

COMPARATIVE STUDY ON PRODUCTION OF BIOETHANOL FROM BANANA AND YAM PEEL

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Abstract

The aim of the study was to compare the production of bioethanol between banana and yam peel. Banana is one of the most popular tropical fruit consumed worldwide. Banana is a common term embracing a number of species or hybrid in the genus of the Musa of the Musaceae and cultivated mainly for its fruits. Yam is also a tuber crop in the plant genus of the Dioscorea of the Family Dioscoreaceae that form edible tubers. The bioethanol from Banana and Yam peels were hydrolysed with *Aspergillus niger* for 5days, the hydrolyses was fermented with *Zymomonas mobilis* at room temperature of 35°C for 5days. The bioethanol from banana peel produced a reducing sugar of 1.047mg/ml, the concentration obtained was 0.109mg/l, the quantity of the bioethanol was 133.53g/l and the density of the bioethanol was 0.843g/ml and for Yam peel the bioethanol produced a reducing sugar of 1.253mg/ml, the concentration obtained was 0.096mg/l, the quantity of the bioethanol produced was 146.33g/l and the density of the bioethanol was 0.793g/ml, This work reveals that Yam peel produces more bioethanol than banana peel.

Keywords: *Banana, Yam, Zymomonas mobilis, Bioethanol*

Introduction

Bioethanol is a renewable energy resource produced through fermentation of simple sugars by yeast. Bioethanol is widely used as partial gasoline replacement in the US and other parts of the world such as Canada, Brazil, Sweden, and China etc. It can also be used in a variety of heating, cooking and light appliance (1). The Federal Government of Nigeria has concluded plans to invest 400 Billion Naira (3.5 Billion US dollars) in Jigawa State for ethanol production programme in order to diversify its sources of revenue (2) The US President George W Bush announced in his state of union speech, an agenda to develop alternative energies such as bioethanol fuel from grains and cellulose in order to terminate Americans dependence on oil (3). The use of bioethanol fuel for automobiles can significantly reduce petroleum use and existing greenhouse gas emission (4). Ethanol is also a safe alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion (5). MTBE is a toxic chemical compound and has been found to contaminate ground water. The search for alternatives to the current, oil based, and fuels is the focus around the world. One of the most attractive alternatives is the Bio-ethanol-alcohol produced from agricultural crops. At present, bio ethanol derived from corncobs, sugar beets, grain and sugar cane, with the help of baker's yeast (6). A great number of by-products result from the cultivation of these crops, such as straw and corn husks. It would be a major step forward if this waste material, which also largely consists of sugar, could be used for the production of Bio-ethanol (6). This would allow agricultural land to be used more efficiently and at the same time prevent competition with food supplies.

The use of ethanol as a fuel was first advocated more than 100 years ago. The first extensive use of ethanol in gasoline was adopted as part of a domestic energy strategy in the 1970s. Ethanol was used as an octane, replacement in the 1980s and as a tool in the battle against air pollution in the 1990s. Today, ethanol meets a host of energy, agricultural, rural development and economic policy objectives. The increasing demand for cleaner transportation fuels creates great opportunities for biofuels –

agricultural renewable fuels such as bioethanol and biodiesel. Production of ethanol in the United States, reached an historic high level in 2006. While sorghum, sugar crops and waste materials such as cassava peel, banana peel, yam peel, rice and wheat straw etc are used to produce ethanol for at least another decade. The implementation of national Renewable Fuels Standard (RFS) is a key factor in expansion of ethanol use nationally (7).

Ethanol is a biofuel that can also be produced from biomass by anaerobic digestion of plant materials with high sugar content. Ethanol can be made from grain such as corn, sorghum and wheat, sugar cane and cassava. Ethanol is compatible with Premium Motor Spirit (PMS), and can be burned directly in automobile engine adapted to use this fuel, or it can be mixed with PMS up to 10 percent to be used in any normal automobile engine. A mixture of PMS and ethanol raises octane ratings and is a good substitute for lead antiknock agents, the major cause of lead pollution. It also helps to reduce carbon monoxide emission in automobile exhaust (8).

Bioethanol produced from renewable biomass has given considerable attention in the present years. Using ethanol as a gasoline fuel additive, as well as transportation fuel helps to reduce global warming and environmental pollution. In the last decade, most research has tended to focus in developing an economical and ecofriendly ethanol production process. Much emphasis is being given to the production of bioethanol from agricultural and forestry residues and other forms of lingo cellulosic biomass (9). In the past disposal and burning of agricultural products residues and waste such as cassava, yam peel, banana peel, wheat straws, rice straws and rice husks etc was an accepted practice. This practice is now being challenged due to concern over health effects from burning fields. Most of these residues and waste contain a reasonable amount of cellulose content which is a good source of fermentable sugars for significant use. Bioconversion of cellulosic biomass into fermentable sugar, for production of ethanol using microorganisms, especially cellulose degrading fungi, makes bioethanol production environmental friendly and also renewable (10).

The world's ethanol production was about 29.9 billion liters in 2000, which was less than 31.4 billion

liters forecasted (5). The benefit of developing biomass-to-ethanol technology are: increased national energy security, reduction in greenhouse gas emissions, use of renewable resources, foundation of a carbohydrate base chemical process industry, macro-economic benefit for rural communities and society at large (11).

Materials and Methods

Sample collection

Banana peel wastes are to be obtained from Kasuwan daji (a local fruit market) in Sokoto State, Nigeria. Similarly, yam peel wastes are also to be obtained from Usmanu Danfodiyo University Mini market, Sokoto.

Sample processing

Both the banana peel and yam peel wastes would be sorted and washed under running tap water to remove sand and other dirt particles. Then both samples are to be sun dried for about two (2) weeks and then milled in to powdered (flour) form using mortar and pestle. Each powdered product is to be sieved several times with different size sieve to obtain fine powder.

Preparation of culture medium

The media that are to be used are Potato dextrose agar (PDA) which is to be used to grown *Aspergillus niger* and Nutrient agar (NA) medium was used to grown the *Zymomonas mobilis*. Both media are to be prepared according to the manufacturer's instruction as the standard procedure as described by Oyeleke and Manga (12). But the PDA medium is to be incorporated with chloramphenicol (antibiotic) to inhibit bacterial growth.

Cultivation of microorganisms used in Enzyme hydrolysis

Aspergillus niger is to be obtained from stock culture in Usmanu Danfodiyo University Laboratory, Sokoto. The culture is to be carefully picked and inoculated on freshly prepared PDA medium. The media plate is to be incubated at 30° C for five (5) days, which would give rise to pure culture of the *Aspergillus niger*. For further clarification, it would be stained with lacto phenol cotton blue dye and observed under the microscope by taking note of

the shape and colour of the conidiospores as described by Oyeleke and Manga, (12).

Cultivation of microorganism used in fermentation

Serial Dilution

Five sterile test tubes containing 9mls of sterile distilled water were labelled for the serial dilution, 1ml of palm wine was aseptically taken and into the first test tube (i.e 10^{-1}) . 1ml was (i.e 10^{-1}) collected using syringe and was transferred into another test tube labelled 10^{-2} , 1ml from 10^{-2} was collected and transferred into another test-tube labelled 10^{-3} , the same procedure was used for the next test-tube labelled 10^{-4} up to 10^{-5} . 0.1ml was later taken from the last test tube (i.e 10^{-5}) and inoculated into freshly prepared nutrient agar medium and incubated at 37°C in an anaerobic jar for 28-48hrs. The fully cultured bacteria will further on be characterized based on colonial morphology, cultural characteristics and biochemical tests as described by Oyeleke and Manga (12).

The *Z. mobilis* will be confirmed by comparing its characteristics and biochemical reactions with those of known taxa using Bergey's Manual of Determinative Bacteriology.

Pretreatment of banana peel and yam peel using acid hydrolysis

Pre-treatment of both banana peels and yam peels was done using dilute H_2SO_4 . Thirty grams of banana peels was weighed and placed into separate 500ml conical flasks and 250mls of dilute H_2SO_4 was added to a conical flask containing the sample. It was then autoclaved at 121°C and was filtered using Whatman paper. The residue was washed for 30minutes until neutral pH and oven dried at 100°C - 105°C.

Enzyme Hydrolysis

The *Aspergillus niger* will be used to hydrolyzed the banana peel and yam peels powder. Thirty grams (30g) of the banana peel powder would be weighed into 500ml sterile conical flask, and 250mls of distilled water would be added to the conical flask to suspend the substrate. A sterilized wire loop was used to pick the growth of the *A. niger* and aseptically inoculated into the solution in the conical flask and stirred very well with a sterile glass rod, so

as to enable the microorganism get access to the substrate. The flask would be covered with cotton wool wrapped in aluminum foil to avoid contamination and kept on the bench at room temperature for seven (7) days, during which on daily basis it was shaken. After seven (7) days, the hydrolysate (solution) would be filtered using the Whatman filter paper No. 1. The filtrate will be collected and sterilized for further use as fermentable sugar (13). The same procedure would be repeated for yam peel.

Fermentation

Fermentation of the hydrolyzed sample was carried out according to the methods described by Oyeleke and Jibrin, (2009). 100mls of the banana peel hydrolysates were suspended into 500mls capacity conical flask and the covered with cotton wool and wrapped with aluminium foil, it was then autoclaved at 121°C for 15minutes. The conical flasks were allowed to cool at room temperature and were then inoculated with the fermentative organism. The conical flask was inoculated with *Zymomonas mobilis*. The flask was then incubated anaerobically at 35°C for 5 days. The Hydrolysates were then distilled according to standards methods (10).

Fractional Distillation

The ethanol produced from fermentation processes contains a significant quantity of water which was removed. This was achieved by dispensing the mixture into round bottom flasks fixed to the other end of the distillation column to collect the distillate. The temperature of the heating mantle was adjusted to 78°C which was used to heat the round bottomed flask containing the ethanol-water-mixture, since ethanol can only be distilled at 78°C. (10)

Determination of Quantity of Bioethanol produced

The distillates collected would be measured using a measuring cylinder, and expressed as the quantity of bioethanol produced in g/l by multiplying the volume of the distillates collected by the density of ethanol (0.8033g/ml). It should be noted that g/l is equivalent to the yield of 200g of dried substrate (13)

Determination of density of Ethanol

Measured 10ml from each sample, weighed it and divide by the volume measured.

$$\text{Density} = \text{Mass/volume}$$

Determination of reducing sugar

The reducing sugar content of hydrolyzed banana peels will be determined using the dinitrosalicylic acid calorimeter method of (Miller, 1959) with glucose as standard. It would be assayed by adding 2ml of 3,5-DNS reagent to 1ml of sample. The mixture will be heated in boiling water for 5 minute to develop the red-brown color. Then 1ml of 40% potassium sodium nitrate solution will be added to stabilize the color, it will then be cooled to room temperature under running tap water. The absorbance of the sample will be measured at 540nm using ultraviolet (UV-VIS) spectrophotometer. The reducing sugar content will be determined by making reference to a standard curve of known glucose (14)

$$\text{Concentration of reducing sugar} = \frac{\text{Absorbance of sample}}{\text{Absorbance of glucose}} \times \text{Conc. of Standard}$$

Determination of percentage ethanol Concentration

Five milliliters (5mls) of each sample would be measured into different test tubes and then 2mls of the prepared potassium dichromate solution was added to each and shaken thoroughly and allowed to stay for 20 minutes. The solution labeled in each test tube was poured into labeled cuticle in the ultra violet (UV) visible spectrophotometer and analyzed to determine the wave length and percentage ethanol concentration which was extrapolated from the standard ethanol curve.

Results

Colonial, Morphological and Biochemical characteristics of the microorganisms used for Hydrolysis and Fermentation

Aspergillus niger was used for Hydrolysis while *Zymomonas mobilis* was used for fermentation. *A. niger* showed a black mycelium on the Potato Dextrose Agar (PDA) medium, if also had septate hyphae, smooth conidiospores and long unbranched sporangiophores with a round head. *Z.*

mobilis was found to be gram negative rod, catalase positive, oxidase and urease negative and it produces gas from glucose. It also has an orange smell when the agar slant bottle containing it was opened. The description of the microorganisms corresponded to the results obtained by Oyeleke and Jibrin (12) (Table 1,2)

Bioethanol produced Banana and Yam peel using enzyme hydrolysis

The tables below show the quantity and concentration of bioethanol produced when enzymes produced from *A. niger* was used for the hydrolysis of the samples and then fermented for seven (7) days after inoculation of *Zymomonas mobilis*.

Table 3 shows the quantity (g/l) and concentration of bioethanol produced from 90g of Yam peel and 90g of banana peel after enzyme hydrolysis with *Aspergillus niger* and then fermented for seven (7) days after the inoculation of *Zymomonas mobilis*. From table 3 below show banana peel produces more bioethanol than yam peel but banana peel has more concentration than yam peel.

Discussion

Comparative study on the production of bioethanol from banana and yam peel through enzyme hydrolysis with *Aspergillus niger* and then fermented with *Zymomonas mobilis* was carried out. Both the Banana and Yam peel were successfully hydrolyzed by the *Aspergillus niger* as was evidenced by the presence of Bioethanol after the inoculations with *Z.mobilis*. A low yield of reducing sugar of 1.047mg/ml and 1.253mg/ml was obtained which is in disagreement with the findings of Rabah et al. (2011), who obtained a high yield of 3.2 % from bacterial hydrolyzed millet husk, which may be because the isolate used for hydrolysis may not be able to break the sugar easily from its structural components. Since a little amount of reducing sugar was available for fermentative organism, less concentration of Bioethanol was produced. The higher the sugar content the more the ethanol can be produced. (15)

A low concentration of 0.109mg/ml and 0.096mg/ml was obtained from banana peel which is in disagreement with the findings of Oyeleke et

al., 2009, who reported a high concentration of 17.6mg/l from cassava peel. This is likely due to the presence of more carbohydrate content in cassava peel than in banana peel. Sanchez et al. (2008) reported that as toxic compounds such as lignin residues, acids and aldehydes accumulated in the fermentation medium, the concentration of bioethanol tends to decrease. Similarly, the fermentative organisms' inability to ferment pentose more especially which is the main component of hemicelluloses fraction of lignocellulose may be another factor for low bioethanol concentration.(16,17)

The volume of Bioethanol produced from Banana peel using *Zymomonas mobilis* was 23g/l higher than the volume of Bioethanol obtained from other biomass such as sawdust (12.30g/l), corn husk (26.31g/l), rice husk (06.22g/l). Bioethanol produced from banana peel was less than that produced from yam peel, Banana peel produced 133.53g/l while yam peel produced 146.33g/l. This is due to the high carbohydrate content in yam peel.(18)

Conclusion

The work reveals the possibility of producing bioethanol from fermentation of banana peel and yam peel which may serve as cheap alternative source of fuel and energy generation. Maximum yield of bioethanol produced through Banana peel was (133.53g/l) and with average concentration of bioethanol produced from banana peel is (0.209mg/l), the reducing sugar concentration obtained was (1.047g/ml) and the density of the bioethanol was (0.793g/ml), while that of Yam peel was (146.33g/l) and with average concentration of bioethanol produced from banana peel is (0.096mg/l), the reducing sugar concentration obtained was (0.843g/ml) and the density of the bioethanol was (1.253g/ml). The use of both banana peel and yam peel is a means of controlling environmental pollution since bioconversion of cellulose of biomass into fermentable sugars for the production of bioethanol was done using cellulose degrading microorganism, which makes bioethanol production environmentally friendly and economically.

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Table 1. The Morphological and biochemical Characteristics of *Zymomonas mobilis* isolated from roselle juice.

Isolate	Gram reaction	Motility	Catalase	Glucose	Urease	Oxidase	Lactose	Organism
1	-ve Rod	+	+	+	-	-	-	<i>Zymomonas mobilis</i>

Table 2. The Morphological and biochemical characterization of *Aspergillus niger* isolated from moist bread

Isolate organism	Colony characterization	Cell shape	Organism
1	black dotted surface as conidia	Filamentous, with septed hyphae	<i>Aspergillus niger</i>

Table 3. The Quality (g/l), Concentration (%), Density (g/ml) and Reducing Sugar (mg/ml) of Bioethanol Produced from Enzyme Hydroslates

Samples	Quantity of bioethanol produced (g/l)	Concentration (%)	Density (g/ml)	Reducing Sugar (mg/ml)
Banana peel	133.53	0.109	0.843	1.047
Yam peel	146.33	0.096	0.793	1.253