OPTIMUM HYDROLYSIS-FERMENTATION PARAMETERS FOR THE PRODUCTION OF BIOETHANOL FROM THE NIGERIAN STEM JUICE OF SWEET SORGHUM

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Published in:
Petroleum Technology Development Journal (ISSN 1595-9104)
An International Journal

July 2011 - Vol. 1
Abstract
This work examines the effect of various the fermentation parameters on the production of Bioethanol from the stem juice of Sweet sorghum using Baker’s yeast (Saccharomyces cerevisiae). It shows that optimal fermentation parameters for optimal yield of Bioethanol from the stem juice of Sweet sorghum are; a pH value of 5.12, temperature of 33°C, Yeast slurry concentration of 10% w/w, sugar concentration (juice sugar) of 86.5g/L and duration of fermentation of five days.

Introduction
Dwindling prices of crude oil coupled with the fear of the petroleum exhaustion made many countries around the world to start looking inwards and utilize their renewable sources of energy. Nigeria is a petroleum producing and exporting country but unfortunately today imports refined petroleum products, chiefly fuels. This brings about the negative consequences whereby queuing for transporting fuels becomes a normal phenomenon. This is because our four refineries don’t function or function far below their capacities. This problem, among others could be one of the reasons the Federal Government has established a Renewable Energy Division (RED) under NNPC, charged with the responsibility of developing biofuels industry in Nigeria. Presently, Nigeria incorporates 10% of bioethanol into Premium Motor Spirit (PMS) consumed throughout the country, and again this bioethanol is imported into the country upon all the abundant agricultural raw materials that can be utilized for its mass production. In view of this, there is therefore the need to develop an indigenous technology for the utilization of our abundant plant sources for biofuels production.

Agricultural Biomass can be converted into a variety of fuels such as bioethanol, biodiesel and biogas. Bioethanol can be made directly from sugar bearing crops and plants and indirectly by converting cellulosic portion of biomass into sugars. Sugar bearing plants such as sugarcane, sweet sorghum and sweet potatoes could be used for the production of Bioethanol. With relatively many sugar bearing plant available for the production of bioethanol, cost implication means that only crops with high yield and lower bioethanol production cost are considered.

Why the Choice of Sweet Sorghum
The question to be asked is: why not using sugar cane or sweet potato but sweet sorghum. Some of the answers include the following:

- Sweet sorghum has a very high photosynthetic rate and produces higher biomass compared to other crops.
  1. It is a high sugar bearing crop, with a lot of simple sugar in its stem juice. This makes fermentation into bioethanol much easier.
  2. It is highly productive as it could be grown all year round.
  3. It has a relatively low cultivation cost.

\* The authors wish to express their appreciation to the Staff and Management of the National Research Institute for Chemical Technology (NARICT), Zaria for the permitting them to use some of their analytical equipment.
1 NNPC magazine (2005): Interview with Group General Manager, RED-NNPC, Abuja, Nigeria.
2 F.D. Yamba (2007): Investigations into the Production and Use of Bio-ethanol from Sweet Sorghum as an Alternative Fuel, Department of Mechanical Engineering, School of Engineering, University of Zambia, Lusaka.

4. The quantity of water needed by sweet sorghum is only one-third (⅓) of that needed by sugarcane.
5. It is seldom consumed by man i.e. it is not consumed by man in commercial quantities. It is not widely used for food production. Therefore the issue of competing plants for food and for fuels does not arise.

One of the major challenges in the production of bioethanol from the stem juice of Sweet sorghum is thought to be the fermentation process, during which the sugars contained in the juice are broken down to Bioethanol by enzymes produced by yeast cells in a complex microbial action. It is imperative to state that works have previously been done on the production of Bioethanol from Sweet sorghum, notably by the International Crop Research Institute for the Semi Arid Tropics (ICRISAT), but none of these works have really studied the fermentation process in detail with a view to optimizing the yield of bioethanol.

This work is thus intended to maximize the yield of Bioethanol from the stem juice of Sweet sorghum by manipulating the variables in the fermentation process so as to ascertain the optimal values of these parameters. These variables are temperature and pH of the reaction mixture, duration of the fermentation reaction, sugar concentration of the juice and the concentration of the yeast slurry.

**Biological characteristics of sweet sorghum**

Sweet sorghum is a variant of sorghum. Sweet sorghum (sorghum bicolor) mainly differs from grain sorghum (broom corn) in that its stalks are taller and juice has a far higher sugar content. It reproduces by seed and produces tillers but it has no rhizomes. It is a perennial grass under tropical conditions, but it doesn’t do well in areas where winter frost occurs. It can be used for many purposes: the juice is used for bioethanol production. The stalks are used as silage or fodder, whereas the fiber of the stalk is one of the best materials for making high quality paper amongst others.

**Sugar content of the stem juice**

Although bioethanol could be produced indirectly from the seed (starch) of sweet sorghum as well as its cellulosic biomass, it is the direct production of bioethanol from the stem juice that is of star attraction. This is because of the high content of reducing or simple sugar in its stem juice. Research has shown that the stem juice contains more sugar than the stem juice of sugar cane. Also, the quality of the fermentable material in the stem juice is better than that of sugar cane.

Research work carried out at institute of Agricultural research, Ahmaddu Bello University, Zaria, has shown that for virtually all the varieties of sweet sorghum, the maximum amount of stem juice occurs between six to seven weeks of growth i.e. just before the flowering stage. This is obvious because after the onset flowering stage, stem juice are partly concerted to starch in the seeds.

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6 Upadhyay S.K. Chemical Kinetics and Reaction Dynamics, page 152-156 Anamaya Publishers, F-154/2, Lado Sarai, New Delhi-110 030 India
7 Kent J. A., Riegel’s Handbook of Industrial Chemistry, CBS Publishes and Distributions 4596/1A. 11 Darya Gang, New Delhi, India.
**Chemistry of the conversion of Sweet sorghum stem juice to Bioethanol**

The stem juice of Sweet sorghum is rich in fermentable sugars mainly glucose and fructose. The yeast cells feed on these sugars and thus the cells grow. As the cells grow, they produce enzymes such as Zymase and Invertase which are necessary for the breakdown of the sugar molecules into bioethanol\(^9\). The reaction is shown below;

\[
C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \epsilon 
\]

Where, \(\epsilon\) represents chemical energy utilized in the growth processes.

This reaction is exothermic as such heat is generated in the system. The reaction occurs anaerobically as under aerobic conditions the yeast cells use the sugar for growth only, producing only carbon dioxide and water\(^{10}\).

**Materials and Methods**

**Materials**

The materials and equipment used for this study included a horse-driven three roller crusher, High Performance Liquid Chromatography machine (HPLC), Analytical weighing balance, 6400 Spectrophotometer (Jenway), Improvised fermenter, Digital pH meter, Distilled water, Stems of sweet sorghum, Baker’s Yeast (*Saccharomyces cerevisiae*), Autoclave, Water bath, Dilute Hydrochloric acid and glassware.

**Methods**

**Collection and Pre-treatment of the stem**

The stems of sweet sorghum were harvested from a farm in Makarfi village, some forty five (45) Kilometres from Zaria, Kaduna state. After harvesting the sweet sorghum stems, the leaves were manually removed from the stems. The stems were then weighed using a weighing balance, after which they were introduced into a Horse-driven three roller crusher one after the other. They were crushed and the juice was collected. It was observed that 48kg of stem gave 18kg (37.5%wt.) of juice.

**Preparation of the juice**

The juice was collected in a clean plastic container and then filtered by passing it through a sieve. This was done so as to ensure that cellulosic materials were removed from the juice. The juice was then refrigerated in order to avoid loss of its sugar content as a result of bacterial activity on the juice at ambient temperature. On removal from the refrigerator, the juice was de-frozen by placing the juice container on a hot water bath at a temperature of 40\(^\circ\)C for about forty five minutes. The juice was then collected in a beaker and placed in an autoclave and heated for fifteen minutes at a temperature of 121\(^\circ\)C. It was then cooled down to 28\(^\circ\)C. This was done so as to ensure that microorganisms that could compete with the desired reaction were eliminated as much as possible. The pH and total sugar concentration of the juice were determined using a digital pH meter and Spectrophotometer respectively.

**Optimization of the Fermentation Conditions**

The following steps were carried out in order to determine the optimum variables under which fermentation could be carried out for the bioethanol production from the stem juice of Sweet Sorghum.

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**Fermentation with variation of the juice pH:** Five different solutions each made up of 500 milliliters of the cooled juice and 500 milliliters of distilled water were made and then using dilute HCl, the pH of the respective solutions was adjusted progressively to get different juice solutions of pH 4.53, 4.72, 4.80, 5.12 and 5.30. Each of the solutions was then fermented with 50 milliliters of 10% yeast slurry at a temperature of 35°C. The fermentation process was carried out for two days and then stopped. An aliquot was taken from each of the solutions and then analyzed with an HPLC machine to determine their respective ethanol concentrations.

**Fermentation with variation of temperature:** Five different solutions each made up of 500 milliliters of juice and 500 milliliters of water were made and a fixed pH of 5.12 (obtained from the previous step) was ensured for each of the solutions. The respective solutions were then fermented with 50 milliliters of 10% yeast at varying temperatures 28°C, 33°C, 35°C, 37°C and 40°C. The fermentation was carried out for two days and then stopped. An aliquot was taken from each of the solutions and then analyzed with an HPLC machine for the respective concentration of ethanol.

**Fermentation with variation of yeast concentration:** Five different solutions each made up of 500 milliliters of juice and 500 milliliters of water were made and a fixed pH of 5.12 was ensured in each of the solutions. The different solutions were then fermented with 50 milliliters of 6%, 8%, 10%, 12% and 15% yeast solutions respectively. The respective solutions were then fermented with the temperature fixed at 33°C (obtained from the previous step). The fermentation process was carried out for two days and then stopped. An aliquot was taken from each of the solutions and analyzed with HPLC for ethanol concentration.

**Fermentation with variation of sugar concentration:** 173 g/L, 86.5 g/L, 57.6 g/L, 43.3 g/L and 34.6 g/L sugar solutions were made out with a fixed pH, temperature and yeast concentration of 5.12, 33°C and 10% yeast respectively. Fermentation of the respective solutions was carried out for two days and then stopped. Sugar concentration of each of the fermented solutions was ascertained using the HPLC machine.

**Fermentation with variation of reaction time:** Five different juice solutions, each with a total sugar concentration of 86.5 g/L were made and then fermented with 10% yeast solution and at pH of 5.12 and temperature of 33°C. The time of the fermentation for the various solutions was varied such that fermentation was made to occur in 2, 3, 4, 5 and 6 days respectively. An aliquot was taken from each of the solutions and then analyzed with an HPLC machine for the respective concentration of ethanol.
Results and Discussion

Results
Table 1 presents results of the fermentation process under various operating conditions. However, for better visualization of the effect of each of the operating conditions on the fermentation process, the tabulated results are separately presented graphically in Figures 1-5.

Table 1: Fermentation Results at various Operating Conditions

<table>
<thead>
<tr>
<th>S/N</th>
<th>Variation of Juice pH</th>
<th>Variation of Temperature</th>
<th>Variation of Yeast Concentration</th>
<th>Variation of Total Sugar Concentration</th>
<th>Variation of Duration of Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>Variation of Juice pH</td>
<td>Evaluation of Temperature</td>
<td>Evaluation of Yeast Concentration</td>
<td>Evaluation of Total Sugar Concentration</td>
</tr>
<tr>
<td></td>
<td>Ethanol Conc., v/v %</td>
<td>Temp., °C</td>
<td>Ethanol Conc., %</td>
<td>Sugar Conc., %</td>
<td>Duration of Fermentation, days</td>
</tr>
<tr>
<td>1</td>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td>Ethanol Conc., v/v %</td>
</tr>
<tr>
<td></td>
<td>4.53</td>
<td>28</td>
<td>6.0</td>
<td>173</td>
<td>4.3</td>
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<tr>
<td></td>
<td>4.72</td>
<td>33</td>
<td>8.0</td>
<td>86.5</td>
<td>7.4</td>
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<tr>
<td></td>
<td>4.80</td>
<td>35</td>
<td>10.0</td>
<td>57.6</td>
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<tr>
<td></td>
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<td>12.0</td>
<td>43.25</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>5.30</td>
<td>40</td>
<td>15.0</td>
<td>34.6</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Table 1 illustrates the effects of varying pH, temperature, yeast concentration, sugar concentration, and duration of fermentation on the fermentation process. The table and accompanying graphs provide a comprehensive view of how these factors influence the ethanol concentration in the fermentation process.
Figure 1: Effect of juice pH on the Fermentation process

![Graph showing the effect of juice pH on the Fermentation process.](image1)

Figure 2: Effect of Temperature on the Fermentation process

![Graph showing the effect of temperature on the Fermentation process.](image2)

Figure 3: Effect of yeast slurry concentration on the Fermentation process

![Graph showing the effect of yeast slurry concentration on the Fermentation process.](image3)
Discussion of Results

From the results obtained, Figure 1 shows that a pH value of 5.12 gave the highest percent yield by volume of ethanol and a pH value of 4.53 gave the lowest yield. The trend was such that the yield increased from a pH value of 4.53 up to a pH value of 5.12 before the decline in yield at pH value of 5.30. Literature values indicate that the optimal pH value for optimal yeast growth is between pH values of 4.50 up to 5.50. The result thus shows a close correlation to literature values.

Figure 2 indicates that the yield of ethanol rose from $28^\circ C$ up to $33^\circ C$ and then declined from $35^\circ C$ down to a very low value at $40^\circ C$, showing the optimal fermentation temperature to be $33^\circ C$. The low yield at $28^\circ C$ could be attributed to the dependence of rate of reaction on temperature, thus a temperature of $33^\circ C$ gave a higher yield of ethanol. At temperatures slightly above $35^\circ C$, findings from literature indicate that bacteria activity tend to increase, thus leading to competing reactions, giving undesirable products. This leads to low yield of ethanol at temperatures above $35^\circ C$. Also, above $35^\circ C$, the temperature becomes destructive to the yeast cells thus leading to reduced activity of the yeast cells.
Figure 3 shows that the optimal concentration of yeast slurry is 10% w/w. The low yield of ethanol recorded at 6% w/w yeast slurry is indicative of the fact that very little yeast cell count gets overwhelmed by a larger substrate concentration thus leading to low yield of ethanol. Also, at high yeast slurry of 15% w/w, the reverse is the case as there is too little food for the larger yeast cell population thus leading to low ethanol yield.

Figure 4 is closely related to Figure 3. Here, there is an abysmal low yield of ethanol at very high sugar concentration of 173g/L. This could be due to the fact that at very high substrate concentration, there is substrate inhibition of the yeast activity. Also at very low sugar concentration of 34.6g/L, there becomes too little food for the yeast cells to feed upon thus leading to low ethanol yield. The optimal sugar concentration is therefore considered to be 86.5g/L.

Figure 5 gives the variation of ethanol yield with number of days that fermentation was carried out. The lowest yield was obtained at two days of fermentation and the highest yield at five days. The result is closely related the plot of Log mass of a biomass versus time of a microbial culture obtained from literature. At two days, the yeast cells are just at the accelerated stage of growth. Below two days, the yeast cells are largely at the lag phase where very little chemical reaction takes place. At day three, the yeast cells were at the exponential growth phase where there is very rapid cell growth and consequently, rapid production of ethanol. At day four to day six, the yeast cell growth is at its peak and so also is the production of ethanol. After six days, the yeast cells die off thus stopping the reaction.

It is imperative to state that the optimal parameters obtained above are synonymous to the yeast strain used and also to the type of substrate. In this case the yeast strain used was Baker’s yeast (*Saccharomyces cerevisiae*), and the substrate is as contained in the stem juice of Sweet Sorghum. Genetically modified yeast cells could give higher or better values.

**Conclusions**

From the results and discussion of results, it is seen that the optimal fermentation conditions for the fermentation of the stem juice of sweet sorghum into Bioethanol using Baker’s yeast (*Saccharomyces cerevisiae*) are; a pH value of 5.12, temperature of 33°C, Yeast slurry concentration of 10% w/w, sugar concentration (juice sugar) of 86.5g/L and duration of fermentation of five days.