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Production of Baker's Yeast (*Saccharomyces cerevisiae*) from Raw Cassava Starch Hydrolyzates in a Bioreactor under Batch Process

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Abstract: Bioconversion of raw cassava starch into value-added product was investigated. This involved hydrolysis of extracted starch from freshly harvested cassava tubers using three different methods. Enzyme-enzyme hydrolysis method gave the highest Dextrose Equivalent (DE) of 34 with starch conversion efficiency of 87.3% followed by the acid-enzyme hydrolysis which gave DE of 29.75 with conversion efficiency of 76%, while the lowest yield of DE 24.5 with conversion efficiency of 63% was recorded when acid hydrolysis method was employed. The cassava starch hydrolyzates obtained were used as feedstocks for the aerobic cultivations of the *Saccharomyces cerevisiae* under batch conditions for 48 h. The results obtained show that the hydrolyzates supported the growth of the microorganism. Biomass yields ($Y_{X/S}$) obtained for the three cultivations carried out using hydrolyzates from acid, acid-enzyme and enzyme-enzyme hydrolysis of raw cassava starch were 0.472, 0.462 and 0.470 g g^{-1}, respectively. Maintenance coefficient (m_e) obtained for the three cultivations were 0.0047, 0.0135 and 0.003 h^{-1} in the same order. These low values of maintenance energy is an indication of non-stressful metabolism by the microorganism in this batch system as the magnitude of the maintenance energy is strongly linked to the environmental conditions and the ability of the microorganism to survive in it.

Key words: Baker's yeast, hydrolysis, biomass yield, maintenance coefficient

INTRODUCTION

Saccharomyces cerevisiae (Baker's yeast) has a very long history in industrial fermentation. It has been extensively used for the production of ethanol and Single-cell Protein (SCP). In addition, it is also employed in the leavening of dough because of its ability to produce ethanol and carbon dioxide from sugars, e.g., maltose, present in the dough (Chen and Chiger, 1985; Jøgensen *et al*., 2002). Furthermore, it is also used in the leavening process because of its contribution to the flavour and aroma of bread (Hoek *et al*., 2000).

Since Yeasts (*S. cerevisiae* inclusive) cannot degrade starch naturally (Mccam and Barnett, 1986), the expression of heterologous amylase genes derived from various microorganisms in yeast has been intensively investigated. For example, amylase genes from *Aspergillus awamori* (Inlow *et al*., 1988), *Schwanniomyces occidentalis* (Wang *et al*., 1989), *S. diastaticus* (Erratt and Nasim, 1986) have been cloned in yeast. Steyn and Pretorius (1991) expressed the genes encoding a bacterial α-amylase, a yeast glucoamylase and a bacterial pullulase in *S. cerevisiae* to produce a direct, one-step bioconversion of starch-rich materials to bioethanol and protein, over the traditional two-step method with glucose as the final product. In addition Solomon *et al.* (1997) reported promising results in one-step synthetic starch degradation ability of *S. cerevisiae* ZC89 in batch processes. However, the starting starch concentrations used in these various investigations are very low. Hence, the need to first hydrolyze the starch (for example from cassava tubers) with high concentration and then use the reducing sugars obtained as substrate (i.e., carbon source) for the cultivation of *S. cerevisiae*.

Cassava (*Manihot esculenta* Crantz.) also known as manioc, tapioca or yucca (Grace, 1977; Tonukari, 2004), is an important starchy staple of lowland tropics and a major source of food chief support of some of the poorest nations of the World (Mba *et al*., 2001). It is a shrubby, tropical perennial plant originating in South America and introduced to Africa and Asia in the 16th century by Portuguese travelers (Alaux and Fauquet, 1990; Guthrie,

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1990). The cassava produced in Africa makes up to 54% of the World production (FAO, 2003), Nigeria being the leading country in the continent. Most of the cassava roots produced in Nigeria (Adewusi *et al*., 1999) and other West African countries are converted into important staple food products like *gari*, *fufu* and *tui*. In spite of its importance, a large proportion of the tubers are lost annually due to ineffective storage techniques. The use of cassava starch hydrolyzates in the production of Baker's yeast, ethanol, reducing sugars (e.g., maltose, glucose etc.), is a way of making value-added products of an abundant and renewable resource. Some of the previous works conducted on cassava starch involved kinetic studies on enzymatic hydrolysis of starch from cassava (Gorinstein, 1993; Gaouar *et al*., 1997a; Paolucci-Jeanjean *et al*., 2000), conversion of cassava starch into reducing sugars e.g., maltose (Gaourar *et al*., 1997b) or glucose (Omemu *et al*., 2005).

Therefore, this study focused on the hydrolysis of cassava starch using three different methods (i.e., acid hydrolysis, acid-enzyme hydrolysis and enzyme-enzyme hydrolysis) and in addition, the cassava starch hydrolyzates obtained were then used as feedstocks (substrates) for the cultivation of *S. cerevisiae* during production of baker's yeast in a bioreactor under batch conditions. The data collected were analyzed to estimate the pertinent parameter like the biomass yield and maintenance requirements.

MATERIALS AND METHODS

Preparation of substrate solution: The three methods used for the preparation of the substrate solution are described as follows:

Acid hydrolysis method: Raw cassava starch (obtained from Ile-Ife market) was dissolved in distilled water to form 35% starch slurry. This was gelatinized at 100°C in the water bath for 10 min. The gelatinized starch slurry was acidified with 0.5 M HCl, to bring the pH to a value of 2.0. The mixture was heated again in the water bath at the same temperature until thinning occurred. The reaction time lasted for 4 h and an iodoform test was used for the starch conversion efficiency (Solomon *et al*., 1997). After the confirmatory test, the acidified mixture was neutralized with 0.5 M sodium hydroxide in order to remove the free acid and the pH was raised to 4.5. Filtration was then carried out after neutralization as the refining process.

Acid-enzyme hydrolysis method: In the acid-enzyme method, the starch slurry was first treated by acidification, neutralization and filtration as it was done in the acid-hydrolysis method before the introduction of the saccharified enzyme, which was Novo amyloglucosidase (AMG) obtained from *Aspergillus niger*. The enzyme (5 mL enzyme kg^{-1} starch) was added with slow agitation. The pH remained 4.5 while the temperature was adjusted to 60°C. The reaction time for the enzyme conversion was 48 h.

Enzyme-enzyme hydrolysis method: Thirty five percent of starch slurry was gelatinized in a water bath by adding α- amylase (5 mL enzyme kg^{-1} starch of Novo Termamyl obtained from *Bacillus licheniformis*) at 105°C for 5 min at pH of 6.5. This was liquefied at a lower temperature of 95°C for 2 h. The liquefied starch was saccharified 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 was the reducing sugars used as substrate for the production of baker's yeast.

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 subculturing.

Preparation of inoculum: 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 to each of the medium in the flasks aseptically. The flasks were transferred to the gyratory incubator shaker (New Brunswick Scientific Co., USA) and growth was achieved at a temperature of 30°C and agitation rate of 200 rpm after incubation for 24 h.

Fermentation: Three aerobic cultivations were carried out on a bench scale bioreactor manufactured by New Brunswick Scientific Co., USA. The starch concentration used in all experiment was 35% slurry. Prior to inoculation in each run, the fermentor vessel containing 1.7 L of the medium and 1 L of the mineral constituents was sterilized by autoclaving at 121°C for 15 min. Thereafter, the vessel was allowed to cool and coupled back to the fermentor assembly. The inoculum was then introduced into the fermentor vessel aseptically. The culture was agitated at 800rpm with an airflow rate of 2 vvm and cultivation was conducted at 30°C and pH of 4.5. The temperature was maintained at 30°C by circulating water at such

temperature from a water bath through the cooling water pot of the fermentor. Few drops of antifoam (Ucolub) were added to the fermentation. Samples were taken at 4 h 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 estimation.

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 0-phenanthroline as indicator.

Biomass concentration: For each run 20 m L of samples was collected at regular interval into pre-weighed tube, which was then centrifuged at 5000 rpm for 10 min. The pellet was washed twice by resuspending them 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 using the methods of Solomon *et al.* (1982) and Akinyemi *et al.* (2003)

RESULTS

The results obtained in the study of batch hydrolysis of raw cassava starch show that the three different methods adopted were very successful (Table 1). Starting with initial 35% (w/v) raw cassava starch slurry, enzyme-enzyme method gave the highest Dextrose Equivalent (DE) of 34 with starch conversion efficiency of 87.3%. This is followed by the acid-enzyme method, which gave DE of 29.75 with starch conversion efficiency of 76%. The lowest yield of DE of 24.5 with starch conversion efficiency of 63% was recorded when acid hydrolysis method was employed.

Table 1: Hydrolysis of raw cassava starch using three different methods

Method of hydrolysis	Dextrose equivalent (DE)	Conversion efficiency (%)
Acid	24.50	63.0
Acid-enzyme	29.75	76.0
Enzyme-enzyme	34.00	87.3

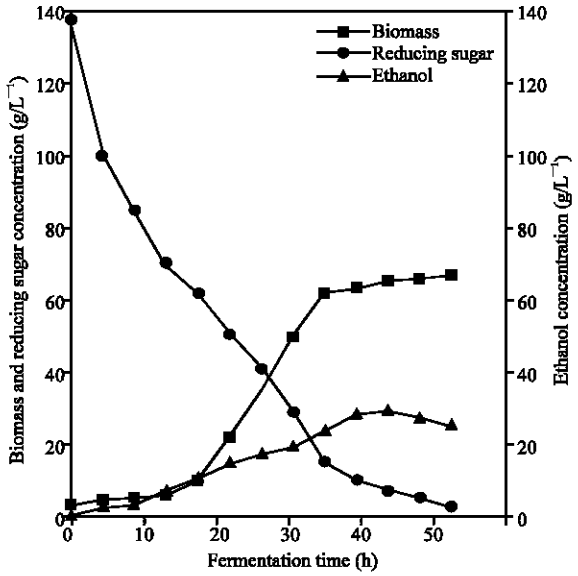

Fig. 1: Plot of biomass, reducing sugar and ethanol concentrations as a function of fermentation time for aerobic growth of baker's yeast on reducing sugar obtained by acid hydrolysis of raw cassava starch

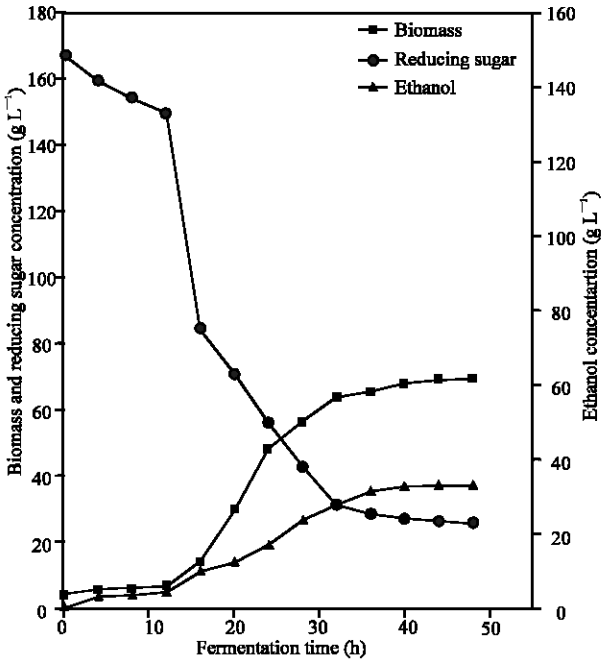

Fig. 2: Plot of biomass, reducing sugar and ethanol concentrations as a function of fermentation time for aerobic growth of baker's yeast on reducing sugar obtained by acid enzyme of raw cassava starch

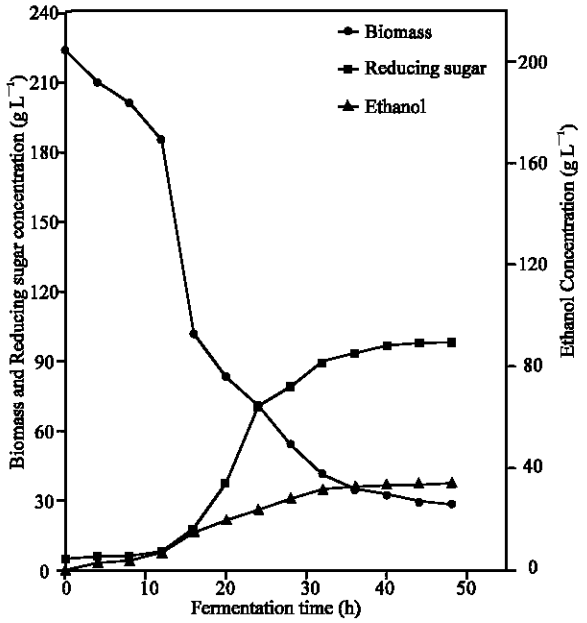

Fig. 3: Plot of biomass, reducing sugar and ethanol concentrations as a function of fermentation time for aerobic growth of baker's yeast on reducing sugar obtained by enzyme-enzyme of raw cassava starch

The results obtained during cultivation of *Saccharomyces cerevisiae* (baker's yeast) on the cassava starch hydrolyzates under batch conditions in a bioreactor are displayed in Fig. 1-3. The results show that the hydrolyzates obtained by hydrolysis of indigenous raw cassava starch via acid (Fig. 1), acid-enzyme (Fig. 2) and enzyme-enzyme (Fig. 3) methods were all able to support the growth of *S. cerevisiae*. Results further display biomass and ethanol production rates with corresponding consumption rate of the substrate (reducing sugar). The results followed the same pattern, as the reducing sugar was been consumed by the baker's yeast, concomitantly production of the biomass and ethanol was observed (Fig. 1-3). The ethanol concentration increased as the reducing sugar concentration used as feedstock for the microorganism increased (Fig. 1-3). Biomass yields ($Y_{X/S}$) obtained for the three cultivations carried out using hydrolyzates from acid, acid-enzyme and enzyme-enzyme hydrolysis of raw cassava starch were 0.472, 0.462 and 0.470 g g^{-1}, with corresponding product yields ($Y_{P/S}$) of 0.168, 0.212 and 0.172 g g^{-1}, respectively. In addition, the integrated biomass energetic yields (η) as well as the integrated product energetic yield (ξ_P) were also estimated. The integrated biomass energetic yields for cultivations made with acid, acid-enzyme and enzyme-enzyme hydrolyzates are 0.585, 0.573 and 0.593 with corresponding integrated product energetic yields of 0.327, 0.416 and 0.335, respectively. Maintenance coefficient (m_e) obtained for the three cultivations are 0.0047, 0.0135 and 0.003 g g^{-1}, respectively.

DISCUSSION

Hydrolysis of synthetic cassava starch using enzymatic method has been previously reported (Gorinstein, 1993; Paloucci-Jeanjean *et al*., 2000; Gaouar *et al*., 1997b). Hydrolysis of raw cassava by amylase of *Aspergillus niger* was reported to give 27.17% conversion efficiency (Omemu *et al*., 2005). In the study of Gaouar *et al*. (1997b) in which, cassava starch was hydrolyzed to maltose using enzymatic method, conversion efficiency of 72% was observed. Hence, the hydrolysis results obtained in this study compare favourably with these previously reported works.

S. cerevisiae baker's yeast has been grown on several substrates like molasses, cashew apple juice, for production of single-cell protein and ethanol (Layokum *et al*., 1986; Solomon and Layokum, 1988; Solomon *et al*., 1991). Although, the production of L-lactic acid from raw cassava starch in a bioreactor using *Aspergillus awamori* and *Lactococcus lactis* sp. has been demonstrated (Roble *et al*., 2003), the growth of *S. cerevisiae* on raw cassava starch hydrolyzates for the production of biomass is novel and the results obtained are promising in making value added products from cassava. The observation of concomitant biomass and ethanol production during cultivation of the baker's yeast is supported by literatures. To corroborate this observation, Petrik *et al*. (1983) reported that oxidative and fermentative glucose catabolism take place simultaneously. Even under fully aerobic conditions a mixed respiro-fermentative metabolism is observed when sugar concentration exceeds a certain threshold value (Verduyn *et al*., 1984; Ejiofor *et al*., 1994a, b). The biomass yields obtained in this study are within the range reported earlier. Solomon *et al*. (1991) reported yield of 0.48 g g^{-1}, in the study of single-cell protein production by cultivation of *S. cerevisiae* on blackstrap molasses and Layokun *et al*. (1986) estimated the biomass yield to be 0.5 g g^{-1}, when this microorganism was cultivated on cashew apple juice for the production of single-cell protein. The low values of maintenance energy obtained in this study is an indication of non-stressful metabolism by the microorganism in this batch system as the magnitude of the maintenance energy is strongly linked to the environmental conditions and the ability of the microorganism to survive in it.

In order to increase volumetric productivity of biomass, it is better to carry out the cultivation of the microorganism using fed-batch process. This will help in preventing formation of ethanol which lowers biomass yield. In the situation where ethanol production is the goal, the cultivation should be run under fully anaerobic conditions.

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