Optimization and Kinetics of Glucose Production via Enzymatic Hydrolysis of Mixed Peels

Ude, Michael U.ᵃ,ᵇ, Oluka, Ikeᵇ, Eze, Paul C.ᵇ

ᵃEngineering Research Development and Production, Projects Development Institute (PRODA), Emene, Enugu State, Nigeria
ᵇDepartment of Agricultural and Bioresource Engineering, Enugu State University of Science and Technology (ESUT), Enugu State, Nigeria

A R T I C L E  I N F O

Article history:
Received 10 May 2020
Received in revised form 30 June 2020
Accepted 21 July 2020
Available online 19 October 2020

A B S T R A C T

The optimization and kinetics of glucose production via enzymatic hydrolysis of mixed peels were carried out. The substrate was characterized using proximate analysis and hydrolysis. The process was optimized using response surface methodology while the kinetics of the hydrolysis was studied using Michaelis-Menten model. The results obtained showed that the mixed peels have high hemicellulose content, hydrolysable carbohydrate and cellulose. The optimum conditions for glucose yield of 79% by enzymatic hydrolysis are temperature of 36 °C, time of five days, pH of 4.55 and enzyme dosage of 0.428 g/50mL. Enzymatic hydrolysis obeyed Michaelis-Menten kinetic model with spontaneous and feasible reactions. Therefore, the results have proved that mixed peels (cassava and potato peels) is a good substrate for glucose production and study of other waste peels as feed-stock is recommended.

Keywords: enzymatic hydrolysis optimization glucose cassava and potato peels

1. Introduction

Energy consumption has increased steadily over the last century as the world population has grown and countries have become industrialized. Crude oil has been the major resource to meet the increased energy demand. It has been predicted by researchers that annual crude oil production will decline drastically by 2050 (Sun and Cheng, 2002). Because the economy of many other nations of the world depends on oil, the consequences of inadequate oil availability could be severe. Therefore, there is a great interest in exploring alternative energy sources. One of the alternative energy sources is glucose to bio-ethanol.

Bioethanol is a principal fuel that can be used as petrol substitute for vehicle (Adeeyo et al., 2015). It is a renewable energy source produced mainly by glucose fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam (Lalitha and Sivaraj, 2011). Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol (Lalitha and Sivaraj, 2011). The main sources of sugar required to produce ethanol come from fuel or energy crops such as maize, cassava and cassava products, wheat crops, waste straw, guinea corn husk, rice husk, millet husk, sawdust and sorghum plant (Adeeyo et al., 2015).

The use of starchy food as feed stocks for glucose (source of bio-ethanol) production in Nigeria will compete with the food chain and make the cost of bio-ethanol expensive. An alternative feed stock from waste/peels thus needs to be found to produce an affordable bio-ethanol for economic empowerment in Nigeria. The production of glucose from lignocellulosic biomass like mixture of cassava and potato peels is a better choice than starch and sugar crops because of the comparative accessibility agricultural, and other cellulosic resources which do not compete with existing food chain (Manikandan et al., 2008). In addition, converting these peels into valuable product like glucose and ethanol provides a potential alternative for treatment and disposal of such materials.

Furthermore, the conversion of waste lignocelluloses and lignocelluloses materials to glucose will lead to diversification of
economy, reduce unemployment and clean environment without altering human food chain (Ajani et al., 2011). The use of combination of different lignocelluloses materials to produce glucose for production of bio-ethanol that will be sufficient for commercial purpose is yet to be explored. Therefore, this study is focused on optimization and kinetics of production of glucose from mixture of cassava and potato peels via enzymatic hydrolysis.

2. Materials and Methods

2.1. Materials

Cassava and potato peels were collected from Abakaliki, Ebonyi State. The *Aspergillus niger* cellulase was purchased from Ogbete Main Market, Enugu.

2.2. Methods

2.2.1. Characterization

The peels were adequately washed with clean water and dried under sunlight for 72 h. They were then grounded with a grinding machine and sieved to particle size of 0.15 mm. The cellulose and hemicellulose composition was measured according to Onyelucheya et al. (2016). The method of Ana et al., (2013) was adopted in determining lignin content of the sample with little modification.

1) Sample De-Fatting

The 50 g of sample was weighed and placed in a 250 mL flat bottom flask. Some quantity of *n*-hexane was added and was thoroughly shaken to proper mixing. The mixture was covered using masking foil and masking tape as well. It was kept for 48 h to achieve complete fat removal. The mixture was filtered and the residue was dried at room temperature.

\[
\text{Fat extracted} \, (\%) = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100\%
\]

2) Bleaching (lignin removal)

The de-fated sample was introduced to a three-neck flat bottom flask fitted with a reflux condenser and 500 mL of 7.5% weight by volume (w/V) aqueous solution of H\(_2\)O\(_2\) was added. The whole solution was refluxed in the condenser at 90 °C for two hours with cold water for complete lignin extraction. The solution was cooled and filtered with a vacuum pump. The hot water was used to wash the solution water to neutralize the residue and then dried at ambient temperature.

\[
\text{Lignin} \, (\%) = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100\%
\]

3) Sodium hydroxide treatment (hemicellulose removal)

The bleached sample was introduced in a flat bottom round flask. The 500 mL of 18% NaOH solution was introduced into it and was mixed at an ambient temperature for 30 min. During mixing, the flask was covered using aluminum foil. After this, the solution was filtered by using a vacuum pump. It was washed with 500 mL of 20% acetic acid in hot water. The solution was washed again with hot distilled water to neutralize the residue. The filtrate was tested with a pH meter to confirm the neutrality of the residue. The residue was allowed to dry. The percentage hemicellulose and other extractives were given as:

\[
\text{Hemicellulose} \, (\%) = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100\%
\]

\[
\text{Cellulose} \, (\%) = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \times 100\%
\]

4) Protein determination (Microkjeldahl method)

The 2 g of sample was weighed and transferred into a 50 mL kjeldahl digestion flask. There 8 g of catalyst mixture was added into the digestion flask. The catalyst mixture was made up of 96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide. The 20 mL of concentrated sulphuric acid was added into the digestion flask. The digestion flask was connected into the macro kjeldahl outfit usually operated within a fume cupboard and heated gently in an inclined position. Then the digest was allowed to cool and washed with 400 mL of distilled water, free of ammonia. The 50 mL of 4% boric acid solution, two drops of 2% methyl red indicator was added to the diluted digest. The distillation apparatus was connected with the delivery tube, dipping below the boric acid solution. The 50% of sodium hydroxide was added to the diluted digest and the solution was distilled into the known volume of boric acid at temperature of 100 °C. The distillate was titrated with 0.05 mol/L sulphuric acid solution.
Protein (%) = \frac{\text{Titre value molarity of titrant conversion factor (6.25)}}{\text{Sample weight}} \times 100\% \quad (5)

Moisture content determination by oven method

The 5 g of sample was weighed into a clean drying petri dish of a known weight. The sample in petri dish was transferred into drying hot air oven already at 105 °C and allowed for two hours. It was later transferred from the oven to functional desiccators and allowed to cool for three hours. Then the dish and the dry matter were weighed.

\text{Moisture} (%) = \frac{\text{Weight of sample before drying} - \text{weight of sample after drying}}{\text{Sample weight}} \times 100\% \quad (6)

Crude fiber determination

The 5 g of sample was weighed into a 500 mL flat bottom round flask and 200 mL of 0.1275 mol/L sulphuric acid (H$_2$SO$_4$) was added to it. The flask was connected to a reflux condenser and the whole fittings connected to a source of heat (heating mantle). The mixture was allowed to boil gently for 30 min. The hot sample acid mixture was carefully filtered (at the end of the 30 min boiling), using the whatman No.1 filter paper and the residue (from the sample acid mixture) was then washed with distilled water. The washed residue was quantitatively transferred back to the round bottled flask. The 200 mL of 0.313 mol/L sodium hydroxide solution was introduced to the acid treated sample in the round bottomed flask (2.50 g of carbon free sodium hydroxide per 200 mL solution). The flask was returned under condenser and boiled again for 30 min. The mixture was filtered through a weighed ash-less filter paper and the residue was washed thoroughly with alcohol (ethanol). The residue and ash-less filter paper were placed in an electric oven (for drying) at 100 °C to constant weight. The moisture free residue and the filter paper were weighed. The fiber and ash less filter paper were transferred into a weighed porcelain crucible and transfer into a muffle furnace at 600 °C for three hours. At three hours, the furnace was switched off and crucible transferred (using thongs) as quickly as possible to desiccators. It was allowed to cool and weigh.

\text{Crude fiber} (%) = \frac{\text{Weight of fibre}}{\text{Sample weight}} \times 100\% \quad (7)

Lipid extraction

The 5 g of sample was weighed into a semi permeable thimble with the ends plugged. The thimble and content were introduced into the central syphon of the soxhlet apparatus. There 200 mL of normal hexane was introduced into the flask and connected to soxhlet siphon and condenser. It was reflux for two hours while water is passed through the condenser. The content of the flask was poured into a clean weighed beaker and dry in oven at 105 °C for two hours. It was allowed to cool in desiccator and reweighed.

\text{Lipid} (%) = \frac{\text{Weight of lipid}}{\text{Sample weight}} \times 100\% \quad (8)

Total ash content determination

The ash content was determined from the total loss in weight that occurred during the incineration of the sample at enough high temperature to allow all organic matter to be burnt off without allowing appreciable decomposition of the ash content or loss by volatilization. It is the inorganic residue remaining after the organic matter has been burnt away. An empty clean porcelain crucible (w$_o$) was weighed. The 5 g of sample was weighed into the crucible and heated in a muffle furnace at 600 °C for three hours. It was allowed to cool in desiccators and weighed (w$_b$).

\text{Ash} (%) = \frac{(w_o + 5) - w_i}{5} \times 100\% \quad (9)

Carbohydrate determination

Total carbohydrate was determined by difference:

\text{Carbohydrate} = 100 - (\text{protein + ash + lipid + crude fibre + moisture}) \quad (10)

2.2.2. Enzyme assay

The method used by Omenu et al. (2005) was adopted with little modification to determine the commercial cellulase activity. This was carried out in a reaction mixture of 3 mL of the enzyme, 3 mL of 2% (w/V) starch solution of mixed peel and 0.3 mL of buffered solution (pH 4) of citrate. It was incubated at 60 °C for 60 min and the reaction was quenched using water boiling at 101 °C for 3 min. The dinitro salicylate (DNS) method was used to evaluate the quantity of reducing sugar produced. The A. niger cellulase activity was expressed as the quantity of enzyme that evolved a given amount of D-glucose from starch in a given volume of a reacting mixture under the conditions for assay.
2.2.3. Enzymatic hydrolysis with *A. niger*

The mixed peel cellulose was hydrolyzed using *A. niger* cellulase enzyme. The hydrolysis was performed in 500 mL conical flask by adding 100 mL of 10% of the enzyme to 100 mL dose of mixed peel cellulose. The mixture was placed on a magnetic stirrer to incubate it and stirred at a rate of 300 r/min for various temperatures, different time intervals and pH. The solution was filtered and the filtrate was examined to determine the soluble sugar yield using the refractometer (RF M960 Model, USA) while the DNS method was used to determine the reducible sugar (Kongkiattikajorn, 2012). The same procedure was repeated for pH of 2.5, 3.5, 4.5, 5.5 and 6.5, and 3, 4, 5, 6 and 7 d at 25 °C, 30 °C, 35 °C, 40 °C and 45 °C. The enzyme dosage was also varied at 0.3, 0.4, 0.5, 0.6 and 0.7 g/50 mL.

2.2.4. Optimization of enzymatic hydrolysis

The Central Composite Rotatable Design (CCRD) was also applied for the optimization enzymatic hydrolysis of the mixed peels. Tables 1 and 2 show the details of the experimental design. The empirical equation is represented as shown below:

\[
Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j
\]  

(11)

Selection of levels for each factor was based on the experiments performed to study the effects of process variables on the enzymatic hydrolysis of mixed peels.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Studied range of each factor in actual and coded form for enzymatic hydrolysis of glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>Low level</td>
</tr>
<tr>
<td>Temperature (A, °C)</td>
<td>30(–1)</td>
</tr>
<tr>
<td>Time (B, d)</td>
<td>4(–1)</td>
</tr>
<tr>
<td>pH (C)</td>
<td>3.5(–1)</td>
</tr>
<tr>
<td>Enzyme dosage (D) (g/50 mL)</td>
<td>0.4(–1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Experimental design Matrix for enzymatic hydrolysis of glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run order</td>
<td>Temperature (A, °C)</td>
</tr>
<tr>
<td>Coded</td>
<td>Real</td>
</tr>
<tr>
<td>1</td>
<td>–1</td>
</tr>
<tr>
<td>2</td>
<td>+1</td>
</tr>
<tr>
<td>3</td>
<td>–1</td>
</tr>
<tr>
<td>4</td>
<td>+1</td>
</tr>
<tr>
<td>5</td>
<td>–1</td>
</tr>
<tr>
<td>6</td>
<td>+1</td>
</tr>
<tr>
<td>7</td>
<td>–1</td>
</tr>
<tr>
<td>8</td>
<td>+1</td>
</tr>
<tr>
<td>9</td>
<td>–1</td>
</tr>
<tr>
<td>10</td>
<td>+1</td>
</tr>
<tr>
<td>11</td>
<td>–1</td>
</tr>
<tr>
<td>12</td>
<td>+1</td>
</tr>
<tr>
<td>13</td>
<td>–1</td>
</tr>
<tr>
<td>14</td>
<td>+1</td>
</tr>
<tr>
<td>15</td>
<td>–1</td>
</tr>
<tr>
<td>16</td>
<td>+1</td>
</tr>
<tr>
<td>17</td>
<td>–2</td>
</tr>
<tr>
<td>18</td>
<td>+2</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>
### 2.2.5. Kinetic modelling of enzymatic hydrolysis of mixed peels

The Michaelis-Menten kinetic model presented in the Equation (12) (Fogler, 1999) was used to study the kinetics of enzymatic fermentation.

\[
-r_s = \frac{V_{\text{max}} C_s}{K_M + C_s}
\]

where \( r_s \) is the reaction rate for enzymatic fermentation; \( V_{\text{max}} \) is the maximum reaction rate; \( K_M \) is the Michaelis-Menten constant; and \( C_s \) is concentration of the substrate. The linearization Equation (12) gives Equation (13).

\[
-\frac{1}{r_s} = \frac{1}{V_{\text{max}}} \frac{K_M}{V_{\text{max}}} + \frac{1}{C_s}
\]

Plotting \(-\frac{1}{r_s}\) versus \(\frac{1}{C_s}\) yielded a straight line where the intercept and the slope were used to deduce \(V_{\text{max}}\) and \(K_M\), respectively.

### 3. Results and Discussion

#### 3.1. Characterization of substrate

Table 3 shows the proximate composition of the mixed peels. The sample was observed to have relatively low moisture content of 0.42%. The proximate analysis of the mixed peels showed that it contained high percentage of hydrolysable carbohydrate and cellulose. It implies that the mixed peel is a good substrate for glucose production which will be converted to bio-ethanol.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Proximate analysis</th>
<th>Composition before treatment (%)</th>
<th>Composition after treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein</td>
<td>5.43</td>
<td>0.96</td>
</tr>
<tr>
<td>2</td>
<td>Ash</td>
<td>3.89</td>
<td>4.35</td>
</tr>
<tr>
<td>3</td>
<td>Fibre</td>
<td>1.28</td>
<td>1.32</td>
</tr>
<tr>
<td>4</td>
<td>Lipid</td>
<td>2.04</td>
<td>0.22</td>
</tr>
<tr>
<td>5</td>
<td>Moisture</td>
<td>0.41</td>
<td>0.38</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrate</td>
<td>86.95</td>
<td>92.77</td>
</tr>
<tr>
<td>7</td>
<td>Cellulose</td>
<td>32.10</td>
<td>34.70</td>
</tr>
<tr>
<td>8</td>
<td>Hemicellulose</td>
<td>39.23</td>
<td>39.48</td>
</tr>
<tr>
<td>9</td>
<td>Lignin</td>
<td>15.19</td>
<td>15.19</td>
</tr>
<tr>
<td>10</td>
<td>Extractive</td>
<td>5.23</td>
<td>6.74</td>
</tr>
</tbody>
</table>
3.2. Enzyme activity

The *A. niger* cellulase has good enzymatic activity for the hydrolysis of the mixed peel cellulose. It gave 800 U/mL enzyme activity. This may be attributed to the fact that little or no difficulty occurred in diffusing the substrate to the enzyme active cites.

3.3. Statistical analysis and optimization of enzymatic hydrolysis of mixed peels

The empirical relationship between yields of glucose produced ($Y$) and the three variables in coded values obtained by using the statistical package Design-Expert 10 version for determining the levels of factors which give optimum glucose yield by the equation below. A quadratic regression equation that fitted the data after the removal of the insignificant terms was started in Equation (14).

$$Y = 80.33 + 1.00A + 1.58B - 0.42C - 0.75AB - 0.75AD - 2.63BC - 1.12BD - 3.50A^2 - 3.00B^2 - 5.13C^2 - 3.37D^2$$  \hspace{1cm} (14)

3.4. Three dimensional surface plot and contour plot for yield of glucose

The 3D response surface plots were generated to estimate the effect of the combinations of the independent variables on the yield of glucose. These plots are shown in Figs. 1, 2, 3 and 4. The figures show the dependency of glucose yield on the interaction of temperature, time, pH and enzyme concentration.
Figure 1 shows the interaction of temperature and time on yield of glucose. It is found that glucose yield increases as both temperature and time increase but a decrease in glucose yield is observed at high temperature above 35 °C. This could be that the high temperature denatured the enzyme thereby reducing its activity. Similar result was obtained by Pramanik and Rao (2015).

Figure 2 presents the interaction of temperature and enzyme dosage on yield of glucose. It is observed that glucose yield increases as both temperature and enzyme dosage increase but a decline in glucose yield is observed at high temperature above 35 °C. This could be that high temperature does not favour enzymatic activity. The mixed peel cellulose yielded about 68% glucose at 35 °C and enzyme dosage of 0.4 g/50 mL.

Figure 3 shows the interaction of pH and time on yield of glucose. It is observed that glucose yield increases as both pH and time increase but a decrease in glucose yield is observed at high pH above 5.5. This could be that the enzyme performs optimally in a mild acidic condition. Figure 4 depicts the interaction of enzyme dosage and time on yield of glucose. It is found that glucose yield increases as both enzyme dosage and time increase but a decrease in glucose yield is observed at high enzyme dosage above 0.4 g/50 mL. This could be that there was enough enzyme to convert the cellulose to glucose. Similar result was obtained by Pramanik and Rao, (2015).

### 3.5. Optimization result

The glucose yield was optimized with the design expert giving 80.70% at optimum conditions of temperature, 35.7 °C, time, 5 d, pH 4.55 and enzyme dosage of 0.482 g/50 mL with desirability of 0.986. The enzymatic hydrolysis under the obtained optimum operating conditions was carried out in order to evaluate the precision of the quadratic model; the experimental value and predicted values are shown in Table 4. The yield of glucose obtained experimentally is 79.10% and the remaining 20.9% could be a side product such as maltose. Comparing the experimental and predicted results, it can be seen that the percentage error between the experimental and predicted is 2.02%, therefore it can be concluded that the generated model has sufficient accuracy to predict the glucose production via enzymatic hydrolysis.
3.6. Kinetics of production of glucose via enzymatic hydrolysis of cellulose

A plot of the reciprocal of rate of decomposition of the substrate \((1/r_s)\), versus the reciprocal of substrate concentration \((1/C_s)\) is expected to yield a straight line with an intercept \(1/V_{\text{max}}\) and slope \(K_M/V_{\text{max}}\) if the data agree with the Michaelis-Menten model. A plot of this using the generated experimental data is shown in Fig. 5, from which it can be seen that the intercept gives a positive value. It implies that the data agree with the Michaelis-Menten model.

Based on the values of the Michaelis-Menten parameters presented in Table 4, the kinetics equation for enzyme hydrolysis is given in Equation (15). The feasibility and ease of reaction can be determined with Gibbs free energy \((\Delta G)\). The lower the \(\Delta G\), the higher the feasibility vice-versa (Badgujar and Bhange, 2015; Jadhav and Gogate, 2014). The lower negative value of \(\Delta G\) for enzyme hydrolysis shows that the hydrolysis is highly feasible suggesting rapid spontaneous disintegration of the enzyme-substrate complex into the product (Hirakawa et al., 2005; Badgujar and Bhange, 2015).

\[
-r_s = \frac{28.2C_s}{60.5 + C_s}
\]  

(15)

4. Conclusions

The results obtained in this study have shown that combination of cassava and potato peels is a good quantity of hydrolysable cellulose which can be hydrolyzed by enzyme. The optimum conditions for glucose yield of 79% by enzymatic hydrolysis are temperature of 36 °C, time of five days, pH of 4.55 and enzyme dosage of 0.482 g/50mL. Enzymatic hydrolysis obeyed Michealis-Menten kinetic model with feasible reactions. It was observed that enzymatic hydrolysis of the mixed peel cellulose was achievable. Therefore, it is recommended that other waste peels as feed-stock can be studied.

References


