Production of Ethanol from Cassava and Yam Peels Using Acid Hydrolysis

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Abstract

Energy from fossil fuels has played a very important role in our lives, but such an important role has been clouded out due to the environment hazards caused from fossil emissions. This has led to a new dimension in energy utilization known as renewable energy fuels. To fully support this type of energy from biological mass, adequate biomass source must be harnessed. This work thus was carried out with a view of utilizing some locally available biomass wastes as an alternative source of ethanol. The production of ethanol from cassava and yam peels was examined using acid hydrolysis at two different temperatures and fermenting with two different strains of \textit{Saccharomyces cerevisiae} (baker's yeast and freshly isolated yeast). Fermentation was allowed for about 5 days after which ethanol was recovered by distillation (at 78°C). Iodoform test and gas chromatography were used, just to confirm that the distillates were ethanol. Cassava peels hydrolyzed at room temperature produced higher yields of ethanol (11.30% and 8.63%). Fermentation with freshly isolated yeast produced more yield of ethanol both at room temperature (11.30%) and at 80°C (6.15%) than those fermented with baker's yeast. Yam peels also produced more ethanol at room temperature than at 80°C using either of the two enzymes for fermentation (21.72% and 27.08%). Moreover, the use of baker's yeast for fermentation produced more yield of ethanol from yam peels. For the mixtures by proportion, only the ratio of 2:1 of cassava to yam peels (C\textsubscript{2}Y\textsubscript{1}) produced a higher yield of ethanol at room temperature (60.52% and 13.39% at room temperature using baker's yeast and freshly isolated enzymes respectively). The other mixtures [(C\textsubscript{1}Y\textsubscript{2}) and (CY) sets of samples] gave higher yields of percentage ethanol at 80°C than at room temperature. Every other sets of samples gave higher yields at room temperature. Generally, most of the samples hydrolyzed better at room temperature except for C\textsubscript{2}Y\textsubscript{1} and CY sets of samples. The highest yield of ethanol was produced by (C\textsubscript{2}Y\textsubscript{1}) when hydrolyzed at room temperature using baker's yeast (60.52%).

\textbf{Keywords:} Ethanol yield; Hydrolysis; Energy; Temperature; \textit{Saccharomyces cerevisiae}.

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1. Introduction

The rapid growth of industries and technological advancement in the world call for development in the chemical sector. The production of industrial chemicals will enhance the economic progress of any nation. Ethanol, one of the important industrial chemicals, can be produced extensively from biomass such as cassava peels and yam peels. The main constituents of this class of crop by-product are cellulose [1] and hemicelluloses, making them lignocelluloses [2] that can be excellent energy sources. Mechanized farming has led to extensive discharge of agricultural wastes that have had negative effects on the environment. As a result of the environmental mess caused the agricultural activities of farmers, the utilization of such wastes has become very important to researchers [3,4,5].

This work was designed to look into the possibility of converting some of such by-products into industrial chemicals of economic importance. Ethanol is one such chemical. Ethanol produced from regeneration sources is an attractive petrochemical feedstock in petroleum for poor countries [6]. The various uses of ethanol and the importance of ridding the environment of the harmful effects of these agricultural by-products (biomass) underscore the significance of this work. Ethanol is produced from palm wine by fermentation process [7]. Fermentation is one of the oldest processes known to man, and it is used in making a variety of products including foods, flavorings, beverages, pharmaceuticals, and chemicals. Ethanol is made from a variety of products such as grain, molasses, fruit, cobs, and shell; its production, excluding that of beverages, has been declining since the 1930s because of the low cost [8]. In 1975, only $76 \times 10^6$ L of proof industrial ethanol were produced by fermentation compared to $7.95 \times 10^6$ L by synthesis.

Fossil fuels, mainly petroleum, coal, and natural gas, were the main energy sources for most industries during the 20th century, and are still the most important feedstocks to produce energy in the world. However, these sources are no longer regarded sustainable, and their availability is much lower. Hence, the need for more research in the production of Bioethanol which can be used as petrol substitute for vehicles.

The objective of this study is to produce ethanol from cassava peels and yam peels using acid hydrolysis at two different temperatures and fermented with two strains of *Saccharomyces cerevisiae* (baker’s yeast and freshly isolated yeast).

2. Materials and methods

2.1 Reagents and apparatus

REAGENTS:

Concentrated sulfuric acid, diethyl ether, sodium hydroxide, distilled water, freshly isolated and industrially made yeasts.

APPARATUS AND EQUIPMENT:
250ml conical flasks, beakers, funnels, whatman filter paper (could be ashless or any other), measuring cylinders, volumetric flasks (or other collector bottles), distillation sets, round bottomed flasks, retort stands, heating source (heating mantles, plates or other sources), water bath, thermometers, refractometer, weighing balance, specific gravity (SG) bottle,

2.2 Methodology (procedure for production)

Collection and Processing of Substrate Used:

COLLECTION OF SAMPLES:

Cassava peels and wastes were collected from the old pilot plant building of the Federal Institute of Industrial Research, Oshodi, FIIRO Lagos state. Yam peels and wastes were also collected from the market premises of a local suburb in Ogba-Iyio Ijoko, Ogun state.

SORTING AND DRYING OF SAMPLES:

The collected samples were taken to a convenient place where they are sorted. The peels of cassava and yam were separately sorted to have mainly the peels for drying. The drying was carried out in open air but due to the weather which delays drying for weeks as a result of frequent rainfall, drying was completed in an oven.

MILLING OR GRINDING OF SAMPLES:

The well dried samples were then milled using a hammer mill in the new pilot plant building of the federal institute of industrial research, Oshodi, FIIRO Lagos state. A serrated disc grinder could also be used to reduce the yam and cassava peels to very small particle sizes.

ISOLATION OF CELLULOSE (PRETREATMENT):

From the milled cassava and yam peels, 120g of five different samples were weighed as follows:

120g of cassava peels

120g of yam peels

120g of a mixture of cassava and yam peels in the ratio of 2:1

120g of a mixture of cassava and yam peels in the ratio of 1:2 and

120g of a mixture of cassava and yam peels in the ratio of 1:1

The cellulose was isolated by the procedure described by [9]. To 30g of each sample of the agricultural waste was added 60ml of diethyl ether in a 250ml conical flask in order to remove extractives and the residue left was
washed with distilled water.

Each of the five weighed samples are done in quadruplet giving rise to twenty different samples \([C_{1-4}, Y_{1-4}, (CY)_{1-4}, (C_1Y_2)_{1-4}, (C_2Y_1)_{1-4}]\).

**HYDROLYSIS:**

30g of the washed residues are then weighed into separate conical flasks and divided into two sets of samples. Each quadruplet being split into duplicates. 150ml of 4.5M sulfuric acid is then added to all the samples in the conical flasks and hydrolysis is allowed to take place at two different temperatures. One set of duplicates (10 samples) are placed in a water bath at 80°C while the other set of ten samples are allowed at room temperature. The stirred 250ml conical flasks serve as reactors. Hydrolysis is then allowed to proceed for about two and half hours. A hand refractometer is used to monitor and analyze the glucose level during the reaction.

The resulting hydrolyzed samples were filtered with filter papers leaving filtrates with high percentage of glucose. These filtrates act as the substrates in the fermentation process. Each filtrate is poured into a portable one liter plastic container which acts as the fermentation medium (fermentation jar).

**FERMENTATION:**

The substrates in the fermentation media were inoculated with *Saccharomyces cerevisiae* as the starter culture. To one of each duplicate, 3g of baker’s yeast was added and to the other, 5ml of freshly isolated yeast was added. This was to determine which of the enzymes was more potent and will ferment which sample best. The sterilized containers were well capped to avoid air entering the reactor medium. The fermentation process was allowed to go on for about five days. At intervals within the fermentation period, the conversion rate of glucose to ethanol was being monitored using a hand refractometer which determined the glucose and ethanol concentration in the sample.

**DISTILLATION:** After the fermentation process, ethanol was recovered using a simple batch distillation method. Distillation sets were mounted and ethanol was recovered at about 78°C. Thermometers were attached to ascertain the temperature of distillation.

Confirmatory tests were then carried out to ascertain that the distillates were actually ethanol (iodoform test and gas chromatography).

**DETERMINATION OF QUANTITY OF ETHANOL PRODUCED**

The distillate collected was measured using measuring cylinder and expressed as quantity of ethanol produced in g/L by multiplying the volume of distillate by the density of ethanol (0.8033g/ml)

**DETERMINATION OF ETHANOL CONCENTRATION**

First Method:
Ethanol concentration was determined by comparing the density of the ethanol produced with the standard ethanol density curve.

Standard ethanol curve was obtained by taking series of percentage ethanol (10, 20, 30, 40 & 50 percent) solution which was prepared in a 100ml volumetric flask and the weight was measured. The density of each of the prepared ethanol was calculated and a standard curve of density against percentage ethanol was plotted.

Second Method Used:

The percentage yield of ethanol was also calculated using the specific gravities of the ethanol solutions produced. Each product (ethanol) was measured using a measuring cylinder and the volume was recorded. It was then weighed with a specific gravity bottle on a weighing balance and the weight noted. The corresponding volume of distilled water was also weighed with the specific gravity bottle and the weight was also recorded. Specific gravity (SG) = weight of ethanol produced (for each sample)/weight of distilled water Percentage ethanol (v/v) = \[8610.6 - (16584 \times SG)] + (7973.3 \times SG \times SG).\] Both the above stated methods were used in this work to calculate the percentage yield of ethanol (with emphasis on the second method). The SG (second) method is used to report the results in this research work

3. Results and discussion

DEFINITION OF TERMS

For proper understanding, the terms used in this chapter are defined below:

RT - room temperature

C₁ and C₃ - cassava peels samples hydrolyzed at 80 °C and RT respectively (using baker’s yeast)

Y₁ and Y₃ - yam peels samples hydrolyzed at 80 °C and RT respectively (using baker’s yeast)

(CY)₁ and (CY)₃ - mixture of cassava and yam peels in the ratio of 1:1 hydrolyzed at 80 °C and RT respectively (using baker’s yeast)

(C₁Y₂)₁ and (C₁Y₂)₃ - mixture of cassava and yam peels in the ratio of 1:2 hydrolyzed at 80 °C and RT respectively (using baker’s yeast)

(C₂Y₁)₁ and (C₂Y₁)₃ - mixture of cassava and yam peels in the ratio of 2:1 hydrolyzed at 80 °C and RT respectively (using baker’s yeast)

C₂ and C₄ - cassava peels samples hydrolyzed at 80 °C and RT respectively (using freshly isolated enzyme)

Y₂ and Y₄ - yam peels samples hydrolyzed at 80 °C and RT respectively (using freshly isolated enzyme)
(CY)₂ and (CY)₄ - mixture of cassava and yam peels in the ratio of 1:1 hydrolyzed at 80 °C and RT respectively (using freshly isolated enzyme)

(C₁Y₂)₂ and (C₁Y₂)₄ - mixture of cassava and yam peels in the ratio of 1:2 hydrolyzed at 80 °C and RT respectively (using freshly isolated enzyme)

(C₂Y₁)₂ and (C₂Y₁)₄ - mixture of cassava and yam peels in the ratio of 2:1 hydrolyzed at 80 °C and RT respectively (using freshly isolated enzyme)

RESULTS

Tables I and II show the volume (cm³), mass (g/cm³), specific gravity and percentage yield of ethanol; (%) of ethanol produced from cassava peels, yam peels and the various mixtures by proportions when hydrolyzed at room temperature and 80 °C respectively, and fermented with baker's yeast. Maximum yield of 3.695g/cm³ with a concentration of 60.52% was produced from the mixture of cassava and yam peels with the ratio 2:1 when hydrolyzed at room temperature (C₂Y₁)₃.

Table I: Samples hydrolyzed at room temperature and fermented with baker's yeast

<table>
<thead>
<tr>
<th>Samples</th>
<th>Volume of Ethanol Produced (cm³)</th>
<th>Mass of Ethanol Produced (g/cm³)</th>
<th>Specific Gravity</th>
<th>Percentage Yield of Ethanol (Conc.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁</td>
<td>18</td>
<td>14.4594</td>
<td>0.99341</td>
<td>4.45</td>
</tr>
<tr>
<td>(CY)₁</td>
<td>8.8</td>
<td>7.0690</td>
<td>0.96555</td>
<td>31.32</td>
</tr>
<tr>
<td>(C₁Y₂)₁</td>
<td>11</td>
<td>8.8363</td>
<td>0.94851</td>
<td>53.86</td>
</tr>
<tr>
<td>(C₂Y₁)₁</td>
<td>4.6</td>
<td>3.6952</td>
<td>0.95561</td>
<td>43.91</td>
</tr>
<tr>
<td>Y₁</td>
<td>11.5</td>
<td>9.2380</td>
<td>0.97146</td>
<td>24.59</td>
</tr>
</tbody>
</table>

Table II: Samples hydrolyzed at 80 °C and fermented with baker's yeast

<table>
<thead>
<tr>
<th>Samples</th>
<th>Volume of Ethanol Produced (cm³)</th>
<th>Mass of Ethanol Produced (g/cm³)</th>
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</tbody>
</table>
Table III: Samples hydrolyzed at room temperature and fermented with freshly isolated enzyme (*saccharomyces cerevisiae*).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Volume of Ethanol Produced (cm³)</th>
<th>Mass of Ethanol Produced (g/cm³)</th>
<th>Specific Gravity</th>
<th>Percentage Yield of Ethanol (Conc.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₄</td>
<td>9.2</td>
<td>7.3904</td>
<td>0.98495</td>
<td>11.30</td>
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<tr>
<td>(CY)₄</td>
<td>6.8</td>
<td>5.4624</td>
<td>0.97944</td>
<td>16.38</td>
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<tr>
<td>(C₁Y₂)₄</td>
<td>9.3</td>
<td>7.4707</td>
<td>0.98915</td>
<td>7.75</td>
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<tr>
<td>(C₂Y₁)₄</td>
<td>6.4</td>
<td>5.1411</td>
<td>0.98262</td>
<td>13.39</td>
</tr>
<tr>
<td>Y₄</td>
<td>6.3</td>
<td>5.0608</td>
<td>0.97414</td>
<td>21.72</td>
</tr>
</tbody>
</table>

Table IV: Samples hydrolyzed at 80 °C and fermented with freshly isolated enzyme (*saccharomyces cerevisiae*).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Volume of Ethanol Produced (cm³)</th>
<th>Mass of Ethanol Produced (g/cm³)</th>
<th>Specific Gravity</th>
<th>Percentage Yield of Ethanol (Conc.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₂</td>
<td>10</td>
<td>8.033</td>
<td>0.99117</td>
<td>6.15</td>
</tr>
<tr>
<td>(CY)₂</td>
<td>5.2</td>
<td>4.1772</td>
<td>0.97358</td>
<td>22.31</td>
</tr>
<tr>
<td>(C₁Y₂)₂</td>
<td>6.0</td>
<td>4.8198</td>
<td>0.96523</td>
<td>31.70</td>
</tr>
<tr>
<td>(C₂Y₁)₂</td>
<td>6.7</td>
<td>5.3821</td>
<td>0.99126</td>
<td>6.08</td>
</tr>
<tr>
<td>Y₂</td>
<td>7.1</td>
<td>5.7034</td>
<td>0.97571</td>
<td>20.09</td>
</tr>
</tbody>
</table>
DISCUSSION

Acid hydrolysis of cassava and yam peels at different temperatures using two different sources of the same enzyme (freshly isolated and baker’s yeast) gave various yields of ethanol.

TEMPERATURE EFFECT

Cassava peels when hydrolyzed at room temperature gave higher yields of ethanol using either freshly isolated yeast or baker’s yeast for fermentation (11.30% and 8.63% respectively). It was also noted that for cassava peels, fermentation with freshly isolated yeast produced more yield of ethanol both at room temperature (11.30%) and at 80 °C (6.15%) than those fermented with ready-made enzymes (baker’s yeast) which produced 8.63% and 4.45% at room temperature and at 80 °C respectively.

Yam peels also produced more ethanol at room temperature than at 80 °C using either of the two enzymes for fermentation (21.72% and 27.08%). But in this case, the use of baker’s yeast for fermentation produced more yield of ethanol from yam peels. Using baker’s yeast, 27.08% and 24.59% were produced at room temperature and at 80 °C respectively while 21.72% and 20.09% ethanol were realized using freshly isolated enzyme.

For the mixtures by proportion, only the ratio of 2:1 of cassava to yam peels (C₂Y₁) produced a higher yield of ethanol at room temperature. (C₂Y₁) gave 60.52% and 13.39% at room temperature using baker’s yeast and freshly isolated enzymes respectively while at 80 °C, it produced 43.91% and 6.08% using baker’s yeast and freshly isolated enzymes respectively. The other mixtures [(C₁Y₂) and (CY) sets of samples] gave higher yields of percentage ethanol at higher temperature (80 °C) than at room temperature as can be seen in tables 3 and 6 above. Every other sets of samples gave higher yields at room temperature. This implies that most of the samples hydrolyzed better at room temperature than at higher temperatures (except for (C₂Y₁) and (CY) sets of...
EFFECT OF ENZYME

It was noticed that in almost all the samples, the percentage yield of ethanol by the samples fermented with baker’s yeast are much higher than the ones gotten from the samples fermented with freshly isolated yeast. The exceptions are for purely cassava (C) samples, (CY)₃ and (CY)₄ which seemed to produce higher yields with the freshly isolated enzyme (yeast). For the other samples, the baker’s yeast proved more effective and fermentation was better with it than with the freshly isolated enzymes while it was vice versa for the purely cassava peels samples and the mixture of cassava and yam peels with ratio 1:1 which was hydrolyzed at room temperature (CY)₃ and (CY)₄. Hence, it implies that baker’s yeast is more effective in the fermentation of most of the samples used for the production of ethanol in this work.

PRETREATMENT

The conversion of cellulose to ethanol requires: pre-treatment (or delignification) to liberate cellulose and hemicelluloses from their complex with lignin; hydrolysis of the carbohydrate polymers to produce free sugars; and fermentation of free sugars (hexose and pentose) to produce ethanol [10]. All the samples were pretreated and they produced appreciable yields of ethanol.

YIELDS

This research work shows that yam peels produce higher concentration (yield) of percentage ethanol either at room temperature or at 80 °C using any of the two enzymes (freshly isolated yeast or baker’s yeast). This invariably shows that it could be more productive using yam peels for ethanol production than cassava peels.

However, the mixture of yam and cassava peels in different proportions also produced good yields of ethanol (in percentage v/v of ethanol). The mixture of cassava and yam peels with ratio 2:1 when hydrolyzed at room temperature and fermented with baker’s yeast gave the highest yield (60.52%) of all the samples worked on in this work. It should be noted that ratio 1:2 of the mixture at 80 °C using same enzyme also gave close value (53.86%).

Based on the results above, it can therefore be seen that production of ethanol from yam and cassava peels (wastes) was most productive when a mixture of both cassava and yam peels are used at ratio 2:1 hydrolyzed at room temperature or ratio 1:2 hydrolyzed at 80 °C using ready-made (baker’s) yeast for fermentation.

4. Conclusion

Ethanol can be produced from biomass in different quantities using either enzyme or acid hydrolysis. However, due to the high cost of production and preservation of enzymes, it is more advisable to carry out the production on a larger scale using acid hydrolysis.
This research work has also shown that it could be more productive producing ethanol from mixture of different sources rather than from the same source. The mixtures of cassava and yam at ratio 1:2 hydrolyzed at 80 °C and 2:1 hydrolyzed at either 80 °C or room temperature gave excellent yield of ethanol when compared to previous works done on the production of ethanol from biomass. This implies that mixtures of different sources of ethanol could be more productive (perhaps, not in all cases).

It can also be concluded from this work that for different samples, hydrolysis is best carried out at different temperatures. Hydrolysis of a particular sample might be complete at a particular temperature while that of the other is best at higher or lower temperatures.

Also, this research work showed that some substrates are best fermented with some enzymes while other substrates will be better fermented using other enzymes as in the case of this work which shows some samples fermenting better with the industrial yeast while others underwent better fermentation with the freshly isolated yeast.

It is also very important to note the right temperature of hydrolysis for each sample. It should be seen from this work that hydrolysis of different samples is better at different temperatures. Some samples hydrolyze better at lower temperatures to produce more glucose while others hydrolyze better at higher temperatures. Hence, temperature of hydrolysis is very important in order to get the best yield of ethanol from the waste cassava, yam or any other peels to be used for ethanol production.

References


[37]. E. Kiran; and H. Balkan. “High-pressure extraction and delignification of red spruce with binary and ternary mixtures of acetic acid, water, and supercritical carbon dioxide”. Journal of Supercritical Fluids 7, 75. (1994).


