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Comparative Study of Bioethanol Yield from Selected Agro-waste Feedstocks Using Fermentative *Saccharomyces Cerevisiae*

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Abstract

Bioethanol is a biofuel produced from renewable biological sources that is currently used in many countries as a safer substitute for fossil fuels. This study investigated the potential of rice husk, cassava, potato and yam peels as biomass for bioethanol production using Saccharomyces cerevisiae. The feedstocks were pre-treated with 50 mL of 0.1 M HCl at 50 °C for 20 min and hydrolysed at 100°C with 100 mL of 2 M HCl. Thereafter, the pH of the samples was adjusted using equal conc. of NaOH and fermented using 10 g of Saccharomyces cerevisiae for 96 h. It was then distilled at 81 – 83 °C. The result showed that the dissolved sugar concentration was significantly higher in potato peels than in yam peels (P = 0.004) and cassava peels (P = 0.045) but insignificant compared to rice husk (P = 0.158). However, the concentration of ethanol produced from rice husk (11.74±6.01% (v/v)) was slightly higher than potato peels (9.75±1.77% (v/v)); cassava peels (5.00±3.53% (v/v)) and yam peels (4.00±4.96% (v/v)), with no significant difference between them (P>0.05). Further investigation showed that, only 47.96% of the hydrolysate from rice husk, 39.06% from cassava peels, 33.27% from yam peels and 25.41% from potato peels were converted to ethanol and other by-products. The selected feedstocks showed good potential for bioethanol production especially the rice husk, and as such; commercial production is recommended for effective utilization and downstream applications.

Keywords: Bioethanol, Agro-based Feedstock, Hydrolysis, Fermentation, Dissolved sugar

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Introduction

Bioethanol is a biofuel that is produced from different plant matter and residues such as agricultural crops, municipal wastes, agricultural and forestry products/wastes **(Manas *et al.*, 2016)**. It is a biodegradable and environmentally friendly fuel which has been seen as a potential replacement for fossil fuels and their associated hazards **(Paniagua-Michael, 2015; Hoang and Nghiem, 2021)**. It is termed "renewable and environmentally friendly" because it is produced by the bioconversion of cellulosic biomass into fermentable sugar and subsequent fermentation by microorganisms in a manner that poses no serious threat to the environment **(Nutawan*et al.*, 2010)**. Its high octane number (108), evaporation enthalpy (38.6KJ/mol), flame speed (35.43 m/s), and wide range of flaming capacity (3.5 to 4 h per 1 L on maximum heat setting), have made it fuel energy of growing interest. It can be used as transportation fuel in many ways either directly or in blended form like gasohol **(Hossain *et al.*, 2014)**. It is produced through an anaerobic fermentation process in the same way as alcoholic beverages; in which microorganisms such as bacteria and fungi produce enzymes that catalyse the embden-meyerhoff (EMP) pathway. This facilitates the breakdown of complex sugars into fermentable sugar hydrolysates which are further fermented to produce ethanol using a specified fermentative agent **(Martin *et al.*, 2012)**.

Several plant materials have been discovered to show potential for bioethanol production and according to **Busic*et al.* (2018)**; their potentials vary with the type of plant material used. The potential of sugar-containing feedstock (Sugar Beet, Sugarcane, Molasses, Whey, Sweet Sorghum), and the starch-containing feedstocks (Grains and tubers) which are classified as first-generation feedstock **(Musatto*et al.*, 2010)** has been overwhelming except for their use as food and feed which may affect their availability and supplies **(Tomas-Pejo *et al., 2011*)**. Crop residues and agricultural wastes (Second generation feedstock) have therefore been considered to offer better alternatives following their low cost, availability, wide distribution and non-competitiveness with food and feed crops **(Tomas-Pejo *et al., 2011;* Busic *et al.*, 2018; Konstantinos *et al.*, 2019)**.

This informed the choice of the agricultural wastes for this study. Prior to now, scientists had the major challenge of complex sugar mixture in lignocellulosic biomass, owing to their strong crystalline structure in the layer of lignin which made it difficult for enzymes to digest them easily **(Gould and Freer, 1984)**. However, the discovery of other pre-treatment options such as acid hydrolysis has proffered a solution to the problem **(Braide *et al.*, 2016)**.

The return to plant materials and other possible wastes for the production of bio-products through environmentally friendly methods is a major breakthrough for humanity in a world that has been bedevilled by environmental pollution. In more recent times, there have been growing global concerns on climate change due to increasing emission of carbon oxides which are the principal greenhouse gases. The combustion of fossil fuels and "land-use change" due to increasing human activities, are the major sources of these emissions; hence the need to reduce dependence on the use of fossil fuels. According to **Busic *et al.* (2018)**, the use of alternative renewable resources which have little environmental and social concerns, is the solution to the current situation of global warming and all fossil-based problems. The bioconversion of wastes does not only produce purer and safer forms of energy, it is also a waste management option that gives the world wealth from her wastes. This study investigated the potentials of the selected feedstocks of Benue State, Nigeria for bioethanol production with the aim of determining the quantity of reducing sugars present in the wastes, as well as to compare their ethanol yield and quality.

MATERIALS AND METHODS

Study Area

The study was carried out in Makurdi, Capital of Benue State, Nigeria. Makurdi is located at the North Eastern part of Benue

State and it lies on latitude 7°30′ N and Longitude 8°5′E **(Amuta *et al.*, 2008).**

Sample collection and processing

The rice husks used for this study were collected from Wurukum Rice Mill, Makurdi. The peels of Cassava, Potato and Yam were collected as wastes from household kitchens and were sorted, washed with distilled water to remove sand and other unwanted materials. The peels were then cut into pieces and sun dried. The samples were further dried in a Laboratory Oven (Uniscope SM9053) at 40 °C for 24 h to ensure dryness and were ground and sieved in order to get a smooth sample of uniform size. The ground samples were stored in properly washed, dried and labelled containers for further analyses.

Acid Hydrolysis

Hydrolysis was carried out according to the method described by **Ezejiofor *et al.* (2018)**. Ten (10) grams each of the samples were first pre-treated with 50 mL of 0.1 M HCl at 50 °C for 20 min. The pre-treated samples were then hydrolysed by boiling them in 100 mL of 2.0 M Hydrochloric acid (HCl) at 100°C. The progress of the hydrolysis was monitored by reacting the boiling samples at intervals on a white tile using Iodine solution. A blue black colouration which kept reducing in intensity until it disappeared completely to give an orange colour, showed complete hydrolysis. The boiled samples were allowed to cool and the pH was adjusted to 5.0 using equal concentrations of NaOH. This was followed by filtration using Whatman filter paper (diam. 45 mm). The filtrates were then subjected to Benedict's test for the presence of reducing sugar. The quantity of reducing sugar produced after hydrolysis was measured using the Refractometer (abbe60/ DR 10-99). The % dissolved sugar was calculated as follows:

$$\% \text{ Dissolved Sugar} = \frac{Mass\ of\ Dissolved\ Sugar}{Volume} \times 100$$

Preparation of Yeast Culture

Ten (10) grams of fermentative *Saccharomyces cerevisiae* (Bakers' yeast) was added to 50 mL of distilled water at 25 °C room temperature. The solution was stirred for 5 min and allowed to stand for 2 h before adding it to the hydrolysates.

Fermentation

The activated yeast was aseptically inoculated into the hydrolysates from the wastes in a flask. The solution was mixed properly and then covered with aluminium foil and left at room temperature for four (4) days. The flask was rocked intermittently daily for the 96 h duration.

Distillation

Distillation was carried out on the hydrosylates after the four-day fermentation. Ethanol was boiled off from the mixture of water and other impurities in a distillation column, where it was monitored from a temperature of 78 °C. The bio-ethanol produced from distillation was assessed for quality using the boiling point and specific gravity. The quantity of the reducing sugar left after fermentation was also measured using the Refractometer in order to determine what percentage of the initial hydrolysate was fermented.

Distinguishing Test for Ethanol

Iodoform test was used to determine the presence of ethanol. Five (5) drops of the distillate were added to 5 mL of Iodine solution in a test tube and NaOH was carefully added until the colour of the Iodine disappeared. The test tube was then placed in a Water Bath (Clifton-61012) at 70 °C for 3 min and was removed and allowed to cool. Pale yellow crystals of Iodoform were observed and the smell was reminiscent of an antiseptic. The Quantity of ethanol produced was measured using a measuring cylinder (100 mL pyrex).

Specific Gravity of the Distillate

The specific gravity of the distillate was measured using the specific gravity bottle (density bottle). The bottle was filled with ethanol sample, weighed and recorded. It was also filled with distilled water, weighed and was recorded. The Specific gravity was then calculated as follows:

$$\text{Specific gravity} = \frac{\text{Weight of alcohol}}{\text{weight of equal volume of water}}$$

The Percentage by volume of the alcohol (ethanol) corresponding to apparent specific gravity at 30 °C was read from the gravity bottle in % (v/v). All the reagents used for this study were supplied from Sigma - Aldrich.

Statistical Analysis

The data collected was analysed using Analysis of Variance (ANOVA) in Completely Randomized Design. The respective variable Means were separated using the Fisher's Least Significant Difference Test (FLSD) where significant differences occurred. Descriptive statistics (Chi-square Test) was also used to check the independence of variables where necessary.

Result

The results of the study are as presented in the Tables 1-3 below. Table 1 showed the percentage dissolved sugar and the quantity of ethanol mixture obtained from the selected agro-based Feedstocks. The percentage concentration of dissolved sugar after hydrolysis of the feedstocks showed that Potato peels had the highest sugar concentration of 5.55±0.07brix, which was significantly higher than those of the yam peels ($P = 0.004$) and cassava peels ($P = 0.045$), but insignificant when compared to rice husk ($P = 0.158$) respectively (Table 1).

The amount of residual dissolved sugar was also evaluated so as to determine the amount of the dissolved sugar converted to ethanol and other by-products. The result showed a significantly higher concentration of the final dissolved sugar in potato peels compared to rice husk ($P = 0.021$), while it was slightly higher but insignificant compared to cassava peels ($P = 0.064$) and yam peels ($P = 0.101$) respectively (Table 1).

Further investigations however revealed that only 25.41 % of the dissolved sugar from potato peels, 33.27 % from yam peels, 39.06% from cassava peels and 47.96 % from rice husk, were converted to the products with no significant difference across these agro wastes ($\div^2 = 7.8$; df = 3; $P = 0.050$) (Table 1).

The result also showed that the ethanol mixture produced from the yam peels had the highest volume (117.00±5.66 cm). This was followed by rice husk (111.00±0.00 cm), potato peels (106.50±37.48 cm) and cassava peels (98.50±20.51 cm). No significant difference was however observed in comparison ($P>0.05$) (Table 1).

Ethanol Yield from the Starch-based Feedstocks

The yield of ethanol from the selected feedstocks was also investigated in this study. The highest ethanol yield was observed in the rice husk (11.74±6.01 %(v/v)) followed by potato peels (9.75±1.77 %(v/v)) and cassava peels (5.00±3.53 %(v/v)). The lowest ethanol yield was observed in yam peels (4.00±4.96 %(v/v)); there was however no significant difference between them ($P>0.05$). These concentrations of the ethanol were validated using the specific gravity of the product. It was observed that, the concentration of the ethanol increased with decrease in specific gravity. The specific gravity of the product from yam peels was slightly higher than the rest of the feedstocks indicating a lower percentage of the product; while it was lower in rice husk indicating a higher percentage (Table 2).

The purity of the product harvested was also tested using the boiling point. The degree of purity obtained in this study was significantly higher in potato peels (81.00±0.00 ^{o}C) than others ($P<0.05$); while it was lower in rice husk (83.5±0.71 ^{o}C) with no significant difference with cassava peels and yam peels respectively ($P>0.05$) (Table 2).

Relationship between Ethanol Yield and Percentage of Fermentable Dissolved Sugar

The study investigated the dependence of ethanol yield on the quantity of dissolved sugar used during fermentation. It was observed that increase in % dissolved sugar did not necessarily lead to corresponding increase in ethanol yield ($\div^2= 4.507$; df = 3; $P = 0.212$) (Table3).

Table 1: Dissolved sugar and ethanol mixture quantity obtained from the selected starch-based feedstocks.

Feedstock	% Dissolved Sugar Concentration (Brix)		% Dissolved Sugar fermented	Quantity of Ethanol mixture (cm^3)
	After hydrolysis	**After Fermentation**		
Cassava Peels	5.30 ±0.00^{ab}	3.23 ±0.18	39.06	98.50±20.51
Potato Peels	5.55 ±0.07^{b}	4.14 ±0.02^{a}	25.41	106.50±37.48
Yam Peels	5.05 ±0.07^{abd}	3.38 ±0.39	33.27	117.00±5.66
Rice husk	5.40 ±0.14^{d}	2.82 ±0.57^{a}	47.96	111.00±0.00
LSD(P<0.05)	**0.051**	**0.068**	**P=0.050**	**NS**

*Values are Mean ± SD in 2 replications. NS = Not significant. Values with the same alphabets in a column are significant

Table 2: The specific gravity, ethanol concentration and boiling points of ethanol produced from the selected Starch-based Feedstocks.

Sample	Specific gravity	Ethanol con. (%v/v)	Boiling points
Cassava peels	0.989±0.005	5.00±3.53	82.50±0.71°C^{a}
Potato peels	0.987±0.002	9.75±1.77	81.00±0.00°C^{abcd}
Yam peels	0.994±0.007	4.00±4.96	83.00±0.00°C^{c}
Rice husk	0.985±0.007	11.74±6.01	83.5±0.71°C^{d}
LSD(P<0.05)	**NS**	**NS**	**0.053**

*Values are Mean ± SD in 2 replications. NS = Not significant. Values with the same alphabets in a column are significant

Table 3: Relationship between ethanol yield and percentage of dissolved sugar fermented

Feedstock	Percentage of Dissolved sugar (Brix)		%Dissolved sugar fermented	Ethanol Con. (%v/v)
	After hydrolysis	**After fermentation**		
Cassava peels	5.30 ±0.00	3.23± 0.18	39.06	5.00 ±3.53
Potato peels	5.55± 0.71	4.14±0.002	25.41	9.75±1.77
Yam peels	5.05±0.07	3.38±0.39	33.27	4.00±4.96
Rice husk	5.40±0.14	2.82±0.57	47.96	11.74±6.01

÷^{2}= 4.507; df = 3; P = 0.212

Discussion

The study of bio-ethanol production from cassava peels, potato peels, yam peels and rice husk was undertaken in this research using acid hydrolysis and fermentation with *Saccharomyces cerevisiae.*

The range of dissolved sugar concentration obtained in this study (5.05 – 5.55%) was within the range of 5-6% recommended by Fakruddin*et al*. (2012) in their study. This was considered optimum for maximum ethanol yield because; high sugar concentration can inhibit the metabolic process of the fermentative organism. The concentration of dissolved sugar obtained from biomass may depend largely on the type of feedstock used as well as the pre-treatment method adopted as demonstrated in many studies including those of **Nutawan *et al.* (2010); Wi *et al.* (2013); Ezejiofor*et al.* (2018); Braide *et al.* (2016) and Sirajo *et al.* (2019)** respectively. The pre-treatment method used in this study was acid hydrolysis (2 M HCl) because it was considered faster and results were achieved within a short time.

After the fermentation process, the weight of the sugar hydrolysate utilized for ethanol production was less than 50 % for the respective feedstocks. The highest conversation rate was 47.96% for Rice husk. It has been noted that, under anaerobic conditions in which *S. cerevisiae* converts glucose to ethanol **(Busic *et al.*, 2018)**; the maximum conversion efficiency is 51% by mass. However, for cell growth and synthesis of other metabolic products by the fermentative organism in which glucose is used, the maximum efficiency is always reduced in practice to about 40-48% **(Lee *et al.*, 2007)**. In this study, only rice husk attained the efficiency level stated above. It could be deduced therefore that the amount of dissolved sugar available for fermentation is not the sole determinant of ethanol yield, but also the ability of the fermentative microorganism to utilize the hydrolysate effectively.

The agro-wastes selected for this study showed very good potential for bioethanol production, with the highest percentage concentration of ethanol observed in rice husk (11.74±6.01 % (v/v)). The use of rice husk and other starch-based feedstocks for bioethanol production has been attributed to their cellulose, hemicelluloses and lignin contents **(Binod *et al.*, 2010)**. The high ethanol yield from rice husk observed in this study is similar to reports from previous studies on rice straw including those of **Nutawan *et al.* (2010); Binod *et al.* (2010) and Wi *et al.* (2013)**. Its high ethanol yield compared to cassava, potato and yam may be as a result of its low moisture content compared to these tubers (that may have some moisture retained in them even after drying). According to Food and Agriculture Organization **(FAO) (1999)**, the energy from tubers is only about one-third of that of an equivalent weight of rice or wheat as a result of the high moisture content of tubers.

The boiling points of the bioethanol obtained in the study were observed to be slightly higher than the normal boiling point (78 ^{o}C) of pure ethanol. This may be due to the presence of some impurities. Hence, further purification may be required for this ethanol during up scaling to enhance its suitability for downstream applications.

The dependence of ethanol yield on the percentage of the sugar hydrolysate used by the fermentative organism was evaluated to establish the relationship between percentage concentration of ethanol produced and the increase in the dissolved sugar utilized. This study showed for instance that potato peels with the lowest percentage of fermented dissolved sugar (25.41%) produced a higher concentration of ethanol than cassava peels (39.06%) and yam peels (33.27%) (Table3). This could be attributed to the possible production of some other unharvested bio-products that could also be of high commercial value. According to **Ellen, (2001)**, many by-products such as heat, carbon dioxide, water, methanol, fuels, fertilizers and alcohols are produced during ethanol fermentation. Majority of these products are however not harvested from the system.

Conclusion

This study revealed that wastes from starch-based feedstocks such as cassava peels, potato peels, yam peels and rice husk are very good sources of biomass for bioethanol production especially where they are produced in abundance. Rice husk had a higher potential for bioethanol production than the other feedstocks. The potential of these feedstocks as biomass for the production of other useful bio-products should be explored. It is notable that higher amounts of bioethanol can be produced from these feedstocks if the environmental and experimental conditions are further optimized.

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