0273-2289/88/1800-0003/$03.00

Semisolid State Fermentation of Baker's Yeast in an Air-Fluidized Bed Fermentor

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ABSTRACT

In an attempt to grow microorganisms other than fungi using a solid-state fermentation process, a model system of Baker's yeast (*Saccharomyces cerevisiae*) was cultured in an air-fluidized bed fermentor. A semisolid potato mixture (pretreated with α-amylase) was used for the substrate in this highly aerated system.

The growth of Baker's yeast in this air-fluidized bed process was easily controllable and very reproducible. Once feasible moisture levels and air flow rates were determined, the independent variables studied were the amount of the enzyme used for digesting the potato starch, the size of the yeast inoculum, and the concentration of the added defined medium.

Index Entries: Solid state fermentation; semisolid substrate fermentation; Baker's yeast fermentation; air-fluidized bed fermentor and potato substrate.

INTRODUCTION

Solid state fermentation processes are among the most interesting types of microbial systems, offering several advantages over conventional submerged culture systems. These advantages include higher volumetric productivity, less purification effort to prepare the fermentation substrate from the solid raw materials, and lower equipment costs (*1–3*). By virtue of the inherently low water content of solid substrates, usually

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only fungi are grown on such solids since only these microorganisms are viable and excrete large amounts of enzymes under these conditions. Other microorganisms, such as yeast and bacteria, potentially offer such high product productivities, if they could be grown on solid or semisolid substrates, perhaps at a somewhat elevated water level.

In an attempt to extend the merits of solid state fermentation to microorganisms other than fungi, Baker's yeast (*Saccharomyces cerevisiae*) is studied here as a model system growing on a semisolid substrate. (A solid substrate of about 80% water content is defined here as a semisolid substrate.) Baker's yeast can be considered to have intermediate characteristics between filamentous fungi and bacteria, providing a first step in generalizing the application of semisolid culture to bacteria and yeast for use in industrial applications.

An air-fluidized bed fermentor containing yeast, growing on a semisolid potato mixture, is employed in this study. The system has a rather high air flow that supplies enhanced aeration to the solids. Such vigorous aeration speeds up the microbial growth rate in the solid so that it approaches the generally more rapidly growing liquid cultures. The system also has enhanced aeration, which can provide better mixing to the semisolid substrate fermentation. The better mixing offers improved humidity and temperature control, particularly when the process is compared to traditional static solid state fermentation systems.

Recently, similar fluidized bed systems have been studied in Germany and Japan. Moebus and Teuber (*4,5*) in Germany studied the production of ethanol using fluidized yeast (*S. cerevisiae*) particles onto which a glucose/nutrient solution was continuously sprayed. The ethanol productivity in their system was comparable to similar submerged culture processes. In another study (*6*), Moebus and Teuber employed an air-fluidized bed fermentor to produce glutathione from a mixture of L-glutamic acid, L-cysteine, and glycine, again utilizing *S. cerevisiae*. In Japan, Akao and Okamoto (*7*) found that their air-fluidized bed system exhibited a several-fold increase in both cell yield and several excreted enzyme levels with *Aspergillus* culture when compared to corresponding conventional solid cultures. Using a similar air-solid fluidized bed system as Akao and Okamoto, Tanaka et al. (*8*) found that the productivities of *S. cerevisiae* cell mass and *Eupenicillum javanicum* enzymes were also increased with the air-fluidized system when compared with traditional static solid fermentation systems.

In this paper, yeast (*Saccharomyces cerevisiae*) growth on a fresh potato semisolid substrate contained in an air-fluidized bed fermentor is described. No internal stirring was required in this system as in (*8*), and no yeast clumping was observed. All of the substrate was added initially, creating a batch process vs the continuous addition of glucose in (*4,8*), a semibatch fermentation. The effects on the yeast cellular growth of certain significant fermentation variables are reported here: the medium concentration, the inoculum size, and the amount of starch hydrolyzing enzyme (α-amylase) added. In addition, the sugar level in the fermenta-

tion mash was regulated in order to control the cell growth rate. Cell growth here was estimated (without separating the cells from the mash) by optical density measurements of diluted samples, following a previously developed procedure (*9*).

MATERIALS AND METHODS

Microorganism

Baker's yeast (*Saccharomyces cerevisiae*, lot no. Oct. 1987) purchased at a local grocery in the form of Fleischmann's brand active dry yeast was used. The inoculum size was 3.6 mg/g potato substrate unless otherwise specified.

Medium

Synthetic Medium C of Maxon and Johnson (*10*), without glucose, was used to supplement the potato substrate described in the next section. The M-J medium was sterilized at 121°C for 15 min before it was added to the solid substrate. The amount of M-J medium used was 0.15 mL/g potato. Appropriately, concentrated medium was used to investigate the effect of the amount of medium on the cell growth with the volume of medium added kept constant. About 60% of the total medium was used to dissolve the α-amylase powder. The resulting solution was mixed with the potato substrate. The rest of the total medium (40%) was used to suspend the yeast cell inoculum. That mixture was added to the potato substrate.

Preparation of the Fermentation Substrate

Potatoes (Golden Crown Brand, B. M. Tibbits & Sons Inc., St. Anthony, ID 83445) purchased at a local grocery, were peeled and washed. They were steamed at 121°C for 15 min, and then mashed to a homogeneous paste with a Waring two speed blender. This paste contained about 80% water content. [The 80% water content level was selected based on a previous study (*9*) on the effect of water content on yeast growth]. The paste was well mixed with an α-amylase (From *Aspergillus oryzae*, Lot no. 31-003, Miles Lab.) solution and stored at room temperature for 12–14 h to allow for the conversion of digestible potato starch into sugars. For the runs without starch predigestion, the enzyme solution and the potato paste were mixed together just before the fermentation. The initial mass of fermentation substrate was 300 ± 10 g.

Apparatus

A schematic diagram of the air-fluidized bed fermentor is shown in Fig. 1. Steam was used to sterilize the entire system. Air was filter-sterilized using glass wool, then passed through an air humidification/

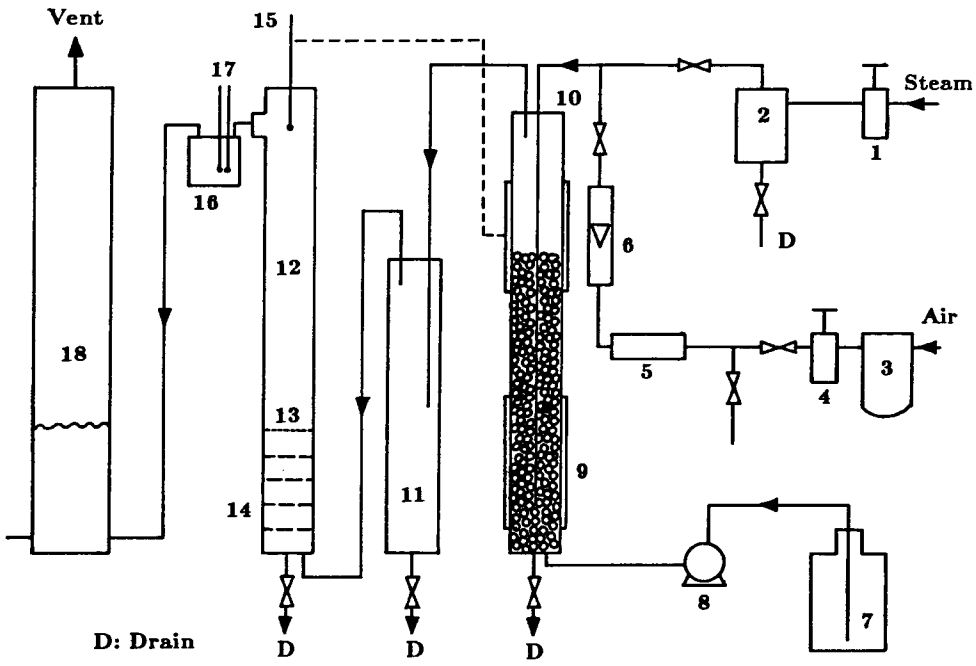

Fig. 1. Schematic diagram of the air-fluidized bed fermentor apparatus. 1. steam regulator; 2. steam trap; 3. air filter; 4. air regulator; 5. air sterilization filter; 6. air flowmeter; 7. water reservoir; 8. water pump; 9. heating tape; 10. air saturation column; 11. air cooling column; 12. fermentation column; 13. fine grid supporting plate; 14. large hole flow distributor; 15. thermoregulator; 16. humidity measuring chamber; 17. wet and dry bulb thermometers; 18. overhead collector.

saturation glass column (number 10 in Fig. 1), where it reached about 95% relative humidity at 50°C. The air was saturated with water (to 99–100%) as it was cooled down to 32°C during passage through the glass cooling column (number 11). The temperature in the glass fermentation column (number 12) was controlled at 32 ± 1°C by a thermoregulator located at the top of the fermentation column, which activated the heating tape along the humidification/saturation column. The fermentation mash within the glass fermentation column was supported by a fine grid Teflon plate.

Treatment of Samples for Analyses

At selected times the upper flange of the fermentation column was loosened and samples were taken with a long-handled spoon. A 0.5 g mash sample was taken each time and prepared for analysis, following the procedure shown in Fig. 2.

Analyses

The growth of yeast in this system was monitored by measuring the optical density of the mash sample, which was suspended in water.

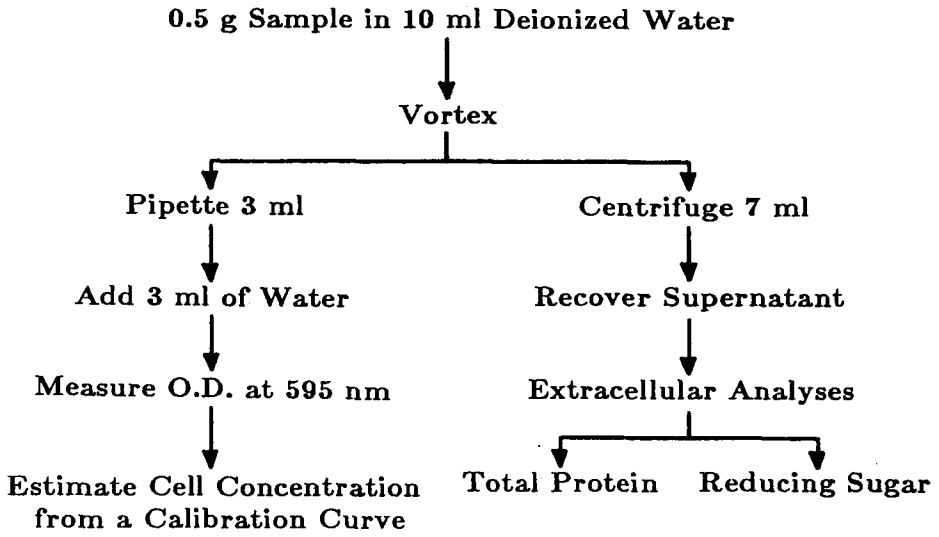

Fig. 2. Preparation of samples for analysis.

Since the water content of a sample changed as the yeast cells grew, the water content of the most diluted sample was used as a basis for measuring the optical densities. This cell estimation method, based on diluting all of the samples to the greatest water content of a given run, was previously cited (*9*).

The water content of each sample was analyzed gravimetrically by measuring the weight of the sample before and after drying at 80 ± 2°C for 48 h. It is noted that volatile organics such as ethanol are included in this "water content" term. A maximum error of ca. 2% is expected in these measurements as a result of the volatile organics.

The reducing sugar level was measured by the Somogyi-Nelson method (*11*) and corrected to include the dilution effect on the semisolid sample caused by the cell growth.

The total extracellular protein was measured by the Bradford Coomassie blue dye binding assay as modified by the Bio-Rad Laboratory Microassay procedure (*12*). The assay was calibrated against the same batch of α-amylase that was used to hydrolyze the potato starch.

RESULTS AND DISCUSSION

Growth of Yeast on Predigested Starch

Results from a typical fermentation experiment of Baker's yeast growing on the semisolid substrate in the air-fluidized bed fermentor are depicted in Fig. 3. The increase in the water content of the fermentation mash is considered to result from the generation of water by respiration as the cells grow, especially during the exponential growth period. The considerable cell growth pictured in Fig. 3 indicates that yeast can be grown easily in this semisolid substrate/air-fluidized bed fermentation

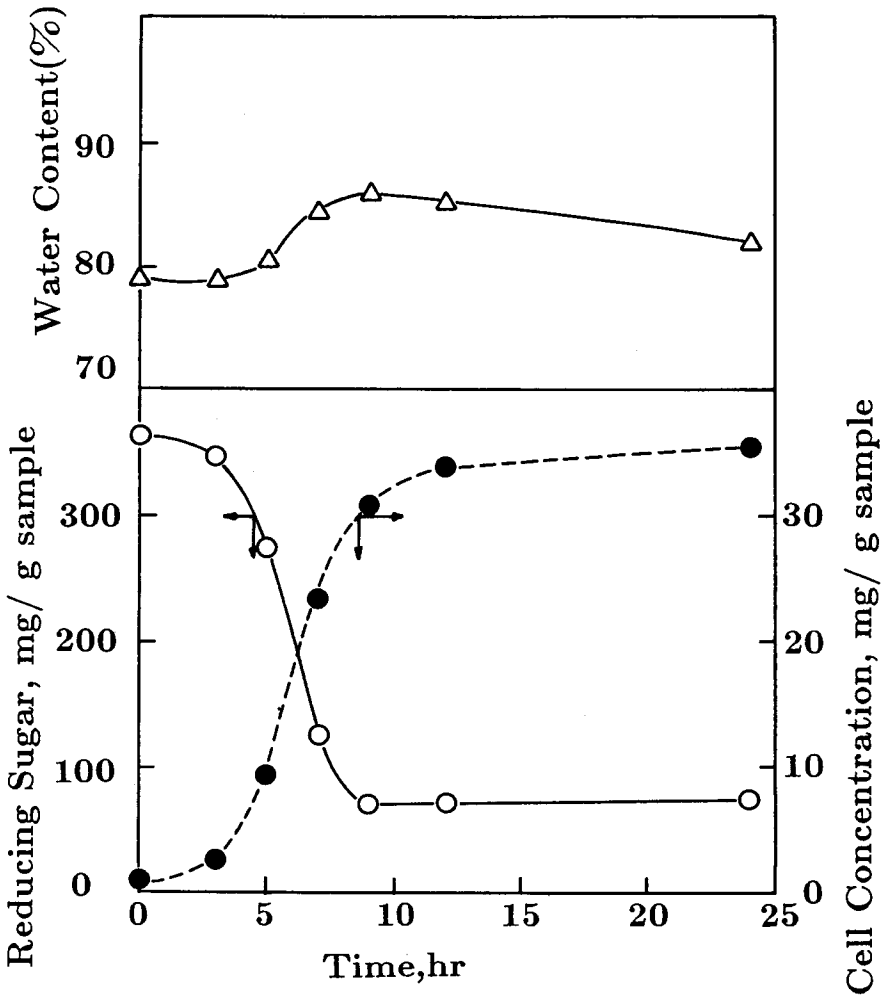

Fig. 3. A typical fermentation experiment of Baker's yeast growing in an air-fluidized bed fermentor.
—△— = water content; —○— = reducing sugar concentration; ----●---- = cell concentration.

system. A residual concentration of reducing sugars of around 70 mg/g sample is observed. This nonfermentable reducing sugar is often called a limit dextrin. These undigestible sugars may be converted further into fermentable sugars by the addition of debranching enzymes such as α-glucosidase.

The amount of synthetic medium added was varied, while holding the total volume of the medium constant at 0.15 mL/g potato. As can be seen in Fig. 4, no appreciable differences in the cell growth profiles were observed. (The threefold concentration run started with less sugar than the other two runs, hence somewhat lower cell growth was observed at late times.) These three runs also demonstrate that the growth in the air-fluidized bed fermentor system with the semisolid substrate is quite reproducible.

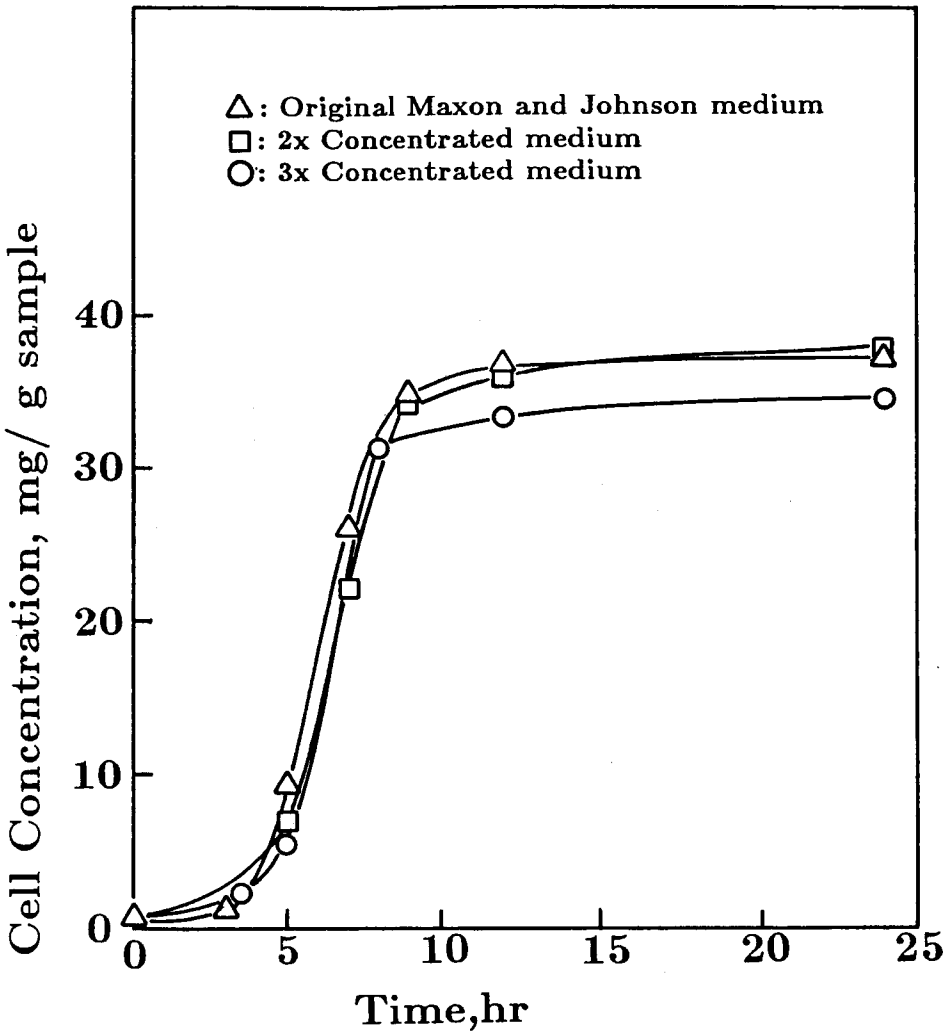

Fig. 4. The effect of the amount of medium on the cell growth.

The effect of a change in the cell inoculum size on the yeast growth is shown in Fig. 5. A 50% reduction in the inoculum size nearly doubles the lag period time, although the final cell level and the specific growth rate during the growth phase remain unchanged.

The amount of enzyme used for predigesting the potato starch was varied in order to determine whether such changes would lead to differences in cell growth (presumably because of differences in the initial sugar levels in the fermentor). Because sufficient time was available for the starch predigestion (12 h), the initial sugar level was nearly constant at the start of the fermentation for all of the predigestion studies. As shown in Fig. 6, the specific growth rates (0.4 /h) and final cell concentrations (35 ± 2 mg/g sample) are also nearly the same for all three enzyme levels. The difference in the final cell concentrations from the previous results (Figs. 4 and 5) is attributed to using potato batches with different

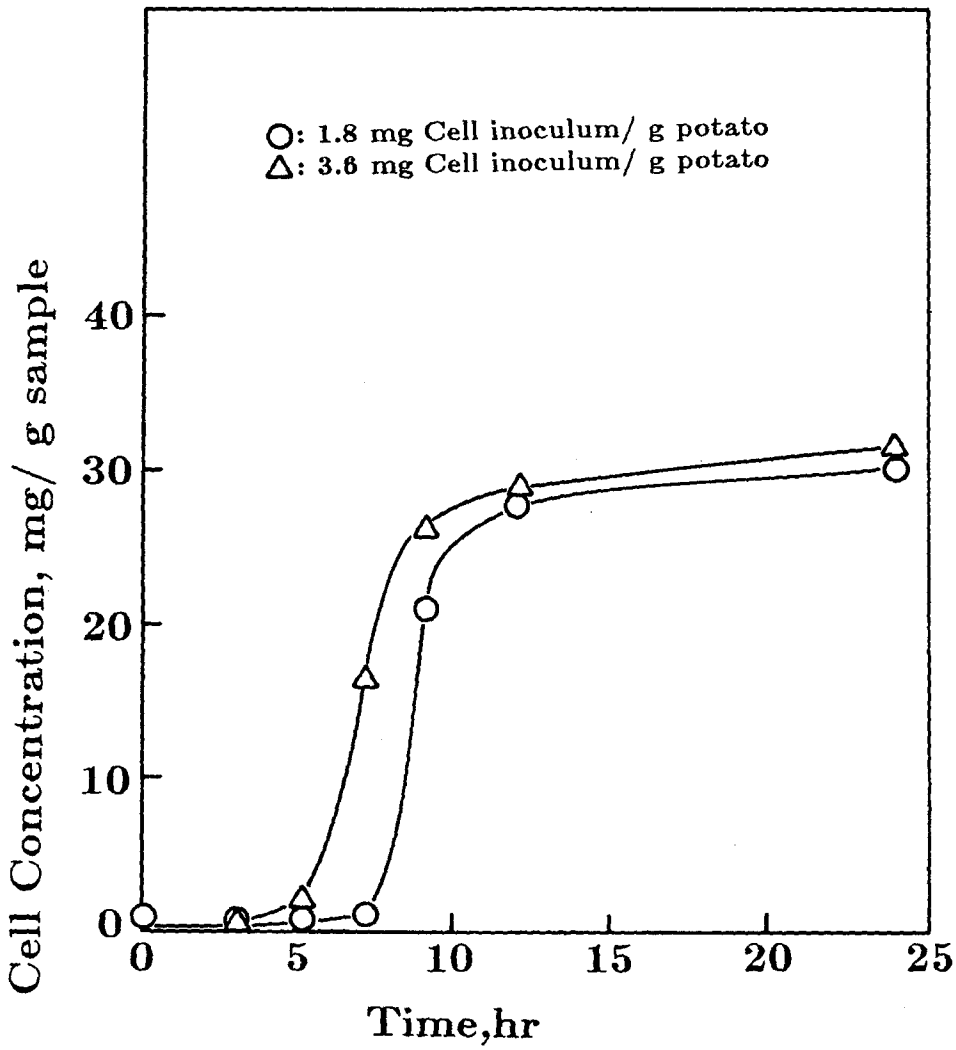

Fig. 5. The effect of the amount of inoculum on the cell growth.

storage times in the different runs. The sugar content of a potato, in general, gradually increases during the storage time (*13*). In addition to the expected natural sugar content increase in the potatoes as a result of storage, the total amount of reducing sugar (obtained by enzymatic hydrolysis of the potato starch) was observed here to be higher for "stored" potatoes than for "fresh" potatoes.

The relative insensitivity of yeast growth to substrate repression/ inhibition over a wide concentration range of reducing sugar seems to be attributable to the finite solubility of sugar in the limited amount of liquid water available to the cells. It is likely that the excess sugar is absorbed on the undigested potato solids (the limit dextrins) and, hence, is in a "storage," rather than in an inhibitory mode. Adding glucoamylases at the end of the fermentation may be desirable to increase the overall cellular

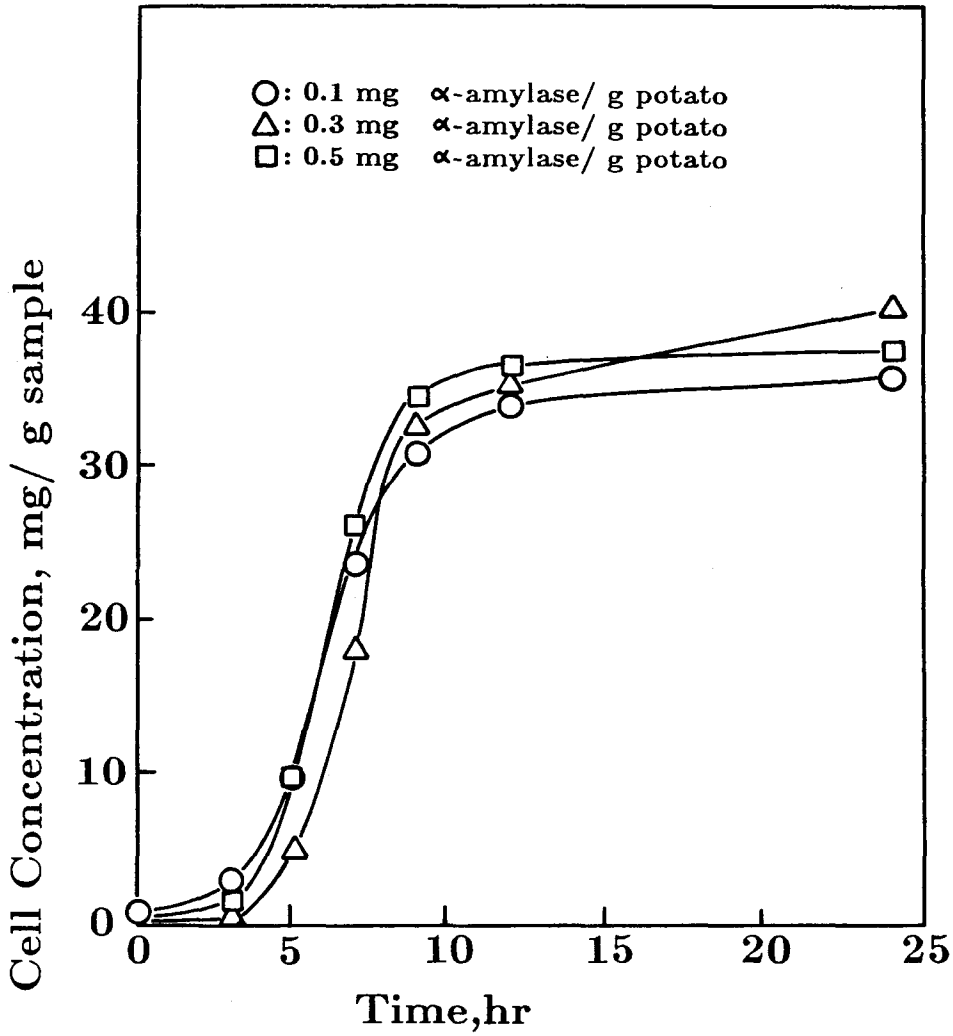

Fig. 6. The effect of the amount of enzyme on the cell growth.

yield without causing early transient inhibition/repression by the substrate.

Growth of Yeast with In-Situ Starch Digestion

The sugar level in the fermentation mash is controlled primarily by adjusting the initial amount of digesting enzyme. This follows from the rate at which the enzyme converts the starch substrate into sugar when the enzyme is administered directly into the potato paste (this is the case of "no enzymatic predigestion"). Examples of such a tandem starch degradation and fermentation are depicted by the cell growth and corresponding reducing sugar profiles in Figs. 7 and 8, respectively.

For the case of no predigestion, the α-amylase was introduced just before the start of the fermentation. Therefore, the potato starch was not completely hydrolyzed during the lag phase of the fermentation. Hence,

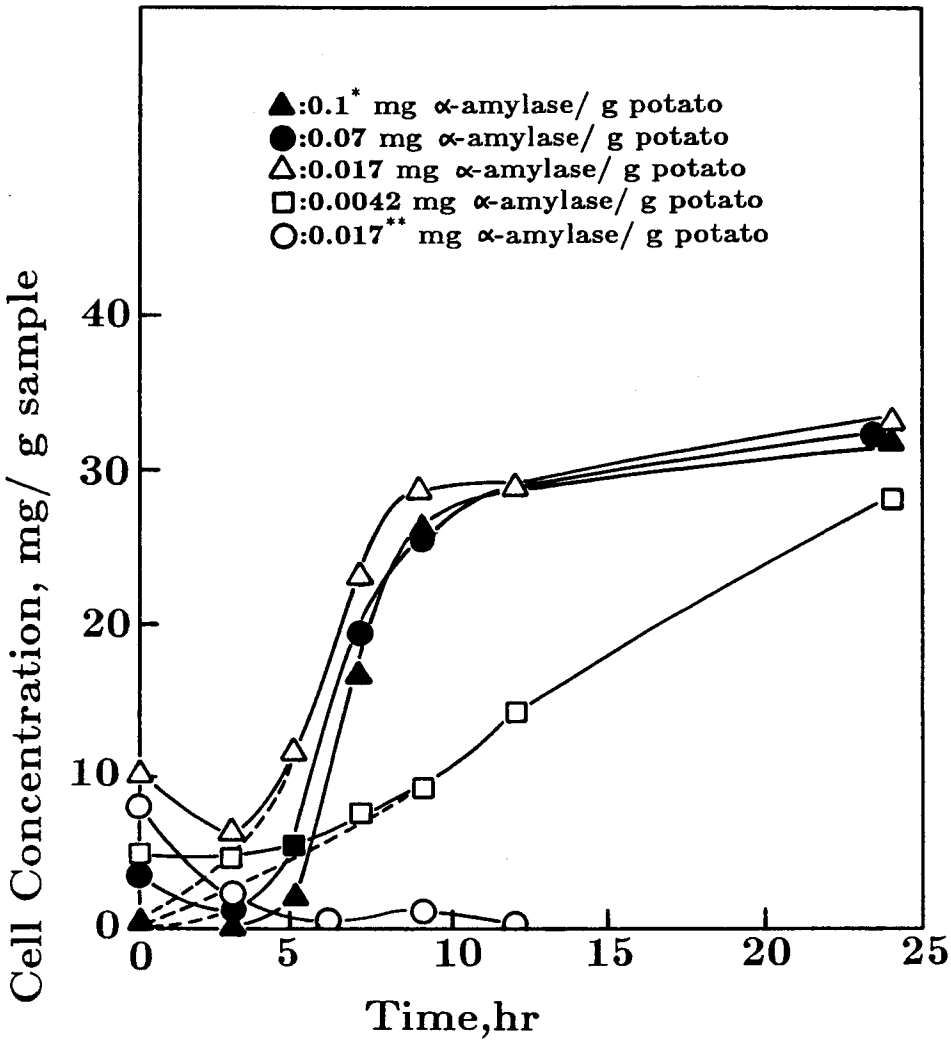

Fig. 7. The effect of the amount of enzyme on the cell growth for tandem starch digestion and fermentation. *Potato starch predigested for 12 h, **No yeast added.

the optical densities (measured for the purpose of estimating cell growth) of the diluted samples during this 0–5 h lag period included contributions from the starch particles as well as the yeast cells. As the potato starch reached its maximum hydrolyzed state, its contribution to the optical density measurement was minimized, reducing its interference in the estimated yeast cell concentration. Since it was not possible to separate yeast cells from the rest of the solid material for a direct measurement of cell concentration for calibration purposes, a baseline run without yeast inoculation was made to determine the underlying component of undigested starch particles to the optical density measurements. As depicted in Fig. 7, the contribution of starch particles to the optically esti-

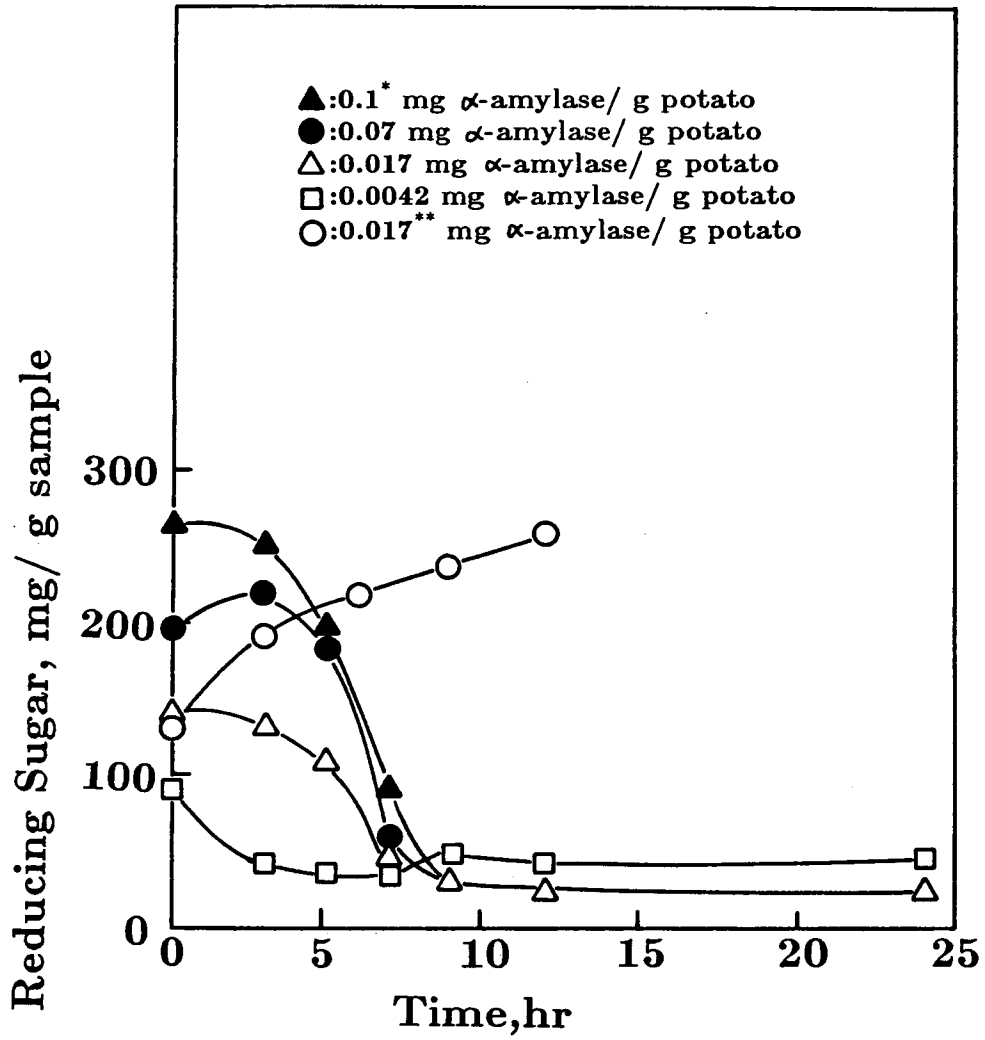

Fig. 8. Reducing sugar profiles for various enzyme levels. *Potato starch predigested for 12 h, **No yeast added.

mated cell concentration decreases rapidly, so that the effect is nearly negligible after approx. 5 h. The expected actual cell concentrations during this early transient period are represented by the dashed lines.

In Fig. 7, the two runs of .07 and .017 mg enzyme/g potato levels show virtually the same growth rates and final cell concentrations as the highest enzyme level run (.1 mg enzyme/g potato with predigested starch). These nearly identical results (within experimental error) hold in spite of the fact that the reducing sugar levels for these three cases are very different (shown in Fig. 8). Thus, the cell growth profile was not significantly affected by differences in the reducing sugar concentration, as long as the initial sugar level was above 130 mg/g sample. The higher (above that in the natural potato) initial reducing sugar levels likely were

the result of enzymatic digestion during the prefermentation mixing of homogenized potato paste and enzyme as well as during the postfermentation offline sample treatment for sugar analysis.

The sugar release rates were calculated from the activity of α-amylase, specified by the manufacturer, in order to select the enzyme levels appropriate for various sugar release rates. Typical sugar release rate values of .07 and .017 mg enzyme/g potato calculated were 85 and 20 mg reducing sugar/h, respectively. For the case of .07 mg enzyme/g potato, the calculated sugar release rate (85 mg reducing sugar/h) was faster than the sugar consumption rate, thus the sugar level reached a maximum (220 mg reducing sugar/g potato, as shown in Fig. 8) at 3 h. As the cells grew faster, the sugar consumption rate exceeded the sugar release rate, consequently lowering the sugar levels in the fermentor. For the case of .017 mg enzyme/g potato, the sugar release rate (20 mg reducing sugar/h) was slower than the sugar consumption rate, resulting in an initial sugar level (140 mg reducing sugar/g, sample in Fig. 8) at its maximum value.

Using the amylase level of .0042 mg/g potato (corresponding to a sugar release rate of 5 mg/h), the specific growth rate dropped from .4 to .1/h, implying that sugar is the growth limiting component here (the lowest enzyme level employed). However, by 30 h (not depicted on Fig. 7), the final cell concentration reached the same level as for the other initial enzyme concentration cases. The cell yield, based on the consumed sugar, held at 12% for all three runs.

Characterizing the Yeast Growth in This System

The cell yield, based on the consumed fermentable sugar, ranged between 12–15%. This yield is lower than the optimal industrial yield (50%) of Baker's yeast. In liquid culture, a typical strategy for maximizing yeast yield is to maintain the sugar concentration at a very low level so that it becomes the growth limiting factor. These low sugar levels generally correspond to specific growth rates between .2 and .25/h, when adequate oxygen is supplied (*14*). In our system, the high air flow rate makes it possible to supply the necessary oxygen partial pressure to the fermentation mash. The lowered sugar levels may be achieved in our process simply by adjusting the initial concentration of the starch hydrolysis enzyme. Controlling the oxygen supply and the sugar level, therefore, provides a feasible strategy for maximizing the yield of Baker's yeast.

The final Baker's yeast cell concentration falls between 30–40 mg yeast cells/g sample, which is comparable to the industrial level of 35–45 g/L in liquid culture (*14*). The industrial cell density level is normally achieved by growing the cells in a succession of ever increasing volumes of submerged batch culture (typically, 5–10 successive batches) as contrasted with the single batch here. The observed high volumetric productivity for the air-fluidized bed fermentor implies that the advantage of the

traditional static solid state fermentation system over the liquid culture holds here as well for our semisolid system.

The specific cell growth rate in this simultaneous saccharification-fermentation system was about .4/h for all of the initial enzyme levels at or above .017 mg α-amylase/g potato. This rate is considerably higher than that required to reach the optimal cell yield from fermentable sugars. It is demonstrated here, however, that the specific growth rate is easily retarded in practice (.1/h in Fig. 7 by reducing the enzyme level to .0042 mg α-amylase/g potato).

Excretion of Yeast Proteins

The amount of extracellular yeast proteins produced under three different conditions is graphed in Fig. 9. These levels correspond to three of the key cell growth patterns of Fig. 7. For the baseline run without yeast, the extracellular protein remains almost constant, as expected. It is noted that these three extracellular protein production rates can be approximately correlated with their corresponding cell growth rates for the studied conditions. In other words, for this air-fluidized bed system, higher cell growth rates lead to higher extracellular protein levels.

The extracellular proteins have also been analyzed using gel electrophoresis. Details of the observed molecular weight distribution will be reported in a separate communication. The possibility of protein separation from the bed by the rapid air stream, capable of carrying specific proteins from the fermentation mash, will also be reported subsequently, focusing on those particular proteins identified by gel electrophoresis. In particular, that report will stress the partitioning of the excreted proteins between the solid phase in the fermentation bed and the gas phase of the rising airstream.

CONCLUSIONS

A process for cultivating Baker's yeast on homogenized potato, pretreated with α-amylase, was developed using an air-fluidized bed fermentor.

The yeast growth was stable and reproducible in this system. No apparent substrate inhibition/repression was observed within the initial reducing sugar range of 130 and 370 mg reducing sugar/g sample. The cell yield ranged between 12–15%, based on the consumed reducing sugar. The final cell concentration averaged 35 mg yeast cells/g fermentation sample, starting from a level of 2.5 mg yeast cells/g fermentation sample (a 14-fold increase). This final cell concentration was comparable to that achieved in industrial Baker's yeast production.

This study demonstrates the possibility of employing solid or semisolid state fermentation for yeast culture. It also serves as a prototype process for cultivating bacteria on solid or semisolid substrates.

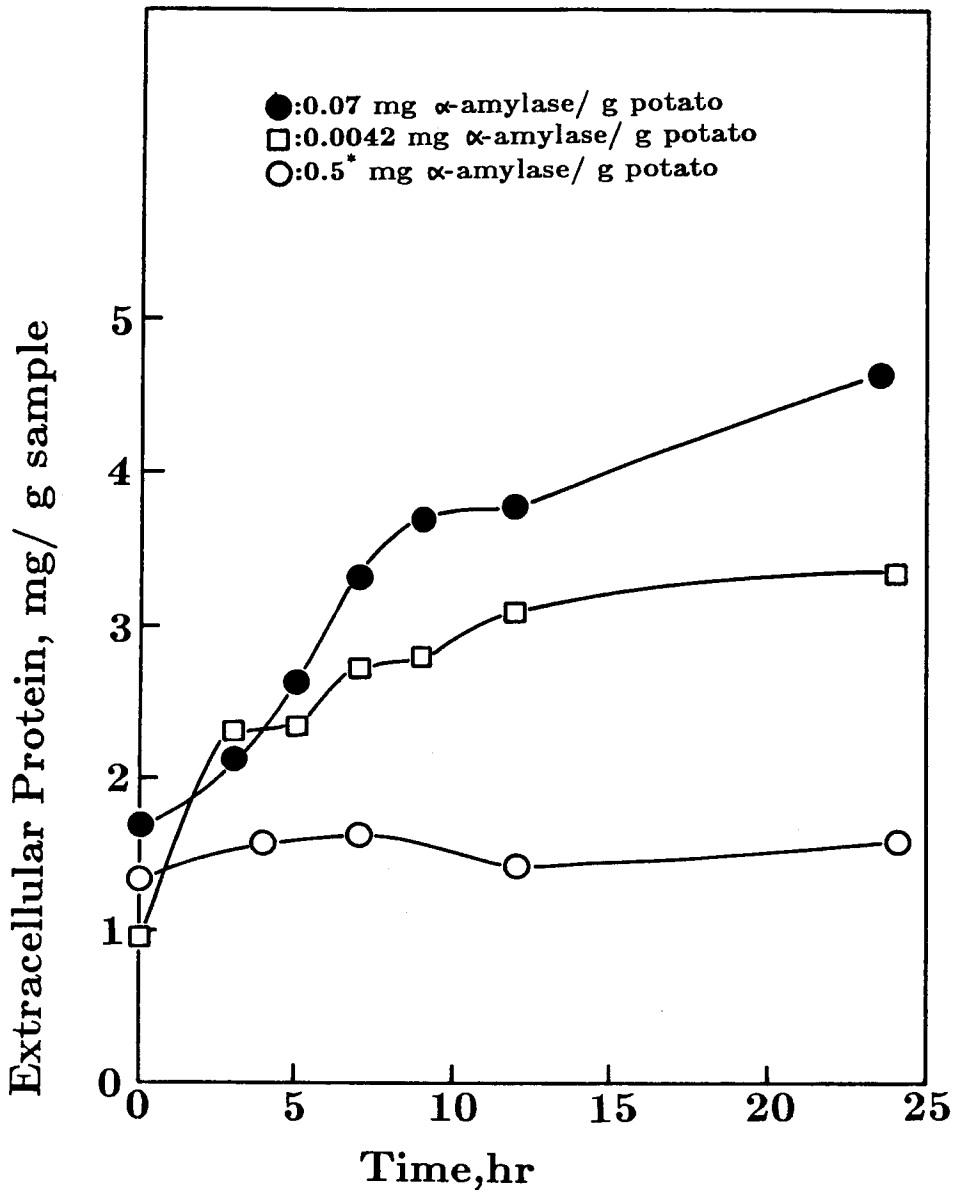

Fig. 9. Extracellular protein profile. *Potato starch predigested for 12 h, **No yeast added.

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