INVESTIGATION INTO THE PROPAGATION OF BAKER'S YEAST

A Laboratory Experiment in Biochemical Engineering

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Nowadays, biocatalysts (including microorganisms and enzymes) and bioreactors are applied not only to the bioprocess industries but also to problems in waste treatment and remediation of contaminated sites. Use of concepts related to them is important to the new generation of chemical engineers, but many students are not exposed to biology through the standard chemical engineering curriculum. In order to introduce these concepts and extend traditional chemical engineering principles, we have designed a laboratory experiment in biochemical engineering that demonstrates a typical growth pattern of microorganisms, as well as the fermentation process involving multistage scale-up.

Saccharomyces cerevisiae, mainly in the form of baker's yeast, represents the largest bulk production of any single-cell microorganism in the world. Several million tons of fresh *S. cerevisiae* cells are produced yearly for use in human food.[1] In several areas of fundamental biological science, *S. cerevisiae* has been extensively studied and serves as a valuable model eukaryotic cell in such studies.[1] The large-scale manufacture of baker's yeast involves a multistage propagation of a specially selected *S. cerevisiae* strain on molasses.[2] The large-scale production of many other microorganisms also follows the same principles that are employed here. The propagation of baker's yeast is applied in this demonstration because it is a widely used and well-studied system using easily obtainable raw material.

In this experiment students become acquainted with practical microbiology techniques such as preparing and sterilizing media and equipment by autoclaving, inoculating yeast into shaker flasks, and inoculation of the bioreactor. The effect of temperature and pH on the propagation of baker's yeast is also studied. Fermentation processes are operated in both batch and fed-batch modes.

Because of the relatively slow response time of biological systems, experiments span one-and-a-half days. Students usually came to the laboratory to prepare the media and autoclave the bioreactor and other stuffs in the afternoon. They began cell cultures in the shake flasks and then transferred to the bioreactor in the following day. We did not expect one student to be in the laboratory for such a long period, so four or five students would work together as a group in shifts. Each group repeated the experiment three times, varying operating conditions (temperature or pH). Students should change their roles in the group to make sure that each of them can go through the whole process.

THEORY

Yeast Propagation Mathematics

The propagation of baker's yeast follows a typical microbial growth pattern: a lag phase where no growth takes place, an exponential phase where the growth follows a first-order reaction scheme, a stationary phase, and then a death phase.[3]

Growth during the exponential phase can be given by

$$\frac{dX}{dt} = \mu X \qquad (1)$$

where X is biomass concentration and μ is the specific growth rate.

As long as μ is constant, the differential equation above can be integrated with the initial condition of $t = 0$, $X = X_0$

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$$X = X_0 e^{\mu t} \quad (2)$$

Taking the natural logarithms of Eq. (2) gives

$$\ell n\, X = \ell n\, X_0 + \mu t \quad (3)$$

This equation indicates that a plot of $\ell n\, X$ versus time will give a straight line with slope μ.

It has been proven[2] that the constant μ is substrate dependent and can be expressed by the Monod equation

$$\mu = \mu_m \frac{S}{K_m + S} \quad (4)$$

where

- S concentration of the growth limiting substrate
- μ_m maximum specific growth rate
- K_m is a constant and the value is very small [*e.g.*, at temperature of 30°C, pH 4.0, with glucose as inhibiting substrate, $K_m = (3.6 \pm 0.5) \times 10^{-4}$ mol/L][2]

Aerobic Growth and Alcohol Fermentation

The presence of oxygen is necessary for yeast cell multiplication, which dispels the general notion that yeast can grow truly anaerobically.[1] This is because, as well as providing a substrate for respiratory enzymes during aerobic growth, oxygen is required for certain growth-maintaining hydroxylations such as those involving the biosyntheses of sterols and unsaturated fatty acids. Oxygen should therefore be regarded as an important yeast growth factor.

Propagation of yeast cells and production of alcohol by yeast are two quite different industrial processes. In the first case, where optimization of respiratory growth is important, sufficient oxygen must be maintained in bioreactors to support rapid yeast growth (*e.g.*, in commercial fermentation, 1 volume of air per fermentor volume is passed through the medium per minute[4]). In contrast, production of alcohol by yeast should be carried out without aeration. In this case, yeast reproduces while producing ethanol and carbon dioxide. The yield of yeast, based on the amount of fermentable sugar, is low—often not more than 10%. But in aerobic systems, the colonies grow up to 20 times faster than those without aeration. A yield of up to 50% of the weight of fermentable sugar can be obtained under some special conditions.

Actual levels of dissolved oxygen (D.O.) in a bioreactor can be determined by oxygen electrodes. They are frequently calibrated by saturating the fermentation medium (without yeast) with air and by equating the instrument response with "air saturation." Instrument readings during the actual fermentation can then be expressed as "% of air saturation." A useful approximation of the actual amount of oxygen present in an air-saturated bioreactor is 7 ppm.[4]

MATERIAL AND METHODS

Microorganism and Medium

Saccharomyces cerevisiae (dried baker's yeast packed for Goodman Fielder Milling & Baking N.Z. Ltd.) was grown in YEPD medium[5] with the following composition: glucose, 20 g/L; yeast extract, 10 g/L; peptone, 20 g/L; and commercial antifoam, 10 drops/L.

Equipment and Experimental Procedure

Shaker Culture • 100 mL of medium was added to a 250-mL flask. After being sterilized at 110°C for 30 minutes, 1 g of dried baker's yeast was added, then cultured in the shaker at 30°C and 200 rpm for 60 to 90 minutes. The broth was sampled at the beginning and at the end of the culture.

Small-Scale Bioreactor Culture • 2 L of medium was added to the 3 L bioreactor (New Brunswick Scientific Co., Inc.) with a working volume of 2.5 L. The complete assembly was sterilized by autoclaving at 110°C for 30 minutes. The pH probe (Ingold Electrodes, Inc.) should be calibrated before sterilization, while the D.O. probe (Mettler-Toledo Process Analytical, Inc.) should be done after sterilization. 400 mL of fermentation broth was then inoculated from flasks into the bioreactor. The agitation rate of the bioreactor was set at 400 rpm. The fermentation process was operated for 4 or 5 hours and sampled every half hour. At the end of the fermentation the air supply was shut off, and the change in D.O. was recorded every 15 seconds until it reached a constant value. One of the student groups repeated the experiment three times, setting the temperature at 28°C, 30°C, and 35°C. The other group ran it without pH control the first time and then added sodium carbonate to keep the pH at 6.0 and 5.5, respectively, for the second and third times. Some of the students tried to operate in a fed-batch mode.

Analytical Methods

Cell Concentration was measured by three methods: 1) by counting numbers of the cells using a hemocytometer; 2) by measuring the wet weight of yeast after centrifuging 10 mL broth samples for 10 minutes at 4500 rpm and decanting the supernatant liquid; and 3) by measuring the dry weight of yeast after dehydrating the remaining pellets in the centrifuge tubes at 65 °C for 48 hours.

Glucose Concentration was determined by reacting the glucose with Glucose Trinder reagent (Sigma Diagnostics, Inc.) to yield a red color solution, and the change in color was then measured by spectrophotometer.

TYPICAL RESULTS AND DISCUSSION

Microorganism Growth Pattern

A series of typical microorganism growth curves was obtained by students from the propagation of baker's yeast in the bioreactor for 4 or 5 hours. A typical result is shown in Figure 1. The growth pattern in which an initial lag phase was followed by an exponential phase and a stationary phase obviously appeared in all three curves, including cell number, wet weight, and dry weight against time.

In the wet weight curve, however, many experimental data

seemed erratic, sometimes in opposition to the increasing trend. This might be caused by variation in the water content of the remaining pellets in the centrifuge tubes. In this instance, the average water content accounted for about 87.5% of the wet weight, and the variation in the water content between different samples was up to 5%. For example, if the dry weight of biomass was 3.5 g/L, the wet weight should be 28 g/L, but the experiment value would vary from 23.33 to 35 g/L with the variation in the water content of 5%. The experimental error was too large to be acceptable.

It was impossible for cells to be absolutely well distributed in the fermentation broth, so the result of counting cells in 0.1 μL of diluted sample could only be statistically representative. In the 4-hour propagation process when the biomass doubled, the cell number did not change as much as observed by counting, using the hemocytometer. This might be the reason for differences between the cell number and the cell weight curves. Another explanation might be the differences in individual cell weight at different times. As shown in Figure 1, the increase in cell number seemed to stop after 2 hours, while the total weight of biomass still went up. The continuous increase of individual cell weight could contribute to the difference. Of the three methods, dry weights seem to be most accurate, although cell counts and wet weights give more rapid results.

According to Eq. (3), a plot of $\ell n\, X$ versus time gave a straight line with slope μ at the exponential phase (X is derived from dry weights). For this experiment, the specific growth rate was found to be 0.3 hr^{-1} (see Figure 1). The doubling time was 2.34 hr.

Substrate Utilization and the Yield

During the 4-hour propagation of baker's yeast, the glucose concentration was seen to decrease corresponding to the growth patterns (Figure 1). Initially, in the period of the lag phase, the rate of glucose consumption was slow, and then a steady decrease in glucose concentration was seen until the glucose was completely consumed.

The total yield of yeast (dry weight), based on the amount of glucose consumed, was 0.18 g/g for this experiment. The result was less than what was expected from the literature.[4] This is because the specific growth rate in this experiment reached 0.30 hr^{-1}, higher than a given value of 0.25 hr^{-1}. Above this value, the fermentable sugars will be fermented into alcohol and the yield of yeast will be reduced.[4] In order to obtain the maximum yield, the supply of fermentable sugars must be limited and their concentrations must be extremely low, generally below 0.0004%.[4] This can be achieved by continuous addition of fermentable sugars to the bioreactor without any removal of fermentation broth, *i.e.,* fed-batch operation mode. Here, in our laboratory, the fermentation process was operated in fed-batch mode only once due to the limiting course schedule. Since no valid conclusions can be drawn from a single experiment, results are not shown.

Change of D.O. and Oxygen Demand

As shown in Figure 2, over the course of the propagation, dissolved oxygen (D.O.) concentration initially increased in the first half hour and then gradually fell for the remainder of the experiment. D.O. concentration fluctuated at the beginning because the contents of the bioreactor were changed when the fermentation broth with yeast was inoculated from flasks. After the lag phase, the consumption of D.O. went up with increasing cell numbers until the amount of aeration was less than the demand of D.O. by yeast in the bioreactor, so D.O. concentration was observed to decrease.

In the fermentation process it is very important to keep a balance between the amount of oxygen supply and de-

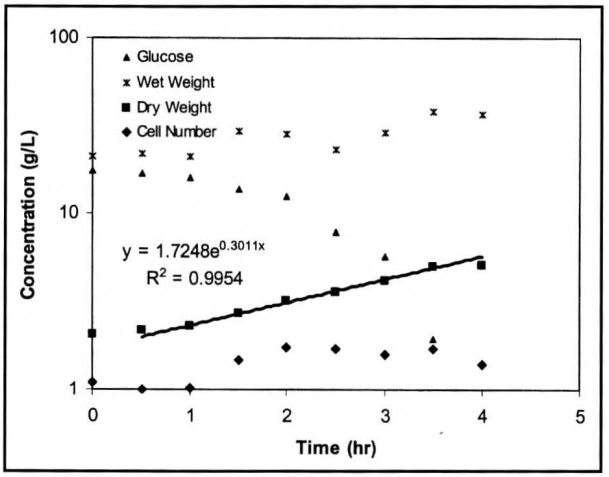

Figure 1. *Ln (concentrations of glucose, wet weight, dry weight, and cell number) versus time over the course of the propagation of baker's yeast in the bioreactor.*

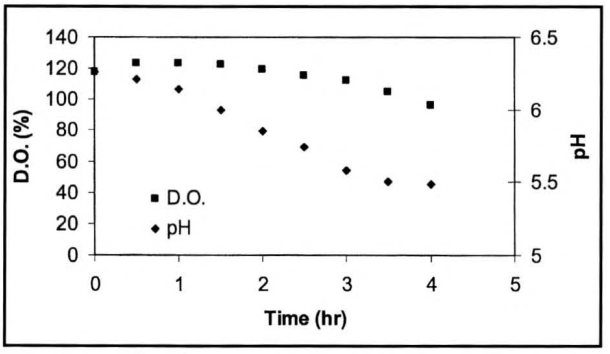

Figure 2. *The change of D.O. and pH over the course of the propagation of baker's yeast in the bioreactor.*

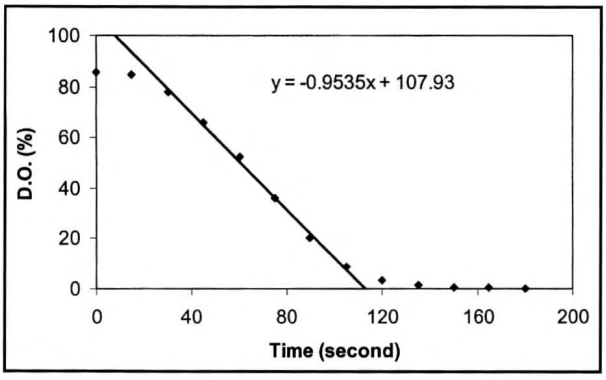

Figure 3. *The change in D.O. after the air supply was shut off in the bioreactor.*

mand. D.O. concentration is always used to judge whether the oxygen supply is enough, as well as to indicate the state of cell growth and abnormal changes in the process. The amount of oxygen required by a given amount of biomass can be estimated by recording the change of D.O. concentration soon after the air supply is shut off. A typical result of this experiment is plotted in Figure 3. Initially, the D.O. concentration decreased slowly due to air bubbles remaining in the fermentation broth continuing to transport oxygen into the solution. Then the D.O. concentration declined rapidly, following a straight line when the decrease of D.O. was equal to the oxygen consumed by yeast. Here, according to Figure 3, the rate of oxygen consumption was about 0.95%/sec. With an approximation of the oxygen in an air-saturated bioreactor of 7 ppm, the oxygen required by a given amount of yeast (dry weight) was 0.041 mmol oxygen/g of yeast/sec for this experiment.

Change of pH over the Propagation

Figure 2 also shows the pH readings over the time course of the experiment. The pH declined from a value of 6.3 to 5.5 over the 4-hour propagation course corresponding to the growth pattern. Normally during the propagation process, apart from yeast cells, carbon dioxide, water, and energy in the form of heat are also produced. Among these, the carbon dioxide produced could form carbonic acid and result in a decrease of pH in the fermentation broth.

In commercial fermentation, pH can be partly controlled by the ratio of ammonia to ammonium sulfate in the feed. When all the required nitrogen for a fermentation has been supplied, the pH can be regulated by adding sodium carbonate.[4] In our experiment, peptone was applied as a nitrogen source. The YEPD medium appeared to be a buffer solution. The pH of the fermentation broth did not change too much during the 4-hour propagation course without adding sodium carbonate, ammonia, or ammonium sulfate.

CONCLUSIONS AND REMARKS

From the propagation of baker's yeast in a 3L bioreactor for four or five hours, we obtained generally good results of typical microorganism growth curves in which an initial lag phase was followed by an exponential phase and a stationary phase. The glucose consumption, pH, and dissolved oxygen curves were also observed to correspond to the growth pattern. Among the results from three kinds of methods to determine the cell concentration in the fermentation broth, dry weights gave the most accurate values, while cell numbers and wet weights could be obtained more rapidly. In this experiment, the total yield was less than the value from literature because the specific growth rate did not fall into the expected region where the best yield can be obtained. The oxygen demand by a given amount of yeast was also estimated by recording the change of D.O. concentration soon after the air supply was shut off.

The fermentation processes were operated in both batch and fed-batch modes. The effects of pH and temperature for the propagation of baker's yeast were studied by different groups of students, but the data were problematic since few data points were available due to the laboratory's time constraints. Further, the requirements for careful attention to detail and sample handling could not be met by every student.

According to the results of the experiment we have run this year, we suggest that the time of each course of the propagation might be extended from four or five hours to over eight hours if there is no strict time constraint for the laboratory. Therefore, a lower inoculum size would be applied at the beginning and a much greater percent increase in cell mass over the course could be observed.

The results for the cell mass concentration might be observed by measuring the optical density (a much easier method) and using a previously prepared calibration curve. If a larger bioreactor (for example, with a volume of 30 L) is employed in the laboratory, the concentration of oxygen and carbon dioxide in the exit gas could also be measured, and further, these data could be used to find the mass transfer coefficients, oxygen uptake, and respiratory quotient.

In summary, processes using living cells are quite different from those with chemicals and materials. The production of cells always involves a multistage propagation of a specially selected strain. An aseptic environment is necessary for the propagation of pure culture, and contamination should be avoided as much as possible. The activities of the cells are affected by many variables and play the most important part in the bioprocesses. We believe that it is necessary to introduce a meaningful, challenging, but practicable microorganism propagation experiment to chemical engineering students who lack any prior exposure to biology. The student response to the experience has been positive.

NOMENCLATURE

K_m	Monod constant
S	concentration of the growth-limiting substrate
t	time
X	biomass concentration
X_0	initial biomass concentration

Greek letters

μ	specific growth rate
μ_m	maximum specific growth rate

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