Improvement of Baker's yeast production using fed-batch fermentation

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Introduction

Yeast metabolism uses nutrients to synthesise new cell material as well as to generate energy for survival. Metabolically, yeast are mostly facultative aerobes, capable of growing either in the absence of air (fermentative) or in its presence (oxidative). Therefore, in the absence of aeration, yeast has the ability to instantaneously change its respiratory metabolism from oxidative to fermentative. This catabolic shift is referred to as the "Pasteur effect" or "Glucose effect".

The growth characteristics of *S. cerevisae* are varied depending on the culture conditions and medium composition. Growth of yeast is greatly influenced by the level of dissolved oxygen concentration in the culture and the different carbon sources used. The growth kinetics of yeast in adaptation to variation in sugar concentration has also been well documented. Growth kinetics information re required for the improvement of Baker's yeast fermentation for production of products in the preparation of bread dough. Subsequently, studies in applications of genetic engineering techniques become very popular due to the increasing demands of the industry to improve strains of yeast.

Control strategies and suitable mode of fermenter operation in industrial aerobic fermentation have been developed to maximize the growth of yeast and minimize the detrimental factors affecting the yeast's growth patterns. For example, fed-batch fermentation, the process whereby nutrients necessary for cell growth are fed into the culture during the fermentation, has been found to be particularly effective for processes in which effects such as substrate inhibition, catabolite repression, and product inhibition are important. In Baker's yeast fermentation, fed-batch culture is devised to prevent a detrimental effect to yeast growth because of high initial glucose concentration as normally employed in conventional batch fermentation. The rate of oxygen transfer from the dispersed air bubbles to the fed-batch culture of high cell density can become the rate limiting factor which changes the growth characteristic of the yeast. This is. another problem associated with Baker's yeast fermentation. The main objective of the study was to investigate the effect of agitation speed; and hence volumetric oxygen transfer rate (K_La) on the performance of yeast cultivation. The feasibility of using fed-batch fermentation technique for improvement of yeast cultivation was also investigated

Materials and Methods

The Baker's yeast, *Saccharomyces cerevisae*, obtained Global Yeast Industry, Turkey was used in this study. Lyophilized culture was rehydrated in YM liquid medium and was incubated for 10 h at 30°C. Stock culture was maintained on a Difco YM agar medium at 4°C and was transferred onto a new medium every month to maintain viability. Starter culture was prepared by transferring two loops of the stock culture to a 500 mL Erlenmeyer flask containing 150 mL of an inoculum medium and incubated at 30°C for 18 h on a rotary shaker at 200 rpm.

Batch fermentation studies of *S. cerevisae* in a 2 L stirred tank fermenter (B. Braun, Biotech., Germany) were undertaken to generate kinetic growth data of the yeast to provide a background information for the design of fed-batch fermentation. The variables studied in batch fermentation include the use of different concentrations of carbon and nitrogen sources and the effect of agitation speed (ranging from 200 to 1200 rpm). The fermentation using a 2 L fermenter was started with the addition of 150 mL of yeast starter culture to 1350 mL of the production medium at 30°C.

The same fermenter was also used for fed-batch fermentation, but it was connected to the multifermenter control system (MFCS) for controlling the feeding rate of the substrate to culture and also to control some of the fermenter operating variables. Control of the peristaltic pump is facilitated using MFCS/win software, which is a fermenter supervisory control and data acquisition system (SCADA) for simultaneous control of multiple fermenters. This 32-bit PC-based application, using algorithm or a profile inputs, was interfaced to the fermenter control unit for controlling the speed of the peristaltic pump according to the given algorithm. The exponential fed-batch fermentation was started after the glucose in the initial batch. 600 mL of culture, became depleted. Several fed-batch fermentations were carried out at different required specific growth rate (μ) of the yeast. In all fermentations, the culture pH was controlled at 5.0, while the level of dissolved oxygen tension (DOT) was monitored. During the fermentation, samples were withdrawn at time intervals for analysis. Cell growth was determined as dry cell weight using filtration and oven dry method. Viability of yeast cell was determined using methylene blue staining method. Glucose was determined using glucose analyzer (), while gas chromatography was used for ethanol determination.

Results and Discussion

In batch fermentation of Baker's yeast, after a very short lag phase (about 3 min), the yeast grew exponentially with concomitant increased in ethanol concentration in the culture. Due to diauxic growth, after glucose in the culture became

depleted, the yeast started to consume the ethanol for second phase of growth. Oxygen requirement for growth using glucose was significantly lower than growth using ethanol as carbon source. Initial concentration of glucose greatly influenced the growth characteristics of the yeast. The final cell concentration attained at the end of fermentation was increased proportionally with initial glucose concentration up to 120 g/L, which gave more or less a constant cell yield of 0.13 g cell/g glucose. However, the specific growth rate of the yeast was reduced with increasing glucose concentration. The amount of ethanol accumulated in the culture was also increased proportionally with increasing glucose concentration. In term of overall productivity, the highest (0.35 g/L.h) was obtained in fermentation using 80-120 g/L glucose.

Nitrogen source used in the medium formulation also greatly influenced growth characteristics of the yeast. Growth was greatly inhibited in fermentation using inorganic nitrogen source (NH_4Cl and NH_42SO_4) as the sole nitrogen source. Growth was enhanced when organic nitrogen source (peptone and yeast) was used as nitrogen source. In this study, the optimize medium formulation for batch cultivation of yeast was proposed.

Different growth characteristics of yeast were also observed at different agitation speeds, which provided different volumetric oxygen transfer rate (K_La) to the culture. The K_La at 200 rpm was only 3.7 h^{-1} and the K_La was increased to 82.46 h^{-1} at 1000 rpm. The final cell concentration attained during the fermentation was increased from 29.8 g/L at agitation speed of 200 rpm to 41.9 g/L at 1000 rpm. However, a slight decrease in cell viability was observed with increasing agitation speed, indicating that high shear rate caused damage to the cells.

High density cell cultivation was achieved in exponential fed-batch fermentation process. During the fed-batch fermentation, the feed rate of the substrate is increased according to the exponential growth of the yeast at specific growth rate below the maximum. Among the specific growth rate (0.1 to 0.4 h^{-1}) employed for the exponential fed-batch fermentation, the highest cell concentration (90.0 g/L) was obtained at specific growth rate of 0.1 h^{-1}, which was associated with very small quantity of ethanol accumulated in the culture during the fermentation. The cell yield (0.5 g cell/g glucose) and overall productivity (6.2 g/L.h) obtained in fed-batch fermentation was significantly higher than those obtained in batch fermentation, with more or less about the same cell viability was maintained.

From this study, it was found that the fed-batch fermentation can be used to avoid engineering limitation and also useful to exert a metabolic control in Baker's yeast fermentation. The sugar limitation that can be maintained during exponential fed-batch fermentation can be utilized to avoid extensive overflow metabolism, which otherwise would result in too high ethanol production and an accompanying reduction of biomass yield. Even if the ethanol is subsequently consumed, the total biomass yield from glucose is reduced when the combustion to carbon dioxide passes via ethanol, as can be concluded from yield calculations. This decrease of the yield has been observed in batch fermentation where growth of yeast was in anaerobic mode, i.e. part of glucose supplied to culture was used for ethanol production. The overflow metabolism of sugar to ethanol is due to a restriction in the carbon metabolism, somewhere after the pyruvate. When subjected to sugar concentrations above a certain critical value, the glycolysis rate exceeds a critical value and the cells can not fully oxidize all the available sugar to CO_2. Some of the sugar uptake is converted to ethanol, even under aerobic conditions. This critical value was determined to be 110 mg/L glucose during Baker's yeast fermentation with feed of molasses. For the cultivation using a defined glucose medium, the critical value of 40 and 50 mg/L have been reported for fed-batch and continuous fermentation, respectively. A sugar concentration above the critical value, ethanol is formed. On the other hand, at concentration below the critical value, if ethanol is present, it will be consumed concomitantly with sugar.

In our study, fed-batch fermentations of Baker's have also been carried out in 100 L fermenter. When cultivation are performed in large scale fermenters, concentration gradient of substrate will arise due to the combined effect of limited mixing and mass transfer, and microbial activities. Limitation in mixing is increased with increasing fermenter size and become more pronounced when the fed-batch fermentation is used, since the rate limiting substrate is fed as a concentrated solution, often from only one point of the fermenter.

Conclusions

Fed-batch fermentation with suitable control strategies has been established for high density cell cultivation of Baker's yeast. Using optimize medium, glucose as a carbon source and yeast extract as a nitrogen source, a final cell concentration of 90.0 g/L was achieved in fed-batch fermentation. This gave a yield and overall productivity of 0.5 g/g and 6.2 g/L.h. This technique of fermentation greatly improved the performance on Baker's yeast fermentation as compared to conventional batch process.

Benefits from the study

Several locally isolated yeasts have been isolated and characterized. Some of which have potentials to be used for commercial applications. Standard operating procedure for industrial production of Baker's yeast, using efficient fed-batch fermentation technique, has been established. Important parameters for the scaling-up of the process have been identified, proposed and described. The same technology could also be used for production of various products from yeast (eg. amino acids, gluthathione, single cell protein and β-glucosidase). This is a strategic and important future technology for the country and the process is environmental friendly. Small scale production for laboratory use is being carried out at UPM

Patent(s), if applicable :

Nil

Stage of Commercialization, if applicable :

Nil

Project Publications in Refereed Journals:
Nil

Project Publications in Conference Proceedings:
Nil

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Tan Li Lung	Characterization of locally isolated yeast strain towards industrial application	Fermentation Technology	MSc	2003
Ahmad Ariff	Development of Fed-batch fermentation for efficient Baker's yeast production	Fermentation Technology	MSc	Expected to be graduated 2004

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