

# CHOOSING AN OPTIMUM FEEDSTOCK FOR YEAST PRODUCTION

## *A Design-Oriented Senior Laboratory Experiment*

SUZANNE M. KRESTA, ANDREE KOENIG, MURRAY R. GRAY  
*University of Alberta • Edmonton, Alberta, Canada T6G 2G6*

While the senior design course is usually perceived as exciting and “real engineering,” the equally important data analysis process in lab courses is often seen as only a necessary hurdle to be overcome. The importance of laboratory results to the design process and to process economics may be completely missed. To bring a greater sense of reality to our senior lab course, we have introduced a fermentation kinetics lab where the students compare five sugar feeds to determine the best candidate for industrial production. Yeast growth offers several

unique advantages in this regard: the stoichiometry is unknown; the kinetics varies over the course of the batch run; and the reaction is inherently safe. Once the equipment is set up, several variations of the experiment are possible, so the assignment can be changed from year to year with very little additional investment from the department.

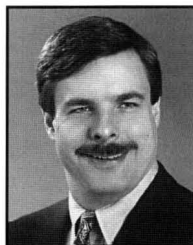
The lab organization (realistically) reduces the amount of duplicate data available to the students for comparison. The five possible feedstocks are rotated continually over the course of the term. Each group of students is assigned one feedstock for their experiment and is provided with data from four other groups for comparison. This provides a new set of data for analysis and comparison each day and encourages the students to critically examine all of the data. This “optimum choice” structure of laboratory organization could be applied equally well to comparisons of catalysts, flocculants, column packings, or other process variables.

The lab assignment requires the students to combine their knowledge of many basic areas (reaction kinetics, mass transfer, steady vs. unsteady state mass balances, process economics, statistical hypothesis testing, and linear regression) and to apply it to one set of experiments. They are required to determine the reaction stoichiometry by completing a mass balance. They use linear regression to determine the first-order rate constant and compare the feedstocks using hypothesis testing. The mass transfer coefficient in the laboratory scale fermenter is estimated, and students discuss the impact that scaleup might have on this variable. They must then select an optimum feedstock based



**Suzanne Kresta** is Associate Professor of Chemical Engineering at the University of Alberta. She obtained her BSc at the University of New Brunswick, her MSc from Leeds University, and her PhD from McMaster University. Since her arrival at the University of Alberta in 1992, she has won two teaching awards. Her research is in the areas of turbulent mixing and multiphase computational fluid dynamics.

**Murray Gray** is Professor of Chemical Engineering and Dean of Graduate Studies and Research at the University of Alberta. He holds a PhD from CalTech, an MSc from the University of Calgary, and a BASc from the University of Toronto. His research program is focused on the study of reaction kinetics, particularly for biological systems and upgrading of heavy oils.



**Andree Koenig** has been a laboratory instructor and chemist in the Department of Chemical Engineering at the University of Alberta since 1980. She has a BSc in chemistry from the Universite de Montreal.

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on cost, growth rate, and any other factors that they think may be important.

## THEORY

### Yeast Growth and Reaction Kinetics

The batch fermentation of yeast proceeds through several phases of growth: the lag phase, during which cells adapt to their new environment; a short accelerating phase; the exponential phase, during which the growth rate is at a maximum and first order kinetics can be applied; a stationary phase, which begins when one of the reactants is depleted; and the death phase.<sup>[1]</sup> During the exponential growth phase, the first-order rate constant,  $\mu$ , can be obtained by linear regression of the cell concentration versus time to fit the familiar first-order rate expression

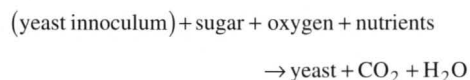
$$\ln[\text{cells}] = \mu t + C$$

While  $k$  is the usual symbol for the rate constant in chemical engineering, the use of  $\mu$  is standard biochemical terminology.

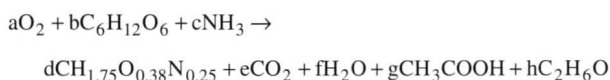
### Stoichiometry

The determination of stoichiometry for biochemical reactions with multiple products and side reactions is an area of active current research.<sup>[2]</sup> The standard biochemical approach examines the intermediate reactions involving ATP and metabolic rates. While the approach used here is approximate, it will give the same result as the more detailed analysis.

Under fully aerated conditions, the yeast production reaction is



Two common side reactions may be observed: ethanol can be produced if there is insufficient oxygen for complete oxidation of the sugar or if the sugar feed is present in significant excess, and common contaminating bacteria may produce acetic acid. The overall stoichiometric equation for a glucose feed with both side reactions is



The formula for yeast,  $\text{CH}_{1.75}\text{O}_{0.38}\text{N}_{0.25}$ , comes from an elemental analysis of yeast cells. For every 12g of carbon, there are 1.75g of hydrogen, 6.08g of oxygen, and 3.5g of nitrogen. Biochemists would not normally include water in the balance equation since its evolution is not normally measured in the lab. Note that the side reactions substantially change the reaction kinetics.

The stoichiometric coefficients  $a$  through  $h$  are determined as part of the laboratory assignment. The amount of sugar consumed, the amount of yeast produced, the amount of ethanol produced, and the amount of acetic acid neutralized can be measured. These values, in moles, are substituted into the above equation to give values for  $b$ ,  $d$ ,  $g$ , and  $h$  for a basis of one mole of primary sugar ( $b=1$ ). It is assumed that the amount of carbon present outside the primary sugar is negligible for the complex feeds (corn syrup and molasses). The remaining four stoichiometric coefficients can they be determined using atomic balances on C, O, H, and N. The yields of the three products and the amount of  $\text{CO}_2$  evolved over the course of the reaction are calculated based on the reaction stoichiometry.

### Mass Transfer

In order to quantify the aeration conditions in the lab, the  $k_L a$  (liquid phase mass transfer coefficient) is measured for the laboratory vessel. It can be approximated from experimental data by the following steady state mass balance when the supply of oxygen to the broth is equal to the rate of oxygen uptake by the cells:

$$\text{rate of oxygen transfer} = \text{rate of oxygen uptake by cells} = k_L a (c^* - c_L)$$

where  $c^*$  is the saturation concentration of oxygen in the broth and  $c_L$  is the actual dissolved oxygen concentration (DO), which is continuously monitored. The oxygen uptake rate can be determined either by combining the stoichiometric ratio with the growth rate of the yeast or from the method described by Roberts, et al.<sup>[3]</sup> Either approach gives an instantaneous value.

Various correlations are available that allow the students to compare their experimental results with the results of other investigations. The classic expression is that of Van't Riet<sup>[4]</sup>

$$k_L a = 2.6 \times 10^{-2} \left( \frac{P}{V} \right)^{0.4} (u_{gs})^{0.5} (s^{-1})$$

where  $P/V$  is in  $W/m^3$  and  $u_{gs}$  is the superficial gas velocity in  $m/s$ . Applying this correlation to the small scales used in the lab, however, may be problematic. Smith gives an excellent and perceptive review of the various approaches in his chapter of the text by Ulbrecht and Patterson.<sup>[5]</sup>

## LAB REQUIREMENTS

The objective of the analysis is to determine which feedstock is optimal by comparing yields (g of yeast/g of sugar), reaction rates, extent of side reactions, and costs. For the lab report, the students are required to determine the five rate constants using linear regression. The statistical significance of differences in the growth rates and yields is determined using statistical hypothesis testing (the student  $t$  test). The stoichiometric equation is balanced, giving yields, the oxygen uptake rate, and production of carbon dioxide. Mass transfer coefficients at various rotational speeds are compared with the results of the correlation(s).

Students are also required to provide, as part of their report, an insight that may either increase my understanding of the area, indicate a future change to the experimental procedure, or reveal a question that might be addressed in future years.

They must use a reference NOT given in the lab handout. This has stimulated many of the students to begin to think independently and to come up with more useful explanations for their results than "human error."

## EXPERIMENTAL

The fermentation is carried out in a 2.25 liter, 150-mm diameter CHEMAP batch fermenter similar to that described by Roberts, et al.<sup>[3]</sup> The vessel is equipped with pH and dissolved oxygen (DO) probes, temperature control, and air sparging. The broth is agitated with a 75-mm diameter Rushton turbine, typically rotating at 500 rpm. Filtered building air is sparged through the vessel at 2.25 liters per minute. The temperature is maintained at 30°C.

Each lab group performs the experiment on one of the five possible sugar feedstocks described in Table 1. Sucrose, glucose, fructose, molasses, and corn syrup are used in rotation. Yeast inoculant and nutrient mixes are available from

home brewing supply stores. The lag phase (about two hours long) is run before the beginning of the lab period. During the four-hour lab, the students observe the accelerating and exponential phases. The onset of the stationary phase is sometimes observed toward the end of the lab. Data are collected on the yeast concentration vs. time, and on the mass transfer characteristics of the fermenter at various rotational speeds. A copy of the yeast growth data is left in the lab, and each lab group is then given five sets of data to analyze, including their own. By rotating the feedstocks and distributing the five most recent sets of results each day, each group analyzes a different set of data.

### Yeast Growth

Two hours before the beginning of the four-hour lab period, the broth is inoculated with 2 g of the yeast *Saccaromyces bayanus*, nutrients, and 10 g of the sugar feed stock for that day. Water (non-chlorinated or distilled) makes up the balance of the 2.25 liters. The yeast concentration (based on optical density of the broth) is determined every fifteen minutes using a spectrophotometer on a 1:10 dilution of 2 ml of broth. Dissolved oxygen and impeller speed are monitored continuously and recorded every five minutes. Measurements are continued for six hours, typically until the end of the exponential growth

phase. The final sugar and ethanol concentrations are measured using bioassays (Boehringer Mannheim, Laval, Province de Quebec).

### Mass Transfer

To calculate  $k_L a$  using the traditional correlation for stirred tanks,<sup>[4]</sup> the *gas hold up* (volume of gas/volume aerated broth) is needed. To obtain this, the height of liquid in the fermenter, with and without aeration, and the diameter of the fermenter are measured.

Over the course of the four-hour lab, the rotational speed is perturbed several times for approximately five minutes. The DO is allowed to stabilize each time so that a steady state mass balance can be performed on the oxygen in the broth. The metabolism of the cells will eventually respond to a change in oxygen concentration, but this takes much longer than the time required to reach steady state and return the

**TABLE 1**  
Description of Sugar Feedstocks Used

<i>Sugar Feed</i>	<i>Primary Sugar</i>	<i>Weight % Carbon</i>	<i>Cost (\$/kg)</i>	<i>Present In</i>
Glucose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	40.0	0.96	fruits (less sweet)
Sucrose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	42.1	0.90	sugar cane, sugar beet
Fructose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	40.0	1.48	honey (very sweet)
Corn Syrup	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	30.26	0.50	
Molasses	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	31.9	0.47	cane sugar

impeller to the original speed. A range of approximately  $\pm 400$  rpm around the initial set point gives an interesting set of results to examine.

### **Troubleshooting**

- ▶ A lot of agitation is required to maintain the concentration of dissolved oxygen at the levels required by aerobic fermentation. We initially installed a 50-mm impeller, but found that the DO consistently dropped to zero before the end of the lab. We have had much better success with a 75-mm impeller. Roberts, et al.,<sup>[3]</sup> used two smaller impellers, with apparently good success.
- ▶ It is easy to contaminate yeast with bacteria that produce acetic acid. By monitoring the pH and adding known amounts of 1 N potassium hydroxide if and when the pH drops below 6.5, the amount of acid produced can be calculated. This side reaction does not appear to have any effect on the growth rate of yeast, but growth of contaminant bacteria does affect the mass balances and the overall stoichiometry.
- ▶ The  $k_L a$  results are very sensitive to the data, especially at high levels of DO.
- ▶ The students usually need some coaching on the use of linear regression and the selection of data to be included in the exponential growth phase. This can be done during the lab period if the concentration vs. time is plotted as the experiment proceeds.

### **TYPICAL RESULTS**

It is not unusual to see variability in the growth rate from one batch fermentation to another. In general, the corn syrup will give the lowest rates ( $0.07$  to  $0.1 \text{ s}^{-1}$ ). Glucose, sucrose, and fructose will give very similar results (around  $0.19 \text{ s}^{-1}$ ), although the glucose rates show more variability. Molasses proves to be the best feedstock, with the highest growth rates ( $0.25$  to  $0.40 \text{ s}^{-1}$ ) and the highest yield of yeast per gram of sugar. Since it is also the cheapest feed, the selection of the optimum feed is straightforward—provided the data and the data analysis are good. Sample student reports are available on request.

### **VARIATIONS**

This laboratory is particularly amenable to variations in the procedure. A variety of other readily available feedstocks (e.g., honey, ethanol) can be substituted for those used here. A single feed could be used at different impeller speeds to examine the impact of oxygen availability on the growth of yeast and the production of ethanol. By removing the air supply, anaerobic production of ethanol can be exam-

## **ChE letter to the editor**

Editor:

The article "ChE Applications of Elliptic Integrals" that appeared in the ChE Classroom section of the Summer, 1996, issue (Volume 30, Number 3) of *Chemical Engineering Education*, did not mention the use of these functions in problems involving simultaneous diffusion and reaction in porous catalysts. In fact, Thiele's classic paper on effectiveness factors,<sup>[1]</sup> published in 1939, predates by fourteen years the earliest reference listed under "Chemical Engineering Problems" in Table 2 of the *Chemical Engineering Education* article. Thiele found that the solution to the differential equation describing diffusion with a second-order reaction in a steady-state catalyst particle could be expressed in terms of elliptic integrals. In addition, certain problems involving multiple reactions in porous catalysts, *i.e.*, problems dealing with the effect of pore diffusion on catalyst selectivity, can be solved using elliptic integrals.<sup>[2]</sup>

#### **REFERENCES**

1. Thiele, E.W., "Relation Between Catalytic Activity and Size of Particle," *Ind. Eng. Chem.*, **31**, 916 (1939)
2. Roberts, G.W., "The Selectivity of Porous Catalysts: Parallel Reactions," *Chem. Eng. Sci.*, **27**, 1409 (1972)

Thank you for your consideration.

George W. Roberts, Professor  
North Carolina State University

ined instead of aerobic production of yeast. A third approach to the mass transfer issue is presented by Roberts, et al.<sup>[3]</sup> The report requirements could include recommendations for the scaleup of the fermenter; alternately, this could be assigned as the subject of a minor report.

#### **REFERENCES**

1. Rose, A.H., and J.S. Harrison, eds., *The Yeasts: Yeasts and the Environment*, Chapter 5, by A. Fiechter, O. Kappeli, and R. Meussdoerffer, Academic Press, Toronto, Canada (1987)
2. Shuler, Michael L., ed., *Chemical Engineering Problems in Biotechnology*, AIChE, New York, NY, p. 29 (1989)
3. Roberts, Ronnie S., et al., "The Effect of Agitation on Oxygen Mass Transfer in a Fermentor," *Chem. Eng. Ed.*, **26**, 142 (1992)
4. Bailey, James E., and David F. Ollis, *Biochemical Engineering Fundamentals*, 2nd ed., McGraw Hill, Toronto, Canada (1986)
5. Ulbrecht, J.J., and G.K. Patterson, *Mixing of Liquids by Mechanical Agitation*, Gordon and Breach Science Publishers, New York, NY, p. 191 (1985) □