The Institute of Biology's
Studies in Biology no. 136

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This book is dedicated to Mr Raymond Jeffrys B.Sc., a young student Biotechnologist tragically killed in a mountaineering accident on 24 May 1980.

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General Preface

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology proposed this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views on biological topics.

The features of the series include the attention given to methods, the selected list of books for further reading and, wherever possible, suggestions for practical work.

Readers' comments will be welcomed by the Education Officer of the Institute.

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Preface

Biotechnology has been defined as the application of biological organisms, systems or processes to manufacturing and service industries. Biotechnology is in reality a subject of great antiquity, having its origins in ancient microbial processes such as brewing, wine making, and fermented milk products such as cheese and yoghurts. However, new developments in fermenter design and fermentation processes, in enzyme technology and more recently in genetic engineering have introduced new and exciting dimensions to the subject.

In particular, biotechnology stresses the integration of microbiology, biochemistry and chemical and process engineering, but above all, it is directed to application.

This small book attempts to highlight some of the important biological components of biotechnology.

Glasgow, 1981 J.E.S.

Contents

1 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 exhibiting a bewildering array of subdisciplines, for example 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 has been derived primarily from the largely post-war introduction of other scientific disciplines such as physics, chemistry and mathematics into biology which have made possible the description of life processes at the cellular and nuclear level. In the last two decades 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 relative insignificance if all the hopes of *biotechnology* can be realized.

Biotechnology has been defined as the application of biological organisms, systems or processes to manufacturing and service industries. Biotechnology is the science which studies the integrated application of microbiology, biochemistry and process technologies on biological systems for their use in the interdisciplinary nature of science. Biotechnology will create wholly novel industries, requiring little fossil energy, and will be responsible for changing the world economy, particularly in the next century. Biotechnological processes will, in most instances, function at low temperature, will consume little energy, and rely mainly on inexpensive substrates for biosynthesis.

The industrial activities to be affected will include human and animal food production, provision of chemical feedstocks to replace petrochemical sources, alternative energy sources, waste recycling, pollution control, agriculture and new products to aid and revolutionize many aspects of medicine, veterinary sciences, and pharmaceutics. Biotechnology is internationally considered to hold as much, if not more, commercial promise as the so-called revolution in micro-electronics. In particular, 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 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 lead 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 which, by way of new intellectual approaches and novel techniques, have done much to giving a clearer understanding of chemical processes and the chemical bases of biological systems.

The term *multidisciplinary* should be considered as describing a quantitative extension of approaches to problems which commonly occur within a given area. It involves the marshalling of concepts and methodologies from a number of separately identifiable and existing disciplines and applying them to a specific problem in another area. In eontrast, *interdisciplinary* application occurs when the blending of ideas which 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 as such is a new discipline in its own right and has arisen through the interaction between various parts of biology and engineering.

A biotechnologist will employ techniques derived from chemistry, microbiology, biochemistry, chemical engineering and computer science. The main objectives will be 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.

A key factor in the distinction between biology and biotechnology is their scale of operation. The biologist, on his way towards understanding life processes, 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 fermentation technology, not only in the production of antibiotics but in many other useful simple or complex chemical products, for example organic acids, polysaccharides, enzymes, vaccines, hormones etc. Inherent in the development of fermentation processes is the growing 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 publication the *Economist* (CHIPS and BUGS, 1979), 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.*'

A prime reason for the increasing awareness of biotechnology is the realization that the supply of fossil fuels is limited. Thus man must now look at alternative methods of converting solar energy, directly and indirectly, utilizing biomass. From this biomass will be derived many of the essential chemicals required for man's existence. The production of these chemicals will come largely through the astonishing synthetic versatility of microorganisms. Although traditional fermentation industries will always occupy a central role in biotechnology, the hopes of present-day biotechnologists lies primarily with two applications of biological discoveries, *viz.*

(a) *the development of enzyme technology or engineering* (Chapter 6) *i.e. the use of isolated biological units or enzymes in industry and medicine*
(b) *genetic engineering* (Chapter 4) *i.e. the newly acquired ability of man to transfer genetic information between quite distantly related organisms such as plants, animals and microorganisms.*

These fields of study basically attempt to exploit the flow of discoveries being made by the molecular biologist and the enzymologist. Together they are increasingly being referred to as *biomolecular engineering.*

1.3 Biotechnology – a two component central core

In essence, biotechnology may be considered as having a two component central core, in which one part is concerned with obtaining the best biological catalyst for a specific function or process while the second part creates, by construction and technical operation, the best possible environment for the catalyst to perform.

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 and, in particular, plant and animal cell culture will play an increasingly important role in biotechnology (Chapter 10).

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 man-made organic molecules. Collectively, they have an immense gene pool which offers almost unlimited synthetic and degradative potential. Furthermore, microorganisms can possess extremely rapid growth rates far in advance 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 by man of microorganisms with totally novel biochemical potentials (Chapter 4). These new techniques have arisen from the fundamental and basically 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 the preservation of organisms, for retaining essential features during industrial processes and, above all, retaining vigour and viability. In many examples the catalyst is used in a separated and purified form, *viz.* as enzyme,

Table 1 The main areas of application of biotechnology.

1. *Fermentation technology*

   Historically, the most important area of biotechnology, *viz.* brewing, antibiotics, etc., extensive development in progress with new products envisaged *viz.* polysaccharides, medically important drugs, solvents, protein enhanced foods. Novel fermenter designs to optimize productivity.

2. *Enzyme engineering*

   To be 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, semi-synthetic penicillins, starch and cellulose hydrolysis etc. Enzyme probes for analysis.

3. *Waste technology*

   Long historical importance but more emphasis now being made to couple these processes with the conservation and recycling of resources; foods and fertilizers, biological fuels.

4. *Environmental technology*

   A 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 new sources of chemical raw materials and energy - ethanol, methane and hydrogen. Total utilization of plant and animal material.

and 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 6).

The second part of the core of biotechnology encompasses all aspects of the system or reactor within which the catalysts must function (Chapter 3). Here the specialist knowledge of the chemical or process engineer will flourish, providing the design and instrumentation for the maintenance and control of the physicochemical environment such as temperature, aeration, pH, etc., thus allowing the optimum expression of the catalyst (Chapter 6). Thus it can be seen that successful involvement in a biotechnological problem must draw heavily

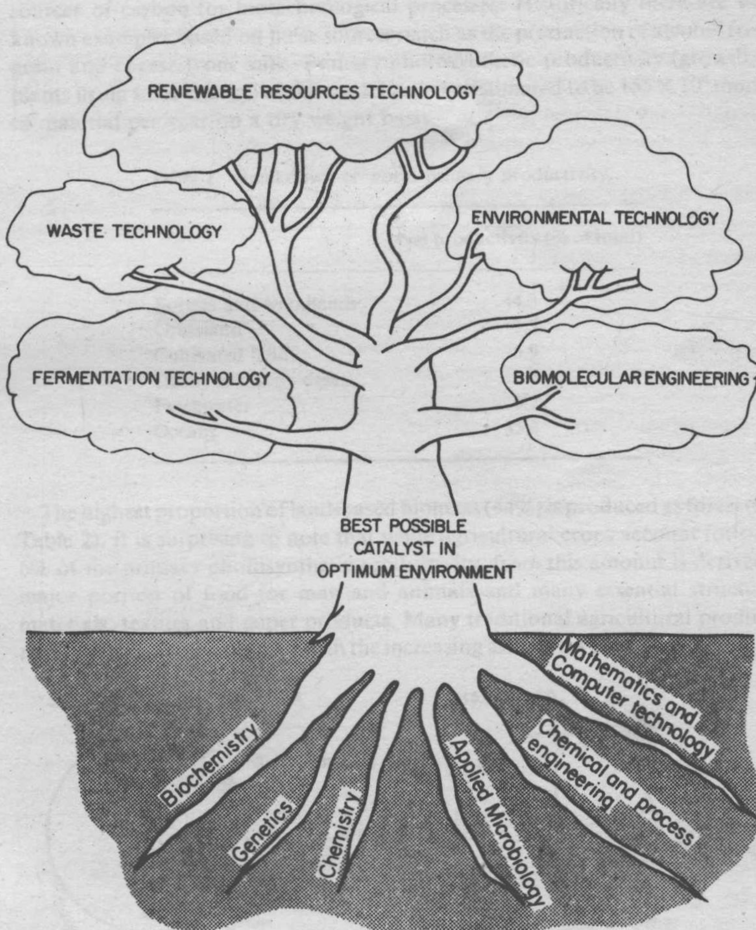

Fig. 1–1 Biotechnology tree.

upon more than one of the input disciplines. The main areas of application of biotechnology are shown in Table 1 while Fig. 1–1 attempts to show how the many disciplinary inputs are realized in biotechnological processes.

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.

2 Substrates for Biotechnology

2.1 The nature of biomass

Plant biomass and, to a lesser extent, animal biomass, represent utilizable sources of carbon for biotechnological processes. Historically there are well known examples based on these sources, such as the production of alcohol from grain and cheese from milk. Primary photosynthetic productivity (growth of plants using solar energy) of the earth has been estimated to be 155×10^9 tonnes of material per year on a dry weight basis.

Table 2 Breakdown of world primary productivity.

	Net productivity (% of total)
Forests and woodlands	44.3
Grassland	9.7
Cultivated land	5.9
Desert and semi-desert	1.5
Freshwater	3.2
Oceans	35.4

The highest proportion of land-based biomass (44%) is produced as forest (see Table 2). It is surprising to note that while agricultural crops account for only 6% of the primary photosynthetic productivity, from this amount is derived a major portion of food for man and animals and many essential structural materials, textiles and paper products. Many traditional agricultural products may well be further exploited with the increasing awareness of biotechnology. In

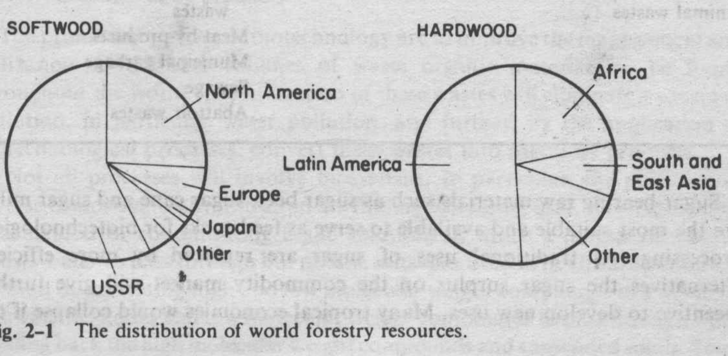

Fig. 2–1 The distribution of world forestry resources.

particular, new technological approaches will undoubtedly be able to utilize the large volume of waste material that presently finds little use with conventional food processing.

Biomass agriculture and forestry may hold great economic potential for many national economies particularly in tropical and subtropical regions (see Fig. 2–1). Indeed the development of biotechnological processes in developing areas where plant growth excels could well see a change in the balance of economic power.

2.2 Natural raw materials

Natural raw materials originate from agriculture and forestry. These are basically carbohydrates of varying chemical complexity and include sugar, starch, cellulose, hemicellulose and lignin. The wide range of by-products obtained from raw materials and of use in biotechnological processes is shown in Table 3.

Table 3 A range of by-products that could be used as substrates in biotechnology.

Agriculture	Forestry	Industry
Straw	Wood waste hydrolysate	Molasses
Bagasse	Sulphite pulp liquor	Distillery wastes
Maize cobs	Bark, sawdust	Whey
Coffee, cocoa and coconut hulls	Paper and cellulose	Industrial waste water from food industries (olive, palm-oil, potato, date, citrus, cassava)
Fruit peels and leaves	Fibres	Wash waters (dairy, canning, confectionery, bakery, soft drinks, sizing, malting corn steep)
Tea wastes		Fishery effluent and wastes
Oilseed cakes		Meat by-products
Cotton wastes		Municipal garbage
Bran		Sewage
Pulp (tomato, coffee, banana, pineapple, citrus, olive)		Abattoir wastes
Animal wastes		

Sugar-bearing raw materials such as sugar beet, sugar cane and sugar millet are the most suitable and available to serve as feedstocks for biotechnological processing. As traditional uses of sugar are replaced by more efficient alternatives the sugar surplus on the commodity market will give further incentive to develop new uses. Many tropical economies would collapse if the market for sugar were removed. Already cane sugar serves as the substrate for the Brazilian gasohol programme (Chapter 7) and many other nations are rapidly seeing the immense potential of these new technologies.

Starch-bearing agricultural products include the various types of grain such as maize, rice and wheat, together with potatoes and other root crops such as sweet potato and cassava. A slight disadvantage of starch is that it must usually be degraded to monosaccharides or oligosaccharides by digestion or hydrolysis before fermentation. However, many biotechnological processes using starch are being developed, including fuel production.

There can be little doubt that cellulose, both from agriculture and forestry sources, must contribute a major source of feedstock for biotechnological processes such as fuels and chemicals. However, cellulose is a very complex chemical and invariably occurs in nature in close association with lignin. The ability of lignocellulose complexes to withstand the biodegradative forces of nature is witnessed by the longevity of trees which are mainly composed of lignocellulose.

Lignocellulose is the most abundant and renewable natural resource available to man throughout the world. However, massive technological difficulties must be overcome before economic use may be made of this plentiful compound. At present, expensive energy-demanding pre-treatment processes are required to open up this complex structure to wide microbial degradation. Pure cellulose can be degraded by chemical or enzymatic hydrolysis to soluble sugars which can be fermented to form ethanol, butanol, acetone, single cell protein (SCP, see Chapter 5), methane and many other products. Exciting advances are being made in the U.S.A. Sweden and Britain and it is only a matter of time before these difficulties are overcome. It has been realistically calculated that approximately 3.3×10^{14} kg CO_2 year^{-1} are fixed on the surface of the earth and that approximately 6% of this, i.e. 22 billion tonnes year^{-1} will be cellulose. On a world-wide basis land plants produce 24 tonnes cellulose person^{-1} year^{-1}. Time will surely show that lignocellulose will be the most useful carbon source for biotechnological developments.

2.2 Availability of by-products

The primary objectives of biotechnology are to improve the management and utilization of the vast volumes of waste organic materials to be found throughout the world. The utilization of these wastes will eliminate a source of pollution, in particular water pollution, and further, by the application of biotechnological processes, convert these wastes into useful by-products.

(Not all processes will involve biosystems. In particular, the processes of reverse osmosis and ultrafiltration are finding increasing uses. Reverse osmosis is a method of concentrating liquid solutions in which a porous membrane allows water to pass through but not the salts dissolved in it. Ultrafiltration is a method of separating high and low molecular weight compounds in a liquid by allowing the liquid and low molecular weight compounds to pass through while holding back the high molecular weight compounds and suspended solids. Some current applications of these technologies include: (a) concentration of dilute factory effluents; (b) concentration of dilute food products; (c) sterilization of water; (d) purification of brackish water; and (e) separation of edible solids from dilute effluents.)

Within this group are products which are important for economic and environmental reasons. Many are the by-products of the food industry and because of their low economic value are discharged into waterways creating, in many cases, serious environmental pollution problems. An attractive feature of carbohydrate waste as a raw material is that, if its low cost can be coupled with suitable low handling costs, an economic process may be obtained. Furthermore, the world-wide trend towards stricter effluent control measures, or parallel increase in effluent disposal charges, can lead to the concept of waste as a negative cost raw material. However, the composition or dilution of the waste may be so dispersed that transport to a production centre may be prohibitive. On these occasions biotechnology may only serve to reduce a pollution hazard.

Each waste material must be assessed for its suitability for biotechnological processing. However, when a waste is available in large quantities and preferably over a prolonged period of time, then a suitable method of utilization can be considered (Table 4).

Table 4 Biotechnological strategies for utilization of suitable organic waste materials.

1.	Upgrade the food waste quality to make it suitable for human consumption.
2.	Feed the food waste directly or after processing to poultry, pigs, fish or other single stomach animals which can utilize it directly.
3.	Feeding the food waste to cattle or other ruminants if unsuitable for single stomach animals because of high fibre content, toxins or other reasons.
4.	Production of biogas (methane) and other fermentation products if unsuitable for feeding without expensive pretreatments.
5.	Selective other purposes such as direct use as fuel, building materials, chemical extraction etc.

Two widely occurring wastes which already find considerable fermentation uses are molasses and whey. Molasses is a by-product of the sugar industry and has a sugar content of approximately 50%. Molasses is widely used as a fermentation feedstock for the production of antibiotics, organic acids and commercial yeasts for baking, and is directly used in animal feeding. Whey, obtained during the production of cheese, could also become a major fermentation feedstock.

More complex wastes such as straw and bagasse are widely available and will be increasingly used as improved processes for lignocellulose breakdown become available (Table 5). Wood wastes will include low grade wood, bark and sawdust, as well as waste liquors such as sulphite waste liquor from pulp production which already finds considerable biotechnological processing in Europe and Communist countries (Chapter 5).

The largest proportion of total volume of waste matter is from animal rearing (faeces, urine), then agricultural wastes, wastes from food industries and finally domestic wastes. The disposal of many waste materials, particularly animal waste, is no problem in traditional agriculture and particularly well exemplified in China where recycling by composting has been long practiced. However, where intensive animal rearing is undertaken, serious pollution problems do arise.

Table 5 Pre-treatments required before substrates are suitable for fermentation.

Substrate	Pre-treatment
Sugary materials	
Sugar cane, beet, molasses, fruit juices, whey	Minimal requirements dilution and sterilization
Starchy materials	
Cereals, rice, vegetables, process liquid wastes	Some measure of hydrolysis by acid or enzymes. Initial separation of non-starch components may be required
Lignocellulosic materials	
Corn cobs, oat hulls, straw, bagasse, wood wastes, sulphite liquor, paper wastes	Normally requires complex pre-treatment involving reduction in particle size followed by various chemical or enzymic hydrolyses. Energy intensive and costly

2.4 Chemical and petrochemical feedstocks

With the development of commercial processes for the production of single cell protein (SCP) and other organic products, a number of chemical and petrochemical feedstocks have become particularly important for fermentation processes since these materials have the advantage of being available in large quantities and in the same quality in most parts of the world. Thus, natural gas or methane and gas oil have been preferred as raw material because of their easy processing and universal availability. Main commercial interest has been concerned with *n*-paraffins, methanol and ethanol. Their involvement in various aspects of biotechnology, but particularly, in SCP production will be considered later. Table 6 summarizes the many technical considerations that must be made when approaching the utilization of waste materials.

Future biotechnological processes will increasingly make use of organic materials which are renewable in nature or occur as low value wastes which may presently cause environmental pollution. Some processes may also more economically utilize specific fractions of fossil fuels as feedstocks for biotechnological processes.

Table 6 Technical considerations for the utilization of waste materials.

Biological availability	*low* (cellulosics)
	moderate (starch, lactose)
	high (molasses, pulping sugars)
Concentration	*solid* (milling residues, garbage)
	concentrated (molasses)
	weak (lactose, pulping sugars)
	very dilute (process and plant wash liquors)
Quality	*clean* (molasses, lactose)
	moderate (straw)
	dirty (garbage, feedlot waste)
Location	*collected* (large installation, small centres)
	collected specialized (olive, palm oil, date, rubber, fruit, vegetable)
	dispersed (straw, forestry)
Seasonality	*prolonged* (palm-oil, lactose)
	very short (vegetable cannery waste)
Alternative uses	*some* (straw)
	none (garbage)
	negative (costly effluents)
Local technology potential	*high* (United States of America)
	middle (Brazil)
	low (Malaysia)