A course in . . .

BIOCHEMICAL ENGINEERING

TERRY K-L. NG, JORGE F. GONZALEZ,
and WEI-SHOU HU
University of Minnesota
Minneapolis, MN 55455

THE DEPARTMENT OF Chemical Engineering and Materials Science at the University of Minnesota has developed a series of courses on biochemical engineering for its senior undergraduate and first-year graduate students. The series includes three lecture courses, which are offered sequentially, and one laboratory course. The lecture courses are entitled Stoichiometry, Energetics and Kinetics of Biological Systems; Biochemical Processing Technology; and Bioseparations. The first course deals with engineering aspects of cellular processes and includes an introduction to the kinetics and mathematical modeling of growth and product formation. The processing technology course covers the reactor aspects of biochemical engineering; topics include kinetics and mass transfer in bioreactors, medium and air sterilization, and enzyme-catalyzed bioreactors. The bioseparations course deals with the unit operations used in the four stages of separation of biomolecules: solids removal, isolation, purification and polishing.

In the Biochemical Engineering Laboratory course, students perform experiments to obtain data for the design of a continuous sterilizer and to compare oxygen uptake rates of yeast cells in free suspension and immobilized in agar beads. They also perform a fermentation experiment in which they use a computer-coupled fermentor to gather kinetic data and to determine the program for feeding rate-limiting nutrient. These four courses give chemical engineering students a relatively complete background in biochemical engineering and also prepare them for meeting challenges in the bioprocessing industries. This communication will discuss the organization and the content of the laboratory course.

ORGANIZATION OF THE COURSE

Typically, a class of fifteen to twenty students is divided into groups of three or four. Each group chooses a leader who is responsible for the coordination and planning of an experiment. The group leader position rotates with each new experiment. Before each experiment, the instructor gives a one-hour lecture on the principles, instrumentation, and methods of chemical analysis needed to carry out the experiment.

The time period required to carry out the experiment varies with different projects. Considerably longer periods (as long as a few days) are required for

Terry K. L. Ng is a PhD student in the Department of Chemical Engineering and Materials Science, University of Minnesota. He received his BS degree from Columbia University. His research interests are liquid-liquid two-phase cultures, oxygenation of mammalian cell cultures with perfluorocarbon liquids, and mass spectrometry of fermentor off gases. (**L**)

Jorge F. Gonzalez is a PhD student in the Department of Chemical Engineering and Materials Science at the University of Minnesota. He has a degree in chemical engineering from the National University of Mar del Plata, Republica Argentina. Prior to being admitted to Minnesota, he did research on wastewater treatment at fisheries. His PhD thesis is a kinetic study of biodegradation of pentachlorophenol in contaminated soil. (**C**)

Wei-Shou Hu is an assistant professor in the Department of Chemical Engineering and Materials Science at the University of Minnesota. He received his PhD in biochemical engineering from Massachusetts Institute of Technology in 1983. (**R**)

the batch fermentation experiment, which is the last experiment in this course. Laboratory hours for this experiment are arranged individually with the teaching assistant to ensure close supervision.

Teaching assistants play an important role in this course. They supervise students on the operation of instruments and equipment and ensure that safety procedures are being followed in the laboratory. Teaching assistants also prepare inocula and sterilize laboratory glassware when sterile operation is needed.

EXPERIMENTS

1. Aseptic techniques

The first experiment is an introduction to sterile techniques during which students practice the aseptic handling of microorganisms. Two strains of *Escherichia Coli* (*E. Coli*) C600 $r^{-}n^{+}$ are used; one harbors the plasmid PDU 1003 which encodes resistance to antibiotic tetracycline, and the other does not. Students prepare two sets of nutrient agar plates; one contains tetracycline (10 μg/ml), and the other does not. Students are given cell suspensions of the two *E. coli* strains and are asked to identify each of them and to determine their cell concentrations. In this experiment, students are exposed to the concept of selective pressure, the principle of gene amplification which is used in modern molecular biology and the new biotechnology industry. This experiment takes a two-hour session.

2. Dissolve oxygen concentration measurement

The second experiment is the construction of a galvanic dissolved oxygen (D.O.) electrode [1, 2] and the measurement of dissolved oxygen concentration. The galvanic electrodes consist of a silver cathode and a lead anode. These electrodes are also to be used in subsequent experiments. The construction of the electrode is completed in the first of the two sessions (total of six hours) assigned to this project.

The overall reactions are:

silver cathode :
$$\frac{1}{2}O_2 + H_2O + 2e^- \rightarrow 2OH^- \quad (1)$$

lead anode :
$$Pb \rightarrow Pb^{++} + 2e^- \quad (2)$$

overall reaction :
$$\frac{1}{2}O_2 + Pb + H_2O \rightarrow Pb(OH)_2 \quad (3)$$

The first course deals with engineering aspects of cellular processes and includes an introduction to the kinetics and mathematical modeling of growth and product formation. The processing technology course covers the reactor aspects of biochemical engineering.

The current generated by the reaction is measured by a microameter. If the resistance to transfer of oxygen from the bulk liquid to the silver cathode resides primarily in the membrane, the output of the electrode at steady state can be described by

$$I = nFA\frac{p_m}{b}C_1 \quad (4)$$

The use of the electrode requires proper calibration. In the range of dissolved oxygen concentrations to be used in the experiment, the output is proportional to the dissolved oxygen concentration. A two-point calibration is usually used. First, the response of the probe in the solution in which the dissolved oxgen is in equilibrium with air at ambient pressure is recorded as the 100% level. The second point is one with all the dissolved oxygen depleted either by the addition of 1.0 M of sodium sulfite (with a trace amount of Cu^{+2} ($\sim 10^{-3}$ M) as a catalyst) or by sparging the fluid with nitrogen gas.

Subsequently students measure the response of the electrode to a step change of dissolved oxygen from depletion to saturation with air. The transient output of the probe can be expressed as an infinite series [3] as

$$I = nFA\frac{p_m}{b}C_1\left\{1 + 2\sum_{n=1}^{\infty}(-1)^n \exp(-n^2 kt)\right\} \quad (5)$$

where $$k = \frac{\Pi^2 D_m}{b^2}$$

The experiment is to be carried out twice; (i) using a magnetic stirrer to stir the oxygen-saturated water in the flask, and (ii) no stirring. After obtaining the response curve, students are asked to examine if the response can be estimated by Eq. (5) and to determine the time constant, k.

3. Oxygen uptake rate of yeast cells in suspension and immobilized in agar gel

A schematic diagram of the set-up for the oxygen

uptake rate measurements, the third experiment in this course, is shown in Figure 1. The device for oxygen uptake measurement is a 250 cm^3 Erlenmeyer flask with a tightly sealed rubber stopper. A dissolved oxygen electrode, previously prepared by the students themselves, is inserted through the stopper to the flask. During the experiment, the flask is placed in a constant temperature water bath. Care should be taken to ensure that the stopper of the flask is tightly sealed and that no gas bubble enters the flask during the experiment. The cell suspension inside the flask is stirred by a magnetic stirrer. Prior to the experiment, the D.O. electrode is calibrated under the experimental conditions to be used. The analogue output of the dissolved oxygen electrode is converted to digital signals and stored in an IBM personal computer.

The students are provided with a suspension of *Saccharomyces cerevisiae* that were growing exponentially in complex medium. The optical density of the culture broth is measured to determine the cell concentration. The suspension is sparged with air to bring the D.O. to a higher concentration and is transferred into the measurement flask, overfilling it slightly. The stopper, along with the D.O. electrode, is quickly inserted and the flask is sealed, avoiding entrapment of air bubbles.

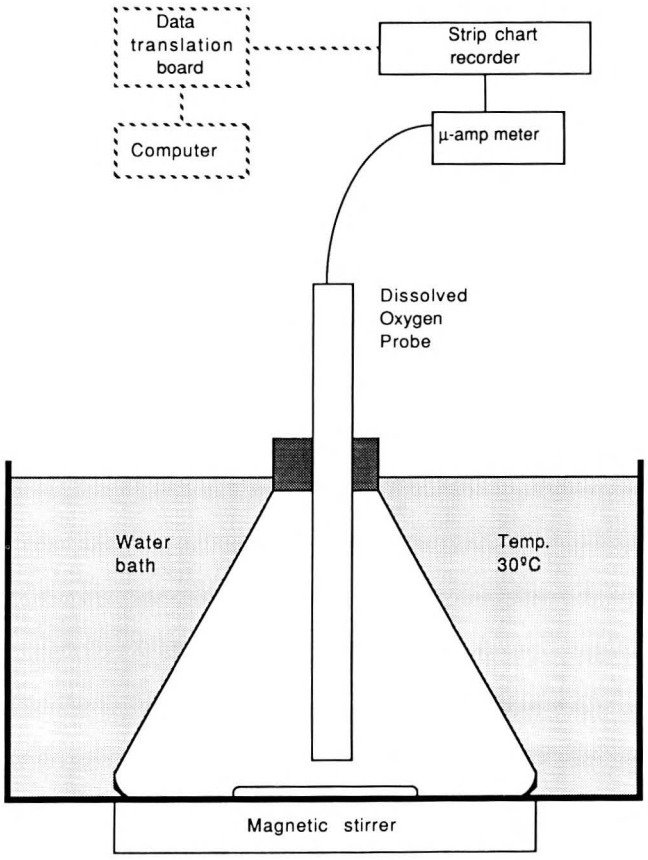

FIGURE 1. Experimental set-up for the oxygen uptake rate measurement

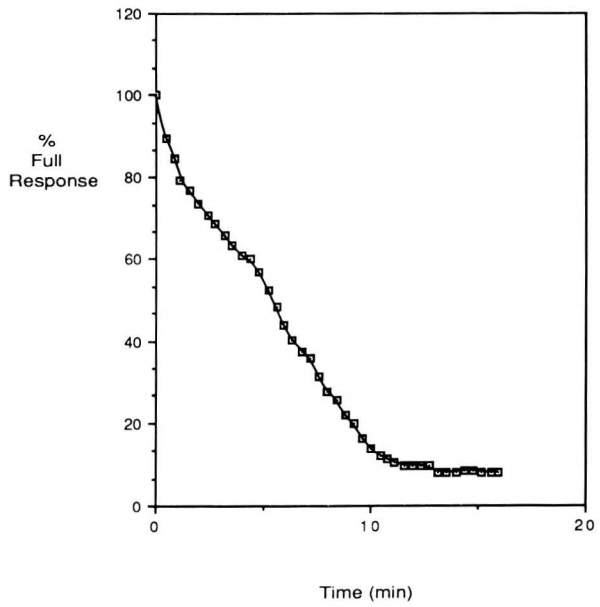

FIGURE 2. Changes of dissolved oxygen concentration during the oxygen uptake rate measurement using suspension of yeast cells

The dissolved oxygen electrode constructed by the students typically has a 90% response time (the time period in which the output of the electrode reaches 90% of the new steady state value after a step change in dissolved oxygen from 0% to 100% saturation with air) ranging from one minute to a few minutes. In the measurement of the oxygen uptake rate it is necessary to ensure that the rate measured is not limited by the electrode response time. This is achieved by measuring the oxygen consumption using cell suspensions of different cell concentration. The proper experimental condition is in the range bounded by (i) the oxygen consumption rate of the suspension being proportional to the cell concentration, and (ii) the total time span needed to acquire an accurate measurement of the oxygen uptake rate being short relative to the doubling time of the cells under the conditions used. The second constraint is needed so that cell concentration can be assumed to be constant. A typical D.O. concentration profile from this experiment is shown in Figure 2. Except for the initial few data points and the period in which the oxygen concentration is very low, the rate of decrease of oxygen is constant. The specific oxygen consumption is then

$$q = (\Delta C/\Delta t)/x \qquad (6)$$

The linear range in the dissolved oxygen concentration curve is used to calculate the oxygen consumption rate. The deviation from linearity at the beginning is due to the switch of the D.O. electrode from a solution in which the electrode is previously submerged to the cell suspension. The consumption of oxygen by yeast cells follows Michaelis Menten kinetics; thus the rate is zero order with respect to the dissolved oxygen concentration only at concentrations above a certain level. The decrease in the oxygen consumption rate at the end of the measurement (longer than ten minutes, as shown in Figure 3) is most likely due to the intrinsic kinetic behavior of yeast cells.

To prepare the immobilized cell system, yeast cells are harvested by centrifugation and subsequently resuspended in a smaller volume of the growth medium. The cell suspension is then mixed with an equal volume of 4% agar solution which has been maintained just above its solidifying temperature (~40°C). This agar-cell suspension is quickly poured into a Petri dish and allowed to solidify. The volume of agar added to each Petri dish is adjusted to give rise to a gel thickness of 2.4 mm. The agar gel disk is then removed from the Petri dish and gently pressed against a screen with an opening of 2.4 mm. The almost cubic agar particles so formed are collected and poured into the oxygen uptake rate measurement flask. The flask is subsequently filled with growth medium and the dissolved oxygen is measured and recorded in the computer. The experiment is repeated with agar cubes containing different concentrations of immobilized cells.

Students are instructed to analyze the mass transfer processes in the immobilized cell system. The dissolved oxygen concentration at the interface of agar beads and liquid is assumed to be the same as that in the bulk liquid. At a high cell concentration and, thus, a high reaction rate in the agar gel, the intraparticle diffusion of oxygen can be limiting. The cell concentrations used in the agar gel are selected to allow students to observe cases of both oxygen transfer limitation and no limitation. Furthermore, students are asked to compare the experimental results to the theoretical analysis using effectiveness factor (η) for substrate utilization with Michaelis-Menten kinetics [6]. The observable modulus Φ is defined as

$$\Phi = \frac{qX_b}{D_{es} C_o} \left(\frac{V_p}{A_p} \right) \qquad (7)$$

In Equation 7, q is obtained from the measurement using free cells in suspension. The diffusion of oxygen in agarose gel needed for the theoretical analysis is obtained from literature [7].

4. Continuous sterilization

The fourth project is the continuous sterilization of *Escherichia coli* cell suspensions. The continuous sterilizer consists of a cell suspension reservoir, a peristaltic pump, and a piece of silicone tubing connecting the reservoir to a four-foot long coiled copper tubing submerged in a constant temperature water bath (Figure 3). The cell suspension stream from the sterilizer is collected in flasks submerged in an ice bath. A three-way valve is installed before the collection flask to allow for rapid switch from one flask to another so that samples from various time points can be taken easily.

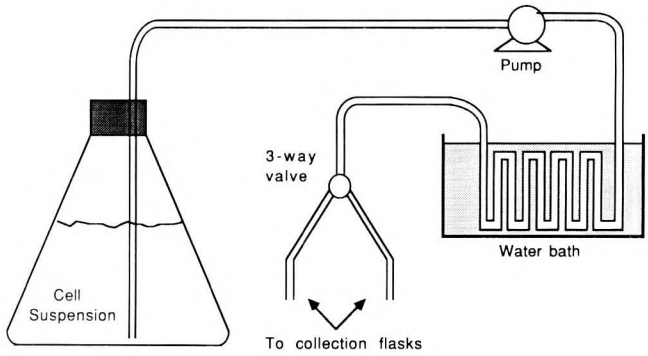

FIGURE 3. Scheme of the apparatus for continuous sterilization

In the first session of this experiment, students determine the thermal death rate constant of the cells. Three water baths are set up at 50°, 60° and 65°C respectively. A series of test tubes containing buffer solution are prewarmed in each water bath. To begin the experiment, small aliquots of cell suspension are added to the test tubes so that the sterilization temperature is reached almost instantaneously. At different time intervals tubes are withdrawn from the water bath and the contents are transferred to bottles containing chilled dilution solution for viable cell count. From the viable count of cells the death rate constant at the three temperatures are determined:

$$\frac{dN}{dt} = -K(T)N \qquad (8)$$

Arrhenius plot is then prepared to estimate the death rate constants as a function of temperature.

The temperature for the continuous sterilization is 65°C. However, with the system employed for this

experiment, the temperature rising period is a significant fraction of the holding time in the sterilizer. Thus, both the heating region and the temperature holding region are important in the killing of bacteria. Students calculate the temperature profile in the sterilizer for a number of flow rates. The heat transfer coefficient of the coil is obtained from the reported value for the same material in literature. Students are also instructed to assume a plug flow behavior for fluid flow inside the sterilizer. Their assignments involve determining sterilization flow rates required to achieve two different degrees of killing (N/N_o) [4, 5, 6] and carrying out the processes.

5. Cultivation of microorganisms in a stirred tank

The last project is a fermentation experiment which is designed to expose students to the tasks involved in fermentation operations. The tasks they carry out include setting up a 2 l or 16 l fermentor and auxiliary systems, preparation of inocula, sterilization of vessel and medium, aseptic inoculation, sampling, data acquisition and analysis. The specifics of the fermentation experiments carried out vary from year to year. Among them is the classical yeast fermentation of glucose. Students are asked to study the production of ethanol and its further oxidation to carbon dioxide and water during different stages of the batch culture. Another experiment is the fed-batch cultivation of *Acinetobacter calcoaceticus* ATCC 31012 using ethanol as the carbon and energy source. In this case a sufficiently high ethanol concentration in the bioreactor is necessary to sustain an optimal growth rate; however, it will inhibit growth if it is allowed to exceed an upper limit. In this experiment, programmed feeding of ethanol is carried out during the cultivation to control ethanol concentration in the tolerable range. Without such a feeding scheme, cell growth ceases after ethanol initially present in the bioreactor is depleted. Students are given kinetic data obtained from a batch culture without programmed feeding. From the data, they determine the specific growth rate and specific ethanol consumption rate or the yield coefficients. The kinetic parameters are used in the growth model to calculate the feeding rate. Students input the feeding rate as a function of process time into the microprocessor. The execution of the feeding is carried out by a microprocessor controlled pump. The temperature, pH, and dissolved oxygen concentration are controlled by simple feedback loops. The oxygen consumption rate, determined by the analysis of off-gas by mass spectrometer, can be used to estimate the specific growth rate, and such information can be used to adjust the feeding rate of ethanol on-line. However, because of the extensive program development needed to implement such on-line adjustment, any adjustment of feeding rate is implemented by the students but not by on-line computer. During the fermentation, samples are withdrawn periodically, and the cell concentration is measured by a colorimeter. A portion of the samples is frozen for the measurement of ethanol concentration by gas chromatography. The experimental results are compared to the prediction.

CONCLUDING REMARKS

One achievement of this laboratory course is the demonstration to our undergraduate students that chemical engineering principles do apply to systems involving living microorganisms. Probably equally important is for the students to realize that the system they deal with is never as simple as it is represented in the textbook. However, it is the simplification or idealization of the complex biological systems that allows us to apply the chemical engineering principles to systematically analyze these systems. In the sterilization experiment, they quickly realize that the thermal death rate constant of microbial cells is affected by many factors, such as growth medium, pH, and culture stage, in addition to temperature. It only takes a few hours into the fermentation experiments for the students to discover that the yield coefficient is not constant in a batch culture, as it is frequently assumed to be in most mathematical growth models. One of the student groups noted in its report: "The overall experiment gave us a very good opportunity to apply knowledge gained in the previous courses of the Biochemical Engineering series, and most importantly to realize that things in the lab are much less ideal than presented by theory!"

Footnote: The student manual, which includes step-by-step instructions for each experiment, is available by writing to W-S. Hu.

NOMENCLATURE

A = area of silver cathode
Δp = area of agar particles
b = membrane thickness
C_l = concentration of O_2 in the bulk liquid
C_0 = oxygen concentration in the bulk of medium in Eq. 7
ΔC = difference in oxygen concentration
D_{es} = oxygen diffusivity in agar particles

D_m = oxygen diffusivity through the membrane
F = Faraday's constant
I = current
k = electrode time constant
K = thermal death rate constant
n = number of electrons
N = number of viable cells
p_m = permeability coefficient of the membrane
q = specific oxygen consumption rate
t = time
T = temperature
Δt = time elapsed between oxygen concentration measurements
V_p = volume of agar particles
x = cell concentration being used in the experiment
X_b = cell concentration in agar particles

REFERENCES

1. Johnson, M. J., J. Borkowsky, and C. Engblom,"Steam Sterilizable Probes for Dissolved Oxygen Measurement," *Biotechnol. Bioen.* **6**:457-468, 1964
2. Borkowsky, J. D., and M. J. Johnson, "Long-Lived Steam Sterilizable Membrane Probes for Dissolved Oxygen Measurement," *Biotechnol. Bioeng.* **9**:635-639, 1967
3. Lee, Y. H., and G. T. Tsao, "Dissolved Oxygen Electrodes," *Adv. Biochem. Eng.* **13**:35-86, ed. by T. K. Ghose, A. Fiechter, and N. Blakebrough. Springer Verlag, Berlin, 1979
4. Wang, D. I. C., *et al.*, Chapter 8, *Fermentation and Enzyme Technology*, J. Wiley & Sons, New York, 1979
5. Aiba, S., A. E. Humphrey, and N. F. Millis, *Biochemical Engineering*, 2nd Ed., Academic Press, New York, 1973
6. Bailey, J., and D. Ollis, Chapters 4 and 8, *Biochemical Engineering Fundamentals*, 2nd Ed., McGraw-Hill, 1986
7. An-Lac Nguyen and J. H. T. Luong, "Diffusion in κ-Carrageenan Gel Beads," *Biotechnol. Bioeng.* **28**:1261-1267, 1986 ❑

In memoriam . . .

ROBERT L. PIGFORD
1917-1988

Professor Robert L. Pigford died on August 4th after suffering a stroke on May 14th from which he never recovered. He was 71 years old and a long-time resident of Newark, Delaware.

He was born and raised in Meridian, Mississippi. He earned his BS degree in chemical engineering from Mississippi State College in 1938, his MS and PhD degrees from the University of Illinois. His next six years were spent in the Engineering Research Laboratory at the DuPont Experimental Station, working on both civilian and military research problems, the latter arising from World War II. With his industrial colleagues, he participated in what was to become one of the national centers for a renaissance in engineering education, in which the group replaced approximate analyses guided by experiment with careful, quantitative models of the chemical and physical processes being considered. Dr. Pigford's association with the University of Delaware began shortly after his arrival in Delaware when he began organizing these new analyses into evening and week-end courses for chemical engineering students on the campus. One result of this activity was a textbook, *Application of Differential Equations to Chemical Engineering Problems*, which he coauthored with the late W. R. Marshall. In 1947 Allan Colburn prevailed upon Bob Pigford to come to the University on a full-time basis as chairman of the fledgling department of chemical engineering. His association with the University of Delaware spanned more than thirty years. From 1966 to 1975 he served on the faculty at the University of California, Berkeley.

He was one of the earliest proponents of the use of computers in engineering and built several for both instruction and research before the widespread availability of such machines. His colleagues remember the numerous hurdles he had to overcome to convince conservative administrators of the need for these expensive new tools of science and technology.

His advice was sought by numerous industrial, academic and governmental institutions. He served as a member of the U.S. Army's Advisory Council, the Scientific Advisory Board of the U.S. Air Force, the Department of Energy and the National Research Council, as well as being a member of the Advisory Committees for Chemical Engineering at Princeton University and Massachusetts Institute of Technology. He received virtually all the national awards of the American Institute of Chemical Engineers and served as a Director of that organization from 1963 to 1966. In 1983, on the occasion of that organization's 75th anniversary, he was named as one of thirty pre-eminent leaders of his profession. He was elected to the National Academy of Engineering in 1971 and to the National Academy of Sciences in 1972. In 1977, the University of Delaware named him as its first Alison Scholar, and in 1983 he was appointed to the University's Board of Trustees.

In addition to serving on numerous editorial advisory boards, he served as editor of the American Chemical Society Journal *Industrial and Engineering Chemistry Fundamentals* for a full quarter century. The Delaware Association of Professional Engineers named him Engineer-of-the-Year in 1988.

Professor Pigford married Marian Pinkston in 1939. Their daughter, Nancy, is a resident of Philadelphia and their son, Robert, lives in Newark, Delaware. There are three grandsons.

Arthur Metzner, Marian Pigford