James M. Lee

Biochemical Engineering

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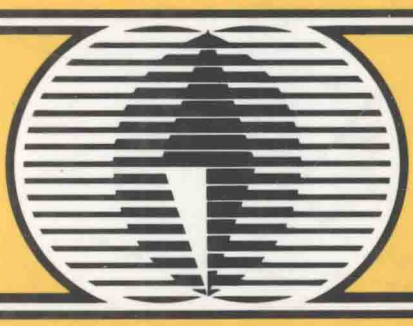

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Biochemical Engineering

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Preface

This book is written for an introductory course in biochemical engineering normally taught as a senior or graduate-level elective in chemical engineering. It is also intended to be used as a self-study book for practicing chemical engineers or for biological scientists who have a limited background in the bioprocessing aspects of new biotechnology.

Several characteristics lacking in currently available books in the area, therefore, which I have intended to improve in this textbook are: (1) solved example problems, (2) use of the traditional chemical engineering approaches in nomenclature and mathematical analysis so that students who are taking other chemical engineering courses concurrently with this course will not be confused, (3) brief descriptions of the basics of microbiology and biochemistry as an introduction to the chapter where they are needed, and (4) inclusion of laboratory experiments to help engineers with basic microbiology or biochemistry experiments.

Following a brief introduction of biochemical engineering in general, the book is divided into three main sections. The first is enzyme-mediated bioprocessing which is covered in three chapters. Enzyme kinetics is explained along with batch and continuous bioreactor design in Chapter 2. This is one of two major chapters which needs to be studied carefully. Enzyme immobilization techniques and the effect of mass-transfer resistance are introduced in Chapter 3 to illustrate how a typical mass-transfer analysis, familiar to chemical engineering students, can be applied to enzyme reactions. Basic biochemistry of carbohydrates is reviewed and two examples of industrial enzyme processes involving starch and cellulose are introduced in Chapter 4. Instructors can add more current examples of industrial enzyme processes or ask students to do a course project on the topic.

The second section of the book deals with whole-cell mediated bioprocessing. Since most chemical engineering students do not have backgrounds in cell culture techniques, Chapter 5 introduces basic microbiology and cell culture techniques for both animal and plant cells. Animal and plant cells are included because of their growing importance for the production of pharmaceuticals. It is intended to cover only what is necessary to understand the terminology and procedures introduced in the following chapters. Readers are encouraged to study further on the topic by reading any college-level microbiology textbook as needs arise. Chapter 6 deals with cell kinetics and

fermenter design. This chapter is another one of the two major chapters which needs to be studied carefully. The kinetic analysis is primarily based on unstructured, distributed models. However, a more rigorous structured model is covered at the end of the chapter. In Chapter 7, genetic engineering is briefly explained by using the simplest terms possible and genetic stability problems are addressed as one of the most important engineering aspects of genetically modified cells.

The final section deals with engineering aspects of bioprocessing. Sterilization techniques (one of the upstream processes) are presented in Chapter 8. It is treated as a separate chapter because of its importance in bioprocessing. The maintenance of complete sterility at the beginning and during the fermentation operation is vitally important for successful bioprocessing. Chapter 9 deals with agitation and aeration as one of the most important factors to consider in designing a fermenter. The last chapter is a brief review of downstream processing.

I thank Inn-Soo, my wife, and Jenny, my daughter, for their support and encouragement while I was writing this book. This book would not exist today without many hours of review and editing of the manuscript by Brian S. Hooker, my former graduate student who is now on the faculty of Tri-State University, Jon Wolf, my previous research associate who is now working for Boeing, and Patrick Bryant, my present graduate student. I also thank Rod Fisher at the University of Washington for using incomplete versions of this book as a text and for giving me many valuable suggestions. I extend my appreciation to Gary F. Bennett at the University of Toledo for providing example problems to be used in this book. I thank the students in the biochemical engineering class at Washington State University during the past several years for using a draft manuscript of this book as their textbook and also for correcting mistakes in the manuscript. I also thank my colleagues at Washington State University, William J. Thomson, James N. Petersen, and Bernard J. Van Wie, for their support and encouragement.

I typeset this book using a software, PC TEX(Personal TEX, Inc., Mill Valley, CA), with macro packages developed by Washington State University, Text1, and myself. Typesetting an entire book requires more work than I had anticipated, but it was a challenging job that I loved.

James M. Lee

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Chapter 1

Introduction

Biochemical engineering is concerned with conducting biological processes on an industrial scale. This area links biological sciences with chemical engineering. The role of biochemical engineers has become more important in recent years due to the dramatic developments of biotechnology.

1.1 Biotechnology

Biotechnology can be broadly defined as "Commercial techniques that use living organisms, or substances from those organisms, to make or modify a product, including techniques used for the improvement of the characteristics of economically important plants and animals and for the development of microorganisms to act on the environment ..." (Congress of the United States, 1984). If biotechnology is defined in this general sense, the area cannot be considered new. Since ancient days, people knew how to utilize microorganisms to ferment beverage and food, though they did not know what was responsible for those biological changes. People also knew how to crossbreed plants and animals for better yields. In recent years, the term *biotechnology* is being used to refer to novel techniques such as *recombinant DNA* and *cell fusion.*

Recombinant DNA allows the direct manipulation of genetic material of individual cells, which may be used to develop microorganisms that produce new products as well as useful organisms. The laboratory technology for the genetic manipulation within living cells is also known as *genetic engineering.* A major objective of this technique is to splice a foreign gene for a desired product into circular forms of DNA (plasmids), and then to insert them into an organism, so that the foreign gene can be expressed to produce the product from the organism.

Cell fusion is a process to form a single hybrid cell with nuclei and cytoplasm from two different types of cells in order to combine the desirable characteristics of the two. As an example, specialized cells of the immune system can produce useful antibodies. However, it is difficult to cultivate those cells because their growth rate is very slow. On the other hand, certain tumor cells have the traits for immortality and rapid proliferation. By

combining the two cells by fusion, a hybridoma can be created that has both traits. The monoclonal antibodies (MAbs) produced from the hybridoma cells can be used for diagnosis, disease treatment, and protein purification.

The applications of this new biotechnology are numerous, as listed in Table 1.1. Previously expensive and rare pharmaceuticals such as insulin for diabetics, human growth hormone to treat children with dwarfism, interferon to fight infection, vaccines to prevent diseases, and monoclonal antibody for diagnostics can be produced from genetically modified cells or hybridoma cells inexpensively and also in large quantities. Disease-free seed stocks or healthier, higher-yielding food animals can be developed. Important crop species can be modified to have traits which can resist stress, herbicide, and pest. Furthermore, recombinant DNA technology can be applied to develop genetically modified microorganisms so that they can produce various chemical compounds with higher yields than unmodified microorganisms can.

1.2 Biochemical Engineering

The recombinant DNA or cell fusion technologies have been initiated and developed by pure scientists, whose end results can be the development of a new breed of cells in minute quantities that can produce a product. Successful commercialization of this process requires the development of a large-scale process that is technologically viable and economically efficient. To scale up a laboratory-scale operation into a large industrial process, we cannot just make the vessel bigger. For example, in a laboratory scale of 100 mℓ, a small Erlenmeyer flask on a shaker can be an excellent way to cultivate cells, but for a large-scale operation of 2,000ℓ, we cannot make the vessel bigger and shake it. We need to design an effective bioreactor to cultivate the cells in the most optimum conditions. Therefore, biochemical engineering is one of the major areas in biotechnology important to its commercialization.

To illustrate the role of a biochemical engineer, let's look at a typical biological process (bioprocess) involving microbial cells as shown in Figure 1.1. Raw materials, usually biomass, are treated and mixed with other ingredients that are required for cells to grow well. The liquid mixture, the medium, is sterilized to eliminate all other living microorganisms and introduced to a large cylindrical vessel, bioreactor or fermenter, typically equipped with agitators, baffles, air spargers, and various sensing devices for the control of fermentation conditions. A pure strain of microorganisms is introduced into the vessel. The number of cells will start to multiply exponentially after a certain period of lag time and reach a maximum cell concentration as the medium is depleted. The fermentation will be stopped and the contents will be pumped out for the product recovery and purification. This process can be operated either by batch or continuously.

Table 1.1 Applications of Biotechnology

PHARMACEUTICALS:
Antibiotics, antigens (stimulate antibody response), diagnostics, endorphin (neurotransmitter), gamma globulin (prevent infections), human growth hormone (treat children with dwarfism), human serum albumin (treat physical trauma), immune regulators, insulin, interferon (treat infection), interleukins, lymphokines (modulate immune reaction), monoclonal antibody (diagnostics or drug delivery), neuroactive peptides (mimic the body's pain-controlling peptides), tissue plasminogen activator (for dissolving blood clots), vaccines, etc.

ANIMAL AGRICULTURE:
Products similar to those being developed in the pharmaceutical industry; development of disease-free seed stocks and healthier, higher-yielding food animals.

PLANT AGRICULTURE:
Transfer of stress-, herbicide-, and pest-resistance traits to important crop species; development of plants with the increased abilities of photosynthesis or nitrogen fixation; development of biological insecticides and nonice nucleating bacterium.

SPECIALTY CHEMICALS:
Amino acids, enzymes, vitamins, lipids, hydroxylated aromatics, and biopolymers.

AGRICULTURAL CHEMICALS:
Pesticides, fungicides, herbicides.

ENVIRONMENTAL APPLICATIONS:
Mineral leaching, metal concentration, pollution control, toxic waste degradation, and enhanced oil recovery.

FOODS AND BEVERAGES:
Alcoholic beverages, sweeteners, single-cell protein.

COMMODITY CHEMICALS:
Acetic acid, acetone, butanol, ethanol, and many other products from biomass conversion processes.

BIOELECTRONICS:
Biosensors, biochips.

To carry out a bioprocess on a large scale, biochemical engineers need to work together with biological scientists:

1. to obtain the best biological catalyst (microorganism, animal cell, plant cell, or enzyme) for a desired process
2. to create the best possible environment for the catalyst to perform by

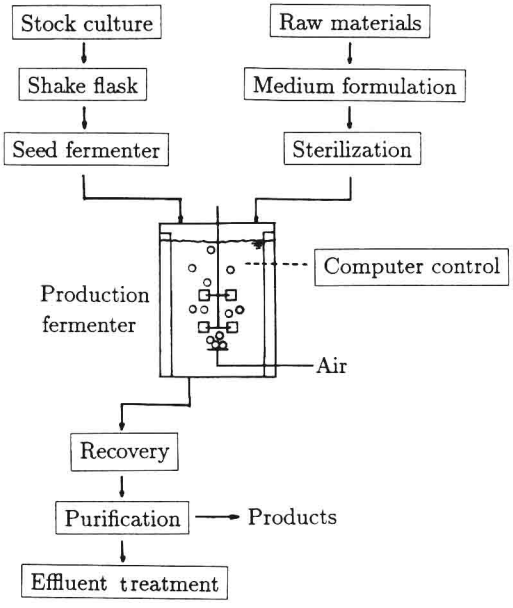

Figure 1.1 Typical biological process.

designing the bioreactor and operating it in the most efficient way

3. to separate the desired products from the reaction mixture in the most economical way

The preceding tasks involve process design and development, which are familiar to chemical engineers for the chemical processes. Similar techniques which have been working successfully in chemical processes can be employed with modifications. The basic questions which need to be asked for the process development and design are as follows:

1. *What change can be expected to occur?*
   To answer this question, one must have an understanding of the basic sciences for the process involved. These are microbiology, biochemistry, molecular biology, genetics, and so on. Biochemical engineers need to study these areas to a certain extent. It is also true that the contribution of biochemical engineers in selecting and developing the best biological catalyst is quite limited unless the engineer receives specialized training. However, it is important for biochemical engineers to get involved in this stage, so that the biological catalyst may be selected or genetically modified with a consideration of the large-scale operation.

2. *How fast will the process take place?*
   If a certain process can produce a product, it is important to know how fast the process can take place. Kinetics deals with rate of a reaction and how it is affected by various chemical and physical conditions. This is where the expertise of chemical engineers familiar with chemical kinetics and reactor design plays a major role. Similar techniques can be employed to deal with enzyme or cell kinetics. To design an effective bioreactor for the biological catalyst to perform, it is also important to know how the rate of the reaction is influenced by various operating conditions. This involves the study of thermodynamics, transport phenomena, biological interactions, clonal stability, and so on.
3. *How can the system be operated and controlled for the maximum yield?*
   For the optimum operation and control, reliable on-line sensing devices need to be developed. On-line optimization algorithms need to be developed and used to enhance the operability of bioprocess and to ensure that these processes are operated at the most economical points.
4. *How can the products be separated with maximum purity and minimum costs?*
   For this step, the downstream processing (or bioseparation), a biochemical engineer can utilize various separation techniques developed in chemical processes such as distillation, absorption, extraction, adsorption, drying, filtration, precipitation, and leaching. In addition to these standard separation techniques, the biochemical engineer needs to develop novel techniques which are suitable to separate the biological materials. Many techniques have been developed to separate or to analyze biological materials on a small laboratory scale, such as chromatography, electrophoresis, and dialysis. These techniques need to be further developed so that they may be operated on a large industrial scale.

1.3 Biological Process

Industrial applications of biological processes are to use living cells or their components to effect desired physical or chemical changes. Biological processes have advantages and disadvantages over traditional chemical processes. The major advantages are as follows:

1. *Mild reaction condition*: The reaction conditions for bioprocesses are mild. The typical condition is at room temperature, atmospheric pressure, and fairly neutral medium pH. As a result, the operation is less hazardous, and the manufacturing facilities are less complex compared to typical chemical processes.
2. *Specificity*: An enzyme catalyst is highly specific and catalyzes only one

or a small number of chemical reactions. A great variety of enzymes exist that can catalyze a very wide range of reactions.

3. *Effectiveness*: The rate of an enzyme-catalyzed reaction is usually much faster than that of the same reaction when directed by nonbiological catalysts. A small amount of enzyme is required to produce the desired effect.
4. *Renewable resources*: The major raw material for bioprocesses is biomass which provides both the carbon skeletons and the energy required for synthesis for organic chemical manufacture.
5. *Recombinant DNA technology*: The development of the recombinant DNA technology promises enormous possibilities to improve biological processes.

However, biological processes have the following disadvantages:

1. *Complex product mixtures*: In cases of cell cultivation (microbial, animal, or plant), multiple enzyme reactions are occurring in sequence or in parallel, the final product mixture contains cell mass, many metabolic by-products, and a remnant of the original nutrients. The cell mass also contains various cell components.
2. *Dilute aqueous environments*: The components of commercial interests are only produced in small amounts in an aqueous medium. Therefore, separation is very expensive. Since products of bioprocesses are frequently heat sensitive, traditional separation techniques cannot be employed. Therefore, novel separation techniques which have been developed for analytical purposes need to be scaled up.
3. *Contamination*: The fermenter system can be easily contaminated, since many environmental bacteria and molds grow well in most media. The problem becomes more difficult with the cultivation of plant or animal cells because their growth rates are much slower than those of environmental bacteria or molds.
4. *Variability*: Cells tend to mutate due to the changing environment and may lose some characteristics vital for the success of process. Enzymes are comparatively sensitive or unstable molecules and require care in their use.

1.4 Definition of Fermentation

Traditionally, fermentation was defined as the process for the production of alcohol or lactic acid from glucose ($C_6H_{12}O_6$).

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2$$