Bioprocessing: from clean-up to preventive strategies

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1. Introduction: Mankind exploiting system Earth

All living organisms in system Earth are living in a competitive interaction for energy and raw materials. Each population can grow as long as its abiotic and biotic boundary conditions allow it to do so. It has been calculated that, if mankind would live in a 'natural' way, which means having a hunting and gathering lifestyle, this would be compatible with a human population of about 10 million people. In 1990, however, the human population was estimated to be 5321 millions and according to forecasts will have grown to 6350 millions in 2000 (1, 2, 3). This large population growth has been possible because mankind exploits system Earth via agricultural and industrial production systems to satisfy its social needs (4, 5).

Agriculture is the oldest human production system, it originated more than 10 000 years ago during the Neolithicum. Therefore the change from a hunting and gathering society to a society based on agriculture is often called the Neolithic Revolution.

The word revolution does not mean, however, that the development was fast — it probably took thousands of years — but it indicates that it had a profound influence on the social structures of human communities. Man also started cattle-breeding by letting tamed, wild animals reproduce in captivity. Together with agriculture and cattle-breeding the need to develop techniques for food conservation arose and this most probably stimulated pottery. In the same period, Man also invented the wheel, which facilitated transportation, and started to use metals for making tools. The use of these new tools further improved, among other things, agricultural techniques.

It is important to realize that in those primitive human communities sunlight was still the primary energy source and the products and processes used were largely identical to those in the natural system. Moreover, the human population was still relatively small and the human-directed activities represented only a modest proportion of the total global activity. The Neolithic Revolution therefore was not a threat to the stability of the biosphere. This cannot be said of an other important revolution in the history of Man, the Industrial Revolution, which again had a very profound influence on human social structures.

The cradle of the Industrial Revolution was the English society of the middle of the eighteenth century (6) and the use of fossil energy played a keyrole in this revolution.

The principle of the steam engine was known already in antiquity but the first practical application was realized by Thomas Newcomen (1663-1729) who developed a steam-driven pump for pumping ground water out of mines. The improved engines — developed subsequently by the Scottish engineer James Watt (1736-1819) — could be used for other purposes as well, for instance, to drive the apparatus in the so-called textile manufacturies, and so energy derived from fossil fuel began to replace muscular strength. The development of the steam engine resulted in an increasing demand for machinery, coal and iron. Industrial production necessitated transport and stimulated both the development of means of transport and the associated infrastructure. To be sure of the supply of fuel and raw materials and to keep the price of transportation as low as possible, factories were built close to mines and blast furnace plants. Houses for workmen were built close to the factories. In such industrial areas working and housing conditions were rather bad, which initiated the striving for social rights. Thus the Industrial Revolution brought Mankind factories and mass production and was accompanied by economic and social changes.

So the industrial revolution again had a profound influence on social structures in industrialised human communities. Differences between highly industrialised and less industrialised societies still illustrate this to some extent. We can no longer say, however, that the results of the Industrial Revolution are not threatening the biosphere. The exponential population growth, the concomittant rapid increase in the need for food, energy and physical resources necessitates Mankind to reconsider social needs and structures; System Earth is our common responsibility.

Figure 1 summarizes the interaction between the human society and system Earth in a highly simplified way (7, 8).

The Earth is a closed system which means that the total amount of matter in the system is constant while energy exchange with the surroundings occurs. In the exchange of energy with the surroundings, the biosphere plays an important role because it is able to capture and convert solar energy and store it in biomolecules.

Traditional agriculture uses the same products and processes that occur in living nature, solar energy again is the direct energetic driving force for agricultural production. In contrast, however, in Man-designed industrial processes practically all elements present on Earth, and fossil energy are used

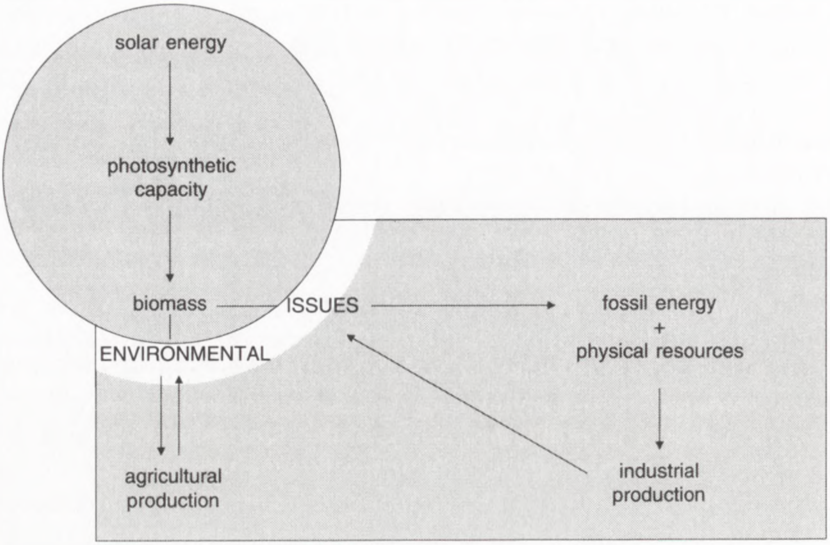

Fig. 1. Schematic representation of the interaction between the human society and system Earth.

in large quantities. Industrial production often implies that the degradation of products and wastes in the biosphere is much less easy than it is for natural products. Furthermore, even with the production of materials involving the elements commonly found in biomolecules, Man often uses production processes yielding products that are virtually non-biodegradable. Plastics produced via radical reactions and halogenated organics used as pesticides are well-known examples of this latter category.

Thus in satisfying its needs Mankind uses the Earth's physical resources and fossil energy and produces products that, after having been used, can not easily be degraded in the biosphere. As to the physical resources we have to realize that, as the earth is a closed system, the amount of matter is constant and can in principle be recycled, although this may cost very large amounts of energy. As to the fossil energy reserves of system Earth the situation is completely different, they will sooner or later simply be exhausted. How soon this will be depends on the growth of the human population and the way it uses the Earth's resources (4).

The "friction" between system Earth and the human social system described above causes what is known to us as environmental problems. The complexity of these problems suggests that they can only be tackled by an integrated approach applying natural sciences, social sciences and technology.

Reports such as "Our Common Future" of the World Commission on Environment and Development (9) (the so-called Brundtland report), "Agenda

21"(10), which is the document summarizing the conclusions from the United Nations Conference on Environment and Development held in Rio de Janeiro in 1992 and the European Fifth Action Plan "Towards Sustainability" (11), all emphasize the need for such a strategy.

2. The potential of biotechnology

Biotechnology can be defined as the use of biological systems for carrying out (production) processes at an economically relevant scale. Mankind has been using biotechnological procedures for thousands of years in the preparation and preservation of food. Also the production of drinking water and waste water treatment are well-known applications of biotechnology. However, until about a century ago the knowledge on which biotechnology was based was largely empirical. During the past decades our knowledge of the molecular and cellular principles of processes occurring in living nature has greatly expanded and this opens the way to deliberately influencing biotechnological processes. It paves the way for optimising existing production processes using micro-organisms like those in the food industry and the pharmaceutical industry. It also opens the way to exchanging genetic information between unrelated organisms, which can have great influence on agriculture and on the development of new drugs and vaccins. Biotechnology can contribute to developments in the field of renewable energy and it can play an important role in designing new and more environmentally friendly industrial production processes which can replace environmentally damaging technology.

3. Biotechnology and agriculture

Let's first turn our attention to the potential of biotechnology in agriculture.

Plants have been important to mankind for a very long time already. They were used for food, for clothing, housing, medical products and fuel. Traditional agriculture depended largely on natural biological processes to produce crops for fulfilling social demands. However, social and economic pressures to increase crop productivity, together with the development of mechanical engineering and chemical technology have changed the complexion of agriculture. Presently on a global scale about 5% of the yearly fossil energy use is invested in agriculture and man-made, potentially environmentally damaging chemicals are used as crop protectants. Continuing economic pressures and the need to feed a fast growing world population encourages further extension of the high technology, energy demanding approach to agriculture, but one feels that in the longer term such an approach will not be sustainable.

It is important to stress that, also in the field of agriculture, technological development and social development should go hand in hand. The results of the Green Revolution have made this clear once more. The Green Revolution, centered on the development and cultivation of new, high-yielding crop varieties has shown that modern agriculture may have a large impact on society. The increase in yield resulted indeed in economic and social developments within agricultural societies and created employment in the food industry and distribution services. However, such developments only occured in areas where an agricultural infrastructure already existed, not in areas where such favourable conditions were absent.

As the development of new high-yielding varieties is expensive and their cultivation goes hand-in-hand with the use of fertilizers, irrigation, herbicides, fungicides and pesticides the new possibilities could easily increase the gap between the rich and poor countries in the world. Of course the use of chemical fertilisers and protectants could also give rise to negative environmental effects while the production of ever larger monocultures could reduce genetic diversity and increase the vulnerability of crops to diseases and pests.

Such considerations make clear that application of new technology in production systems needs our concern and attention, both from the scientific and the social point of view. The application of new biotechnological procedures in agriculture should be placed in this context.

The new technologies could contribute to increasing the yield of crops, to generating devices for preventing the ravages of parasites and pathogens, to improvements in product quality such as food value and shell-life and to changes in the way we use land. These developments could be very useful to mankind indeed as crops are extremely important for the food supply of the world population. In addition to their role in food supply, crops can be used for energy production and as raw materials in a number of branches of industry. We can think, for instance, of microbiological conversion of plant materials to basic chemicals and of the production of special compounds by plants, plant tissues or plant cells. Examples are gums, glues, starch, dyes, ink, detergents, cosmetics, flavouring compounds, preservatives, emulsifiers, oils, textile fibres and fibres in composites. An advantage of using raw materials derived from plants is that such compounds can easily be degrated in the biosphere, and therefore agro-industrial production could be interesting from the environmental point of view. In turning these possibilities into realities research in the science and technology field must go hand-in-hand with research in economics and social sciences.

4. Biotechnology and energy

Solar energy is the most important driving force for social activity in our society. Man uses stored solar energy directly in agricultural production and indirectly in fossil fuel based industrial production. The world-wide dependency on fossil fuels poses a major global problem as we are rapidly exhausting the Earth's fossil energy reserves on the one hand while we cause environmental problems on the other. Therefore there is an urgent need for both renewable energy sources and cleaner production procedures; biotechnology could contribute to both (12, 13).

According to an 1993 estimate $24 \cdot 10^{11}$ GJ solar energy is stored per year through the process of photosynthesis, while $2 \cdot 10^{11}$ GJ fossil energy is used per year (14). It can be calculated that, on a global scale, about 5% of this yearly fossil energy use is invested in agriculture for mechanical treatment of soil, for harvesting and for the production of fertilisers and chemical protection agents. These data make clear that, under appropriate agricultural conditions energy-production from biomass is feasible. Thus energy conversion and storage in photosynthetic organisms may have potential as a source of renewable energy. An additional advantage of crop-production in the context of renewable energy is that the same amount of carbon dioxide is liberated during combustion as is used during photosynthesis, thus no negative environmental carbondioxide effects should be anticipated.

It is generally believed that an energy crop must produce at least five times the amount of (fossil) energy invested in it during its growth to make the cultivation of energy crops interesting from the commercial point of view. In regions with a temperate climate energy production from biomass is feasible. For example, in the Netherlands the ratio energy-yield/energy invested is 6 for hemp, 5 for wood, 4.5 for beet, 2.5 for potatoes and wheat and about 2 for grass. In (sub) tropical regions where climatological conditions enable agricultural production all the year round, energy crops could be more interesting. Of course we have to realize that using agriculture for energy production may result in a conflict of interests (and economics) between energy production and food production.

Theoretically crops can convert about 7.5% of the available solar energy because the pigments involved in trapping solar energy can capture radiation from only a limited range of wavelengths. From the trapped energy 40% is used for metabolic housekeeping, so the net energy yield of biomass production is about 4.5%. There are, however, large differences amongst the crop species and some crops are more promising as potential energy crops than others. Presently the main energy crop options are fast growing trees, starch and sugar crops, and oil and hydrocarbon crops.

Obviously, the idea of improving crops in a way that enables them to trap solar energy more efficiently or makes it possible to use energy from a larger range of wavelengths in the solar spectrum is exciting. Also the possibilities offered by photosynthetic organisms in oceans could be considered in energy forecasting

When we consider the possibility to replace fossil fuel by biomass we also have to think of the conversion of biomass to a suitable fuel such as, for instance, alcohol from wheat or sugar cane and biodiesel from rape seed (12). Factors that have to be taken into account are thermal content, moisture content, bulk density and the possibility of automatic feeding of combustion plants. At present two well-known examples of the production of gaseous or liquid biofuels from biomass are biogas and bioethanol. Biogas is a mixture of gases, mainly methane and carbondioxide, produced upon degradation of organic material by micro-organisms under anaerobic conditions. The feedstocks used for the production of bioethanol usually have a high starch content such as maize, cassava, millet and potatoes, or a high sugar content such as sugar cane and sugar beet. During a fermentation process such substrates are converted to bioethanol by yeast.

Furthermore hydrogen, an important energy carrier in biological systems, could have the potential to replace petrol in transport because it can be stored as a metal hydride which could be portable and safe for use as a fuel in cars. Hydrogen can be produced by the electrolysis of water, by reaction of biomass with steam and by hydrogen-producing bacteria. Also some microscopic algae produce hydrogen and there has been research into the possibility of growing these organisms in photobioreactors driven by solar radiation. Also linking the photosynthetic apparatus of plants to a hydrogen producing system could in principle result in the generation of hydrogen from water using solar energy. Presently, such a system has been made to work, but until now it is not stable enough for economic operation.

Finally, in addition to cultivating energy crops, we must also pay attention to the fact that in normal agricultural production mostly only parts of the crop are used, and thus part of the biomas energy is present in wastes. The same holds true for human and animal wastes. If such wastes are degraded by micro-organisms under controlled conditions in a bioreactor, biogas can be produced. In small-scale biogas production the gas is often used on the location where it is produced. In larger municipal processes the methane produced at a landfill site can be collected and burnt to produce steam used to generate electricity.

5. Biotechnology and industry

The advent of the use of non-renewable resources in the Industrial Revolution and the subsequent diversification of the chemical industries has enabled the fulfilment of a wide range of human needs and through this contributed to the growth of the world population. Yet, it is recognised by many that these developments are not, in the longer term, sustainable. For industrial production Mankind is withdrawing fossil energy and raw materials from the Earth's reserves. Products that have been used for shorter or longer times and are not needed any more are brought back into the environment. Furthermore, during production processes, unwanted products are released into the environment. Except for biologically produced materials, the composition of the compounds that are returned to the environment via discharge, emissions or wastes is often very different from the composition of the raw materials used. Recycling is possible, but most of the time recycling is rather energy demanding. This depletion of non-renewable resources and the accumulation of materials which are not readily returned to natural geocycling are of major concern.

A first obvious consequence of such considerations is that we should consider the costs of the production process in a broad sense and not only look at the costs of the product from an economic point of view. We must take into account the raw materials used, the amount of energy invested, the possibility of recovering raw materials and energy during recycling and the possibility to design alternative, more environmentally friendly processes. In other words we must consider the total life cycle of the product. The design of a production process taking into account these aspects is often referred to as integral life cycle management (15). In the context of integral life cycle management biotechnology offers potential solutions for replacing environmentally damaging processes and products. Biotechnology is based on our knowledge of the interactions taking place in living nature. It is thus founded upon systems which utilise the processes and products which occur in nature and which are components of the sustainable geochemical cycling of materials.

In biotechnological processes the conversion of raw material to product usually takes place in a bioreactor an is catalysed by micro-organisms, parts of micro-organisms (enzymes) or sometimes by other cell types. The conditions in the bioreactor are mild with respect to temperature, pressure and pH. Furthermore, the reactions take place with high specificity and selectivity, resulting in fewer by-products. Finally biotechnological production processes are closely related to naturally occurring processes and therefore can be considered as environmentally friendly.

Within the chemical industry biochemical unit operations are used in the production of for example organic chemicals, solvents, polymers, pharmaceuticals, fragrancy and aroma compounds.

Alcohol is a well-known example of an industrial solvent which can be made from sugars by microbial fermentation and distillation and by a petrochemical process. Economically the latter process still is the most attractive one, but oil prices on the world market may influence this. Other examples are the production of biodegradable plastics and rheological agents. Biodegradable plastics are based on polyhydroxybutyrate produced by bacteria from renewable feed stocks such as sugar. Biologically produced polymeric carbohydrates such as xanthan are used as rheological agents in products and processes as far apart as cosmetics and oil extraction. Also the use of enzymes and micro-organisms to carry out regio- and stereospecific transformations are interesting.

Ammonia serves as an example of how biotechnology might also have implications in the processing and production of inorganic materials. Ammonia is currently produced by the Haber-Bosch process in which gaseous nitrogen and hydrogen are combined at high temperatures and pressures. The process consumes large amounts of fossil energy. Ammonia is used as a feedstock in many processes and it is an important fertiliser in agriculture which has undoubtedly increased the yield of crops, however, at an appreciable environmental price. Much of the applied ammonium salt leaches out from soil into waterways where it causes eutrophication leading to increased algal blooms and subsequent anoxia of the water. So the water loses amenity value because it will no longer support fish life and becomes more difficult to treat to become potable.

The conversion of nitrogen gas to ammonia can also be brought about by nitrogen-fixing bacteria and only very little of the nitrogen fixed in this way is leached from the soil. Biological nitrogen fixation therefore can be seen as an environmentally sounder approach to provide nitrogen for agriculture. Nitrogen-fixing bacteria do supply certain plants with nitrogen; biotechnology offers the potential to widen the range of crops that can be supported using such *in situ* nitrogen fixation. In principle the biological nitrogen fixation process could also be used to provide ammonia for chemical synthesis. However, currently the economics favour the use of the environmentally damaging Haber-Bosch process, which shows once more that it would be desirable to add the environmental costs to the cost price as well.

A second example in the inorganic context would be the production of metals. Traditionally the production of metals involves consumption of considerable amounts of fossil fuels for mining and smelting. This consumption of non-renewable energy can be reduced by using acid- generating *Thiobacilli* for the leaching of metals from ores (acid/mining).

We can conclude from the foregoing that biotechnology may offer a range of environmentally favourable alternatives for classical industrial production. However, the development of new products based on cleaner production processes and on alternative raw materials is not only a question of technology. It is not only the technical production that matters, products also have to be sold, which implies calling in an extensive transport network and a complex tertiary sector. It also calls on public acceptance of the new products and processes. Moreover, acceptance of a new product should not simply depend on economics, but energy and environmental factors should play a role as well. Thus the penetration of biotechnological procedures in industrial production is not only dependant on technological and scientific advance, but also on politicians, legislators, investors and the general public; to cut a long story short, on society as a whole.

6. Biotechnology and the environment

In the foregoing sections the contribution of biotechnology to new developments in agriculture, energy management and chemical industry was discussed. We now will turn our attention to the application of biotechnology in the context of environmental management. Emphasis will be on the application of biotechnology in relation to the consequences of industrial production and distribution systems. We must not forget however, that wastes and pollutants derived from housekeeping, housing and processing of domestic wastes are also increasingly important (16), Table 1 illustrates this.

TABLE 1
WASTES AND POLLUTANTS DERIVED FROM HOUSEKEEPING, HOUSING AND PROCESSING OF DOMESTIC WASTES

	Source/compartment	Components
wastes	housekeeping	sewage (faeces, urine, wash-water) food scraps, garbage, scrapped domestic appliances
	housing	building and demolition refuse (wood, stone, asbestos, synthetics)
	processing	incineration residues (fly ash, scoriae) effluent sewage treatment, sewage sludge
pollutants	atmosphere	CO_2, CO, SO_x, NO_x (burning fossil fuels) hydrochloric acid, polyclorinated biphenyls, heavy metals, dioxines (refuse incineration) volatile organic solvents (painting) chlorofluorohydrocarbons (refrigerator, insulating materials) methane (landfill)
	surface waters	oxygen binding components eutrophicating compounds (nitrate, phosphate) percolation water (landfill sites)
	soil	solid residues, heavy metals, dioxines (refuse incineration) percolation waters (landfill sites)

Source: Biotechnological Innovations in Energy and Environmental Management, Butterworth Heinemann, Oxford, UK, 1994.

From a technological point of view we can distinguish three different ways by which biotechnology can contribute to environmental management (13). In the first place biotechnology can be used to eliminate or reduce existing environmental problems caused by conventional technology. By means of this so-called clean-up biotechnology polluted air, water and soil are cleaned to an acceptable level. The second application is focused on the emissions and wastes produced during the product life cycle and is called end-of-pipe or add-on biotechnology. By this approach environmentally hazardous materials are removed by (selected) micro-organisms directly after they have been produced and as a result these chemicals do not enter the environment. The third strategy is to use biotechnology to replace existing, environmentally-damaging technology. This application is called preventive biotechnology or, as this technology often only replaces a part of the total production process, process-integrated biotechnology. The different places of these technologies with respect to the product life cycle are shown in Figure 2.

In the past the product and its cost price have been the primary objectives of production, the environmental costs were mostly not taken into account. Presently we are confronted with the immense damage that has already been done to the environment. This not only holds true for industrial production, but also for agriculture, forestry and fishery, although the problems associated with the different production systems vary. In forestry and fishery exhaustion, extinction and biodiversity are important items. In agriculture biodiversity and pollution are key factors while pollution seems to be the main problem associated with industrial production. But we should of course realize that there is much interaction between the different production systems. Therefore sustainable development asks for an integrated approach.

The remainder of this paragraph will mainly deal with pollution. Lets first consider environmental biotechnology within the context of bio-remediation. There are many regions on earth which are heavily polluted with all kinds of chemicals. In such situations there are two choices: to leave the biodegradation to nature or to deliberately clean the polluted air, water or soil. When the waste streams are not deliberately taken care of by mankind, the biodegradable components will be subjected to spontaneous microbial degradation. Some components, such as some organic compounds, will degrade rapidly, while the degradation of others, such as plastics, may take signifi-

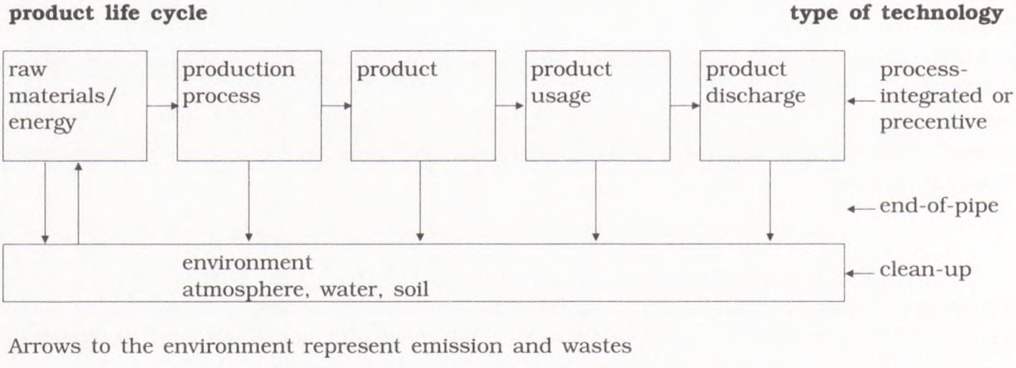

Fig. 2. Schematic representation of the relation between the product life cycle and different applications of environmental biotechnology.

cantly longer. Microbial diversity and adaptive ability are of utmost importance here and also the short generation times of micro-organisms contribute to the capacity of nature to deal with recalcitrant compounds. Therefore many compounds can be dealt with in the biosphere but at a very low speed. In many cases, especially if the pollutant is harmful to living nature and/or there is a chance of extension of the pollution, it is desirable not to leave biodegradation to nature, but to remove the pollutants by a faster method. To accomplish this, one or a combination of different mechanical, physical, chemical and biological technologies can be used. The usefulness of these technologies is determined by various factors such as costs, efficacy, reliability, energy consumption, need for process additives, production of secondary wastes, legal standards and so on.

Biotechnology, as all technologies, has its own advantages and drawbacks. The main attractiveness of biotechnology is the fact that biotechnology is founded on systems which utilise processes and products that occur in nature and which are components of the global elemental cycles. Table 2 gives some examples of the application of biotechnology in an environmental context.

TABLE 2
APPLICATION OF BIOTECHNOLOGY IN AN ENVIRONMENTAL CONTEXT

Application field	Examples
1	2
prevention of environmental pollution by replacement of (physio-)chemical processes by (micro)biological/biocatalytic processes	— biocatalytic production of fine chemicals such as amino acids and optical pure chiral compounds — microbial production of monomers — hydrogenation and chlorination reactions with microbial enzymes
prevention of environmental pollution by biotechnological crop protection	— incorporation of resistance and toxin genes in agricultural crops — use of microorganisms to combat pest organisms; baculo-viruses are used to control insects pests (*Lepidoptera* and *Diptera* family)
biotechnological processes as alternative for other environmental technologies	microbial removal of ammonia by nitrification/denitrification; *alternative for steam or air stripping and absorption in acid* — microbial removal of organic pollutants from soils; *alternative for thermic or extractive cleaning* — microbial removal of phosphates from waste water; *alternative for chemical precipitation*
combination of biotechnological processes with other environmental technologies	— enhancement of reactor biomass concentration by immobilization of microorganisms — pretreatment of waste streams prior to biodegradation with (physico)chemical techniques in order to degrade or convert toxic compounds — use of active coal to absorb peak loads in gas and water purification — pH-adjustment; removal of particles by sedimentation; reduction of particle seize in soil remediation

1	2
production of valuable materials from waste streams	— biogas (methane) formation from liquid and solid wastes (landfill; organic household scraps) — production of chemicals such as ethanol, etanol and acetic acid — production of biomass as Single Cell Protein and biofuel — production of amino acids, citric acid from (beet)melasses

Source: M.C.E. van Dam-Mieras et al., in: *Cleaner and Economic Production for Performance*, Ed. K.B. Misra, Springer Verlag, Heidelberg, Germany, to be published.

7. Examples of clean-up biotechnology

In practise clean-up biotechnology is mostly used to restore the quality of polluted (underwater) soils and ground waters.

It is important to emphasize that cleaning-up is only useful if the pollutant is concentrated and localised. In other words clean-up biotechnology is only applied to decontaminate very polluted soils and ground waters. We can distinguish two strategies to remove the pollutant: without removal of the polluted soil/water or by its removal. These methods are referred to as respectively *in-situ* or *ex-situ* techniques. Here we will discuss two examples of biotechnological decontamination: *in-* and *ex-situ* cleaning of oil-polluted soil and *ex-situ* removal of heavy metals and sulphate from ground water.

Currently, a lot of sites in the Netherlands (mostly petrol stations and leaking oil-tanks) have to be cleaned because they are polluted with oil. The choice of cleaning technique used for the treatment of such regionally located and very concentrated bio-degradable pollution depends on the factors given above and on the type of soil. Sand is easier to clean than clay, as clay retains more water and forms agglomerates. Therefore it is not possible to treat clay soils *in-situ*, and even *ex-situ* cleaning often turns out to be difficult. More sandy soils can be cleaned by both approaches.

What *in-situ* and *ex-situ* methods essentially do is optimising the conversion conditions for the natural biodegradation process by increasing the availability of nutrients and oxygen for the micro-organism. Sometimes selected micro-organisms, for instance descendent from soil containing a similar type of pollution, are added to the process as well. With an *in-situ* technique such as biorestoration, the microbial activity at the polluted site is stimulated by injection of micro-organisms, nutrients and oxygen. Major difficulties in *in-situ* techniques are the adhesion of the pollutant to the solid matrix, the hydro-geological situation, the ambiguity of the decontamination level reached, and the long treatment time (periods over 1-2 years).

For *ex-situ* techniques, such as land farming and the use of bioreactors, the soil has to be excavated first. In land farming the polluted soil is brought in depots and can be treated there by ploughing the soil from time to time to assure availability of oxygen and/or raining upon the soil to enhance the transport of added nutrients and micro-organisms. Bioreactors make use of the same principle, but the type of treatment has a more continuous character: the soil is brought in the reactor together with an amount of water and nutrients and in the reactor it is stirred and aerated. In this way the time for biodegradation is reduced as compared to land farming, which still suffers from long treatment times and low conversion rates, but as the cleaning process consumes more energy the operational costs increase (17, 18).

Another example of applied clean-up biotechnology is the removal of heavy metals and sulphate from the ground water beneath a 100 year old zinc refining plant. In order to avoid future contamination of the nearby drinking water aquifers, a geohydrological containment system (GCS) was developed. The GCS was designed to contain the contaminated ground water within the site boundaries by pumping up ground water from strategically located wells. The ground water was treated by a new three step process based on the use of sulphate-reducing bacteria. In the first step the sulphate in the water was converted to sulphide in an anaerobic reactor by sulphate-reducing bacteria resulting in the precipitation of metal sulphides. In the second step the excess sulphide was biologically oxidised to elemental sulphur by the colourless sulphur bacteria. In the last step, the solids were removed by a tilted plate separator and a sand filter. In this process sulphate, zinc, cadmium, copper, lead, arsenic and nickel were efficiently removed. The metals sulphide sludge and the sulphur slurry were mixed and could be fed to the zinc refinery. The metal could be reclaimed and the sulphur compounds were converted to sulphuric acid.

8. Examples of end-of-pipe biotechnology

Although this type of technology can treat all kinds of emissions and wastes generated along the product life cycle, it is mainly applied to treat gases and waste water (19-23). Currently, many applications of environmental biotechnology focus on the removal of hydrogen sulphide (H_2S) from industrial emissions. Hydrogen sulphide has the characteristic smell of rotten eggs and thus is a nuisance to mankind. It also gives rise to acid rain as it oxidises readily in the atmosphere to sulphur dioxide and sulphuric acid.

In principle the biological methods used to purify the waste gases are similar to those used in waste water purification, but the micro-organisms used differ. An additional difference is that in the treatment of waste gasses the pollutants first have to be transferred from the gas phase to the liquid phase. In bioscrubbing adsorption of the pollutants takes place in a wash column and subsequently the water is treated with micro-organisms suspended in a bioreactor.

In biological air treatment with biofilters the micro-organisms used are grown on support material. The polluted air originating from the production process is blown through the support layer and the available micro-organisms break down the undesired components. Problems with such biofilters are that the biological activity of the filter is difficult to control and that not all pollutants are converted. Humidity, a very important factor for biological activity, is difficult to regulate. Also pollutants giving rise to acid production upon biodegradation cause difficulties. Therefore the process result tends to be variable and unpredictable.

The so-called trickle-bed bioreactor or trickling filter, consisting of a three phase reactor system, appears to be more efficient. In this filtertype gas and liquid are flowing co- or counter currently through the support material on which the micro-organisms are immobilised. The biofilm can be controlled to a certain extent (nutrients, pH) via the circulating water phase. The pollutants are transferred from the gas phase into the water phase, from which they diffuse into the immobilised biofilm, where degradation takes place. Because of the possibility to control the pH in the biofilm the trickling filter can also be used to treat waste streams producing acid upon biodegradation. For example, the sulphuric acid that is produced by a micro-organism such as *Thiobacillus* on the supply of hydrogen sulphide can easily be neutralized in such a trickling filter. The trickling filter is, however, less effective in the removal of compounds with a lower water solubility. For the elimination of such contaminants, a new system has been developed based on a two phase (gas/liquid) system in which the phases are separated by a membrane on which selected micro-organisms are immobilised on the liquid side. In this membrane bioreactor the polluted components of the gas phase enter the biofilm on the liquid side of the membrane and are consumed by the micro-organisms.

9. Examples of process-integrated or preventive biotechnology

It is self-evident that the process-integrated or preventive biotechnology would be the strategy of choice from an environmental point of view, but this strategy sometimes has further reaching consequences for the total process than end-of-pipe biotechnology, which of course has economic implications. The key factor for changing production methods in industry is profitability, until now defined almost exclusively in an economic sense. Changing the production system for environmental reasons usually has not been economically feasible. However, growing public concern about environmental issues, stricter environmental legislation, and financial tools for preventing waste emissions may make environmentally sound behaviour economically sound as well.

Traditionally, micro-organisms and enzymes are used in production processes in the food and pharmaceutical industry, where process optimisation has led to increases in yield and product quality, reduced energy use and a reduction of waste and emissions. Bioleaching, the use of sulphur-oxidising bacteria in mineral resource winning and processing forms another example of applied microbiology. More recent examples are the production of optically pure chiral compounds, alternatives for specific chemical reaction(steps), dehairing and degreasing of hides by enzymes in the leather industry, enzymatic extraction of vegetable oils, the use of enzymes (rather than chlorine) in pulp and paper industry and degreasing in the electroplating industry with the use of enzymes and micro-organisms. Mineral resource winning by bioleaching and a biotechnological alternative for chlorine bleaching in the pulp and paper industry will be used as an example here.

The recovery of metals from ores is usually done by pyrometallurgy. The ores are milled and smelted at high temperatures before the metal can be recovered. This procedure involves a very high energy consumption, emission of gaseous pullutants and mineral containing solid wastes. Bioleaching can be a more environmentally friendly alternative.

Bioleaching makes use of the capability of specific bacteria to liberate metal ions (copper, zinc, lead, uranium) from their insoluble sulphides by oxidising these to soluble sulphates. In addition to this biological activity chemical oxidation of sulphide with ferric ions also plays a role. Presently the major commercial application of bioleaching is in the leaching of copper ores, but it may also become of economic significance for the extraction of uranium and gold (24).

Bioleaching is used to obtain copper from low grade ores arisen from conventional mining practises. The copper ore is sprayed with dilute sulfuric acid containing ferric ions and bacteria (*Thiobacillus ferreoxidans*). During the percolation of this solution through the ore, copper sulphate is formed. Copper can be obtained from the solution by reduction, for instance with scrap iron. The elemental copper precipitates out of the solution, which is regenerated by aerating it in order to stimulate *T. ferroxidans* to oxidise ferrous sulphate back to ferric sulphate. The regenerated solution is then recycled. In principle, high grade ores can be leached in a similar way.

Another example of introducing a more environmentally friendly production step is the preparation of pulp for paper production. To obtain a colourless pulp the lignin, responsible for the brownish colour, has to be removed, which involves a cooking and a bleaching stage. In the multistep bleaching stage chlorine and chlorine dioxide are usually applied. An obvious disadvantage of the procedure is the formation of toxic chlorinated compounds (e.g. dioxins) during the bleaching stage. To minimize the use of chlorine during the process several improvements have been introduced, among others increased delignification by extended cooking and oxygen treatment, and replacement of chlorine and chlorine dioxide with hydrogen peroxide. However, the use of hydrogen peroxide results in lower paper brightness and increased process costs. A new development is the use of xylanases in the prebleaching stage. The enzyme hydrolyses the bond between lignin and cellulose, which promotes lignin extraction in the subsequent chemical bleaching steps. In the near future further process development may make complete replacement of chlorine compounds possible in a cost effective way.

10. Concluding remarks

Growing public concern and increasing stringency of environmental regulations will probably stimulate the application of biotechnology in the context of environmental management. It is obvious that in this respect process-integrated or preventive technology is preferred to end-of-pipe technology as emissions and wastes are undesirable and treatment of these hazardous materials is energy-demanding. However, it should be realised that zero-waste technology will never exist as there will always be a waste stream. For example, during the cleaning of liquid wastes we usually produce solids as a by-product. The main issue is that the production process should be evaluated and the waste streams minimised before the implementation of an end-of-pipe technology.

Biotechnology has many advantages as compared to other technologies, for example:

- bioconversions have high specificity and selectivity and therefore fewer by-products are formed;
- reactions take place at normal temperature and pressure;
- relatively few reaction additives and only low energy input are necessary (sometimes oxygen and nutrients to support microbial growth are added).

Of course bioprocesses also have disadvantages, for example:

- a relatively slow velocity of conversion which implies the need for a large reactor volume;
- the process is susceptible to disturbances (formation of acid, overloading, toxic substances);
- the water phase is necessary for microbial activity;
- sometimes long adaptation times are needed, which makes it difficult to deal with changes in composition and volume of waste streams;
- a huge amount of (toxic) sludge (biomass) is produced.

However, the increasing application of the principle that 'the polluter pays' together with the gathering public and political conception of the need for proper environmental management must be seen as supportive of the replacement of environmentally damaging processes by cleaner production strategies. Biotechnology will most certainly play a role in such strategies.

An implication of this way of thinking for environmental education is that there will be a gradual shift from an emphasis on environmental policy and legislation to an emphasis on science and technology as well. In our opinion, biotechnology should have a place in environmental science curricula. The BIOTechnology by Open Learning programme (BIOTOL) developed by the Open

University of the Netherlands, the University of Greenwich, UK, and the De Montfort University at Leicester, UK, reflects such an approach. In the 33 books and 7 computer programmes belonging to the series basic principles and enabling techniques are the basis for understanding biotechnological innovations in agriculture, food industry, pharmaceutical industry, chemical industry and environmental and energy management. The materials can easily be adapted in different learning environments and hopefully will contribute in enabling society to make knowledge based decisions.

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Bioprocessing: from clean-up to preventive strategies

Summary

In satisfying its needs Mankind uses the Earth's physical resources and fossil energy and produces products that often are recalcitrant to biogradation. Therefore the production strategies employed in agriculture and industry need reconsideration Biotechnology paves the way for optimising production processes. Molecular breeding strategies can contribute to agriculture. Biotechnology can contribute to developments in the field of renewable energy. Environmental biotechnology can be of value in environmental management. Biotechnology thus can contribute to both bio-remediation and making technology in our society more sustainable, it therefore deserves a place in (higher) education programmes in the environmental sciences field.

Key words:
biotechnology, agriculture, industry, environment, energy.

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