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Fed-batch production of baker's yeast using millet (*Pennisetum typhoides*) flour hydrolysate as the carbon source

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A fermentation medium based on millet (*Pennisetum typhoides*) flour hydrolysate and a four-phase feeding strategy for fed-batch production of baker's yeast, *Saccharomyces cerevisiae*, are presented. Millet flour was prepared by dry-milling and sieving of whole grain. A 25% (w/v) flour mash 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, pH 6.1–6.3, for 1 h. The liquefied mash was saccharified with 1,4-α-D-glucan glucohydrolase (EC 3.2.1.3) at 55°C, pH 5.5, for 2 h. An average of 75% of the flour was hydrolysed and about 82% of the hydrolysate was glucose. The feeding profile, which was based on a model with desired specific growth rate range of 0.18–0.23 h^{-1}, biomass yield coefficient of 0.5 g g^{-1} and feed substrate concentration of 200 g L^{-1}, was implemented manually using the millet flour hydrolysate in test experiments and glucose feed in control experiments. The fermentation off-gas was analyzed on-line by mass spectrometry for the calculation of carbon dioxide production rate, oxygen up-take rate and the respiratory quotient. Off-line determination of biomass, ethanol and glucose were done, respectively, by dry weight, gas chromatography and spectrophotometry. Cell mass concentrations of 49.9–51.9 g L^{-1} were achieved in all experiments within 27 h of which the last 15 h were in the fed-batch mode. The average biomass yields for the millet flour and glucose media were 0.48 and 0.49 g g^{-1}, respectively. No significant differences were observed between the dough-leavening activities of the products of the test and the control media and a commercial preparation of instant active dry yeast. Millet flour hydrolysate was established to be a satisfactory low cost replacement for glucose in the production of baking quality yeast.

Keywords: Millet; *Pennisetum typhoides*; liquefaction; saccharification; baker's yeast; *Saccharomyces cerevisiae*; fermentation

Introduction

Molasses is an inexpensive and commonly used source of sugars for producing baker's yeast. In regions where molasses is produced, it costs about $120–150 per metric tonne [21]. In other regions, where it must be imported, transportation costs and the usually high custom duty add to the price, 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 essential. One possible source is millet (*Pennisetum typhoides*) flour. Millet is among the least known cereal crops of the world produced in tropical and sub-tropical countries [6] as well as in India and Egypt [13] and Germany and Hungary [16]. The millet plant survives drought-stricken areas more effectively than any other cereal crop. Millet is a small grain weighing approximately 5.5–6 mg, 60–67% of which is starch. Millet starch granules consist of unbranched amylose (22.5 ± 2.5%) and branched amylopectin (77.5 ± 2.5%) both of which can be hydrolysed with acid or enzymes (either with pure enzymes or amylase-producing microorganisms) to release their constituent glucose and malto-oligosaccharides. Both products are easily transported across the cell membrane and metabolized by yeasts. Cooked millet starch granules have a lower tendency to thicken on cooling than sorghum or cassava starch granules. Millet also has a high protein content of 10.6–15% most of which occurs as endogenous α- and β-amylases. Consequently, millet has a high diastatic power in malting, greatly enhancing the α-amylase activity of the malt and, to a lesser degree, the β-amylase activity [6]. World production of millet in 1968 was 30 million metric tonnes. Of this, 7.4% entered international trade for use in animal feed production while 0.6–5% was utilized as food in developing countries [14,18].

Metabolism of glucose by *Saccharomyces cerevisiae* can be either respiratory or fermentative depending on culture conditions and availability of the limiting substrate when adequate and continuous supply of mineral supplements, nitrogen, growth factors and other physical inputs are available [8,9]. 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 model used to guide the feeding. Either linear or exponential models may be used and these must anticipate the needs of the exponentially increasing cell mass concentration. A fed-batch operation controls the specific growth rate at a value below the critical growth rate so that fermentative metabolism is suppressed. The glucose concentration is held within the desirable range of 0.1–0.25 g L^{-1}. Above a glucose concentration of 0.25 g L^{-1}, metabolism becomes increasingly fermentative as growth rate increases and the Crabtree effect or respiratory bottleneck sets in [2,3,11,19,20,22].

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This work aims to develop a low energy-requiring process to convert millet flour to a fermentable medium and an exponential feeding strategy for the production of baker's yeast on the novel medium. The medium and the feeding scheme are expected to provide a cost-effective alternative to molasses for producing baking quality yeast in regions where millet is cultivated but molasses is not locally available. While other molasses replacements such as cassava flour hydrolysates have been shown to be able to support baker's yeast production [7], cultivation of cassava is generally confined to wetter climates compared to those where millet is grown.

Materials and methods

Development of fermentation medium

Millet grains were dry-milled and sieved to 12–60 mesh. A 25% (w/v) mixture of the flour in deionized water was hydrolysed by the low-temperature long-time cooking procedure shown in Figure 1. The procedure was an adaptation of the schemes previously used for hydrolysing cassava starch [1,7]. The hydrolysis procedure involved gelatinization of the mash in the presence of 100 ppm of Ca^{2+} (provided as $CaCl_2 \cdot 2H_2O$) by cooking at 80°C for 1 h 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 mash 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 Co, St Louis, MO, USA) per kg of mash. The process was allowed to continue with stirring (1 h, pH 6.1–6.3). The liquefied broth was saccharified (pH 5.5, 55°C, 2 h) with 0.5 ml of glucoamylase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3, from *Aspergillus niger*, Sigma Chemical Co, St Louis, MO, USA) per liter of liquefied broth. Samples were taken every 20 min. The enzyme reaction was stopped with addition of hydrochloric acid (1 M; 0.5 ml acid per ml of sample) during liquefaction and 3,5-dinitrosalicylic acid (DNS) reagent (1 ml per ml of sample) during saccharification. The samples were analyzed with a Waters (Millipore, Milford, MA, USA) HPLC equipped with an external differential refractometer (model R401). 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-μl 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 *et al* [17] in which the vitamin solution was replaced with 0.3% Bacto yeast extract (Difco Laboratories, Detroit, MI, USA). Sigma 289 antifoam (Sigma Chemical Co, St Louis, MO, USA) was used for automatic foam control in response to a foam sensor.

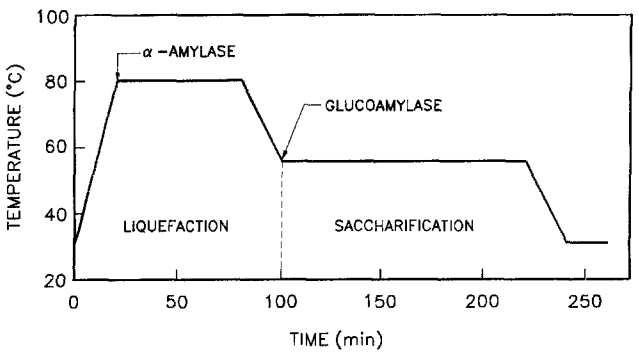

Figure 1 Low-temperature long-time process profile for millet flour hydrolysis. Arrows indicate the points of addition of enzymes.

The microorganism and inoculum preparation

Saccharomyces cerevisiae was isolated from a commercial strain of Fleischmann baker's yeast ATCC 7754 (Philip Burns Foods Inc, Canada). The yeast was maintained on slants containing (g L^{-1}): glucose, 10; yeast extract, 0.1; biotin, 0.01; and agar (BDH, Darmstardt, Germany), 20, as direct stock culture from which 3-staged inocula were prepared. Pure slant cultures were inoculated into two 500-ml baffled Erlenmeyer flasks containing 100 ml of glucose (10 g L^{-1}) and yeast extract (3 g L^{-1}) broth. The flasks were incubated on an orbital shaker (30°C, 150 rpm, 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, Wetzikon, Switzerland) containing 3 L of glucose (30 g L^{-1}), mineral salts and yeast extract medium detailed previously [8]. The last stage was run as a batch process at 30°C, pH 4.5, 400–700 rpm agitation and air flow of 2 vvm and 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

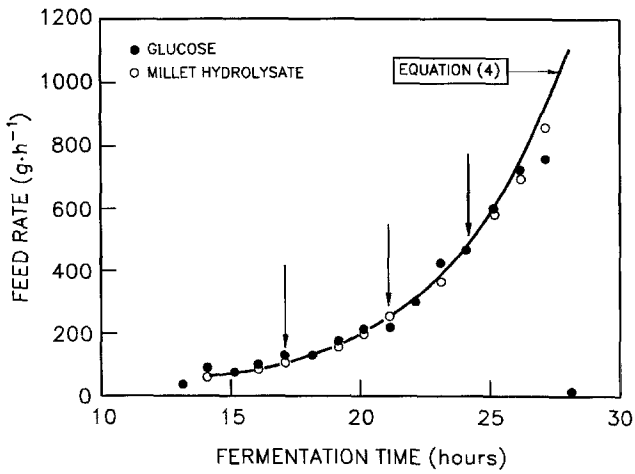

Figure 2 Calculated (Equation 4) and experimental substrate flow rates for fermentations using millet flour hydrolysate and glucose feeds. Arrows indicate the points at which the specific growth rate values (Equation 4) were changed.

was generated with Equation 4 presented in the next section. The feeding profile was executed manually with a peristaltic pump (Watson Marlow, Cornwall, UK; type MHRE 100) which was calibrated with Masterflex 6424-14 tubing. To compensate for possible pumping errors, on-the-spot on-line re-calibration 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}. Fermentations were run with glucose (S_0 = 0.2 g g^{-1}) and millet flour hydrolysate (S_0 = 0.206 g g^{-1}) feeds that were held on a balance and continuously stirred with a magnetic stirrer. Other conditions were: temperature 30°C; 400–950 rpm agitation; and 2–2.5 vvm aeration rate. Figure 3 shows the specific patterns of variations in air flow rate, the dissolved oxygen concentration and the agitation speed that occurred during the fermentations. Agitation was controlled automatically in response to the level of dissolved oxygen. The aeration rate was varied manually as in Figure 3. Aeration and agitation ensured a dissolved oxygen level of ≥20% of air saturation. Air flow was monitored with a thermal mass flow meter (Sierra Instruments, CA, USA) having a range of 0–20 L min^{-1}. The pH was controlled with 12.5% NH_3 held on a balance. The fermentation off-gas was analyzed for oxygen and carbon dioxide using a quadrupole mass spectrometer (PEGASUS VG, Cheshire, UK) equipped with an SX-PC interface. The PROCESS SOFT applications package was used for on-line calculation of the oxygen up-take rate (*OUR*), the carbon dioxide production rate (*CPR*) and the respiratory quotient (*RQ*).

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 250 × *g* at 10°C. The solids were washed twice by resuspending them 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, 24 h), allowed to cool in a desiccator and weighed. The difference

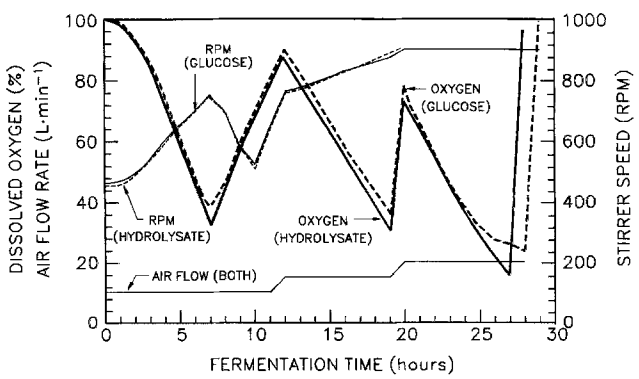

Figure 3 On-line measurements of dissolved oxygen concentration, air flow rate and stirrer speed during fermentation runs using millet flour hydrolysate and glucose feeds.

was the dry cell weight in 20 ml which was used to calculate the total dry cell weight in the fermentation vessel.

Optical density was determined by diluting broth culture samples by appropriate dilution factors to obtain the linear response range of absorbance. The optical density of the diluted sample was measured at 600 nm. The result was related to the cell dry weight and used in subsequent fermentations to estimate the cell dry weight. Increase in biomass and optical density were approximately directly proportional with a constant factor of 1.88 ± 0.05.

The first supernatant phase 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 Co, St Louis, MO, USA). The colour developed following the assay procedure was read at 520 nm. Ethanol concentration was determined by gas chromatography using a Hewlett-Packard (Rockville, MD, USA) 5880A series gas chromatograph. The supernatant fluid (1 ml) was mixed with 50 μl of an internal standard containing 2.5% isopropanol in hydrochloric acid (0.5 M). The processed sample (2.4 μl) was injected manually. Two ethanol standards containing 1 mmol L^{-1} and 20 mmol L^{-1}, respectively, were used to calibrate the chromatograph. Peak areas 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 method [23] 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 Inc, Markham, Canada; type 405), 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 of a 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 to 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.

Growth can be represented as

$$X_t = X_0 e^{\mu t}, \tag{1}$$

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; μ is the specific growth rate. Substrate consumption can be represented with the equation

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

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 Equation 2 represent, respectively, the rates of consumption of the carbon substrate for cell mass production, product formation and maintenance of the cells. In Equation 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 little or no ethanol production. Consequently, the product formation and maintenance terms are negligible compared to the growth term; hence, Equation 2 may be rewritten as

$$F_s = \frac{\mu}{Y_{x/s}S_0} X_t \tag{3}$$

Substitution of Equation 1 in Equation 3 gives

$$F_s = \frac{X_0}{Y_{x/s}S_0} \mu e^{\mu t}, \tag{4}$$

Equation 4 is used to guide the feeding rate for 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 ($Y_{x/s}$) 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 and 0.30 h^{-1} [4,15].

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

$$q_s = q_{s.max} \frac{C_s}{C_s + K_s}, \tag{5}$$

where C_s is the substrate concentration and K_s is the Monod constant. The glucose up-take rate (q_s) follows Equation 5 when the substrate concentration exceeds the critical value $C_{s.crit}$. The respiro-fermentative metabolism of the yeast [9,10] 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

$$\mu_E = \mu_{max.E} \frac{E}{E + K_e}, \tag{6}$$

where E is the concentration of ethanol and K_e is the ethanol concentration at half the maximum ethanol up-take 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 of which can be represented by

$$q_{ox} = q_{ox.max} \frac{C_{ox}}{C_{ox} + K_o}, \tag{7}$$

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 millet flour hydrolysate.

In a fed-batch operation, nutrient mixture continuously flows into the fermenter without corresponding withdrawal except for sampling. For this system cell, substrate and product mass balances lead to

$$\mu = \frac{Q}{V} + \frac{1}{x}\frac{dx}{dt}, \tag{8}$$

$$q_s = \frac{1}{x}\left(\frac{Q(C_{s0} - C_s)}{V} - \frac{dC_s}{dt}\right), \tag{9}$$

and

$$q_p = \frac{1}{x}\left(\frac{QE}{V} + \frac{dE}{dt}\right), \tag{10}$$

respectively, where x, C_s and E are, respectively, the dry biomass, glucose and ethanol concentrations, Q is the volumetric feed rate of glucose at time t. The volume, V, of the broth in the fermenter is given by

$$V = V_0 + \int_0^t Q dt - \sum_{i=1}^{i=n} V_{si}, \tag{11}$$

where V_0 is the starting volume of the medium in the fermenter, V_{si} is the volume of the ith sample withdrawn (i = 1, 2, ..., n) and n is the number of samples taken. The experimental specific growth rate (μ), specific glucose uptake rate (q_s) and ethanol production rate (q_p) were calculated using Equations 8, 9 and 10, respectively.

Results and discussion

The time course of the conversion of millet starch to sugars is shown in Figure 4 for the liquefaction and saccharification stages. In three experiments, conversion of starch to sugars was consistently 75%. Glucose constituted 82% of the converted material; the rest were malto-oligosaccharides comprising essentially maltose and isomaltose. At 75% the conversion was significantly lower than the 98% observed with cassava starch under identical conditions [7]. Association of some of the starch with the relatively larger quantity of protein in millet apparently affected the conver-

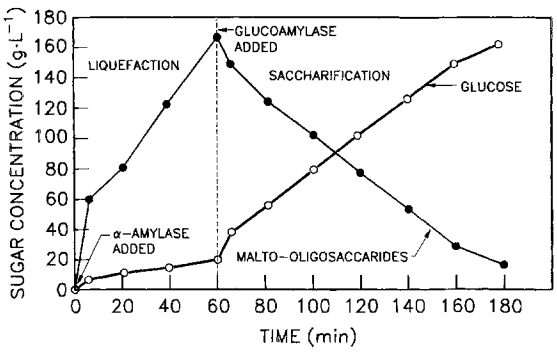

Figure 4 Time course of enzymatic hydrolysis of millet flour.

sion. 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 malto-oligosaccharides. Addition of glucoamylase rapidly converted the maltose and malto-oligosaccharides to glucose. The long-time low-temperature cooking procedure used (Figure 1) was beneficial in that high pressure steam was not required to attain the high temperatures needed in the alternative short-time high-temperature procedure [1]. 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 [25]. Concentration of the hydrolysate yielded 206 g sugar per litre of hydrolysate which was 3% 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 high 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 modelled but was designed simply for the production of the initial total cell mass (X_0) of about 30 g. The objective value was achieved, 29.1 g being obtained with the millet flour 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

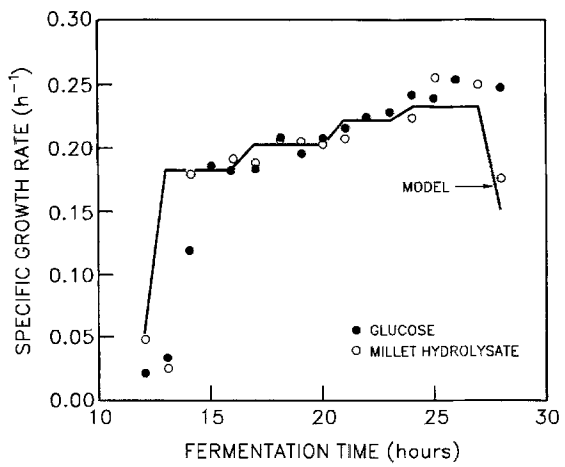

Figure 5 Specified and measured specific growth rates during fermentation runs with millet flour hydrolysate and glucose feeds.

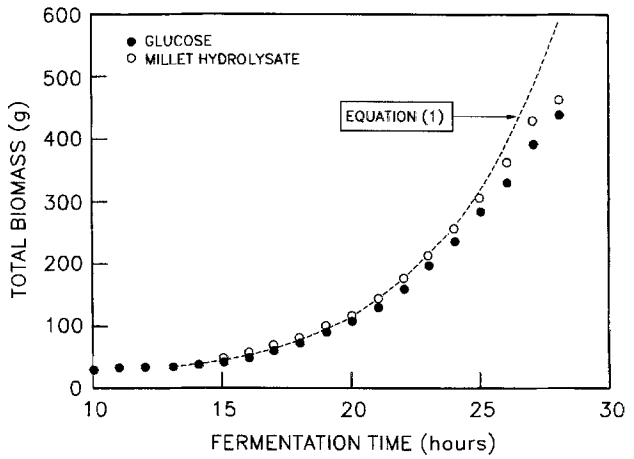

Figure 6 Estimated (Equation 1) and actual total biomass in the fermenter for runs using millet flour hydrolysate and glucose feeds.

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 agree reasonably well for the two substrates. Throughout the fed-batch phase, the residual glucose was maintained between 0.09 and 0.15 g L^{-1} (Figure 7), thus avoiding the Crabtree effect and ensuring low ethanol concentrations, between 9.5 mmol L^{-1} (or 0.43 g L^{-1}) and 21.3 mmol L^{-1} or (0.98 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 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 (Figure 5) and the corresponding sharp changes were seen in the composition of the exhaust gas (Figure 8). 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, 459.4 g for the millet flour hydrolysate and 468.3 g for glucose (Figure 6) at the end of the fermentation. These

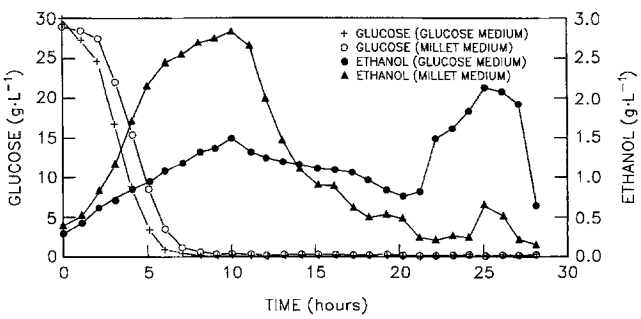

Figure 7 Glucose and ethanol concentrations during fermentation runs using millet flour hydrolysate and glucose feeds.

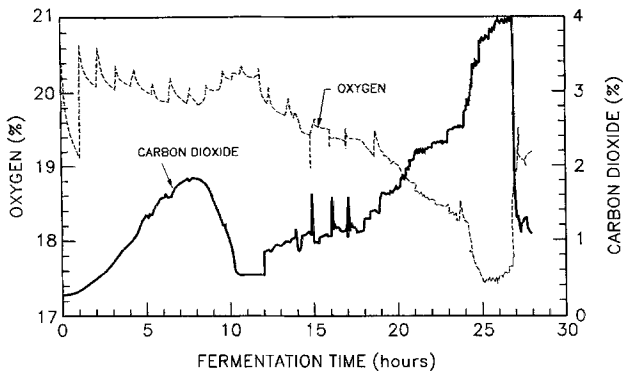

Figure 8 On-line measurements of carbon dioxide and oxygen in the exhaust gas during fermentation runs using millet flour hydrolysate feed.

yielded, respectively, 51.9 g L^{-1}, 50.0 g L^{-1} and 49.9 g L^{-1} when fermenter volumes were considered. Cell mass growth rate, which ranged from 1.7 to 71.3 g h^{-1}, for millet flour hydrolysate and 0.5 to 69 g h^{-1} for glucose, also reflected the pattern of glucose consumption. The ammonia used in pH control was an additional source of nitrogen necessary for the metabolism of glucose; the ammonia feed rates were virtually the same for the two systems.

The on-line measurements of carbon dioxide and oxygen in the exit gas are presented in Figure 8. The calculated oxygen consumption rate (*OUR*), the carbon dioxide production rate (*CPR*) and respiratory quotient (*RQ*) are shown in Figure 9. The respiratory quotient remained at 1.0 ± 0.1 throughout the fed-batch phase. This is further indication that the metabolism was oxidative through the fermentation. Figure 3 provides additional confirmation that although the agitation and aeration were held constant, respectively, at 950 rpm and 20 L min^{-1} from 20 h of fermentation, no oxygen limitation occurred as the level of dissolved oxygen remained about 20% of air saturation.

For further comparison of the fermentations that used two 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). The fermentations on glucose and on millet flour hydrolysate had comparable performance. In addition, the data on glucose compared well with previously published information (Table 1), validating the experimental and the computational techniques used in this work. The elemental composition of the yeast product,

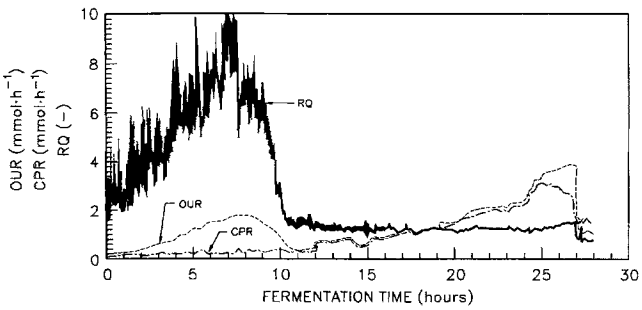

Figure 9 On-line measurements of carbon dioxide production rate (*CPR*), oxygen up-take rate (*OUR*) and respiratory quotient (*RQ*) during fermentation using millet flour hydrolysate feed.

Table 1 Experimental parameter values and published ranges

Parameter	Experimental value		Range	Units
	Glucose	Millet		
$q_{s.max}$	3.46	3.48	3.0–3.75	g g^{-1} h^{-1}
$Y_{x/s}$ oxidative	0.49	0.48	0.47–0.5	g g^{-1}
$Y_{x/s}$ fermentative	0.10	0.07	0.05–0.15	g g^{-1}
$Y_{x/e}$	0.68	0.70	0.67–0.72	g g^{-1}
K_s	0.25	0.35	0.1–0.5	g L^{-1}
K_o	0.1	0.1	0.08–0.12	mg L^{-1}
K_e	0.1	0.1	0.1–0.11	g L^{-1}
α	1.0	1.0	1.0	mol mol^{-1}
β	1.72	1.70	1.7–1.85	mol mol^{-1}
γ	0.60	0.60	0.54–0.63	mol mol^{-1}
δ	0.165	0.18	0.16–0.18	mol mol^{-1}

$C_\alpha H_\beta O_\gamma N_\delta$, obtained on glucose and millet flour hydrolysate are also presented in Table 1 with the published ranges for glucose-grown yeast. Once again, the millet flour 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 decreased allowing the cells to use up residual glucose and ethanol. This was accompanied by a reduced fraction of budding cells to 6–10% of the population which increases the leavening activity of the yeast product [5,24]. 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 10). The millet flour hydrolysate-grown yeast fully attained 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; yeast cells utilize about 1% of the intracellular storage carbohydrates per day at 4°C [15].

Although the focus of this work was on production of baking quality yeast on millet flour hydrolysate, the comparable composition, fermentation performance and quality attributes of the glucose-grown and the millet flour

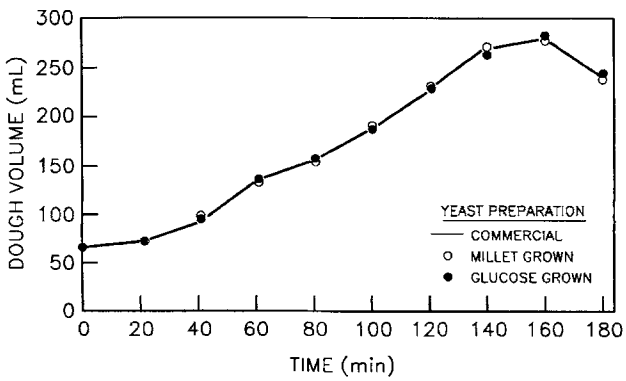

Figure 10 Comparison of dough-leavening activities of the glucose-grown yeast, millet flour hydrolysate-grown yeast and a commercial baker's yeast.

hydrolysate-grown yeasts suggest wider use potential for the millet flour hydrolysate-based media. For example, the cost of production of the many recombinant proteins in genetically modified strains of baker's yeast [12] may be lowered by employing the less expensive millet flour hydrolysate in the fermentation medium.

Conclusion

A strategy for utilization of millet flour in production of baking-quality yeast was demonstrated. Millet flour 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 millet flour hydrolysate and the control media were identical, agreeing closely also with the activity of a commercial baking yeast preparation.

With the demonstration of millet as a good source of hydrolysable carbohydrate, ability of the cereal to thrive on marginal land and in arid climates and the low level of its current utilisation for other purposes, a strong potential for its large-scale use in the fermentation industry is indicated. The cost of processing associated with the use of enzymes may be reduced by using microorganisms that are capable of hydrolysing 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.

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Nomenclature

C_{ox}	Dissolved oxygen concentration (mg L^{-1})
CPR	Carbon dioxide production rate (mmol h^{-1})
C_{s0}	Glucose concentration in the feed (g 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})
i	Sample number (–)
K_e	Constant in Equation 6 (g L^{-1})
K_o	Constant in Equation 7 (mg L^{-1})
K_s	Constant in Equation 5 (g L^{-1})
m	Specific maintenance term (h^{-1})
OUR	Oxygen up-take rate (mmol h^{-1})
q_{ox}	Specific oxygen up-take rate (h^{-1})
$q_{ox.max}$	Maximum specific oxygen up-take rate (h^{-1})
q_p	Specific product formation rate (h^{-1})
q_s	Specific substrate up-take rate (g g^{-1} h^{-1})
$q_{s.max}$	Maximum specific substrate up-take rate (g g^{-1} h^{-1})
RQ	Respiratory quotient (–)
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)
V	Instantaneous volume of the broth in the fermenter (L)
V_0	Starting volume in the fermenter (L)
V_{si}	Volume of sample i (L)
x	Biomass concentration in the fermenter (g L^{-1})
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 (–)
$Y_{x/e}$	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 up-take rate (h^{-1})
$\mu_{max.E}$	Maximum specific ethanol up-take rate (h^{-1})

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