COMPREHENSIVE BIOTECHNOLOGY

The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine

EDITOR-IN-CHIEF
MURRAY MOO-YOUNG

Volume 2

The Principles of Biotechnology: Engineering Considerations

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Preface

In his recent book, entitled 'Megatrends', internationally-celebrated futurist John Naisbitt observed that recent history has taken industrialized civilizations through a series of technology-based eras: from the chemical age (plastics) to an atomic age (nuclear energy) and a microelectronics age (computers) and now we are at the beginning of an age based on biotechnology. Biotechnology deals with the use of microbial, plant or animal cells to produce a wide variety of goods and services. As such, it has ancient roots in the agricultural and brewing arts. However, recent developments in genetic manipulative techniques and remarkable advances in bioreactor design and computer-aided process control have founded a 'new biotechnology' which considerably extends the present range of technical possibilities and is expected to revolutionize many facets of industrial, agricultural and medical practices.

Biotechnology has evolved as an ill-defined field from inter-related activities in the biological, chemical and engineering sciences. Inevitably, its literature is widely scattered among many specialist publications. There is an obvious need for a comprehensive treatment of the basic principles, methods and applications of biotechnology as an integrated multidisciplinary subject. *Comprehensive Biotechnology* fulfils this need. It delineates and collates all aspects of the subject and is intended to be the standard reference work in the field.

In the preparation of this work, the following conditions were imposed. (1) Because of the rapid advances in the field, it was decided that the work would be comprehensive but concise enough to enable completion within a set of four volumes published simultaneously rather than a more encyclopedic series covering a period of years to complete. In addition, supplementary volumes will be published as appropriate and the work will be updated regularly via *Biotechnology Advances*, a review journal, also published by Pergamon Press with the same executive editor. (2) Because of the multidisciplinary nature of biotechnology, a multi-authored work having an international team of experts was required. In addition, a distinguished group of editors was established to handle specific sections of the four volumes. As a result, this work has 10 editors and over 250 authors representing 15 countries. (3) Again, because of the multidisciplinary nature of the work, it was virtually impossible to use a completely uniform system of nomenclature for symbols. However, provisional guidelines on a more unified nomenclature of certain key variables, as provided by IUPAC, was recommended. (4) According to our definition, aspects of biomedical engineering (such as biomechanics in the development of prosthetic devices) and food engineering (such as product formulations) are not included in this work. (5) Since the work is intended to be useful to both beginners as well as veterans in the field, basic elementary material as well as advanced specialist aspects are covered. For convenience, a glossary of terms is supplied. (6) Since each of the four volumes is expected to be fairly self-contained, a certain degree of duplication of material, especially of basic principles, is inevitable. (7) Because of space constraints, a value judgement was made on the relative importance of topics in terms of their actual rather than potential commercial significance. For example, 'agricultural biotechnology' is given relatively less space compared to 'industrial biotechnology', the current raison d'être of biotechnology as a major force in the manufacture of goods and services. (8) Finally, a delicate balance of material was required in order to meet the objective of providing a comprehensive and stimulating coverage of important practical aspects as well as the intellectual appeal of the field. Readers may wish to use this work for initial information before possibly delving deeper into the literature as a result of the critical discussions and wide range of references provided in it.

Comprehensive Biotechnology is aimed at a wide range of user needs. Students, teachers, researchers, administrators and others in academia, industry and government are addressed. The requirements of the following groups have been given particular consideration: (1) chemists, especially biochemists, who require information on the chemical characteristics of enzymes, metabolic processes, products and raw materials, and on the basic mechanisms and analytical techniques involved in biotechnological transformations; (2) biologists, especially microbiologists and molecular biologists, who require information on the biological characteristics of living organisms involved in biotechnology and the development of new life forms by genetic engineering techniques; (3) health scientists, especially nutritionists and toxicologists, who require information on biohazards and containment techniques, and on the quality of products and by-products of biotechnological processes, including the pharmaceutical, food and beverage industries; (4) chemical engineers, especially biochemical engineers, who require information on mass and energy balances and rates of processes, including fermentations, product recovery and feedstock pretreatment, and the equipment for carrying out these processes; (5) civil engineers, especially environmental engineers, who require information on biological waste treatment methods and equipment, and on contamination potentials of the air, water and land within the ecosystem, by industrial and domestic effluents; (6) other engineers, especially agricultural and biomedical engineers, who require information on advances in the relevant sciences that could significantly affect the future practice of their professions; (7) administrators, particularly executives and legal advisors, who require information on national and international governmental regulations and guidelines on patents, environmental pollution, external aid programs and the control of raw materials and the marketing of products.

No work of this magnitude could have been accomplished without suitable assistance. For guidance on the master plan, I am indebted to the International Advisory Board (J. D. Bu'Lock, T. K. Ghose, G. Hamer, J. M. Lebault, P. Linko, C. Rolz, H. Sahm, B. Sikyta and H. Taguchi). For structuring details of the various sections, the invaluable assistance of the section editors is gratefully acknowledged, especially Alan Bull, Charles Cooney, Harvey Blanch and Campbell Robinson, who also acted as coordinators for each of the four volumes. For the individual chapters, the 250 authors are to be commended for their hard work and patience during the two years of preparation of the work. For checking the hundreds of literature references cited in the various chapters, the many graduate students are thanked for a tedious but important task well done. A special note of thanks is due to Jonathan and Arlene Lamptey, who acted as editorial assistants in many diverse ways. At Pergamon Press, I wish to thank Don Crawley for originally suggesting this project and Colin Drayton for managing it. Finally, I am pleased to note the favourable evaluations of the work by two distinguished authorities, Sir William Henderson and Nobel Laureate Donald Glaser, who provided a foreword and a guest editorial, respectively, to the treatise.

MURRAY MOO-YOUNG
Waterloo, Canada
December 1984

Foreword

This very comprehensive reference work on biotechnology is published ten years after the call by the National Academy of Sciences of the United States of America for a voluntary worldwide moratorium to be placed on certain areas of genetic engineering research thought to be of potential hazard. The first priority then became the evaluation of the conjectural risks and the development of guidelines for the continuation of the research within a degree of containment. There had hardly been a more rapid response to this type of situation than that of the British Advisory Board for the Research Councils. The expression of concern by Professor Paul Berg and the committee under his chairmanship, and the call for the moratorium, was published in *Nature* on 19 July 1974. The Advisory Board agreed at their meeting on the 26 July to establish a Working Party with the following terms of reference:

> 'To assess the potential benefits and potential hazards of the techniques which allow the experimental manipulation of the genetic composition of micro-organisms, and to report to the Advisory Board for the Research Councils.'

Because of the conviction of those concerned that recombinant DNA techniques could lead to great benefits, the word order used throughout the report of the Working Party (Chairman, Lord Ashby) always put 'benefits' before 'hazards'. The implementation of the recommendations led to the development of codes of practice. This was followed by the establishment of the Genetic Manipulation Advisory Group as a standing central advisory authority operating within the framework of the Health and Safety at Work, *etc.* Act 1974 and, later, more specifically within the framework of the Health and Safety (Genetic Manipulation) Regulations 1978. Similar moves took place in many other countries but the other most prominent and important activity was that of the US National Institutes of Health. This resulted in the adoption by most countries of the NIH or the UK guidelines, or the use of practices based on both.

The significant consequence of the debates, the discussions and of the recommendations that emerged during these early years of this decade (1974–1984) was that research continued, expanded and progressed under increasingly less restriction at such a pace that now makes it possible and necessary to devote the first Section of Volume 1 of this work to genetic engineering. Many chapters of the subsequent Sections and Volumes are of direct relevance to the application of genetic engineering.

The reason for identifying today's genetic engineering for first mention in this foreword is its novelty. It was being conceived barely more than ten years ago. Ten years by most standards is a short time. Although in the biological context it represents at least 10^4 generation times of the most vigorous viruses, it is less than one of man even for the most precocious. The current developments in biotechnology, whether they be in recombinant DNA, monoclonal antibodies, immobilized enzymes, *etc.* are mostly directed towards producing a better product, or a better process. This is commendable and is supportable by the ensuing potential commercial benefits. The newer challenge is the application of the new biotechnology to achieve what previously could scarcely have been contemplated. Limited biological sources of hormones, growth regulators, *etc.* are being, and will be increasingly, replaced by the use of transformed microorganisms, providing a vastly increased scale of production. Complete safety of vaccines by the absence of ineffectively inactivated virus is one of the great advantages of the genetically engineered antigen. This is quite apart from the ability to prepare products for which, at present, there is a technical difficulty or which is economically not feasible by standard methods.

A combination of advances in recombinant DNA research, molecular biology and in blastomere manipulation has provided the technology to insert genetic material into the totipotent animal cell. The restriction on the application of this technology for improved animal production is the lack of knowledge on the genetic control of desirable biological characteristics for transfer from one breed line to another.

There are probably greater potential benefits to be won in the cultivation of the domesticated plants than in the production of the domesticated animals. In both cases, the objectives are to increase the plant's or the animal's resistance to the prejudicial components of its environment and to increase the yield, quality and desired composition of the marketable commodities. These include the leaf, the tuber, the grain, the berry, the fruit or the milk, meat and other products of animal origin. This is not taking into account the other valuable products of horticulture, of oil or wax palms, rubber trees and forestry in general. Genetic engineering should be able to provide short-cuts to reach objectives attainable by traditional procedures, for example by by-passing the sequential stages of a traditional plant breeding programme by the transfer of the genetic material in one step. Examples of desirable objectives are better to meet user specifications with regard to yield, quality, biochemical composition, disease and pest resistance, cold tolerance, drought resistance, nitrogen fixation, *etc.* One of the constraints in this work in plants is the scarcity of vectors compared with the many available for the transformation of microorganisms. The highest research priority on the plant side is to determine by one means or another how to increase the efficiency of photosynthesis. The photosynthetic efficiency of temperate crop plants is no more than 2–2.5% in terms of conversion of intercepted solar energy. These plants possess the C_3 metabolic pathway with the energy loss of photorespiration. Tropical species of plants with the C_4 metabolic pathway have a higher efficiency of photosynthesis in that they do not photorespire. One approach for the breeder of C_3 plants is to endow them with a C_4 metabolism. If this transformation is ever to be achieved, it is most likely to be by genetic engineering. Such an advance has obvious advantages with regard, say, to increased wheat production for the ever-increasing human population. Nitrogen fixation as an agricultural application of biotechnology is given prominence in Section 1 of Volume 4. Much knowledge has been acquired about the chemistry and the biology of the fixation of atmospheric nitrogen. This provides a solid foundation from which to attempt to exploit the potential for transfer of nitrogen-fixing genes to crop plants or to the symbiotic organisms in their root systems. If plants could be provided with their own capability for nitrogen fixation, the energy equation might not be too favourable in the case of high yielding varieties. Without an increase in the efficiency of photosynthesis, any new property so harnessed would have to be at the expense of the energy requirements of existing characteristics such as yield.

Enzymes have been used for centuries in the processing of food and in the making of beverages. The increasing availability of enzymes for research, development and industrial use combined with systems for their immobilization, or for the immobilization of cells for the utilization of their enzymes, is greatly expanding the possibilities for their exploitation. Such is the power of the new biotechnology that it will be possible to produce the most suitable enzymes for the required reaction with the specific substrate. An increasing understanding at the molecular level of enzyme degradation will make it possible for custom-built enzymes to have greater stability than those isolated from natural sources.

The final section of Volume 4 deals with waste and its management. This increasingly voluminous by-product of our society can no longer be effectively dealt with by the largely empirical means that continue to be practised. Biological processes are indispensable components in the treatment of many wastes. The new biotechnology provides the opportunity for moving from empiricism to processes dependent upon the use of complex biological reactions based on the selection or the construction of the most appropriate cells or their enzymes.

The very comprehensive coverage of biotechnology provided by this four-volume work of reference reflects that biotechnology is the integration of molecular biology, microbiology, biochemistry, cell biology, chemical engineering and environmental engineering for application to manufacturing and servicing industries. Viruses, bacteria, yeasts, fungi, algae, the cells and tissues of higher plants and animals, or their enzymes, can provide the means for the improvement of existing industrial processes and can provide the starting points for new industries, for the manufacture of novel products and for improved processes for management of the environment.

SIR WILLIAM HENDERSON, FRS
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and *Celltech Ltd., London, UK*

Guest Editorial

Since 1950, the new science of molecular biology has produced a remarkable outpouring of new ideas and powerful techniques. From this revolution has sprung a new discipline called genetic engineering, which gives us the power to alter living organisms for important purposes in medicine, agriculture and industry. The resulting biotechnologies span the range from the ancient arts of fermentation to the most esoteric use of gene splicing and monoclonal antibodies. With unprecedented speed, new scientific findings are translated into industrial processes, sometimes even before the scientific findings have been published. In earlier times there was a more or less one-way flow of new discoveries and techniques from scientific institutions to industrial organizations where they were exploited to make useful products. In the burgeoning biotechnology industry, however, developments are so rapid that there is a close intimacy between science and technology which blurs the boundaries between them. Modern industrial laboratories are staffed with sophisticated scientists and equipped with modern facilities so that they frequently produce new scientific discoveries in areas that were previously the exclusive province of universities and research institutes, and universities not infrequently develop inventions and processes of industrial value in biotechnology and other fields as well.

Even the traditional flow of new ideas from science to application is no longer so clear. In many applications, process engineers may find that the most economical and efficient process design requires an organism with new properties or an enzyme of previously unknown stability. These requirements often motivate scientists to try to find in nature, or to produce through genetic engineering or other techniques of molecular biology, novel organisms or molecules particularly suited for the requirements of production. A recent study done for the United States Congress* concluded that "in the next decade, competitive advantage in areas related to biotechnology may depend as much on developments in bioprocess engineering as on innovations in genetics, immunology, and other areas of basic science."

These volumes bring together for the first time in one unified publication the scientific and engineering principles on which the multidisciplinary field of biotechnology is based. Following accounts of the scientific principles is a large set of illustrations of the diverse applications of these principles in the practice of biotechnology. Finally, there are sections dealing with important regulatory aspects of the potential hazards of the growing field and of the need for promoting biotechnology in developing countries.

Comprehensive Biotechnology has been produced by a team of some of the world's foremost experts in various aspects of biotechnology and will be an invaluable resource for those wishing to build bridges between 'academic' and 'commercial' biotechnology, the ultimate form of any technology.

Donald A. Glaser
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*"Commercial Biotechnology: An International Analysis," Office of Technology Assessment Report, U.S. Congress, Pergamon Press, Oxford, 1984.

Executive Summary

In this work, biotechnology is interpreted in a fairly broad context: the evaluation and use of biological agents and materials in the production of goods and services for industry, trade and commerce. The underlying scientific fundamentals, engineering considerations and governmental regulations dealing with the development and applications of biotechnological processes and products for industrial, agricultural and medical uses are addressed. In short, a comprehensive but concise treatment of the principles and practice of biotechnology as it is currently viewed is presented. An outline of the main topics in the four volumes is given in Figure 1.

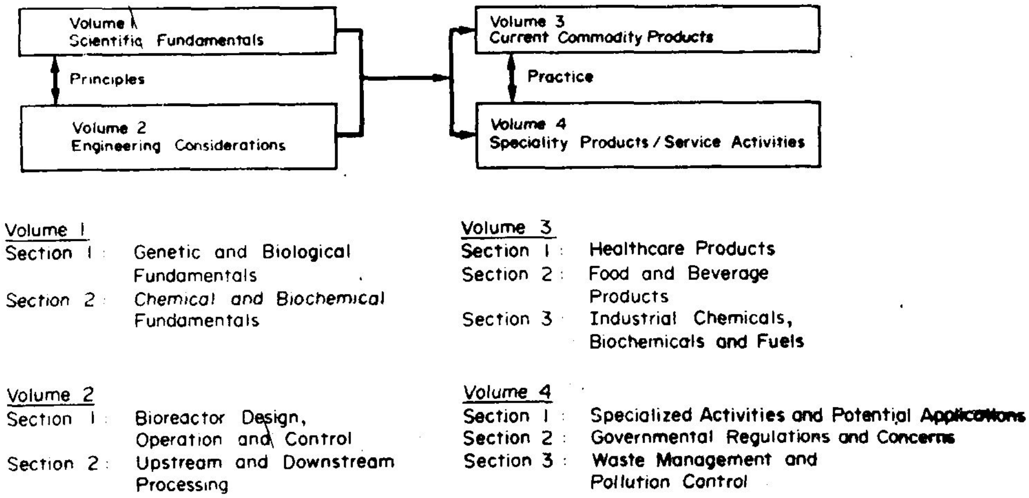

Figure 1 Outline of main topics covered.

As depicted in Figure 2, it is first recognized that biotechnology is a multidisciplinary field having its roots in the biological, chemical and engineering sciences leading to a host of specialities, *e.g.* molecular genetics, microbial physiology, biochemical engineering. As shown in Figure 3, this is followed by a description of technical developments and commercial implementation,

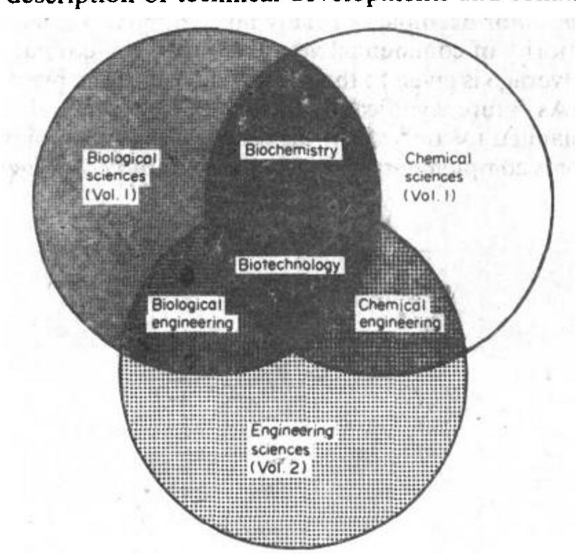

Figure 2 Multidisciplinary nature of biotechnology.

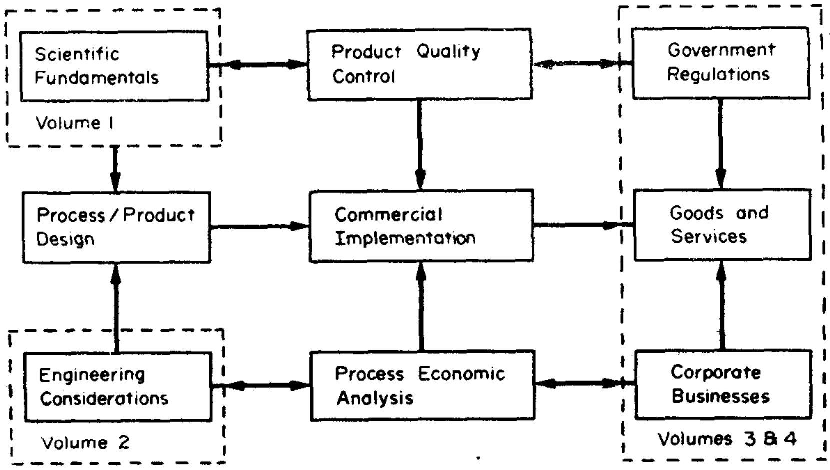

Figure 3 Interrelationships between biotechnology principles and applications.

the ultimate form of any technology, which takes into account other important factors such as socio-economic and geopolitical constraints in the marketplace.

There are two main divisions of the subject matter: a pedagogical academic coverage of the disciplinary underpinnings of the field (Volumes 1 and 2) followed by a utilitarian practical view of the various commercial processes and products (Volumes 3 and 4). In the integration of these two areas, other common factors dealing with product quality, process economics and government policies are introduced at appropriate points throughout all four volumes. Since biotechnological advances are often ahead of theoretical understanding, some process descriptions are primarily based on empirical knowledge.

The four volumes are relatively self-contained according to the following criteria. Volume 1 delineates and integrates the unifying multidisciplinary principles in terms of relevant scientific fundamentals. Volume 2 delineates and integrates the unifying multidisciplinary principles of biotechnology in terms of relevant engineering fundamentals. Volume 3 describes the various biotechnological processes which are involved in the manufacture of bulk commodity products. Volume 4 describes various specialized services, potential applications of biotechnology and related government concerns. In each volume, a glossary of terms and nomenclature guideline are included to assist the beginner and the non-specialist.

This work takes into account the relative importance of the various topics, primarily in terms of current practice. Thus, bulk commodity products of the manufacturing industries (Volume 3) are accorded more space compared to less major ones and for potential applications (part of Volume 4). This proportional space distribution may be contrasted with the expectations generated by the recent news media 'biohype'. For example, virtually no treatment of 'biochips' is presented. In addition, since the vast majority of commercial ventures involve microbial cells and cell-derived enzymes, relatively little coverage is given to the possible use of whole plant or animal cells in the manufacturing industries. As future significant areas of biotechnology develop, supplementary volumes of this work are planned to cover them. In the meantime, on-going progress and trends will be covered in Pergamon's complementary review journal, *Biotechnology Advances*.

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