Bioethanol Production and Proximate Composition of Waste Potatoes

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\textbf{Authors’ contributions}

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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\textbf{ABSTRACT}

Bioethanol can be produced from biological matter through processing of food wastes or crops meant for bioethanol production. This study used potato wastes from food vendors in Sokoto, Nigeria as a cheap and renewable carbon source for fermentation of ethanol. \textit{Saccharomyces cerevisiae} was used to optimize the growth parameters and hydrolysis of potato wastes of the ethanol fermentation aimed at achieving maximum production of bioethanol. Following the analysis, results indicated that, a combination of 0.5, 1.0, 1.5, 2.0, 2.5\% of H\textsubscript{2}SO\textsubscript{4} at 121\,°C for 20 min in an autoclave can yield complete hydrolysis of all starch contents of potato wastes. The average proximate composition of the potato wastes showed 13.94\%,1.42\%, 1.72\%,1.38\%,0.43\% and 81.11\% of Moisture, Ash, Fat, Crude protein, Fiber and Carbohydrate contents respectively. Positive confirmation of reducing sugars and bioethanol was achieved by using benedicts and Jones’ reagents respectively, Quantitative Test for reducing sugars indicated 124.9 mg/gm, 88.6 mg/gm, 61.45 mg/gm, 53.22 mg/gm, 47.23 mg/gm for 0.5\%, 1.5\%, 2.0\%,2.5\% and 3\% concentrations respectively.

\textbf{Keywords:} Bioethanol; potato; fermentation; \textit{Saccharomyces cerevisiae}.

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1. INTRODUCTION

Energy is very vital resource to living generations as it is the main social and economic development of the societies, and lack of energy affects socioeconomic problems [1]. Energy has been associated with most global crisis of nowadays [2]. Today, fossil fuels are the major source of energy presenting large amount of the energy demand at about 80% globally, hence 58% is used by transport sector [3]. They have played an indispensable role in humanity’s recent history, providing a vast energy source which has fueled much of society’s development and industrialization. These fuels are still the primary source of energy for the world’s developed nations, and yet it is agreed that these traditional sources of energy cannot continue to power humanity’s growth into the future [4]. The declining of fossil fuels and its increase in health threat and overall global warming became worldwide challenge [5].

Ethanol has proven to be a great alternative fuel and can complement the current fossil fuels thereby reducing the incessant negative environmental pollutions posed by fossils. Fermentation is a bioprocess catalyzed by cells to produce a chemical change in an organic substrate [6]. For ethanol production; carbon is mainly sourced from the following crops rice, corn, wheat, potato, cassava, yam, sorghum, banana and plantain. These starchy crops are used for ethanol production via fermentation. [7,8]. The use of agricultural wastes provides a means of cheap, reliable and most abundant lignocellulosic resources that can be utilized for the production of bioethanol production, among these fermentable crops, potato tends to be cheap, available and requires simple pretreatment operations and it has rich composition of simple starch, sugar and complex carbohydrate. More so, the world’s potato production is over 300 million tonnes, which accounts for about 7000 million liters of fuel ethanol per year hence chosen as desirable feedstock for bioethanol production [9,10,11].

Saccharomyces cerevisiae are microscopic organisms which converts carbon sources to ethanol aimed at producing energy. An example is in a brewery, where Saccharomyces cerevisiae are used in the production of alcohol which simply involves the conversion of sugar to energy. During the fermentation process as the yeast flocculate, they attach to the carbon dioxide being produced and float to the top of the wort, this allowed brewers to collect the yeast and create more colonies for later beers. However, the organism is also used by bakers to make dough hence named baker’s or brewer’s yeast [7]. The study was conducted to use Saccharomyces cerevisiae; a low-cost fermentative microorganism in producing bioethanol from waste potato aimed at recovering energy from wasted potato and also to study its proximate composition. Saccharomyces cerevisiae was selected in this study for being the most utilized organism for industrial application.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Substrate (Waste Potato)

Waste potato was collected from fruits and vegetable market, Ramin Kura, Sokoto. The sample (rejected/spoil tubers) were washed from dust and debris, peeled off and chopped off to small pieces, grounded and sieved through a 2-3mm diameter size mesh, the powder was later stored in an air tight container.

2.1.2 Organism (Saccharomyces cerevisiae)

Saccharomyces cerevisiae: It was prepared and maintained according to [12]. The broth was prepared following the Peptose dextrose sugar manufacturer’s guide. Saccharomyces cerevisiae was obtained from Microbiology Department, Usmanu Danfodiyo University Sokoto, Nigeria, it was later fed into homogenized solution and was maintained in Malt-extract agar at 5-10°C.

2.2 Methods

2.2.1 Fermentation

For the fermentation, the hydrolysate of the starch was adjusted to a pH of 6 using 2M NaOH and was supplemented with additional nutrients (per L): 1 g MgSO4.7H2O, 1 g yeast extract, 5 g KH2PO4 and 2 g (NH4)2SO4. This was used as bioethanol production medium, a working volume of 100 mL was transferred into a 250 mL flask and was sterilized in an autoclave at 121°C, 15 psi for 30 min. S. cerevisiae cells were later loaded into the sterilized medium (10% v/v), subsequently, the fermentation process the operating conditions were maintained at 30 °C, pH was adjusted to 6 with 2M NaOH and incubation time of 84 hrs while the broth was collected at an interval of 6-hour basis.
2.2.2 Hydrolysis

For the hydrolysis, 0.5%, 1%, 1.5%, 2%, and 2.5% (w/v) of H\textsubscript{2}SO\textsubscript{4} was added to the 30g of powdered Waste potato in a 1:1 solid-liquid ratio which were later autoclaved at 121°C for 15 min. this is done in order to breakdown the starch into fermentable sugars, and the H\textsubscript{2}SO\textsubscript{4} will attack α – 1,4 – glycosidic bonds and breaks them to glucose unit [13].

2.2.3 Proximate Analysis

Determination of Moisture, Ash, Protein, Fibre and Fat content followed Standard methods of the Official Methods of Analytical Chemist [14].

2.3 Test for Reducing Sugars

2.3.1 Qualitative

Benedict’s test was used to test for the reducing sugars; the Benedict’s reagent was composed of mixture of copper (II) sulphate and a filtered mixture of hydrated sodium citrate and hydrated sodium carbonate which were added to the test solution followed by boiling. Red precipitate indicates the presence of high concentration of reducing sugars while yellow precipitate shows low concentration.

2.3.2 Quantitative

Dinitrosalicylic acid (DNS) colorimetric method was used to quantify the total reducing sugars in the hydrolysate of the waste potato using. 0.50 mL DNS acid was inserted into a test tube loaded with 0.50 mL of the hydrolysate. It was taken to boil at 100°C for about 10mins, it was allowed to cool and 5mL distilled water was added, well shaken and left to settle for 5mins. A UV-Visible spectrophotometer (Model: Nicolet-evolution300- Thermo Electron Corporation, Wavelength: 520 nm) was used to measure the absorbance against reagent blank. For each g/100 g substrate of the reducing sugar yield, the following equation was used:

\[
\text{Reducing Sugar (g/100g)} = \frac{RC \times V1 \times 100g}{1000mL \times M1}
\]

Where; \( RC \) stands for reducing sugar concentration (g/L), \( V1 \) stands for volume of Acid (mL), and \( M1 \) is the mass of substrate added (g). [15].

2.4 Test for Bioethanol

Following the successful fermentation process, Jones’s reagent was used to confirm the bioethanol; Jones reagent oxidizes ethanol into acetic acid in the presence of potassium dichromate and sulfuric acid and in turn will produce green color.

3. RESULTS AND DISCUSSIONS

3.1 Results

a. Proximate composition of waste potato

Table 1. Proximate composition of waste potato

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dry weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content (%)</td>
<td>13.94%</td>
</tr>
<tr>
<td>Ash Content (%)</td>
<td>1.42%</td>
</tr>
<tr>
<td>Fat Content (%)</td>
<td>1.72%</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>1.38%</td>
</tr>
<tr>
<td>Fibre Content (%)</td>
<td>0.43%</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>81.11%</td>
</tr>
</tbody>
</table>

b. Test for reducing sugars

- Qualitative

Table 2. Results for Qualitative Test for reducing sugars

<table>
<thead>
<tr>
<th>Hydrolysis (H\textsubscript{2}SO\textsubscript{4}) (%)</th>
<th>Color change (With Benedict’s Reagent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. at 0.5%</td>
<td>Red Precipitate</td>
</tr>
<tr>
<td>Conc. at 1.5%</td>
<td>Red Precipitate</td>
</tr>
<tr>
<td>Conc. at 2%</td>
<td>Orange Precipitate</td>
</tr>
<tr>
<td>Conc. at 2.5%</td>
<td>Orange Precipitate</td>
</tr>
<tr>
<td>Conc. at 3%</td>
<td>Yellow Precipitate</td>
</tr>
</tbody>
</table>

- Quantitative

Table 3. Results for Quantitative Test for reducing sugars

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total Reducing sugar (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. at 0.5%</td>
<td>124.9 mg/gm</td>
</tr>
<tr>
<td>Conc. at 1.5%</td>
<td>88.6 mg/gm</td>
</tr>
<tr>
<td>Conc. at 2%</td>
<td>61.45 mg/gm</td>
</tr>
<tr>
<td>Conc. at 2.5%</td>
<td>53.22 mg/gm</td>
</tr>
<tr>
<td>Conc. at 3%</td>
<td>47.23 mg/gm</td>
</tr>
</tbody>
</table>

3.2 Discussion

The waste potato has shown a high composition (81.11%) of carbohydrate which is the main driver for bioethanol production. The content is a
bit higher than those reported by [16,17]. However, alongside fat and proteins; wasted food can be a good feedstock for bioenergy especially potato. However, the rich carbohydrates content also indicates it potentials in nutrition if properly managed, because carbohydrates play key roles in human body other than energy supply, which includes fueling kidney, brain, heart muscles and central nervous systems. Carbohydrate is sometimes used as flavors and sweeteners especially in pediatric medicines. And most importantly regulate our blood glucose and breakdown fatty acids thereby shielding us from ketosis [18,19].

The results for reducing sugars as in Table 2 indicates that as concentration of the H2SO4 increases with respect to hydrolysis; so also, the color changes towards lesser amounts of reducing sugars present as shown in figure 1 which indicates the benedicts reagent in tracing the reducing sugars. Hence Conc at 0.5 and 1.5 have moderate (1.5-2g%) reducing sugars, conc. at 2.0% and 2.5% have low (1.1-1.5g%) reducing sugars while conc. at 3% have 0.5-1g% traceable grams of reducing sugars.

![Fig. 1. Benedicts Test results for reducing sugars](image)

In the same vein, quantitate estimation for reducing sugars also decreases by increase in H2SO4 conc. used for hydrolysis. It showed the following trend 0.5% > 1.5% > 2% > 2.5% > 3% as shown in table 3. the bioethanol was finally confirmed using Jones Reagent that oxidized ethanol into acetic acid in the presence of potassium dichromate and sulfuric acid and in turn produced a green color as a confirmation for bioethanol according to [20,21].

### 4. CONCLUSION

Waste potato from a famous fruit and vegetable market in Sokoto was studied aimed at recovering value from waste. Sulphuric acid was used for hydrolysis in an autoclave in a spread concentrations of 0.5%, 1.5%,2%,2.5% and 3%. The lower concentrations were found to have better concentrations of reducing sugars as shown by both qualitative and quantitative estimations. The highest concentration achieved was 124.9 mg/gm while the lowest was 47.23 mg/gm. The fermentation operating conditions were maintained at 30˚C at a pH of 6.0 following incubation time of 84 hours. *Saccharomyces cerevisiae* in this study has shown great potential for bioethanol production from waste potato, it was reported to be the most ideal and used organism for ethanol production [22]. Potato waste is cheap, reliable and easy to utilize for bioethanol production in order to produce alternative energy source aimed at meeting up the current and future.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

### REFERENCES


6. Soares MCP, Vit FF, Suzuki CK, de la Torre LG, Fujiwara E. Perfusion


