

1

Introduction

1.1	Introduction	1
1.2	Environment	1
1.3	Biotechnology	2
1.4	Areas of application for biotechnology	4
1.5	Genetically manipulated organisms	9
1.6	Legislation	11
1.7	Conclusions	13
1.8	Further reading	14

1.1 Introduction

Environmental biotechnology is the application of biotechnology to all aspects of the environment. Biotechnology deals with the use of living organisms or their products and has been around for some time. Biotechnology has been recently invigorated by the development of genetic engineering, which now has applications in all areas of biotechnology. This book is concerned with how biotechnology can be applied to environmental problems and issues. This will cover existing pollution in terms of the detection and bioremediation of the contaminants. In addition, treatment is required for industrial, agricultural, and domestic process wastes, some of which cannot be eliminated. There is also a need to reduce the production of pollutants at source. One of the most important pollutants is carbon dioxide, which is a cause of global warming. The reduction in the production of industrial pollutants is known as ‘**clean technology**’. The chapters cover these subjects and also included is a chapter on microbiology because it is one of the core disciplines in biotechnology and genetic engineering.

1.2 Environment

It would appear that the public’s awareness of the environment was only noticeable after a number of disastrous environmental accidents. Examples

are the oil spills from the tankers *Amoco Cadiz*, off the coast of Brittany in 1978, and *Exxon Valdez*, in Alaskan water in 1989, the toxic gas release from a pesticide plant in Bophal, India, in 1984, and the nuclear power station accident at Chernobyl in 1986. These events achieved great publicity and increased the awareness of environmental issues. However, problems with the environment were recognized earlier, with Rachel Carson's book *Silent Spring* in 1963 dealing with the problems caused by pesticides. This book and others lead to a gradual change in the perception of the environment as unchanging and capable of dealing with any amount of pollution and contamination to one of the environment being a delicate balance of many components. Unfortunately not all the problems in the environment develop rapidly; often the changes are slow and can remain unnoticed until a critical stage has been reached. These types of problems are much more difficult to publicize and deal with. Spectacular environmental accidents do sometimes have positive outcomes. One of the most publicized environmental catastrophes was the grounding of the supertanker *Exxon Valdez* on the Bligh Reef in Prince William Sound, Alaska, in 1989. The tanker released 35 500 tonnes (41.6 million litres) of crude oil, polluting 1700 km of coastline. The spill was subjected to intense media coverage because of its visible impact on this pristine and unspoiled area of Alaska. Pictures of oiled seabirds and otters caused such concern that the oil spill was directly responsible for the passing of the US Oil Pollution Act of 1990.

1.3 Biotechnology

Although the term 'biotechnology' has been in existence for a considerable time since it was first used in 1919, and again in 1938 (Kennedy, 1991), the term was only recognized much later. Probably the first recognition by the wider scientific community was with the publication of the *Journal of Biotechnology and Bioengineering* in 1962 and the *Journal of Biotechnology* in 1979. The general public may have first heard of biotechnology with the publication of the Spinks report in 1980 (*Biotechnology, Report of a Joint Working Party*). The working party was set up to study the industrial applications of biological knowledge and were to review the opportunities in biotechnology, to recommend action by the government and to prepare a report. In this report biotechnology was defined as "The application of biological organisms, systems or processes to manufacturing and service industries". More recently the European Federation of Biotechnology defined biotechnology as "The integrated use of biochemistry, microbiology and engineering sciences in order to achieve applications of the capabilities of microorganisms, cultured animal cells or plant cells or parts thereof in industry, agriculture, health care and in environmental processes" (European Federation of Biotechnology, 1988). The Organization for Economic Cooperation and Development (OECD) defined biotechnology

as “the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services”.

The term biotechnology may imply a degree of coherence as a separate subject or discipline but, in practice, it embraces a set of disparate but often interrelated technologies relevant to a broad range of industries and requiring a wide range of science and engineering expertise, often to produce a single development. The choice of subjects combined depends on the individual industrial or scientific problem. Thus biotechnology acts as an interface between scientific disciplines and their areas of application. The relationship between environmental biotechnology and biotechnology is shown in Fig. 1.1.

It was thought that biotechnology would create novel industries with low demands on fossil-fuel energy and the technology would affect industries involved with food and feed, animal feed, energy sources, waste recycling, pollution control, and medical and veterinary care (Houwink, 1989). In this report it was stated that biotechnology would “launch an industry as characteristic of the twenty first century as those based on Physics and Chemistry

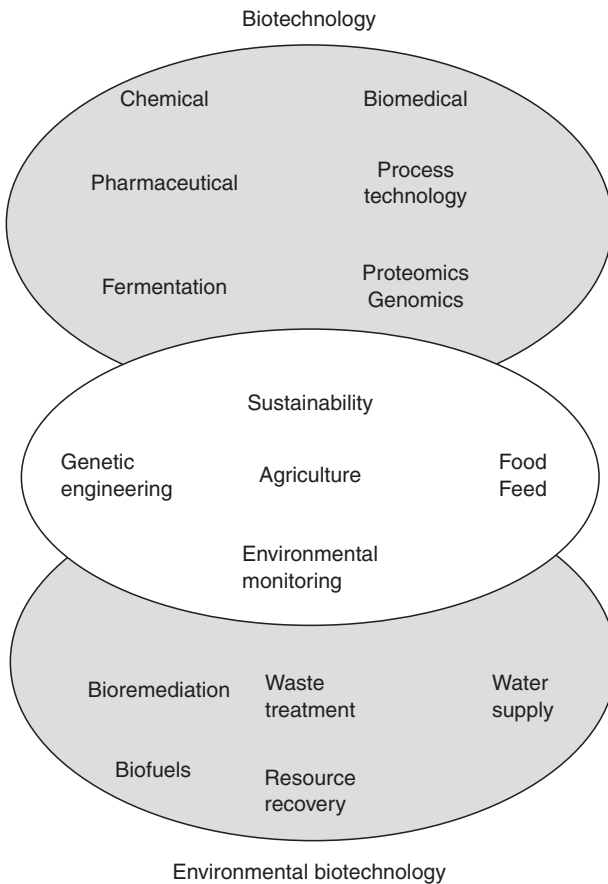


Figure 1.1 The link and crossover between biotechnology and environmental biotechnology.

have been for the twentieth century”. Moreover it was to be an industry based in large part on renewable and recyclable materials and thus adapted to the needs of a society in which energy is expensive and scarce. Many of these predictions have come true and genetic manipulation has had a significant impact on almost all industries from food to forensics. Some predictions have not come true to date. For example, the appreciation that the supply of fossil fuels is limited and their use contributes to global warming has only just begun to be a national and international issue. Thus the development of biological energy supplies has only begun recently.

Despite the somewhat later start, environmental biotechnology — the application of biotechnology to environmental issues — has a predicted market in excess of US\$300 million worldwide (Golub, 1997) and \$84–94 billion dollars in Europe.

1.4 Areas of application for biotechnology

In contrast to medical biotechnology, environmental biotechnology has to deal with high volumes of low-value wastes, products, and services. A list of environmental pollutants that can be released into the environment is given in Table 1.1 and these can be very diverse, ranging from metals to gases. These pollutants present three main problems in the environment, which can be addressed by biotechnological processes, and these are as follows.

- The problem of existing pollution of land, water, and air. The pollution is often a legacy from old industries and industrial processes often long defunct.
- Current industrial, agricultural, and domestic processes still producing pollutants and to this can be added accidents and spills. Some of these pollutants, such as sewage, cannot be reduced or eliminated so that they will have to be treated.
- Industrial, agricultural, and transport sectors create pollutants which could be reduced by introducing a more sustainable, renewable source of raw materials and energy. The application of biotechnology to industrial processes is known as ‘**clean technology**’.

1.4.1 Existing pollution

Central to the treatment of pollution and the implementation of any environmental act is the ability to determine what chemicals are present or being released, their concentration, and their potential environmental risk. It is estimated that some 60 000 chemicals are in common use and hundreds are introduced each year. Many of these chemicals are released into the environment during processing, use, and accidental spills. These pollutants can be found in

Table 1.1 Environmental pollutants

Type of pollutant	Examples
Inorganic	
Metals	Cd, Hg, Ag, Co, Pb, Cu, Cr, Fe
Radionucleotides	
Nitrates, nitrites, phosphates	
Cyanides	
Asbestos	
Organic	
Biodegradable	Sewage, domestic agricultural, and process waste
Petrochemical	Oil, diesel, BTEX*
Synthetic	Pesticides, organohalogenes, PAHs [†]
Biological	
Pathogens	Bacteria, viruses
Gaseous	
Gases	Sulphur dioxide, carbon dioxide, nitrous oxides, methane
Volatiles	CFCs [‡] , VOCs [§]
Particulates	

*BTEX, benzene, toluene, ethylbenzene, xylene.

[†]PAH, polyaromatic hydrocarbon.

[‡]CFC, chlorofluorocarbon.

[§]VOC, volatile organic compound.

air, water, or soil and can be metals or organic compounds not normally found in nature. Chemical techniques are often used to determine the concentration of pollutants in samples but **bioindicators** and **biomarkers** can be used to monitor pollution *in situ*. Bioindicators are whole organisms that are representative of their environment and whose population changes are used to estimate the effects of pollutants. Biomarkers are physiological, biochemical, and molecular characteristics of organisms removed from the environment and the characteristics chosen are those that will be affected by pollutants. Micro-organisms containing a green fluorescent protein under the control of a gene that responds to pollutants *in vivo* have been developed using recombinant gene technology. Genetic manipulation has also produced luminescent micro-organisms that can be used to test for toxicity in addition to tests like the Ames test. Molecular techniques can now be used to follow microbial populations *in situ* and from extreme habitats such as deep-sea hydrothermal vents and volcanic springs. **Biosensor** technology can be used to measure biological oxygen demand (BOD) and follow processes online and in real time (see Chapter 3).

Once the pollution has been detected and quantified, it will need to be removed and degraded, or degraded *in situ*. Micro-organisms provide an alternative to chemical methods in a process known as **bioremediation** (see Chapter 5). A specific problem is the release of heavy metals into the environment. Metals from a number of industrial processes can pollute air, water, and land. Metals are clearly not degraded by biological systems but a number of micro-organisms and plants can accumulate metals in a process known as **bioaccumulation** (see Chapter 5). The ability of biological material to accumulate metals is being put to use in the removal of heavy metals from water and contaminated sites.

Organic industrial waste such as that from food processing can be degraded biologically and treated both aerobically and anaerobically, in a similar way to domestic sewage. However, many of the synthetic chemicals produced by industry are not found in nature and are known as **xenobiotic**, from the Greek *xenos*, meaning new. The Environmental Protection Agency (EPA) and European Union (EU) list the chemicals which can cause problems if released into the environment. These are listed in Chapter 3 (Tables 3.3 and 3.5). The degradation of these synthetic chemicals is dependent on their structure, solubility, and toxicity. Often the lower the solubility of a compound in water the greater its solubility in cellular lipids and thus the greater the accumulation in the fatty tissues of the organism in a process known as **bioaccumulation** or **bioconcentration**. The relationship between the aqueous solubility of organic compounds and the bioconcentration factor is given in Fig. 1.2. As the compound becomes more water soluble the less it is accumulated. Therefore, these types of compounds have a greater toxic potential, as the bioaccumulation may be in an organism that is part of a food chain. In general organic compounds containing halogen atoms are slow to degrade, and the rate of degradation is influenced by the type of halogen atom, its position in the molecule, and the number of atoms present. In some cases it takes up to 15 years or more for these types of compound to be reduced by 50% and therefore, in essence, they are permanent and will accumulate in the environment. Some fungi and bacteria have been isolated that can degrade xenobiotics and although the degradation rate is slow, work is in progress to harness these organisms to treat waste streams and contaminated sites (see Chapter 5).

Another group of industrial wastes are the petrochemicals; oil, petrol, and diesel. Pollution of the environment with petrochemical wastes is often the result of disposal, tank leakage, and the more spectacular major marine spills. There are natural micro-organisms that occur widely in the environment that are capable of degrading petrochemicals. This is not unusual, as petrochemicals were organic material many millions of years ago. Biotechnology is developing ways of enhancing the petrochemical-degrading activity of micro-organisms either *in situ* or *ex situ* (off site).

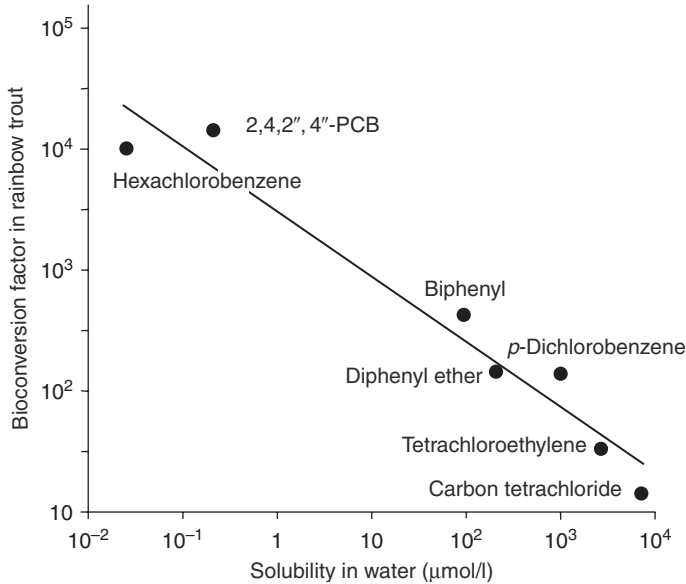


Figure 1.2 The relationship between the water solubility of organic compounds and their bioconcentration factor (Reeve, 1994). PCB, polychlorobiphenyl.

1.4.2 Bioremediation

Bioremediation is the biological treatment and removal of pollution from the environment. A long history of industrialization in developed countries has left a legacy of industrial pollution of land, water, and air. Industries such as petrochemical, smelting, mineral extraction, and gasworks, and other sources such as landfill have left a long list of polluted sites. Legislation will now require local authorities to list contaminated sites and perhaps oversee the cleaning up. It has been estimated that in the UK there are some 100 000 sites, which will take between £10 000 million and £20 000 million to clean up. The use of micro-organisms for the removal of pollution is not a new method, as sewage treatment using activated sludge has been with us since 1914. The principal organisms in bioremediation are bacteria and fungi that have the ability to degrade hydrocarbons such as oil and coal tar, and xenobiotics such as pesticides. Although metals cannot be degraded they can be accumulated by micro-organisms and therefore removed from the environment. Various bioremediation strategies have been developed (see Chapter 5) to treat polluted land and water, although there is only a limited knowledge of the processes involved. As bioremediation uses mixed populations of micro-organisms the dynamics of such populations are complex. In addition, the bioremediation process depends greatly on the quality and quantity of the pollution and is affected by other factors such as the presence of toxic agents,

temperature, presence of nutrients, bioavailability of the compounds, and oxygen limitation.

1.4.3 Removal or reduction of pollution from current processes

Bioremediation can be applied to the wastes from various processes in an 'end-of-pipe' system. Biotechnology offers some of the most environmentally friendly solutions. Examples are the removal of metals and radionucleotides from waste streams, reed beds for the degradation of organic pollutants and the accumulation of metals, and the reduction of volatile organic compounds (VOCs) from waste gases by passing through a biofilter (see Chapter 5).

Gaseous pollution consists also of particulates, and gases like carbon dioxide, sulphur dioxide, and nitrous oxide produced by industrial processes. There are physical methods for trapping particulates and removing sulphur dioxide and nitrous oxide but under development are biofiltration units which are capable of removing VOCs, such as dichloromethane (see Chapter 5).

Burning of fossil fuels is responsible for most of the production of sulphur dioxide, nitric oxide, and carbon dioxide and the main source of these pollutants is power stations. Sulphur dioxide and nitric oxide released into the atmosphere will produce acid rain, which has the effect of reducing the pH of waters and soils, killing plants, and corroding buildings. Legislation now seeks to reduce the levels of sulphur and nitrous oxide emissions from power stations. A number of approaches have been made to reduce these:

- burn less fossil fuel, switch to gas or other energy sources;
- use low-sulphur coal or reduce the sulphur content by a biological process of desulphurization (biological; see Chapter 5);
- improve combustion; and
- flue gas desulphurization using an alkali such as limestone.

The land and the seas/oceans exchange carbon dioxide in a balanced manner. Plants fix carbon dioxide during photosynthesis while respiration and decomposition release carbon dioxide back to the atmosphere. The oceans act as a sink, by dissolving carbon dioxide and by algae in the ocean fixing carbon dioxide photosynthetically. The burning of fossil fuel releases large amounts of carbon dioxide which was fixed millions of years ago when the plants, which formed coal, were alive. It is the release of this carbon dioxide that is causing a significant increase in the amount of carbon dioxide in the atmosphere. The carbon dioxide is known as a **greenhouse gas** as it traps heat radiated from the Earth, so that an increase in carbon dioxide will cause **global warming**. An alternative to fossil fuels, other than fission or fusion, would be the use of biologically derived fuels which would result in no net gain in carbon dioxide as the carbon dioxide released was previously removed from the atmosphere during photosynthesis (carbon dioxide-neutral).

1.4.4 Prevention of pollution (clean technology)

Biotechnology can contribute towards the cleaner production of existing products. The drive towards greener processes, which are environmentally sustainable, is a preferred option to the clean up of the pollution once formed, but this approach has not received the attention it deserves. The use of enzymes or bacteria instead of chemical processes has the potential to reduce the use of feedstock and the energy required for the processes, as lower temperatures are used. In addition, the enzymes and bacteria themselves are also biodegradable. One example is the microbial removal of sulphur compounds from coal prior to combustion. Another is the use of fungi to pretreat logs before pulp and paper production, which reduces the use of energy and bleaching materials. The development of biodegradable plastics would be of considerable use in the reduction of solid waste, as much (20%) of the material deposited in landfill sites is plastic. Micro-organisms are capable of producing biopolymers from sustainable resources that can be used as plastics and these are biodegradable. At present the cost of biopolymers has restricted their application (see Chapter 6).

The demand for energy shows no evidence of declining and electricity generation uses mainly coal, oil, and gas in addition to nuclear and hydropower. Coal, oil, and gas supplies are limited, and all produce greenhouse gases on combustion and therefore alternatives are required to replace these fossil fuels and to reduce greenhouse gases. Biological alternatives to fossil fuels are the combustion of rapidly growing plants (willow, *Miscanthus*) for electricity generation, plant oil (rapeseed oil), and ethanol as liquid fuel (**biofuels**) for transport (see Chapter 7). Ethanol can be used in petrol engines with little modification to the engine and ethanol can be produced from sugar and starch by fermentation. Plant oil can be modified so that it can be used in diesel engines. Both fuels represent a sustainable, renewable, and carbon dioxide-neutral alternative to fossil fuels.

To combine the protection of the environment with the operation of an industrial process is known as **integrated pollution control**. The aims of integrated pollution control are to prevent, minimize, or render harmless releases of prescribed substances using the best available technology not entailing excessive cost ('BATNEEC'). The concept was that pollutants could transfer from air to land and to water, using complex routes. Therefore, any civil and industrial development should consider both public and expert advice as to the effect on the environment as a whole.

1.5 Genetically manipulated organisms

The last era recognized by Houwink (1989) was the 'new biotechnologies', which are principally involved with the application of genetic engineering to

all areas of biotechnology including environmental biotechnology. Genetic engineering or recombinant DNA technology was developed in the 1970s and the techniques have revolutionized our ability to isolate, manipulate, and express genes and therefore proteins. The ability to isolate a particular gene, to multiply it if required, and to insert it into another organism means that traditional species barriers can be crossed in ways not possible by traditional breeding. An example is the production of human proteins in bacteria, animal cells, and 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 micro-organism and the 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 (Flavr Savr™) 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 designed to alter the characteristics of the organism; instead, the recipient 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 *Escherichia coli*. The recipient can be bacterial, yeast, insect, plant, or mammalian cultures depending on the product required and the posttranslational processing.

The first medical transgenic product was produced in 1982 and 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. Human growth hormone is now 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 products that have been developed are for medicine, providing material which was difficult to extract, carries 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 first genetically engineered crop was introduced in 1994 and was the Flavr Savr™ tomato. This tomato has a prolonged shelf life due to inhibition of any enzyme which delays ripening. Since that time the adoption of genetically modified (GM) crops has increased continuously, with the main crops being soybean, maize (corn), rapeseed (canola) cotton, potato, and tomato. The characteristics transferred

have been principally herbicide resistance, insect resistance, and delayed ripening. 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.

Despite the successful introduction into the UK of GM tomato puree by Zeneca in 1996–97 there has been as backlash against GM crops and food (Moses, 2002; Lassen et al., 2002). This is despite the safety evaluation of foods for GM crops laid out by organizations like the OECD, which introduced the Principle of Substantial Equivalence (Kok and Kuiper, 2003). There is public concern over GMOs in food production as the public perceives the development of genetically engineered plants is being mainly for profit, and believes that the crops and animals are unnatural and carry unknown risks, and that transgenic organisms or products should be labelled. The introduction of GM food also has occurred at an unfortunate time with the worries about bovine spongiform encephalopathy (BSE), food-contamination scares, and foot and mouth, which means that GM foods are regarded in the same light. It may be that GM crops and GM foods have become the scapegoat for other issues like globalization, the power of multinational companies, US dominance, ethical values, etc. (Braun, 2002).

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” (Kareiva and Stark, 1994). Environmental newsletters have had titles such as “Are you ready for frankenfood?” (Kareiva and Stark, 1994). All this publicity has seen sales of genetically engineered tomatoes staying at low levels in the USA (Golub, 1997). This resistance may be overcome if the technology seeks to solve the perceived problems rather than being introduced just for profit. As a consequence some supermarkets in the UK have dropped any GM foods or have labelled products as not containing genetically engineered ingredients. Companies involved in the use of transgenic plants have run a series of explanations of biotechnology in newspapers in the UK, including a web site (www.monsanto.co.uk) with links to the web site for Friends of the Earth (www.foe.co.uk). In contrast, the application of genetic modification to the medical and screening sector remains popular.

1.6 Legislation

There has been legislation concerning the environment in terms of public health in the UK since 1848. The Public Health Act (1848) and many of the subsequent acts produced were in response to continued industrialization and were related mainly to sanitation and housing. The response to present concerns about the environment in the UK initiated the Environmental Protection Act (1990) (Table 1.2), which saw the setting up of the Environment Agency

for England and Wales and the Scottish Environment Protection Agency in the 1995 Environmental Act. These two acts define the environment as “consisting of the air, water and land and the medium of air includes the air within buildings and the air within other natural or man-made structures above or below ground” and pollution as “the release into any environmental medium from any process of substances which are capable of causing harm to man or any other living organisms supported by the environment”. In the USA acts dealing with clean air, clean water, and hazardous waste were passed between 1977 and 1982 and in 1994 the Maritime Pollution Treaty was signed.

However, pollution is not constrained within individual countries and therefore many attempts at cleaning up the environment have had to be carried out on an international scale. One of the most public international meetings was the United Nations Conference on Environment and Development (UNCED), held in Rio de Janeiro in 1992, which resulted in conventions on biodiversity and climate change; however, these have achieved little in real terms to date.

The Rio Declaration also included a number of principles for future action, which are:

- access to environmental information;
- that the precautionary principle should be widely applied, where national authorities should adopt the policy that the polluter pays.

Conventions are really international treaties under international law, which may also contain subsidiary agreements called protocols. The convention on climate change asked developed countries to submit plans to reduce the production of greenhouse gasses. The Montreal Protocol agreed in 1987 covered the production and use of chlorofluorocarbons (CFCs) which were reducing the ozone layer around the world. There have been a number of meetings since Rio de Janeiro, including at Kyoto in 1997, which have ratified the previous agreements and ‘polluter pays’ has been adopted in some countries but otherwise little progress has been made in these meetings on greenhouse gas emissions. The World Summit on Sustainable Development in Johannesburg in 2002 made commitments to water and sanitation, energy, health (air pollution), agriculture, and biodiversity. The hope is to reduce natural resource degradation, restore fisheries, introduce ozone friendly chemicals, and to stop marine pollution. There was another UNCED in South Korea in 2004.

In the Treaty of Maastricht (1991) obligations were added to the original Treaty of Rome (1957) which included to preserve, protect, and improve the quality of the environment, contribute to the protection of the health of individuals, the prudent and rational use of natural resources, and to promote at an international level methods to deal with environmental problems. These activities have seen the adoption of a number of acts in various countries and the setting up of agencies to monitor specific environments such as clean beaches.

Table 1.2 Legislation and summits on environmental biotechnology

Date	UK and International
1848	Public Health Act
1957	Treaty of Rome
1969	USA National Environmental Policy Act (NEPA)
1970	Environmental Protection Agency (EPA) established
1970	USA Clean Air Act (CAA)
1972	Stockholm Declaration established United Nations (UN) Environmental Programme
1973	USA Endangered Species Act (ESA)
1974	UK Control of Pollution Act
1977	USA Clean Water Act
1987	Montreal Protocol (UN) elimination of CFC* use
1990	USA Oil Pollution Act
1990	UK Environmental Protection Act
1990	USA Pollution Prevention Act
1991	UK Water Resources Act
1991	Treaty of Maastricht (European Union)
1991	UK Water Industries Act
1992	UN Conference on Environment and Development (UNCED), Rio de Janeiro
1994	UN Maritime Pollution Treaty (MARPOL)
1995	UK Environment Act
1996	USA Food Quality Protection Act
1997	UN Conference on Environment and Development, Kyoto
2002	UN World Summit on Sustainable Development, Johannesburg
2004	UN World Environmental Summit, South Korea

*CFC, chlorofluorocarbon.

1.7 Conclusions

Thus, in conclusion, environmental biotechnology will be applied in the following areas.

- Transgenic organisms will continue to be generated but with more emphasis on improved food quality rather than just agricultural advantages. Acceptance of GM food will depend on whether the public

can see benefits to society rather than to multinational corporations. An example of this type of advantage is the golden rice which has vitamin A engineered into the grain.

- Bioremediation will continue to expand as a cheap, environmentally sustainable method for the clean up of contaminants.
- The monitoring of environmental contamination is essential.
- Biofuels and clean technology will become increasingly important as fuel stocks diminish and global warming threatens to change the climate in significant ways.

1.8 Further reading

Creuger, W. and Creuger, A. (1991) *Biotechnology – A Textbook of Industrial Microbiology*. Freeman, New York.

Glazer, A.N. and Nikaido, N. (1994) *Microbial Biotechnology*. Freeman, New York.

McEldowney, S., Hardman, D.J., and Waite, S. (1993) *Pollution: Ecology and Biotreatment*. Longman Scientific & Technical, Harlow.

McEldowney, J.F. and McEldowney, S. (1996) *Environment and the Law*. Addison Wesley Longman, Harlow.

Moses, A. and Cape, R.A. (1992) *Biotechnology, the Science and Business*. Harwood Academic Press, London.

Moses, A. and Moses, S. (1995) *Exploiting Biotechnology*. Harwood Academic Publishers, Chur, Switzerland.