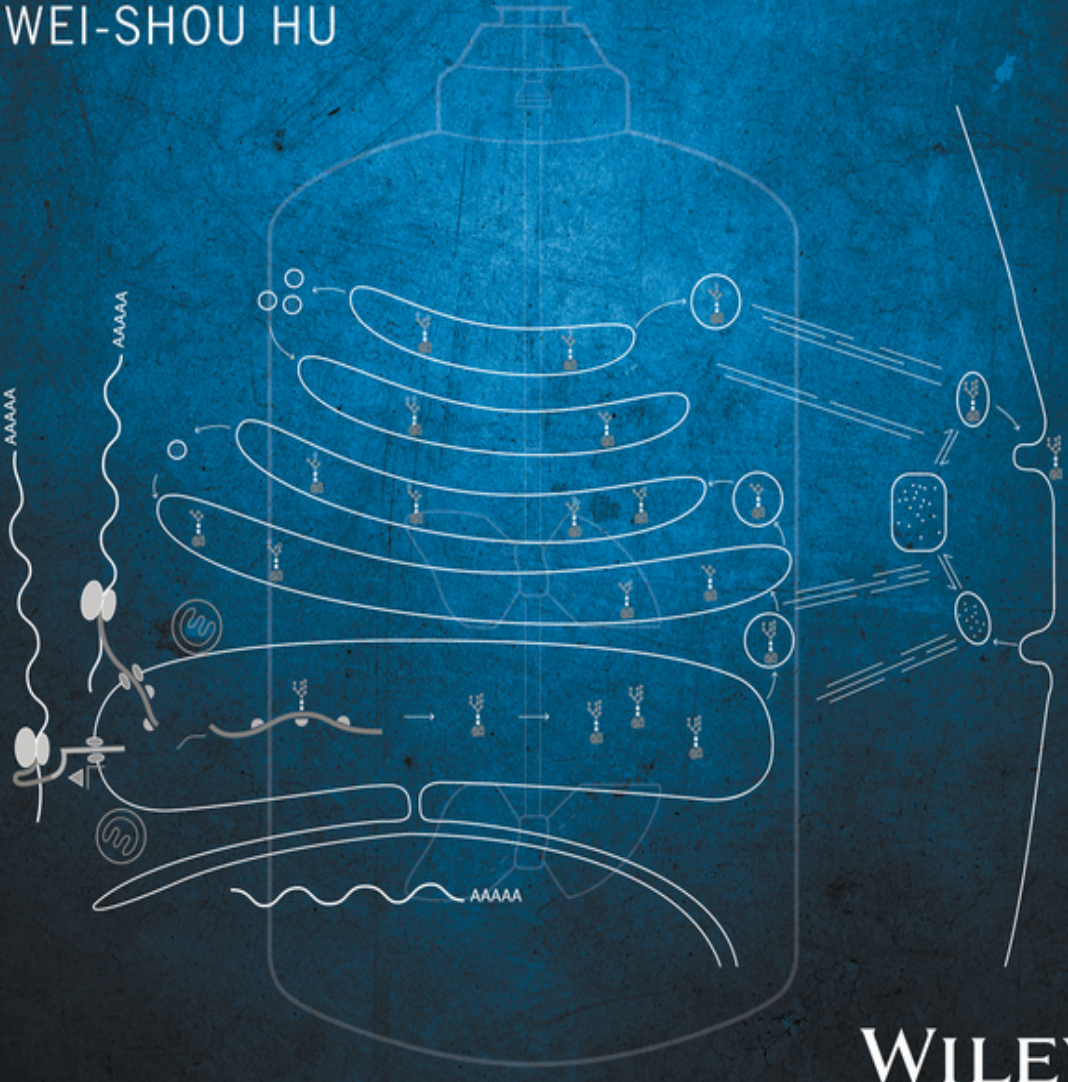
ENGINEERING PRINCIPLES IN BIOTECHNOLOGY

WEI-SHOU HU



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This book is dedicated to Jenny, Kenny, and Sheau-Ping.

Contents

Preface

Bioprocesses use microbial, plant, or animal cells and the materials derived from them, such as enzymes or DNA, to produce industrial biochemicals and pharmaceuticals. In the past two decades, the economic output from bioprocesses has increased drastically. This economic growth was the result of the translation of numerous discoveries to innovative technologies and manufactured products. The success has brought together numerous scientists and engineers of different disciplines to work together to break new ground. The task of taking biotechnological discoveries to a successful product or process requires a multidisciplinary team consisting of engineers and chemical and biological scientists to work synergistically. The success of a project, a team, or even a company in biotechnology often hinges on the ability of scientists and engineers of different specialties to work effectively together. This book has been written with this important characteristic of the bioprocess industry in mind. A major goal of the book is to give students the necessary vocabulary and critical engineering knowledge to excel in bioprocess technology.

This textbook is based on a biochemical engineering course that has been offered at the University of Minnesota for a number of years. The contents are intended for a semester course of about 14 weeks of three lecture-hours a week. Although the majority of the students taking this course are senior undergraduate and graduate students from chemical engineering and bioengineering, nearly one-third are graduate students from a life science background. An emphasis of the content and writing of the book is thus the fundamental engineering principles, the quantitative practice, and the accessibility of analysis for students of different backgrounds. The target audience of the book is not only students taking the biochemical engineering or bioprocess engineering courses given in chemical engineering or bioengineering programs but also students in biotechnology programs that are outside of the chemical engineering disciplines, especially in countries outside North America.

In writing this book, I assumed that the students have had at least one biology course, and have fundamental knowledge of carbohydrates, DNA, RNA, proteins, and other biomolecules, as is the case for most engineering students nowadays. Nevertheless, students from both engineering and life science backgrounds will encounter new vocabulary and new concepts that will help them in cross-disciplinary communication once they join the biotechnology workforce.

Chapters 1 and 2 give an overview of organisms, cells and their components, how they become the product, and what the bioprocesses that produce them look like. Chapters 3 and 4 use basic biochemical reactions, especially the energy metabolism pathways, to

familiarize engineering students with analysis of biochemical systems and to introduce the concepts of material balance and reaction kinetics to students with a life science background. For all students, these chapters introduce them to kinetic analysis of binding reactions, gene expression, and cellular membrane transport.

Chapters 5 and 6 cover the quantitative description of cell growth and the steady-state behavior in a continuous bioreactor. This paves the way for dealing with different types of bioreactors. Chapters 7, 8, and 9 are the core of bioreactor engineering, dealing with subjects important to process development. These chapters draw upon extensive practical interactions with industry to make them more relevant to bioprocess technology.

The next three chapters – 10, 11, and 12 – discuss three segments of bioprocesses. Cell culture processes, the subject of Chapter 10, currently produce goods valued over US$100 billion per annum. After introducing cell culture processes, the evolution of biomanufacturing and its life cycle is discussed. Chapters 11 and 12 look to the future on cell-based therapy and on the technologies arising from synthetic biology. In dealing with stem cells, the kinetic description of cellular differentiation is also introduced, and in discussing synthetic pathways the importance of using a stoichiometric relationship to determine the maximum conversion yield is reiterated. The last two chapters, 13 and 14, highlight the bioseparation processes. The overall strategy and the key concepts of various unit operations in bioseparation are covered briefly in Chapter 13. Chapter 14 focuses on the basic quantitative understanding of chromatography.

Writing this book has been a long undertaking. Many of my former and current graduate students have helped in formulating the problem sets and the examples. In preparing the book, I also took ideas from many textbooks on biochemical engineering, especially *Bioprocess Engineering: Basic Concepts* by Shuler and Kargi; *Biochemical Engineering Fundamentals* by Bailey and Ollis; *Fermentation and Enzyme Technology* by Wang, Cooney, Demain, Dunnill, Humphrey, and Lilly; and *Biochemical Engineering* by Aiba, Humphrey, and Millis. I extend my gratitude to my colleagues at the University of Minnesota, especially Arnold G. Fredrickson, Friedrich Srienc, Edward Cussler, Ben Hackel, Kechun Zhang, Samira Azarin, Efie Kokkoli, Yiannis Kaznessis, and Prodromos Daoutidis, for their stimulating discussion that helped shape the book. Finally, I thank Kimberly Durand for her editorial devotion to this book.

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An Overview of Bioprocess Technology and Biochemical Engineering

1.1 A Brief History of Biotechnology and Biochemical Engineering

For thousands of years, humans have harnessed the metabolic activities of microbes. Microbes are important contributors to the generation of many foods, including bread, cheese, and pickled vegetables. Microbes are our unwitting life partners, but humans were not even aware of their existence until Antony van Leeuwenhoek discovered microorganisms. In the 1860s, Louis Pasteur discovered that microbes are responsible for lactic acid and the ethanol fermentation of sugar. He directly linked microbial metabolism to the synthesis of products.

Before the turn of the twentieth century, both researchers and food producers began to use microbes more purposefully. They were increasingly used to ferment milk and wine. This period is now considered to be the dawn of microbiology, or applied microbiology. In the early years, microbiology as a scientific field was closely linked to food microbiology, due to the positive roles of microbes in food fermentation as well as food spoilage and resulting illness.

1.1.1 Classical Biotechnology

In the beginning of the twentieth century, the use of microorganisms was extended beyond fermenting foods to the production of chemical compounds. Lactic acid was produced by fermentation using *Lactobacillus* spp. This marked the start of genuine industrial microbial fermentation. One of the first workhorses was the anaerobic bacterium *Clostridium acetobutylicum*, which was used to ferment sugar to acetone, ethanol, and butanol. Citric acid production using the mold *Aspergillus niger* also came about in the 1920s.

Penicillin production, the predecessor of modern fermentation, did not start until the 1940s. Alexander Fleming discovered penicillin after observing the inhibition of bacterial growth by a compound produced by a green mold. This observation led to the development of the bioprocess we know today. In nature, the compound was produced only at low concentrations. Thus, a large volume of culture was needed to generate the amount needed for clinical trials.

The demand for large quantities of penicillin led to the development of submerged culture in liquid medium, as opposed to the traditionally used agar-surface culture.

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Along with the use of liquid-submerged culturing techniques came the search for a better medium composition and the utilization of corn steep liquor, a practice that continued for more than five decades.

The successful use of microbial fermentation to produce chemicals and natural products began a long and prosperous period. During this time, research laboratories and pharmaceutical and food companies isolated microorganisms from various sources (e.g., soil, gardens, and forests) to look for microbial species that produce various useful compounds. In addition to penicillin, many other microbial natural products with antibiotic activities were discovered. In the 1950s, *Corynebacterium glutamicum* began to be used to produce glutamic acid, which was used in a common food seasoning, monosodium glutamate. This led the way to a large amino acid industry. This period is considered to be the "classical period" of biotechnology (Figure 1.1).

Unlike the earlier solvent-producing bacteria, the molds and bacteria used in the production of antibiotics, amino acids, and other biochemicals are aerobic microorganisms. In order to produce a large quantity of a product, the volume of the culture and cell concentration must be increased, which in turn increases the demand for oxygen by microbes in culture. The demand for oxygen during scaling up led to the use of stirred-tank bioreactors with continuous sparging of air. A scaled-up process also produces more heat from the increased cellular metabolism and mechanical agitation.

The technical challenges associated with process scale-up spurred a golden period of bioprocess research. Many technical advances were made in bioreactor design to enhance oxygen transfer, sterility control, and process performance during the 1950s and 1960s.

In the second half of the twentieth century, microbiology became the core of industrial biotechnology. Many more antibiotics were discovered. The spectra of new secondary metabolites expanded from antibacterial (e.g., streptomycin, actinomycin, and cepharosporin) products to antifungal (e.g., nystatin and fungicidin), anticancer (e.g., mitomycin), and immunomodulating (e.g., cyclosporin) products. Many other microbial metabolites found their applications in the food, chemical, agricultural, and pharmaceutical industries and were successfully commercialized. Nucleosides produced by microorganisms were used to season food. Fermentation-derived lysine became an important additive to animal feed, and supported a vast industry of farm animals. Citric acid fermentation became a bioproduced commodity chemical.

In addition to metabolites, enzymes produced by microorganisms or isolated from plant and animal tissues have been utilized in food processing. For instance, amylases are used in starch processing, renin is used in cheese fermentation, and various proteases are used for protein hydrolysis. Many enzymes are also used in biocatalytic processes to produce new products. Glucose isomerase catalyzes the isomerization of the glucose molecules derived from cornstarch into high-fructose corn syrup, a staple ingredient in many processed foods. Penicillin isomerases are used to convert the side chain in penicillin from a phenylacetyl group to different acyl groups. Unlike the original penicillin G discovered by Fleming, these new penicillin-derived antibiotics are not sensitive to acid hydrolysis within the human digestive system. They also have expanded antimicrobial spectra.

During the decades following World War II, there was an unprecedented expansion of government-funded research in universities and other research institutions. The advances in fundamental biochemistry, biophysics, and molecular biology enhanced

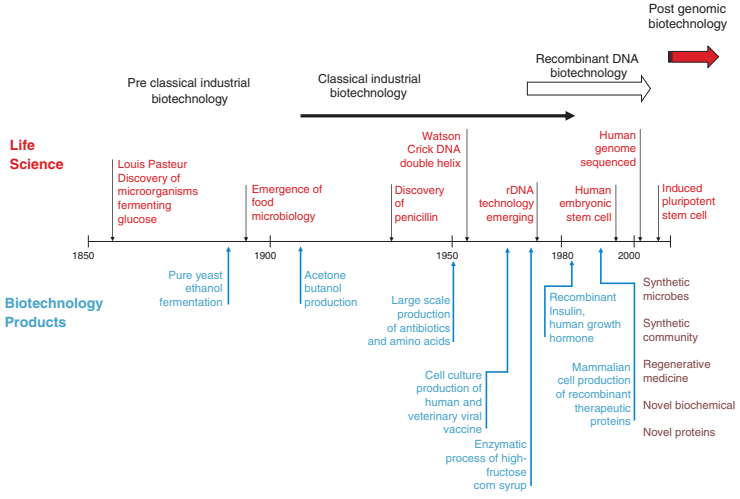

Figure 1.1 Milestones in biotechnology and historical advances in biochemical engineering.

our understanding of the nature of life and the universe. These fundamentals enabled mechanism-based discoveries that paved the way for the modern field of biotechnology, touched upon many aspects of human life, and spurred economic growth. The statins, which inhibit the rate-controlling enzyme in cholesterol biosynthesis, became star drugs for controlling cholesterol metabolism. Another example is mitomycin, an anticancer drug that is toxic because it crosslinks DNA molecules.

In the second half of the twentieth century, researchers gained a basic understanding of the structure and biochemistry of DNA and its role in genetics. These advances led to contemporary molecular biology. New understanding of the regulation of gene expression transformed industrial biotechnology and many other economic sectors. The invention of recombinant DNA technology then allowed the never-before-imagined insertion of engineered DNA sequences into a host organism for expression.

1.1.2 Recombinant DNA

Recombinant DNA (rDNA) technology enabled a new generation of products. It enabled a human protein to be produced in a host cell, be it a bacterium or a cultured human or hamster cell (Figure 1.2). It allowed us to modify the metabolic pathway of an organism by amplifying, deleting, or changing an enzyme in the pathway. Importantly, this enabling technology also spurred many venture-capital-financed startups like Genentech, Cetus, and Biogen. Thus began a new era of entrepreneur-driven innovation, forming the early stages of the next rapid expansion phase of industrial biotechnology.

Using rDNA technology, human insulin was produced in *Escherichia coli* by Genentech and licensed by Eli Lilly Company in 1981. Prior to that, type I diabetes patients had to use insulin isolated from pig pancreas, which has one amino acid different from the human form. After 1981, the insulin being used by patients was identical to the insulin that is produced in humans. A new era of producing human proteins for disease treatment then followed. Although difficult to produce before the age of recombinant technology, many of these proteins could now be cloned into a host cell and produced in sufficient quantities for treating patients. The benefit of producing these therapeutic proteins in a host cell lies not only in their increased availability; they also have increased purity. Proteins isolated from pooled donor blood might harbor harmful contaminants such as bloodborne viruses. By using rDNA technology for protein production, such danger is eliminated.

The list of heterologous proteins (or proteins produced using a different species of host cell) expressed in *E. coli* and yeast cells includes: interferon, human growth hormone, and cytokines for therapeutic uses; hepatitis B surface antigen as a vaccine; and bovine growth hormone for use in cows. However, many human proteins need to be modified after they are translated to produce the final biologically active form (Figure 1.3). Such posttranscriptional modifications, including glycosylation, complex disulfide bond formation, phosphorylation, and γ-carboxylation, are not carried out in microbes in the same way as they are in humans. To ensure their faithful expression with the requisite posttranslational modifications, mammalian cells are often employed.

Prior to the rDNA era, mammalian cells were used primarily for research and to produce viruses for use as vaccines. The use of Chinese hamster ovary (CHO) cells to produce recombinant therapeutic proteins (e.g., coagulation factor VIII for hemophiliac patients, and erythropoietin, a stimulator for red blood cell formation, for severe

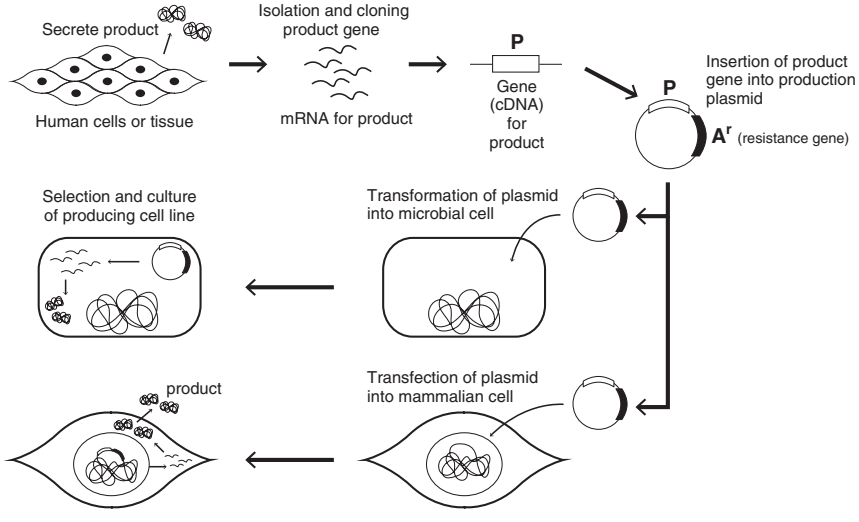

Figure 1.2 Expression of recombinant DNA (rDNA) proteins in host cells.

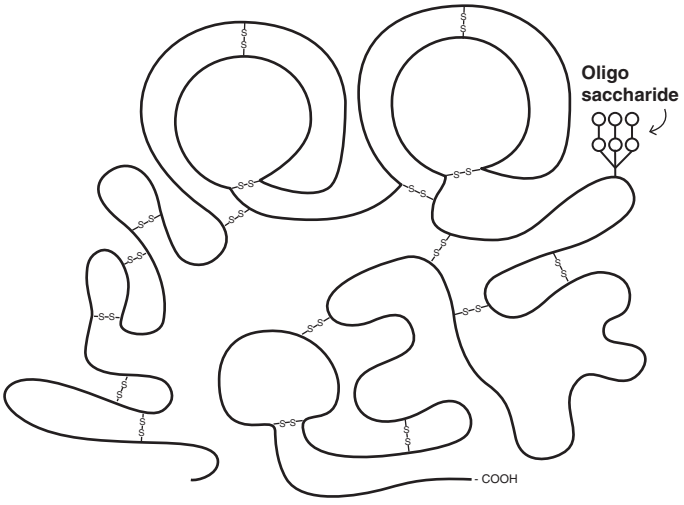

Figure 1.3 Examples of posttranslational modifications that necessitate the expression of heterologous therapeutic proteins in mammalian cells. The posttranslational modifications frequently encountered include disulfide bond formation and glycosylation.

anemia patients) led to an expansion of the use of these factors in the clinic and their commercial success. In the past quarter century, the commercial value of these so-called "biologics" grew to over US$80 billion per annum worldwide by 2015. Most notably, the success of antibody-based proteins and, more recently, fusion proteins containing the constant region of immunoglobulin molecules (Fc proteins) has primarily contributed to this expansion.

1.1.3 A Typical Bioprocess

Most biotechnological processes using microbes or mammalian cells for producing products follow a similar *modus operandi* (Panel 1.1). At the heart of the process are the "producing cells." The producing cells, once established and characterized, are replicated in large quantities and aliquoted into smaller quantities. Each aliquot is used to start a separate production process, sometimes called a "production train" or "production run." Typically, microbial-producing cells are lyophilized (i.e., freeze-dried), and mammalian cells are cryopreserved using liquid nitrogen.

Panel 1.1 Unit Operations of a Typical Bioprocess

- Sterilization
- Raw materials preparation
- Innoculum preparation (cell expansion)
- Production in bioreactors
- Recovery
- Derivatization
- Formulation
- Packaging

To start a production process, cells in a vial are reactivated after being placed into a test tube or flask of media (Figure 1.4). This starter culture is serially transferred into larger culture volumes, until the necessary number of cells needed for inoculation into production fermenters is reached. During serial expansion, the volume ratio for consecutive cultures ranges from 1:20 to 1:50 for microbial cells and from 1:5 to 1:10 for mammalian cells (Figure 1.5). During the cell expansion phase, cells are maintained in a state of rapid growth.

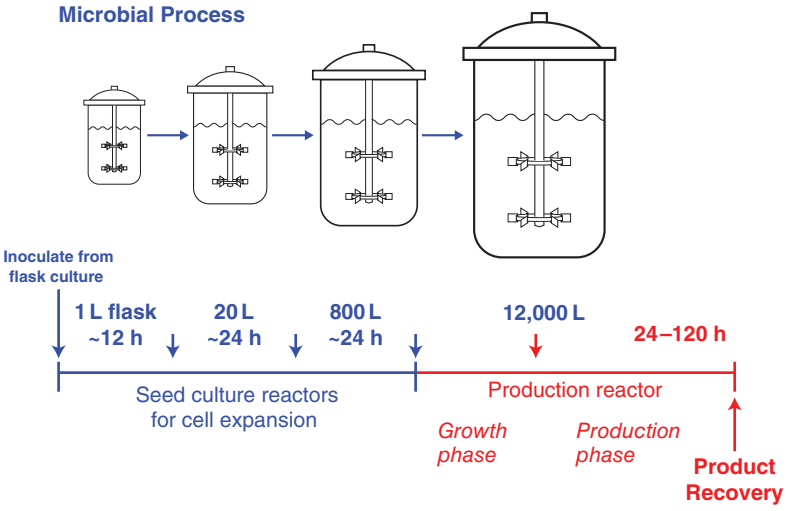

Figure 1.4 A typical manufacturing process of microbial product.

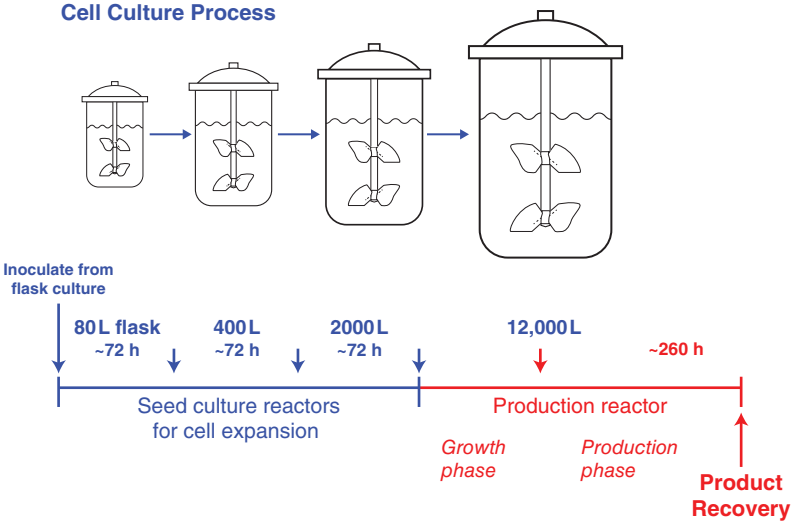

Figure 1.5 A typical manufacturing process of therapeutic proteins by mammalian cells. The duration of each expansion stage is longer and the size ratio of consecutive bioreactors is smaller than those in microbial fermentation.

In the production reactor, cells are further expanded until the cell density reaches a high level. In many cases, the product is formed when cells are in the expansion stage. In other cases, the majority of product is formed in the production stage, after the cell expansion stage is over. The production phase may be triggered by adding an inducer for the desired product to induce gene expression, or otherwise changing culture conditions to favor production formation. To enhance productivity, researchers often prolong the duration of the viability and activity of cells in the production phase.

The running time of the production reactor is typically longer than it was in each seed culture reactor. Upon the completion of fermentation, culture broths are processed to recover product. In many cases, the fermentation process produces an intermediate product. After recovery, the intermediate product has to be further modified to become the final product.

1.1.4 Biochemical Engineering and Bioprocess Technology

In the 1940s, the emergence of submerged cultures for microorganisms led to the adoption of stirred-tank reactors. The subsequent research on bioreactor development coincided with a period of new trends for reactor analysis in chemical engineering. For example, the need to sterilize the media and the reactor before fermentation led to research on the kinetics of thermal cell death. The search for better media supported high-productivity stimulated studies on microbial growth requirements and inspired the application of stoichiometric principles to microbial fermentation.

Like the chemical reaction engineering research, kinetic models of cell growth and product formation for microbial culture also began to take shape (Figure 1.6). A mathematical formula describing the response of growth rate of *E. coli* to glucose concentration, often called the Monod model, began to be adopted in the 1950s.

Driven by the wide application of continuous stirred-tank reactors in chemical reaction engineering research, continuous culture became a powerful tool to explore the dynamic behavior of microorganisms grown either as a single-population pure culture or as a mixed culture of multiple microorganisms. These studies set the foundation for quantitative analysis of cell cultivation for the next few decades.

Sustaining a sufficient oxygen level is pivotal when scaling up a microbial culture. Studies in gas–liquid interfacial mass transfer led to the development of principles for scaling up. As they grow, many molds and mycelial bacteria generate a culture broth that is essentially a non-Newtonian fluid. Alternatively, they may grow as an aggregated cell mass (i.e., a pellet), with some pellets being relatively large in size. The diffusion of oxygen inside a microbial pellet is more limited than diffusion through culture medium. For this reason, intensified studies have been devoted to transport phenomena in fermentation.

In some processes, cells or enzymes are immobilized in porous solids so that they can be retained in a continuous biocatalytic reactor and reused. The transfer of substrate and product in the solid phase may restrict the production rate. This interplay between reaction and diffusion is another area of study for biochemical engineers.

Following the development of models for describing the kinetics of microbial growth came the need to better control bioprocesses for maximal productivity. In the late 1960s, much effort was devoted to online instrumentation, leading to the use of computer-controlled bioreactors in the 1970s. Online nutrient feeding based

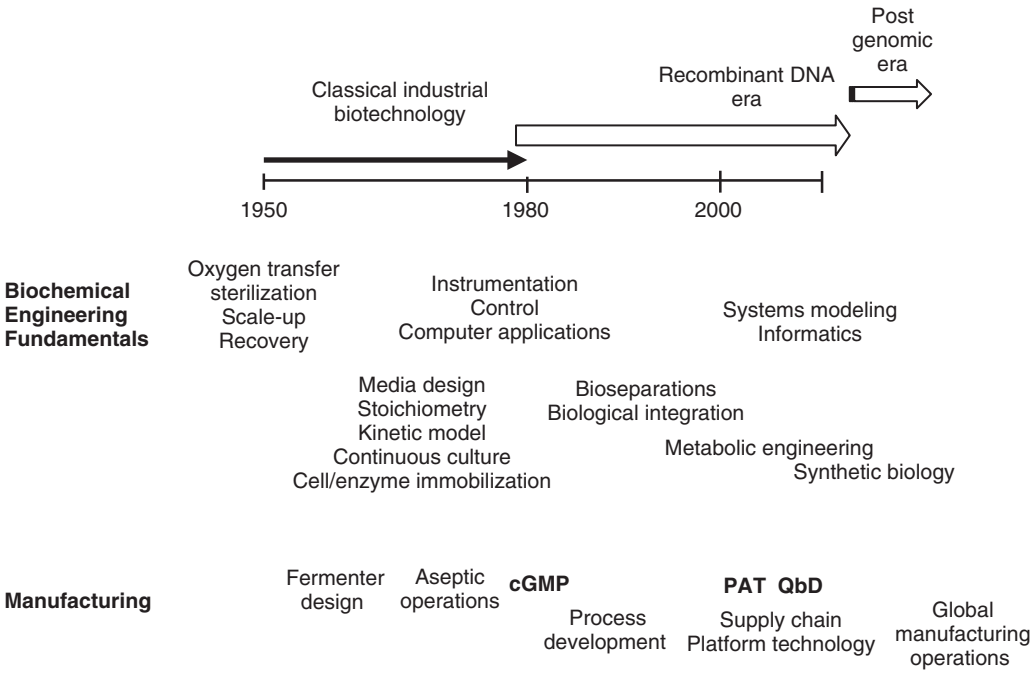

Figure 1.6 Subjects of studies in biochemical engineering as bioprocessing advances. In the manufacturing of industrial products, technological development evolved from reactor design to aspect control (to reduce the contamination rate in the 1950–1970s) to implementing current Good Manufacturing Practice (cGMP) and establishing process development infrastructure in the 1980s–1990s. More recently, emphasis has been on developing Process Analytical Technology (PAT), Quality by Design (QbD), and platform technology that will be suitable for different products. Current interests include the search for transformative technology that may further cut the process development timeline and reduce the cost of goods.

on computer-administered algorithms enabled the exploration of controlling a cell's physiological state. Such efforts extended into the 1980s. Increased computational power and the drive to further enhance productivity also facilitated the development of mathematical models, online data acquisition, signal processing, and control strategies.

The beginning of recombinant protein research triggered major changes throughout the field of biochemical engineering. The demand for the new products posed two challenges: first, the purification of product proteins from recombinant *E. coli* cells; and second, the cultivation of mammalian cells in large-scale bioreactors. Many new conceptual frameworks for bioreactor and product isolation were explored in the 1980s and the first half of the 1990s. These increased research efforts and expanded corps of biochemical engineers contributed significantly to the rapid expansion of the rDNA protein-based industry.

Genome sequencing in the past two decades has had huge impact on biological sciences and health care, as well as many technological areas. Importantly, genomic information has facilitated the rapid discovery of proteins and enzymes from remote species. Some of these have proven useful for modifying a pathway or even synthesizing a new pathway, and they have led to advancements in metabolic engineering and

synthetic biology. Advances in desktop computing have also made mathematical analysis readily accessible to engineers. This has led to the permeation of systems analysis into biochemical engineering research.

Along with the development of biotechnology and biochemical engineering, manufacturing bioprocess technology has also evolved over the years. The field's early focus was on bioreactor design and operation. The subsequent need for generating more sophisticated protein molecules (e.g., for injection into patients) has since facilitated the development of cGMP (current Good Manufacturing Practice) in biomanufacturing. This ensures a consistently high-quality product. cGMP practices have led to the emphasis on PAT (Process Analytical Technology) and QbD (Quality by Design) in product development, to better monitor process variables in real time and to implement control strategies to ensure the quality of final products.

Biochemical engineers in industrial environments have also been increasingly engaged in ensuring a steady and consistent global supply of raw materials and product inventory. Market globalization has resulted in a more distributed manufacturing process, meaning that many products are produced in multiple sites over the globe, but that all sites must produce products of the same quality. For this reason, contract manufacturing organizations (CMOs), which specialize in making products for the "innovator" or the originator of products, have become more common. This allows the originator to focus on the function it performs better, such as the research and development of new technologies and products.

To speed up product development, many firms have adopted the strategy of platform technology. In this scenario, the same platform is used for different products. For example, a host cell that has already been optimized to secrete enzyme products and is being actively used in product development may also be used to produce all future industrial enzymes, even if another host cell may be theoretically better. This is because the time and effort required to optimize a new host cell can be very high and costly.

1.2 Industrial Organisms

The workhorses of biotechnology encompass a wide array of organisms, from prokaryotic and eukaryotic cells to multicellular organisms, like plants or animals. The vast majority of industrial cultures carried out in bioreactors employ unicellular organisms (like bacteria, yeast, and mold) or cells derived from tissues of animals and plants. In some cases, somatic embryos or shoots of plants may be propagated in a bioreactor. In a few rare cases, an entire organism (such as insect larvae) may be grown in a reactor environment to produce vaccines or bio-insecticides.

Until the introduction of rDNA, virtually all industrial products were derived from their native producers. However, few of these natural organisms are able to produce their product at a level that is suitable for economical industrial production. To overcome this limitation, the organisms are mutated or otherwise improved to enhance their productivity.

In the rDNA era, this changed. Now, many proteins can be easily expressed in host cells for which genetic tools are widely available, such as *E. coli*, the yeast *Saccharomyces cerevisiae*, and even some mammalian cells. An entire metabolic pathway can be introduced into a microorganism to make it produce a new and nonnative compound at a very high level.

Industrial microorganisms can be classified according to the class of products they produce or by their taxonomy (Panels 1.2 and 1.3). Some genera have a certain propensity for producing certain classes of compounds. For example, members of *Streptomyces* are known for being producers of antibiotics, while species in *Corynebacterium* are most amenable for producing amino acids.

Panel 1.2 Industrial Organisms

Prokaryote
- Eubacteria
  - *Unicellular*
  - *Mycelial – Actinomycetes*
    - *(Streptomyces, Nocardia)*

Archaea (Extremophiles)

Eukaryotic Cells
- Fungi
  - *Yeast*
  - *Mold*
- Algae
- Protozoa
- Insect cells
- Plant cells, tissues, and organs
- Animal cells, tissues, and organs

Transgenic Animals and Plants

Panel 1.3 Biotechnological Products

Metabolic Process
- Food
- Waste treatment
- Mineral leaching
- Bioremediation
- Degradation of toxic compounds

Metabolites
- Primary metabolites
- Secondary metabolites

Secreted Biopolymers
- Extracellular polysaccharides
- Extracellular proteins

Cells, Tissues, and Their Components
- Cells
- Enzymes
- Polysaccharides
- Viruses

The boundaries among those product-based classes became blurred with the introduction of rDNA. A few microorganisms (such as *E. coli*, a few species of yeast, as well as CHO cells) have become the favorite hosts for industrial production for a wide range of products. However, the overall vast majority of products are still produced by their native producers after undergoing some strain improvement work in the laboratories.

1.2.1 Prokaryotes

1.2.1.1 Eubacteria and Archaea

Prokaryotic microorganisms constitute the majority of industrial organisms. Industrially important unicellular microorganisms include eubacteria ("true bacteria") and archaea. Microorganisms in both orders lack a nucleus, and their cytosol and its chromosome are enwrapped in a cytoplasmic membrane, which is encased in a cell wall.

The industrially important bacteria are very diverse. A common classification of bacteria is based on Gram staining of their cell wall. They are generally classified as Gram-positive (e.g., *Bacillus*) or Gram-negative (e.g., *E. coli*) bacteria, both of which are represented among industrial bacteria. They may also be aerobic and require oxygen to grow (e.g., *Corynebacterium*), or be anaerobic (e.g., *Clostridium*). For example, *Clostridium acetobutylicum* was used to produce acetone and butanol over a hundred years ago, while *Corynebacterium glutamicum* has been used in the production of glutamic acid since the 1950s.

Some bacteria are capable of differentiation; for instance, *Bacillus* can differentiate to form spores. One family of microorganisms, the Actinomycetaceae, includes many species in the *Streptomyces* genus and produces many important antibiotics. The Actinomycetaceae bacteria grow as mycelia. The mycelia protrude into the air, where they become aerial mycelia, and on a solid agar medium, mycelia protrude into the agar, where they form substrate mycelia. The aerial mycelia can fragment and form spores, which help the bacteria to counter adverse environmental conditions.

Microorganisms in the Archaea domain resemble eubacteria in most respects. They are morphologically similar and can be Gram-positive or negative. Members of the Archaea domain have rather unusual metabolic characteristics. Many are extremophiles, meaning they thrive in very extreme and hostile environments. They can be found living in conditions with high salt concentrations (extreme halophile) or high temperatures (extreme thermophile).

The methanogenic archaea, which grow under strict anaerobic conditions, use CO_2 and H_2 to produce CH_4 and derive energy. Other than photosynthetic organisms, few living systems are capable of fixing CO_2; these archaea use H_2 as an energy source instead of light for this process. Microorganisms in this order produce many useful enzymes that are stable in extreme conditions, such as high-temperature stable DNA polymerases, used in polymerase chain reactions (PCRs), and other high-temperature industrial enzymes. However, their preference of growing under extreme conditions also poses challenges for their cultivation.

1.2.2 Eukaryotic Microorganisms

Eukaryotes all share a common characteristic: a true nucleus. Eukaryotic organisms encompass microorganisms like yeasts, molds, and algae. Cells of plants and animals are

also eukaryotic cells. In eukaryotic cells, lipid bilayer membranes are present not only on the cell surface, but also surrounding the various organelles. These lipid bilayers segregate the cell into different compartments, and allow each compartment to have different kinds of chemical environments for carrying out specialized functions. For example, the highly oxidative environment of the lumen of mitochondria, from which most cellular energy [adenosine triphosphate (ATP)] is derived, is segregated from other parts of the cell. Protein molecules that are destined for secretion are translocated into the endoplasmic reticulum (ER) while they are being translated, so that protein folding can proceed with the help of the various chaperone proteins in the ER. Such compartmentalization gives eukaryotes functional complexity and sets them apart from prokaryotes.

1.2.2.1 Fungi

Both yeasts and filamentous molds are fungi. The best-known industrial yeast is the ethanol-fermenting *S. cerevisiae*. Some yeast strains are used to produce citric acid, enzymes, and specialized lipids. Others are capable of growing on methanol or hydrocarbons.

Molds, as commonly seen in spoiled foods, are widely used in the food industry. Many industrial enzymes for hydrolyzing protein (proteases), starch (amylases), or cellulose (cellulases) are produced by molds. Molds produce many important antibiotics, including penicillin and cephalosporin. Recently, yeast cells have also been used for producing heterologous recombinant proteins. A notable one is the hepatitis B surface antigen, which is used as vaccine against hepatitis B virus.

1.2.2.2 Algae

Algae are used for the industrial production of specialty compounds, such as carotenoids. They are more frequently used as a dried cell mass for health food supplements and animal feed, without extracting the product out of cells. Different from traditional bioprocesses, algae are also used in environmental processes, often in an open environment (such as open ponds or circulating pipes). In some cases, the cultivation is carried out as a mixed culture of algae and other microorganisms. These cultures have the capability of performing photosynthesis and fixing CO_2, and they are being explored as a vehicle for biodiesel production.

1.2.3 Multicellular Organisms and Their Cells

1.2.3.1 Insect Cells

Insect cells can be used for the production of an insect virus, which is used as an insecticide. Some insect cells are also good vehicles for expressing different proteins that can subsequently be used as vaccines, especially in veterinary applications. The use of insect cells for producing human protein pharmaceuticals, however, has not been successful. Most human therapeutic proteins are glycosylated, but the glycosylation carried out in insect cells gives rise to glycans of different structures than those synthesized in mammals. Thus, proteins made in insect cells are unsuitable for administration to patients.

1.2.3.2 Plant Cells, Tissues, and Organs

Plant tissues have long been the source of many medicinal substances, pigments, fragrances, and enzymes. It is thus natural to try to isolate and cultivate plant cells, or

even tissue, for the production of those compounds. Isolated plant cells may be grown as relatively disperse cells, or as cell clusters, called "callus." Some plant metabolites are produced in differentiated tissues and transported to another tissue for maturation. The complexity of biosynthesis in plant tissues is greater than it is in microbial metabolites.

Although much progress has been made over the past couple of decades, the concentrations of product that can be achieved using cultured plant cells are still generally low. However, the successful transformation of plant metabolite production to an industrial bioreactor operation will ensure steady supplies and minimize the susceptibility to supply chain interruption due to natural or social-political incidents.

Plant tissues or organs are cultured for propagation purposes. Bioprocess methods may be applied to grow somatic embryos or shoots (stems to allow for generating cultivars), a process often called "micropropagation." By using somatic embryos, it is possible to generate clones of progeny of the same somatic cell, thus greatly enhancing the consistency of the product.

1.2.3.3 Animal Cells, Tissues, and Organs

Animal tissues have long been used to produce many enzymes for use in medicine. Insulin was first isolated from pig pancreas before its recombinant product became available. The pituitary gland of cadavers was the source of human growth hormone to treat dwarf syndrome. Factor VIII isolated from human blood was used to treat hemophilic patients. Animal cells isolated from tissues and cultivated *in vitro* are now used to produce virus particles for vaccines. Prior to the wide application of cultured animal cells, virus production was often carried out using animals. The shift of viral vaccine production to cultured animal cells has made the production process more robust and the product more amenable to quality control.

Many human proteins of therapeutic value are now produced as heterologous proteins in host cells. Some are produced in *E. coli* (such as insulin and human growth hormone) or yeast (such as cytokines and granulocyte stimulating factor). However, many human proteins require glycosylation and other posttranslational modifications to be clinically effective. The production of those proteins typically requires the use of mammalian cells as the production vehicle.

Unlike traditional biochemical products, which are produced by a large array of microorganisms, the host mammalian cells used for rDNA production are limited to a handful of cell lines. In fact, CHO cells are used for the production of over 80% of mammalian cell-based rDNA products. Other cells used include mouse myeloma-derived cells and Syrian hamster kidney cells.

1.2.4 Transgenic Plants and Animals

Genetically modified organisms (GMOs), especially transgenic plants, have been used in the field since the 1990s. An engineered resistance to insects and herbicide, carried in transgenic plants like corn, soybeans, and cotton, has greatly increased the yield of those products. The resistance to insecticide can be accomplished by cloning a protein, often derived from *Bacillus thuringiensis*, into the plant genome. The protein is lethal to many insects once ingested. Herbicide tolerance allows for selective removal of weeds and preservation of the engineered plant (e.g., soybean) upon herbicide application.

For corn and cotton, GMOs are planted in more acreage than nonengineered crops in the United States.

Transgenic farm animals, on the other hand, have faced more resistance in customer acceptance and regulatory approval. Many cows are given bovine growth hormone (BGH) to increase their efficiency of feed consumption and milk production. Transgenic cows that endogenously produce a higher level of BGH similarly produce more milk. Farmed salmon have been engineered to express higher levels of growth hormone, to extend their growth season and speed their growth. Pigs have also been engineered to produce less polluting excretes. These modifications, however, are not acceptable to most consumers, so the use of GMO animals in the consumer market is still being debated.

In the past decade, transgenic organisms, including plants and animals, have been explored as a production vehicle for biopharmaceuticals. Antitrypsin and protein C (a clotting factor) have been produced in the milk of goats and pigs, respectively. Recombinant antibody has been produced in corn. It is anticipated that the costs of goods for products produced in transgenic organisms will be substantially lower than those produced by cultured cells in bioreactors.

Transgenic technology, while widely used in crops, is still in the exploratory stage for animals. Environmental concerns are a lingering issue. This is even more applicable to plants, as they are cultivated in open fields and it is unavoidable that escaped pollen will eventually cross-contaminate native plant species.

1.3 Biotechnological Products

Biotechnological products can be classified by their chemical nature (e.g., carbohydrates or proteins), application (e.g., agricultural or medical), or source (e.g., animal or microbial products) (Panel 1.3). Tables 1.1 to 1.6 list examples of biotechnological products by their areas of application. In this section, we will group and discuss products by their source or chemical nature.

1.3.1 Metabolic Process

Humans have been cohabitating with microbes since the dawn of existence. For thousands of years, humans have unwittingly taken advantage of their metabolic processes while making wine, bread, and cheese. In more recent decades, with specific knowledge of the microorganisms involved, we intentionally select specific variants of microbes to better control those processes. For example, by using a yeast strain that has a particular pattern of carbon dioxide release, one can better control the texture of bread. In such applications, no particular compound is isolated as a product; rather, it is the process of metabolism that is being exploited.

Metabolic process is the centerpiece of a number of biotechnological applications. In wastewater treatment, a mixture of microbes grows and metabolizes the organic materials in a waste stream to reduce the level of organics below a threshold. Likewise, in some detoxification processes, microbes make certain compounds less toxic, such as chlorinated organics, pesticides, and herbicides. In other cases, microorganisms reduce the metallic oxide in mineral ore to facilitate the extraction of metals from the ore.

Table 1.1 Microorganisms used in food processing.

Food and beverages	
Yeast	
Saccharomyces cerevisiae	Baker's yeast, wine, ale, sake
Bacteria	
Lactobacillus sanfranciscensis	Sour French bread
Streptococcus thermophilus	Yogurt
Lactobacillus bulgaricus	Yogurt
Gluconobacter suboxidans	Vinegar
Mold	
Penicillium roquefortii	Blue-veined cheeses
Penicillium camembertii	Camembert and brie cheeses
Aspergillus oryze	Sake (rice starch hydrolysis)
Agriculture	
Entomopathogenic bacteria	
Bacillus thuringiensis	Bio-insecticides

Note: Their metabolic process contributes to the product. Microbial cells are also used directly as product, as in insecticide and baker's yeast.

Table 1.2 Some primary metabolites and their producers.

Industrial chemicals	
Yeast	
Saccharomyces cerevisiae	Ethanol
Bacteria	
Clostridium acetobylicum	Acetone and butanol
Xantomonas campestris	Polysaccharides
Mold	
Aspergillus niger	Citric acid
Bacteria	
Corynebacterium glutamicum	L-lysine, monosodium glutamate

Note: Many are industrial chemicals or bulk biochemicals.

Table 1.3 Examples of additional metabolites as industrial chemicals.

Other industrial chemicals and producing organisms	
Polysaccharides	
Bacteria	
Leuconostoc mesenteroides	Dextran
Xanthomonas campetris	Xanthan gum
Vitamins	
Yeast	
Eremothccium ashbyi	Riboflavin
Bacteria	
Pseudomonas denitrificans	Vitamin B_{12}
Propionibacterium	Vitamin B_{12}

Table 1.4 Examples of industrial proteins produced in native producers and in recombinant hosts.

Secreted protein products and producing organisms	
Industrial proteins	
Enzymes	
Mold	
Aspergillus oryzae	Amylases
Aspergillus niger	Glucoamylase
Trichoderma reesii	Cellulase
Saccharomycopsis lipolytica	Lipase
Aspergillus	Pectinases and proteases
Endothia parasitica	Microbial rennet
Bacteria	
Bacillus	Proteases
Heterologous industrial proteins	
Aspergillus niger	Albumin (for industrial use)
E. coli	Bovine growth hormone

1.3.2 Metabolites

Microbial metabolites constitute the bulk of bioproducts. In the first half of the twentieth century, microbiologists learned how to harness microbes to make metabolites. Natural microbes make metabolites for their own needs, usually only at the time and in the exact quantity that is needed. In the laboratory, microbiologists first identify and isolate microbes that produce those useful metabolites, then introduce mutations in them to make them produce those metabolites, typically at levels thousands of times above what is natural in order for the production to be economical. For almost every common metabolite that is useful to humans (e.g., acetic acid, ethanol, citric acid, and lysine), scientists have isolated organisms to produce it.

Metabolites are largely divided into primary metabolites and secondary metabolites. "Primary metabolites" are terminal products or intermediates of the cell's catabolism and anabolism. These are generally the intermediates of energy metabolism (such as ethanol, citric acid, or succinic acid) or products of the biosynthetic pathways of cellular building blocks (such as glutamic acid and lysine) (Table 1.2). Primary metabolites are essential for the growth of the producers.

In contrast, many natural products produced by microorganisms and plants are seemingly nonessential, meaning that if cells were not able to synthesize the product, their growth would not be affected. These metabolites are called "secondary metabolites" (Table 1.5). Many pharmaceutical drugs, such as antibiotics, fall into this category. These secondary metabolites are usually produced after the period of rapid growth is over. In nature, antibiotic biosynthesis is possibly a mechanism of the microbe's self-defense. Under adverse conditions, when growth ceases, antibiotics are produced to retard the invasion of other microorganisms into the producer's habitat.

Following the discovery of penicillin, scientists began to search for microbes in soils, lakes, oceans, and plants that produce metabolites with antimicrobial activities. These products are often called natural products. These activities have greatly expanded the

Table 1.5 Some secondary metabolite producing organisms and their products as drugs.

Pharmaceuticals	
Drugs	
Mold	
Penicillium chrysogenum	Penicillins
Cephalogporium acremonium	Cephalosporins
Rhizopus nigricans	Steroid transformation
Bacteria	
Streptomyces	Amphotericin B, kanamycins, neomycins, streptomycin, tetracyclines, and others
Bacillus subtilis	Bacitracin
Bacillus polymyxz	Polymyxin B
Mycobacterium	Steroid transformation
Norcadia autotrophica	Pravastatin

Table 1.6 Examples of pharmaceutically important biologics, including viral vaccines and recombinant proteins.

Pharmaceuticals **Biologic products and their sources**	
Mammalian cells for virus vaccines	
Human lung fibroblast MRC-5	Attenuated hepatitis C virus
Monkey kidney epithelial (Vero) cell	Inactivated polio virus
Heterologous expression of human proteins	
Mammalian cells as hosts	
Chinese hamster ovary (CHO) cells	Antibodies Interferon, tissue plasminogen (tPA) activator, erythropoietin (EPO)
Human kidney 293 cell lines	Adenovirus for gene therapy
Bacteria as hosts	
Escherichia coli	Insulin, human growth hormone, bovine growth hormone
Yeast as hosts	
Saccharomyces cerevisiae	Hepatitis B virus surface antigen (vaccine against hepatitis B)
Saccharomyces cerevisiae, *Pichia pastoris*	Serum albumin

repertoire of bioactive metabolites. Many have found medical and industrial applications, including tetracyclines as antibiotics, mitomycin and taxol as anticancer drugs, cyclosporin as an immune suppressor, and various statins for suppressing cholesterol biosynthesis.

1.3.3 Cells, Tissues, and Their Components

A large number of biotechnological products are the biomass of the organism. Baker's yeasts are notable industrial products (Table 1.1). *B. thuringiensis* (BT) is used as a bio-insecticide (Table 1.1). Plant tissues are used widely for the extraction of secondary metabolites for medical use, such as the malaria drug quinine. Plant shoots are used as cultivars. Human cartilage procured from cadavers is used for joint repair.

The potential of producing human tissues through *in vitro* cultivation or tissue engineering has been a growing field. The US Food and Drug Administration (FDA) has approved the culture of a human skin equivalent for patients with severe ulcers or burns. *In vitro* expansion of somatic plant embryos, and their application as artificial seed, has been a reality for nearly three decades.

1.3.3.1 Viruses

Viral vaccines are important to human and animal health care. Most vaccines are produced by infecting cultured animal cells and harvesting the virus released into the culture fluid (Table 1.6). Two major kinds of viruses are used to produce vaccine: attenuated or inactivated. Attenuated viruses are unable to elicit a pathological response, but can still replicate and induce an immunogenic response.

Inactivated viruses are viruses that have been rendered harmless by chemical treatments, such as formalin. Attenuated viruses are live, but have been adapted to become harmless. They are more potent and are required only in very small doses to elicit an immunogenic response. However, with attenuated viruses, there is always a small risk of genetic reversion to render the virus pathogenic. Another disadvantage of attenuated viruses is that they need to be shipped at low temperature to preserve biological activity.

1.3.4 Secreted Enzymes and Other Biopolymers

Many microorganisms secrete proteins, especially enzymes that convert the resources available in the environment to a form that can be used as nutrients. For example, *Bacillus* and *Aspergillus* produce proteases outside of the cell to hydrolyze proteins into amino acids, which are then used as nutrients by those organisms. A mold, *Trichoderma reesei*, produces cellulase to hydrolyze cellulose and use the hydrolysis product for growth (Table 1.4).

In addition to enzymes, microbes also produce other polymers and secrete them into the extracellular space. For instance, *Xanthomonas campestris* produces a polysaccharide called xanthan gum, which makes solutions very viscous and is used as a thickening agent in ketchup and some processed foods (Table 1.2). Many microorganisms that are capable of using hydrocarbon as a carbon source also produce emulsifiers (often lipid-conjugated polysaccharides) to help dispense hydrocarbon droplets and to increase the surface area of this carbon source for their consumption.

1.3.5 Recombinant DNA Products

rDNA technology opened a new era in biotechnology (Table 1.6). While the transformation has been visible in medicine, agriculture, and chemistry, its impact was most immediate in medicine. Many therapeutic proteins, which were at one point in short supply, became more widely available through rDNA technology. Success in the agriculture sector was also apparent through the cloning and engineering of the gene responsible for a desired trait, such as insect resistance, into a plant.

rDNA technology also enables one to alter the metabolic pathway of an organism to enhance the production of a metabolite, or even to introduce a new pathway for a novel product. Such metabolic engineering is now common practice in both microorganisms and plants.

1.3.5.1 Heterologous rDNA Proteins

The deficiency of some biologically active proteins has been at the root of many congenic diseases and has been known to be the cause of many pathological conditions. Before the rDNA era, many therapeutic proteins, including Factor VIII, insulin, and human growth hormone, were isolated from human blood, cadavers, or animal tissues. Production relied on pooling a large number of procured human or animal

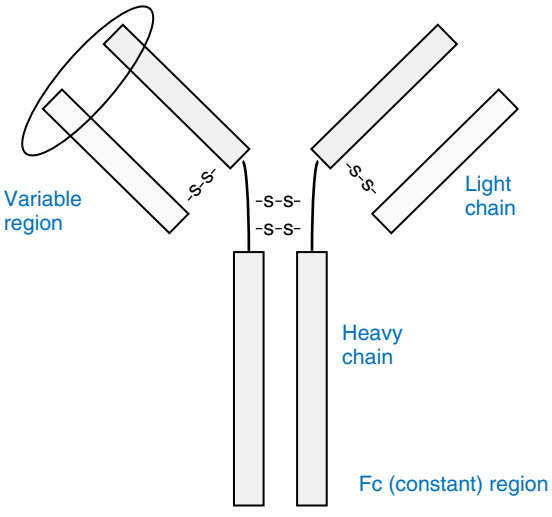

Figure 1.7 Structure of an immunoglobulin-G (IgG) molecule. A major class of therapeutic proteins that has emerged in the past 15 years is antibodies, in particular IgG. For example, anti-VEGF (vascular endothelial growth factor) suppresses blood vessel formation in some tumors. Each IgG molecule has two heavy chains and two light chains that are linked by disulfide bonds and segregated into a constant region and variable region. The variable region recognizes antigen. Each IgG has two antigen-binding sites.

tissues. The detection and exclusion of contaminated or contagious tissues was not always easy. Compounding this difficulty were previously unknown or undetectable infectious agents, such as HIV in the 1980s. rDNA technology allows these proteins to be produced under well-controlled conditions in large quantities, free of human virus contamination.

The use of a recombinant protein as a therapeutic agent quickly expanded beyond supplementing a deficiency in patients. Antibodies and other binding proteins are now also used to block disease-causing processes (Figure 1.7). For example, trastuzumab (trade name, Herceptin), an immunoglobulin G (IgG) against epidermal growth factor receptor that is overexpressed in some breast cancers, is used to treat HER2-positive breast cancer patients.

Many of those proteins are not native to the human body, but are designer proteins made of domains of human proteins (such as the Fc region of the IgG molecule) and an activity domain for the induction of a biological response. An example of such a fusion protein is etanercept (trade name, Enbrel), which is the fusion product of tumor necrosis factor receptor 2 (TNFR2) and the Fc fragment of IgG. It binds to tumor necrosis factor α (TNFα), the master regulator of the inflammatory response, and is used to treat rheumatoid arthritis.

These protein medicines, along with viruses and cells, are classified as "biologics" by drug regulatory agencies. This is to distinguish them from the traditional chemical drugs, which are called "drugs." Traditional drugs can be very well characterized in terms of their chemical composition, structure, purity, and contaminants. Once characterized, the quality of the product is not in doubt. Biologics, on the other hand, cannot be completely defined by their chemical composition, and sometimes a complete chemical characterization is not even possible. For example, the chemical composition of cells or viruses cannot be entirely defined due to their enormous complexity. Furthermore, their chemical composition may differ in different growth conditions. For instance, a therapeutic protein may have the same primary amino acid sequence, but may have a different glycosylation profile depending on how it was produced. Biologics are thus much more prone to variations caused by process changes. The regulation on their commercial availability is thus much more stringent than it is for drugs.

The use of rDNA for protein production goes beyond human applications. It has also become a powerful vehicle for the production of industrial proteins and enzymes. Many microbes that produce attractive industrial proteins are not easily cultured at an industrial scale because of any one of a number of factors, such as slow growth, low productivity, special nutrient requirements, unfavorable temperature, or possible pathogenicity. Those proteins are instead produced in a number of industrial hosts, including *E. coli*, yeasts, and fungi. Notable products include enzymes for food processing, proteases used in detergents, BGH for the cattle industry, and human collagen and serum albumin for medical use.

1.3.6 Metabolic Engineering and Synthetic Pathways

The repertoire of metabolite products grew dramatically during the classical period of industrial biotechnology. The capability to produce a metabolite is largely dependent on the producing microorganism. Often, a particular species or strain is used for a particular compound because of the characteristics of the organism. For example, *C. glutamicum* is used to produce glutamic acid and *A. niger* is used to produce citric acid.

Once the organism producing a compound is isolated, extensive strain improvement is performed to increase its production by orders of magnitude higher than what the microorganism would normally produce. This is often accomplished by a series of treatments by chemicals (mutagens) or irradiation, to increase the mutation frequency so that mutants with the desired properties can be found and isolated (Figure 1.8).

rDNA technology changed the landscape of metabolite production. With rDNA technology, one or several genes can be introduced to change a cell's metabolic reactions in a specific and planned manner. This approach is often referred to as metabolic engineering.

One of the first examples of metabolic engineering was the introduction of an enzyme into a methanol-utilizing bacterium to enable it to use a more energy-efficient pathway to incorporate ammonium into organic nitrogen. Similarly, rDNA was used to enhance amino acid productivity in *Corynebacterium*, by replacing its enzymes with ones that are less prone to feedback inhibition and more energetically favorable, and by changing its transporters for better substrate uptake and product secretion.

The reach of rDNA goes beyond enhancing the productivity in traditional producers like *Corynebacterium*. It also enabled a number of amino acids, including phenylalanine and lysine, to be produced in *E. coli*. Amino acid biosynthesis in *E. coli* is tightly regulated to minimize wasteful overproduction. To engineer *E. coli* to overproduce and excrete an amino acid to a level sufficient for industrial production, biotechnologists replace *E. coli*'s rate-controlling enzymes with ones that are not subject to feedback inhibition or repression.

Through metabolic engineering, microbes can also be made to shunt their metabolite to a new branch to generate a new metabolite. For example, *E. coli* has been engineered to produce ethanol at an efficiency only previously seen from the yeast *S. cerevisiae*.

Metabolic engineering has also been applied to create a synthetic pathway to produce novel compounds. For instance, *E. coli* has been engineered to synthesize the antimalaria drug artemisinin, which is normally produced only in the plant *Artemisia*.

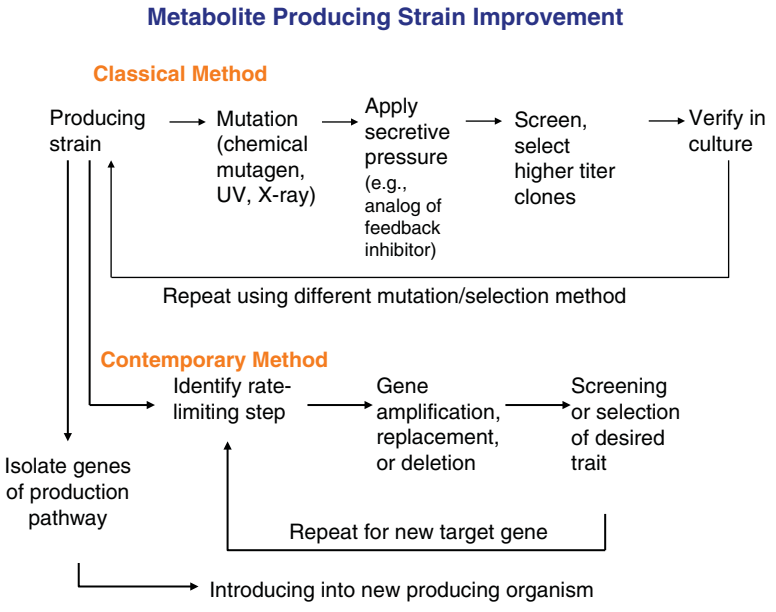

Figure 1.8 Classical and contemporary scheme of strain improvement for the producing microorganisms of industrial chemicals. The classical way is to introduce mutations in the producing organism through mutagenesis to increase the frequency of mutations on the genes that may affect the synthesis of the target metabolite. Sometimes, a selective strategy is available to enrich the mutated higher producing cells. For example, an analog (a compound that has a similar structure to a native compound, has some similar chemical properties such as exerting inhibition on an enzyme, but cannot be metabolized) may be used to cause inhibition of biosynthetic enzyme. Consequently, its biosynthesis in the wild-type microorganism is suppressed, causing cell growth to be retarded. Any mutant that is no longer inhibited by the analog will survive. Such an analog can thus selectively enrich feedback inhibition deregulated mutants. In any case, extensive screening of higher producers after mutagenesis is necessary. The modern method uses direct cloning to introduce, enhance, or eliminate a gene (or genes) to enhance the high-productivity trait. It can speed up the process of obtaining a high producer dramatically compared to the classical method.

1.4 Technology Life Cycle, and Genomics- and Stem Cell-Based New Biotechnology

1.4.1 The Story of Penicillin and the Life Cycle of Technology

Discoveries in microbiology (from the late nineteenth century through the first half of the twentieth century) paved the way for classical biotechnology. Penicillin has proven to be one of the most influential discoveries of this period. The campaign that translated this discovery into a groundbreaking therapeutic medicine also laid the foundation for many other products and new technologies.

Alexander Fleming's discovery in the laboratory was only one piece of this impressive story. Chemists and biochemists pushed forward to isolate and characterize the

compound. Medical researchers and clinicians crusaded to test its effectiveness in animal models and patients. These visionaries had to lead the way and gather the necessary resources to convince manufacturers to make sufficient quantities for use in clinical trials. Only through this combined effort did penicillin become available for more widespread use throughout medicine.

At the time of its discovery, the amount of penicillin attainable in culture was extremely low, making the cost of goods extremely high (Figure 1.9). This initial limited availability made penicillin a high-profit-making drug. Subsequent efforts in strain improvement and process development resulted in a rapid increase in productivity and expanded the capacity of production. Meanwhile, the cost of the drug decreased substantially, but generally remained high and profitable for a couple of decades.

Over time, all products enter a second phase with the end of a product's patent and the entry of generic versions of the drug into the market. At this point, competition begins to drive down the price and profit margin for drugs. In the case of penicillin, no patent was ever applied for. Nevertheless, the maturation of technology allowed the cost of goods to decrease substantially. The lower price allowed more people in more countries to have access to it, and the market expanded quickly. Usually, the total profit from making and marketing a drug is still sufficiently attractive to keep the original lead makers in the market. In this period, increasing competition spurs process intensification and enhances productivity and robustness.

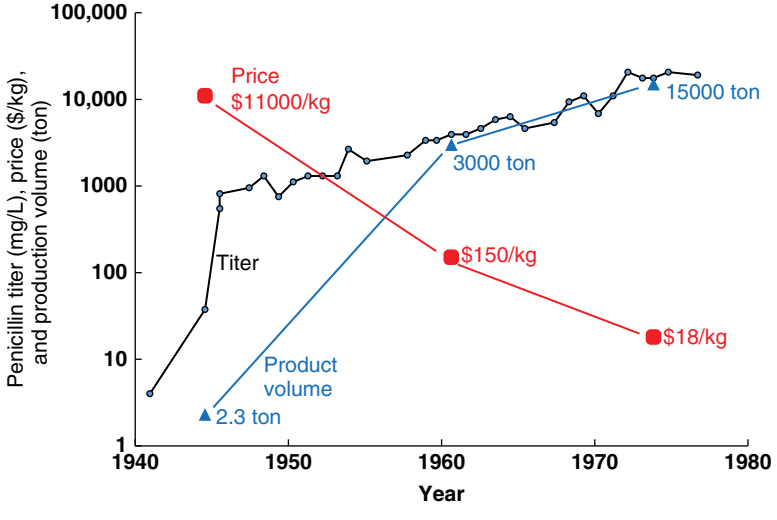

Figure 1.9 The evolvement of penicillin as a technological product. Initial low productivity and production level were quickly enhanced by orders of magnitude due to the high demand created by the revolutionary nature of the product. This is followed by a slower but steady growth driven by the reduced price and wider affordability in different regions of the world. The latter slower growth period entails a much larger total volume of goods and sales. Eventually, the commercial value of penicillin is too low to be profitable for major pharmaceutical companies. The history of penicillin is a general reflection of natural products in classical biotechnology. The decline in classical biotechnological products is supplanted by recombinant DNA (rDNA) technology. The same life cycle will be repeated for rDNA technological products in the years to come.

As a class of drugs, antibiotics (and natural products in general) inspired many discoveries. The number of drugs in this class increased drastically in the 1960s and 1970s. Initially being profitable, the landscape of competitions subsequently drove down the price of antibiotics, and the technology used to produce them became widely practiced. In the 1990s, traditional Western pharmaceutical companies found it more profitable to move on to the new class of enabling biopharmaceutical products. This life cycle pattern is generally applicable for a successful product or class of products and will likely be replayed in the rDNA era.

After the emergence of classical biotechnology, one witnessed an infusion of research funds in life and biochemical sciences from governments and commercial firms that drove the push for new technologies. Rather than traditional pharmaceutical companies, alliances of venture capitals and scientists propelled rDNA into a transformative technology. In the 1980s, the growth opportunity for startup companies across North American campuses was unprecedented. These companies are now global giants like Genentech, Amgen, and Biogen.

Two decades after the introduction of the first wave of rDNA-based biologics, we are seeing the emergence of "biosimilars." This term is used to describe the copy version of therapeutic proteins. Because of the complexity of biologic drugs, regulatory agencies do not permit the same substance produced by companies other than the original producer to enter the market without at least some clinical trials after the original patent expires. These "follow-on" biologics are thus called biosimilars. This distinguishes them from generic small-molecule drugs, which can generally be introduced without conducting clinical trials.

Because of the complexity of the product proteins and the regulatory requirements for product entry, the impact of biosimilars on the landscape of biologics will be more gradual than that of traditional chemical drugs. However, the transition of rDNA biotherapeutics from the exponential expansion phase to a slower growing and momentous stage is inevitable. This transition represents technological maturation in advanced countries but bestows arising opportunities and challenges in other regions of the world.

1.4.2 Genomics, Stem Cells, and Transformative Technologies

While rDNA technology is propelling technological advancement, huge strides have also been made in new areas of science. Mouse embryonic stem cells were isolated in the beginning of the 1980s. Human embryonic stem cells were not established until 1997. These embryonic stem cells are able to differentiate into cells constituting all adult tissues. This ability is called pluripotency.

A decade later, scientists discovered that they could reprogram somatic cells to become pluripotent cells [called induced pluripotent cells (iPSCs)]. This made it possible to generate pluripotent cells from any individual's somatic cells, like skin or liver cells, for possible tissue regeneration. Stem cell technology will likely transform the face of medicine in the future.

Another significant advancement is in the field of genomics. The human genome was sequenced at the dawn of the twenty-first century. Prior to that, a number of yeasts, fungi, and bacteria had their genomes sequenced. Simultaneously, methods were being developed to engineer genomes. While rDNA accomplished the insertion and deletion

of genes, altered segments constitute only a tiny fraction of the genome, even for the smallest bacterial genomes. We are now on the verge of being able to reorganize the cellular genome globally, rather than locally, and to understand how the genome is globally regulated and controlled.

In the twenty-first century, biotechnologists and biochemical engineers will need to learn and relearn systems analysis of biochemical sciences, as new technology is likely to involve regulations at both genomic and biochemical levels in everything from microbes to plant and stem cells to genome-engineered synthetic organisms and communities.

Further Reading

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Problems

A1 List three products for each of the following categories:
- rDNA protein by microbiological process
- Industrial specialty chemicals
- Industrial commodity chemicals

A2 Give a short answer to each of the following:
- Explain the reasons for using mammalian cells to produce therapeutic proteins.
- Describe how animal cells are engineered to synthesize a heterologous rDNA protein.
- Give the names of two primary metabolite products and two secondary metabolite products. Explain what primary and secondary metabolites are.

A3 Do most secondary metabolites exhibit growth-associated or non-growth-associated production kinetics?

A4 Match each term in the first column in Table P.1.1 to a term in the second column. Each term in the second column can be used only once.

A5 Many IgG antibody molecules have important pharmaceutical applications. Are they produced in animal cells or in bacteria? What structural characteristics of IgG dictate the selection of producing cells?

Table P.1.1 Matching terms with close relationships.

A. Penicillin	a. *Saccharomyces cerevisiae*
B. Lysine	b. Primary metabolism
C. Chinese hamster ovary cell	c. Antibody
D. Ethanol	d. Secondary metabolism
E. Glucose isomerase	e. *Clostridium acetobutylicum*
	f. Tissue-type plasminogen activator
	g. High-fructose corn syrup

A6 List two examples of classical biotechnology products produced using each category of microorganism: bacteria, yeast, and mold. Write down the name of the genus and species.

A7 Microbial processes are used to produce product G from substrate A, as shown in Figure P.1.1. Part of the pathway is also used to produce product E. Both E and G are required for cell growth. The first enzyme is inhibited by G and E individually, and repressed by E.

- Discuss briefly how microbiologists use traditional mutation, selection, and screening approaches to direct more substrate A to product G.
- Describe how to use modern metabolic engineering approaches (on genes or proteins) to enhance the production of G.

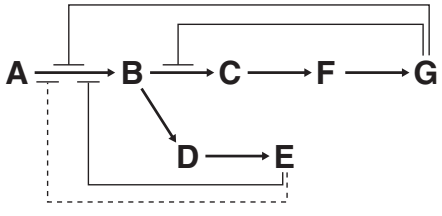

Figure P.1.1 A metabolic pathway producing *G* from *A* with feedback inhibition (solid curve) and feedback repression (dashed curve).