TEACHING BIOCHEMICAL SEPARATIONS TO ENGINEERS

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A course in bioseparations for chemical engineers must be approached from a unique interdisciplinary perspective; a course developed from traditional chemical engineering and applied to, for example, proteins, like a course built on bench-scale biochemistry scaled to industrial quantities, is insufficient. Creating a course that synthesizes these traditional perspectives while embracing the uniqueness of the biochemical engineering environment and the materials it processes is the subject of a collaboration among the institutions of the three authors. A course package consisting of classroom instruction, syllabus, homework problems, multimedia, and laboratory exercises has been developed for the training of chemical engineers, environmental engineers, and biochemists in the field of Biochemical Separation Science and Technology.

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The intense interdisciplinary nature of the subject raises a number of questions, but an integrated assessment of the three institutions reveals that more than one of the issues has been addressed in each case. Some pertinent questions and their answers, gleaned from the past few years of teaching the subject at the senior and graduate levels, are:

- **How much fundamental biochemistry should be taught to chemical engineers?** *Engineers need to learn about biological molecules of all types, together with a little about the cells and tissues that produce them; ordinary organic molecules are in their normal repertoire.*
- **How much engineering, and at what level, should be taught to scientists?** *Scientists can quickly pick up the basic principles of engineering analysis by using equilibria, material balances, and transport phenomena in a universal analytical paradigm.*
- **How much breadth to provide in the form of a variety of unit operations?** *One or two important unit operations in the following categories should be subjected to engineering analysis: solid-liquid separations; solute-solid separations; solute-solute separations; and solute-liquid separations.*
- **How much depth to provide in single operations, especially chromatography?** *Each operation presented should be covered in enough depth to enable critical engineering calculations (yield, purity, etc.).*
- **How can too much or too little emphasis on proteins as target products be avoided?** *Every opportunity should be seized to illustrate the unique requirements of macromolecule separations.*
- **How much process development, plant design, and economics should be included?** *The basic rules for*

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calculating cost of steps and process and for sequencing operations should be incorporated in process design.

- **What should be taught in the laboratory?** *Standard unit operations should be covered in laboratory exercises; for example, filtration, sedimentation, extraction and chromatography; electrophoresis is also a good experience for engineers.*

In a continuing effort, additional lectures, notes, homework, text, and laboratory exercises are being developed to implement the above issues.

TABLE 1
1994 Survey of 84 Chemical Engineering Departments in the United States and Canada

Departments with a course in biochemical engineering	65
Total annual enrollment in biochemical engineering courses	1214
Departments with a course in bioseparations	29
Total annual enrollment in bioseparations courses	211

STATUS OF TEACHING BIOCHEMICAL SEPARATIONS

Courses Offered • In 1994, a survey of 84 North American chemical engineering departments was conducted. The results indicate that while over one thousand students in North American universities are taking a course in biochemical engineering, only about two hundred enroll in biochemical separations courses. Because about half as many departments offer biochemical separations courses as offer biochemical engineering courses, there is evidence that an unmet demand exists. These important findings from the survey are summarized in Table 1.

Textbooks and Literature • While volumes on the subject of biochemical engineering separations are abundant,[i.e.,1-4] to date only one has been specifically written as a textbook for undergraduate and graduate students in chemical engineering and other technical majors.[5] Text material also exists in chapters of more general biochemical engineering textbooks.[6,7]

Economic Significance • There is a strong and growing likelihood that intrinsically value-added biotech products will stop working economic miracles, and income from selling them will have to be earned (as it is from standard products) for at least four reasons: trends in health-care legislation, regulatory costs, competition, and recovery of research costs. Processing in general, and downstream processing in particular, will have to be performed on a competitive basis. Humulin™ (recombinant human insulin) is already purified by a modified process, but Epogen™ (recombinant erythropoietin) and Abokinase™ (natural human urokinase) are still produced in roller-bottle animal cell cultures. Likewise, much of the purification of these products depends on increasing the volume at which elution chromatography is practiced—the workhorse of the bench-scale biochemist. The trend toward ever larger chromatographs has at least three effects on engineering education: scale-up is becoming more sophisticated and must be learned (hence taught), scale-up is getting too expensive and must be carefully costed, and chromatography should be replaced by a more efficient process in high-volume applications. All are reasons for creating a population of engineers to support the forthcoming downstream processing infrastructure in which traditional chemical engineering unit operations cannot be applied and traditional biochemical procedures cannot be afforded.

The issues faced by the engineering educator in this field are the items mentioned in the first part of this article. The following paragraphs attempt to respond to those issues with a syllabus and a style that is suitable for the preparation of engineering students for the downstream biochemical engineering profession.

INTRODUCTORY MATERIAL

Biochemistry for Engineers

Process engineers do not need to know a great deal about intermediate metabolism, but the physical properties of the materials they must purify constitute essential knowledge. In the field of traditional pharmaceuticals this means: antibiotics (the birthplace of bioprocessing), hormones (from natural to synthetic), vitamins, neurotropics, chemotherapeutics, and vaccines. In the field of biotechnology and biopharmaceutical products this means proteins, nucleic ac-

ids, complexes, antibodies, subcellular particles, and whole cells. A sufficiently detailed understanding of cell structure and function is necessary for the intelligent choice of early post-fermentation processes.

Review of Engineering Analysis

An attempt should be made to make this subject accessible to biochemists, who need to be introduced to principles of engineering analysis. It has also been found useful to review principles for seasoned engineering students. In general, this means reviewing the derivation and solution of simple differential equations, including the establishment of initial and boundary conditions, and the definitions of a few relevant dimensionless numbers. Three main elements are emphasized: equilibria (equations of state are not directly useful), material balances (energy balances are seldom needed), and flux and transport relationships, including non-equilibrium driving forces and chemical potentials. Examples of each, as applied to biochemical separations, are:

Equilibria: partition coefficients, adsorption isotherms, solubility

Material Balances: shell balances on a column element; balances on solutes between phases

Transport: Ohm's law, Hagen-Poiseuille flow, Darcy's law, and Stokes flow in inertial fields; brief review of dimensionless numbers

The goal of engineering analysis should be made clear: how do the above elements combine to analyze a process for how much per what, how fast, what cost, how big, etc. Cost of step calculations can be introduced at this stage to stimulate interest in the unit operations that are to follow and in process synthesis, which will require process analysis.

UNIT OPERATIONS

Sequence • An approach that foreshadows process synthesis can be used, and there is a choice of the order in which to present the unit operations of biochemical purification. One could didactically build one process on the lower complexity of another—simplest first, or one could present them in the order in which they would typically be applied in a process scheme. There are, however, no hard rules about the order (Asenjo says, for example, to use the highest-purification-factor process first[1]). But one must also teach that particulates and impurities might foul high-purification units, such as chromatography, ultrafiltration, differential precipitation, etc. A reasonable choice of sequence appears to be liquid-solids separations followed by solute-solute separations and solute-liquid separations, similar to the recovery-isolation-purification-polishing (RIPP) paradigm.[5]

Content for each unit operation • In each case, an outline is used that combines descriptive and analytical learning. The following subjects are covered in most cases:

- Objective: *the purpose of the unit operation, product examples*
- Underlying physics and chemistry: *what reactions and physical and statistical principles are applied*[2,3]
- Governing equations: *what analytical relationships describe the process*
- Engineering analysis: *"bottom-line" calculations using the governing equations*[5]
- Applications: *examples of applications to specific products at specific stages*
- Example problems: *worked problems amplifying engineering analysis and numerical examples using the governing equations*
- Homework: *routine and mind-stretching calculations, spreadsheets, and/or programming problems*
- Literature

A few unit operations not readily familiar to engineers are included. Preparative and analytical electrophoresis and extraction using aqueous two-phase systems, for example, enable new professionals to consider meaningful alternatives when scale-up becomes costly or purification factors become inadequate. They also learn novel ways of using membranes to replace or augment major unit operations, such as adsorption and crystallization.

PRACTICUMS

Laboratory exercises are chosen to try to span the range of basic separations and engineering problems: separating liquids from solids involves filtration and centrifugation; separating solutes from solutes with liquid handling means extraction; and purification (solute-solute separation) means chromatography and preparative electrophoresis.

Each of five practicums we have used is summarized below.

1. **Filtration** Measure flow rates and flux decline using pilot-scale equipment (ca. 50 liters/hour) filtering a suspension of microorganisms in batch concentration mode. Scale this process for a large fermentation plant in feed-and-bleed mode.
2. **Sedimentation** Measure flow rates and particle breakthrough using a pilot-scale disk-stack centrifuge at constant speed with and without flocculation of a suspension of microorganisms. Scale this process for a large fermentation plant in continuous mode.
3. **Extraction** Determine partition coefficients of a protein in an aqueous two-phase system at bench (10-gram) scale. Write a report about the properties of this process and its governing equations.
4. **Chromatography** Obtain ion-exchange or size-

exclusion chromatograms of two or three proteins in a mixture at pilot (1-gram) scale using three combinations of column dimensions, elution gradient, and elution flow rate. Scale this process to a specified level of production and resolution using Yamamoto's principles.[8]

5. **Electrophoresis** Purify milligram quantities of an oligonucleotide from a mixture of in vitro transcripts and evaluate purity and yield using preparative polyacrylamide gel electrophoresis. Scale this process to the kilogram level and calculate the cost of step.

The above scaling exercises are, of course, calculations only. The students are expected in each case to write a formal report as if it were intended for a client.

ADDITIONAL ASSIGNMENTS

Homework is assigned just as it is in any engineering course. A few open-ended problems are assigned in which students must find or estimate extensive and/or intensive properties; spreadsheet calculations and some derivations are included. In some cases a term paper must be written in the form of a critique of a single published paper or as an in-depth summary of a single subject (chosen by the student) based on the reading of recent literature. Both descriptive and analytical questions are included on examinations.

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Computing

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of steps set out in the lab handout. The applications should be interactive and open-ended, requiring the students to use engineering analysis and methodology during the lab, *e.g.,* they should figure out which one of several possible control schemes for level and/or temperature in tank 1 of Figure 1 is "best" for their application.

- ▶ Integrating the RTSB computing with other course material, *e.g.,* using real-time Simulink software in the lab if the students have used Matlab/Simulink in other courses.
- ▶ The applications, *i.e.,* the process instrumentation and computer system, should be realistic enough that the relevance to course material and industrial requirements is obvious. However, the application should be more flexible and easier to program than most commercial systems designed for industrial operations.

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APPENDIX

The specifications of the computer-process interface used to generate the results presented in this paper are:

Hardware

- CIO-DAS16/F (primary data acquisition board with 16 single-ended (8 differential) 12-bit A/D, 2 D/A, 32 DIO and 3, 16-bit counters
- CIO-EXP16: Expansion board with 16 A.I. multiplexing and thermocouple signal conditioning
- CIO-DAC02: (add-on board with 2 12-bit D/A for voltage or current output) (Source: Computer Boards, Inc.)

Software

- Labview drivers (Source: National Instruments)
- Matlab/Simulink drivers (Source: Mathworks)
- C++ drivers (Source: Mathworks, Inc., and user written)

Host Computer

- PC (486 with 16Mb memory, 1.2Gb HDD)
- Running Microsoft Windows 3.1 ❐