Chemical Engineering education. The experience gained by the practice teacher is not incompatible with developing professional engineering experience. By considering a broad definition of engineering, the profession of developing available resources to be useful to men,[3] the services rendered in assisting developing colleges and training under-achieving students are definitely legitimate egineering activities. In addition, the experienced teachers that the program can provide will be an asset in whatever positions they may assume after graduate school. As beginning faculty in universities, the trial of initial teaching duties will not be unduly burdensome at a time when some new research interests are being explored.

The small Chemical Engineering Departments that may participate in this program will be benefited in the short term simply with respect to more faculty. In addition, possibly some of the "practice teachers" will be induced to return to the same college and aid its development. The benefits to the students of a practicing teacher may be more psychological than educational. The new teacher will make mistakes in academic matters at the expense of the students, but as a symbol of youth and scholarly excitement, the new teacher can favorably motivate many students to higher goals. □

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ChE laboratory

Chemical Reactor Laboratory

BIOLOGICAL REACTIONS: KINETICS OF YEAST GROWTH

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This is the third in a series[1] of articles describing an undergraduate chemical reactor laboratory designed for seniors in the Department of Chemical Engineering at Princeton. Professor Richard H. Wilhelm provided the inspiration for the successful development of the laboratory. The basic objectives are outlined in the first article of the series.

The experiment described here provides students with an introduction to biological processes and techniques by demonstrating the transfer of reaction engineering knowledge learned in non-biological systems to the kinetics of yeast growth. The increasing understanding of biological systems and recognition of their importance in chemical processing indicate the value of familiarity with biological processes. The growth of yeast under aerobic conditions is a relatively simple experiment for which the kinetics of growth may be compared with theoretical behavior.

The experiment is patterned after the commercial process for growing yeast in which an initial charge of yeast in a nutrient solution is allowed to multiply and grow. An excess of all nutrients except sugar is provided. Under these conditions the rate of growth is a function of the yeast present and the amount of sugar present. After an induction period the yeast growth rate is rapid. As the sugar present is depleted the growth rate decreases and falls to zero at the end of the experiment. The yeast cell volume and sugar concentrations are measured over the period of the experiment and the results compared with predictions.

The growth of yeast is carried out over a ten-hour period. An additional three hours is required for analysis of samples for sugar concentration. Yeast cell volume is determined by centrifugation while sugar concentration is de-

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The experiment is patterned after the commercial process for growing yeast . . . and it demonstrates the transfer of reaction engineering knowledge learned in non-biological systems to the kinetics of yeast growth.

termined by a modification of a colorimetric method commonly used in analysis for sugar in blood.

THEORETICAL BACKGROUND

Several general reviews of the application of engineering techniques to biological processes are available[2]. The kinetics of reactions in microorganisms have been treated in books by Hinshelwood[3] and Bray and White[4]. Humphrey and Diendoerfer[5] have furnished an excellent review of fermentation.

Yeasts are formally classified as plants, but like animal organisms they utilize the oxidation of carbon in the form of sugar to carbon dioxide as a source of energy. Under anaerobic conditions yeast is able to use the oxygen in the sugar molecule, giving off alcohol and carbon dioxide as waste products, but growth is relatively slow. Under aerobic conditions yeast growth is greatly enhanced and the waste products are primarily acids and aldehydes together with carbon dioxide. In addition to sugar and oxygen yeast requires a nitrogen source in the form of ammonia or amino acids and several minerals and vitamins in order to grow and multiply.

The kinetics of yeast growth follow a pattern similar to that of adsorption and catalysis in that a large number of parameters, each with saturation effects, is involved. After an initiation period in which the yeast becomes accustomed to a new environment, the growth rate is first order in yeast concentration and also depends on the concentrations of sugar, oxygen, available nitrogen, minerals, vitamins, hydrogen ion concentration and temperature. Hinshelwood[3] formulated the rate equation as a product of terms for each of the vital substances,

$$\frac{dY}{dt} = kY \left(\frac{b_s C_s}{1 + b_s C_s}\right)\left(\frac{b_o C_o}{1 + b_o C_o}\right)\left(\frac{b_n C_n}{1 + b_n C_n}\right) \cdots \left(\frac{b_x C_x}{1 + b_x C_x}\right) \qquad (1)$$

where Y is the yeast cell volume per unit volume of solution, k is a rate constant, C_s is the sugar concentration, b_s is a constant for sugar, and so forth for oxygen (o), nitrogen (n) and others. It is assumed that other variables such as temperature and pH are constant. For an excess of any of the nutrients the product bC becomes large compared to unity and the term in brackets approaches unity.

In the experiment considered all nutrient substances except sugar are supplied in large excess so that the rate expression becomes

$$\frac{dY}{dt} = kY \frac{b_s C_s}{1 + b_s C_s} \qquad (2)$$

Since yeast growth occurs both by the growth of individual cells and by cell division with further growth and division of new cells, a rate equation for the number of cells per unit volume may differ slightly from that for the volume of yeast per unit volume.

A material balance for the sugar may be used to relate sugar and yeast concentrations:

$$R(Y - Y^o) = C_s^o - C_s \qquad (3)$$

where 1/R is the yeast cell volume which results from the utilization of a unit amount of sugar and the superscript o indicates an initial value. Combining with Eqn. (2) to eliminate C_s yields

$$\frac{dY}{dt} = kY \left[\frac{b_s(C_s^o - R(Y - Y^o))}{1 + b_s(C_s^o - R(Y - Y^o))}\right] \qquad (4)$$

which may be integrated for the initial condition, $Y = Y^o$ at $t = 0$, to give

$$Y = Y^o e^{kt \left[\frac{Y^o}{Y}\left(1 - \frac{R}{C_s^o}(Y - Y_o)\right)\right]^{\frac{1}{b_s(C_s^o + RY^o)}}} \qquad (5)$$

The growth curve of Eqn. (5) will not include the induction period and transition at the start of growth nor the loss of cell volume after the sugar has been consumed.

APPARATUS

The reaction is carried out in a 30-liter fermentor equipped with an agitator, water coils, air sparger and sampling ports. Temperature is controlled by circulating water from an auxiliary constant-temperature bath through the fermentor coils. A photograph of the fermentor is shown in Figure 1.

Air supplied to the sparger located below the agitation blades is passed through a 1-inch diameter, 0.8 micron pore-size Millipore filter to remove contaminants. A 1.2-SCFM rotameter is included in the air feed line.

Samples of the medium are placed in 12-ml, tapered, graduated centrifuge tubes which are subsequently placed in a high speed centrifuge. A lower cost centrifuge would probably be adequate.

If samples to be analyzed for sugar are to be stored, a freezer is required. The colorimeter

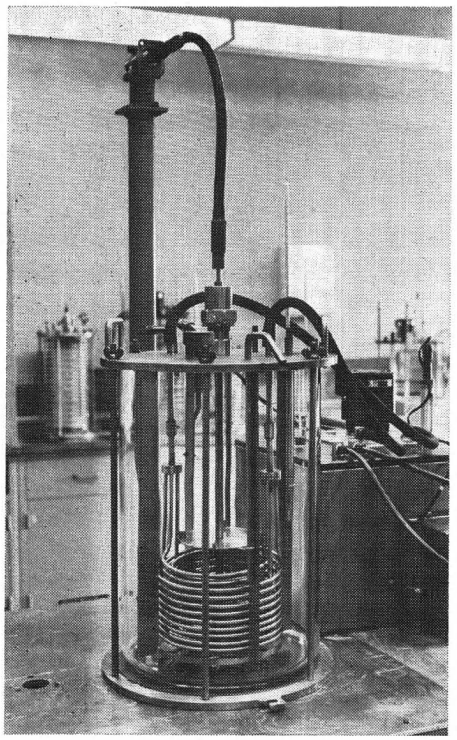

Figure 1. Fifteen-liter fermentor for yeast growth.

analysis is carried out with a Beckmann Model DU Spectrophotometer. In addition an assortment of specialized laboratory glassware is required.

PROCEDURE

The growth medium used is 500 g of dextrose (cane sugar) and 200 g of nitrogen base (Bacto, dehydrated) containing nitrogen and necessary trace elements and vitamins all dissolved in 15 liters of water. Since yeast is fairly resistant to disease, complete sterilization is unnecessary but the sugar and nitrogen base are heated to 90°C for 5 minutes prior to use. Distilled water is used. The solution is placed in the fermentor and heated to 29°C. The air flow is started and 20 g of Fleischmann's dry yeast is added.

Samples are taken with a pipette at intervals of one-half to one hour for approximately ten hours or until yeast growth has ceased. Since the yeast tends to concentrate in the foam produced by sparging, care must be taken to obtain representative samples.

To eliminate its effect on growth the pH of the medium is kept constant in the range of 5.0 to 5.5 units. The pH is tested at 15-minute inervals with pH paper and excess acidity is neutralized with concentrated ammonium hydroxide.

Samples are placed in centrifuge tubes and centrifuged to produce a compact yeast mass at the bottom of the tubes. Cell volume is recorded as the volume of the yeast mass produced. Since the yeast may continue to produce carbon dioxide which may swell the volume of the yeast mass, readings of cell volume must be taken immediately after centrifugation.

The supernatant liquid is collected for subsequent sugar analysis. Some yeast remains in this liquid and must be prevented from decreasing the sugar content. A drop of phenol is added and the solution is frozen.

The sugar determination is made by the colorimetric method of Nelson[6-10] which is not affected by the presence of other compounds. Samples are thawed and diluted to give sugar concentrations of about 0.2 mg per ml. Duplicates of each unknown are desirable. Both samples and standard solutions are treated by boiling with a copper reagent and adding an arsenomolybdate color reagent. The absorbances of the resulting solutions are measured at 500 mu with a Beckmann Model DU Spectrophotometer using standard techniques.

Results are compared with Eqns. (2) and (5) with allowance for an initiation period of one to two hours. The constants $C_S°$ and $Y°$ are known from direct measurement. The value of k is determined from the rate during the initial growth period when sugar is present in excess and Eqn. (2) becomes

$$\frac{d \ln Y}{dt} = k \qquad (6)$$

When sugar concentration affects the rate the term $\left[\frac{b_s C_s}{1 + b_s C_s}\right]$ becomes important and b_S may be evaluated from the slope of a plot of $\left(k \frac{d \ln Y}{dt}\right)^{-1}$ vs. C_s^{-1} vs. C_S^{-1}. The value of R is determined from yeast and sugar balances. Once the constants are known the integrated rate equations may be tested.

STUDENT PERFORMANCE

Students are enthusiastic about this experiment and attack it as an adventure in a new area. The yeast growth and measurements of cell volume are carried out without difficulty. Left on their own the students usually fail to obtain consistent results for sugar concentration. Close supervision and detailed recipes are required for successful analyses.

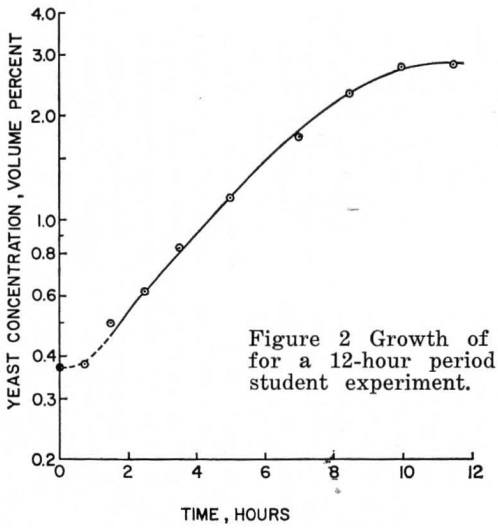

Figure 2 Growth of yeast for a 12-hour period in a student experiment.

Typical results of a student experiment are shown in Figures 2, 3 and 4 in which cell volume and sugar concentration are plotted *vs.* time and the agreement with Eqn. (5) is indicated.

DEVELOPMENT OF THE EXPERIMENT

Yeast was chosen for this experiment because of its rapid growth rate, its insensitivity to experimental conditions and its resistance to disease. A number of trials were made before suitable temperature and concentration variables were determined. Several batches of yeast were killed (obvious from the smell) for unknown reasons in the development runs.

Attempts to measure yeast concentration by counting cells in a small volume under a microscope were unsuccessful. The combined problems of representative sampling and the time required for counting eliminated use of this method of analysis. Turbidity measurements were attempted briefly but eliminated because differences in sample transmittance were small.

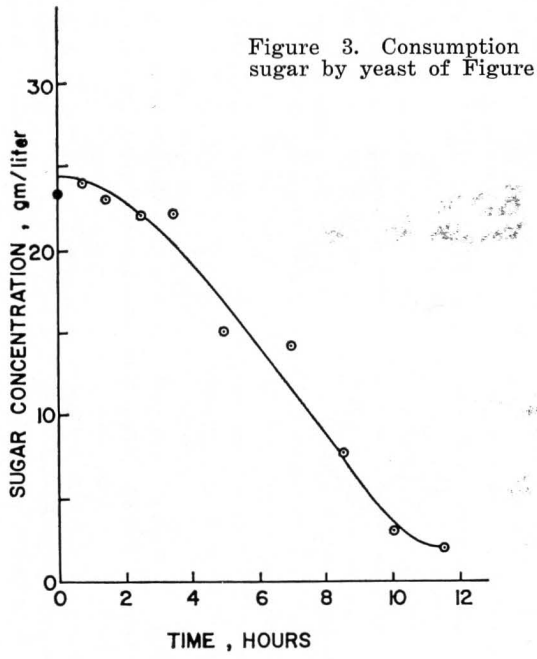

Figure 3. Consumption of sugar by yeast of Figure 2.

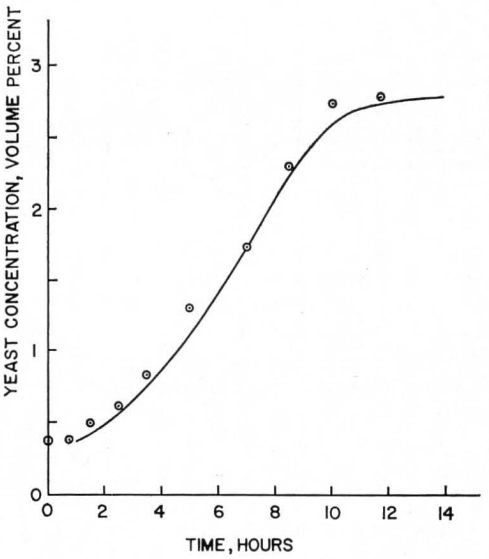

Figure 4. Comparison of theoretical growth curve with data of student experiment. Experimental points are those of Figure 2. The solid line is the theoretical behavior as calculated from Eqn. (5) with $Y^\circ = 0.0037$, $C_S^\circ = 22.5$ gm/liter, $R = 935$ gm/liter, $b_S = 0.105$ liter/gm, $k = 0.4$ hr^{-1}. The curve is displaced one hour to allow for the induction period.

Foam formed in the latter stages of growth creates problems in taking representative samples. An anti-foam agent added at the end of a run eliminated the foam within seconds. The effect on yeast growth has not been tested but such agents are used routinely in biological laboratories. □

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