# *Environmental Biotechnology*

- ***Bioremediation***
- ***Prevention***
- ***Detection and Monitoring***
- ***Genetic Engineering***

EUROPEAN FEDERATION OF BIOTECHNOLOGY

EFB

TASK GROUP ON PUBLIC PERCEPTIONS OF BIOTECHNOLOGY

Biotechnology is "the integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services" (EFB General Assembly, 1989). Environmental biotechnology as discussed in this briefing paper is the application of these processes for the protection and restoration of the quality of our environment.

**This briefing paper reviews the various areas of environmental biotechnology together with their related issues and implications. The overall aim is to provide balanced information and advance public debate. This paper results from the combined contributions of scientists, industrialists, and governmental and environmental organisations across Europe. It is intended to supply information and does not represent the views or policy of the European Federation of Biotechnology or any other body.**

## INTRODUCTION

Biotechnological processes to protect the environment have been used for almost a century now, even longer than the term 'biotechnology' exists. Municipal sewage treatment plants and filters to purify town gas were developed around the turn of the century. They proved very effective although at the time, little was known about the biological principles underlying their function. Since that time our knowledge base has increased enormously. This briefing paper describes the state-of-the-art and possibilities of environmental biotechnology. It also deals with the societal aspects of environmental biotechnology.

Biotechnological techniques to treat waste before or after it has been brought into the environment are described and exemplified in the section on bioremediation. Biotechnology can also be used to develop products and processes that generate less waste and use less non-renewable resources and energy. In this respect biotechnology is well positioned to contribute to the development of a more sustainable society, a principle which was advocated in the Brundtland Report in 1987 and in Agenda 21 of the second Earth Summit in Rio de Janeiro in 1992 and which has been widely accepted in the mean time. The section on prevention deals with this subject. Biotechnological techniques to monitor the quality of the environment are presented in the section on detection and monitoring. Recombinant DNA technology has improved the possibilities for the prevention of pollution and holds a promise for a further development of bioremediation. These topics will be discussed in the section on genetic modification. The development of modern biotechnology has been accompanied by the establishment or adaptation of regulations to deal with genetically modified organisms. What this means for environmental biotechnology is embodied in the section on legislation. The section on public opinion, dialogue and debate highlights how people feel about environmental biotechnology and ways in which their opinion is influenced.

## BIOREMEDIATION

Bioremediation is the use of biological systems for the reduction of pollution from air or from aquatic or terrestrial systems. Micro-organisms and plants are the biological systems which are generally used. Biodegradation with micro-organisms is the most frequently occurring bioremediation option. Micro-organisms can break down most compounds for their growth and/or energy needs. These biodegradation processes may or may not need air. In some cases, metabolic pathways which organisms normally use for growth and energy supply may also be used to break down pollutant molecules. In these cases, known as co-metabolism, the micro-organism does not benefit directly. Researchers have taken advantage of this phenomenon and use it for bioremediation purposes. A complete biodegradation results in detoxification by mineralising pollutants to carbon dioxide, water and harmless inorganic salts. Incomplete biodegradation will yield breakdown products which may or may not be less toxic than the original pollutant. Incomplete biodegradation of tri- or tetrachloroethylene for instance can yield

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vinylchloride, which is more toxic and carcinogenic than the original compounds.

Biodegradation may occur spontaneously, in which case the expressions “intrinsic bioremediation” or “natural attenuation” are often used. In many cases however the natural circumstances are not favourable enough for this to happen due to the lack of enough nutrients, oxygen or suitable bacteria. Such situations may be improved by supplementing one or more of these prerequisites. Extra nutrients were for instance disseminated to speed up the breakdown of the oil spilled on 1000 miles of Alaskan shoreline by the super tanker Exxon Valdez in 1989. The future trend in bioremediation increasingly is to look at the speed of unaided biodegradation first and act only if there is insufficient activity to remove the pollutant quickly enough to prevent any expected risks of the pollutant.

Bioremediation techniques can be used to reduce or to remove hazardous waste which has already polluted the environment. They can also be used to treat waste streams before they leave production facilities: end-of-pipe-processes. Some applications of bioremediation are discussed below.

**Waste water and industrial effluents:** Micro-organisms in sewage treatment plants remove the more common pollutants from waste water before it is discharged into rivers or the sea. Increasing industrial and agricultural pollution has led to a greater need for processes that remove specific pollutants such as nitrogen and phosphorus compounds, heavy metals and chlorinated compounds. New methods include aerobic, anaerobic and physico-chemical processes in fixed-bed filters and in bioreactors in which the materials and microbes are held in suspension. The costs of waste water treatment can be reduced by the conversion of wastes into useful products. For example, heavy metals and sulphur compounds can be removed from waste streams of the galvanisation industry by the aid of sulphur metabolising bacteria and reused. Another example is the production of animal feed from the fungal biomass which remains after the production of penicillin. Most anaerobic waste water treatment systems produce useful biogas.

**Drinking and Process Water:** Abundant supplies of water are vital for modern urban and industrial development. By the turn of this century, it is estimated that two-thirds of the world’s nations will be water stressed - using clean water faster than it is replenished in aquifers or rivers. A very important aspect of biotechnology is therefore its potential for the reclamation and purification of waste waters for re-use. Public concern has also increased over the quality of drinking water. Not only does water need to be recycled in the development of sustainable use of resources, overall quality must also be improved to satisfy consumers. In many agricultural regions of the world, animal wastes and excess fertilisers result in high levels of nitrates in drinking water. Biotechnology has provided successful methods by which these compounds can be removed from processed water before it is delivered to customers.

**Air and waste gases:** Originally, industrial waste gas treatment systems were based on cheap compost-filled filters that removed odours. Such systems still exist. However, slow processing rates and the short life of such filters drove research into better methods such as bioscrubbers, in which the pollutants are washed out using a cell suspension and biotrickling filters, in which the pollutant is degraded by micro-organisms immobilised on an inert matrix and provided with an aqueous nutrient film trickling through the device. The selection of micro-organisms that are more efficient at metabolising pollutants has also led to better air and gas purifying biofilters. Examples are a bioscrubber based system for the simultaneous removal of nitrogen and sulphur oxides from the flue gas of blast-furnaces which has been developed as an alternative to the classical limestone gypsum process, and the elimination of styrene from the waste gas of polystyrene processing industries by a biofilter containing fungi.

**Soil and land treatment:** Both in situ (in its original place) and ex situ (somewhere else) methods are commercially exploited for the cleanup of soil and the associated groundwater. In situ treatments may include the introduction of micro-organisms (bio-augmentation), ventilation and/or adding nutrient solutions (biostimulation). Ex situ treatment involves removing the soil and groundwater and treating it above ground. The soil may be treated as compost, in soil banks, or in specialised slurry bioreactors. Groundwater is treated in bioreactors and either pumped back into the ground or drained into surface water. Bioremediation of land (biorestoration) is often cheaper than physical methods and its products are harmless if complete mineralisation takes place. Its action can however, be time-consuming, tying up capital and land. The in situ bioremediation of the ground under petrol stations has already become common practice but also for chlorinated solvents like tri- and tetrachloroethylene in situ bioremediation is possible. The applicability of in situ bioremediation is and probably will remain dependent on the physical parameters of the soil, mainly its transport properties. Bioremediation using plants is called phyto-remediation. This technique is presently already used to remove metals from contaminated soils and groundwater and is being further explored for the remediation of other pollutants. The combined use of plants and bacteria may also be possible. Certain bacteria live closely associated with the roots of plants and depend on substances excreted by the roots. Such rhizobacteria, whose numbers are much higher than those of other soil bacteria, may be genetically modified to break down pollutants. Research is being conducted to test this hypothesis.

**Solid waste:** Domestic solid wastes are a major problem in our consumption society. Their elimination is both costly and warrants constant surveillance in terms of groundwater and air pollution. Yet, for a major part they are composed of readily biodegradable organics. In this respect, source separated bio-wastes can be converted to a valuable resource by composting or anaerobic digestion. In recent years, both processes have seen remarkable developments in terms of process design and control. Particularly, anaerobic digestion of solid wastes in high-rate anaerobic digesters has gained increasing public acceptance because it permits the recovery of substantial amounts of high-value biogas together with a high quality stable organic residue and this without giving rise to environmental nuisance. Moreover, anaerobic digestion of mixed solid wastes is under intensive development because in the near future it may be an important step in recycling of solid wastes and constitute an alternative to incineration.

## PREVENTION

Progressively more industrial companies are developing processes with reduced environmental impact responding to the international call for the development of a sustainable society. There is a pervading trend towards less harmful products and processes; away from “end-of-pipe” treatment of waste streams. Biotechnology is pre-eminently suitable to contribute to this trend and it has already done so in many cases, both by the improvement of existing processes and by the development of new ones [6,7].

**Process improvement:** Many industrial processes have been made more environmentally friendly by the use of enzymes. Enzymes are biological catalysts that are highly efficient and have numerous advantages over non-biological catalysts. They are non-toxic and biodegradable, work best at moderate temperatures and in mild conditions, and have fewer side reactions than traditional methods because they are highly specific. Production methods that employ enzymes are generally not only cleaner and safer compared with other methods, but mostly also more economic in energy and resource consumption (see box). Their specificity does however mean that it is not always easy to find the appropriate enzyme for a given application. Enzymes are already widely employed in industry and have been for many years. New techniques and approaches to protein design and molecular modelling are enabling researchers to develop novel enzymes active at high temperatures, in non-aqueous solvents and as solids.

**Product innovation:** Biotechnology also can help to produce new products which have less impact on the environment than their predecessors. The production of new biomaterials like bioplastics avoids the use of non-renewable resources like fossil fuels. Potatoes normally contain 80% amylopectin but also 20% amylose which is unwanted in many applications. For the isolation of pure amylopectin large amounts of water and energy are consumed. A Dutch company has developed a genetically modified potato variety which no longer contains amylose and hence can be processed with less impact on the environment. The use of genetically modified plant varieties that are resistant against insects and/or diseases may considerably diminish the use of pesticides which not only prevents the use of the -mostly non-renewable- raw materials, energy and labor necessary for their

**More sustainable industrial processes through the use of enzymes.**

The leather processing industry has introduced enzymes to replace harsh chemicals traditionally used for cleaning the hide. In textile production, enzymes have superseded chemicals for bleaching, including the “stone washing” of jeans. Chlorine consumption by the pulp and paper industry may soon also be reduced considerably by the use of enzymes. The grease and protein digesting enzymes in washing powders significantly reduce the quantity of detergents needed for a given washing effect. They also mean that the washing temperature can be reduced. Lowering the temperature 20°C saves more than a third of the energy used by the machine. Since in many Western European countries up to 5% of household energy consumption was used for washing, these molecules have made a significant contribution to energy conservation.

production but also will reduce the negative impacts of their residues. Many more of these biotechnological solutions for pollution have been developed (see box). Future developments may involve things which at this moment seem science-fiction to most people such as the replacement of chemically produced super-fibres by microbially produced spider web silk.

One thing that should not be forgotten however is that the increased use of biological systems in industry should be accompanied by adequate training and protection of workers handling these systems, just like in other sections of industry.

## DETECTION AND MONITORING

**Detection and monitoring of pollutants:** A wide range of biological methods are already in use to detect pollution incidents and for the continuous monitoring of pollutants. Long established measures include: counting the number of plant, animal and microbial species, counting the numbers of individuals in those species or analysing the levels of oxygen, methane or other compounds in water. More recently, biological detection methods using biosensors and immunoassays have been developed and are now being commercialised.

Most biosensors are a combination of biological and electronic devices - often built onto a microchip. The biological component might be simply an enzyme or antibody, or even a colony of bacteria, a membrane, neural receptor, or an entire organism. Immobilised on a substrate, their properties change in response to some environmental effect in a way that is electronically or optically detectable. It is then possible to make quantitative measurements of pollutants with extreme precision or to very high sensitivities. The sensors can be designed to be very selective, or sensitive to a broad range of compounds. For example, a wide range of herbicides can be detected in river water using algal-based biosensors; the stresses inflicted on the organisms being measured as changes in the optical properties of the plant’s chlorophyll.

Microbial biosensors are micro-organisms which produce a reaction upon contact with the substance to be sensed. Usually they produce light but cease to do so upon contact with substances which are toxic to them. Both naturally occurring light emitting micro-organisms as well as specially developed ones are used. Positively acting bacterial biosensors have been constructed which start emitting light upon contact (and subsequent reaction) with a specific pollutant. In the USA such a light emitting bacterium has been approved for the detection of polyhalogenated aromatic hydrocarbons in field tests.

Immunoassays use labelled antibodies (complex proteins produced in biological response to specific agents) and enzymes to measure pollutant levels. If a pollutant is present, the antibody attaches itself to it; the label making it detectable either through colour change, fluorescence or radioactivity. Immunoassays of various types have been developed for the continuous, automated and inexpensive monitoring of pesticides such as dieldrin and parathion. The nature of these techniques, the results of which can be as simple as a colour change, make them particularly suitable for highly sensitive field testing where the time and large equipment needed for more traditional testing is impractical. Their use is however limited to pollutants which can trigger biological antibodies. If the pollutants are too reactive, they will either destroy the antibody or suppress its activity and so also the effectiveness of the test.

**Biotechnological solutions for pollution**

Pigs and chickens cannot utilize phosphate from phytate in their feed, which therefore ends up in their manure. By adding the enzyme phytase to their feed the amount of phosphate which is excreted by these animals can be reduced by more than 30 %.

In South Africa bacteria are used for the isolation of gold from gold-ore. This so-called biomining saves an enormous amount of smelting energy and generates much less waste.

The chemical production of indigo, the dye which is used for blue jeans, takes eight steps, the use of very toxic chemicals and special protection measures for the process operators and the environment. The biotechnological production of indigo, which uses a genetically modified bacterium containing the right enzymes, takes only three steps, proceeds in water, uses simple raw materials like sugar and salts and generates only indigo, carbon dioxide and biomass which is biodegradable.

**Detection and monitoring of micro-organisms used for bioremediation:** When laboratory grown micro-organisms are inoculated into a bioremediation site (bio-augmentation) it often becomes necessary to monitor their presence and/or multiplication to check the progress of the process. This is especially true and even required when genetically modified micro-organisms are involved.

The traditional technique to detect the presence of micro-organisms in soil is direct plating on selective media. This is greatly facilitated if the organism contains a marker which can be selected for. Newer techniques include the abovementioned immunological and light-based bioreporter techniques. The spatial distribution of specific micro-organisms in a sample can be determined microscopically and non-invasively by using fluorescent in situ hybridisation (FISH) of micro-organisms. The most sensitive and specific technique is the direct isolation and amplification of DNA from soil, which is increasingly being used.

**Detection and monitoring of ecological effects:** Bioremediation is aimed at improving the quality of the environment by removing pollutants. However, the disappearance of the original pollutant is not the only criterion by which the success of a bioremediation operation is determined. (Even more) toxic metabolites may be produced from the pollutant or the biodegrading bacterium may cause diseases or produce substances that are harmful to useful micro-organisms, plants, animals or humans. All these negative effects, are of course, excluded as much as possible in advance by getting as familiar as possible with the organism through extensive literature searches and microcosm studies in which the bioremediation process is simulated in the laboratory. To avoid unexpected effects, especially after the release of new member of the eco-system like a genetically modified organism, the monitoring of the ecological effects of a bioremediation operation may be required. The problem with monitoring ecological effects is what to monitor. Numerous ecological effects are possible but not all of them may be relevant or permanent or even the result of the bioremediation operation. The parameters to be monitored are usually determined case-by-case. Monitoring techniques may include all of those mentioned in the two previous subsections on detection and monitoring.

## GENETIC ENGINEERING

Recombinant DNA technology has had amazing repercussions in the last few years. Molecular biologists have mapped entire genomes, many new medicines have been developed and introduced and agriculturists are producing plants with novel types of disease resistance that could not be achieved through conventional breeding. Several of the previously mentioned examples like the amylose-free potato and the indigo-producing bacterium also involve the use of organisms genetically modified by recombinant DNA technology. Many enzymes are routinely produced by genetically modified organisms too.

Given the overwhelming diversity of species, biomolecules and metabolic pathways on this planet, genetic engineering can in principle be a very powerful tool in creating environmentally friendlier alternatives for products and processes that presently pollute the environment or exhaust its non-renewable resources. Politics, economics and society will ultimately determine which scientific possibilities will become reality.

Nowadays organisms can also be supplemented with additional genetic properties for the biodegradation of specific pollutants if naturally occurring organisms are not able to do that job properly or not quickly enough. By combining different metabolic abilities in the same micro-organism bottlenecks in environmental cleanup may be circumvented. Until now this has not been done on any significant scale. The main reason being the fact that in most cases naturally occurring organisms can be found or selected for, which are able to clean up a polluted site. Examples have been found where soil bacteria have developed new properties in response to the introduction of xenobiotics (that is, man-made chemicals that are normally not found in nature). In some cases they even appear to have acquired properties from other species. In the USA some genetically modified bacteria have been approved for bioremediation purposes but large scale applications have not yet been reported. In Europe only controlled field tests have been authorized.

Because new organisms can be created by genetic engineering that may never be produced by spontaneous or selection driven evolution, concerns exist about the unpredictability of their possible interactions with the eco-system. Genetically modified organisms which are properly kept within the confines of their approved production facilities are much less a concern than genetically modified organisms which are meant to be released into the environment like disease-resistant plants or soil bacteria for bioremediation.

The possible ecological effects of the latter are even more difficult to evaluate due to the fact that it is well known that soil bacteria frequently exchange genetic material (also between species). This together with the fact that we know little about the great majority of soil inhabiting bacterial species, makes it almost impossible to predict the fate of every DNA copy of a newly introduced genetic property in a soil bacterium. If the extra DNA is derived from another soil bacterium, it may on the other hand be reasonable to argue that the genetically modified bacterium might also have evolved spontaneously some day due to the frequent exchange of genetic material in the soil.

## LEGISLATION

Regulation to ensure safe application of novel or modified organisms in the environment is important, not least to maintain public confidence. The European Union has two Directives[1,2] on the contained use of genetically modified micro-organisms, and on the deliberate release of genetically modified organisms into the environment. These have been implemented in the national legislation of most EU Member States. They require that a detailed experimental protocol, including assessment of potential risks, is approved by competent authorities before a genetically modified organism is released into the environment. The nature and sometimes even the site of the release has to be published in the local press in some countries. After several years' experience using the legislation, the procedures involved are now being revised. Ammendments to clarify and revise Directive 90/219/EEC were published in December 1998. The aim of the European Commission is to maintain the EU's competitiveness globally - both in research and commercial applications- without compromising safety.

## PUBLIC OPINION, DIALOGUE AND DEBATE

In spite of the fact that traditional biotechnology already is of great value to bioremediation and modern biotechnology may enhance this even further, there are no recent data on what Europeans specifically think about environmental biotechnology. Generally speaking, Europeans tend to take an "optimistic" view of the developments they expect from modern biotechnology, according to the most recent European Commission public opinion survey which was published in 1997[3]. Unfortunately this survey did not investigate the attitude of the public towards environmental biotechnology. The only environmentally related question in this survey was whether people believed that modern biotechnology would substantially reduce environmental pollution, which 47 % did. Whether or not this is only wishful thinking remains to be determined. Ultimately, hard proof expessed in the form of improved environmental parameters will be needed for full acceptance of environmental biotechnology.

Conferences, public debates, seminars and round table meetings have been held to bring people from the public, government, environmental organisations, science and industry together to discuss critical issues. These lively debates do not always lead to consensus, but they can provide a fuller appreciation of all the aspects in a particular issue, facilitating a better understanding of the problems involved. A recent example is the workshop 'How can biotechnology benefit the environment' [4].

Public information aimed at advancing dialogue and debate is provided by many organisations. A compilation of these can be found in the handbook of information sources which has been published by and can be ordered from the EFB Taskgroup on Public perceptions of Biotechnology [5].

## CONCLUSION

Environmental biotechnology has a career extending back into the last century. As the need is better appreciated to move towards less destructive patterns of economic activity, while maintaining improvement of social conditions in spite of increasing population, the role of biotechnology grows as a tool for remediation and environmentally sensitive industry. Already, the technology has been proven in a number of areas and future developments promise to widen its scope. Some of the new techniques now under consideration make use of genetically modified organisms designed to deal efficiently with specific tasks. As with all situations where there is to be a release of new technology into the environment, concerns exist. There is a potential for biotechnology to make a further major contribution to protection and remediation of the environment. Hence biotechnology is well positioned to contribute to the development of a more sustainable society. As we move into the next millennium this will become even more vitally important as populations, urbanisation and industrialisation will continue to climb.

**References**

1 Council Directive on the Contained Use of Genetically Modified Micro-organisms. 1990, 90/219/EEC

2 Council Directive on the Deliberate Release into the Environment of Genetically Modified Organisms. 1990, 90/220/EEC

3 Eurobarometer 46.1, *The Europeans and modern biotechnology,* CEC DG XII, 1997

4 *How can Biotechnology benefit the Environment* (1997) Report of a Workshop organized by the EFB Taskgroup on Public Perceptions of Biotechnology and The Green Allience on 13 January 1997 at the Science Museum in London, ISBN 90 76110 02 6.

5 *Biotechnology for non-specialists, a handbook of information sources*. (1997) EFB Taskgroup on Public perceptions of Biotechnology. ISBN 90-76110-01-8

6 *Biotechnology for a clean environment*. (1994) OECD. ISBN 92-64-14257-6

7 *Biotechnology for clean industrial products and processes: Towards industrial sustainability*. (1998) OECD. ISBN 92-64-16102-3