

A PROJECT-ORIENTED APPROACH

to an Undergraduate Biochemical Engineering Laboratory

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Although many chemical engineering programs offer lecture courses covering various topics of biotechnology, relatively few undergraduate students receive meaningful laboratory exposure to experimental work in this important field. One of the major prohibitive factors in offering this type of educational experience is time. Although some of the pertinent technologies have been incorporated into laboratory instruction,^[1-3] many of the new biological methods cannot be adequately introduced and thoroughly investigated in a traditional laboratory course format that consists of, perhaps, one or two three-hour laboratory sessions per topic. Most fields of study in biotechnology, such as microbial fermentation or plant and mammalian tissue cultivation, require experimental durations of up to one month to obtain meaningful data. In addition, many of these technologies require extensive training before comprehensive investigation can take place.

To rectify this problem, we developed a biochemical engineering laboratory experience that includes long-term experimental projects in areas of plant cell cultivation, *in situ* bioremediation of hazardous wastes, enzymatic cellulose hydrolysis, and microbial fermentation. The course is distinctive in its use of single experimental projects (completed over the duration of one instructional quarter) that demonstrate many engineering principles related to biotechnology. The one credit-hour laboratory is offered in conjunction

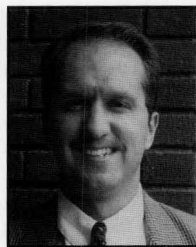
with a three credit-hour lecture titled, "Fundamentals of Biochemical Engineering."

COURSE ORGANIZATION

Considering the time constraints of a ten-week quarter, it was immediately evident to those planning the course contents that it would not be feasible to provide student exposure to all available laboratory projects. So we split the students into four research groups, each comprised of two to three juniors and seniors, which were then assigned to one of the available experimental modules for the duration of the course. Each group was expected to invest a minimum of ten student-hours per week in its research project. Although the university catalog list the lecture course as 3.0 credit-hours and the laboratory course as 1.0 credit-hour, the laboratory work actually comprised nearly fifty percent of the total course effort. The instructor was available for consultation at "set" laboratory hours and, in addition, each group was given a room key, thus allowing for project work at any time of the day.

To assure that all students received essentially the same educational experience, we formulated common overall objectives for all the experimental modules. These objectives, split into two groups titled "software" and "hardware," are listed in Tables 1 and 2, respectively. Hardware objectives refer to tasks completed specifically in the laboratory facility, whereas software objectives involve necessary research steps completed outside, but in support of, laboratory efforts.

We formulated software objectives as a guide for students through the necessary planning steps of any research endeavor, not merely the projects at hand. They began fulfilling these objectives in the library with a list of recommended journal articles and book chapters to read, and this material provided a foundation for a more comprehensive literature search using available on-line and off-line library data bases. This activity also enabled the students to formulate their own experimental objectives as well



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as to set tentative dates for completion. The instructor reviewed each group's final objectives before any experimental design could be initiated.

To assure proper communication, both with the instructor and within the group, we held weekly project-planning meetings and required that bi-weekly progress reports be completed by the groups. During the weekly planning meeting, the group informed the instructor of the previous week's progress and presented a tentative plan for its upcoming activities. Bi-weekly written progress reports followed the same basic format: details of previous results, including tabular and graphic data with appropriate discussion, along with a comprehensive plan for the group's efforts over the next two weeks, including detailed designs of upcoming experiments. In addition, during the lecture portion of the course we required the students to explain facets of their project work as related to concepts studied by the entire class. This gave all the students some exposure to each project area.

The end of the quarter culminated in final oral and written presentations. Written reports had to describe the results obtained over the entire project, including an overview of the initial literature search, while oral reports focused on the group's progress toward planned experimental objectives.

Also, since each group researched a unique topic, the final oral report had to include a brief demonstration of the studied technology in order to inform the other students of the techniques that were used. We asked students who presented exceptional written and oral reports to participate in the regional AIChE Student Paper Competition.

Hardware objectives (see Table 2) were formulated to assure that although each group was involved in a different subject, all students were exposed to the same basic principles of biochemical engineering. These objectives included training in many facets of sterile technique, along with media preparation, contamination detection, and organism identification methods. The students also learned how to perform necessary measurements for substrate, biomass, and product concentration, and all the groups had to complete an analysis of data gathered through experimental studies in order to obtain estimates of kinetic parameters and to predict performance of proposed reactor configurations. The mathematical modeling and parameter estimations were completed using SimuSolv* modeling and simulation software.

INDIVIDUAL EXPERIMENTAL MODULES

Using the four experimental modules available for investigation, we separated laboratory project work into two areas: preliminary studies and objectives. Preliminary studies, to be completed within the first three weeks of the course, are designed to orient students to both literature material and routine laboratory tasks associated with the subject area. Project objectives are open-ended experimental tasks which incorporate training gained from the preliminary studies and knowledge from biochemical engineering lecture material as well as prior chemical engineering coursework.

1. Plant Cell Cultivation

This project focuses on batch studies for the measurement of substrate, biomass, and secondary metabolite concentrations in suspensions of *Nicotiana tabacum* and *Catharanthus roseus*. To provide a literature background for the study, students read portions of the text *Plant Propagation by Tissue Culture*^[4] as well as a number of pertinent articles giving an overview of plant cell culture advances,^[5-7] outlining necessary cultivation and analytical techniques,^[8-11] and discussing kinetic modeling in cell culture systems.^[12] While completing this literature search, students learn techniques fundamental to plant tissue cultivation, such as sterile sub-

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TABLE 1

Overall "Software" Objectives for all Groups

- Topic-based literature search using on-line or off-line data bases, such as Chemical Abstracts
- Formulation of experimental objectives and target dates for completion
- Design of experiments
- Bi-weekly progress reports; weekly project planning meetings
- Final oral and written reports with experimental demonstration

TABLE 2

Overall "Hardware" Objectives for all Groups

- Sterile cultivation techniques, including culture initiation
- Assay techniques for substrate and product concentration
- Media preparation, contamination detection, and organism identification
- Biomass concentration measurements (weight analysis, hemocytometer, optical density)
- Kinetic analysis, modeling, and reactor design considerations

cultivation, fresh weight and dry weight concentration determination, media preparation, and assays of substrate (sucrose, glucose, and fructose) and secondary metabolite (phenolics and indole alkaloids) concentrations. All of these techniques were previously developed by either the instructor or chemical engineering students involved in biotechnology research at Tri-State University.

After the literature search and preliminary study activities are completed, the group begins fulfilling the project objectives, starting with the formulation of a GC/MS assay for ajmalicine concentration determination. This determination is considered a main objective of the project, rather than a preliminary study activity, because details of the technique have not yet been completely developed. After this objective has been completed, students initiate batch, shake flask cultures of both cell lines, measuring concentrations of substrate, biomass, and secondary metabolites over the culture duration. The *N. tabacum* culture is subsequently scaled up to a 2-L bioreactor, while students determine the same parameters as before. All studies are then modeled mathematically, using simple Monod kinetics for the prediction of all measured responses. Kinetic parameters are estimated using SimuSolv, allowing for direct comparison between the two species studied as well as between the shake flask and bioreactor cultures. In addition, students learn the steps of culture formation by initiating callus culture from seedlings of *Capsicum frutescens*.

2. In Situ Bioremediation

This project involves remediation of gasoline components benzene, ethylbenzene, toluene, and xylene (BTEX) in liquid systems by a pure strain of *Pseudomonas stutzeri* and a consortium grown from local vadose zone soil. As a review of pertinent literature, students read excerpts from *Environmental Biotechnology for Waste Treatment*,^[13] as well as several journal articles covering the use of various biological remediation techniques.^[14-17] Different microbiological methods necessary for project completion are also reviewed.^[18] Laboratory preliminary studies consist of learning compulsory techniques, including preparation of solid and liquid bacterial culture medium, sterile inoculation of cultures, bacterial identification methods such as gram staining, and quantitative assays consisting of viable cell counts using a hemocytometer and toluene concentration determination using GC/MS.

Project objectives begin with a series of batch studies using both *P. stutzeri* and the "local" microbial consortium to degrade 500 ppb toluene in a nutrient salt solution under different redox (aerobic and anaerobic) regimes and agitation levels. This initial test serves as a basis for designing further experiments to investigate the destruction of BTEX under optimal conditions. These experiments require that the group develop purge-and-trap GC/MS assays for all BTEX components. All batch degradation studies are then

modeled mathematically, using SimuSolv, to obtain estimates for kinetic parameters and to suggest optimal conditions for BTEX destruction.

3. Enzymatic Cellulose Hydrolysis

This project focuses on the hydrolysis of cellulosic substrates using pure cellulase enzyme (a preparation from fungal cultures which produce different types of cellulases) and sulfuric acid. Literature for this project includes significant portions of the text *Biochemical Engineering*^[18] and a number of important articles discussing enhanced enzymatic cellulose hydrolysis,^[19-22] cellulase production by fungal culture,^[23-24] and kinetic modeling of hydrolysis reactions.^[25-26] In laboratory preliminary studies, students master sterile fungal culture techniques using both solid and liquid media. An enzymatic glucose analysis technique is also demonstrated for future use in determining hydrolysis product formation. Students then complete a trial hydrolysis experiment using cellobiose (the β -1,4 dimer of glucose) as a substrate. Product formation data from this study is fit with a Michaelis-Menten response curve as estimates are made for the appropriate kinetic parameters.

The main objective of this study is to compare hydrolysis rates in long-term (eight-hour) studies using (1) pure Cellulase* addition, (2) addition of fungal preparation of *T. reesei*, and (3) addition of a known concentration of sulfuric acid. The extent of hydrolysis is experimentally determined by measuring glucose concentration periodically throughout each study. Hydrolysis rate constants, estimated using the SimuSolv program, are then directly compared for each method.

4. Microbial Fermentation

This project involves the study of substrate uptake, biomass formation, and product formation in two bacterial species, *Escherichia coli* and *Micrococcus luteus*. Reference materials consist of excerpts from both microbiology textbooks and laboratory manuals.^[27-29] Preliminary studies consist of learning basic techniques such as preparation of solid and liquid bacterial culture mediums, sterile inoculation and culture sampling, cell strain identification methods, and quantitative assays including viable cell counts as well as glucose and ammonia concentration determinations. Because of the similarity between the two projects, student groups investigating *in situ* bioremediation and microbial fermentation are allowed to collaborate in completing preliminary tasks.

The overall objective of this project is to formulate a reactor configuration to maximize microbial cell density in the two cell strains studies. Several tasks leading to this main objective include initiating shake flask and 2-L bioreactor cultures of either *M. luteus* or *E. coli* and moni-

* Sigma Chemical Co., St. Louis, Missouri

toring concentrations of glucose, cells, and metabolic product (ammonia for *M. luteus* or pH for *E. coli*) throughout a batch culture cycle. In addition, semi-batch cultures of both cell strains are completed with periodic medium replacement to eliminate toxic waste products from the broth in an effort to boost cell density. To aid in cell concentration determination, students also correlate microbial cell counts to measured optical density. In addition, a portion of the batch studies is modeled mathematically to determine kinetic parameters for the individual tests. Given this information, the group then proposes and tests a reactor configuration specifically designed to yield a maximum cell density.

STUDENT EVALUATION

The overall student impression of the laboratory course format was extremely favorable. A survey taken at the end of the course yielded a rating of 4.5/5.0 for overall course evaluation, while laboratory teaching methods were rated at 4.7/5.0. Students specifically enjoyed the freedom afforded by the project-oriented approach of the course, as they were directly responsible for planning and scheduling experimental activities. Several participants chose to continue their projects through independent study over the next two quarters, and many students showed an interest in the possibility of a continuation course focusing primarily on laboratory methods in biotechnology.

Criticism of the course was reserved to two points: first, because of long hours spent on project tasks, students felt that the laboratory should qualify for more course credit-hours, and second, several students did not feel adequately prepared to initiate laboratory investigation in biotechnology. To rectify this concern, in a second offering of the biochemical engineering laboratory, both faculty and students from the biology and chemistry departments assisted the participants.

CONCLUSIONS

Using a project-oriented approach to biochemical engineering laboratory education proved to be successful in motivating students to produce quality experimental work. Participants were willing to take "ownership" of the investigations because they were intimately involved in all project planning and development steps and were able to conduct a number of experiments in a single research area over an extended period of time. This approach also stimulated students to better integrate previously acquired chemical and biochemical engineering knowledge into decisions pertinent to their project objectives. In addition, the quarter-long investigative projects gave students a more realistic picture of the research world, and they were able to use research tools such as on-line and off-line data bases and mathematical modeling software. By working for extended time periods in a research group, students received more exposure to group accountability in completing delegated experimental

tasks. This type of fundamental change in approach to laboratory education has enhanced the quality of instruction in biochemical engineering and may be applicable to other fields of study within the chemical engineering discipline.

ACKNOWLEDGMENTS

I would like to thank Dr. James M. Lee (Washington State University) and Dr. Michael L. Shuler (Cornell University) for the respective, generous donations of *Nicotiana tabacum* and *Catharanthus roseus* plant cell suspensions. I also want to acknowledge Dr. Ira F. Jones (Tri-State University) for his assistance in developing several GC/MS assays. Partial support for this project was provided by the National Science Foundation Instrumentation and Laboratory Improvement Program. The National Science Foundation is in no way responsible for or endorses the contents of this paper. Partial support for this project was also provided by the Olive B. Cole Foundation. The SimuSolv modeling and simulation software was donated by the Dow Chemical Company.

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ChE book review

ACCIDENT AND EMERGENCY MANAGEMENT

by Louis Theodore, Joseph P. Reynolds, and Francis B. Taylor

John Wiley & Sons, New York, NY 10158-0012; 478⁺ pages, \$65.95 (1989)

Reviewed by

Robert M. Bethea

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Although the authors state that this book is "intended primarily for regulatory officials, company administrators, (practicing) engineers, . . . industry maintenance personnel, and both undergraduates and first-year graduate students," I believe that it is much better suited as a reference than as a text for chemical engineering students. The primary objective of the book is to provide a diverse audience with a broad overview of the scope and interrelations of the parts and functions of accident and emergency management programs. The authors have been successful in meeting this objective.

The book is divided into thirteen chapters, each with references, a summary, and problems for discussion or homework (or term papers!). The chapters are divided into four parts: an overview of accident and emergency managements (Part I, 76 pages), process and plant accidents (Part II, 181 pages), dispersion (Part III, 142 pages), and hazard and risk assessment (Part IV, 79 pages). The index is reasonably detailed and is easy to use.

Chapter 1, "Past History," presents brief descriptions of early and recent major accidents (Flixborough, Three Mile Island, Chernobyl, Bhopal, etc.) to illustrate the scope and breadth of emergencies for which the reader may need to plan.

Chapter 2, "Legislation," discusses significant Federal laws regarding air and water pollution and hazardous and toxic wastes.

Chapter 3, "Emergency Planning and Response," is a continuation of Chapter 2. It presents brief descriptions and lists some of the items to be considered in the various stages of the development and implementation of emergency response plans. I have used this material as part of a graduate course on chemical process safety for practicing chemical and environmental engineers and safety professionals.

Chapter 4, "Process Fundamentals and Plant Equipment," contains elementary and descriptive material (remember the intended audience) from stoichiometry, thermodynamics, unit operations, and design. This chapter is designed to familiarize the non-chemical engineer with terminology, equipment, processes, and concepts used in examples in Part III.

Chapter 5, "Fires, Explosions, and Other Accidents," presents an overview of fire fundamentals, types, and sources with some physical property data. Appropriate calculation procedures are presented. (Caution: the f_i in Eqs. 5.2.1 and 5.2.2 are not correctly defined; they must be on an air/oxygen-free basis, *i.e.*, a combustibles-only basis.) The sections on fire hazards, and especially on fire prevention and protection, are altogether too brief. The section on explosion fundamentals is overly short and will require consider-