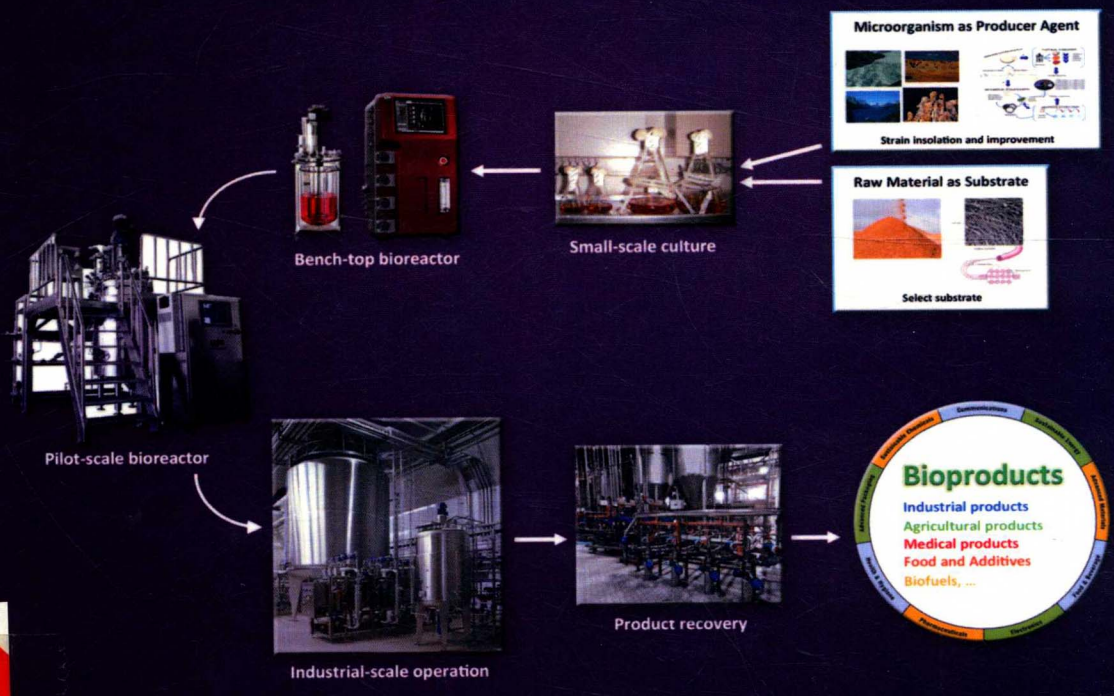
MICROBIAL BIOTECHNOLOGY

Progress and Trends



Edited by
Farshad Darvishi Harzevili
Hongzhang Chen

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Preface

Microbial biotechnology is a technology based on microbiology. Microbial biotechnology is the use of microorganisms and their derivatives to make or modify specific products or processes. Microbial biotechnology is closely related to applied and industrial microbiology. Microbial biotechnology is sometimes considered synonymous with modern industrial microbiology.

The microorganisms used in industrial processes are natural, laboratory-selected mutants, or genetically engineered strains to obtain an economically valuable product or activity on a commercial and large scale. Natural and mixed microbial strains were used in ancient or traditional industrial microbiology, whereas pure or mutant microbial strains are used in classic industrial microbiology. The use of genetically engineered microbial strains began with modern industrial microbiology or microbial biotechnology.

Microbial biotechnology is an interdisciplinary field, and successful development in this field requires major contributions in a wide range of disciplines, particularly microbiology, biochemistry, genetics, molecular biology, chemistry, biochemical engineering, bioprocess engineering, and so on.

Recently, new methods of metabolic engineering, industrial systems biology, bioinformatics, and X-omics science such as genomics, metagenomics, transcriptomics, proteomics, metabolomics, fluxomics, and even nanobiotechnology, have been used to find and modify microorganisms with industrial capacity and their valuable products.

In general, the production of products in industrial microbiology and microbial biotechnology are typically investigated under upstream processes, fermentation processes, and downstream processes.

Microbial Biotechnology: Progress and Trends covers recent developments in some fields of microbial biotechnology. Chapter 1 reviews microbial biotechnology from its historical roots to its different processes. Chapters 2 through 5 discuss some of the new developments in upstream processes. Chapter 6 considers solid-state fermentation as an interesting field in fermentation processes. Chapters 7 through 12 argue about recent developments in the production of valuable microbial products such as biofuels, organic acids, amino acids, probiotics, healthcare products, and edible biomass. Chapters 13 through 15 discuss important microbial activities such as biofertilizer, biocontrol, biodegradation, and bioremediation.

The book is written in simple and clear text, and we also used many figures and tables to make the book easier to understand. Furthermore, case studies are included at the end of some chapters.

Overall, this book will serve as a suitable reference for students, scientists, and researchers at universities, industries, corporations, and government agencies interested in biotechnology, applied microbiology, bioprocess/fermentation technology, healthcare/

pharmaceutical products, food innovations/food processing, plant agriculture/crop improvement, energy and environment management, and all disciplines related to microbial biotechnology.

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On behalf of the authors, I would like to thank Michele Smith (editorial assistant), Marsha Pronin (project coordinator), Rachael Panthier (production editor), Scott Shamblin (cover designer) and their respective teams at CRC Press/Taylor & Francis Group for their valuable efforts to develop our manuscript into a high-quality book.

Farshad Darvishi Harzevili
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Editors

Dr. Farshad Darvishi Harzevili earned a BSc in biology at the University of Guilan, Iran. He earned his MSc and PhD in industrial microbiology and microbial biotechnology from the University of Isfahan, Iran. He is currently a faculty member and head of the microbial biotechnology and bioprocess engineering (MBBE) group at the University of Maragheh, Iran. His main interest is in the biotechnological and environmental applications of yeasts, especially the use of agro-industrial wastes and renewable low-cost substrates in the production of biotechnologically valuable products such as microbial enzymes, organic acids, single-cell oils, biofuels, and so forth. He is also interested in the expression of heterologous proteins, metabolic engineering and synthetic biology of yeasts.

Dr. Hongzhang Chen earned his master's degree in microbiology from Shandong University in 1991, and his PhD in biochemical engineering from the Institute of Process Engineering, Beijing, China in 1998. He is currently a member of the Chinese Academy of Sciences and the vice director of the State Key Laboratory of Biochemical Engineering. His research is focused on ecological biochemical engineering and cellulose biotechnology. Currently, his research explores new types of solid-state fermentation techniques, devices and processes, and clean fractionation using steam explosion techniques for efficient pretreatment in cellulose conversion to useful biorenewable materials.

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chapter one

Microbial biotechnology

An introduction

Farshad Darvishi Harzevili

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1.1 Introduction

There are many definitions for biotechnology. Simply, biotechnology is a technology based on biology, or the use of living systems and organisms to make or develop useful products. The most comprehensive definition was given by the United Nations Convention on Biological Diversity at a meeting held in Rio de Janeiro, Brazil in 1992. In this convention, biotechnology was defined as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use." This definition was signed and accepted by 168 countries up to now.

The biological processes of microorganisms have been used to make and preserve useful food products for more than 6000 years. Microbial biotechnology or industrial microbiology is the use of microorganisms to obtain an economically valuable product or activity at a commercial or large scale. The microorganisms used in industrial processes are natural, laboratory-selected mutant or genetically engineered strains. Economically valuable products such as alcohols, solvents, organic acids, amino acids, enzymes, fermented dairy products, food additives, vitamins, antibiotics, recombinant proteins and hormones, biopolymers, fertilizers, and biopesticides are produced by microorganisms that are used in chemical, food, pharmaceutical, agricultural, and other industries. Biodegradation and biotransformation of complex compounds, domestic and industrial wastewater treatment, biomining, and enhanced oil recovery are examples of microbially valuable activities. According to the UN Convention on Biological Diversity, microbial biotechnology can be defined as any technological application that uses microbiological systems, microbial organisms, or derivatives thereof, to make or modify products or processes for specific use.

1.2 A brief history

Industrial microbiology has a long history and its roots can be traced back to the ancient times of human life, when microbiology had not yet been accepted and developed as a science. Approximately 7000 years BC, the Sumerians and Babylonians used yeast to convert sugar to alcohol. By 4000 years BC, the Egyptians used leaven containing yeast to improve bread quality. Moreover, the ancients knew how to use bacteria and molds for vinegar and cheese production.

The Chinese used molds as antibiotics for the treatment of purulent wounds approximately 500 years BC. This first period, from several thousand years to 150 years ago, is known as the ancient or traditional industrial microbiological period, in which mixed and impure cultures of microorganisms were used in nonsterile conditions to make products (El-Mansi et al. 2012; Soetaert and Vandamme 2010).

Approximately 150 years ago, Louis Pasteur proved the microbial source of fermentation and established industrial microbiology as a science based on scientific principles. During World War I, Chaim Weizmann used *Clostridium* bacterium for the production of acetone and butanol. Then *Aspergillus* mold was used to produce citric acid.

Following Alexander Fleming's discovery of the antibiotic properties of *penicillium* mold, Florey and Chain were able to prepare a pure form of penicillin during the Second World War. In the 1940s, Waksman discovered several aminoglycoside antibiotics such as streptomycin and neomycin. In the late 1950s and 1960s, microorganisms were used to produce amino acids and single-celled proteins, respectively. This second period, 150 years to 40 years ago, is known as the classic industrial microbiological period, in which pure cultures of microorganisms were used in sterile conditions for the manufacture of products. Furthermore, the microbial strains were improved by classic genetic methods such as protoplasm fusion and mutagenesis with physical and chemical mutagens (Glazer and Nikaido 2007).

The third period, which began in the 1970s and continues to the present, is known as the modern industrial microbiology or microbial biotechnology period. The prominent features of this period are its use of recombinant DNA or genetic engineering methods for the improvement of industrial strains and the production of recombinant proteins (Figure 1.1).

1.3 Nature of industrial microbiology and microbial biotechnology

In some sources, the term *biotechnology* was incorrectly substituted for genetic engineering or modification. This mistake originated in the United States where, several years ago, new genetic methods were considered as awful and demonic procedures. Therefore, the term biotechnology was used instead of genetic engineering and producing transgenic organisms to reduce worry and diversion of public opinion. Later, the term was used by the media and politicians, and thus it entered legislation and government documents.

Humans have been using genetic modification with the selective breeding of plants and animals for more productivity over tens of thousands of years. For more than 50 years, classic methods such as protoplasm fusion and mutagenesis have been used for the genetic modification of organisms. Genetic engineering and its equivalent terms, genetic modification or genetic manipulation, are advanced molecular biology techniques that have been used since the 1970s (Smith 2009).

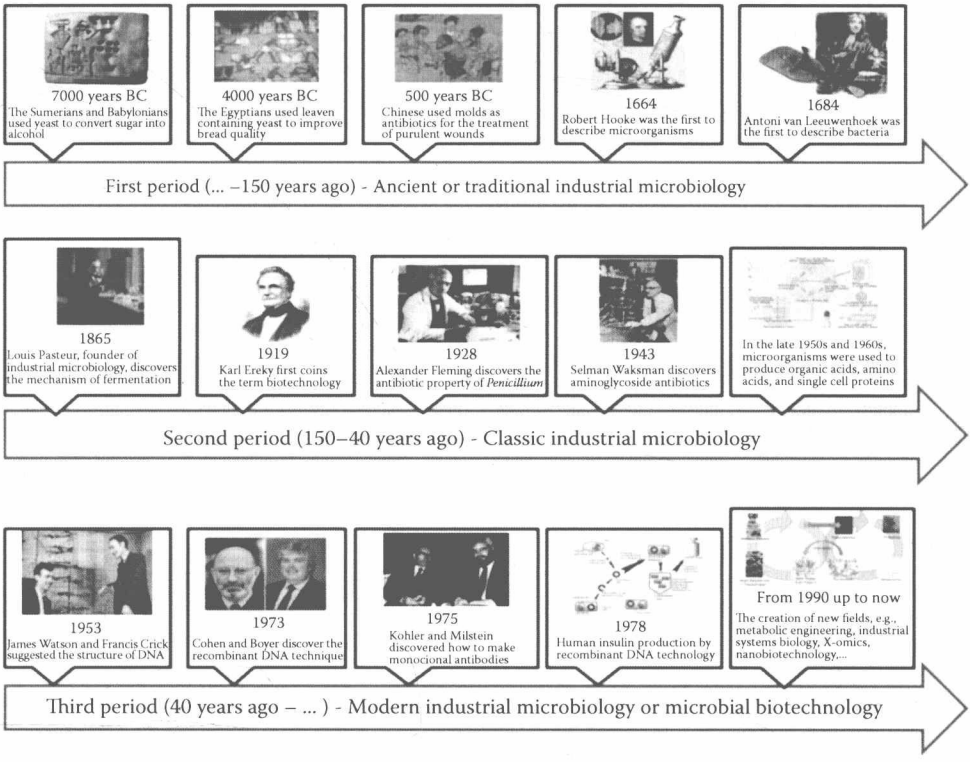

Figure 1.1 Timeline of industrial microbiology and microbial biotechnology.

The term biotechnology was first coined by Karl Ereky in 1917, when molecular genetics and genetic engineering had not yet been discovered. By 1919, in his book entitled *Biotechnology of Meat, Fat and Milk Production in an Agricultural Large-scale Farm*, Ereky described biotechnology as a technology based on converting raw materials into useful products.

Afterward, many definitions were proposed for biotechnology. The application of biological systems, living organisms, or derivatives thereof, to make or modify products is the most comprehensive definition for biotechnology. Biotechnology is not only a science or a set of methods but it is also the interdisciplinary science that encompasses microbiology, plant and animal science, biochemistry, cellular and molecular biology, genetic modification, and engineering fields with biological perspectives such as mechanics, electronics, information technology, robotics, and so on.

The European Federation of Biotechnology considers biotechnology in two categories—"traditional or old" and "new or modern" biotechnology. Several thousand-years–old traditional methods are used to produce beverages, foods, and dairy in traditional or old biotechnology, which is the equivalent of traditional industrial microbiology and classic industrial microbiology. New methods of genetic engineering, which were used from the 1970s to the early 1980s, began the development and evolution of traditional biotechnology to new or modern biotechnology, which is the equivalent of modern industrial microbiology or microbial biotechnology (Smith 2009).

Today, the third wave of biotechnology, known as industrial biotechnology or white biotechnology, is expanding. It has made considerable progress in comparison with the second wave, namely, red biotechnology or medical biotechnology and the first wave, namely, green biotechnology or agricultural biotechnology.

Industrial biotechnology uses biological systems, especially microorganisms, in industrial fermentation processes to produce large quantities of pure materials and energy including alcohols, organic acids, amino acids, vitamins, solvents, antibiotics, biopolymers, biopesticides, enzymes, alkaloids, steroids, and others. Industrial biotechnology is founded on biological catalysts and fermentation technology, and it is associated with advances in molecular genetics, protein engineering, and metabolic engineering of microorganisms and cells.

Recently, new methods of metabolic engineering, industrial systems biology, bioinformatics, X-omics such as genomics, metagenomics, transcriptomics, proteomics, metabolomics, fluxomics, and even nanobiotechnology, have been used to find and modify microorganisms with industrial capacity and their valuable products (Soetaert and Vandamme 2010).

1.4 *Review of main processes in microbial biotechnology*

In the past, many diverse products were derived from natural sources or synthesized through chemical processes; nowadays, some of these products are commercially produced through microbial fermentation and biological conversion processes. The benefits of using microorganisms in such processes includes the ease of mass production, the high growth rate, and the use of cheap substrates which, in many cases, are considered waste products of some industries. In general, the products of industrial microbiology and microbial biotechnology are typically investigated under upstream processes, fermentation processes, and downstream processes (Stanbury et al. 1995).

1.4.1 *Upstream processes in microbial biotechnology*

The upstream processes consider the isolation and screening of microorganisms, strain improvement to produce cheap and abundant amounts of the desired product, industrial strain preservation, development of inoculum, substrate selection, optimization and development of appropriate media, and industrial sterilization of media (Figure 1.2).

Microorganisms (as producing agents) and raw materials (as substrates of fermentation) are important in the upstream processes. Strain improvement is critical in the development of most fermentation industries because it increases production efficiency and reduces production costs. In many cases, strain improvement is performed by conventional mutagenesis and recombinant DNA techniques. Then, a suitable strain for the industrial fermentation process is selected through mutant and recombinant strains.

The choice of medium depends on the scale of fermentation. In the small laboratory scale, mostly pure chemicals are used in media composition. This is not possible for large-scale fermentation due to the high costs. Hence, low-cost complex substrates are mainly used in large and commercial-scale fermentations. Most of these compounds are obtained from plant and animal residues, as well as from other industrial wastes that have variable compositions. The effects of fluctuation must be considered between each group of substrates. These effects on performance and product recovery are examined in the small scale with each group of substrate (Waites et al. 2009).

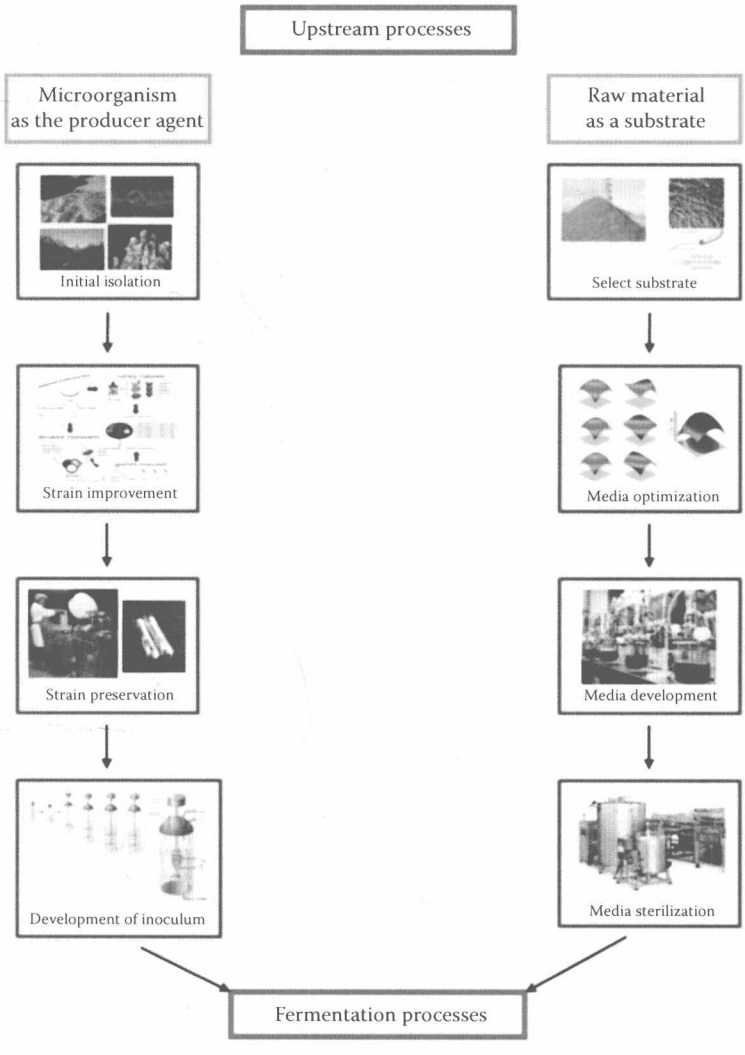

Figure 1.2 Upstream processes in microbial biotechnology.

1.4.2 Fermentation processes in microbial biotechnology

The root word of fermentation is derived from the Latin verb *fervere*, meaning to boil, which describes the boiling appearance of the action of yeast on extracts of fruit or grain during fermentation. Fermentation, from the viewpoint of an industrial microbiologist, is the production of a product by the mass culture of microorganisms under aerobic or anaerobic conditions. Fermentation is usually carried out in a fermentor or bioreactor set, whose main objective is to provide a suitable environment for organisms to produce biomass and metabolites. Fermentation system performance depends on many factors, but the main physicochemical properties that need to be controlled are temperature, pH, oxygen transfer, agitation, and foam level.

Fermentation is performed in simple to complex fermentors consisting of a tank equipped with (or without) an agitator controlled by an integrated computer system.

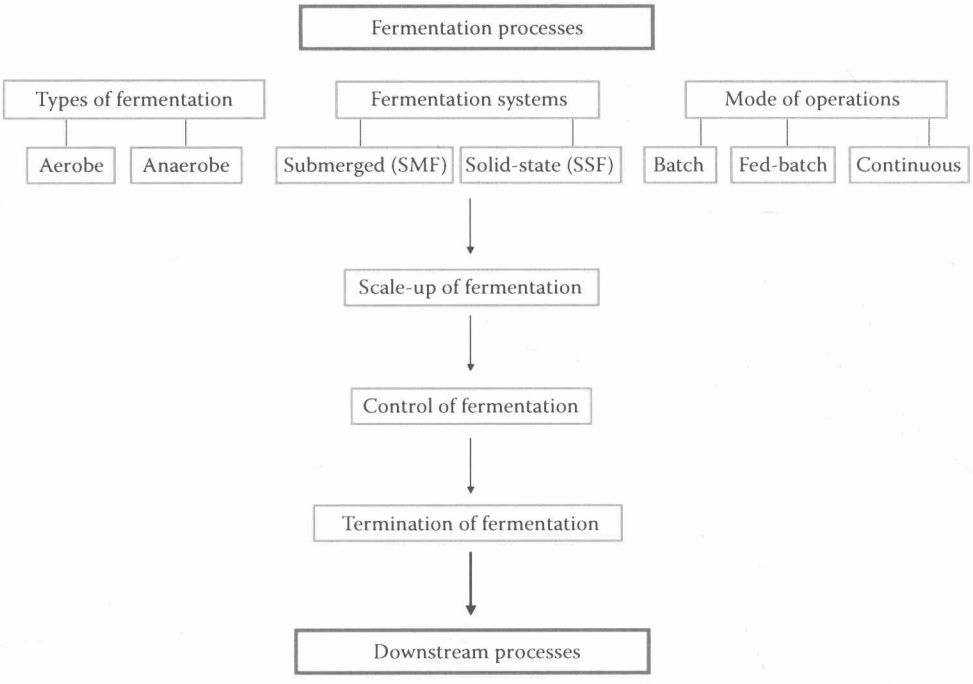

Figure 1.3 Fermentation processes in microbial biotechnology.

Industrial fermentation, depending on the type of end product, is operated by batch, semi-continuous, or continuous models (Stanbury et al. 1995).

The type of fermentation, fermentation system, mode of operation, fermentation scale-up, characteristics of the fermentor, physicochemical control conditions, addition of inoculum and additives to sterile medium, maintenance of sterile conditions during fermentation, and choice of a suitable time to stop fermentation are generally investigated in the fermentation processes (Figure 1.3).

1.4.3 *Downstream processes in microbial biotechnology*

Downstream processes involve all operations after fermentation, the main aim of which is to increase efficiency and ensure product recovery with the desired purity and biological activity. The primary process includes cell separation and cell wall disruption, followed by isolation and purification of the product from the cell extract or culture medium.

Biomass with insoluble bodies are separated by centrifugation or filtering from the liquid part of the medium. When the goal is the isolation of intracellular material, cells are disrupted using chemical, physical, and biological methods. The amount of desired product in the fermented medium is generally low, therefore a concentration process is required.

Afterward, purification methods such as different types of chromatography, dialysis, reverse osmosis, distillation, and solvent extraction are used depending on the type and desired purity level of the final product. In the final stages, evaporation, the addition of chemicals, or drying are used to make the powdered or crystal form of the product.

The downstream processes should use effective and rapid methods for the isolation and purification of the product. This is very important when impure products are