Pilot plant production of ethanol from cassava starch and fermentation kinetic studies

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ABSTRACT
In this study, hydrolyzed cassava starch (containing glucose) was fermented in a fabricated 25.8 L pilot scale batch fermenter kept at a temperature of 30-34°C and 4-6 pH for 108 h to study their fermentation kinetics. Acid treatment of the cassava starch hydrolysis was carried out using diluted sulphuric acid (1.5M) such that the maximum concentration of sugar obtained was found to be 42 g/mL after 180 min of hydrolysis, which is used as the initial sugar concentration for ethanol production using Baker’s yeast (Saccharomyces cerevisiae). Kinetic parameters (\( \mu_{\text{max}} = 0.0465 \, \text{h}^{-1} \) and \( K_s = 15.375 \, \text{g/mL} \)) were determined from and studied using the Monod kinetic model and the Logistic kinetic model, simultaneously. Findings reveals that the maximum biomass concentration, ethanol concentration, ethanol yield and the maximum % sugar utilization rate are 7.3 g/mL, 29 g/mL, 69% and 47.62 %, respectively after 96 h of fermentation. Specific growth rate based on the use of the Logistic kinetic model gave \( \mu = 0.0225 \, \text{h}^{-1} \). This study applied the dry weight spectrophotometric analysis and refractive index method respectively to analyze the biomass and sugar amounts, while the ethanol concentrations were derived from the specific gravity method. Diversification of yeast type (alternatively, Zymomonas mobilis, Candida albicans, Cryptococcus neoformans, Saccharomyces bouardii, Pichia pastoris, Kluyveromyces lactis etc.) in the production of ethanol and their separate examination to checkmate their resultant yield should be carried out in the future. Presently, the development of a universal kinetic model that is valid for an extensive range of fermentation methods, which is capable of providing a theoretical basis for the existing empirical models is essential in this field. By studying more fermentation processes and by integrating extra factors that affect fermentation, research will get closer to a more general model.

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1. Introduction:
Increased demand for ethanol (C\(_2\)H\(_5\)OH) for several industrial drives for example, as alternative source of energy, solvents, cleansing agents, preservatives and in pharmaceuticals and beverage industries, has brought about an upsurge in the manufacture of the alcohol [1]. Incorporation of C\(_2\)H\(_5\)OH into the fuel market for energy generation had already begun, in order to augment depleting reserve all over the world, and there is currently a shift to microbial fermentation process for its production [2]. More than half of C\(_2\)H\(_5\)OH manufacturing cost goes to raw materials alone triggering the exploitation of cheap renewable agricultural resources (sugar, starchy or lignocellulose) such as cassava, maize, sugarcane, molasses, sugar beet, sweet sorghum and sawdust, to mention a few. Genuine effort to now translate these raw materials through aerobic or

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anaerobic bio-fermentation must start from the laboratory where kinetic data could be easily generated and analyzed before large-scale conversion. Yeast is an agent that directly consumes simple sugars in the feedstock and convert them to C₂H₅OH [3]. Basically, C₂H₅OH production from cassava feedstock involves 5 main steps which are feedstock preparation, cooking (at 140–180°C), starch hydrolysis, yeast fermentation and lastly, distillation and dehydration [4]. During hydrolysis, the complex starch molecules are broken down into short chain molecular compounds like glucose and fructose. This process helps the hydrolyzed solution to be susceptible to attack by Saccharomyces cerevisiae, which convert reducing sugar into alcohol and carbon dioxide. The produced C₂H₅OH in its pure form will be a colorless liquid, miscible with water, ether, acetone and benzene. Other properties are 78.39°C boiling point, –114.15°C freezing point, 13°C flash point in a closed vessel and 425°C auto ignition temperatures [5-6].

Currently, Nigeria, Brazil and Thailand are ranked highest in world cassava production [7]. Cassava pulp contains 61.84-69.90% starch, 10.61-27% fiber, 72-85% moisture and other carbohydrates such as cellulose, galactain, arabinan, xylantirhamnan and mannan. Its starch content will facilitate hydrolysis followed by sugar fermentation, while its water content makes it easily degraded by microorganisms [8]. Cassava starch is produced primarily by the wet milling of fresh cassava roots or from dry cassava chips and a preferred choice for adhesive production. Fermentation of starch is to a certain degree more intricate than fermentation of sugars because starch must first be converted into sugar and then into C₂H₅OH [9]. Several manuscripts have been published on production of C₂H₅OH fermentation by microorganisms, and several yeasts, bacteria, and fungi have been reportedly used for the production [10-12]. The terms fermenter and bioreactor are used interchangeably, generally categorized into two main classes of bioreactors: anaerobic and aerobic. A fermentation pilot plant is a small fermentation processing system which is operated to generate information about the behavior of the fermentation system for use in design of larger industrial facilities [13]. The most common type of bioreactor used in industry is the stirred tank reactor, where a draught is fitted to provide a defined circulation pattern. The first problems encountered in studying fermentation kinetics are the establishment of consistent rate expression and the selection of meaningful rate processes to be measured. Some primary process variable in fermentation are temperature, pH and nutrient (or reactant) concentration. Jacques Monod growth kinetic model developed in 1942 [14] can also be used under the above suitable process conditions to study the growth stages of the microorganism present in the pilot plant. Four factors, namely substrate limitation, substrate inhibition, product inhibition and cell death, influence cell growth and C₂H₅OH fermentation. Most of the models discussed in this study account for only one or two of these factors and a model that accounts for all four of these factors will most likely divulge an enhanced picture of fermentation [15].

The aim of embarking on this work is to obtain C₂H₅OH from cassava using a pilot plant and to study the kinetics involved in the pilot plant production of C₂H₅OH from the raw material. Other specific objectives are to hydrolyze cassava starch to glucose by acid hydrolysis and assess the feasibility of the production of C₂H₅OH by fermentation. Consequently, the bioethanol produced using the pilot scale plant design may be used in institutions and technological transfer programs for research work. This work followed a different approach comparable with reports of Akande & Mudi (2005) [16], in which the kinetic model for production from cassava starch by Saccharomyces cerevisiae yeast strain was also studied. Their results indicated that the model could predict optimum fermentation performance using sugar in cassava as the substrate alternative to the use of Monod herein. Regarding hydrolysis, literature reviewed show that the main acid used are hydrochloric, sulphuric, acetic acid and oxalic acid. According to Thongchul et al. (2010) [17], either acid or enzymatic hydrolysis of cassava pulp gives a high glucose concentration (>100 g/L); however, acid hydrolysis produced a higher glucose concentration (85% of theoretical yield) than did enzymatic hydrolysis (<40% of theoretical yield) using cellulase, a-amylase and glucoamylase. Wojciechowski et al. (2002) [18] highlights the beneficial and economical merit that acid hydrolysis had over enzymatic hydrolysis, while Srinorakutra et al. (2006) [19] preferred enzymatic hydrolysis to acid hydrolysis due to the unwanted browning compounds and the higher cost of acid hydrolysis. Their findings and view forms partially, the reason for the selection of sulphuric acid (H₂SO₄) of hydrolysis in this work.

2. Materials and methods:
2.1. Instruments and Reagents:
This study utilizes the following equipment: namely, beaker, 250 mL Erlenmeyer flask, hot plate, weighing balance, condenser, GP 353 ATC pH meter, fermenter, thermometer, refractometer, hydrometer, Cole Parmer UV-7504 Spectrophotometer and a 0.5 hp pump. For the study, fresh cassava tubers were purchased from a local farm in Adamawa State. Reagents including, Benedict’s solution, cassava starch, 4 N sodium hydroxide (NaOH) and 98% H₂SO₄ were used together with the laboratory instruments mentioned to serve different roles at various stages of the work.

2.2. Sample Preparation:
2.2.1. Feed Preparation:
Fresh cassava tubers were peeled, washed and grated, leading to the formation of a pulp. The resulting pulp was sieved through a 300 μm diameter sieve. Resultant liquor after sieving was allowed to settle for 30 min and the water decanted.
Subsequently, the starch slurry was bagged and pressed. To conclude the preparatory stage, the wet starch was sun dried and stored.

2.2.2. Acid Hydrolysis:
2400g of the cassava starch was hydrolyzed by dispersing in 3.6 L of H₂SO₄ with solution strength of dilute 1.5M. At a temperature of 95°C and for 4 h, the slurry obtained was heated in the hydrolysis vessel using a hot plate. The chosen temperature falls within the range examined by Srinorakutara et al. (2006) [19] during acid hydrolysis of casava waste. Reaction mixture was stirred at regular intervals of 30 min to maintain the reaction medium in suspended state. The mixture was observed to attained a desired temperature of 95°C within 30 min after which 30 mL of the reaction mixture was taken out at a 30 min interval to analyze the total reducing sugar. After the specified time of the hydrolysis, 2L of 4N NaOH was used to neutralize the activity of H₂SO₄ that may be present. In Wojciechowski et al. (2002) [18], hydrochloric acid was used to hydrolyze cassava bagasse without the need to neutralize it with a base, differently as carried out in this research. Also, Yoonan et al. (2007) [20] used neutralized hydrolysates of sugar and glucose for ethanol production. The sugar concentration of clear glucose syrup obtained was then determined using a refractometer.

2.2.3. Yeast Preparation:
Forty percent yeast inoculum (40g of dry baker’s yeast rehydrated in 100 mL of distilled water at 37°C for 10 min) was prepared accordingly.

2.3. Fermentation and Distillation:
Under an anaerobic condition, the fermentation process was carried out with hydrolyzed cassava starch (glucose) as substrate and saccharomyces cerevisiae (bakers’ yeast) as fermenting micro-organism. A designed and constructed pilot fermenter was used for the fermentation. Precisely 10L of hydrolyzed cassava starch was fed to the fermenter. Also added to the fermenter was 40g of activated baker’s yeast. Air tight conditions were ensured during fermentation by applying grease to air prone closures and ensuring that the sampling line was tightly shut. Agitation of the fermentation broth was done regularly using a stirrer attached to an electric motor to increase fermentation efficiency. Samples were taken from the fermenter at an interval of 6 hours for 5 days to measure the yeast cell concentration, sugar and C₆H₁₂O₆ concentration. A thermometer and pH meter were used to measure the samples’ temperature and pH, respectively. An optimum pH value of 4-5 with temperatures between 30-35°C were maintained during the fermentation. By the use of 4N NaOH, the pH was maintained in the region of 4-6. The fermented broth was distilled using a distillation set up.

2.4. Concentrations Measurement:
2.4.1. Determination of Biomass Concentrations:
A Spectrophotometer (Cole Parmer UV-7504) was used to determine the yeast cell concentration at a 12 h interval. The spectrophotometer measured the optical density of the sample. A small quantity of the sample was then transferred to a cuvette placed in the spectrophotometer, and the absorbance of the sample was taken at a wavelength of 630 nm. Water was used as blank. A calibration curve to relate the absorbance measured by the spectrophotometer to the amount of yeast cells present was prepared by using known amounts of yeast cells. Common sense reveals that the maximum biomass concentration is the highest value of the biomass concentration in g/L. This idea was derived from Chavan et al. (2013) [21] and used to find the highest/maximal biomass concentration.

2.4.2. Ethanol Concentration Measurement:
Ethanol concentration was measured using a hydrometer and a calibration curve of different concentrations of C₆H₁₂O₆. Using this value, ethanol yield (g/g), was calculated using Equation 1 given by Abdullahi (2013) [22] and Irfan et al. (2014) [23].

\[
\text{Ethanol Yield} = \frac{\text{Weight of Ethanol Produced}}{\text{Mass of Glucose Utilized}} \quad (1)
\]

2.4.3. Quantifying Sugar Concentration:
Refractometer was used to determine the concentration of sugar via the refractive index. First, the sample was transferred to the refractometer and the samples’ refractive index was read including the corresponding sugar concentration. To determine the % sugar conversion, Equation 2 by Chavan et al. (2013) was used.

\[
\% \text{ Sugar Conversion} = \left( \frac{\text{Initial Sugar Conc.} - \text{Final Sugar Conc.}}{\text{Initial Sugar Conc.}} \right) \times 100 \quad (2)
\]
2.5. Kinetic Parameters Computation:

2.5.1. Specific Growth Rate:
Values of fermentation kinetic parameters can be respectively determined from the Monod model or Equation 3 given by Manikandan & Viruthagiri (2010) [24]. Equation 4 or the Monod Logistic model (growth) was used to determine the specific growth rate, \( \mu \) at different biomass concentration.

\[
\mu = \mu_{\text{max}} \frac{S}{K_s + S} \quad (3)
\]

\[
\mu = \frac{1}{t} \ln \left( \frac{X}{X_0} \right) \quad (4)
\]

Where \( \mu \) = specific growth rate \( (\mu = \mu_{\text{max}}/2) \), \( \mu_{\text{max}} \) = maximum specific growth rate, \( S \) = substrate concentration, \( t \) = time taken, \( X_0 \) = initial biomass/cell concentration, \( X \) = biomass concentration at time \( t \), and \( K_s \) = substrate saturation constant (i.e., substrate concentration at half \( \mu_{\text{max}} \)). The said kinetics parameters are \( \mu_{\text{max}} \) and \( K_s \). In order to compute them, the holding time was first calculated.

2.5.2. Calculation of Holding Time for the Batch System:

\[
t = -\int_{S_0}^{S_g} \frac{dS_g}{-r_G} \quad (5)
\]

Monod growth rate, \( \mu \) in Equation 1 was taken as \( -r_G \) = rate of glucose utilization, where we arrived at Equation 6 (customized for this study).

\[
-r_G = \mu_{\text{max}} \frac{S}{K_s + S} \quad (6)
\]

Equation 6 was then fixed into Equation 5 to produce Equation 7.

\[
t = -\int_{S_0}^{S_g} \frac{K_s + S}{\mu_{\text{max}} S} dS_g \quad (7)
\]

Applying the Simpson’s rule on Equation 7, \( t \) was determined using Table 1 auxiliary information.

In Table 1, the ‘c’ term values in column 4 are function coefficients in the original Simpson’s 1/3 formula while ‘f’ is the function itself whose values are in column 3. Based on the rule, the product of the ‘cf’ term values sum and the ‘S’ interval (2.5), was divided by 3 to get \( t \). Therefore, \( t = 239.208 \text{ h} = 9.967 \text{ days} \) \( (\cong 10 \text{ days}) \) was used as the length of time to keep the substrate.

Table 1. Simpson’s Rule tables.

<table>
<thead>
<tr>
<th>( S )</th>
<th>( \frac{\mu_{\text{max}} S}{K_s + S} = -r_A )</th>
<th>( 1/-r_A )</th>
<th>( c )</th>
<th>( cf )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.039</td>
<td>25.38</td>
<td>4</td>
<td>101.54</td>
</tr>
<tr>
<td>5.0</td>
<td>0.068</td>
<td>14.62</td>
<td>2</td>
<td>29.23</td>
</tr>
<tr>
<td>7.5</td>
<td>0.091</td>
<td>11.03</td>
<td>4</td>
<td>44.10</td>
</tr>
<tr>
<td>10.0</td>
<td>0.108</td>
<td>9.23</td>
<td>2</td>
<td>18.46</td>
</tr>
<tr>
<td>12.5</td>
<td>0.089</td>
<td>11.23</td>
<td>4</td>
<td>44.92</td>
</tr>
<tr>
<td>15.0</td>
<td>0.134</td>
<td>7.44</td>
<td>2</td>
<td>14.87</td>
</tr>
<tr>
<td>17.5</td>
<td>0.144</td>
<td>6.92</td>
<td>4</td>
<td>27.39</td>
</tr>
<tr>
<td>20.0</td>
<td>0.153</td>
<td>6.54</td>
<td>1</td>
<td>6.54</td>
</tr>
<tr>
<td>Sum:</td>
<td></td>
<td></td>
<td></td>
<td>287.05</td>
</tr>
</tbody>
</table>

2.6. Pilot Plant Fermenter Design:
Using literature cylindrical volume expression shown in Table 2, the tank sizing was carried out. Parameters without expression were measured using tape or a distance/length measuring device as shown in Figure 1. The Paint and Microsoft Word AutoShapes tools were used concurrently to produce Figure 1 which was then translated to Figure 2 using appropriate fabrication gadgets.
Table 2. Design Parameters of a Pilot Scale Fermenter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Dimension</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing Vessel Diameter</td>
<td>$D_t$</td>
<td>0.25 m</td>
<td>$2D_t$</td>
</tr>
<tr>
<td>Mixing Tank Height</td>
<td>$H_t$</td>
<td>0.5 m</td>
<td>-</td>
</tr>
<tr>
<td>Height of Conical Base</td>
<td>$H_C$</td>
<td>0.5 m</td>
<td>-</td>
</tr>
<tr>
<td>Height of Cylindrical Part</td>
<td>$H_S$</td>
<td>500 mm</td>
<td>-</td>
</tr>
<tr>
<td>Volume of Cylindrical Part</td>
<td>$V_C$</td>
<td>0.0245 m$^3$ (24.5 L)</td>
<td>$\pi \left(\frac{D_t}{2}\right)^2H_C$</td>
</tr>
<tr>
<td>Mixing Tank Radius</td>
<td>$r$</td>
<td>0.125 m</td>
<td>$\frac{D_t}{2}$</td>
</tr>
<tr>
<td>Volume of Hemispherical Base</td>
<td>$V_H$</td>
<td>0.00409 m$^3$ (4.09 L)</td>
<td>$\frac{2}{3}\pi r^3$</td>
</tr>
<tr>
<td>Total Volume of Mixing Vessel</td>
<td>$V_T$</td>
<td>0.02859 m$^3$ (28.59 L)</td>
<td>$V_C + V_H$</td>
</tr>
<tr>
<td>Clearance of Impeller</td>
<td>$C$</td>
<td>0.833 mm</td>
<td>-</td>
</tr>
<tr>
<td>Width of Impeller</td>
<td>$W$</td>
<td>0.0625 mm</td>
<td>-</td>
</tr>
<tr>
<td>Length of Impeller</td>
<td>$L$</td>
<td>0.5 mm</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: $\pi = 3.142$ and the ratio of $D_t$ : $L$ : $W$ = 20: 5: 4

Figure 1. Schematic Representation of the Pilot Plant Fermenter.

Figure 2. Experimental Setup Consisting of the Fermenter and a Hydrolysis Vessel.

3. Results and Discussion:

3.1. Outcome of Hydrolysis:

Table 3 contains the results obtained during the pilot scale hydrolysis of cassava starch using dilute $\text{H}_2\text{SO}_4$ and the trends is shown in Figure 3.

Table 3 and Figure 3 show the increase in concentration of glucose produced as the hydrolysis progresses. If the rate of glucose concentration increases with time after the hydrolysis of cassava starch, it means that the process of breaking down cassava starch into glucose is ongoing and becoming more efficient, attaining a maximum value. In this case, the maximum glucose obtained is 42 g/L. Factors that can influence this process include the temperature, pH, concentration of chemical used and the initial starch concentration.

Table 3. Dilute $\text{H}_2\text{SO}_4$ Hydrolysis of Cassava Starch at 95°C.

<table>
<thead>
<tr>
<th>Runs</th>
<th>Reaction Time (min)</th>
<th>Concentration Glucose Produced with Time (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>60</td>
<td>23</td>
</tr>
<tr>
<td>4.</td>
<td>90</td>
<td>25</td>
</tr>
<tr>
<td>5.</td>
<td>120</td>
<td>35</td>
</tr>
<tr>
<td>6.</td>
<td>150</td>
<td>40</td>
</tr>
<tr>
<td>7.</td>
<td>180</td>
<td>42</td>
</tr>
</tbody>
</table>

3.2. Sugar Utilization Rate:

The sugar utilization rate (g/min) is the slope obtained by plotting sugar utilized (g) against time (min) [21].
3.2. Cassava Starch Fermentation Kinetics:

3.2.1. General Fermenter Condition:
Table 4 displays results obtained for the pilot plant fermentation of cassava starch carried out for a period of 4 days maintained at pH 4-6 and temperatures between 30-34°C. During the course of the laboratory investigation, the broth was regularly agitated to facilitate the reaction.

Table 4. Pilot Scale Fermentation of Cassava Starch.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>pH</th>
<th>Temp. (°C)</th>
<th>Biomass (X)</th>
<th>Alcohol</th>
<th>Sugar (S)</th>
<th>ln (X/X₀)</th>
<th>μ (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.98</td>
<td>30</td>
<td>1.1</td>
<td>0</td>
<td>42</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>4.37</td>
<td>32</td>
<td>1.9</td>
<td>1.0</td>
<td>40</td>
<td>0.55</td>
<td>0.045833</td>
</tr>
<tr>
<td>23</td>
<td>4.13</td>
<td>30</td>
<td>3.2</td>
<td>3.0</td>
<td>38</td>
<td>1.07</td>
<td>0.046522</td>
</tr>
<tr>
<td>36</td>
<td>4.01</td>
<td>32</td>
<td>5.2</td>
<td>5.2</td>
<td>32</td>
<td>1.55</td>
<td>0.043056</td>
</tr>
<tr>
<td>46</td>
<td>5.98</td>
<td>34</td>
<td>6.0</td>
<td>7.5</td>
<td>25</td>
<td>1.70</td>
<td>0.036957</td>
</tr>
<tr>
<td>60</td>
<td>3.60</td>
<td>34</td>
<td>6.9</td>
<td>12.5</td>
<td>21</td>
<td>1.84</td>
<td>0.030667</td>
</tr>
<tr>
<td>70</td>
<td>5.87</td>
<td>34</td>
<td>7.1</td>
<td>15.8</td>
<td>18</td>
<td>1.86</td>
<td>0.026571</td>
</tr>
<tr>
<td>82</td>
<td>3.40</td>
<td>34</td>
<td>7.3</td>
<td>20.0</td>
<td>15</td>
<td>1.89</td>
<td>0.023049</td>
</tr>
<tr>
<td>96</td>
<td>5.60</td>
<td>32</td>
<td>7.2</td>
<td>29.0</td>
<td>10</td>
<td>1.88</td>
<td>0.019583</td>
</tr>
<tr>
<td>108</td>
<td>5.40</td>
<td>32</td>
<td>7.2</td>
<td>28.8</td>
<td>10</td>
<td>1.88</td>
<td>0.017407</td>
</tr>
</tbody>
</table>

Table 4 shows the C₂H₅OH yield, reducing sugar, biomass yield as well as pH and temperature variations obtained during the fermentation of cassava starch. It points to a gradual increase in yield of C₂H₅OH from the first 12 h (1.0 g/mL) and a time beyond 96 h when a maximum production equivalent to 29 g/mL was obtained. This represents 29% of the starch utilized. Optimum length of the fermentation selected was therefore 4 days. Also observed is that the biomass yield (growth) increased throughout the length of fermentation from 1.1-7.2 g/mL while the reducing sugar concentration declined from 42 g/mL in the first 12 h of fermentation to 10 g/mL after 96 h. The pH of the medium decreased from 5.98 at the beginning to 4.01 after 36 h, the time at which the pH was increased to 5.98 by adding 4N NaOH to the broth so it does not go below or above the optimum pH (4-6) as stated in the literature. Temperature happens to increase gradually from 30-34°C as a result of an exothermic fermentation, even though there are fluctuations as shown in Table 3, influenced by changes in ambient air temperature.

3.2.2. Yeast Cells Calibration Curve:
Originally, the calibration curve in Figure 4 was prepared using known standard concentrations of yeast cells. The corresponding absorbance (optical density) of the samples was determined using a Spectrophotometer (Cole Parmer). The various biomass concentrations, X, reported in Table 4 were extrapolated from the curve.

This is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Figure 5 shows that the microorganisms have only two phases; the exponential phase and the stationary phase. The absence of the lag phase may have resulted because the sample may have been taken after the lag phase while the absence of the death phase may have resulted because the fermentation was stopped before the complete exhaustion of the substrate. It also shows that as the cells consume substrate (which is initially maximum), it leads
to cell growth, which results in the increase in cell concentration. The maximum % sugar utilization rate determined from the curve is 47.62%. Figure 6 corresponds to logarithmic cell concentration with respect to fermentation time. The experimental data fitted well with the theoretical predictions that were shown in Table 3. The slope of Figure 6 shows the specific growth rate is $\mu = 0.0225 \, \text{hr}^{-1}$.

In view of that, microbial growth in the pilot fermenter during the first 90 h of the fermentation, after which *Saccharomyces cerevisiae* stop increasing, can be shown using Figure 5. Figure 6 indicates that the specific growth rate increases as the substrate concentration increases, which are due to abundant nutrients that promote and sustain microbial growth. Maximum specific growth rate was identified in the plot of $\mu$ against $S$ (Figure 7) during the exponential phase, which is $0.0465 \, \text{h}^{-1}$. Compared to Nadir *et al.*, (2009) [26] who studied the comparison of sweet sorghum and cassava for $\text{C}_2\text{H}_5\text{OH}$ production using *Saccharomyces cerevisiae*, their $\mu_{\text{max}}$ value for cassava was $0.0506 \, \text{h}^{-1}$, slightly close to this work’s value. There was a relative % error of $\pm 10\%$ when their maximum $\text{C}_2\text{H}_5\text{OH}$ yield was $34.07 \, \text{g/L}$; whereas for this study, the maximum yield of $\text{C}_2\text{H}_5\text{OH}$ obtained was $29 \, \text{g/mL}$. This difference may have been due to initial sugar concentration.

![Figure 4](image1.png)  
*Figure 4. Calibration Curve for Biomass Concentration.*

![Figure 5](image2.png)  
*Figure 5. Growth Profile for Saccharomyces cerevisiae with Depleting Sugar (Substrate) Concentration.*

![Figure 6](image3.png)  
*Figure 6. Graphs of Dependent and Independent Terms in the Logistic Kinetic Model.*
Estimated $K_s$ which is the substrate saturation constant was obtained as stated by Puyate & Yelebe (2012) [27], who defined it as substrate concentration at $\mu = \frac{\mu_{\text{max}}}{2}$. Manikandan & Viruthagiri (2010) [24] arrived at $K_s = 14$ g/mL using Monod equation while in this work, $K_s = 15.375$ g/mL. The former studied kinetic and optimization of ethanol production from corn flour, whose deviating value may be attributed to the use of different material.

The parameters witnessed above (and as determined from Figure 7) are experimental. ORIGIN software reveals new estimates of $\mu_{\text{max}}$ and $K_s$ through user defined model (Equation 3) fit to the empirical $\mu$ and $S$ shown in Figure 8.

**Figure 7.** Specific Growth Rate Against Substrate Concentration for the Pilot Fermentation.

**Figure 8.** Mond Curve for the Microbial Activity in the Batch Process.
Heavy initial guess of the unknown parameters leads to a satisfactory fit shown in Figure 8, based on high $R^2$ and adjusted $R^2$ given by the software. Invariably, a deviating new set of $\mu_{\text{max}}$ and $K_S$ that are respectively, 0.108 h$^{-1}$ and 51.42 g/mL were obtained. These new set of parameters cannot be adopted simply because the predicted $\mu$ line (red color) is not typical of the graph of $\mu$ against $S$ in growth kinetic study. Ignoring the estimates, if $\mu_{\text{max}}$ and $K_S$ are to be determined using similar approach in Figure 7, closer estimates of the parameters to the experimental results may be obtained. During ethanol concentration from corn flour, Manikandan & Viruthagiri (2010) [24] arrived at $\mu_{\text{max}} = 0.26$ h$^{-1}$, which is still $>50\%$ higher than the predicted result.

3.2.3. Evaluation of Ethanol-Cell Concentration Trends:
Figure 9 relates $C_2H_5OH$ concentrations in conjunction with cell dry weights versus time.

![Figure 9. Cell Growth and Ethanol Concentrations with Fermentation Period.](image)

It is clearly observed from Figure 9 that, as the cell concentration increases, there is progressive formation of $C_2H_5OH$. This implies a progressive trend between the two measurements with time. Fuel ethanol production embarked upon by Srinorakutara et al. (2006) [19] represent are divergent trends with time between the two concentrations, where, as the glucose concentration decreases, the biomass concentration increases. In order to calculate the ethanol productivity (g/ mL h), determine the slope of the graph obtained by plotting ethanol concentration (g/mL) against time (h). This slope is the ethanol productivity. In addition, Equation 2 gives 69.05% as the ethanol yield, based on the amount of ethanol produced (29 g/mL) and the glucose amount utilized (42 g/mL). Under optimum condition at 135°C for 90 min dilute-acid hydrolysis, Yoonan et al. (2007) [20] reported an ethanol productivity and yield in the range of 0.29-0.51 g/L h and 0.27-0.43 g/g.

4. Conclusion:
Batch fermentation of $S. \text{cerevisiae}$ in a 25.8 L fermenter produced a maximum bioethanol concentration of 29 g/mL after 96 h. With the aid of two different kinetic models based on substrate utilization and microbial growth during anaerobic fermentation; namely, Monod and Monod Logistic model, $\mu_{\text{max}}$ and $K_S$ were determined. They are 0.0465 hr$^{-1}$ and 15.375 g/mL, respectively. Substrate depletion and $C_2H_5OH$ oxidation to ethanoic acid may have caused to decline beyond 96 h of fermentation. Also observed is a prolonged period of fermentation which led to accumulation of $C_2H_5OH$, inhibiting its formation due to deactivation of the yeast cells. Reduction of the inhibitory effect is possible if the $C_2H_5OH$ produced is constantly removed from the fermenter without altering the rate constant. By inference, $C_2H_5OH$ can be effectively produced from cheap, easily available cassava at industrial scale, resulting in the manufacture of alternative clean fuel for energy production. Next work should deal seriously with the same task by setting up an aerobic scheme of production and analysis.

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