# FED-BATCH PRODUCTION OF BAKER'S YEAST (*S.CEREVISIAE*) FROM CASSAVA (*MANIHOT ESCULENTA*) STARCH HYDROLYSATE

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**Abstract**

Yeasts are microorganisms in which the unicellular form is conspicuous and outstanding. In this group are the versatile strains of *Saccharomyces cerevisiae* (Baker's yeast). The local production of Baker's yeast becomes necessary in Nigeria to replace imported ones being used currently in the industries.

The utilization of cassava as a feedstock for the production of baker's yeast using fed-batch fermentation process was investigated. Starch was extracted from freshly harvested cassava tubers and hydrolyzed into fermentable sugar using the enzyme-enzyme method. From the initial starch slurries of 8.84% and 12.40% concentrations, Sugar concentrations of 8.57% and 12.0% respectively were attained. These hydrolysates were used as substrates for the aerobic cultivation of *S. cerevisiae* under fed-batch conditions for 24 hours with a constant feed rate of 1.1174 L/h.

The result obtained revealed that biomass, reducing sugar as well as ethanol concentration increased with fermentation time. The highest ethanol and biomass obtained were 0.060 g ethanol/g glucose and 0.508 g biomass/g glucose. It is noteworthy that the maintenance coefficients of 0.0585 $h^{-1}$ and 0.0598 $h^{-1}$ for the two runs respectively are low, which is an indication of non stressful metabolism by the micro organism.

**Key words**: Baker's yeast, hydrolysis, biomass yield, ethanol yield and maintenance coefficient.

## 1. Introduction

Baker's yeast (*Saccharomyces cerevisiae*) has long been a subject of both biochemical and genetical research. As a eukaryote, *Saccharomyces cerevisiae* offers advantages in producing recombinant proteins. It has a secretory pathway, which is very similar to that of higher organisms. In comparism to mammalian tissue culture system it is cheaper, more convenient and gives higher product yields. It is an indispensable raw material for food, pharmaceutical and chemical industries because of its GRAS (Generally Regarded as Safe) status (Solomon *et al.*, 1997; Hak, 2006).

The baker's yeast propagation is carried out on carbon sources which consist of assimilable sugars like glucose, fructose, and sucrose. For this work, the major carbon source is hydrolyzed cassava starch. Cassava (*Manihot esculenta* Crants) also known as manioc, tapioca or yucca is a shrubby, tropical perennial plant that is not well known in the temperate zone. For most people, cassava is commonly associated with tapioca in Portuguese (Alaux and Fauquet, 1990; Guthrie, 1990). Sailors introduced the plant to Africa from Brazil in the 16th century. It is being grown in Northern and Southern Nigeria. In fact, starch sources such as cassava chips are available in abundance and can act as substitute for other sugar sources like sorghum, maize and molasses in the production of baker's yeast. Though, cassava is also competitive with local production of starch, garri and fufu (garri and fufu are both local staple foods), it has an advantage over the other sources as it is cheap, easily cultivated and thrives better on poor soils than any other food plant which makes fertilization rarely necessary. It is also a heat-loving plant that requires a minimum temperature of 80 °F to grow. Since many cultivars are drought resistant, cassava can survive during the dry season, when the soil moisture is low, but humidity is high.

Some of the previous works conducted on cassava starch involved kinetic studies on enzymatic hydrolysis of starch from cassava (Gorinstein, 1993), Fed-batch production of yeast using millet (Ejiofor *et al.*, 1995) and production of baker's yeast from raw cassava starch hydrolysates in a bioreactor under batch process (Aransiola, 2006). Therefore this study focused on the enzymatic hydrolysis of cassava starch to obtain the hydrolysates used as substrate for the fed-batch cultivation of baker's yeast in a bioreactor.

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The data collected were analyzed to estimate the pertinent parameters like the biomass yield, production yield and maintenance coefficient.

## 2. Materials and Methods

*Preparation of Feedstock Solution:* The enzyme-enzyme hydrolysis method was used for the preparation of the feedstock solution. Aransiola *et al.,* (2006) showed that this method gave the best yield (output) amidst the three methods of hydrolysis (acid, acid-enzyme and enzyme-enzyme methods of hydrolysis).

Starch slurries of 8.84% and 12.40% were gelatinized in a water bath respectively by adding α-amylase (5 ml enzyme $kg^{-1}$ starch of Novo Termamyl obtained from *Bacillus licheniformis*) at 105 °C for 5 min at a pH of 4.5 and 60 °C for 72 h by adding Novo amyloglucosidase (AMG) obtained from *Aspergillus niger* (5 ml enzyme $kg^{-1}$ starch). Agitation was done continuously throughout the period of hydrolysis. After saccharification, the mixture was filtered and the syrup obtained contained the reducing sugars used as feedstock (substrate) for the baker's yeast production.

*Microorganism:* The yeast, *S. cerevisiae*, used in this study was collected from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The microorganism was grown on YPD (Yeast extract, peptone and dextrose) agar slant at 30 °C for 4-5 days and was stored at 4 °C with regular sub culturing.

*Preparation of Inoculums:* One hundred milliliters of a mineral salts solution, which is a modification of the synthetic medium developed by O'Connor *et al.,* (1992), was measured into three 250 ml Erlenmeyer flasks. The flasks were cotton plugged and autoclaved at 121 °C for 15 min. The sterilized medium was allowed to cool to ambient temperature and large amount of the microorganism (*S. cerevisiae*) was scraped from the cultured slant and added aseptically to each of the medium in the flasks. The flasks were transferred to the gyratory incubator shaker (New Brunswick Scientific Co., USA) and growth was achieved at a temperature of 30 °C at agitation rate of 200 rpm for 24 h.

*Fermentation:* Two aerobic cultivations were carried out on a bench scale bioreactor manufactured by New Brunswick Scientific Co., USA. In the fed-batch fermentation, the fermentor vessel containing 2 liters of the mineral salts solution and 3.5 liters of the feed (glucose solution) in the feed reservoir were sterilized in the autoclave at 121 °C for 15 minutes. The starting volume in the fermentor vessel was inoculated with an actively growing inoculum with a volume of 450 ml and was properly agitated and aerated for about 30 minutes so that the yeast cells got adapted to the new environment before feeding. After the time elapsed, the pumping of the feed commenced. Constant feeding strategy was applied and the pumping of the feed took 24 hours. The feed rate was 0.1174 liter per hour.

The fermentor was agitated at 800 rpm with an airflow rate of 2 vvm (volume of air per volume medium per minute) at 30 °C by circulating water at such temperature from a water batch through the cooling water pot of the fermentor. Small drops of antifoam were added to the fermentation. In each of the runs, samples were taken at 2 hour interval. Each of the samples collected was centrifuged and the supernatant analyzed for reducing sugar and ethanol content. The residue at the bottom was used for biomass.

*Analysis of the Sample*

*Reducing Sugar Concentration:* The reducing sugar concentration in the broth was estimated by analyzing the glucose in the supernatant using DNS method of Miller (1959).

*Ethanol Concentration:* The ethanol present in the culture medium was analyzed by using the method of James (1996). The supernatant of sample collected was steam distilled into acidified $K_2Cr_2O_7$ solution of known volume and concentration. Oxidation of ethanol to acetic acid was completed by heating and unreacted dichromate is determined by titrating with standard $FeSO_4$ $(NH_4)_2$ $SO_4.6H_2O$ solution using 1,10-phenanthroline as indicator.

*Biomass Concentration:* For each run 20 ml of samples were collected at regular intervals into a pre-weighted tube, which was then centrifuged at 5000 rpm for 10 min. The pellets were washed twice by resuspending in an equal amount of distilled water and repeating the centrifugation process. The washed cells were then dried to constant weight in an oven at 105 °C, allowed to cool in a desiccator and the final weight was recorded.

*Data Analysis:* Data analysis was carried out, to obtain biomass and product energetic yields, using the methods of Solomon *et al., (1982)* and Akinyemi *et al.*, (2003).

## 3. Results

The enzyme-enzyme method of hydrolysis using starch slurries of 8.8% and12.4% gave reducing sugar concentrations of 8.57% and 12.0% with starch conversion of 87.3% respectively.

In this fed-batch method, the substrate was being fed at a constant feeding rate of 0.1174 L/h. The results obtained for the production of biomass, reducing sugar consumption and ethanol are shown in Fig. 1 and 2. The results showed that biomass and ethanol concentration increased with time as the reducing sugar concentration increased.

For the two runs of 8.57% and 12% of reducing sugar concentrations, the highest biomass concentrations were 11.72 g/L and 16.30 g/L while the highest ethanol

**Table 1**:Values of Specific Growth, Substrate Consumption and Ethanol Production Rates for the Aerobic Fed-Batch Growth of S. Cerevisiae on 8.5% Reducing Sugar Concentration Obtained by Enzyme-Enzyme Hydrolysis of Cassava Starch

| Fermentation Period (H) | Specific Growth Rate $\mu$ ($H^{-1}$) | Specific Rate Of Substrate Consumption ($Q_S$) ($H^{-1}$) | Specific Rate Of Product Formation ($Q_P$) ($H^{-1}$) | Biomass Energetic Yield ($\eta$) | Product Energetic Yield ($\zeta_p$) |
|---|---|---|---|---|---|
| 0 | - | - | - | - | - |
| 2 | | | | | |
| 4 | 0.259 | 0.4797 | | 0.669 | |
| 6 | 0.213 | 0.268 | | 0.985 | |
| 8 | 0.143 | 0.1629 | 0.0246 | 1.088 | 0.294 |
| 10 | 0.109 | 0.1066 | 0.0223 | 1.267 | 0.408 |
| 12 | 0.052 | 0.1620 | 0.0109 | 0.398 | 0.131 |
| 14 | 0.0407 | 0.1488 | 0.01085 | 0.339 | 0.142 |
| 16 | 0.053 | 0.093 | 0.01375 | 0.706 | 0.288 |
| 18 | 0.043 | 0.086 | 0.01046 | 0.620 | 0.237 |
| 20 | 0.0465 | 0.0597 | 0.00835 | 0.965 | 0.273 |
| 22 | 0.0423 | 0.0496 | 0.00746 | 1.057 | 0.293 |

Integrated $\eta = 0.630$ $Y_{x/s} = 0.508$g/g

$\zeta_p = 0.116$ $Y_{p/s} = 0.060$g/g

$M_e = 0.0585h^{-1}$

**Table 2**: Values of Specific Growth, Substrate Consumption and Ethanol Production Rates for the Aerobic Fed-Batch Growth of S. Cerevisiae on 12% Reducing Sugar Concentration Obtained by Enzyme-Enzyme Hydrolysis of Cassava Starch

| Fermentation Period (H) | Specific Growth Rate $\mu$ ($H^{-1}$) | Specific Rate Of Substrate Consumption ($Q_S$) ($H^{-1}$) | Specific Rate Of Product Formation ($Q_P$) ($H^{-1}$) | Biomass Energetic Yield ($\eta$) | Product Energetic Yield ($\zeta_p$) |
|---|---|---|---|---|---|
| 0 | - | - | - | - | - |
| 2 | - | - | - | - | - |
| 4 | 0.262 | 0.482 | - | 0.673 | - |
| 6 | 0.210 | 0.266 | - | 0.978 | - |
| 8 | 0.144 | 0.163 | 0.015 | 1.095 | 0.179 |
| 10 | 0.109 | 0.107 | 0.015 | 1.262 | 0.273 |
| 12 | 0.053 | 0.163 | 0.013 | 0.403 | 0.156 |
| 14 | 0.042 | 0.149 | 0.012 | 0.349 | 0.157 |
| 16 | 0.054 | 0.093 | 0.012 | 0.719 | 0.252 |
| 18 | 0.043 | 0.087 | 0.008 | 0.612 | 0.179 |
| 20 | 0.047 | 0.060 | 0.008 | 0.971 | 0.260 |
| 22 | 0.042 | 0.050 | 0.007 | 1.041 | 0.173 |

Integrated $\eta = 0.626$ $Y_{x/s} = 0.505$g/g

$\zeta_p = 0.096$ $Y_{p/s} = 0.049$g/g

$M_e = 0.0598h^{-1}$

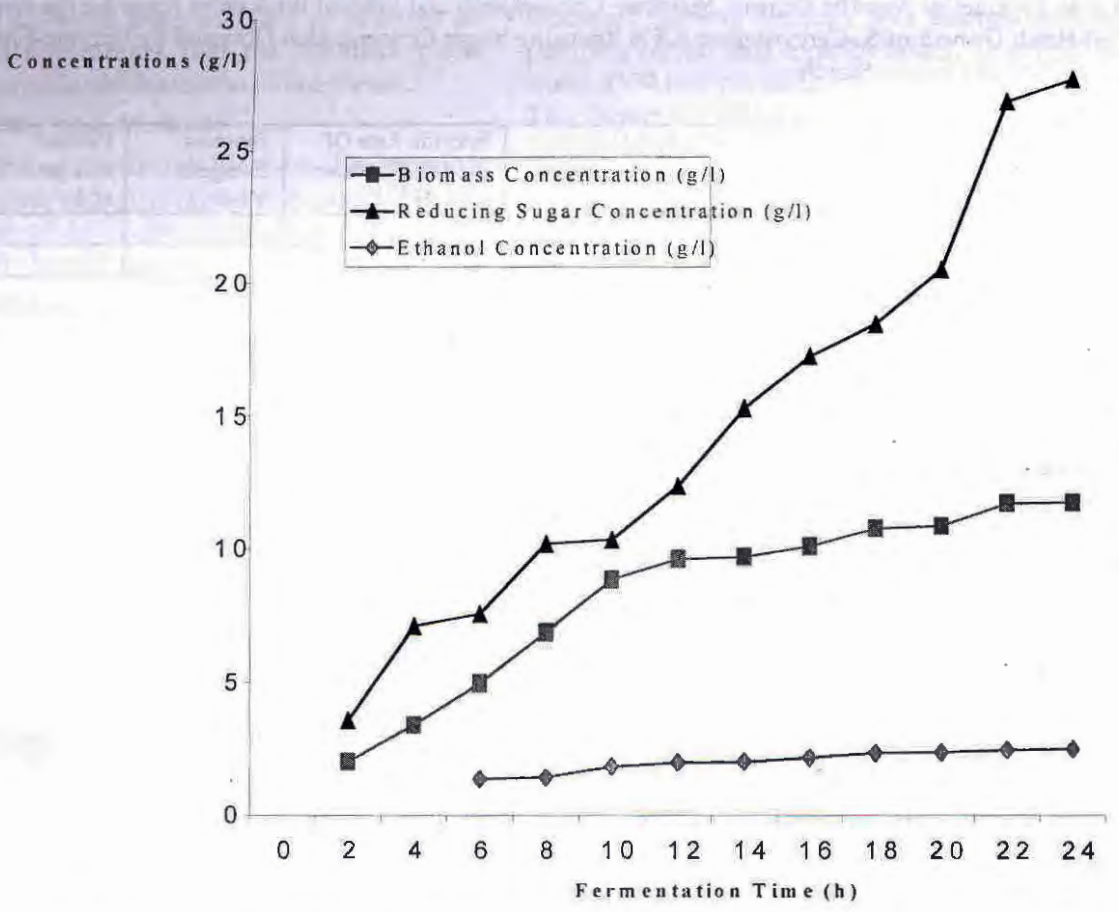


**Fig.1**: Plot of Biomass, Reducing Sugar and Ethanol Concentrations as a function of Fermentation Time for Aerobic Fed-batch growth of S.Cerevisiae on 8.57% Reducing Sugar Concentration obtained by Enzyme-Enzyme Hydrolysis of Cassava Starch

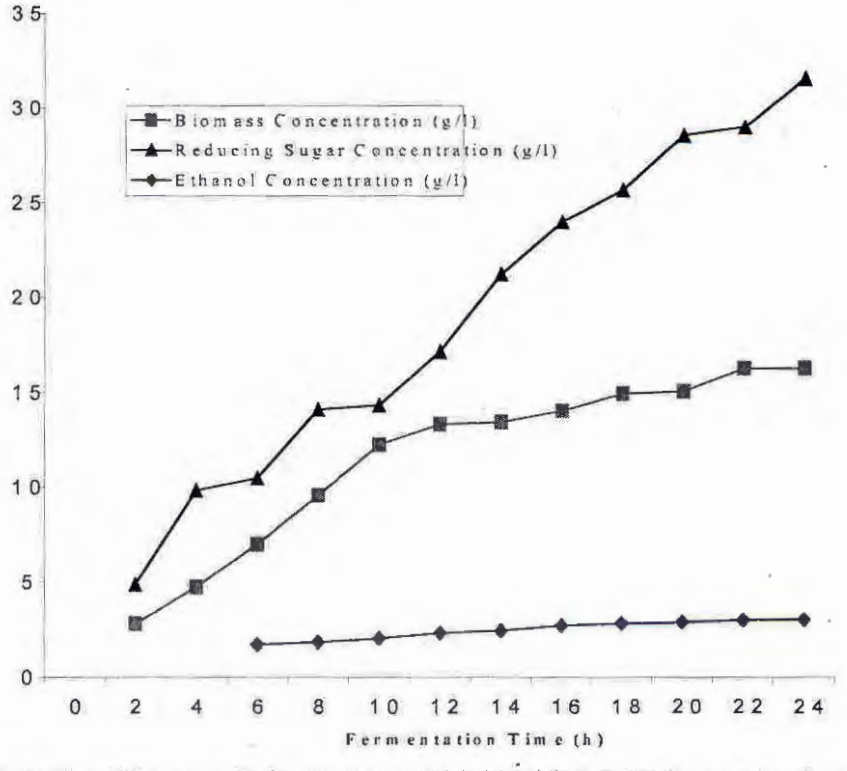


**Fig.2**: Plot of Biomass, Reducing Sugar and Ethanol Concentrations against Fermentation Time for the Aerobic Fed-batch growth of S.Cerevisiae on 142% Reducing Sugar Concentration obtained by Enzyme-Enzyme Hydrolysis of Cassava Starch

concentrations were 2.49 g/L and 3.04 g/L respectively.

The values of specific growth, substrate consumption and ethanol production rates for the aerobic fed-batch growth of *S. cerevisiae* are presented in Tables 1 and 2. The biomass yields obtained were 0.508 g/g and 0.505 g/g for 8.57% and 12% reducing sugar feed concentrations respectively. These values correspond to biomass energetic yield (ç) values 0.630 and 0.626 respectively.

The production of ethanol did not start till after the 6th hour when the specific growth rate is almost close to the critical specific growth rate. This ethanol production may be as a result of respiro-fermentative growth that had set in. The yields obtained are 0.060 g/g and 0.049 g/g for 8.57% and 12% reducing sugar concentrations respectively. These corresponded to product energetic yield values of 0.116 and 0.096 respectively. It was noteworthy that the maintenance coefficients were low at 0.0585 $h^{-1}$ and 0.0598 $h^{-1}$ for the two runs respectively.

## 4. Discussion

The residual reducing sugar concentration rate which consequently resulted in the glucose effect is referred to as Crabtree effect. This Crabtree effect is the phenomenon whereby the yeast, *Saccharomyces cerevisiae,* produces ethanol (alcohol) aerobically in the presence of high external glucose concentrations rather than producing biomass via tricarboxylic acid cycle, the usual process occurring aerobically in most yeast. Nevertheless, a very reasonable amount of the reducing sugar, which was believed to be mostly glucose, was converted to biomass while small amount of it was converted to ethanol and the rest was used up as energy. Drawing inferences from the specific growth rate values of Tables 1 and 2 as cell yield is a function of specific growth rate (μ). Meyerburg Von (1969) reported that lower substrate yield results when ì exceeds 0.23 $h^{-1}$ and this was attributed to the Crabtree effect which is a consequence of the limited respiratory capacity of *S. cerevisiae*. From the results it was observed that value of μ at the 4th hour for the two runs exceeded 0.23 $h^{-1}$ and from the 6th hour of the two runs downwards, the specific growth rate were much lower than the critical growth rate of 0.23 $h^{-1}$ while the production of ethanol did not start till after the 6th hour when the specific growth rate is almost close to the critical specific growth rate. All these were as a result of Crabtree effect that had set in and the growth became respiro-fermentative (Akinyemi *et al.*, 2003). The biomass yields obtained agreed with those of the previous workers (Ejiofor *et al.*, 1994 and Akinyemi *et al.*, 2003) who reported values in the range of 0.50 g/g.

Finally, the low values of the maintenance coefficients were indications of non stressful metabolism by the miero organism.

In conclusion, Cassava Starch can now be seen as one of the best local suitable substrate for baker's yeast production in Nigeria. The medium and fermentation conditions supported a high rate of fermentation and high yield efficiency while the fed batch process gave high yield of biomass and lower yield of ethanol.

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