

**DEVELOPMENT of an INTRODUCTORY
LABORATORY COURSE in
BIOCHEMICAL ENGINEERING**

by

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Abstract

A one-semester undergraduate laboratory course in biochemical engineering has been developed at The University of Maryland College Park Campus. Experiments covering most of the basic and commonly used laboratory techniques in enzyme and microbial technology are introduced at the rate of one per week. Enzymatic experiments conducted during the first half semester include demonstration of common commercial and household usage, enzyme kinetics, purification, and immobilization. Microbial experiments during the second half semester include cell fractionation and fermentation kinetics in different operation modes. In addition to aseptic techniques, topics on computer data acquisition and control are also covered. The course is concluded with individual laboratory projects. The criteria used for the selection and design of the experiments are discussed.

Introduction

The Introductory Biochemical Engineering Laboratory (ENCH485) is a three-credit, one-semester senior technical elective offered by the Chemical Engineering Program at The University of Maryland. It is generally taken after one has completed the lecture course of Introductory Biochemical Engineering (ENCH482). Approximately 30 students out of a senior class of 50 enroll for the lecture course at College Park, and about half of these students advance to the laboratory course. This laboratory also represents an ideal training opportunity for first year graduate students who plan to pursue their research in the area of biochemical engineering and enzyme/fermentation technology. Partially because only in recent years have we witnessed the rapid expansion of the field of biochemical engineering, only a handful of departments in this country offer a laboratory course in biochemical engineering, and we are very proud to be one of them.

ENCH485 is designed to introduce chemical engineering students to a wide spectrum of fundamental biochemical engineering concepts, with an emphasis on quantitative engineering analysis. Along with each experiment, students are presented with not only biochemical engineering concepts but also basic, essential laboratory techniques. In overview, the first half of the semester is devoted to cell-free enzymatic processes; the topics include enzyme kinetics, production, purification, and immobilization. The second half focuses on microbial processes; the objective is to expose students to aseptic techniques, cell fractionation, fermentation kinetics, sensor automation, and on-line computer data acquisition/control. Finally, considerable time

is allocated to individual projects. Numerous practical applications are interjected to stimulate the students' interest. Experiments in enzyme and microbial technology are introduced at the rate of one per week. During the fifteen-week semester of Spring 1988, experiments are conducted according to the following schedule:

- Week 1. Cheese Production from Milk
- Week 2. Enzymes in Laundry Detergents / Wine Fermentation
- Week 3. Digestion of Protein into Amino Acids
- Week 4. Cellulose Degradation to Glucose
- Week 5. Starch Degradation to Dextrin and Glucose
- Week 6. Enzyme/Protein Purification
- Week 7. Enzyme Immobilization
- Week 8. Yogurt Production and Aseptic Culture Techniques
- Week 9. Batch Submerged Fermentation of Yeast in Shaker Flasks
- Week 10. On-Line Monitoring of Beer Fermentation
- Week 11. Immobilized Cell Bioreactor
- Week 12. Individual Lab Projects
- Week 13. Individual Lab Projects
- Week 14. Individual Lab Projects
- Week 15. Presentation of Lab Projects

The syllabus is by no means rigid; students are encouraged to suggest ideas for experiments so that the class's interests may also be incorporated. In response to popular demand, as an example that comes as no surprise to the author, a scheduled experiment on the continuous fermentation of phenol with cell recycle has been replaced by beer fermentation this year.

As part of our ongoing emphasis on implementing computers in chemical engineering courses, microcomputers are utilized to the fullest extent at different levels. For instance, with few exceptions, all required weekly experimental reports are generated by computers. The students also have built hardware interface and written appropriate programs to carry out on-line data acquisition, analysis, and control of various fermentation experiments. Due to the inherent process time constants, some fermentation experiments last many days. In these cases, the responsibilities must be divided among each team member, and the entire effort must be coordinated by a designated project manager.



A wide range of experiments are covered in this course, reflecting the versatile applications of biotechnology. Many of the experiments are original and are being offered to the students for the first time. More importantly, they cover the fundamental laboratory techniques involved in modern biotechnology research. The students find these experiments exciting and challenging, are highly motivated, and seem to enjoy the course immensely. The author firmly believes that the current students are learning a great deal more about biochemical engineering than those in the previous years who have not been taught systematically. Thus far, the author has received overwhelmingly positive feedback from the students.

Course Description

Prerequisite

Satisfactory performance of the laboratory course work in biochemical engineering requires that one has had previous experience in freshman chemistry or physics laboratories. In addition, familiarity in biochemical engineering at the introductory level is assumed. The student should have taken or is concurrently enrolled in an introductory undergraduate course in biochemical engineering. Because this is a senior elective course offered by the department, the student should be able to apply meaningfully what one has learned from the previous fundamental courses in transport, kinetics, thermodynamics, and engineering mathematics.

Course Format

Immediately preceding each experiment, there is a lecture ranging between 10 to 45 minutes, depending on the subject. This discussion briefly covers the background principles, cautions avoidable pitfalls, answers any questions that may arise, and demonstrates techniques that have not been explicitly discussed in the instruction manual. It is the teaching staff's responsibility to perform the experimental procedure in its entirety at least once before the class to ensure that all the necessary equipment and supplies are available for the smooth execution of each experiment. This is especially important because, unlike other chemicals, enzymes may not be active and cultures may not be viable. Conducting a trial run is the key to the success of a laboratory course because there are always many components that can be overlooked, even when one has performed it during previous years.

As with any other laboratory course, it is imperative that students likewise prepare for each experiment beforehand. Each student is to read not only the instructions but also comprehend all the procedures and concepts. This is to be achieved by requiring that the objectives and procedures for that experiment be clearly stated in a bound laboratory notebook in one's own words prior to coming to the class. Because various chemicals and equipment can be potentially dangerous if used incorrectly, admission to the laboratory may be denied to those students who are uncertain of the procedures and precautions for any particular experiment. Since the course is tightly scheduled, sufficient time is not available to repeat the experiment should it fail due to an individual's lack of preparation.

Because this course is offered in a chemical engineering program, analytical aspects of the experiment as well as

the conceptual aspect are emphasized. The thorough analytical treatment of the data ensures that the engineering aspect is preserved; whereas, the questions for discussion at the end of each experiment are designed to enhance the conceptual understanding of the underlying principles of the subject. Students must master the laboratory techniques to yield acceptable results, and the correct analytical methods must be applied to reduce the raw laboratory data to a set of more meaningful parameters. Above all, students must understand, not merely memorize, the guiding principles. Consequently, they are allowed to deviate from some of the procedures. If better procedures can be found, let them be followed. Healthy collaborative effort among fellow classmates and creativity in each individual are both highly encouraged.

Accordingly, except for the 2 or 3 short 10-minute quizzes on laboratory techniques, there are no formal examinations. Grades are partially based on the pre-experiment handwritten entries in the laboratory notebook and the post-experiment typewritten reports, including answers to questions for discussion. In addition, the individual project at the end of the semester counts heavily toward the final grade. The individual project is evaluated based on both the quality of the proposal submitted before the experiment is conducted and the experimental results presented in the final report.

Course Schedule

Week 1. Besides demonstrating the use of rennet in casein coagulation from milk and studying the effect of pH in cheese making, the first week is allocated to familiarize students with the use of general equipment such as a spectrophotometer and to refresh general laboratory techniques such as pipetting.

Because a typewritten report is required for each experiment, every student is to use a text editor or word processor for its preparation. An introduction to the use of the operating system, text editors, and plotting routines on departmental personal computers is presented in addition to the regularly scheduled laboratory period during this week to aid those few students who have not yet mastered the techniques of computer-assisted document preparation.

Week 2. Small pieces of fabric stained with two types of dyed proteins are subjected to bacterial and fungal proteases and a range of household laundry detergents. The blue dye attached to the protein is released into the solution as the protein is hydrolyzed. The hydrolysis rate is measured by monitoring the color intensity of the washing liquid with a spectrophotometer, and the effectiveness of each detergent is evaluated by inspecting the cleaned fabric. The objective is to demonstrate the common use of enzymes around us, which few students are aware of.

Week 3. This is the second experiment in the enzymatic hydrolysis of proteins. The amino acid concentration is analyzed with the ninhydrin colorimetric method. The emphasis is on the competent quantitative use of spectrophotometers with the generation of the absorbance spectra and calibration curves. In addition, kinetic rate constants for enzymatically catalyzed reactions are obtained.

Week 4. A comparison of enzymatic and acid hydrolysis of cellulose is made. The effect of cellulose source (paper, CMC, wood chip, cotton, etc.) and temperature



are studied with cellulase and hydrochloric/sulfuric acid by measuring the glucose concentration. The existence of alternative competing processes is emphasized, and students are asked to evaluate economically the relative merit of each degradation process.

Week 5. The reaction kinetics of starch degradation to dextrin and glucose, another industrially important process, is studied as a function of enzyme and inhibitor concentrations by measuring the product (glucose) concentration with the dinitrosalicylic colorimetric method and the starch concentration with the iodine method. The enzyme used originated from a variety of sources: bacterial amylase, fungal amylase, amyloglucosidase, saliva, etc. Furthermore, the specificity of an enzyme is demonstrated by subjecting starch to cellulase and contrasting the results from the previous week's. Temperature and pH optima are identified for each enzyme. Enzyme stabilization with calcium ions is studied by subjecting enzymes to different intervals of heat treatment at 90°C. Because a large number of samples must be analyzed, collaborative class effort is necessary in this experiment.

Week 6. Proteins and enzymes are recovered from a solution by

- a) Salt (ammonium sulfate) precipitation,
- b) Acetone precipitation, and
- c) Isoelectric precipitation.

The percent saturation at which the protein begins to precipitate is first determined for a known protein solution. Fractionation is then performed on a protein mixture to recover individual components. Percent recovery is reported.

Week 7.

The effectiveness of enzyme immobilization by gel entrapment are evaluated using three commonly used media:

- a) Polyacrylamide
- b) Calcium alginate
- c) Gelatin

Amylase activities before and after immobilization are measured. Shifts in the optimum operating conditions as well as the increased resistance to adverse pH and temperature conditions are studied. Furthermore, enzyme leakage is monitored over a period of one week.

Week 8. As a demonstration on the use of microorganisms in food processing, yogurt fermentation is carried out with *Lactobacillus* cultures in milk. The proper steam autoclave operating procedures, microscope usage, nutrient formulation, Petri dish preparation, plate streaking, and other basic aseptic microbiological culture techniques are taught. To emphasize the necessity to exercise aseptic techniques and to dramatize the fact that microorganisms are present everywhere, Petri dishes are briefly exposed to various outside environmental conditions, followed by 48-hour incubation. The proliferation of a large number microbial colonies is inevitable in every case, and colonial morphological characteristics are further examined based on the resulting profusion of contaminants on these plates.

Week 9. Microscopic techniques practiced are cell differentiation by gram's stain and negative staining. addition to the direct counting of cell numbers under a microscope with a hemocytometer, the measurement of cell biomass concentration is carried out by a) dry/wet weight, b) optical density, and c) plate count with successive dilutions. Lastly, aseptic nutrient preparation and inoculation are taught. During the week, the batch submerged fermentation of yeast is carried out in a traditional manner using shaker flasks. The batch growth kinetics are studied with a) glucose, b) ethanol, c) sucrose, and d) mixed substrate of glucose and sucrose. Samples are taken at three-hour intervals, 24 hours a day, until the stationary phase is reached.

Week 10. After an exhausting week of sampling and analysis, the students can truly appreciate the conveniences of on-line computer data acquisition. A/D and D/A conversions, hardware interfacing, and automation are covered. Carbon dioxide evolution rate, specific gravity, cell density, and sugar concentration are each measured continuously with a separate spectrophotometer for the duration of the primary beer fermentation (about a week). In addition, ethanol concentration is monitored with expanded Teflon tubing coupled to a GC's FID; the pH is also measured and controlled. Students are asked to identify the most useful measurement to monitor the beer fermentation if only one is to be chosen. The product is consumed at a later date to compare and contrast with commercial brands.

Week 11. The industrially important process of glucose to ethanol conversion by entrapped yeast cells in a continuous bioreactor is investigated. Other cell immobilization techniques are also discussed.

Weeks 12 - 14. (Individual Project)

The last three weeks are devoted to special individual projects where each student proposes and executes one's own experimental topic and plan. It is not to be unrealistically ambitious, nor should it be trivial. Each student is required to consult with the instructor in planning the experiment to ensure the availability of the necessary reagents and instruments. A typewritten proposal is due at the middle of the semester; this allows the instructor ample time for the procurement of necessary reagents or for the arrangement of required equipment. The objectives must be clearly stated, and the procedures are iterated until the plan is judged feasible. Like the instruction manual, each proposal must contain well defined objectives, clearly stated methods of attack, relevant background information, and suitable references. Otherwise, it is rejected. The experiment itself should be similar in difficulty to those performed in class but with a slightly more ambitious goal. Because students must execute the experimental part of the individual project within a two-week period, the choice of suitable objectives and carefully planned steps are extremely critical in determining the eventual success. The semester is concluded with a short oral presentation of the individual projects while the audience samples the product from the wine fermentation experiment initiated at the beginning of the semester.

This part of the course is considered to be the most enjoyable to both the students and the author. To ensure



the proper planning, the schedule outlined below should be closely followed:

- Week 8. Initial project proposal due
- Week 9. Initial project proposal returned for modifications
- Week 10. Final project proposal due
- Week 11. Final project proposal returned with comments
- Week 12. Laboratory phase, equipment setup
- Week 13. Laboratory phase, data acquisition
- Week 14. Laboratory phase, data analysis
- Week 15. Final project report due and presentation

Description of the Ongoing Effort

In recent years, biotechnology programs are increasingly being emphasized nationally, and The University of Maryland has also identified the field of biotechnology and biochemical engineering as one of the thrust areas of excellence. As the biotechnology industry matures, the demand for qualified, trained graduates will increase. Furthermore, because biotechnology is fast evolving into a major technological force in the U.S., the development of a comprehensive introductory laboratory course in biochemical engineering is essential to expose our general undergraduate students to such important concepts. For many years, such an introductory biochemical engineering laboratory course has been offered by the Chemical and Nuclear Engineering Department.

Despite the State of Maryland's enthused emphasis on biotechnology, our biochemical engineering laboratory has been traditionally ill equipped due to the high cost of equipment, and that fact has severely limited the type of experiment one can offer to students. In certain recent past semesters, as few as only one experiment was introduced to the students during the entire semester in the form of a demonstration performed by a graduate teaching assistant, with the rest of the time devoted to computer simulation. The author was given the opportunity to instruct the course for the first time in Spring 1987. Within the limited resources, a range of inexpensive experiments that are both interesting and educational have been designed and subjected to preliminary testings and quickly adopted in class. The course is aimed at enhancing hands-on biotechnology training that the author wishes he himself could have been exposed to as a student.

This biochemical engineering laboratory has been further developed systematically during the past year. Extensive effort has been spent designing each experiment because there is currently no established textbook available anywhere for a laboratory course in biochemical engineering, although there are lab manuals for microbiology and biochemistry that can be used to provide part of the basic techniques. As a result, a 250+ page laboratory manual has been prepared for the experiments listed previously. A biochemical engineering laboratory text based on this manual is planned after further revisions as each course offering in the coming years brings improvement and refinement. Unworkable experiments will be eliminated, and those that are of more interest to the students will be expanded. Many additional experiments suitable for an undergraduate course are being tested and the corresponding instructions are in

the process of being written. The author's objective is to cover various fundamental aspects of biochemical engineering, from the food/nutritional science to the highly delicate recombinant DNA technology.

Selection and Design Criteria

The experiments are selected and designed subject to the following limitations, including budgetary constraints:

- * Reagents must be inexpensive and relatively nontoxic.
- * They can be completed in a short time period (3 hours per lab).
- * The topics are chosen to cover the basic fundamentals of biochemical engineering.
- * They are stimulating, interesting, and thought-invoking.
- * Minimum preparation is required.
- * They should yield definite quantitative results that are highly reproducible.
- * The difficulty of the material is designed to be comprehensive, implementing what the students have learned from previous experiments.

The experiments may be easily modified to address the specific interests of the class or to satisfy the constraints on equipment availability. (These experiments use only a minimal amount of equipment, e.g., Spec 20, centrifuge, balance, and water bath.) Other required materials are common glassware and consumables. Some of the experiments can be used as short demonstrations by the instructor in a biochemical engineering lecture course. They have been planned in an interactive mode; comments have been actively solicited from the currently enrolled students to improve each experiment in the coming years. Because these experiments have been developed under a severe budgetary constraint, they should be attractive to various other departments in our country who also experience similar practical budget limitations.

Need for a Laboratory Course

Laboratory experience is indispensable in the training of engineering students because it offers them the rare opportunity of practical experience rather than pure abstract which is traditionally encountered in a lecture course. A well designed laboratory course can help remove the student's mental block toward a subject. What hampers the understanding of biochemical engineering concepts, as in any other discipline, is the lack of feel for a real system. Without an experimental course, most students have no feel for, for example, enzymes because they think they have never seen the substance, no matter how strongly an instructor emphasizes the contrary. But the entire concept is suddenly understood once they see the enzyme in action. It is no surprise that the smell of protease is similar to that of rotten eggs or decomposing meat because the protein degradation in nature is often accomplished by the presence of protease. Discovering this fact, the student suddenly feels at ease with the subject because he has just realized that he has always known the material and it no longer seems so alien and threatening.

There is a conscious effort throughout the course to provide experiments that students can relate to their daily experience. The transformation of an abstract idea



to a concrete experience is the key to a true understanding and the lifelong appreciation of the subject in any field of study, and biochemical engineering is certainly no exception. Because biochemical engineering deals with materials that closely relate to one's everyday experience as opposed to, say, nuclear physics or electronics, which is difficult to perceive using one's five senses, a well designed laboratory course can ensure that one retains and later applies what one has learned long after graduation. Laboratory courses that complement traditional lecture courses are essential in engineering, as it is in chemistry or physics. The author strongly believes that fruitful laboratory experiences are an extremely important part of chemical engineering education.

Acknowledgement

The author sincerely thanks the encouragement and support given by Drs. George E. Dieter and Marvin L. Roush during the development of this course. Constructive comments given by the students in ENCH485 are also appreciated.

