

Culture of *Saccharomyces cerevisiae* on hydrolyzed waste cassava starch for production of baking-quality yeast

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A fermentation medium based on waste cassava starch hydrolysate and a four-phase feeding strategy for a fed-batch culture of Baker's yeast *Saccharomyces cerevisiae* are presented. Cassava starch isolated from the wastewater produced in processing of cassava mash into gari was liquefied with a thermostable 1,4- α -D-glucanohydrolase (EC 3.2.1.1) in the presence of 100 ppm Ca^{2+} at 80°C and pH 6.1–6.3 for one h. The liquefied material was saccharified with 1,4- α -D-glucan glucohydrolase (EC 3.2.1.3) at 55°C and pH 5.5 for two h. Over 98% of the starch was hydrolyzed; about 80.7% of the hydrolysate was glucose. The fermentation feeding profile which was based on a desired specific growth rate range of 0.18–0.23 h^{-1} , a biomass yield coefficient of 0.5 g g^{-1} , and a feed substrate concentration of 200 g l^{-1} was implemented manually using the cassava hydrolysate feed in test experiments and glucose feed in control experiments. The fermentation off-gas was analyzed on-line by mass spectrometry for the calculation of the oxygen uptake rate, the carbon dioxide evolution rate, and the respiratory quotient. Off-line determinations of biomass, ethanol, and glucose were done, respectively, by dry weight, gas chromatography, and spectrophotometry. Cell mass concentrations of 50–58 g l^{-1} were achieved in all experiments within 28 h of which the last 15 h were in the fed-batch mode. The average biomass yields for the cassava and glucose media were identical at 0.49 g g^{-1} . No significant differences were observed between the leavening activities of the products of the test, the control media, and a commercial preparation of instant active dry yeast. Waste cassava starch hydrolysate was established as a suitable low cost replacement for glucose in the production of baking-quality yeast.

Keywords: Waste cassava starch; liquefaction; saccharification; Baker's yeast; *Saccharomyces cerevisiae*; fermentation

Introduction

The least expensive and most common source of metabolizable sugars for Baker's yeast fermentation is molasses. This is true in regions where molasses is produced and the cost is about \$120–150 per metric tonne.¹ In other regions where it must be imported, transportation costs and the usually high customs duty add to the price, thereby making molasses expensive. Because fermentation raw materials are major contributors to the cost of production of low value products such as Baker's yeast, exploration of other inexpensive and locally available fermentable sources is essen-

tial. One possible source is cassava (*Manihot esculenta*, Crantz) starch. Cassava is a tropical root crop produced in more than 80 countries with a world production in the mid-1980s of 137 million metric tonnes and a yield of 60 tonnes per hectare.¹ About 30 million tonnes or 22% was incorporated into animal feed. A similar amount was converted into starch for industrial use and another portion to human food in some of the developing countries. The rest was lost since cassava is perishable after harvest. Harnessing the lost portion in addition to gains from new high-yielding varieties with outputs of 100 tonnes per hectare could provide the fermentation industry with an abundance of raw material.

Cassava starch is composed of unbranched amylose (20 \pm 5%) and branched amylopectin (80 \pm 5%) both of which can be hydrolyzed acidically or enzymatically (either with pure enzymes or amylase-producing microorganisms) to release their constituent glucose and maltooligosaccharides. Both products are easily transported across the cell membrane and metabolized by yeasts.²

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Metabolism of glucose by *Saccharomyces cerevisiae* can be either aerobic or anaerobic depending on culture conditions, the availability of the limiting substrate, and the continuous supply of mineral supplements, nitrogen, growth factors, and other physical inputs. Baker's yeast production aims at providing aerobic conditions and eliminating or minimizing ethanol production. In practice, this is normally achieved by fed-batch operation which allows for the control of a key nutrient concentration. The degree of success depends on the ability to anticipate the needs of the exponentially increasing cell population. Hence, the nutrient feeding is usually guided by a process model. Either linear or exponential models may be used. The aim is to control the specific growth rate at a value below the critical growth rate at which metabolism becomes fermentative. The growth rate is controlled by fed-batch operation by keeping the glucose concentration within the range of 0.1–0.25 g l⁻¹. Above a concentration of 0.25 g l⁻¹, metabolism becomes increasingly fermentative as growth rate increases and the Crabtree effect as well as respiratory bottleneck occur.³

The goal of this work is to develop a low energy-requiring process to convert waste cassava starch to a fermentable medium and an exponential feeding strategy for the production of Baker's yeast on the novel medium. The medium and feeding strategy are expected to reduce the expenditure on raw material procurement and relax the dependence on molasses in cassava-producing regions of the world.

Materials and methods

Development of fermentation medium

Cassava starch was obtained from the wastewater expressed from pulverized cassava starch in *gari* processing.⁴ A 20% (w/v) mixture of the dry starch was made and a low temperature long cooking procedure (Figure 1) was employed in the liquefaction and saccharification processes. The procedure involved cooking the mash for 1 h in the presence of 100 ppm Ca²⁺ (provided as CaCl₂ · 2H₂O) at 80°C in a stirred fermenter (MBR Bioreactor AG, Wetzikon, Switzerland; 15 l nominal, 0.2 m vessel diameter equipped with two downward pumping axial flow impellers, 0.1 m impeller diameter, fully baffled with four baffles) at 900 rpm. The gelatinized starch was liquefied with 0.3 g of a thermostable α -amylase (1,4- α -D-glucanohydrolase, EC 3.2.1.1, from *Bacillus*

licheniformis, Sigma Chemical, St. Louis, MO, USA) per kg of starch. The process was allowed to continue with stirring (1 h at pH 6.1–6.3). The liquefied broth was then saccharified (pH 5.5 at 55°C for 2 h) with 0.5 ml of glucoamylase (1,4- α -D-glucan glucohydrolase, EC 3.2.1.3, from *Aspergillus niger*, Sigma Chemical) per liter of liquefied broth. Samples were taken every 20 min. The enzyme reaction was stopped by addition of hydrochloric acid (1 M; 0.5 ml acid ml⁻¹ sample) during liquefaction, and by addition of 3,5-dinitrosalicylic acid (DNS) reagent (1 ml ml⁻¹ sample) during saccharification. The samples were analyzed with a Waters (Milipore, Milford, MA, USA) HPLC equipped with an external differential refractometer (Model R 401). The Aminex HPX-87P column (300 mm × 7.8 mm; Biorad Laboratories, Melville, NY, USA) was used with automatic sample injection (Waters 700 Saterlite WISP, 20 μ l injection volume). Chromatograms were acquired and integrated with a Baseline 810 Chromatography Workstation. Double-deionized water was the eluent (85°C, 0.6 ml min⁻¹). The approximate elution times were 12.5 min for maltose and 15.1 min for glucose. The final broth was concentrated, as required, by evaporation and used with a modification of the synthetic medium of O'Connor and coworkers⁵ in which the vitamin solution was replaced with 0.3% Bacto yeast extract (Difco Laboratories, Detroit, MI, USA). The Sigma 289 antifoam (Sigma Chemical) was used for foam control in response to a foam sensor.

The microorganism and inoculum preparation

Saccharomyces cerevisiae was isolated from a commercial strain of Fleischmann Baker's yeast (ATCC 7754, Philp Burns Foods, Canada). The yeast was maintained on slants containing: glucose, 10 g l⁻¹; yeast extract, 0.1 g l⁻¹; biotin, 0.01 g l⁻¹; and agar (BDH, Darmstadt, Germany), 20 g l⁻¹ as direct stock culture from which 3-stage inocula were prepared. Pure slant cultures were inoculated into two 500-ml baffled Erlenmeyer flasks containing 100 ml glucose (10 g l⁻¹) and yeast extract (3 g l⁻¹) broth. The flasks were incubated on an orbital shaker at 30°C and 150 rpm for 22 h and used to inoculate two 5-l baffled Erlenmeyer flasks containing 1.9 l of glucose (25 g l⁻¹) and yeast extract (3 g l⁻¹) broth. These flasks were incubated as described above, pooled, and used to inoculate a 15 l stirred fermenter (MBR Bioreactor AG) containing 3 l of glucose (30 g l⁻¹), mineral salts, and yeast extract medium.⁶ The last stage was run as a batch process at 30°C and pH 4.5 at 400–700 rpm with an air flow of 2 vvm; this ensured an actively growing total dry cell weight of approximately 30 g and less than 0.5 g l⁻¹ ethanol at the beginning of the fed-batch operation.

Fed-batch operation

All fermentations were carried out with a starting volume of 5 l. The exponential feeding profile shown in Figure 2 was generated with Eq. (4) presented later. The feeding profile was executed manually with a peristaltic pump (Watson Marlow, Type MHRE 100) which was calibrated with Masterflex 6424–14 tubing. To compensate for possible pumping errors, on-the-spot on-line recalibration was done at the beginning of each feeding phase. The feeding profile (Figure 2) was based on four different selected values of the desired specific growth rate (μ); these were 0.18, 0.20, 0.22, and 0.23 h⁻¹. The arrows in Figure 2 denote the points at which the values of the specific growth rates change. Fermentations were run with glucose ($S_0 = 0.2 \text{ g g}^{-1}$) and cassava hydrolysate ($S_0 = 0.217 \text{ g g}^{-1}$) feeds that were held on a balance (Mettler, PE11) and continuously stirred with a magnetic stirrer. Other conditions were 30°C at 400–950 rpm and 2–2.5 vvm aeration. Figure 3 shows the specific patterns of variations in air flow rate, the dissolved oxygen concentration, and the agitation speed

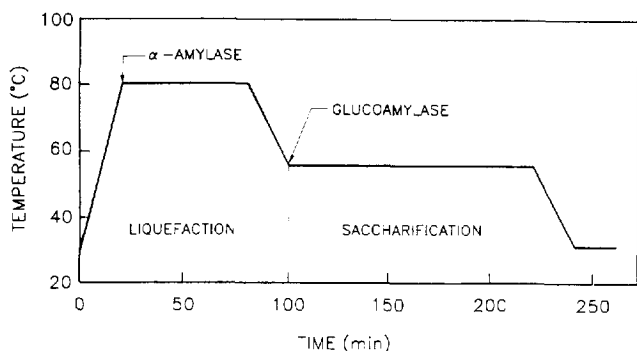


Figure 1 Long low-temperature process profile for cassava flour hydrolysis. Arrows indicate the points of addition of enzymes

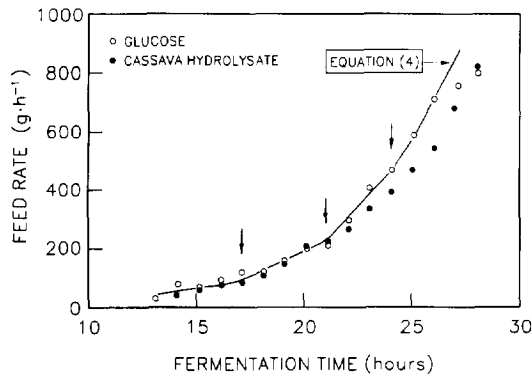


Figure 2 Calculated (Eq. 4) and experimental substrate flow rates for fermentations using cassava hydrolysate and glucose feeds. Arrows indicate the points at which the specific growth rate values (Eq. 4) were changed

that occurred during the fermentations. Agitation was controlled automatically in response to the level of dissolved oxygen. Aeration rate was varied manually as in *Figure 3*. Aeration and agitation ensured a dissolved oxygen level of $\geq 20\%$ of air saturation. Air flow was monitored with a thermal mass flow meter (Sierra Instruments, CA, U.S.) having a range of 0–20 l min⁻¹. The pH was controlled with 12.5% NH₃ held on a balance (Mettler, PM3000). The fermentation off-gas was analyzed for oxygen and carbon dioxide using a quadrupole mass spectrometer (PEGASUS VG, Cheshire, England) equipped with an SX-PC interface. The PROCESS-SOFT applications package was used for on-line calculation of the oxygen uptake rate, the carbon dioxide production rate, and the respiratory quotient.

Off-line analytical procedures

Samples collected every hour were used for off-line determinations of cell dry weight, optical density, and glucose as well as ethanol concentration. Cell dry weight was determined with 20 ml of the sample broth which was pipetted into each of two pre-weighed centrifuge tubes and spun at 10,000 rpm in an IEA B-20A refrigerated (10°C) centrifuge. The solids were washed twice by resuspending in 20 ml of sterile deionized water and repeating the centrifugation as described. The washed cells were dried to constant weight in an oven (80°C at 24 h), allowed to cool in a desiccator, and weighed. The difference was the dry cell weight in 20 ml which was used to calculate the total dry cell weight in the fermentation vessel.

Optical density (OD) was determined by diluting broth culture

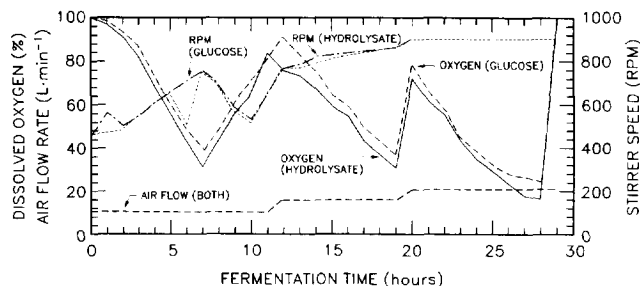


Figure 3 On-line measurements of dissolved oxygen concentration, air flow rate, and the stirrer speed during fermentation runs using cassava hydrolysate and glucose feeds

samples by appropriate dilution factors to obtain the linear response range of absorbance. The optical density of the diluted sample was measured in a spectrophotometer (LKB Biochrome, Model 4057) at 600 nm. The result was related to the cell dry weight by the Beer's law and used in subsequent fermentations to estimate the cell dry weight. Increase in biomass and the optical density were approximately directly proportional with a constant factor of 1.88 ± 0.05 .

The first supernatant from the cell dry weight determination was used for the estimation of glucose and ethanol concentrations. Glucose was determined using a glucose enzymatic assay kit (Sigma Chemical, St. Louis, MO). The color developed following the assay procedure was read at 520 nm using a Shimadzu UV-160 spectrophotometer. Ethanol concentration was determined by gas chromatography using a Hewlett-Packard 5880A series gas chromatograph. The supernatant (1 ml) was mixed with 50 μ l of an internal standard containing 2.5% isopropanol in hydrochloric acid (0.5 M). The processed sample (2.4 μ l) was injected manually. Two levels of ethanol standards containing 1 mmol l⁻¹ and 20 mmol l⁻¹ were used for calibration of the chromatograph. The chromatograms were integrated automatically by the series integrator.

Because the baking use properties of the product were important, the leavening activity of the products was determined in accordance with the dough raising method⁷ in comparison with the commercial instant dry yeast from which the cells used in this work were isolated. The well-mixed dough contained 40 g wheat flour (Robin Hood Multifoods, Markham, Canada) 4 g sucrose, 2% yeast relative to the flour, and 30 ml water. For the experimental wet yeast cells, 2% was equivalent to 30 ml suspension of the cells in water with an optical density of 1.7. Measurements of dough volume in a 500 ml measuring cylinder, lubricated with vegetable oil to prevent sticking to the walls while the dough was being deposited on the bottom, were taken every 20 min.

The fermentation model

The fed-batch part of the fermentation operation depended on a model-based feeding strategy. The basis of the model used is presented here.

In the presence of adequate nutrients, suitable environmental conditions (temperature, pH) and the absence of inhibitory concentrations of ethanol, the yeast cell growth is autocatalytic; growth can be represented as

$$X_t = X_0 e^{\mu t} \quad (1)$$

where X_0 and X_t are the total amounts of biomass in the fermentation vessel at time zero and any other time t , respectively. In Eq. (1), μ is the specific growth rate. Cells consume the carbon source for growth, product formation, and maintenance. This can be represented with the equation

$$-\frac{dS}{dt} = F_s S_0 - \frac{\mu}{Y_{x/s}} X_t - \frac{q_p}{Y_{p/s}} X_t - mX_t \quad (2)$$

where S is the total amount of carbon source in the fermenter at time t , F_s is the mass flow rate of the carbon source containing feed, and S_0 is the mass fraction of the carbon source in the feed. The second, third, and fourth terms on the right-hand side of Eq. (2) represent, respectively, the rates of consumption of the carbon substrate for cell mass production, product formation, and maintenance of the cells. In Eq. (2), $Y_{x/s}$ and $Y_{p/s}$ are the biomass and the product yield coefficients based on the carbon source, q_p is the specific product formation rate, and m is the maintenance coefficient.

Under aerobic conditions in the fed-batch mode, if the flow rate

is set in such a way that the glucose concentration in the fermenter is less than or equal to 0.2 g l^{-1} and all the added glucose is consumed, then dS/dt is zero. Usually under growth conditions, the maintenance term is relatively small. Because the growth rate is controlled below the critical value, there will be no ethanol production. Consequently, the product formation and maintenance terms are negligible compared to the growth term; hence, Eq. (2) may be rewritten as

$$F_s = \frac{\mu}{Y_{s/x} S_0} X_t \quad (3)$$

Substitution of Eq. (1) into Eq. (3) gives

$$F_s = \frac{X_0}{Y_{s/x} S_0} \mu e^{\mu t} \quad (4)$$

Eq. (4) is used to guide the feeding rate for any specified values of the specific growth rate (μ), the initial quantity of biomass (X_0), and the mass fraction S_0 of the substrate in the feed. For this work, the batch fermentations were used to provide an initial biomass (X_0) value of 30 g. The yield coefficient ($Y_{s/x}$) value was 0.5 g g^{-1} and the S_0 value was 0.2 g g^{-1} . The μ values were 0.18, 0.20, 0.22, and 0.23 h^{-1} . These values were selected to be lower than the critical specific growth rate (μ_{crit}) which has been reported to be between $0.25\text{--}0.30 \text{ h}^{-1}$.^{3,8}

For further comparison of the glucose-fed and cassava hydrolysate-fed fermentations, the maximum substrate uptake rate ($q_{s,max}$) and other fermentation characteristics were compared. Thus, the $q_{s,max}$ was calculated with the equation.

$$q_s = q_{s,max} \frac{C_s}{C_s + K_s} \quad (5)$$

where C_s is the substrate concentration and K_s is the Monod constant. The glucose uptake rate (q_s) follows Eq. (5) when the substrate concentration exceeds the critical value $C_{s,crit}$. The respiro-fermentative metabolism of the yeast⁹ switches to the fermentative mode when the substrate concentration exceeds the critical value and under other conditions such as oxygen limitation. When the substrate concentration is lower than the critical value, glucose and ethanol uptake can occur simultaneously. Ethanol uptake is described as

$$\mu_E = \mu_{max,E} \frac{E}{E + K_E} \quad (6)$$

where E is the concentration of ethanol and K_E is the ethanol concentration at half the maximum ethanol uptake rate ($\mu_{max,E}$). However, ethanol uptake can be inhibited by glucose when present above 0.3 g l^{-1} . Further, the utilization of ethanol is completely dependent on the availability of oxygen; the rate of consumption can be represented by

$$q_{ox} = q_{ox,max} \frac{C_{ox}}{C_{ox} + K_o} \quad (7)$$

where C_{ox} is the concentration of dissolved oxygen.

Comparison of the fermentation parameters $q_{s,max}$, K_s , K_E , and K_o is a useful means of demonstrating the equivalence of the fermentations carried out on pure glucose and the cassava hydrolysate.

Results and discussion

The time course of the conversion of cassava starch to sugars is shown in Figure 4 for the liquefaction and the saccharification stages. Conversion of starch to sugars ex-

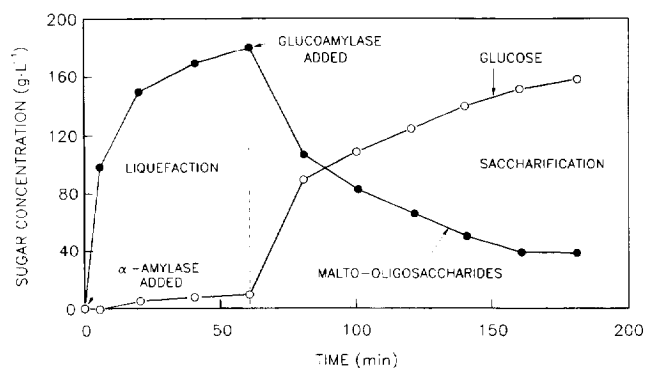


Figure 4 Time course of enzymatic hydrolysis of waste cassava starch

ceeded 98%. Glucose constituted 80.7% of the converted material; the rest were maltooligosaccharides comprising essentially maltose and isomaltose. The production of sugars in Figure 4 followed the expected pattern: α -amylase liquefied the starch by random hydrolysis of the α -1,4 glycosidic linkages, yielding some glucose but mostly maltose and maltooligosaccharides. Addition of glucoamylase rapidly converted the maltose and maltooligosaccharides to the monosaccharide, glucose. The long low-temperature cooking procedure used (Figure 1) was considered beneficial in that high pressure steam was not required to attain the high temperatures needed in the alternative short high-temperature procedure.¹⁰ Consequently, the technology requirements for steam generation were simplified. The cost of enzymatic hydrolysis can be minimized by substitution of pure enzymes with amylase-producing microorganisms.⁴ Concentration of the hydrolysate yielded $217 \text{ g sugar liter}^{-1}$ of hydrolysate which was about 10% higher than the value used for the simulations. Concentration of the hydrolysate would not have been necessary if the cooking had been done in a mash cooker because a higher concentration of the starch could have been used while ensuring thorough mixing at a lower agitation rate of about 100 rpm.

The batch phase of fermentation was not modeled but was designed simply for the production of the initial total cell mass concentration (X_0) of about 30 g. The objective value was achieved: 30.0 g being obtained with the cassava hydrolysate run and 29.8 g for the glucose run. Figures 2, 5, and 6 show simulations of a fed-batch feeding strategy with the features described above. The experimental data for the feed flow rate (F_s), the specific growth rate (μ), and the total biomass (X_t) in the fermenter are also presented and show good fit with the simulations. The feeding pattern during the fed-batch operation consisted of four phases each with a different, constant, specific growth rate (μ). The phases are indicated with arrows in Figure 2. As a result of the manual execution of the profile, the points of change in the specific growth rate are not sharply discernible in Figure 2, but the general upward trend is clear. The selected and experimentally measured values of specific growth rates in Figure 5 agreed reasonably well for the two substrates. Throughout the fed-batch phase, the residual glucose varied between $0.09\text{--}0.21 \text{ g l}^{-1}$ (Figure 7), thus avoiding the Crabtree effect and ensuring low ethanol concentrations between 10.1

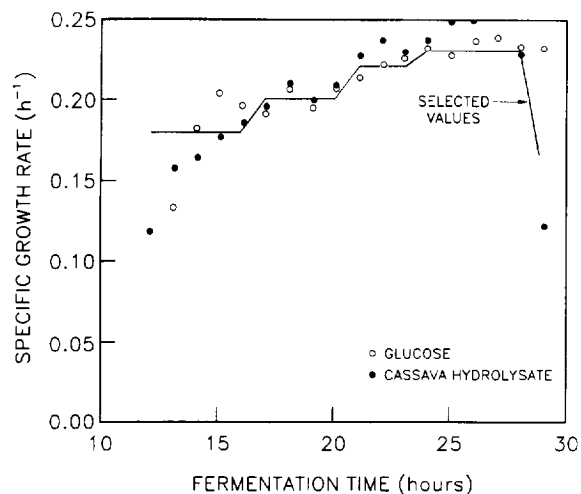


Figure 5 Specified and measured specific growth rates during fermentation runs with cassava hydrolysate and glucose feeds

mmol l⁻¹ (or 0.47 g l⁻¹) and 30.6 mmol l⁻¹ (or 1.41 g l⁻¹) throughout the fermentation (Figure 7). A sharp decline in ethanol concentration near the end of the fermentation (Figure 7) occurred after the feeding had ceased but aeration continued (for about an hour). Ethanol was being utilized as a carbon source in this stage. A rapid decline in the specific growth rate in this final stage of the fermentation was confirmed in Figure 5; corresponding sharp changes were seen in the composition of the exhaust gas. Because there were no significant amounts of ethanol (Figure 7), there were no losses in yield or total cell mass production which were 518.6 g simulated, 496.2 g for the cassava hydrolysate, and 468.3 g for glucose (Figure 6) at the end of the fermentation process. These yielded, respectively, 51.9 g l⁻¹, 58.5 g l⁻¹, and 49.9 g l⁻¹ when fermenter volumes were considered. Cell mass growth rate which ranged from 0.9–74 g h⁻¹ for cassava hydrolysate and 0.5–69 g h⁻¹ for glucose also reflected the pattern of glucose consumption. The ammonia

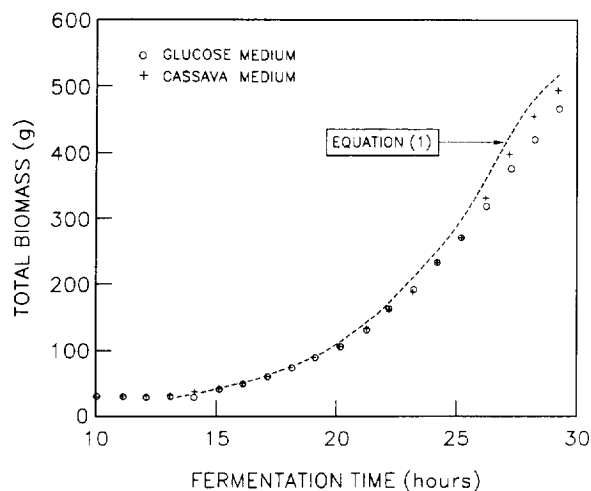


Figure 6 Estimated (Eq. 1) and actual total biomass in the fermenter for runs using cassava hydrolysate and glucose feeds

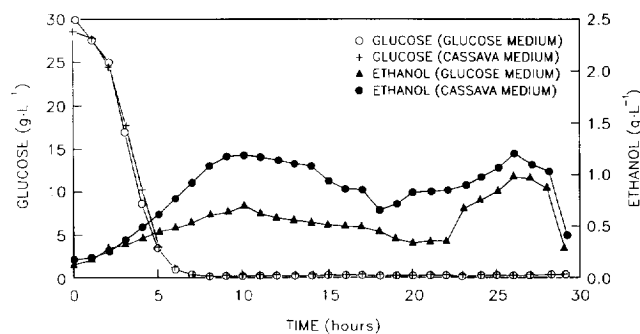


Figure 7 Glucose and ethanol concentrations during fermentation runs using cassava hydrolysate and glucose feeds

used in pH control was an additional source of nitrogen necessary for the metabolism of glucose; the ammonia feed rates were identical for the two systems.

On-line measurements confirmed that the respiratory quotient remained at 1.0 ± 0.1 throughout the fed-batch phase. This was further indication that the metabolism was oxidative through the fermentation. Figure 3 provides additional confirmation that although the agitation and aeration were held constant at 950 rpm and 20 l min⁻¹, respectively, from 20 h, no oxygen limitation occurred since the level of dissolved oxygen remained about 20% of air saturation.

Although the glucose feed rate was controlled manually during the fed-batch operation, the control strategy was quite effective in maintaining the respiratory quotient close to unity. This provides strong evidence that a control strategy not dependent on sophisticated computerization can produce requisite results, thus being useful for process implementation in the low-technology environments of many of the cassava-producing regions.

For further comparison of the fermentations that used two different substrates, Table 1 presents the parameters $q_{s,max}$, $Y_{x/s}$, $Y_{x/e}$, K_s , K_e , and K_o for the two cases. Previously published ranges for these parameters in glucose media are also presented (Table 1). Clearly, the fermentations on glucose and cassava hydrolysate had comparable performance.

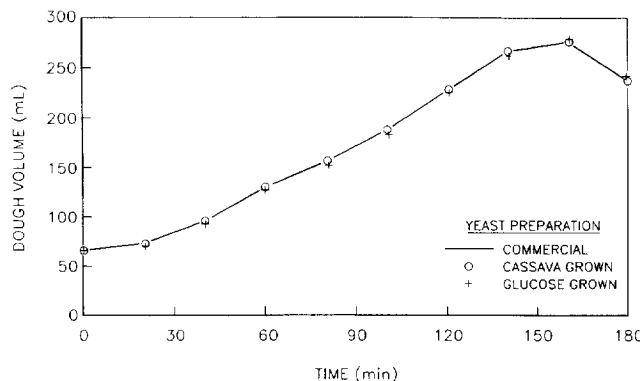


Figure 8 Comparison of dough-leavening activities of the glucose-grown yeast, cassava hydrolysate-grown yeast, and a commercial Baker's yeast

Table 1 Experimental parameter values and published ranges

Parameter	Experimental values		Range	Units	Reference
	Glucose	Cassava			
$q_{s,max}$	3.46	3.48	3.0–3.75	$g\ g^{-1}\ h^{-1}$	2
$Y_{x/s}$ oxidative	0.49	0.49	0.47–0.5	$g\ g^{-1}$	2, 11
$Y_{x/s}$ fermentative	0.10	0.09	0.05–0.15	$g\ g^{-1}$	2, 11
$Y_{x/e}$	0.68	0.72	0.67–0.72	$g\ g^{-1}$	2, 11
K_s	0.25	0.3	0.1–0.5	$g\ l^{-1}$	2
K_o	0.1	0.1	0.08–0.12	$mg\ l^{-1}$	2
K_e	0.1	0.1	0.1–0.11	$g\ l^{-1}$	2
α	1.0	1.0	1.0	$mol\ mol^{-1}$	
β	1.72	1.77	1.7–1.85	$mol\ mol^{-1}$	2, 11
γ	0.60	0.62	0.54–0.63	$mol\ mol^{-1}$	2, 11
δ	0.165	0.17	0.16–0.18	$mol\ mol^{-1}$	2, 11

In addition, the data on glucose compared well with previously published^{2,11} information (Table 1), hence validating the experimental and the computational techniques used in this work. The elemental composition of the yeast product, $C_{\alpha}H_{\beta}O_{\gamma}N_{\delta}$, obtained on glucose and cassava hydrolysate are also presented in Table 1 alongside the published ranges for glucose-grown yeast. Once again, the cassava hydrolysate fermentations give a product that is comparable to that obtained on the glucose medium.

Feeding was terminated and pH adjusted to 5.0 1 h before the end of the process for product maturation and quality. During this period, the specific growth rate decreases allowing the cells to use up residual glucose and ethanol. This is accompanied by a reduced fraction of budding cells to 6–10% of the population which increases the leavening activity of the yeast product.¹² This is confirmed by a comparison of the leavening activities of the products obtained in this work with the activity of the commercial instant active dry yeast (Figure 8). Clearly, the cassava hydrolysate-grown yeast fully attained the baking quality standards. Cessation in budding is an indication of cessation of biosynthesis of informational macromolecules and accumulation of intracellular carbohydrates which the cells use as energy supply during storage. This makes for stability during storage. It has been shown that yeast cells utilize about 1% of the intracellular storage carbohydrates per day at 4°C.²

Although the focus of this work was on production of baking quality yeast on cassava hydrolysate, the comparable composition, fermentation performance, and quality attributes of the glucose- and cassava hydrolysate-grown yeasts suggest wider use potential for the cassava hydrolysate-based media. Thus, for example, the cost of production of the many recombinant proteins in genetically modified strains of Baker's yeast¹³ may be lowered by employing the less expensive cassava hydrolysate in the fermentation medium.

Conclusion

A strategy for utilization of the waste cassava starch in production of baking-quality yeast was demonstrated. Waste cassava starch could be liquefied, saccharified, and

formulated into a suitable medium that was equivalent to the glucose control medium in supporting the requisite growth rate and yield of Baker's yeast. The important dough leavening activities of the yeast products from the cassava hydrolysate and the control media were identical, agreeing closely also with the activity of a commercial baking yeast preparation.

With the demonstration of cassava as a rich source of hydrolyzable carbohydrate, established worldwide production of the tuber, the development of new high-yielding, early-maturing, and pest-resistant varieties, and the low level of its current utilization for other purposes, suggest a strong potential for its large-scale use in the fermentation industry. The cost of processing associated with the use of enzymes may be reduced by using microorganisms that are capable of hydrolyzing starch in alternative processing schemes.

For accurate parameter estimation, screening new yeasts and media as well as the design of production-scale operations, processes should be based on simple and robust models capable of implementation in a low technology environment. This work has shown that the substrate concentration and the growth rate can be controlled below the critical ranges even with limited automation without losing yield and product quality.

List of symbols

C_{ox}	Dissolved oxygen concentration ($mg\ l^{-1}$)
C_s	Substrate concentration in the fermenter ($g\ l^{-1}$)
$C_{s,crit}$	Critical substrate concentration ($g\ l^{-1}$)
E	Ethanol concentration ($g\ l^{-1}$)
F_s	Substrate flow rate ($g\ h^{-1}$)
K_s	Constant in Eq. (6) ($g\ l^{-1}$)
K_o	Constant in Eq. (7) ($mg\ l^{-1}$)
K_e	Constant in Eq. (5) ($g\ l^{-1}$)
m	Specific maintenance term (h^{-1})
q_{ox}	Specific oxygen uptake rate (h^{-1})
$q_{ox,max}$	Maximum specific oxygen uptake rate (h^{-1})
q_p	Specific product formation rate (h^{-1})
q_s	Specific substrate uptake rate ($g\ g^{-1}\ h^{-1}$)
$q_{s,max}$	Maximum specific substrate uptake rate ($g\ g^{-1}\ h^{-1}$)

S	Total substrate in the fermenter at time t (g)
S_0	Substrate mass fraction in the feed (g g^{-1})
t	Fermentation time (h)
X_0	Total amount of initial biomass (g)
X_t	Total amount of biomass at time t (g)
$Y_{p/s}$	Product yield coefficient on substrate (-)
$Y_{x/c}$	Biomass yield coefficient on ethanol (-)
$Y_{x/s}$	Biomass yield coefficient on substrate (-)

Greek letters

α	Moles of carbon per mole of yeast (-)
β	Moles of hydrogen atom per mole of yeast (-)
γ	Moles of oxygen atom per mole of yeast (-)
δ	Moles of nitrogen atom per mole of yeast (-)
μ	Specific growth rate (h^{-1})
μ_{crit}	Critical specific growth rate (h^{-1})
μ_E	Specific ethanol uptake rate (h^{-1})
$\mu_{\text{max-E}}$	Maximum specific ethanol uptake rate (h^{-1})

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References

1. Sasson, A. *Feeding Tomorrow's World*. UNESCO, Paris, 1990, 500-510
2. Kristiansen, B. *Integrated Design of a Fermentation Plant: The*

- Production of Baker's Yeast*. VCH Verlagsgesellschaft, Weinheim, 1994, 21
3. Sonnleitner, B. and Käppeli, O. Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis. *Biotechnol. Bioeng.* 1986, **28**, 927-937
 4. Okafor, N. and Ejiofor, A. O. Rapid detoxification of cassava starch fermenting for gari production following inoculation with a yeast simultaneously producing linamarase and amylase. *Proc. Biochem.* 1990, **25** (3), 82-86
 5. O'Connor, G. M., Sanchez-Riers, F. and Cooney, C. L. Design and evaluation of control strategies for high cell density fermentations. *Biotechnol. Bioeng.* 1992, **39**, 293-304
 6. Ejiofor, A. O., Posten, C., Solomon, B. O., and Deckwer, W.-D. A robust fed-batch feeding strategy for optimal parameter estimation for Baker's yeast production. *Bioproc. Eng.* 1994, **11**, 135-144
 7. Staples, D. G. *An Introduction to Microbiology*. Macmillan, London, 1983, 98
 8. Dantigny, P., Ziouras, K., and Howell, J. A. A structured model of Baker's yeast fed-batch growth. Proc. IFAC Symp. Control Biotech. Proc. (Karim, M. N. and Stephanopoulos, G., Eds.). Keystone, CO, 1992, 223-226
 9. Ejiofor, A. O., Solomon, B. O., Posten, C., and Deckwer, W.-D. Analysis of the respiro-fermentative growth of *Saccharomyces cerevisiae* on glucose in a fed-batch fermentation strategy for accurate parameter estimation. *Appl. Microbiol. Biotechnol.* 1994, **41**, 664-669
 10. Atthasampunna, P., Somchai, P., Eur-aree, A., and Artjarayasripong, S. Production of fuel ethanol from cassava. *MIRCEN J. Microbiol. Biotechnol.* 1987, **3**, 135-142
 11. Atkinson, B. and Mavituna, F. *Biochemical Engineering and Biotechnology Handbook*. Macmillan, New York, 1983, 115-203
 12. Yuan, J.-Q., Bellgardt, K.-H., Deckwer, W.-D., Jiang, W.-S. Modification and verification of the dynamic cell cycling model for Baker's yeast. *Bioproc. Eng.* 1993, **9**, 173-182
 13. Garrido, F., Banerjee, U.C., Chisti, Y., and Moo-Young, M. Disruption of a recombinant yeast for the release of β -galactosidase. *Bioseparation* 1994, **4**, 319-328