# Industrial Biotechnology

Dr. Rita Singh • Dr. S.K. Ghosh

# INDUSTRIAL BIOTECHNOLOGY

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# INDUSTRIAL BIOTECHNOLOGY

# Preface

This book *Industrial Biotechnology* is an attempt to deal with the use of living organisms or their products in large-scale industrial processes. It is an old field that has been rejuvenated in recent years due to the development of genetic engineering techniques. At present biotechnology is in an amazing growth phase whose end is nowhere in sight.

The major achievement in industrial biotechnology came through the Weizmann's discovery of acetone-butanol fermentation during First World War. The era of modern industrial microbiology was marked with the biosynthesis of penicillin using fermentation methods in the 1940s. In this book we have given an illustrative account on the industrially used microorganisms, different methods of industrial fermentation and the quick recovery process of the end products. One can find a detail description of those enzymes, normally used for industrial fermentation, their structure, biosynthesis, etc. In case of Chemical and pharmaceutical industry biotechnology has brought a tremendous change in its product synthesis, and cost effective processes. In case of pharmaceutical industry, not only are new classes of substances being sought for human therapy, but also cost-effective processes are being developed for major organic chemicals. We have dealt with this area in detail in this book.

The production of different kinds of antibiotics has been broadly emphasized in one of the chapters. After the discovery of antibiotic penicillin, the era of antibiotic research begins. Intensive screening programs in all industrial countries continue to increase the number of antibiotics. The chapter will be of immense importance to those who are working in field of antibiotics.

Microbial production of food and food related substances are an ancient practice. Many of the processes are of commercial importance. Not only that microbial colors have been produced from a number of microorganisms as a natural source of color. The food industry is increasingly interested in these colors due to large concern shown to alter dyes having cancerous implication. One can find many interesting and up-to-date information in food flavour and fragnance industry.

Although there are a number of books and research monographs dealing with industrial biotechnology, there has been no up-to-date information suitable for scholars in this field. We hope that this book would meet the need of the scholars and professionals and would be used widely by scholars, teachers as well as professionals interested in this area.

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# 1

# Introduction

Biotechnology deals with the use of living organisms or their products in large-scale industrial processes. It is an old field that has been rejuvenated in recent years because of the development of genetic engineering techniques. At present, biotechnology is in an amazing growth phase whose end is nowhere in sight. Microbiology is a central part of Industrial Biotechnology, matured as a science of industrial microbiology in the antibiotic era, as the large-scale manufacture of microbial products became a multibillion dollar industry. Genetic engineering has now made possible the directed construction of microorganisms that will do almost anything, and new products are being announced almost daily. Not only are new classes of substances being sought for human therapy, but cost-effective processes are being developed for major organic chemicals. Yet the mere engineering of a microbe is not enough. Large-scale, economically viable production must be attained. The industrial microbiologist knows that there are vast difficulties transfering a laboratory process to the production plant. There are some well-established principles of industrial microbiology. On the basis of these principles Industrial Biotechnology has arisen as a new area in the field of science.

Biotechnology is the use of microbiology, bio-chemistry, and engineering in an integrated fashion with the goal of using microorganisms and cell and tissue cultures (or their parts) to manufacture useful products. Biotechnology can be divided into two categories which are sometimes called "traditional biotechnology" and "new biotechnology". The major products of the traditional biotechnology industry are food and flavor ingredients, industrial alcohol, antibiotics, and citric acid. These products amount, on a world-wide basis, to about 300 billion dollars annually. The new biotechnology, which involves the use of the newer techniques of genetic engineering and cell fusion to produce organisms capable of making useful products provides at present products with a total value of less than a billion dollars. In the future, however, it is predicted that the new biotechnology will account for a much larger fraction of the total biotechnology industry.

Industrial biotechnology arose out of empirical developments in the production of wine, vinegar, beer, and sake, and with the traditional fungal fermentations used in Asia and Africa for the production of food. An experimental approach to the production of microbial metabolites only began at the beginning of the 20th century. Up until the time of World War II, the main microbial products that had developed from this experimental approach were enzymes such as proteases, amylases, and invertase.

A major breakthrough in biochemical and microbial engineering occurred after World War II as a result of the large-scale production of the first antibiotic, *penicillin*. In order to produce this antibiotic economically, important engineering developments had to be made, including the development of techniques for large-scale sterilization, aeration, and growth of microorganisms. In addition, genetic methods for microbial strain improvement were perfected.

From World War II up until about 1960, the major new biotechnology products were antibiotics. Through intense efforts of the pharmaceutical industry, numerous new antibiotics were discovered and of these around 20 were put into commercial production. In addition, in this early post-World-War-II period, processes were developed for the chemical transformation of steroids, and the culture of animal cells for the production of virus vaccines was perfected.

In the period from 1960 through 1975, new microbial processes for the production of amino acids and 5'-nucleosides as flavor enhancers were developed, primarily in Japan. In addition, numerous processes for enzyme production for industrial, analytic, and medical purposes were perfected. In this same period, successful techniques for the immobilization of enzymes and cells were developed. During this time a further development was the use of continuous fermentation for the production of *single-cell protein* from yeast and bacteria for use as human and animal food. Single-cell protein processes were developed using microorganisms capable of using petroleum-based starting materials such as gas oil, alkanes, and methanol. In this same period, microbial biopolymers such as xanthan and dextran, used as food additives, were also developed into commercial processes. Somewhat distinct processes that were advanced during this period were the use of microorganisms for tertiary oil recovery (an aspect of geomicrobiology) and the perfection of techniques for anaerobic cultivation of microorganisms, derived out of studies on the sewage treatment process.

Since 1975 biotechnology has entered some important new phases. First was the development of the hybridoma technique for the production of monoclonal antibodies, of interest primarily in the medical diagnosis field. Soon after was the production of human proteins using genetically engineered *Escherichia coli*.

The first product, human insulin was introduced in 1982, followed soon by Factor VIII, human growth hormone, interferons, and urokinase. At present, a vast array of human proteins are in the development stage.

Although the production of human proteins by engineered bacteria is generally recognized as the major "highlight" of the period since 1975 in actuality other products are economically more important. For instance, the production of ethanol by immobilized cells has become a major process. The enzyme *glucose isomerase* has become a 27 million dollar industry and is used to produce high-fructose syrup which itself has a value of 2.5 billion dollars. *Aspartame*, a major artificial sweetener, is produced microbially. Many new antibiotics have been introduced. Cheap fats are being increased in value by enzymatic esterification, the enzymes being microbial products. The biodegradation of persistent chemicals using specially developed microbial strains as starter cultures is being field-tested.

TABLE 1.1

**Patent applications for three countries with major biotechnology industries.**

| *Country* | *Molecular biology patents* | *Fermentation patents (including enzymes)* |
|---|---|---|
| USA | 100 | 160 |
| Japan | 90 | 700 |
| Federal Republic of Germany | 12 | 55 |

To place in perspective research activities in traditional biotechnology and the "new" biotechnologies (using genetic engineering, etc.), Table 1.1 provides a comparison of the number of patent applications in the whole field of biotechnology for three major industrial countries U.S.A., Japan, and the Federal

Republic of Germany in 1984. As can be seen, traditional biotechnologies still dominate, especially in Japan.

**Screening for Production of Metabolites**

The biochemical capabilities of microorganisms are vast, and a wide variety of new or unusual compounds may be produced by various microbial isolates. One of the main tasks of the microbiologist is to develop procedures for obtaining new microbial metabolites. There are five distinct approaches:

**1. Screening** for the production of new metabolites with new isolates and /or new test methods. This is the only way to obtain completely new classes of substances.

**2. Chemical modification** of known microbial substances.

**3. Biotransformation** which results in change in a chemical molecule by means of a microbial or enzymatic reaction.

**4. Interspecific protoplast fusion** which is a means of recombining genetic information from rather closely related producer strains. New or hybrid substances are expected and the method is widely used in the antibiotic industry.

**5. Gene cloning** in which genes, may be transferred between unrelated strains which are producers of known substances. Alternatively, transfer may be to nonproducers which contain "silent" genes, leading to the generation of modified or even new substances. This will undoubtedly be the method of choice in the future.

In order to be successful, screening must be an inter-disciplinary activity, combining the activities of microbiology, chemistry, biochemistry, engineering, and bibliographies. Microbiology is involved in the isolation and identification of microorganisms, strain preservation, testing for biological activity, and fermentation practice. Biochemistry provides the analytical procedures needed as well as the approaches to purification of

the biologically interesting molecules whereas synthesis of substrates and inhibitors falls under the purview of the chemist. The bibliographic specialist searches the literature and information related to this subject. The engineer's activity focuses on the development of technical equipment needed for the successful process.

Screening can never be considered a routine activity, since the methods must always be adapted to the newest techniques and knowledge. The goal is always to detect and identify new substances of commercial interest and to separate them in the quickest possible way from the numerous easily detected substances that are of no commercial interest. Some of the most intelligent screening methods that are currently used are the products of Japanese scientific activity, particularly the group of Omura. For instance, 42 completely new compounds were found by Omura using systems that detected microbially produced substances with antibacterial, antimycoplasmal, antianaerobe, antifungal, antiparasite, and antitumor activity. Substances were also found that acted as herbicides and as inhibitors of penicillin, elastase, and adenosine deaminase.[1]

**Primary and Secondary Metabolites**

Bu'Lock borrowed the term "secondary metabolite" from plant physiology in 1961 and applied it to microbiology. While primary metabolites are essential for life and reproduction of cells, "and primary metabolism functions similarly in all microorganisms, the following is true for secondary metabolites:

- Every secondary metabolite is formed by only a few organisms.
- Secondary metabolites are seemingly not essential for growth and reproduction.
- Their formation is extremely dependent on environmental conditions.

- Some secondary metabolites are produced as a group of closely related structures; one strain of *Streptomyces*, for example, produces 32 different anthracyclines.
- Some organisms form a variety of different classes of substances as secondary metabolites.
- The regulation of the biosynthesis of secondary metabolites differs significantly from that of the primary metabolites.

There are several hypotheses about the role of secondary metabolites, of which Hans Zähner's, illustrated in Figure 1.1, is the most elegant.[2] Besides the five phases of the cell's own metabolism (intermediary metabolism, regulation, transport, differentiation and morphogenesis), secondary metabolism is considered a "playing field" for the evolution of further biochemical development, which can proceed without damaging primary metabolism. Genetic changes leading to the modification of secondary metabolites would be expected not to have any major effect on normal cell function. If a genetic change leads to the formation of a compound that in some way is beneficial, then this genetic change would be fixed in the cell's genome, perhaps becoming essential. In this way the former secondary metabolite would be converted into a primary metabolite.

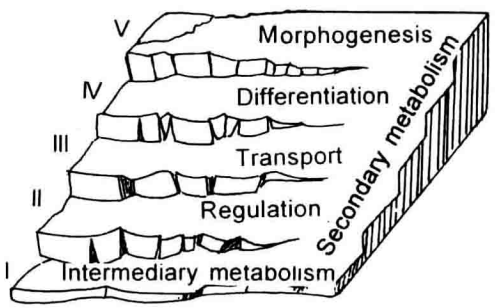


**Figure 1.1:** The five levels of primary metabolism with the "playing field" of secondary metabolism.

**Screening Methods**

There are no universal screening methods. The success of a screening programme depends upon the selection of appropriate tests as well as appropriate microorganisms to be tested. The capacity of an industrial screening group for isolation of microorganisms and thorough testing is around 1000–2000 strains per year.

Today, most screening programmes focus on chemotherapeutically useful products for the following areas: activity against antibiotic-resistant strains, tumors, and viruses, as well as a search for enzyme inhibitors and pharmacologically active substances (hormones, etc.). Better starter cultures for the food industry as well as microorganisms that are capable of degrading hazardous and persistent chemicals are also sought.

Of the 10,000 antibiotically active compounds known in the late 1980's, 67% are produced by microorganisms (67% by actinomycetes, 9% by other bacteria, and 15% by fungi). Additionally, about 2000 other biologically active secondary metabolites are known, as well as a large number of enzymes.

**Strains used in Screening**

The success of a screening programme depends on both the kinds of organisms used and the methods for detection of activity. Currently, the choice of strain has a 30–40% influence on the outcome, the test procedure a 60–70% influence.

A gram of soil contains between $10^6$–$10^8$ bacteria, $10^4$–$10^6$ actinomycete spores, and $10^2$–$10^4$ fungal spores. Less than 1% of the world's microorganisms have been intensively studied. Above all, the approximately 100,000 known fungi have been poorly studied, so that a vast number of new natural products can be expected from this group in the future.

In the isolation of new metabolic products, researchers try to isolate strains from extreme or unusual environments in the hope