Biotechnology

EDITED BY I. J. HIGGINS, D. J. BEST & J. JONES

principles and applications

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Preface and acknowledgements

The purpose of this volume is to present a broad, informed view of contemporary biotechnology, a technology with ancient roots and enormous future potential. We hope that it will prove valuable both to students of the subject and practitioners of research in specific aspects seeking a broader view. Clearly, it is not intended to be an advanced treatise but we believe that it is an authoritative introduction to most major aspects of biotechnology written by leading researchers. A substantial collection of references has been included after each chapter for those wishing to pursue more specialist studies.

The fact that this volume was first conceived by one of the editors some four years prior to publication is a measure of the pressure of time on many practising biotechnologists in recent years rather than of their slothfulness. During that period the number of editors grew from one to three and some manuscripts are still awaited! Perhaps they will make the next edition.

The editors are most grateful for the extreme forebearance shown by some of the contributors to this volume who originally offered manuscripts some three years prior to publication. We would also like to thank all contributors and the publishers for their efforts to make a success of a difficult project. We are delighted to have eventually 'got the act together' and sincerely hope that our readership will derive both knowledge and pleasure from this book.

The authors of Chapter 3 wish to thank Dr J. Edelman, Director of Research, RHM PLC, for permission to publish, and the management of the Frish Dytes Partnership for the release of information on MycoProtein. The authors of Chapters 4 and 6 acknowledge the assistance of Mrs D.P. Fowler in the preparation of diagrams.

The author of Chapter 9 is greatly indebted to the following colleagues at Rothamsted Experimental Station for photographs to illustrate the text of this chapter: Dr Barbara Mosse for Figs. 9.1 and 9.2; Dr D.S. Hayman for Figs 9.3–9.6; Dr M.G.K. Jones for Figs. 9.7 and 9.8; and Dr R. Nelson for Figs. 9.9 and 9.10.

Biotechnology Centre
Cranfield Institute of Technology
September 1984

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Contents

1 What is Biotechnology?

I. J. HIGGINS

1.1 Introduction

Biotechnology is not simply, as some sceptics would have it, a latter-day buzz-word for man's oldest industry, but it is a word, the acceptance of which into common parlance is highly symbolic. This acceptance reflects the widely, but not universally held view that many facets of industry and society will be revolutionized over the next ten to fifty years by the application of biological principles and materials. There is no doubt that there has been an extraordinary increase in interest and activity in the subject in the last few years. This has manifested itself in many ways. from the development of countless small entrepreneurial biotechnology companies to the setting up of committees by national governments to examine the potential for the subject and the widespread introduction of university courses in biotechnology. The governments of most advanced nations and many less advanced ones have already committed substantial funds to help catalyse the development of biotechnology, although there are substantial differences in the level of commitment and efficiency of use of available funds. It is a commonly held view amongst professionals concerned with the development of biotechnology that, in general, for a nation to be successful in this endeavour, a commitment from central government is essential to facilitate the evolution of a technology of such a complex and uniquely multi-disciplinary nature. Many aspects of biotechnology involve long lead times from ideas to product and only a few nations, most notably the USA, have fully adequate, free-enterprise financial mechanisms to enable optimum development of such technology, largely independent of government.

Biotechnology is perhaps best defined as the industrial exploitation of biological systems or processes and it is largely based upon the expertise of biological systems in recognition and catalysis. This is reflected in the ability to recognize other biological systems and specific chemicals and an extraordinary ability of enzymes to catalyse a vast range of specific chemical reactions under moderate conditions. Even today, the catalytic chemist cannot compete with the efficiency and specificity of biological catalysis and our understanding of the processes of enzymic catalysis is very limited. In spite of our achievements in chemistry, upon which so much of our industry is based, we are still quite naive in our understanding of catalysis.

Clearly, therefore, man has exploited biotechnology for thousands of years in such activities as brewing, wine-making, bread-making, food preservation and modification by fermentation (e.g. cheese, vinegar and soy sauce), the manufacture of soap from fats, primitive medications and waste treatment. It is, however, the discovery of genetic engineering techniques via recombinant DNA technology (see Chapter 7) which is responsible for the current 'biotechnology boom'. Not only do these techniques offer the prospect of improving existing processes and products, but they are enabling us to develop totally new products which were not previously possible, and facilitate the realization of other processes. The development of genetic engineering is a dramatic example of the difficulty of predicting those areas of basic science which are most likely to lead to important applications. The discovery of recombinant DNA technology is a consequence of the large amount of support given to research into molecular biology for more than forty years. Yet even as recently as the late sixties, a common criticism of giving relatively so much support to this 'glamour' area of chemistry and biology was that nothing directly useful had come from it. Now, however, it is clear that it has led to discoveries that will radically affect humanity.

Whilst recombinant DNA technology has doubtless been the main cause of much of the recent publicity for biotechnology, it must be emphasized that there have also been important recent developments in other areas of activity which are essential for the development of the technology. The most important subjects in this regard are shown in Table 1.1 and their respective contributions to the various different areas of biotechnology are discussed in the following chapters in this book.

In no area of scientific endeavour is the following quotation more appropriate: 'No a thousand times no, there does not exist a category of science to which one can give the name, applied science. There are science and the applications of science, bound together as the fruit to the tree which bears it' (Pasteur, 1871, translated from *Revue Scientifi-*

Table 1.1. Subjects in which there have been recent advances of importance to biotechnology

Genetic engineering (recombinant DNA technology)	
Biocatalysis	Enzymes (isolation, immobilization, stabilization)
	Intact microorganisms and cells from macro-organisms (immobilization, stabilization)
Immunology (especially monoclonal antibodies)	
Fermentation technology	Production
	Waste treatment
Bioelectrochemistry	

que). In biotechnology the applications of the science are intimately linked with fundamental work and are following closely behind the frontiers of the science. The extraordinary multi-disciplinary nature of biotechnology alluded to above is emphasized in Fig. 1.1. Not all products or processes involve all the disciplines depicted but, in any one case, several are involved.

Another interesting aspect of the current stage of development of the subject is the need in many promising areas for close international

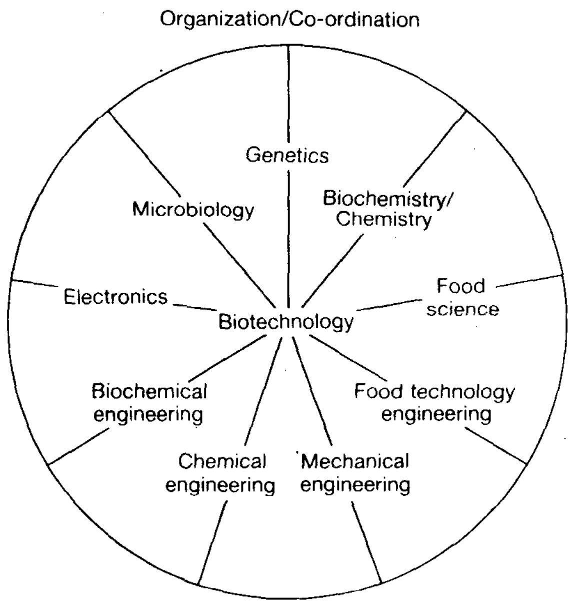

Fig. 1.1. The interdisciplinary nature of biotechnology.

co-operation between the scientists and technologists involved. This is because there are often only a few research groups in the world having the appropriate expertise. This phenomenon has already been exemplified most obviously by the highly multinational nature of several of the larger entrepreneurial biotechnology companies established over the last few years.

The remainder of this chapter is devoted to a brief survey of the development and future potential of the major areas of biotechnology, together with economic considerations, as a prelude to the more detailed discussions of the various areas in subsequent chapters.

1.2 Historical perspectives

Until the recent general acceptance of the all-embracing term, biotechnology, most biologically based technology was encompassed by terms such as applied microbiology, applied biochemistry, enzyme technology, bioengineering, applied genetics and applied biology. Apart from the manufacture of soap, the first of these 'technologies' to develop was the forerunner of applied microbiology. Although ignorant of the processes involved and, therefore, practising an art rather than a science, our ancestors have for thousands of years exploited microbial fermentations for food preservation (e.g. cheese, vinegar), flavour enhancement (e.g. bread, soy sauce) and alcoholic beverage production. Indeed, brewing still remains today in monetary terms the most important biotechnology industry, with a world annual production of about 10^{11} l, worth approximately M£100 000. All these processes depend upon the mechanisms used by some microorganisms to grow and reproduce under anaerobic conditions. It was Pasteur who, in the late nineteenth century, really formed the basis for future developments in applied (industrial) microbiology and, hence, much of biotechnology. He realized that microbes were responsible for fermentation and showed that different types gave different products. His work laid the foundations for the subsequent development of industrial processes for fermentative production of organic solvents (e.g. acetone, ethanol, butandiol, butanol, isopropanol) and other chemicals by different species in the late nineteenth century and early twentieth century. These all involved the conversion of plant carbohydrates to useful chemical products by microbes in the absence of oxygen. Under these conditions, the microbe uses the entropy change in the interconversion as the source of energy for growth. This is distinct from aerobic processes, in which living organisms get far more energy from the controlled oxidation of chemicals to carbon dioxide and water.

Such processes using biomass, i.e. renewable resource feedstocks, for making chemicals constituted the first phase of modern biotechnology. Many were replaced by chemical processes, however, as the petrochemical industry developed. Some chemicals, e.g. citrate, vinegar acetate and itaconate, particularly where used extensively in food processing, have continued to be produced by fermentation, which is the most economic route. Even industrial alcohol in some parts of the world (e.g. Italy) continued to be made by fermentation and now, of course, as a result of the energy crisis, ethyl alcohol production from plant material by fermentation is increasingly important in the USA and Brazil and may well become so in the Far East.

There is likely to be a return to the manufacture of organics by fermentation, but in Europe it may well be limited to higher value chemicals. An obvious exception is the conversion of the EEC wine lakes to industrial alcohol. This is a strange case of politics, biotechn logy and, to say the least, unconventional economics offering unfair competition to the chemical industry.

Fig. 1.2 depicts the biochemical basis of an early example of 'modern' biotechnology, of particular interest since it involves 'engineering' a modification of the normal metabolism involved in the yeast ethanolic fermentation. Addition of bisulphite to the fermentation broth prevents acetaldehyde from acting as an acceptor for reducing equivalents derived from the Embden–Meyerhof pathway. Dihydroxyacetone phosphate then acts as the acceptor, with the consequent generation of glycerol in place of ethanol. This process was particularly important for meeting the demands for glycerol for explosive manufacture during World War I.

The next major milestone in biotechnological generation of valuable products was the development of the antibiotic industry, arising initially from the discovery of the chemotherapeutic properties of penicillin by Fleming, Florey & Chain in 1940. Worldwide, the turnover of this industry is now about £2 × 10^9/a.

Both fermentative production of chemicals and food additives and antibiotic synthesis have always involved aseptic operation of plant, although some more recent processes (e.g. single cell protein (SCP) production) are more demanding in this respect. This is an interdisciplinary problem for the chemical engineer and microbiologist and is discussed in Chapter 10. In contrast, the use of microorganisms in waste

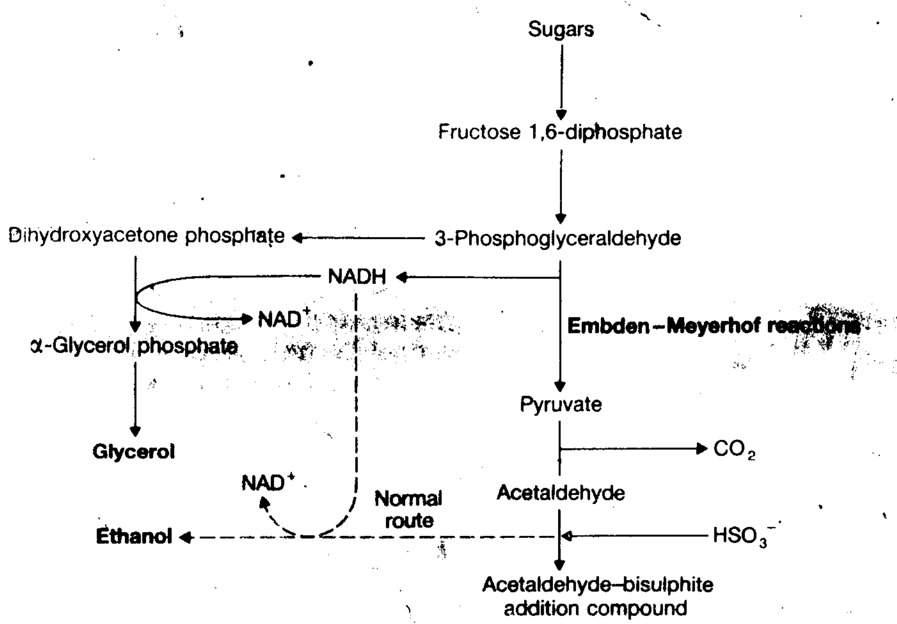

Fig. 1.2. Modification of the yeast ethanolic fermentation to generate glycerol.

treatment processes (Chapter 6) does not involve aseptic operation (in general, the more species present, the better) but the design and manufacture of waste treatment plants presents the chemical engineer and microbiologist team with a different set of problems. The activated sludge process for mineralizing organic waste was first developed in 1914 and since then it has developed dramatically in size and sophistication and is exploited worldwide for treatment of sewage.

The treatment of wastes by anaerobic digestion by mixed microflora, eventually generating biogas (mainly methane and CO_2), has become an increasingly important process throughout this century. It is energetically highly efficient in terms of conserving and concentrating the energy available in the waste (over 80% of the free energy is recovered in the gas). In some parts of the world, particularly in rural communities, the process represents a substantial part of overall energy consumption. There are, for example, over eighteen million biogas generators in rural China. In advanced nations, with high energy consumption, the conversion of wastes to biogas could only meet a few per cent of the energy demands. Nevertheless, in large municipal waste treatment plants, biogas is often burnt in heat engines which in turn drive electricity generators. Small systems for treatment of agricultural wastes have also been developed in recent years.

1.3 Developments in the biotechnology industry since World War II

In addition to continuing improvements in processes discussed above, there have been many other important practical developments over the last forty years, some of which are shown in Table 1.2. The examples given are discussed in subsequent chapters but four particularly interesting examples—amino acids, SCP (single cell protein) steroid transformations and the cultivation of plant and animal cells—are briefly examined further in this section.

During the last thirty years, the production of amino acids by aerobic microbiological processes has expanded rapidly. The two produced in the largest amounts are monosodium glutamate (annual world production of about 150 000 t), used primarily as a flavour enhancer, and lysine (annual world production of about 15 000 t) as a food supplement. The total annual world amino acid sales represent about £1×10^9, with Japanese companies fulfilling most of the demand. The rather special position of Japan in some aspects of biotechnology is discussed later in this chapter under 'Economic considerations'.

Microorganisms have the ability to upgrade low protein plant

Table 1.2. Some recent important products and services based on biotechnology

Industry	Examples
Agriculture	Strain selection, plant and animal breeding techniques (including cloning)
Chemicals	Organic acids (e.g. citric, itaconic), use of enzymes in detergent formulations
Energy	Increasing use of biogas, large-scale production of ethanol as a liquid fuel
Environment	Improved test and monitoring procedures. Prediction of the fate of xenobiotic chemicals via increasing understanding of microbial biochemistry. Improvement in waste treatment techniques, especially for industrial wastes
Food	New methods of food treatment and preservation. Food additives (e.g. microbial polymers, microbiologically derived amino acids), use of enzymes in food processing. Single cell protein
Materials	Mineral extraction, improved knowledge and control of microbial biodeterioration
Medicine	Improved diagnosis using enzymes, enzyme sensors, use of microorganisms and enzymes in manufacture of complex drugs (e.g. steroids), new antibiotics, use of enzymes in therapy

materials to high protein food, and the large-scale industrial exploitation of this phenomenon to grow *Saccharomyces cerevisiae* for human consumption was exploited in Germany during World War I. The yeast was incorporated mainly into sausages and soups and in this way about 60% of the country's pre-war food imports were replaced. Similar processes using the food yeasts, *Candida arborea* and *Candida utilis* were used during World War II. A number of oil and chemical companies began research and development work during the 1960s aimed at developing new processes for making SCP for animal or human consumption (see Chapter 3). This was, in part, a response to the world shortage of protein foodstuff. Substrates include petroleum, methane, methanol and starch, and most products so far have been developed for animal feed. In general, methanol and starch processs have proved most viable. In western countries, ICI have developed the largest plant, making about 70 000 t/a of feedstuff (Pruteen) from methanol in a single fermenter using the methanol-utilizing bacterium, *Methylophilus methylotrophus*. Modifications of the nitrogen assimilating mechanism of this bacterium by recombinant DNA technology, leading to an improved yield, was one of the earliest practical demonstrations of the potential industrial value of genetic engineering (Chapter 7). In the USSR, over 1 Mt/a of SCP is produced, largely from hydrocarbons and vegetable waste. One of the few examples of a high quality SCP product for direct human consumption is being test marketed in Britain by Rank Hovis McDougall and is derived from a fungus grown on carbohydrate feedstocks (Chapter 3).

Whilst there has been a steady increase in the use of the enzymes in industrial processing, particularly over the last twenty years, growth of the enzyme industry has been disappointingly slow. Their use is mainly limited to a small number of enzymes used primarily in food processing. However, the range of enzymes used in medicine, mainly for diagnosis, has been increasing quite rapidly over the last few years. The total enzyme market nevertheless remains relatively small, about £250×10^6/a. The main reasons for this rather slow development are lack of stability, product recovery and problems of cofactor supply or replacement. In some cases, however, these problems have been circumvented by exploiting the specificity of enzymes in intact microorganisms. This technique became extremely important during the 1950s for the large-scale production of steroid drugs. It became established that many microorganisms are capable of hydroxylating the complex steroid nucleus with great regio- and stereo-specificity. An early and particularly important finding was that the mould, *Rhizopus arrhizus*, could hydroxylate the female sex hormone, progesterone, stereospecifically in the 11-position. This greatly simplified the procedure for manufacturing cortisone, used for treating arthritis. This compound had previously been manufactured by a 37-step chemical process with 0·02% yield, leading to a cost of about $200/g. The introduction of the microbial biotransformation step reduced the cost to about 68 c/g by facilitating a much simpler synthetic procedure. Subsequently, a range of microorganisms were isolated capable of specific hydroxylation of the other ring carbon atoms and, more recently, microbial systems have been used for converting phytosterols to the smaller C-19 steroid hormones, used, amongst other things, in oral contraceptives.

There is currently considerable interest in the chemical industry, particularly in the USA and Japan, in the possibility of devising analogous processes for the production of lower cost chemicals, exploiting microbial oxidative biocatalysis as a basis for more economic processes than the conventional chemical one (see Chapter 4).

Our ability to cultivate plant and animal cells on a large scale has already had important industrial consequences, e.g. in the mass production of vaccines. Techniques for fusing different cell lines have