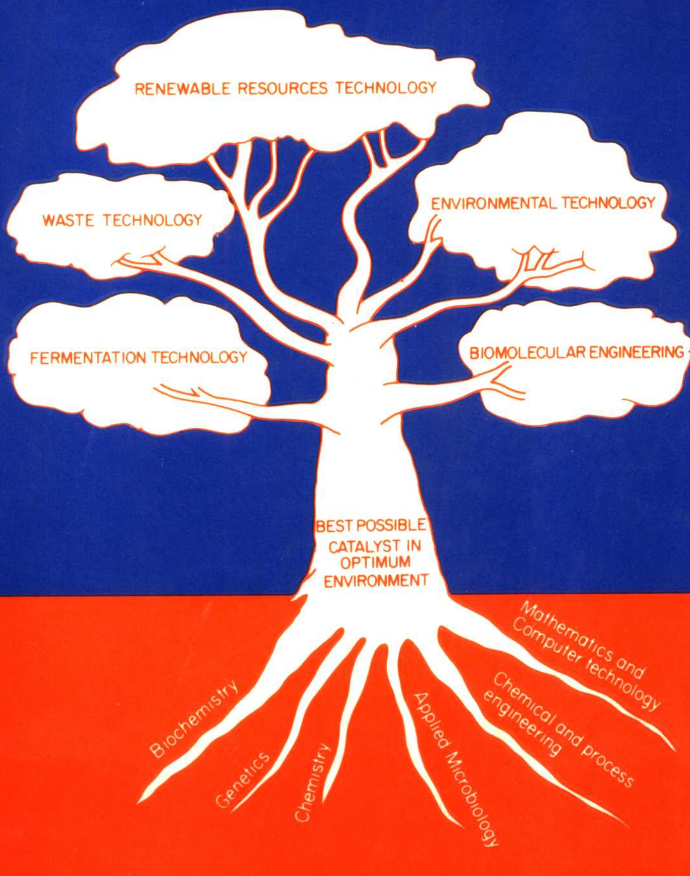
NEW STUDIES IN BIOLOGY

Biotechnology

Second Edition

John E. Smith



New Studies in Biology

Biotechnology

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General Preface to the Series

Recent advances in biology have made it increasingly difficult for both students and teachers to keep abreast of all the new developments in so wide-ranging a subject. The New Studies in Biology, originating from an initiative of the Institute of Biology, are published to facilitate resolution of this problem. Each text provides a synthesis of a field and gives the reader an authoritative overview of the subject without unnecessary detail.

The Studies series originated 20 years ago but its vigour has been maintained by the regular production of new editions and the introduction of additional titles and new themes become clearly identified. It is appropriate for the New Studies in their refined format to appear at a time when the public at large has become conscious of the beneficial applications of knowledge from the whole spectrum from molecular to environmental biology. The new series is set to provide as great a boon to the new generation of students as the original series did to their fathers.

1986

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Preface

Biotechnology is in reality a series of enabling technologies and involves the practical application of biological organisms, or their subcellular components, to manufacturing and service industries and to environmental management. Biotechnology is a subject of great antiquity having its origins in ancient microbial processes such as brewing, wine making and fermented milk products, such as cheeses and yoghurts. However, new developments in enzyme technology, fermentation processes, monoclonal antibodies, and in genetic engineering, have introduced new and exciting dimensions to the subject. It is becoming increasingly important to develop in young people an awareness of the existence and future role of biotechnology in modern society. This new and much expanded edition of *Biotechnology* is aimed to give an integrated overview of biotechnology and for some students to point the way forward for exciting and satisfying careers.

I am deeply indebted to Miss Liz Clements for typing the manuscript.

I dedicate this edition to my grandaughters Christy, Emma, Lucy and Kim.

John E. Smith
1988

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An Introduction to Biotechnology

1.1 What is biotechnology?

There is little doubt that modern biology is the most diversified of all the Natural sciences. It exhibits a bewildering array of subdisciplines, microbiology, plant and animal anatomy, biochemistry, immunology, cell biology, plant and animal physiology, morphogenesis, systematics, ecology, palaeobotany, genetics and many others. The increasing diversity of modern biology derived primarily from the largely post war introduction into biology of other scientific disciplines, such as physics, chemistry and mathematics, which have made possible the description of life processes at the cellular and molecular level. In the last two decades well over twenty Nobel prizes have been awarded for discoveries in these fields of study.

This newly acquired biological knowledge has already made vastly important contributions to the health and welfare of man. And yet, what has gone before may well pale into insignificance if all the hopes of *biotechnology* can be realized.

Biotechnology has been defined in many forms (Table 1.1) but in essence implies the use of microbial, animal or plant cells or enzymes to synthesize, break down or transform materials. This requires the integration of biochemistry, biology, microbiology, chemical engineering and process engineering, together with other disciplines, in a way that optimizes the exploitation of their potential (Fig. 1.1). Biotechnology is not itself a product or range of products like microelectronics: rather it should be regarded as a range of enabling technologies which will find significant application in several industrial sectors. As will be seen in later sections, it is a technology in search of new applications and the main benefits lie in the future. New biotechnological processes will, in most instances, function at low temperature, will consume little energy and will rely mainly on inexpensive substrates for biosynthesis.

However, it should be recognized that biotechnology is not new but represents a developing and expanding series of technologies with roots established (in many cases) thousands of years ago. Biotechnology includes many traditional processes such as brewing, baking, winemaking, cheesemaking, the production of oriental foods such as soy sauce and tempeh, and sewage treatment where the use of microorganisms has been developed somewhat

Table 1.1 · Some selected definitions of biotechnology.

'The application of biological organisms, systems or processes to manufacturing and service industries.'
'The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissue cells and parts thereof.'
'A technology using biological phenomena for copying and manufacturing various kinds of useful substances.'
'The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.'
'The science of the production processes based on the action of microorganisms and their active components and of production processes involving the use of cells and tissues from higher organisms. Medical technology, agriculture and traditional crop breeding are not generally regarded as biotechnology.'
'Biotechnology is really no more than a name given to a set of techniques and processes.'
'Biotechnology is the use of living organisms and their components in agriculture, food and other industrial processes.'

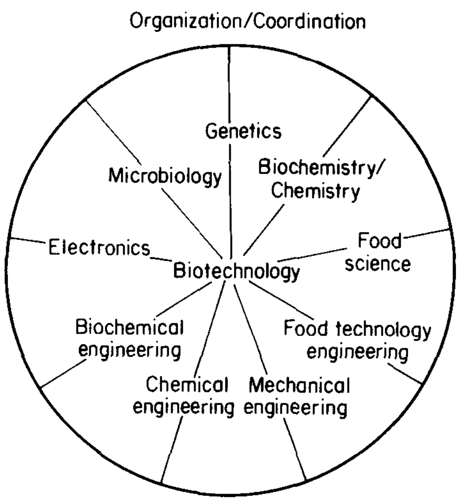

Fig. 1.1 The interdisciplinary nature of biotechnology (from Higgins *et al.*, 1985).

empirically over many years (Table 1.2). It is only recently that these processes have been subjected to rigorous scientific scrutiny and analysis; even so it will surely take some time for modern scientifically based practices fully to replace traditional empiricism.

There is also a considerable danger that biotechnology will be viewed as a coherent, unified body of scientific and engineering knowledge and thinking, to

Table 1.2 Historical development of biotechnology.

1	***Biotechnological production of foods and beverages*** Sumerians and Babylonians were drinking beer by 6000 BC; Egyptians were baking leavened bread by 4000 BC; wine was known in the Near East by the time of the book of Genesis. Microorganisms first seen in 17th century by Anton van Leeuwenhoek who developed the simple microscope; fermentative ability of microorganisms demonstrated between 1857–1876 by Pasteur – ***the father of biotechnology***; cheese production has ancient origins, also mushroom cultivation.
2	***Biotechnological processes initially developed under nonsterile conditions*** Ethanol, acetic acid, butanol and acetone produced by end of 19th century by open microbial fermentation processes; waste-water treatment and municipal composting of solid wastes, the largest fermentation capacity practised throughout the world.
3	***Introduction of sterility to biotechnological processes*** Introduction in the 1940s of complicated engineering techniques to the mass cultivation of microorganisms to exclude contaminating microorganisms. Examples include antibiotics, amino acids, organic acids, enzymes, steroids, polysaccharides and vaccines.
4	***Applied genetics and recombinant DNA technology*** Traditional strain improvement of important industrial organisms has long been practised; recombinant DNA techniques together with protoplast fusion allow new programming of the biological properties of organisms.

be applied in a coherent and logical manner. This is not so; the range of biological, chemical and engineering disciplines that are involved have varying degrees of application to the industrial scene.

Traditional or 'old' biotechnology has established an expanding market. 'New' or modern aspects of biotechnology, founded in recent advances in molecular biology, genetic engineering and fermentation process technology, are not yet finding wide industrial application. A wide range of knowledge and expertise is ready to be put to productive use; but the rate at which it will be applied will depend less on scientific or technical considerations and more on such factors as adequate investment by the relevant industries, improved systems of biological patenting, marketing skills and the economics of the new methods in relation to technologies currently employed.

The industrial activities to be affected include human and animal food production, provision of chemical feedstocks to replace petrochemical sources, alternative energy sources, waste recycling, pollution control and agriculture. The new techniques will also revolutionize many aspects of medicine, veterinary science and pharmaceutics.

Biotechnological industries will be based largely on renewable and recyclable materials and so can be adapted to the needs of a society in which energy is ever-increasingly expensive and scarce. In many ways, biotechnology is an embryonic technology and will require much skilful control of its development, but the potentials are vast and diverse, and it undoubtedly will play an increasingly important part in many future industrial processes.

1.2 Biotechnology — an interdisciplinary pursuit

Biotechnology is *a priori* an interdisciplinary pursuit. In recent decades a characteristic feature of the development of science and technology has been the increasing resort to multidisciplinary strategies for the solution of various problems. This has led to the emergence of new interdisciplinary areas of study, with the eventual crystallization of new disciplines with identifiable, characteristic concepts and methodologies.

Chemical engineering and biochemistry are two well-recognized examples of disciplines that have done much to clarify our understanding of chemical processes and the chemical basis of biological systems.

The term *multidisciplinary* describes a quantitative extension of approaches to problems that commonly occur within a given area. It involves the marshalling of concepts and methodologies from a number of separate disciplines and applying them to a specific problem in another area. In contrast, *interdisciplinary* application occurs when the blending of ideas that occur during multidisciplinary cooperation leads to the crystallization of a new disciplinary area with its own concepts and methodologies. In practice, multidisciplinary enterprises are almost invariably mission-orientated. However, when true interdisciplinary synthesis occurs the new area will open up a novel spectrum of investigations. Biotechnology has arisen through the interaction between various parts of biology and engineering.

A biotechnologist employs techniques derived from chemistry, microbiology, biochemistry, chemical engineering and computer science (Fig. 1.1). The main objectives are the innovation, development and optimal operation of processes in which biochemical catalysis has a fundamental and irreplaceable role. Biotechnologists must also aim to achieve a close working cooperation with experts from other related fields such as medicine, nutrition, the pharmaceutical and chemical industries, environmental protection and waste process technology.

The application of biotechnology will increasingly rest upon the ability of each of the contributing disciplines to understand the technical language of the others and – above all – to understand the potential as well as the limitations of the other areas.

A key factor in the distinction between biology and biotechnology is the scale of operation. The biologist usually works in the range between nanograms and milligrams. The biotechnologist working on the production of vaccines may be satisfied with milligram yields, but in most other projects aims at kilograms or tonnes. Thus, one of the main aspects of biotechnology consists of scaling-up biological processes.

Many present-day biotechnological processes have their origins in ancient and traditional fermentations such as the brewing of beer and the manufacture of bread, cheese, yoghurt, wine and vinegar. However, it was the discovery of antibiotics in 1929 and their subsequent large-scale production in the 1940s that created the greatest advances in fermentation technology. Since then we have witnessed a phenomenal development in this technology, not only in the

Table 1.3 World markets for biological products, 1981.

Product	*Sales (£ million)*
Alcoholic beverages	23 000
Cheese	14 000
Antibiotics	4 500
Penicillins	500
Tetracyclines	500
Cephalosporins	450
Diagnostic tests	2 000
Immunoassay	400
Monoclonal	5
Seeds	1 500
High-fructose syrups	800
Amino acids	750
Baker's yeast	540
Steroids	500
Vitamins	
All	330
C	200
B_{12}	14
Citric acid	210
Enzymes	200
Vaccines	150
Human serum albumin	125
Insulin	100
Urokinase	50
Human factor VIII protein	40
Human growth hormone	35
Microbial pesticides	12

(From P. Dunnill (1984) SERC Biotechnology Directorate Newsletter, 1(1)).

production of antibiotics but in many other useful, simple or complex chemical products, for example organic acids, polysaccharides, enzymes, vaccines, hormones, etc. (Table 1.3). Inherent in the development of fermentation processes is the close relationship between the biochemist, the microbiologist and the chemical engineer. Thus, biotechnology is not a sudden discovery but rather a coming of age of a technology that was initiated several decades ago. Looking to the future, the *Economist* when reporting on this new technology stated that it may launch 'an industry as characteristic of the twenty-first century as those based on physics and chemistry have been of the twentieth century'.

If it is accepted that biotechnology has its roots in distant history and has large successful industrial outlets, why then has there been such public awareness of this subject in recent years? The main reason must derive from the rapid advances in molecular biology – in particular recombinant DNA technology – which are giving humans dominance over nature. By these new techniques (discussed in Chapter 4) it is possible to manipulate directly the heritable material (DNA) of cells between like and unlike cells, creating new combinations of characters and abilities not previously present on this planet. The potential of this series of techniques, first developed in academic laboratories, is being rapidly exploited in industry. The industrial benefits are immense but the inherent dangers of tampering with nature must always be appreciated and respected.

The developments of biotechnology are proceeding at a speed similar to that of the microelectronics industry in the mid-1970s. Although the analogy is tempting, any expectations that biotechnology will develop commercially at the same rate should be tempered with considerable caution. While the potential of 'new' biotechnology cannot be doubted, meaningful commercial realization and benefits are not expected until well into the 1990s. New biotechnology will have a considerable impact across a wide range of chemical substances. In each case the economics of competing means of production will influence the development of a biotechnological route. Biotechnology will undoubtedly have great benefits in the long term.

The growth in awareness of modern biotechnology parallels the serious worldwide changes in the economic climate arising from the escalation of oil prices since 1973. There is a growing realization that fossil fuels (although at present in a production glut period) and other non renewable resources will one day be in limited supply. This will result in the requirement of cheaper and more secure energy sources and chemical feedstocks, which biotechnology could perhaps fulfil. Countries with climatic conditions suitable for rapid biomass production could well have major economic advantages over less climatically suitable parts of the world. In particular, the tropics must hold high potential in this respect.

Another contributory factor to the growing interest in biotechnology has been the current world recession, in particular the depression of the chemical and engineering sections, in part due to increased energy costs. Biotechnology has been considered as one important means of restimulating the economy, whether on a local, regional, national or even global basis, using new biotechnological methods and new raw materials. In part, the industrial boom of the 1950s and 1960s was due to cheap oil; while the information technology advances in the 1970s and 1980s resulted from developments in microelectronics. It is quite feasible that the 1980s and 1990s will be the era of biotechnology. There is undoubtedly a worldwide increase in molecular biological research, the formation of new biotechnological companies, large investments by nations, companies and individuals and the rapid expansion of data bases, information sources and, above all, extensive optimistic media coverage.

It is perhaps unfortunate that there has been excessive concentration on the new implications of biotechnology and less interest paid to the very large biotechnological industrial bases that already function throughout the world and contribute considerably to most nations' gross national profits. Indeed, many of the innovations in biotechnology will not appear *a priori* as new products but rather as improvements to organisms and processes in long-established biotechnological industries, e.g. brewing and antibiotics production.

New applications are likely to be seen earliest in the area of health care and medicine, followed by agriculture and food technology. Applications in chemical production, fuel and energy production, pollution control and resource recovery will possibly take longer to develop and will depend on changes in the relative economics of currently employed technologies. Biotechnology-based industries will not be labour-intensive; and although they will create valuable new employment, the need will be more for brains than muscle.

Biotechnology is high technology *par excellence*. The most exciting and potentially profitable facets of new biotechnology in the 1990s will involve research and development at the very frontiers of current knowledge and techniques.

In the late 1970s biotechnologists were putting forward vague promises about the wonders of their subject, while the realizing technologies were still being developed and still required immense levels of research and product development fundings. Biotechnologists now make predictions with more confidence since many of the apparently insurmountable problems have been overcome more easily than had been predicted, and many transitions from laboratory experiments to large-scale industrial processes have been achieved. Truly, new biotechnology has come of age.

1.3 Biotechnology — a three-component central core

Many biotechnological processes may be considered as having a three-component central core, in which one part is concerned with obtaining the best biological catalyst for a specific function or process, the second part creates (by construction and technical operation) the best possible environment for the catalyst to perform, and the third part (downstream processing) is concerned with the separation and purification of an essential product or products from a fermentation process.

In the majority of examples so far developed, the most effective, stable and convenient form for the catalyst for a biotechnological process is a whole organism, and it is for this reason that so much of biotechnology revolves around microbial processes. This does not exclude the use of higher organisms; in particular, plant and animal cell culture will play an increasingly important role in biotechnology (Chapter 9).

Microorganisms can be viewed both as primary fixers of photosynthetic energy and as systems for bringing about chemical changes in almost all types of natural and synthetic organic molecules. Collectively, they have an immense gene pool which offers almost unlimited synthetic and degradative potential. Furthermore, microorganisms possess extremely rapid growth rates far in excess of any of the higher organisms such as plants and animals. Thus immense quantities can be produced under the right environmental conditions in short time periods.

The methodologies that are in general use enable the selection of improved microorganisms from the natural environmental pool, the modification of microorganisms by mutation and, more recently, the mobilization of a spectacular array of new techniques, deriving from molecular biology, which may eventually permit the construction of microorganisms and plants with totally novel biochemical potentials (Chapter 4). These new techniques have arisen from the pure scientific efforts in molecular biology over the last two decades.

These carefully selected and manipulated organisms must be maintained in substantially unchanged form, and this involves another spectrum of techniques for preserving organisms, for retaining essential features during industrial processes and, above all, for retaining vigour and viability. In many examples the catalyst is used in a separated and purified form, i.e. as enzymes; a huge amount of information has been built up on the large-scale production, isolation and purification of individual enzymes and on their stabilization by artificial means (Chapter 5).

The second part of this central core of biotechnology encompasses all aspects of the containment system or bioreactor within which the catalysts must function (Chapter 3). Here the specialist knowledge of the chemical or process engineer provides the design and instrumentation for maintaining and controlling the physicochemical environment – temperature, aeration, pH, etc. – thus allowing the optimum expression of the catalyst (Chapter 3).

Having achieved the successful formation of a biotechnological product within a bioreactor, in most cases it will be necessary to separate the product from the aqueous environment. This can be a technically difficult and expensive procedure, and is the least understood area of biotechnology. Down-

Table 1.4 The main areas of application of biotechnology.

1 ***Fermentation technology***
Historically, the most important area of biotechnology (brewing, antibiotics, etc.); extensive development in progress with new products envisaged – polysaccharides, medically important drugs, solvents, protein-enhanced foods. Novel fermenter designs to optimize productivity.

2 ***Enzyme technology***
Used for the catalysis of extremely specific chemical reactions; immobilization of enzymes; to create specific molecular converters (bioreactors). Products formed include L-amino acids, high-fructose syrup, semisynthetic penicillins, starch and cellulose hydrolysis, etc. Enzyme probes for analysis.

3 ***Waste technology***
Long historical importance, but more emphasis now on coupling these processes with the conservation and recycling of resources; foods and fertilizers, biological fuels.

4 ***Environmental technology***
Great scope exists for the application of biotechnological concepts for solving many environmental problems – pollution control, removing toxic wastes; recovery of metals from mining wastes and low-grade ores.

5 ***Renewable resources technology***
The use of renewable energy sources, in particular, lignocellulose, to generate chemical raw materials and energy-ethanol, methane and hydrogen. Total utilization of plant and animal material.

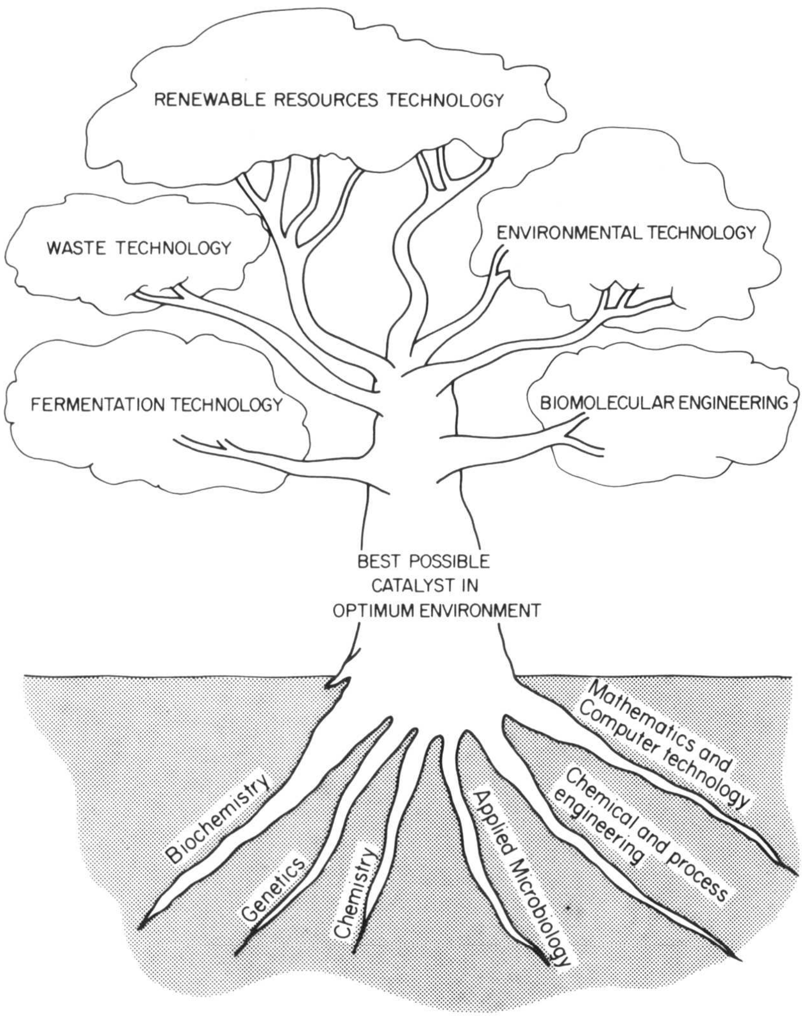

Fig. 1.2 Biotechnology tree.

stream processing is primarily concerned with initial separation of the bioreactor broth or medium into a liquid phase and a solids phase, and subsequent concentration and purification of the product. Processing will usually involve more than one stage. Downstream processing costs (as approximate proportions of selling prices) of fermentation products vary considerably; e.g. with yeast biomass, penicillin G and certain enzymes, processing costs as percentages of selling price are 20%, 20 to 30% and 60 to 70% respectively.

Successful involvement in a biotechnological problem therefore draws heavily upon more than one of the input disciplines. The main areas of application of biotechnology are shown in Table 1.4, while Fig. 1.2 shows how the many disciplines input into the biotechnological processes.

Biotechnology will continue to create exciting new opportunities for commercial development and profits in a wide range of industrial sectors, including health care and medicine, agriculture and forestry, fine and bulk chemicals production, food technology, fuel and energy production, pollution control and resource recovery. Biotechnology offers the hope of solving many of the problems our world faces.

In the following chapters some of the most important areas of biotechnology are considered with a view to achieving a broad overall understanding of the existing achievements and future aims of this new area of technology. However, it must be appreciated that biotechnological development will not only depend on scientific and technological advances, but will also be subject to considerable political and economic forces.