environmentally friendliness and sustainability (Saha, 2020). This produces the second-generation bioethanol which is the most acceptable liquid biofuel. This feedstock does not compete with food or feeds and thereby reducing scarcity of food and fuel. Though, it is very difficult to utilize compare to agricultural crops. These polysaccharides, varies with percentages of hemicelluloses, cellulose, and lignin (Sharma *et al.*, 2019) along with minor pectin, protein, ash, and extractives. The proportion differs based on the biomass species. However, hardwood has more cellulose content, while leaves and grasses have more hemicelluloses. The recalcitrant of lignocelluloses require the need for a pretreatment process to breakdown the matrix to enhance the solubilization of the hemicelluloses for hydrolysis for the production of sugars (Jaffer *et al.*, 2021). Several pretreatment methods such as physical, chemical, physicochemical, and biological have been developed to degrade the crystalline structure to enhance hydrolysis. During the pretreatment, many toxic compounds are generated which results in reduction of production efficiency. The occurrence of these inhibitors is as a result of high temperature, use of chemicals, and long pretreatment duration. These inhibitors can be classified into organic acid aldehydes, sugar derived aldehydes, and aromatic compounds. The organic acid aldehydes such as acetic acids are derived from all parts of the biomass. The sugar-derived aldehydes such as furfural, hydroxymethylfurfural occur during degradation of hexose and pentose. The aromatic compounds such as phenolic compounds are derived

when lignin degrades. The occurrence of these toxic compounds reduces the activity of the enzymatic hydrolysis and resulting in low sugar production. Mango (*Mangifera indica*) of the family anacardiaceous, is a tropical, subtropical and forest fruits (Bally *et al.*, 2009). It is the fifth largest fruit crops produces worldwide and produced annually with about 2.7 million tons. It is originated from the foothills of the Himalayas of India and Burma and has been cultivated in that origin for about 4,000 years. In Kenya, it has been the third most important fruit terms of areas and production for the last ten years after banana and apple (HCDA, 2010). Mango seeds, a lignocelluloses biomass contains between 30-50% cellulose content, 15-25% hemicelluloses content, and 15-30% lignin content.

II. MATERIALS AND METHODOLOGY

a. Sample Collection

Mango seeds were collected from Dalinger area in Kebbi State.

b. Sample Treatment

The mango seeds were washed thoroughly to remove impurities. The samples were then sundried for four (4) weeks. The mango husk was first removed to get the seeds. The seeds were then grounded into fine powdered using mechanical grinder. This treatment was done to increase the surface area of the mango seed powder and also to enhance the contact between hemicelluloses and cellulose with dilute acid to reduce the cellulose crystallinity. The powdered samples were stored at room temperature in an air tight container prior to usage (Tambuwal *et al.*, 2018).

c. Determination of the Physico-chemical Analysis of the Sample

This was achieved according to the standard method recommended by the Association of Official Analytical Chemist (AOAC, 2006).

Moisture content determination of the sample

This is based on loss of weight of sample in an oven.

Moisture content (%) = $\frac{w_1 - w_2}{w_1 - w_{00}} \times 100$.

$$w_1 - w_{00}$$

Where w_0 is the mass of evaporating dish, w_1 is the mass of sample + evaporating dish and w_2 is the mass of dry sample + evaporating dish.

Ash content determination of the sample

This is the residue that remains on ignition of the sample in muffle furnace at 550°C for 4 hours, cooled in desiccators and weighed until the weight is constant.

w_0 is the mass of empty evaporating dish, w_1 is the mass of evaporating dish + dried sample, and w_2 is the mass of evaporating dish + ash content

Percentage ash content (%) = $\frac{w_1 - w_2}{w_1 - w_0} \times 100$.

$$w_1 - w_0$$

Crude Protein Content determination of the sample

Protein content can be determined by converting organic nitrogen into protein.

Two gram (2.0 g) each of the sample will be weighed and transferred into a clean dry 100cm³ micro Kjeldahl flask and some quantity of selenium tablet (Kjeldahl catalyst) will be added, followed by the 10ml of freshly prepared concentrated H₂SO₄. The content will then be heated continuously in a fume cupboard until it becomes clear. The mixture will be allowed to cool and will be slowly made up to 50cm³ by volume with distilled water and shaken vigorously. Ten-centimetre cube of the aliquot will be taken into 500cm³ Kjeldahl flask and 20ml of 40% sodium hydroxide will be added followed by 20cm³ of distilled water. The mixture will be placed on the distillation apparatus, in a flask containing 20cm³ of 2% boric acid which turns from pink to green on distillation. The colour will change due to the ammonia produced during the process. A freshly prepared 0.01M tetraoxosulphate (vi) acid will be titrated against the distillate. A colour change from green to pink indicates the end point. The initial and final burette reading will be recorded.

Total organic nitrogen (%) = $\frac{\text{titre value} \times \text{normality of acid} \times 0.014 \times 50}{\text{Weight of sample} \times \text{value of aliquot}} \times 100$.

Weight of sample \times value of aliquot

Crude protein (%) = % total organic nitrogen \times 6.25

Determination of Crude lipid content

This is based on continuous extraction with extracting solvent.

A cleaned and dried bottom flask will be weighed as W_1 . 2.0g each of the dried sample will be packed into the Soxhlet extractor. A sufficient quantity of n-hexane will be added into a Soxhlet extractor. The extraction will be run for 6hrs then the apparatus set up will be disconnected. The round bottom flask will be allowed to cooled and reweighed as W_2 . The sample will be repeated in triplicate.

W_1 is the weight of empty flask, W_2 is the Weight of the flask + oil

Crude lipid content (%) = $\frac{W_2 - W_1}{2} \times 100$

Determination of crude fibre content

This is determine on the principle of loss of fibre on ignition after digestion with acid and base. Crude fibre will be determined by acid-base digestion. The sample 5.0g processed will be placed in a 100ml conical flask and weighed (W_0). The quantity 200ml of boiled 1.25% of H_2SO_4 solution will be added to the sample and boiled for 30min under reflux. The mixture will be filtered, and then rinsed with water. The fibre will be collected into a conical flask and 200ml of 1.25% NaOH will be added and boil for 30minutes. The sample will again be filtered through muslin cloth. The sample will then be washed with water and then with 10% HCl and methylated spirit. The sample will then again be rinsed with petroleum ether (BP 40-60°C). The sample will be air dried and weighed (W_1). It will then be ash at 55°C for 90minutes in a muffle furnace, allowed to cooled and weighed as (W_3).

W_0 is the mass of dish, W_1 is the mass of sample, W_2 is the mass of dish + ash

Crude fibre content (%) = $\frac{W_1 - W_2}{W_0} \times 100$

Carbohydrate Content Determination

The carbohydrate content will be calculated to percentage by adding ash content, protein content, lipid content and crude fibre (Udo and Ogunwale, 1986)

% carbohydrate content = $\% 100 - (\% \text{ ash} + \% \text{ crude protein} + \text{cude lipid} + \text{crude fibre})$

Extractives:

2.5g of dried raw sample will be loaded into the cellulose thimble of the soxhlet extractor. 150ml of acetone will be added and the extractor adjusted to 70°C for 4hr run on the heating mantle. After extraction, the sample will be air dried at room temperature for few minutes. The residue (extractive) will be placed in an oven 105°C for 1hr and air dried until a constant weight was achieved. The percentage weight (w/w) of the extractives was evaluated as the difference in weight between the raw biomass and the extractive-free biomass (Lin *et al.*, 2010).

Hemicellulose

The 1.0g of the extracted dried biomass was added into a 250ml flask. 150ml of 500mol/m³ was added. The mixture was boiled for 3hrs with distilled water.

It was allowed to cool and filtered through vacuum filtration. The residue was washed under running tap water until neutral pH. The residue was dried to a constant weight at 105°C in an oven. The percentage hemicelluloses content is the difference between the weight before and after treatment (Lin *et al.*, 2010).

Lignin content

The 0.3g of dried extracted raw biomass was weighed in a test tube. 3ml of 72% H_2SO_4 was added. The test tube was kept for 2hr at room temperature with careful shaking at 30min interval for of complete hydrolysis. After initial hydrolysis, 84ml of distilled water was added. The mixture was kept in an autoclave at 121°C for 1hr for the second hydrolysis. The slurry was cooled at room temperature. The hydrolyzates was

filtered through vacuum using a filtering crucible. The acid insoluble lignin was determined by drying the residue at 105°C and accounting for ash by incinerating the hydrolyzates at 575°C in a muffle furnace. The acid soluble lignin fraction was determined by measuring the acid hydrolysate absorbance at 320nm. The lignin content calculated as the summation of acid insoluble lignin and acid soluble lignin (Sluter, 2001).

Cellulose content

This will be calculated by difference, assuming the extractives, hemicelluloses, lignin, ash (Lin et al., 2010).
Percentage cellulose = 100 - (sum of hemicelluloses, lignin and ash) content

d. Pretreatment of the Sample

The sample will be pretreated with mixture of 0.8% formic acid and hydrochloric acid solution in the ratio 5%/5% v/v, 10%/5% v/v, 15%/5% v/v, and 15%/10% v/v. 6g of the sample will be pretreated with 50ml of each ratio of the mixture in 500ml conical flask at a temperature of 55 - 70 °C for 5hrs. The mixture will be autoclave at 121 °C for 30min. The mixture will be filtered through a whatman filter paper to separate the solid residue. The residue will be washed with distilled water until neutral pH will be obtained. The residue will be oven dried at 105 °C and stored in tightly sealed bottle for further use (Tambuwal, 2016). The chemical composition of the pretreated residue will be determined.

e. Acid hydrolysis

This is carried out to separate the simple sugars (Cai *et al.*, 2017).
2g of the pretreated sample will be weighed in a 500ml conical flask containing 40ml of formic acid and hydrochloric acid solution in the ratio 5%/5% v/v, 10%/5% v/v, 15%/5% v/v, and 15%/10% v/v respectively. The flask will be covered with aluminium foil and heated for 2hr at 65°C on a water bath and autoclave for 30min at 121°C. The flask will be allowed to cool and filter through whatman filter paper. The solid residue will be washed with distilled water and neutralized with 1M sodium hydroxide solution. This is further filtered and dried at 600°C for 5hr. The sample will be stored in a desiccators until further use.

f. Determination of Reducing Sugars

The concentration of the reducing sugars in the acid hydrolysis will be analysed by the DNS Assay (Itelima *et al.*, 2013). 1ml (50μ) of the hydrolysed supernatant will be mixed with 3ml (150μ) of DNS solution. The resulting mixture will be incubated at 95°C in a water bath for 5min. The mixture will be allowed to cooled for 5min on ice. 1ml of distilled water will be added to the mixture. The concentration of the reducing sugars will be determined by measuring the absorbance at 540nm using UV-Visible Spectrophotometer. The concentration will be calculated using the glucose standard curve.

Reducing sugar conc. = Absorbance of sample/Absorbance of standard x concentration. of standard.

g. Fermentation of the Hydrolyzates

The fermentation of the hydrolyzate of the detoxified and undetoxified will be carried out as described by Rabah *et al.*, (2011) using commercial yeast.
The sample hydrolyzates 100ml will be transferred into 500ml conical flask. The conical flask will be enclosed with cotton wool, wrapped with aluminium foil, autoclaved for 15min at 121 °C, and cooled at room temperature. The pH of the medium will be adjusted to 6.5. The flask will then be inoculated 1% (w/v) with the yeast. The conical flask will then be incubated anaerobically at 30°C to 35°C for 24hrs to 120hrs. The fermented broth from the flask will be removed for every 24hrs incubation period for a period of 5days to determine the amount of ethanol using UV-visible spectrophotometer at a wavelength of 280nm or 290nm. Concentration of bioethanol (v/v) = Absorbance of sample/slope of calibration curve x dilution factor. The fermented broth produced will be subjected to distillation.

h. Distillation

Bioethanol from the process of fermentation will possess a considerable amount of water which must be eliminated. The removal of the water will be achieved by using distillation process according to (Romano, 2011). The process will be done by boiling the fermentation broth. Since bioethanol has a lower boiling point of 78.3°C compared to water's boiling point of 100°C. The bioethanol will turn into vapour state before water and will subsequently be subjected to condensation and separation. The resulting liquid that was collected will be measured using measuring cylinder (Oyeleke and jibril, 2009).

Bioethanol yield = Volume of bioethanol produced/ Volume of sample used x 100.

i. Qualitative test of bioethanol

Two (2) cm³ of acetone in a test tube was followed by four (4) drops of distilled bioethanol, and then two (2) drops of chromic acid. The mixture was shaken vigorously.

j. Quantitative yield of bioethanol

The value of the bioethanol yield was measured using a measuring cylinder, which was expressed in g/l.

III. RESULTS AND DISCUSSION

Table 1 shows the physico-chemical analysis of mango seeds. It revealed that there is high percentage of carbohydrate 53.90%, moisture content 10.56%, and significant ash content of 5.62%, 22.60% of crude fibre and 2.7% extractives. The structural composition of the mango seeds indicates high hemicelluloses content and cellulose content of 24.30% and 40.77% respectively. The results of table 1 agreed when compared with the reported values of literatures that mango seeds has high chemical composition and a viable potential for bioethanol production.

Table 1: Physico-Chemical Composition Analysis of Mango (*Mangifera indica*) seeds before pretreatment

Parameters	Value %
Moisture content	10.56
Ash content	5.62
Crude fibre	22.60
Protein content	6.12
Carbohydrate content	53.90
Extractives	2.7
Cellulose content	40.77
Hemicellulose content	24.30
Lignin content	20.33

Table 2 : Physico-Chemical Composition Analysis of Mango (*Mangifera indica*) seeds after pretreatment 0.8% 5%/5% v/v Fomic acid /Hydrochloric acid

Parameters	Value %
Ash content	3.45
Extractives	1.42
Cellulose content	50.36
Hemicellulose content	19.18
Lignin content	16.43
Moisture content	7.13

Table 3: Physico-Chemical Composition Analysis of Mango(*Mangifera indica*) seeds after pretreatment 0.8% 10%/5% Fomic acid /Hydrochloric acid

Parameters	Value %
Ash content	2.84
Extractives	0.93
Cellulose content	60.42
Hemicellulose content	16.13
Lignin content	14.10
Moisture content	5.28

Table 4 : Physico-Chemical Composition Analysis of Mango(*Magnifera indica*) seeds after pretreatment 0.8% 15%/5% v/v Fomic acid /Hydrochloric acid

Parameters	Value %
Ash content	1.45
Extractives	0.62
Cellulose content	66.36
Hemicellulose content	14.18
Lignin content	10.46
Moisture content	3.46

Table 5 : Physico-Chemical Composition Analysis of Mango (*Magnifera indica*) seeds after pretreatment 0.8% 15%/10% v/v Fomic acid /Hydrochloric acid

Parameters	Value %
Ash content	0.25
Extractives	0.42
Cellulose content	70.36
Hemicellulose content	6.18
Lignin content	8.16
Moisture content	2.91

Table 2-5 shows the chemical composition of the mango seeds after pretreatment. It shows that both the lignin and hemicelluloses decreasing while the cellulose content is increasing down the table. The results indicates that the combined dilute acid pretreatment removes more hemicelluloses content. This is in agreement of Zhang *et al.*, (2012) who reported that hemicelluloses are reduce during dilute acid pretreatment to enhance digestibility of cellulose.

Table 6: Determination of Reducing Sugars of Mango(*Magnifera indica*) seeds hydrolyzates

S/N	Wgt of Spl (g)	Conc. of Formic/ Hcl acid	Abs. of Spl.	Hydroly Zate vol. ml	Abs. of Std	Abs. of blank	Conc. of Std (g)	Reducing sugars (g)	Total RS (g)
1	5	5%/5%	0.590	18.80	0.846	0.07	2.0	16.75	314.90
2	5	10%/5%	0.608	24.20	0.846	0.07	2.0	17.83	479.39
3	5	15%/5%	0.684	28.70	0.846	0.07	2.0	19.78	567.69
4	5	15%/10%	0.721	32.50	0.846	0.07	2.0	20.97	681.53

Table 6 shows the effects of different concentration of dilute acid pretreatment on the amount of reducing sugar produced. The results of the reducing sugars are very significant for the values of 16.75%, 17.83%, 19.78% and 20.97%. As the concentration is increasing, the amount of reducing sugar increases. Ahmad *et al.*,(2012), reported that combined pretreatment can be a promising method for high recovery of sugars.

Table 7: Determination of bioethanol produced

S/N	Conc. of Fomic acid/Hcl (%)	Volume of hydrolyzate (cm ³)	Volume of distillate (cm ³)	Percentage yield (%)
1	5%/10%	64	21	32
2	10%/5%	68	23	33
3	15%/5%	72	27	37
4	15%/10%	82	32	39

Table 8: Qualitative Analysis of all the bioethanol produced

Test	Observation	Inference
Distillate + Acidified KMnO ₄	Purple colouration of KMnO ₄ declolorized	OH confirmed
Distillate + Acidified K ₂ Cr ₂ O ₇ + conc. H ₂ SO ₄ + warm	Orange colour of K ₂ Cr ₂ O ₄ changes to green	OH confirmed
Distillate + drop of acetic acid +conc. H ₂ SO ₄	A pleasant smell	OH confirmed

From the results obtained during fermentation as shown in table 7, there is an increase in the bioethanol formed on the basis of increase in dilute acid concentration. The highest amount of bioethanol was obtained from the 15%/10% concentration of dilute acid hydrolysis. However, maximum amount of bioethanol production was 39%. The high percentage shows a great potential for bioethanol production from mango seed which from a local plant that can thrive in every part of Nigeria.

Table 8 shows the qualitative analysis of the bioethanol produced. A pleasant smell when a few drops of acetic acids were added followed by concentrated sulphuric acid. However, when a few drops of acidified potassium heptaoxodichromate vi were added to the distillate, the orange colour of the dichromate turns greenish, indicating the presence of hydroxyl group. Moreso, a few drops of acidified potassium tetraoxomanganate vii were added the distillate, it decolourize the purple colour of the permanganate, indicating the presence of the hydroxyl group.

IV. CONCLUSION AND RECOMMENDATIONS

Production of bioethanol, a high octane biofuel is a better replacement for gasoline. The present study was done with the objective to produce bioethanol from mango seeds which will solves the waste disposal problem and prevent environmental degradation. In a country like Nigeria, it is very difficult to do proper disposal of wastes and thus generates of infectious disease and other challenge issues. So, using these wastes will help in the development and sustainability of the economy of a nation. Therefore, mango seeds is a suitable resources for the production of bioethanol as it is abundant, affordable, renewable and cheap feedstock for industrial production of bioethanol.

From what was observed, it is recommended that further research should be carried out using a weaker organic acids than formic acid in order to examine the structure and quantify the bioethanol produced. The industry should collaborate with energy institution, universities, polytechnics, colleges of education and other private sectors to come up with a better ways of producing bioethanol in large quantities to serve as alternatives to gasoline thereby creating room for job empowerment in the society.

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