Environmental Biotechnology

Alan Scragg

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作　　者：A.Scragg

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Preface

This book covers the influence and application of biotechnology on many aspects of the environment. The influence will be dominated, as is biotechnology in general, by the advances in recombinant DNA technology which have seen application in all areas of biotechnology. Environmental applications of biotechnology have been in four areas: the monitoring of pollution, the treatment of waste, the treatment of already polluted sites and waterways, and the prevention of pollution.

In order to enforce pollution legislation and to determine levels of contamination, the detection and estimation even of very low levels of pollution is required. Biosensors can be used to give real-time on-line measurements. Genetic engineering can be used to generate biomarkers which can provide sophisticated and specific methods for the detection of contaminants. This will have a considerable influence on pollution control, just as recombinant DNA technology has profoundly affected forensic science.

The disposal of organic wastes from domestic, agricultural and industrial sources cannot be eliminated. Many of the advances in the methods of disposal of these organic wastes have been based on a better knowledge of the biological processes involved. The traditional methods of disposal are described, as are the more recent process developments such as fluidised beds and membrane reactors. The book discusses the application of recombinant DNA technology to the better understanding of the aerobic and anaerobic processes involved, and to developments in the removal of nitrates and phosphates.

Industrialisation and the widespread use of chemicals have left a legacy of polluted sites and waterways. These sites need to be cleaned up to comply with current legislation, and biological methods are a viable alternative to chemical methods. The use of biological material, known as bioremediation, may be a slower process than chemical methods, but bioremediation can mineralise even very low levels of contamination and can be cheaper. The underlying metabolic pathways are described along with a number of processes. The use of biological material for the removal of metals and for the extraction of metals from ores is also described.

The prevention of pollution has in the past often been the last option to be considered, but the medical axiom 'prevention is better than cure' is also true of pollution. In this area the use of microorganisms or enzymes to replace

chemical synthesis will reduce side-products and energy requirements. Cultivation of genetically engineered plants will reduce the use of agrochemicals, and there is considerable potential in the improvement of plant and animal characteristics. Biotechnology can also be used to produce biofuel which should reduce the production of greenhouse gases, and the development of biodegradable plastics should help to reduce waste. The application of transgenic plants and animals is not without its problems, however, as there is much public concern over the release of such transgenic material into the environment and its use as a food product.

In explaining the influence of biotechnology on environmental sciences, it is inevitable that some areas will see greater application of biotechnology than others. The book does not cover all aspects of environmental science and therefore some areas, such as landfill, will be covered only briefly, as biotechnology has seen greater application in such areas as environmental monitoring and agriculture.

Many thanks to Pat Bonham for the considerable time and effort that he put into editing this text.

Alan Scragg
September 1998

SI units

Prefix	Symbol	Factor
exa	E	10^{18}
peta	P	10^{15}
tera	T	10^{12}
giga	G	10^{9}
mega	M	10^{6}
kilo	k	10^{3}
hecto	h	10^{2}
deka	da	10^{1}
deci	d	10^{-1}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}
atto	a	10^{-18}

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Chapter 1

Overview

1.1 Introduction

To set environmental biotechnology in context it is perhaps useful to define biotechnology itself. Biotechnology can be described as the application of biological organisms, systems or processes to manufacturing or services. The European Federation of Biotechnology defines biotechnology as 'the integrated use of biochemistry, microbiology, and engineering sciences in order to achieve applications of the capabilities of microorganisms, cultured animal or plant cells or parts thereof in industry, agriculture, health care and in environmental processes' (European Federation of Biotechnology, 1988), which is a somewhat expanded version of their 1982 definition.

The use of the word 'biotechnology' to describe a subject might seem to infer a marked degree of coherence, as in biochemistry, but there is an argument that biotechnology cannot really be regarded as a single subject. Biotechnology, in practice, combines a set of different but interrelated subjects which are applied to a broad spectrum of industries. The choice of subjects which are combined depends on the individual industrial problems. This application to various industries requires a wide range of science and engineering expertise, often concentrated on the development of a single product. Houwink (1989) perhaps best describes biotechnology briefly as 'the controlled use of biological information'. As Houwink pointed out, the study of more than one subject is not itself biotechnology, but when such study is directed towards an application it becomes biotechnology. Thus, biotechnology is in essence multidisciplinary, combining subjects such as microbiology, molecular biology, cell biology and engineering. The relationships between the various disciplines and biotechnology are best illustrated by the hourglass model (Houwink, 1989) where biotechnology acts as an interface between individual scientific disciplines and their various areas of application (Fig. 1.1).

The term 'biotechnology' was first used in 1919 and later in 1938 (Kennedy, 1991). The journal *Biotechnology and Bioengineering* was named in 1962 and the journal *Biotechnology* was launched in 1979. The public in the UK probably first heard of biotechnology in the early 1980s with the publication of the Spinks report, *Biotechnology: Report of a Joint Working Party* (Spinks, 1980), and with the start-up of a number of small biotechnology companies, often specialising in genetic engineering.

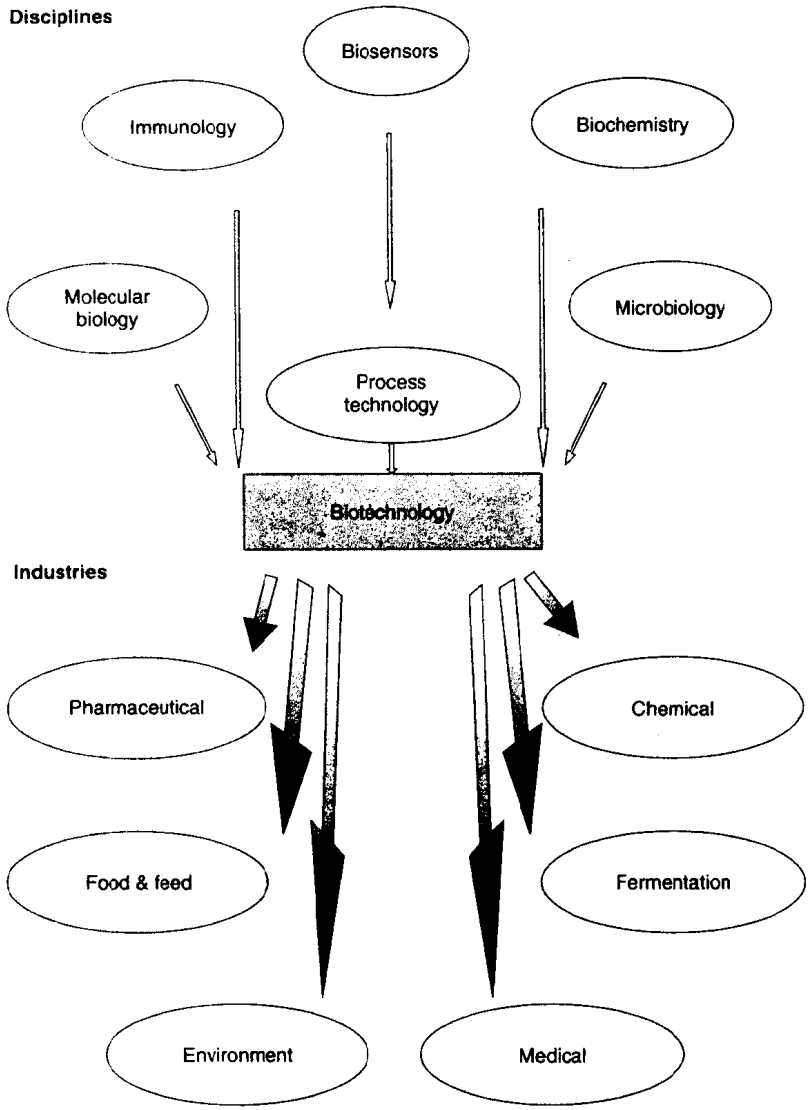

Fig. 1.1 The hourglass model for biotechnology, adapted from Houwink (1989).

1.1.1 Environmental biotechnology

This book attempts to cover the application of biotechnology to the wide range of environmental problems under the title of environmental biotechnology. One definition of environmental biotechnology is 'the specific application

of biotechnology to the management of environmental problems, including waste treatment, pollution control, and integration with non-biological technologies'. The application of biotechnology to environmental problems has developed alongside biotechnology in general and in many ways its application has been as an extension of natural processes. Current estimates of the market for environmental biotechnology are in the region of 300 billion dollars worldwide (Golub, 1997) and some 84–94 billion dollars in Europe (Glass *et al.*, 1995). Investment in environmental biotechnology will continue, particularly in the processes of environmental clean-up and remediation and the development of a sustainable technology.

1.1.2 Biotechnology past

Over the centuries microorganisms have been used unwittingly for the preparation of various foods and beverages. This was probably the first example of biotechnology. Such operations for food and drink preparation were carried out in a highly empirical manner without any real knowledge of the microorganisms or processes involved. As our understanding of the science underlying biological processes has increased so the nature of biotechnology has shifted from the empirical to the controlled. To understand this development, the evolution of biotechnology can be divided into a series of eras (Houwink, 1984) as shown in Fig. 1.2. The first biotechnology process was probably the production of alcoholic beverages over 5000 years ago. This was first documented in Egypt from 4000 BC. Recently the Scottish and Newcastle Brewery in the UK produced a beer similar to an ancient Egyptian beer prepared from an emmer wheat strain similar to that found in Egyptian tombs. The beer proved quite palatable. At the same time as brewing was evolving, fermented milk products such as cheese and yogurt were used as a method of preserving milk. As anyone who has attempted alcoholic fermentations will know, it is easy for some microorganisms to convert ethanol to acetic acid, so that vinegar production also developed before 3000 BC (Prave *et al.*, 1987).

The empirical approach changed with the realisation, from the work of Anton van Leeuwenhoek in 1650, that microorganisms existed and that these microbes were the causative agent of such processes as fermentation. The latter advance was based on the work of people such as Pasteur, Koch and Buchner in the nineteenth century. The slow developments in the study of microorganisms led to what is entitled the Pasteur era, where specific products were produced using selected microorganisms. The first was perhaps the microbial production of lactic acid in 1881. Acetone and butanol are important bulk chemicals as solvents, hydraulic fluids and plasticisers, and the production of butanol by bacteria was first discovered by Pasteur. Later Weizmann, working at the University of Manchester in 1914, investigated the use of the anaerobic bacterium *Clostridium acetobutylicum* for the production of butanol to be used in rubber manufacture. However, the bacterium produced acetone, butanol and ethanol. Acetone, apart from being used as a solvent, was also

Pre-Pasteur Era: before 1865
Alcoholic beverages (beer, wine)
Dairy products (cheese, yogurt)
Other fermented foods

Pasteur Era: 1865–1940
Ethanol, butanol, acetone, glycerol
Organic acids (citric acid)
Aerobic sewage treatment

Antibiotic Era: 1940–1960
Penicillin: submerged fermentation
Large variety of antibiotics
Animal cell culture technology; virus vaccines
Microbial steroid transformations

Post-antibiotic Era: 1960–1975
Amino acids
Single cell protein (SCP)
Enzymes (detergents)
Immobilised enzyme and cell technology
Anaerobic wastewater treatment (biogas)
Bacterial polysaccharides (xanthan)
Gasohol

New Biotechnology Era: 1975–
Hybridoma technology – monoclonals
Monoclonal diagnostic tests (1980)
Genetic engineering (1974)
Animal diarrhoea vaccines (1982)
Human insulin (1982)
Release of genetic engineered plants (1992)
Genetic engineered food (1996)

Fig. 1.2 Biotechnology calendar of events, adapted from Houwink (1984).

used for the production of the smokeless explosive cordite. Acetone was normally produced by the pyrolysis of wood, which formed calcium acetate which on heating released acetone. About 100 kg of wood was required to produce each kilogram of acetone. With the outbreak of World War I the demand for cordite far outstripped the supply of acetone from wood pyrolysis. Therefore, the microbial process for the production of butanol and acetone using *C. acetobutylicum* went from laboratory scale to production very rapidly in 1915 due to the need for explosives. The biological process was more efficient and cheaper than wood pyrolysis, forming 12 tonnes of acetone per 100 tonnes of molasses used to grow the microorganism. This process continued until the 1950s when it was replaced by the production of acetone from polypropylene produced by the petrochemical industry. The chemical process replaced the biological process as it was cheaper, because the price of molasses had risen, and the biological process produced considerable quantities of waste. The last biological process was operating in South Africa until 1982 because of the embargo on the importation of petrochemical products to that country. This is not the only example of a biological process being rapidly developed due to the pressure of war. This also happened with the antibiotic penicillin. Recent

advances in biochemistry, molecular biology and waste treatment have suggested that the biological process for acetone production could be reintroduced as it uses renewable resources and not petrochemicals which have a finite supply (Girbal and Soucaille, 1998). In this period other significant developments were the start of the biological treatment of sewage in 1914, with filter beds and activated sludge plants in Manchester and a number of continental European cities.

Although penicillin was discovered by Alexander Fleming in 1928–29, its large-scale production was not achieved until 1941. Research and development since 1928 by Florey and Chain had made the extraction of penicillin possible, and its ability to treat infection was demonstrated, but its large-scale production was still not possible at the start of World War II. The penicillin-producing organism *Penicillium notatum* was normally grown as a mat on the surface of the medium, which made scale-up very difficult and labour intensive. In 1941 Florey's team moved to the USA where submerged deep fermentation was developed for the cultivation of a related but higher-producing *Penicillium* strain, *P. chrysogenum*. The submerged deep fermentation allowed the large-scale growth of *P. chrysogenum* and required the combined efforts of microbiologists, biochemists and process engineers. However, by 1944 there was sufficient penicillin to treat a great many military casualties. This is another example of the development of a product driven by the needs of a world war.

Although large-scale cultivation of bakers' yeast for baking and of *Aspergillus niger* for citric acid was possible before the development of the penicillin process, the ability to grow microorganisms on a large scale began the 'antibiotic era'. Thus the production of a large variety of antibiotics, such as streptomycin, tetracyclines and cephalosporins, followed.

Other areas of scientific advancement at this time were animal cell culture and microbial steroid transformation. The cultured animal cells were used for virus isolation and vaccine production, for example the first polio vaccine. In the 1950s it was found that microorganisms were able to transform a wide range of compounds, and in most cases these transformations were very specific. Thus microorganisms were able to carry out transformations which would be difficult to carry out chemically. Microbial transformation has been extensively used in the production of steroids.

Knowledge gained in the development of the antibiotic industry was applied to other problems in what is referred to as the post-antibiotic era. Microorganisms were used to produce individual amino acids and vitamins; some 30,000–40,000 tonnes of amino acids such as lysine and glutamic acid (monosodium glutamate) are produced by microbial processes annually. The mass production of enzymes, in particular extracellular enzymes, allowed enzymes to be used in detergents and for the production of glucose from starch.

The production and use of enzymes can be expensive as they have only a limited active life and when added to the substrate they cannot easily be recovered. However, if enzymes are immobilised they can be used in a continuous process and their active life extended. This has meant that some

enzyme-based processes can be adopted as immobilisation has reduced the cost of the enzymes to acceptable levels. One example of the use of immobilisation has been the development of a process for the production of high-fructose syrups. Fructose is 1.7 times as sweet as sucrose and therefore can be used in smaller quantities as a sweetener in low-calorie foods and drinks. The production of high-fructose (55%) sweeteners was costly, as the enzyme glucose isomerase which converts glucose to fructose was expensive to prepare. However, if glucose isomerase was immobilised it could be used in a continuous process for the production of high-fructose sugars from glucose syrups, thus reducing the costs. Techniques of immobilisation of both cells and enzymes have also been applied to the development of biosensors.

In the 1960s there was much concern that, with the increase in population in the world, there would be a shortage of food and in particular protein. Alternative sources of protein were sought and these included microorganisms such as algae, bacteria, yeasts and fungi. Algae and yeast had previously been used as both human and animal foods. The main aim was to use microorganisms to produce a high-protein animal feed, and the term 'single cell protein' (SCP) was used to describe this. One feature of SCP production as an animal feed was that its cost had to be low to compete with existing animal food ingredients such as soya bean meal. One of the major costs in the production of microbial biomass (cells) is the substrate, which can account for up to 60% of the cost. Therefore, the substrates used for SCP production should be cheap and available in large quantities. Substrates investigated included either renewable substrates like agricultural wastes, crops containing starch and cellulose, or non-renewable substrates from the petrochemical industry (Sharp, 1989). At that time the petrochemical industry had a surplus of cheap methane, methanol and alkanes and for this reason many of the large petrochemical companies became involved in the production of SCP from these substrates. In order to keep the cost of SCP to a minimum the SCP process needs to be carried out on a large scale, as large-scale processes bring cost reductions.

Perhaps the best known SCP process was developed by ICI for the production of Pruteen, an animal food. ICI had a bacterium, *Methylophilus methylotrophus*, which was capable of growth on methanol. Methanol was produced cheaply from methane coming from the North Sea gas fields. The bacterium passed tests of safety and nutritional value and proved successful as an animal food. ICI built a large plant in the early 1980s for the production of Pruteen with a 1.5 million litre bioreactor which was run in a continuous manner. This was a prodigious technological feat representing a considerable advance in bioprocess technology, and in 1981 6000 tonnes of Pruteen were produced per month. Despite this early technical success the process has now been abandoned for the following reasons:

- Oil/gas and therefore methanol prices increased much more than expected.
- The production of fish and soya bean meal, the main competitor, expanded considerably.

- Food shortages did not develop as expected, due to improvements in the organisation of storage and distribution.
- The 'green revolution', the development of high-yielding crops, has reduced demand for synthetic protein foods.

In an attempt to improve the efficiency of the process, *M. methylotrophus* was genetically engineered to enhance its uptake of ammonia, which was one of the first examples of the application of genetic engineering to a process. However, it is not clear whether a genetically engineered animal food would have been accepted. There remains one successful SCP product, Quorn, which is a human food based on a fungus, *Fusarium graminarium*, which is grown on molasses or hydrolysed starch. Quorn was developed initially by Rank Hovis McDougall and approved for human use in 1982–83. Its sales were initially slow, but changes in eating habits in the late 1980s and early 1990s saw more interest in low-fat, high-fibre vegetarian foods and thus the sales of Quorn increased. The production of Quorn is now carried out by Marlow Foods which is mainly owned by ICI.

The 1970s saw a crisis in oil production due to hostilities in the Middle East which reduced the supply of crude oil to developed countries. Therefore, alternative supplies of fuel were investigated, and one of the products which was examined was ethanol which could be used as a supplement (10–20%) or as a complete replacement for petrol without major changes to car engines. Ethanol can be produced by the fermentation of sugars in the same process as brewing or wine production. Ethanol was introduced as a 100% petrol replacement in Brazil in order to reduce the country's reliance on imported oil, and to develop an ethanol industry. The process used was simple fermentation using yeast and sugar from sugarcane. The fermentation produced 8–10% ethanol which was extracted and purified by distillation. In the 1980s some 75% of the cars in Brazil used ethanol either exclusively or as an additive to petrol. The percentage using ethanol has declined because of the continued low price of crude oil, and at present 50% of the cars in Brazil use alcohol either alone or as a 10% addition to petrol. Another biofuel, methane (biogas), can be formed by anaerobic digestion of sewage or other wastes. Methane has also been developed as a fuel for heating and cooking, particularly in developing countries.

Another microbial product developed at this time was the microbial polysaccharide xanthan which is used in foods to increase their viscosity and in drilling muds due to its viscoelastic properties.

1.1.3 Biotechnology present

The last era recognised by Houwink was named after the 'new biotechnologies' which are principally involved with the application of genetic engineering to all areas of biotechnology, including environmental biotechnology. The other novel development during this era has been that of monoclonal antibodies.

Genetic engineering or recombinant technology developed in the 1970s and the techniques have revolutionised our ability to isolate, manipulate and express genes and therefore proteins virtually at will. The ability to isolate a particular gene, to multiply the gene if required, and to insert the gene into another organism means that traditional species barriers can be crossed in ways not possible by traditional breeding, so that for instance human proteins can be made in plants. The ability to manipulate and transfer genes has had a dramatic impact on all areas of biotechnology. The transformed organism can be animal, plant or microorganism and the form of genetic engineering irrespective of the organism can be of three main types:

- The insertion of a single gene which gives the recipient a new characteristic, such as herbicide resistance in cotton plants, or starch degradation (amylase) in *Saccharomyces cerevisiae*.
- The alteration of the operation of existing genes which may change the characteristics of the recipient, for example the change in fruit ripening by reducing the activity of polygalacturonase by antisense technology in tomato plants (e.g. Flavr Savr™ – see Section 8.2) or changes in oil quality in rapeseed.
- The insertion of a gene so that the recipient produces a specific product, usually a protein, which is not aimed at altering the characteristic of the organism but acts as a supply of the product. This is often known as biopharming and an example is the production of human insulin in the bacterium *E. coli*. The recipient can be bacterial, yeast, insect, plant or mammalian cultures depending on the product required and the post-translational processing.

Some examples of the development of transgenic technology since 1982 in three industries – medical, agriculture and food – are given in Table 1.1. The first medical transgenic product was human insulin (humulin) where the gene was cloned into the bacterium *E. coli* and the bacterium used to produce insulin for the treatment of diabetes. This product has the advantage of being identical to human insulin rather than pig insulin which had previously been used. There is an alternative supply of human insulin from a process in which pig insulin is converted enzymatically into human insulin by the alteration of one amino acid. Human growth hormone has been produced in bacteria and, although the market for this hormone is limited, the hormone was previously extracted from human pituitary glands and any extraction from human material carries a risk of viral contamination. Thus many of the transgenic materials produced for medicine have been developed to provide material which was either difficult to extract, carried other risks or was very expensive. In contrast agriculture has seen the genetic engineering of common crops such as maize, cotton and tomato. In these cases the transgenic plants have been given characteristics such as herbicide and insect resistance or changes to fruit quality. The food industry has developed a diverse range of transgenic products but only a few have been adopted. One transgenic product which has been used is the enzyme chymosin produced by transgenic yeast. Chymosin is the starter enzyme in cheese production and the transgenic product has been used to replace calf chymosin (rennet). The cheese is sold as a vegan product as the

Table 1.1 Applications of genetic engineering in the medical, agricultural and food industries

Year Product	Disease/property/action
Medical	
1982 Human insulin (humulin)	Diabetes
1985 Human growth hormone (prototropin)	Growth deficiency
1987 Human growth hormone (humatrope)	Growth deficiency
1991 Intron A	Hepatitis C
1992 Recombinate (factor VIII)	Hemophilia A
1993 Pulmozyme (DNase)	Cystic fibrosis
1994 Albunex	Diagnosis of heart disease
Agriculture	
1992 Flavr Savr™ tomato	Altered ripening
1994 Cotton	Herbicide resistance, glyphosate
1994 Rapeseed/Canola	Altered oil content
1994 Squash	Virus resistance
1995 Potato	Insect resistance
1995 Maize	Insect resistance
1997 Chicory	Male sterile
Food	
1990 Bakers' yeast	Faster carbon dioxide liberation
1991 Bovine chymosin from GM yeast	Cheese production
1994 Brewers' yeast	Starch degradation
1997 Hemicellulase	Xylanase
1997 Riboflavin	Vitamin B_2

enzyme was from yeast and it has been labelled as a genetically engineered product.

It is clear that there are tremendous advances to be made with transgenic organisms (genetically manipulated organisms, GMOs) in all areas of biotechnology including environmental biotechnology. However, there is public concern over modern biotechnology as the public perceives that the development of genetically engineered products is motivated mainly by profit, that such crops and animals are unnatural and carry unknown risks, and that transgenic organisms or products should be labelled as such. This feeling of distrust in biotechnology is also involved with the notion of 'playing God', particularly when confronted with animal experiments, with tales of cloned animals and humans. It has been stated that 'genetic engineering is not some minor biotechnology development. It is a radical new technology that violates fundamental laws of nature'. Environmental newsletters have carried articles with titles such as 'Are you ready for frankenfood?' (Kareiva and Stark, 1994). All this unfavourable publicity has seen sales of genetically engineered tomatoes staying at low levels in the USA (Golub, 1997) and there have been calls to