Dynamic Biochemistry, Process Biotechnology and Molecular Biology ©2010 Global Science Books

Environmental Biotechnology: Achievements, Opportunities and Challenges

Maria Gavrilescu*

"Gheorghe Asachi" Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection,
Department of Environmental Engineering and Management, 71 Mangeron Blvd., 700050 Iasi, Romania
Correspondence: * **mgav@ch.tuiasi.ro**

ABSTRACT

This paper describes the state-of-the-art and possibilities of environmental biotechnology and reviews its various areas together with their related issues and implications. Considering the number of problems that define and concretize the field of environmental biotechnology, the role of some bioprocesses and biosystems for environmental protection, control and health based on the utilization of living organisms are analyzed. Environmental remediation, pollution prevention, detection and monitoring are evaluated considering the achievements, as well as the perspectives in the development of biotechnology. Various relevant topics have been chosen to illustrate each of the main areas of environmental biotechnology: wastewater treatment, soil treatment, solid waste treatment, and waste gas treatment, dealing with both the microbiological and process engineering aspects. The distinct role of environmental biotechnology in the future is emphasized considering the opportunities to contribute with new solutions and directions in remediation of contaminated environments, minimizing future waste release and creating pollution prevention alternatives. To take advantage of these opportunities, innovative new strategies, which advance the use of molecular biological methods and genetic engineering technology, are examined. These methods would improve the understanding of existing biological processes in order to increase their efficiency, productivity, and flexibility. Examples of the development and implementation of such strategies are included. Also, the contribution of environmental biotechnology to the progress of a more sustainable society is revealed.

Keywords: biological treatment, bioremediation, contaminated soil, environmental biotechnology, heavy metal, natural attenuation, organic compound, phytoremediation, recalcitrant organic, remediation

Abbreviations: BOD_5, five-day biological oxygen demand; **CNT**, carbon nanotube; **MBR**, membrane bioreactor; **MSAS**, membrane separation activated sludge process; **MTBE**, methyl tert-butyl ether; **TCE**, trichloroethylene; **VOC**, volatile organic compounds

CONTENTS

INTRODUCTION

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" (van Beuzekom and Arundel 2006). Biotechnology is versatile and has been assessed a key area which has greatly impacted various technologies based on the application of biological processes in manufacturing, agriculture, food processing, medicine, environmental protec-

Received: 17 September, 2008. Accepted: 29 September, 2009.

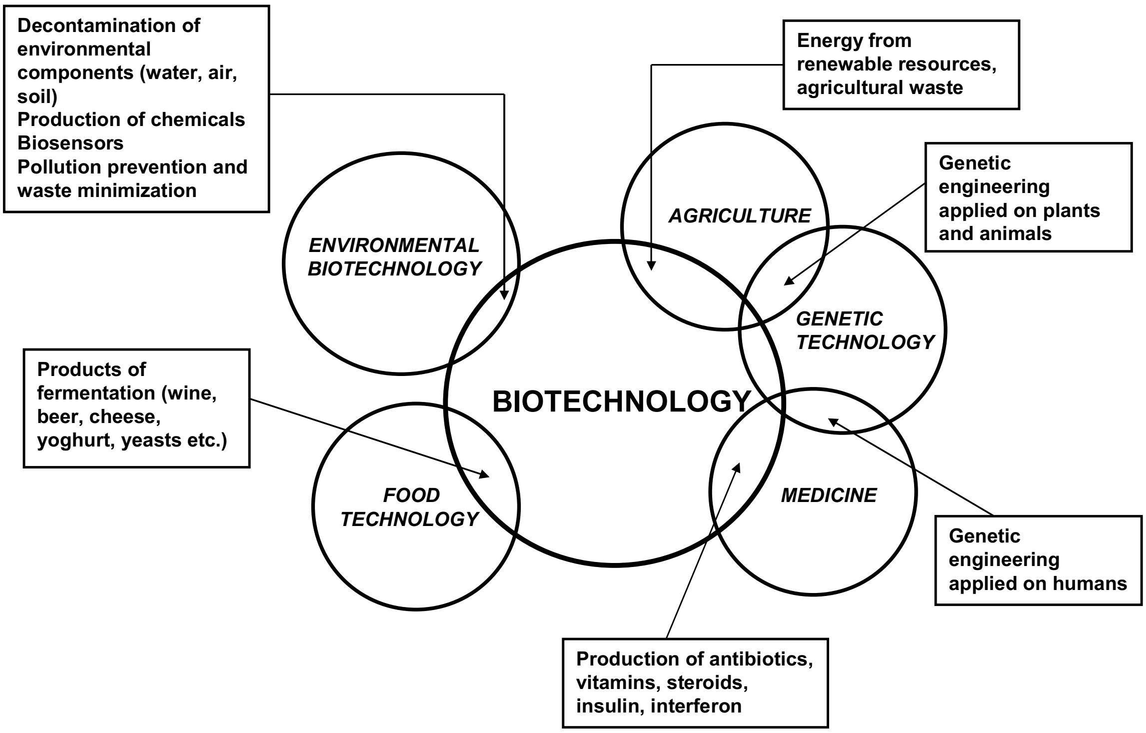


Fig. 1 Application of biotechnology in anthropogenic activities (industry, agriculture, medicine, health, environment). (Adapted from Sukumaran Nair 2006).

tion, resource conservation (**Fig. 1**) (Chisti and Moo-Young 1999; EC 2002; Evans and Furlong 2003; Gavrilescu 2004a; Gavrilescu and Chisti 2005). This new wave of technological changes has determined dramatic improvements in various sectors (production of drugs, vitamins, steroids, interferon, products of fermentation used as food or drink, energy from renewable resources and waste, as well as genetic engineering applied on plants, animals, humans) since it can provide entirely novel opportunities for sustainable production of existing and new products and services (Johnston 2003; Das 2005; Gavrilescu and Chisti 2005). In addition, environmental concerns help drive the use of biotechnology not only for pollution control (decontamination of water, air, soil), but prevent pollution and minimize waste in the first place, as well as for environmentally friendly production of chemicals, biomonitoring.

ROLE OF BIOTECHNOLOGY IN DEVELOPMENT AND SUSTAINABILITY

The responsible use of biotechnology to get economic, social and environmental benefits is inherently attractive and determines a spectacular evolution of research from traditional fermentation technologies (cheese, bread, beer making, animal and plant breeding), to modern techniques (gene technology, recombinant DNA technologies, biochemistry, immunology, molecular and cellular biology) to provide efficient synthesis of low toxicity products, renewable bioenergy and yielding new methods for environmental monitoring. The start of the 21st century has found biotechnology emerging as a key enabling technology for sustainable environmental protection and stewardship (Cantor 2000; Gavrilescu 2004b; Arai 2006). The requirement for alternative chemicals, feedstocks for fuels, and a variety of commercial products has grown dramatically in the early years of the 21st Century, driven by the high price of petroleum, policies to promote alternatives and reduce dependence on foreign oil, and increasing efforts to reduce net emissions of carbon dioxide and other greenhouse gases (Hettenhaus 2006). The social, environmental and economic benefits of environmental biotechnology go hand-in-hand to contribute to the development of a more sustainable society, a principle which was promoted in the Brundtland Report in 1987, in Agenda 21 of the Earth Summit in Rio de Janeiro in 1992, the Report of the World Summit on Sustainable Development held in Johannesburg in 2002 and which has been widely accepted in the environmental policies (EIBE 2000; OECD 2001).

Regarding these domains of application, four main subfields of biotechnology are usually talked about:

- *green biotechnology*, the oldest use of biotechnology by humans, deals with plants and growing;
- *red biotechnology*, applied to create chemical compounds for medical use or to help the body in fighting diseases or illnesses;
- *white biotechnology* (often green biotech), focusing on using biological organisms to produce or manipulate products in a beneficial way for the industry;
- *blue biotechnology* – aquatic use of biological technology.

The main action areas for biotechnology as important in research and development activities can be seen as falling into three main categories (Kryl 2001; Johnston 2003; Gavrilescu and Chisti 2005):

- *industrial supplies* (biochemicals, enzymes and reagents for industrial and food processing);
- *energy* (fuels from renewable resources);
- *environment* (pollution diagnostics, products for pollution prevention, bioremediation).

These are successfully assisted by various disciplines, such as biochemical bioprocesses and biotechnology engineering, genetic engineering, protein engineering, metabolic engineering, required for commercial production of biotechnology products and delivery of its services (OECD 1994; EFB 1995; OECD 1998; Evans and Furlong 2003; Gavrilescu and Chisti 2005).

This review focuses on the achievements of biotechnological applications for environmental protection and control and future prospects and new developments in the field, considering the opportunities of environmental biotechnology to contribute with new solutions and directions in remediation and monitoring of contaminated environments, minimizing future waste release and creating pollution prevention alternatives.

ENVIRONMENTAL BIOTECHNOLOGY - ISSUES AND IMPLICATIONS

As a recognition of the strategic value of biotechnology, integrated plans are formulating and implementing in many countries for using biotechnology for industrial regeneration, job creation and social progress (Rijaux 1977; Gavrilescu and Chisti 2005).

With the implementation of legislation for environmental protection in a number of countries together with setting of standards for industry and enforcements of compliance, *environmental biotechnology* gained in importance and broadness in the 1980s.

Environmental biotechnology is concerned with the application of biotechnology as an emerging technology in the context of environmental protection, since rapid industrialization, urbanization and other developments have resulted in a threatened clean environment and depleted natural resources. It is not a new area of interest, because some of the issues of concern are familiar examples of "old" technologies, such as: composting, wastewater treatment etc. In its early stage, environmental biotechnology has evolved from chemical engineering, but later, other disciplines (biochemistry, environmental engineering, environmental microbiology, molecular biology, ecology) also contribute to environmental biotechnology development (Hasim and Ujang 2004).

The development of multiple human activities (in industry, transport, agriculture, domestic space), the increase in the standard of living and higher consumer demand have amplified pollution of air (with CO_2, NO_x SO_2, greenhouse gasses, particulate matters), water (with chemical and biological pollutants, nutrients, leachate, oil spills), soil (due to the disposal of hazardous waste, spreading of pesticides), the use of disposable goods or non-biodegradable materials, and the lack of proper facilities for waste (**Fig. 2**).

Studies and researches demonstrated that some of these pollutants can be readily degraded or removed thanks to biotechnological solutions, which involve the action of microbes, plants, animals under certain conditions that envisage abiotic and biotic factors, leading to non-aggressive products through compounds mineralization, transformation or immobilization (**Fig. 3**).

Advanced techniques or technologies are now possible to treat waste and degrade pollutants assisted by living organisms or to develop products and processes that generate less waste and preserve the natural non-renewable resources and energy as a result of (Olguin 1999; EIBE 2000; Gavrilescu and Chisti 2005; Chisti 2007):

- improved treatments for solid waste and wastewater;
- bioremediation: cleaning up contamination and phytoremediation;
- ensuring the health of the environment through biomonitoring;
- cleaner production: manufacturing with less pollution or less raw materials;
- energy from biomass;
- genetic engineering for environmental protection and control.

Unfortunately, some environmental contaminants are refractory with a certain degree of toxicity and can accumulate in the environment. Furthermore, the treatment of some pollutants by conventional methods, such as chemical degradation, incineration or landfilling, can generate other contaminants, which superimposed on the large variety of noxious waste present in the environment and determine increasing consideration to be placed on the development of combination with alternative, economical and reliable biological treatments (OECD 1994; EFB 1995; Krieg 1998; OECD 1998; Futrell 2000; Evans and Furlong 2003; Kuhn *et al.* 2003; Chen *et al.* 2005; Gavrilescu 2005; Betianu and Gavrilescu 2006a, 2006b).

At least four key points are considered for environmental biotechnology interventions to detect (using biosensors

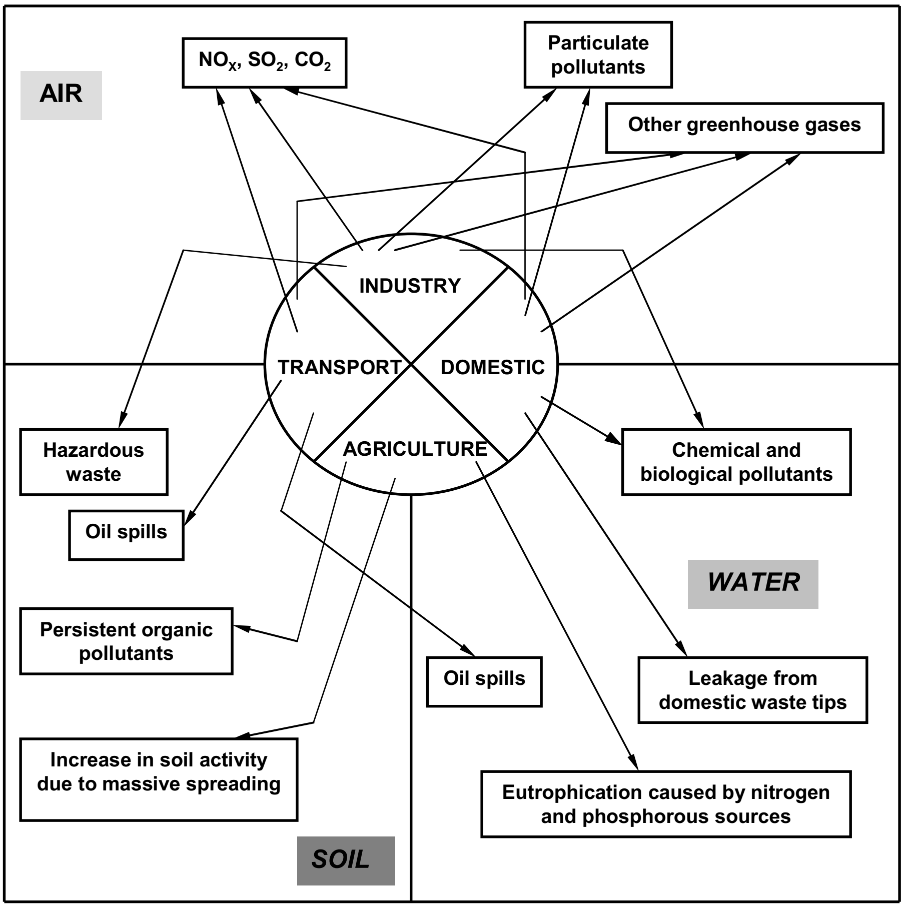


Fig. 2 The spider of environmental pollution due to anthropogenic activities. (Adapted from EIBE 2000).

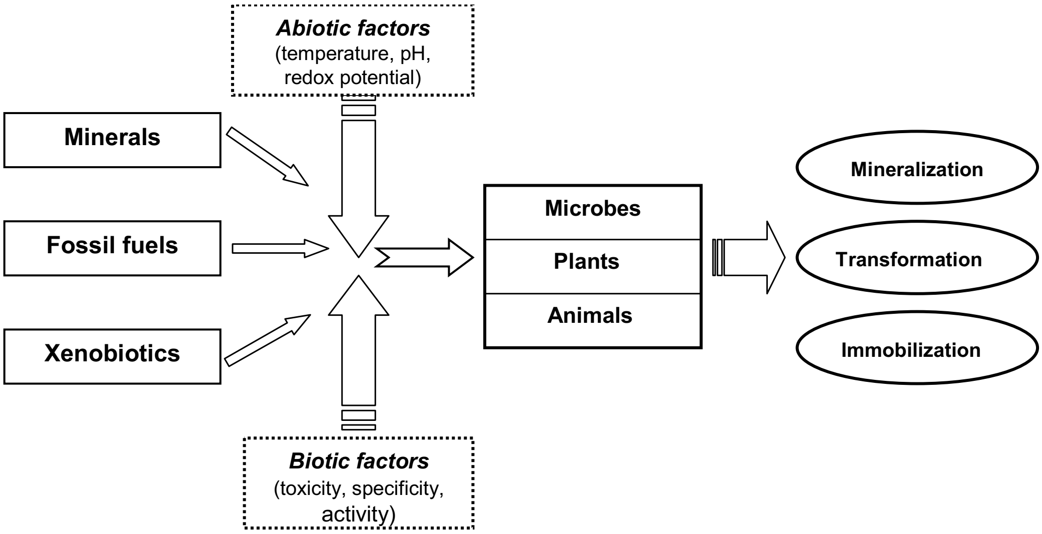


Fig. 3 Sources of environmental pollutants and factors that influence their removal from the environment. (Adapted from Chen *et al* 2005).

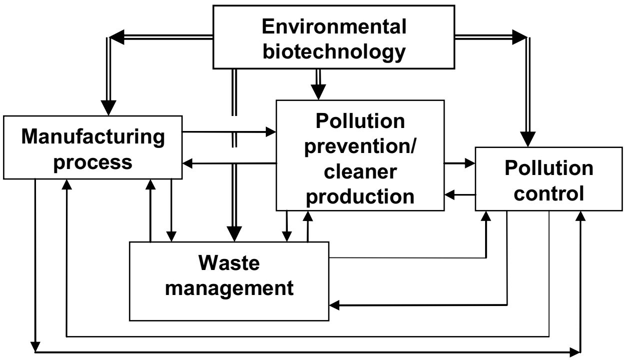


Fig. 4 Key intervention points of environmental biotechnology.

and biomonitoring), prevent in the manufacturing process (by substitution of traditional processes, single process steps and products with the use of modern bio- and gene technology in various industries: food, pharmaceutical, textiles, production of diagnostic products and textiles), control and remediate the emission of pollutants into the environment (**Fig. 4**) (by degradation of harmful substances during water/wastewater treatment, soil decontamination, treatment and management of solid waste) (Olguin 1999; Chen *et al.* 2005; Das 2005; Gavrilescu 2005; Gavrilescu and Nicu 2005). Other significant areas where environmental biotechnology can contribute to pollution reduction are production of biomolecules (proteins, fats, carbohydrates, lipids, vitamins, aminoacids), yield improvement in original plant products. The production processes themselves can assist in the reduction of waste and minimization of pollution within the so-called clean technologies based on biotechnological issues involved in reuse or recycle waste streams, generate energy sources, or produce new, viable products (Evans and Furlong 2003; Gavrilescu and Chisti 2005; Gavrilescu *et al.* 2008).

By considering all these issues, biotechnology may be regarded as a driving force for integrated environmental protection by environmental bioremediation, waste minimization, environmental biomonitoring, biomaintenance.

ENVIRONMENTAL REMEDIATION BY BIOTREATMENT/ BIOREMEDIATION

Environmental hazards and risks that occur as a result of accumulated toxic chemicals or other waste and pollutants could be reduced or eliminated through the application of biotechnology in the form of (bio)treatment/(bio)remediating historic pollution as well as addressing pollution resulting from current industrial practices through pollution prevention and control practices. Bioremediation is defined by US Environmental Protection Agency (USEPA) as “a managed or spontaneous practice in which microbiological processes are used to degrade or transform contaminants to less toxic or nontoxic forms, thereby remediating or eliminating environmental contamination” (USEPA 1994; Talley 2005).

Biotreatment/bioremediation methods are almost typical “end-of-pipe processes” applied to remove, degrade, or detoxify pollution in environmental media, including water, air, soil, and solid waste. Four processes can be considered as acting on the contaminant (Asante-Duah 1996; FRTR 1999; Khan *et al.* 2004; Doble and Kumar 2005; Gavrilescu 2006):

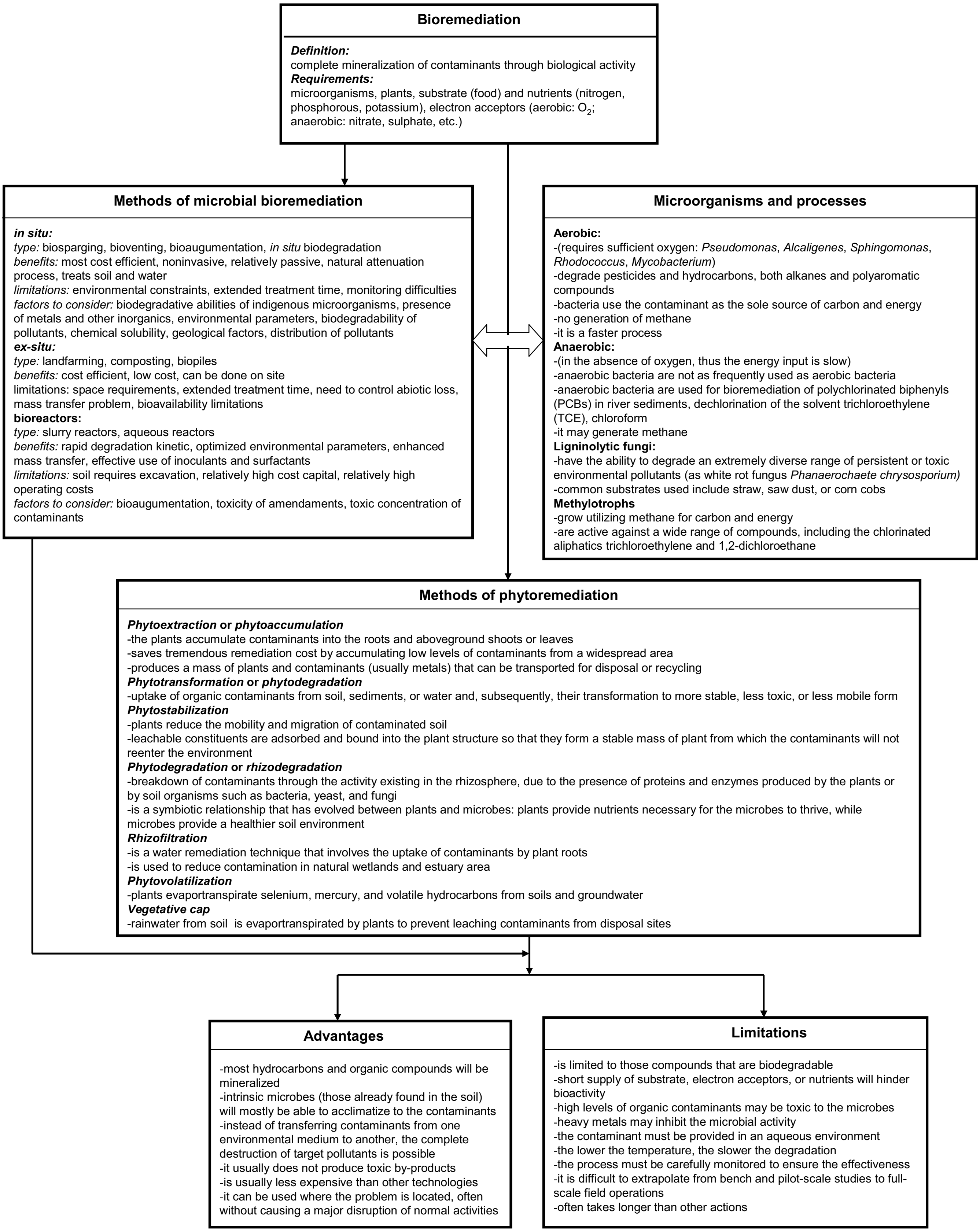
1. *removal:* a process that physically removes the contaminant or contaminated medium from the site without the need for separation from the host medium;
2. *separation:* a process that removes the contaminant from the host medium (soil or water);
3. *destruction/degradation:* a process that chemically or biologically destroys or neutralizes the contaminant to produce less toxic compounds;
4. *containment/immobilization:* a process that impedes or immobilizes the surface and subsurface migration of the contaminant;

Removal, *separation*, and *destruction* are processes that reduce the concentration or remove the contaminant. *Containment*, on the other hand, controls the migration of a contaminant to sensitive receptors without reducing or removing the contaminant (Watson 1999; Khan *et al.* 2004; Gavrilescu 2006).

Removal of any pollutant from the environment can take place on following two routes: degradation and immobilization by a process which causes it to be biologically unavailable for degradation and so is effectively removed (Evans and Furlong 2003). A summary of processes involved in bioremediation as a generic process is presented in **Fig. 5** (Gavrilescu 2004).

Immobilization can be carried out by chemicals released by organisms or added in the adjoining environment, which catch or chelate the contaminant, making it insoluble, thus unavailable in the environment as an entity. Sometimes, immobilization can be a major problem in remediation because it can lead to aged contamination and a lot of research effort needs to be applied to find methods to turn over the process.

Destruction (biodegradation and biotransformation) is carried out by an organism or a combination of organisms (consortia) and is the core of environmental biotechnology, since it forms the major part of applied processes for environmental cleanup. Biotransformation processes use natural



Bioremediation

Definition:
complete mineralization of contaminants through biological activity
Requirements:
microorganisms, plants, substrate (food) and nutrients (nitrogen, phosphorous, potassium), electron acceptors (aerobic: O_2; anaerobic: nitrate, sulphate, etc.)

Methods of microbial bioremediation

in situ:
type: biosparging, bioventing, bioaugumentation, *in situ* biodegradation
benefits: most cost efficient, noninvasive, relatively passive, natural attenuation process, treats soil and water
limitations: environmental constraints, extended treatment time, monitoring difficulties
factors to consider: biodegradative abilities of indigenous microorganisms, presence of metals and other inorganics, environmental parameters, biodegradability of pollutants, chemical solubility, geological factors, distribution of pollutants
ex-situ:
type: landfarming, composting, biopiles
benefits: cost efficient, low cost, can be done on site
limitations: space requirements, extended treatment time, need to control abiotic loss, mass transfer problem, bioavailability limitations
bioreactors:
type: slurry reactors, aqueous reactors
benefits: rapid degradation kinetic, optimized environmental parameters, enhanced mass transfer, effective use of inoculants and surfactants
limitations: soil requires excavation, relatively high cost capital, relatively high operating costs
factors to consider: bioaugumentation, toxicity of amendaments, toxic concentration of contaminants

Microorganisms and processes

Aerobic:
-(requires sufficient oxygen: *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, *Mycobacterium*)
-degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds
-bacteria use the contaminant as the sole source of carbon and energy
-no generation of methane
-it is a faster process
Anaerobic:
-(in the absence of oxygen, thus the energy input is slow)
-anaerobic bacteria are not as frequently used as aerobic bacteria
-anaerobic bacteria are used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), chloroform
-it may generate methane
Ligninolytic fungi:
-have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants (as white rot fungus *Phanaerochaete chrysosporium)*
-common substrates used include straw, saw dust, or corn cobs
Methylotrophs
-grow utilizing methane for carbon and energy
-are active against a wide range of compounds, including the chlorinated aliphatics trichloroethylene and 1,2-dichloroethane

Methods of phytoremediation

Phytoextraction* or *phytoaccumulation
-the plants accumulate contaminants into the roots and aboveground shoots or leaves
-saves tremendous remediation cost by accumulating low levels of contaminants from a widespread area
-produces a mass of plants and contaminants (usually metals) that can be transported for disposal or recycling
Phytotransformation* or *phytodegradation
-uptake of organic contaminants from soil, sediments, or water and, subsequently, their transformation to more stable, less toxic, or less mobile form
Phytostabilization
-plants reduce the mobility and migration of contaminated soil
-leachable constituents are adsorbed and bound into the plant structure so that they form a stable mass of plant from which the contaminants will not reenter the environment
Phytodegradation* or *rhizodegradation
-breakdown of contaminants through the activity existing in the rhizosphere, due to the presence of proteins and enzymes produced by the plants or by soil organisms such as bacteria, yeast, and fungi
-is a symbiotic relationship that has evolved between plants and microbes: plants provide nutrients necessary for the microbes to thrive, while microbes provide a healthier soil environment
Rhizofiltration
-is a water remediation technique that involves the uptake of contaminants by plant roots
-is used to reduce contamination in natural wetlands and estuary area
Phytovolatilization
-plants evaportranspirate selenium, mercury, and volatile hydrocarbons from soils and groundwater
Vegetative cap
-rainwater from soil is evaportranspirated by plants to prevent leaching contaminants from disposal sites

Advantages

-most hydrocarbons and organic compounds will be mineralized
-intrinsic microbes (those already found in the soil) will mostly be able to acclimatize to the contaminants
-instead of transferring contaminants from one environmental medium to another, the complete destruction of target pollutants is possible
-it usually does not produce toxic by-products
-is usually less expensive than other technologies
-it can be used where the problem is located, often without causing a major disruption of normal activities

Limitations

-is limited to those compounds that are biodegradable
-short supply of substrate, electron acceptors, or nutrients will hinder bioactivity
-high levels of organic contaminants may be toxic to the microbes
-heavy metals may inhibit the microbial activity
-the contaminant must be provided in an aqueous environment
-the lower the temperature, the slower the degradation
-the process must be carefully monitored to ensure the effectiveness
-it is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations
-often takes longer than other actions

Fig. 5 Characteristics and particularities of bioremediation. (Adapted from Vidali 2001; Gavrilescu 2004a).

and recombinant microorganisms (yeasts, fungi, bacteria), enzymes, whole cells. Biotransformation plays a key role in the area of foodstuff, pharmaceutical industry, vitamins, specialty chemicals, animal feed stock (**Fig. 6**) (Trejo and Quintero 1999; Doble *et al.* 2004; Singhal and Shrivastava 2004; Chen *et al.* 2005; Dale and Kim 2006; Willke *et al.* 2006). Metabolic pathways operate within the cells or by enzymes either provided by the cell or added to the system after they are isolated and often immobilized.

Biological processes rely on useful microbial reactions including degradation and detoxification of hazardous organics, inorganic nutrients, metal transformations, applied to gaseous, aqueous and solid waste (Eglit 2002; Evans and Furlong 2003; Gavrilescu 2004a).

A ***complete biodegradation*** results in detoxification by mineralizing pollutants to carbon dioxide, water and harm-

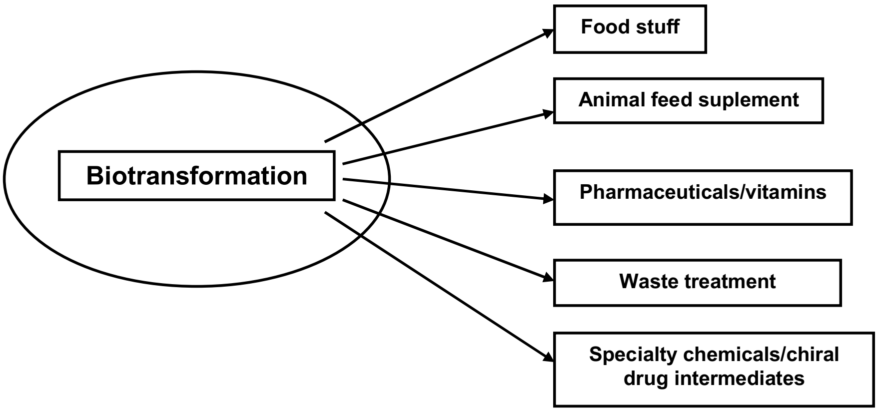


Fig. 6 Applications of biotransformations.

less inorganic salts.

Incomplete biodegradation will yield breakdown products which may or may not be less toxic than the original pollutant and combined alternatives have to be considered, such as: dispersion, dilution, biosorption, volatilization and/ or the chemical or biochemical stabilization of contaminants (Lloyd 2002; Gavrilescu 2004a).

In addition, *bioaugmentation* involves the deliberate addition of microorganisms that have been cultured, adapted, and enhanced for specific contaminants and conditions at the site.

Biorefining entails the use of microbes in mineral processing systems. It is an environmentally friendly process and, in some cases, enables the recovery of minerals and use of resources that otherwise would not be possible.

Current research on *bioleaching* of oxide and sulfide ores addresses the treatment of manganese, nickel, cobalt, and precious metal ores (Sukla and Panchanadikar 1993; Smith *et al.* 1994).

Fig. 7 provides some bioprocess alternatives for heavy metals removal from the environment (Lloyd 2002; Gavrilescu 2004a).

Biological treatment processes are commonly applied to contaminants that can be used by organisms as carbon or energy sources, but also for some refractory pollutants, such as:

- organics (petroleum products and other carbon-based chemicals);
- metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, zinc);
- radioactive materials.

Microbes and plants in environmental remediation

All forms of life can be considered as having a potential function in environmental biotechnology. However, microbes and certain plants are of interest even as normally present in their natural environment or by deliberate introduction (Evans and Furlong, 2003).

The generic term “microbe” includes prokaryotes (bacteria or arcaea) and eukariotes (yeasts, fungi, protozoa, and unicellular plants, rotifers).

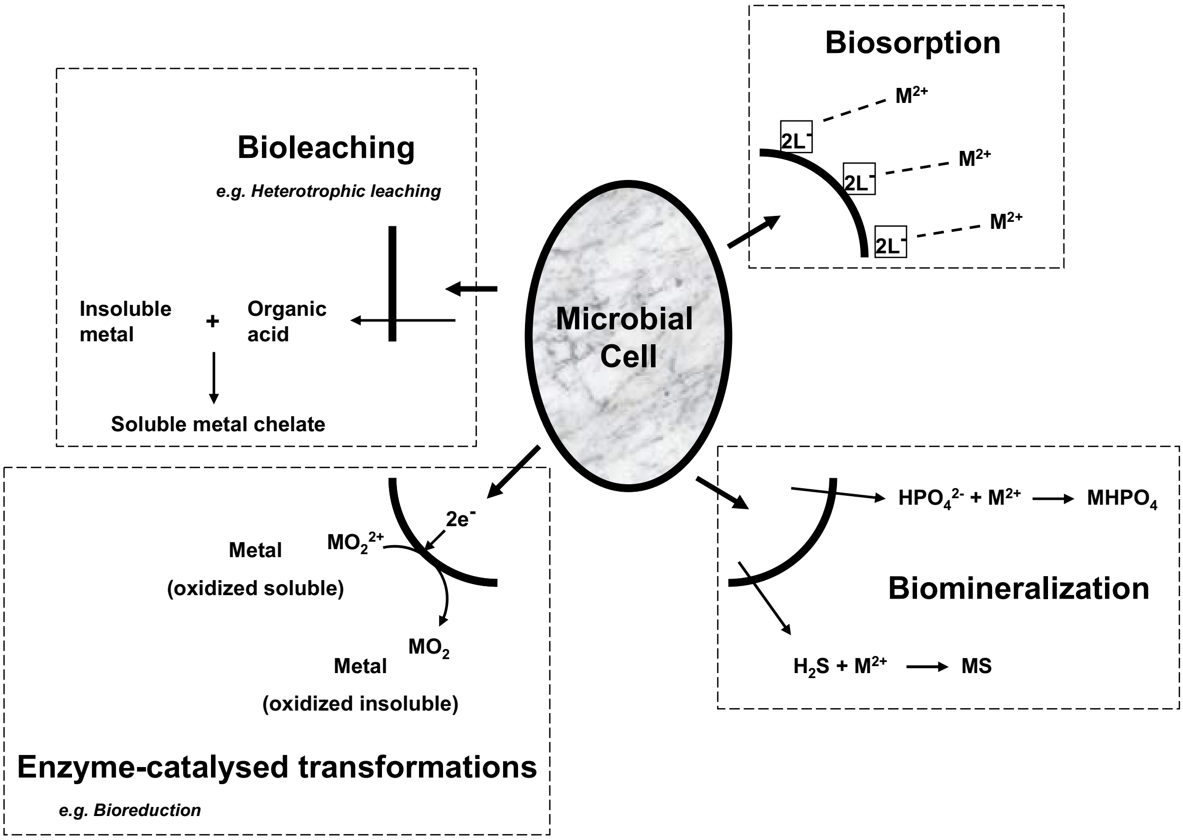


Fig. 7 Mechanisms of metal-microbe interactions during bioremediation applications. (Lloyd 2002; Gavrilescu 2004a).

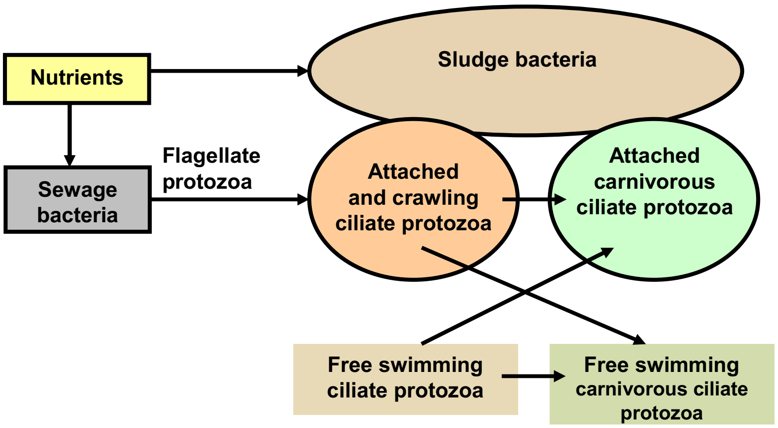


Fig. 8 Structure of microbial community in activated sludge. (Adapted from Wagner *et al.* 2002; Bitton 2005).

Some of these organisms have the ability to degrade some of the most hazardous and recalcitrant chemicals, since they have been discovered in unfriendly environments where the needs for survival affect their structure and metabolic capability.

Microorganisms may live as free individuals or as communities in mixed cultures (consortia), which are of particular interest in many relevant environmental technologies, like activated sludge or biofilm in wastewater treatment (Gavrilescu and Macoveanu 1999; Gavrilescu and Macoveanu 2000; Metcalf and Eddy 1999). One of the most significant key aspects in the design of biological wastewater treatment systems is the microbial community structures in activated sludges, constituted from activated sludge flocs, which enclose various microorganism types (**Fig. 8, Table 1**) (Wagner and Amann 1997; Wagner *et al.* 2002).

The role of plants in environmental cleanup is exerted during the oxygenation of a microbe-rich environment, filtration, solid-to-gas conversion or extraction of contaminants.

The use of organisms for the removal of contamination is based on the concept that all organisms could remove substances from the environment for their own growth and metabolism (Hamer 1997; Saval 1999; Wagner *et al.* 2002; Doble *et al.* 2004; Gavrilescu 2004; Gavrilescu 2005):

- *bacteria and fungi* are very good at degrading complex molecules, and the resultant wastes are generally safe (fungi can digest complex organic compounds that are normally not degraded by other organisms);
- *protozoa*
- *algae and plants* proved to be suitable to absorb nitrogen, phosphorus, sulphur, and many minerals and metals from the environments.

Microorganisms used in bioremediation include aerobic (which use free oxygen) and anaerobic (which live only in the absence of free oxygen) (**Fig. 5**) (Timmis *et al.* 1994; Hamer 1997; Cohen 2001; Wagner *et al.* 2002; Gray 2004; Brinza *et al.* 2005a, 2005b; Moharikar *et al.* 2005). Some have been isolated, selected, mutated and genetically engineered for effective bioremediation capabilities, including the ability to degrade recalcitrant pollutants, guarantee better survival and colonization and achieve enhanced rates of degradation in target polluted niches (Gavrilescu and Chisti 2005).

They are functional in activated sludge processes, lagoons and ponds, wetlands, anaerobic wastewater treatment and digestion, bioleaching, phytoremediation, land-farming, slurry reactors, trickling filters (Burton *et al.* 2002; Mulligan 2002). **Table 1** proposes a short survey of microbial groups involved in environmental remediation (Rigaux 1997; Pandey 2004; Wang *et al.* 2004; Bitton 2005).

Factors affecting bioremediation

Two groups of factors can be identified that determine the success of bioremediation processes (Saval 1999; Nazaroff and Alvarez-Cohen 2001; Beaudette *et al.* 2002; Wagner *et al.* 2002; Sasikumar and Papinazath 2003; Bitton 2005; Gavrilescu 2005):

- nature and character of contaminant/contamination, which refers to the chemical nature of contaminants and their physical state (concentration, aggregation state: solid, liquid, gaseous, environmental component that contains it, oxido-reduction potential, presence of halogens, bonds type in the structure etc.);
- environmental conditions (temperature, pH, water/air/soil characteristics, presence of toxic or inhibiting substances to the microorganism, sources of energy, sources of carbon, nitrogen, trace compounds, temperature, pH, moisture content.

Also, bioremediation tends to rely on the natural abilities of microorganisms to develop their metabolism and to optimize enzymes activity (**Fig. 9**).

The prime controlling factors are air (oxygen) availability, moisture content, nutrient levels, matrix pH, and ambient temperature (**Table 2**) (Vidali 2001).

Usually, for ensuring the greatest efficiency, the ideal range of temperature is 20-30°C, a pH of 6.5-7.5 or 5.9-9.0 (dependent on the microbial species involved). Other circumstances, such as nutrient availability, oxygenation and the presence of other inhibitory contaminants are of great importance for bioremediation suitability, for a certain type of contaminat and environmental compartment, the required remediation targets and how much time is available. The selection of a certain remediation method entails non-engineered solutions (natural attenuation/intrinsic remediation) or an engineered one, based on a good initial survey and risk assessment.

A number of interconnected factors affect this choice (as is also illustrated in **Figs. 5, 10**):

- contaminant concentration
- contaminant/contamination characteristics and type
- scale and extent of contamination
- the risk level posed to human health or environment
- the possibility to be applied in situ or ex situ
- the subsequent use of the site
- available resources

Bioremediation technologies offer a number of advantages even when bioremediation processes have been established for both *in situ* and *ex situ* treatment (**Fig. 10**), such as (EIBE 2000; Sasikumar and Papinazath 2003; Gavrilescu 2005; Gavrilescu and Chisti 2005):

- operational cost savings comparative to other technologies
- minimal site disturbance
- low capital costs
- destruction of pollutants, and not transferring the problem elsewhere
- exploitation of interactions with other technologies

These advantages are counterbalanced by some disadvantages (Boopathy 2000; Sasikumar and Papinazath 2003):

- influence of pollutant characteristics and local conditions on process implementation
- viability needs to be improved (time consuming and expensive)
- community distress for safety of large-scale on-site treatment
- other technologies should be necessary
- may have long time-scale

The biotreatment is applied above all in wastewater treatment, soil bioremediation, solid waste treatment, biotreatment of gaseous streams.

(Bio)treatment of municipal wastewater by activated

Table 1 Survey of microbial groups involved in environmental remediation.

Microorganisms	**Type**	**Shape**	**Example**	**Abilities**	**References**
Bacteria	cocci	spherical shape	*Streptococcus*	hydrocarbon-degrading bacteria heavy oil degrade dairy industry waste (whey)	Atlas 1981 Leahy and Colwell 1990 Ince 1998 Donkin 1997 Grady *et al.* 1999 Marques-Rocha *et al.* 2000 Blonskaya and Vaalu 2006 Kumar *et al.* 2007 Mohana *et al.* 2007 Xu *et al.* 2009
	bacilli	rods	*Bacillius subtilis*	degrade crude oil bioremediation of chlorpyrifos-contaminated soil	Gallert and Winter 1999 Eglit 2002 Das and Mukherjee 2007 Lakshmi *et al.* 2009
		spiral forms	*Vibrio cholera* *Spirillum volutans*	heavy metals	Bitton 2005
	sheated bacteria	filamentous (gram-negative rods that become flagellated)	*Sphaeratilus* *Leptothrix* *Crenothrix*	reduce iron to ferric hydroxide (*Sphaeratilus natans, Crenothrix*) reduce manganese to manganese oxide (*Leptothrix*) found in polluted streams and wastewater treatment plants	Sukla and Panchanadikar 1993 Smith *et al.* 1994 Sasaki *et al.* 2001 Gray 2004 Bitton 2005 Fitzgiblon *et al.* 2007
	ptalked bacteria	flagellated	*Caulobacter*	aerobic, aquatic environments with low organic content	Poindexter *et al.* 2000 Bitton 2005
			Gallionella	*G. ferruginea*, present in iron rich waters and oxidizes Fe^{2+} to Fe^{3+}. can be formed in water distribution systems	Benz *et al.* 1998 Blanco 2000 Smith *et al.* 2004 Bitton 2005
	budding bacteria	filaments or hyphae	*Hyphomicrobium*	soil and aquatic environments requires one-carbon compounds to grow (e.g. methanol)	Trejo and Quintero 1999 Gallert and Winter 2001 Burton *et al.* 2002 Duncan and Horan 2003
			Rhodomicrobium	phototrophic	Bitton 2005
	gliding bacteria	filamentous (gram-negative)	*Beggiatoa* *Thiothrix*	oxidize H_2S to S^0	Droste 1997 Guest and Smith 2002 Reddy *et al.* 2003
	bdellovibrio	flagellated (predatory)	*B. bacteriovorus*	grow independently on complex organic media	Bitton 2005 Saratale *et al.* 2009
	actinomycetes	filamentous (gram-positive) mycelial growth	*Micromonospora* *Streptomyces* *Nocordia (Gordonia)*	• most are strict aerobes • found in water, wastewater treatment plants, soils (neutral and alkaline) • degrade polysaccharides (starch, cellulose), hydrocarbons, lignin • can produce antibiotics (streptomycin, tetracycline, chloramphenicol) • *Gordonia* is a significant constituent of foams in activated sludge units	Grady *et al.* 1999 Lema *et al.* 1999 Olguin 1999 Saval 1999 Duncan and Horan 2003 Gavrilescu 2004 Bitton 2005 Dash *et al.* 2008 Joshi *et al.* 2008
	cyanobacteria (blue-green algae)	unicellular, colonial or filamentous organisms	*Anabaena*	• prokaryotic organisms • able to fix nitrogen • have a high resistance to extreme environmental conditions (temperature, dessication) so that are found in desert soil and hot springs • responsible for algal blooms in lakes and other aquatic environments • some are quite toxic	Blanco 2000 Burton *et al.* 2002 Bitton 2005 Brinza *et al.* 2005a El-Sheekh *et al.* 2009
Archea	crenarchaeotes euryarchaeotes korarchaeotes (more closely related to eukaryotes than to bacteria)	extremophyles	thermophiles hyperthermophiles psychrophiles acidophiles alkaliphiles halophiles	• prokaryotic cells • use organic compounds as a source of carbon and energy (organotrophs) • use CO_2 as a carbon source (chemoautothrophs)	Eglit 2000 Burton *et al.* 2002 Gavrilescu 2002 Dunn *et al.* 2003 Bitton 2005 Doble and Kumar 2005

Table 1 (Cont.)

Microorganisms	Type	Shape	Example	Abilities	References
Eukaryotes	fungi	long filaments (hiphae) which form a mass called mycellium		• use organic compounds as carbon source and energy, and play an important role in nutrient recycling in aquatic and soil environments • some form traps that capture protozoa and nematodes • grow under acidic conditions in foods, water or wastewater (pH 5) • implicated in several industrial application (fermentation processes and antibiotic production)	Hamer 1997 Burton *et al.* 2002 Brinza and Gavrilescu 2003 Gupta *et al.* 2004 Bitton 2005
			Phycomycetes (water molds)	• occur on the surface of plants and animals in aquatic environments some are terrestrial (common bread mold, Rhizopus)	Duncan and Horan 2003 Bitton 2005
			Ascomycetes *(Neurospora crassa, Saccharomyces cerevisiae)*	some yeasts are important industrial microorganisms involved in bread, wine, beer making	Bitton 2005
			Basidiomycetes (mushrooms - *Agaricus*, *Amanita* (poisonous))	wood-rotting fungi play a significant role in the decomposition of cellulose and lignin	Hernández-Luna *et al.* 2007 Bitton 2005
			Fungii imperfecti (ex. *Penicillium)*	can cause plant diseases	Gadd 2007
	algae	floating unicellular microorganisms filamentous colonial	phyloplankton *Uhlothrix* *Volvox*	• play the role of primary producers in aquatic environments (oxidation ponds for wastewater treatment) • carry out oxygenic photosynthesis and grow in mineral media with vitamin supplements (provide by some bacteria) and with CO_2 as the carbon source • some are heterotrophic and use organic compounds (simple sugars and organic acids) as source of carbon and energy	Chavan and Mukherji 2010 Tuzen *et al.* 2009 Duncan and Horan 2003 Feng and Aldrich 2004
			Phylum Chlorophyta (green algae) *Phylum Chrysophyta* (golden-brown algae) *Phylum Euglenophyta* *Phylum Pyrrophyta* (dinoflagellates) *Phylum Rhodophyta* (red algae) *Phylum Phaeophyta* (brown algae)		Bitton 2005 Gadd 2007
	Protozoa	unicellular organisms		important for public health and process microbiology in water and wastewater treatment	
			Sarcodina (amoeba) *Mastigophora* (flagellates) *Ciliophora* (ciliates) *Sporozoa*	• resistant to desiccation, starvation, high temperature, lack of oxygen, disinfection in waters and wastewaters • found in soils and aquatic environments • some are parasitic to animals and humans	Bitton 2005
Viruses	Belong neither to prokaryotes nor to eukaryotes (carry out no catabolic or anabolic functions)		Animal viruses Algal viruses Bacterial phages	• some are indicators of contamination • distruct host cells • infect a wide range of organisms (animals, algae, bacteria)	Duncan and Horan 2003

sludge method was perhaps the first major use of biotechnology in bioremediation applications. Municipal sewage treatment plants and filters to treat contaminated gases were developed around the turn of the century. They proved very effective although at the time, the cause for their action was unknown. Similarly, aerobic stabilization of solid waste through composting has a long history of use. In addition, bioremediation was mainly used in cleanup operations, including the decomposition of spill oil or slag loads containing radioactive waste. Then, bioremediation was found as the method of choice when solvents, plastics or heavy metals and toxic substances like DDT, dioxins or TNT need to be removed (EIBE 2000; Betianu and Gavrilescu 2006a).

General advantages associated with the use of biologi-

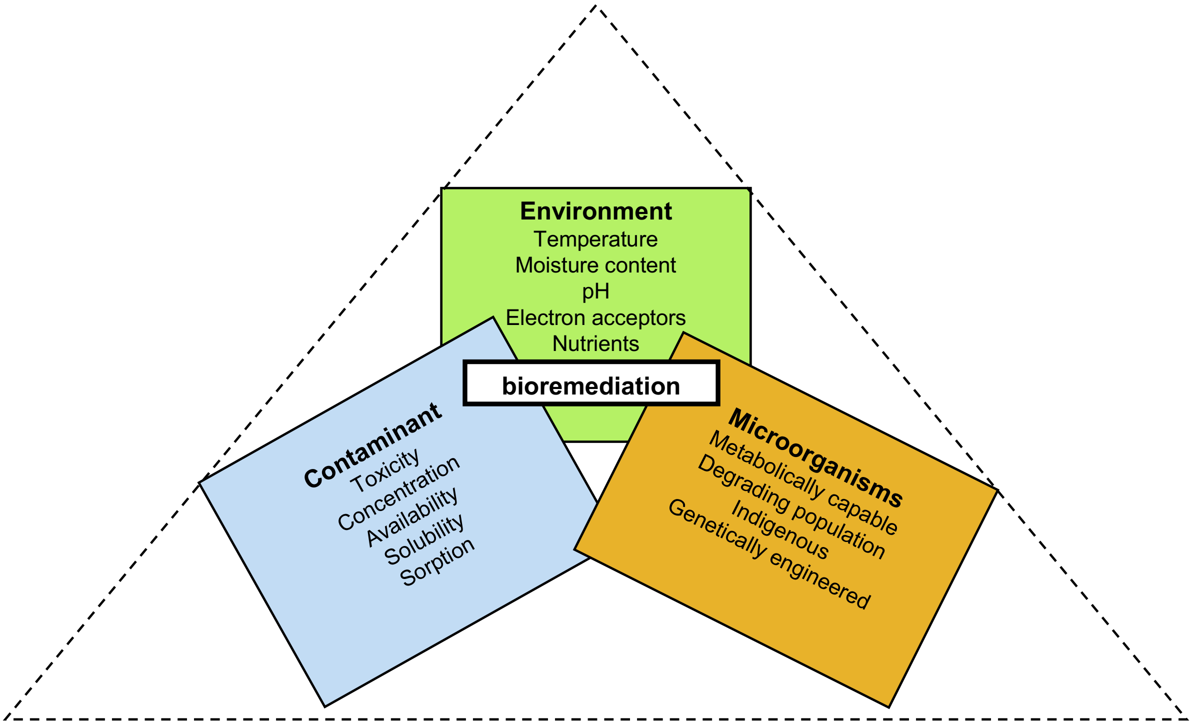


Fig. 9 Main factors of influence in bioremediation processes. (Adapted from Beaudette *et al.* 2002; Bitton 2005).

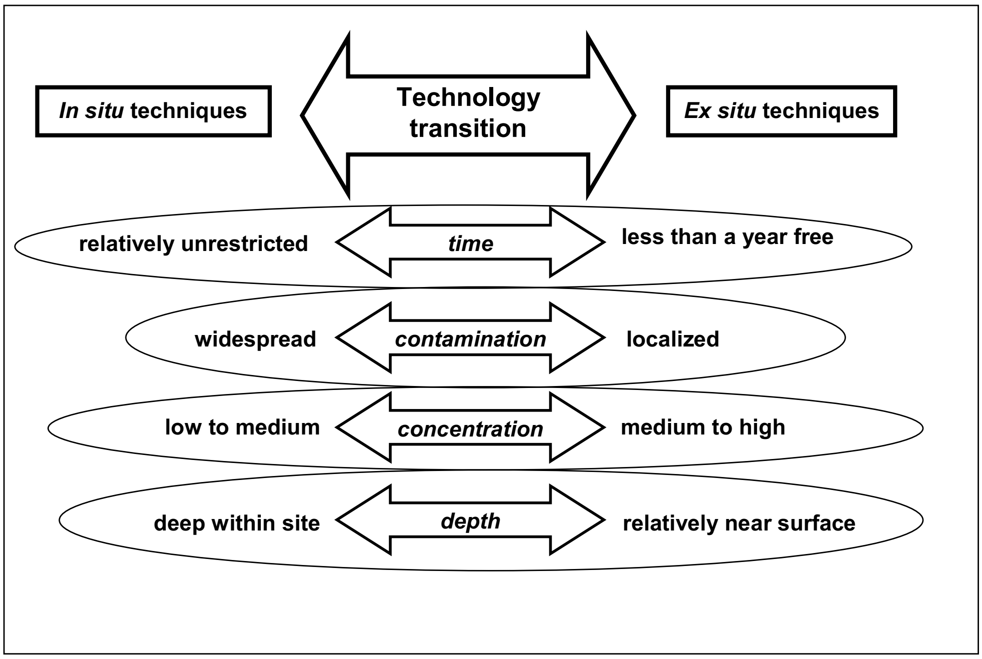


Fig. 10 Factors involved in the choice of a remediation technology.

Table 2 Environmental factors affecting biodegradation.

Parameters	Condition required for microbial activity	Optimum value for an oil degradation
Soil moisture	25-28% of water holding capacity	30-90%
Soil pH	5.5-8.8	6.5-8.0
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10-40%
Nutrient content	N and p for microbial growth	C:N:P = 100:10:1
Temperature (°C)	15-45	20-30
Contaminants	Not too toxic	Hydrocarbon 5-10% of dry weight of soil
Heavy metals	Total content 2000 ppm	700 ppm
Type of soil	Low clay or silt content	

cal processes for the treatment of hazardous wastes refer to the relatively low costs, simple and well-known technologies, potential for complete contaminant destruction (Nazaroff and Alvarez-Cohen 2001; Sasikumar and Papinazath 2003; Gavrilescu 2005).

Wastewater biotreatment

The use of microorganisms to remove contaminants from wastewater is largely dependent on wastewater source and characteristics.

Wastewater is typically categorized into one of the following groups (Wiesmann *et al.* 2007):

- municipal wastewater (domestic wastewater mixed with effluents from commercial and industrial works, pre-treated or not pre-treated)
- commercial and industrial wastewater (pre-treated or not pre-treated)
- agricultural wastewaters

The effluent components may be of chemical, physical or biological nature and they can induce an environmental impact, which includes changes in aquatic habitats and species structure as well as in biodiversity and water quality. Some characteristics of municipal and industrial wastewaters are presented in **Tables 3** and **4**.

It is evident that the quality parameters are very diverse, so that the biological wastewater treatment has to be adequate to pollution loading. Therefore, it is a difficult task to find the most appropriate microorganism consortia and treatment scheme for a certain type of wastewater, in order to remove the non-settleable colloidal solids and to degrade specific pollutants such as organic, nitrogen and phosphorus compounds, heavy metals and chlorinated compounds contained in wastewater (**Fig. 11**) (Metcalf and Eddy 1991; Bitton 2005).

Since many of these compounds are toxic to microorganisms, pretreatment may be required (Burton *et al.* 2002). Biological treatment requires that the effluents be rich in unstable organic matter, so that microbes break up these unstable organic pollutants into stable products like CO_2, CO, NH_3, CH_4, H_2S, etc. (Cheremisinoff 1996; Guest and Smith 2002; Dunn *et al.* 2003).

To an increasing extent, wastewater treatment plants have changed from "end-of-pipe" units toward module systems, most of them fully integrated into the production

Table 3 Typical characteristics of wastewater from various industries.

Process/source	**Parameters (mg/L)**														**References**
	pH	**TSS**	**BOD_5**	**COD**	**N**	**P**	**S**	**Carbohydrate**	**Acetic acid**	**Methanol**	**Cl^-**	**Na^+**	**Ca^{2+}**	**K^+**	
	Pulp and paper industry														
Thermo mechanical pulping (TMP)	4.2	810	2800	5600	12	2.3	72	2700	235	25					Pokhrel and Viraghavan 2004
Chemi-thermomechanical pulping	-	500	3000-4000	6000-9000	-	-	167	1000	1500	-					Bajpai 2000
Kraft bleaching	10.1	37-74	128-184	1124-1738	-	-	-	-	-	40-76					Bajpai 2000; Pokhrel and Viraghavan 2004
Spent liguor	-	253	13,300	39,800	86	36	315	6210	3200	90					Bajpai 2000; Das and Jain 2001
Chip wash	-	6095	12,000	20,600	86	36	315	3210	820	70					Bajpai 2000
Paper mill	-	800	1600	5020	11	0.6	97	610	54	9					Bajpai 2000; Pokhrel and Viraghavan 2004
	Pharmaceutical industry														
	3.98	407		3420		10 as PO_4^{3-}	160 as SO_4^{2-}		1,900		2800	2000	20		Sirtari *et al.* 2009
Synthetic drug plant (1)	2.3-11.1	11-126	2980-3780	5480-7465	262-512	7.95-45.8					2900-4500				Murthy *et al.* 1984
Chemical synthesis-based pharmaceutical	7-8	800-900		40,000-60,000		3-6 PO_4-P									Oktem *et al.* 2007
Synthetic drug plant (2)	7-8	7130	5900	12370	3200 as NO_3^{2-}	-	9000 as SO_4^{2-}	-	-	-	1150	-	-		
	Dairy industry														
	5.5-7.5	250-600	350-600	1500-3000											Sarkar *et al.* 2006
Cheese industry	6.2-11.3	326-3560	565-5722	785-7619		29-181						263-1265	1.4-58.5		Danalevich *et al.* 1998; Hwang and Hansen 1998
Milk processing plant	8-11	350-1100	1200-1400	2000-6000		20-50 PO_4-P						170-200	35-40	35-40	Ince 1998; Samkutti and Gough 2002
Butter/milk powder plant	5-7		1500	1908		35						560	8	13	Donkui 1997; Strydom *et al.* 1997
	Textile industry														
Textile finishing industry	8.6-8.8	250-750	150-170	1700	5-45 N-NH_4	14-30	525-590 SO_4^{2-}				1650-1750				Eremektar *et al.* 2007
Cotton textile wastewater	9.12-9.60		500-900	800-1200	7-21 NH_4-N	1.95-2.49	15-32				17750-34000				Kapdan and Alparslan 2005
Textile wastewater	10	150	170	1150			680 SO_4^{2-}				1820				Selcuk 2005

Table 4 Typical loading of municipal wastewater (Bitton 2005).

Wastewater characteristics	**Concentration (mg/L)**		
	Strong	**Medium**	**Weak**
Suspended solids	350	220	100
Total solids	1200	720	350
Biochemical Oxygen Demand (BOD_5)	400	220	110
Chemical Oxygen Demand (COD)	1000	500	250
NH_3-N	50	25	12
Total N	85	40	20
Organic N	35	15	8
Total P	15	8	4

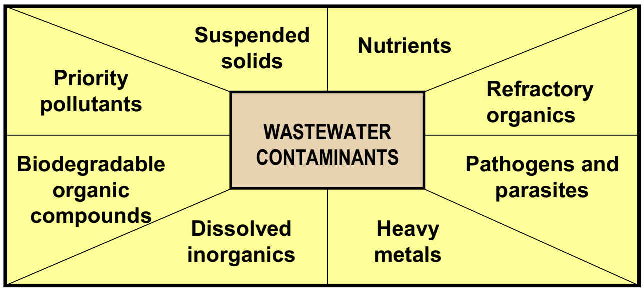


Fig. 11 Categories of contaminants in wastewater. (Adapted from Metcalf and Eddy 1991; Bitton 2005).

process (production integrate environmental protection) (Rosenwinkel *et al.* 1999).

The three major groups of biological processes: aerobic, anaerobic, combination of aerobic and anaerobic can be run in combination or in sequence to offer greater levels of treatment (Grady *et al.* 1999; Burton *et al.* 2002; Gavrilescu 2004a). The main objectives of wastewater treatment processes can be summarized as:

- reduction of biodegradable organics content (BOD_5)
- reduction/removal of recalcitrant organics
- removal of heavy/toxic metals
- removal/reduction of compounds containing p and n (nutrients)
- removal and inactivation of pathogenic microorganisms and parasites

1. Aerobic biotreatment

Aerobic processes are often used for municipal and industrial wastewater treatment.

Easily biodegradable organic matter can be treated by this system (Wagner *et al.* 2002; Doble and Kumar 2005; Gallert and Winter 2005; Russell 2006).

The basic reaction in aerobic treatment plant is represented by the reactions (1, 2):

$$Organic\ material + O_2 \xrightarrow[other\ nutrients]{cells} CO_2 + H_2O + new\ cells \quad (1)$$

Microbial cells undergo progressive auto-oxidation of the cell mass:

$$Cells + O_2 \rightarrow CO_2 + H_2O + NH_3 \quad (2)$$

Lagoons and low rate biological filters have only limited industrial applications.

The processes can be exploited as suspended (activate sludge) or attached growth (fixed film) systems (Gavrilescu and Macoveanu 1999; Grady *et al.* 1999; Gavrilescu *et al.* 2002a; Lupasteanu *et al.* 2004; Pavel *et al.* 2004) (**Fig. 12**). Aeration tanks used for the activated sludge process allows suspended growth of bacterial biomass to occur during biological (secondary) wastewater treatment, while trickling filters support attached growth of biomass (Burton *et al.* 2002; Gavrilescu and Macoveanu 2000; Gavrilescu *et al.* 2002b; Gavrilescu and Ungureanu 2002; Gallert and Winter 2005) (**Fig. 12**). Advanced types of activated sludge systems use pure oxygen instead of air and can operate at higher biomass concentration.

Biofilm reactors are applied for wastewater treatment in variants such as: trickle filters, rotating disk reactors, airlift reactors. Domestic wastewaters are usually treated by aerobic activated sludge process, since they are composed mainly of proteins (40-60%), carbohydrates (25-50%), fats and oils (10%), urea, a large number of trace refractory organics (pesticides, surfactants, phenols (Bitton 2005) (**Table 4**).

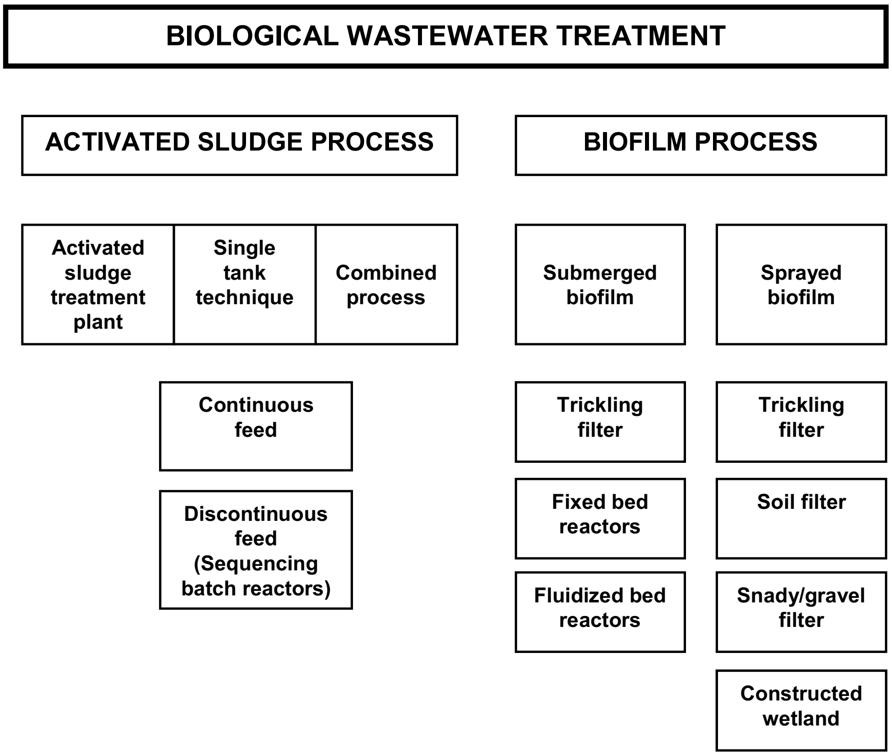


Fig. 12 Processes and equipment involved in biological wastewater treatment.

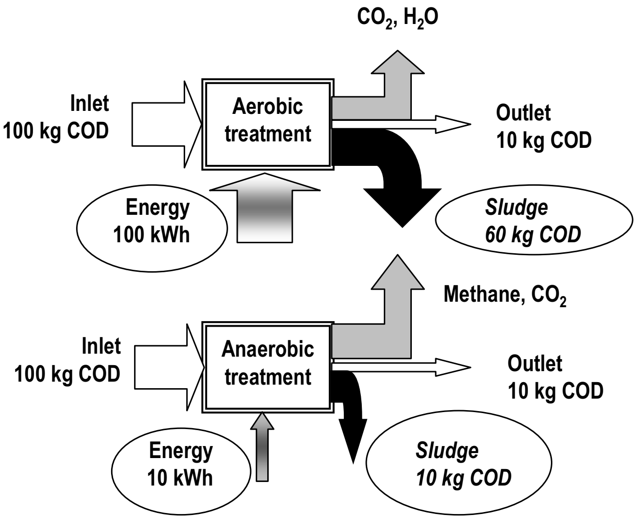


Fig. 13 Comparison of aerobic and anaerobic biological treatment. (Blonskaya and Vaalu 2006).

2. Anaerobic biotreatment

Anaerobic treatment of wastewater does not generally lead to low pollution standards, and it is often considered a pretreatment process, devoted to minimization of oxygen demand and excessive formation of sludge. Highly concentrated wastewaters should be treated anaerobically due to the possibility to recover energy as biogas and low quantity of sludge (Gallert and Winter 1999).

Research and practices have demonstrated that high loads of wastewater treated by anaerobic technologies generates low quantities of biological excess sludge with a high treatment efficiency, low capital costs, no oxygen requirements, methane production, low nutrient requirements (**Fig. 13**) (Blonskaya and Vaalu 2006).

New developments in anaerobic wastewater treatment

High rate anaerobic wastewater treatment technologies can be applied to treat dilute concentrated liquid organic wastewaters which are discharged from distilleries, breweries, paper mills, petrochemical plants etc. Even municipal wastewater can be treated using high rate anaerobic technologies. There are also a number of established and emerging technologies with various applications, such as:

- sulphate reduction for removal and recovery of heavy metals and sulphate denitrification for the removal of nitrates
- bioremediation for breakdown of toxic priority pollutants to harmless products.

Sulphate reducing process

The characteristics of some sulphur-rich wastewaters (temperature, pH, salinity) are determined by discharging process. Often, they have to meet constraints imposed by restrictive environmental regulations so that a growing interest to extend the application of sulphate reducing anaerobic reactions in conditions far from the optimal growth conditions of most bacteria is obvious (Droste 1997; Guest and Smith 2002).

The mechanism of the sulphate reduction for removal of organics, heavy metals and sulphur is illustrated by reactions (3 – 5):

$$\underset{\text{sulphate}}{SO_4^{2-}} + \underset{\substack{\text{organic}\\\text{substrate}}}{COD} \xrightarrow[\text{sulfate reducing bacteria}]{} \underset{\text{disulfide}}{HS^-} + \underset{\substack{\text{carbon}\\\text{dioxide}}}{CO_2} \qquad (3)$$

$$\underset{\text{sulfide}}{S^{2-}} + \underset{\substack{\text{heavy metal}\\\text{[soluble]}}}{M^{2+}} \longrightarrow \underset{\substack{\text{metal sulfide}\\\text{[insoluble]}}}{MS} \downarrow \qquad (4)$$

$$\underset{\text{disulfide}}{HS^-} + \underset{\text{oxygen}}{O_2} \xrightarrow[\substack{\text{chemotropic bacteria}\\\text{(eg. Thilobacillus)}}]{} \underset{\substack{\text{elemental}\\\text{sulfur}\\\text{[insoluble]}}}{S^0} \downarrow + \underset{\text{water}}{H_2O} \qquad (5)$$

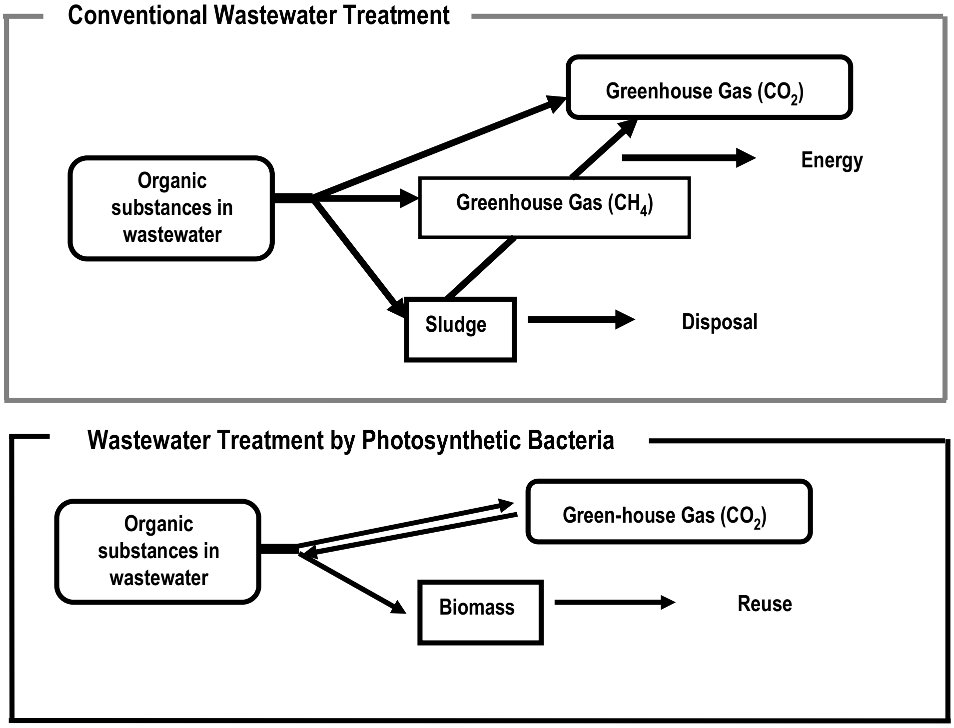


Fig. 14 Comparison of carbon conversion pathways during conventional wastewater treatment and wastewater treatment by photosynthetic bacteria (Nakajima *et al.* 2001).

Upflow anaerobic sludge blanket (UASB) reactors can be used to treat sulphur-rich wastewaters (Tuppurainen *et al.* 2002; Lens *et al.* 2004).

Wastewater treatment using purple nonsulphur bacteria, a sort of photosynthetic bacteria under light and anaerobic conditions is applied to produce a large amount of useful biomass with little carbon dioxide, one of the major greenhouse gases (**Fig. 14**) (Nakajima *et al.* 2001). The biomass of these bacteria can be utilized for agricultural and industrial purposes, such as a feed for fish and animals, fertilizers, polyhydroxyalkanoates.

3. Advanced biotreatment

Advanced wastewater biotreatment must be considered in accordance with various beneficial reuse purposes as well as the aspect of human and environmental health. This is especially important when the treated wastewater is aimed to use for the rehabilitation of urban creak and creation of water environment along it.

Membrane technology is considered one of the innovative and advanced technologies which rationally and effectively satisfy the above mentioned needs in water and wastewater treatment and reuse, since it combines biological with physical processes (Yamamoto 2001; Bitton 2005).

In combination with biological treatment, it is reasonably applied to organic wastewaters, a large part of which is biodegradable. In fact, this is the combination of a membrane process like microfiltration or ultrafiltration with a suspended growth bioreactor (Ben Aim and Semmens 2003; Bitton 2005) (**Fig. 15**).

It is widely and successfully applied in an ever increasing number of locations around the world for municipal and industrial wastewater treatment with plant sizes up to 80,000 population equivalent (Membrane Separation Activated Sludge Process, MSAS). The process efficiency is dependent on several factors, such as membrane characteristics, sludge characteristics, operating conditions (Bitton 2005; Judd 2006).

A new generation of MSAS is the submerged type where membrane modules are directly immersed in an aeration tank (**Fig. 15**). This aims to significantly reduce the energy consumption by eliminating a big circulation pump typically installed in a conventional MSAS (Judd 2006).

Membrane bioreactors (MBR) can be applied for removal of dissolved organic substances with low molecular weights, which cannot be eliminated by membrane separation alone, can be taken up, broken down and gasified by microorganisms or converted into polymers as constituents of bacterial cells, thereby raising the quality of treated water. Also, polymeric substances retained by the membranes can be broken down if they are still biodegradable, which means that there will be no endless accumulation of the substances within the treatment process. This, however, requires the balance between the production and degradation rates, because the accumulation of intermediate metabolites may decrease the microbial activities in the reactor (Yama-

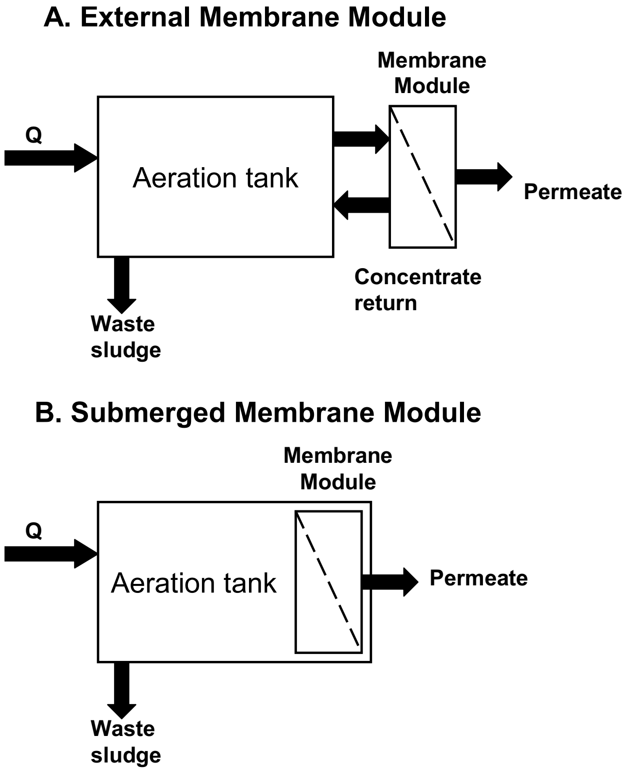


Fig. 15 Membrane bioreactors with (a) external module and (b) internal (submerged) module. (Bitton 2005; Ben Aim and Semmens 2003).

Table 6 Sustainability criteria for MBR technology (Balkema *et al.* 2002; Fane 2007).

Criteria	Indicators	Improvement needed	Applied now with good results
Economic	Cost and affordability	X	
Environmental	Effluent water quality		
	Microorganism		X
	Suspended solids		X
	Biodegradable organics		X
	Nutrient removal		X
	Chemical usage	X	
	Energy	X	
	Land use		X
Technical	Reliability		X
	Ease of use	x	
	Flexible and adaptable		X
	Small-scale systems		X
Socio-cultural	Institutional requirements	X	
	Acceptance	X	
	Epertise	X	

Table 5 Expected performance of MBR for wastewater treatment.

Wastewater loading	Expected performance
Suspended solids (SS)	Complete removal
	No influence of sludge settle ability on effluent quality
	Removal of particle-bound micropollutants
Virus, bacteria, protozoa	Reliable removal by size exclusion, retention by dynamic membrane, a high removal along with SS retention
Nitrogen	Stable nitrification due to high retention of nitrifying bacteria
	Low temperature nitrification is attained
	A high effectiveness factor in terms of nitrification due to relatively small size floc
	Endogenous denitrification is highly expected due to high concentration of biomass
Sludge stabilization	Minimize excess sludge production due to long SRT
	Sludge treatment is possible together with wastewater treatment
	Use of higher tropic level of organism is expected to control sludge
Degradation of hazardous substances	Selective growth of specific microorganisms is expected for hardly degradable hazardous substances
	Almost pure culture system is easily operated

moto 2001).

MBRs can be operated aerobically or anaerobically for organic compounds and nutrients removal.

Due to its hybrid nature, MBRs offer advantages and gain merits (**Table 5**) (Yamamoto 2001).

The technology meets water sustainability criteria, discusses by Bitton (2005) and shown in **Table 6** (Balkema *et al.* 2002; Fane 2007).

The main advantages of biological processes in comparison with chemical oxidation are: no need to separate colloids and dispersed solid particles before treatment, lower energy consumption, the use of open reactors, resulting in lower costs, and no need for waste gas treatment (Langwaldt and Puhakka 2000; Wiesmann *et al.* 2007).

4. Molecular techniques in wastewater treatment

Although molecular technique applications in wastewater biotreatment are quite new, being developed during the 1990s and not appearing to be more economically than the established technologies, major applications may include the enhancement of xenobiotics removal in wastewater treatment plants and the use of nucleic acid probes to detect pathogens and parasites (COST 624 2001; Khan *et al.* 2004; Bitton 2005; Sanz and Kochlung 2007). Among these techniques, the most interesting proved to be cloning and creation of gene library, denaturant gradient cell electrophoresis (DGGE), fluorescent *in situ* hybridization with DNA probes (FISH) (Sanz and Kochlung 2007).

Wastewater treatment processes can be improved by selection of novel microorganisms in order to perform a certain action. However, the use of DNA technology in pollution control showed to have some disadvantages and limitations (Timmis *et al.* 1994; Bitton 2005), such as: multistep pathways in xenobiotics biodegradation, limited degradation, instability of the recombinant strains of interest in the environment, public concern about deliberate or accidental release of genetic modified microorganisms etc.

5. Metals removal by microorganisms from wastewaters

Heavy metals come in wastewater treatment plants from industrial discharges, stormwater etc. Toxic metals may damage the biological treatment process, being usually inhibitory to both areobic and anaerobic processes. However, there are microorganisms with metabolic activity resulting in solubilization, precipitation, chelation, biomethylation, volatilization of heavy metals (Bremer and Geesey 1991; Bitton 2005; Gerardi 2006).

Metals from wastewater such as iron, copper, cadmium, nickel, uranium can be mostly complexed by extracellular polymers produced by several types of bacteria (*B. licheniformis*, *Zooglea ramigera*). Subsequently, metals can be accumulated and then released from biomass by acidic treatment. Nonliving immobilized bacteria, fungi, algae are able to remove heavy metals from wastewater (Eccles and Hunt 1986; Bitton 2005) (**Table 7**).

The mechanisms involved in metal removal from wastewater include (Kulbat *et al.* 2003; Bitton 2005; Gerardi 2006): adsorption to cell surface, complexation and solubilization of metals, precipitation, volatilization, intracellular accumulation of metals, redox transformation of metals, use of recombinant bacteria. For example, Cd^{2+} can be accumulated by bacteria, such as *E. coli*, *B. cereus*, fungi (*Aspergillus niger*). The hexavalent chromium (Cr^{6+}) can be reduced to trivalent chromium (Cr^{3+}) by the *Enterobacter cloacae* strain; subsequently Cr^{3+} precipitates as a metal hydroxide (Ohtake and Hardoyo 1992). Some microorganisms can also transform Hg^{2+} and several of its organic compounds (methyl mercury, ethyl mercuric phosphate) to the volatile form Hg^0, which is in fact a detoxification mechanism (Silver and Misra 1988).

The metabolic activity of some bacteria (*Aeromonas*, *Flavobacterium*) can be exploited to transform Selenium to volatile alkylselenides as a result of methylation (Bitton 2005).

Table 7 Organisms involved in metal removal/recovery from wastewaters.

Metal	Organism
Yeasts	
Cd(II)	*Saccharomyces cerevisiae*
	A. pullulans
	Cr. laurentii
	Cy. capitatum
	H. anomala
	P. fermentans
	R. rubra
	S. cerevisiae
	Sp. roseus
	S. cerevisiae entrapped in polyurethane foam
	S. cerevisiae modified by crosslinking cystine with glutaraldehyde
Cr(VI)	*S. cerevisiae*
Pb(II)	
Ni(II)	
Cr(VI)	*Candida utilis*
Cr(VI)	*S. cerevisiae*
Cr(III)	*S. cerevisiae*
Living microalgae free in solution	
Cd(II)	*Chlorella vulgaris*
	Chlorella salina
	Chlorella homosphaera
	Scenedesmus obliquus
	Chlamydomonas reinhardtii
	Asterionella formosa
	Fragilaria crotonensis
	Thalassiosira rotula
	Cricosphaere elongate
Pb(II)	*Chlorella vulgaris*
	Euglena sp.
Zn(II)	*Chlorella vulgaris*
	Chlorella regularis
	Chlorella salina
	Chlorella homosphaera
	Euglena sp.
Au(I)	*Chlorella vulgaris*
U(II)	*Chlorella regularis*
	Chlorella sp.
	Scenedesmus obliquus
	Scenedesmus sp.
	Chlamydomonas sp.
	Dunaliella tertiolecta
	Ankiistrodesmus sp., *Selenastrum* sp.
Cu(I)	*Chlorella regularis*
	Euglena sp.
	Cricosphaere elongate
Ni(I)	*Chlorella regularis*
	Thalassiosira rotula
Co(II)	*Chlorella regularis*
	Chlorella salina
Mn(II)	*Chlorella regularis*
	Chlorella salina
	Euglena sp.
Mo(I)	*Chlorella regularis*
	Scenedesmus sp.
	Chlamydomonas reinhardtii
Tc(II)	*Chlorella emersonii*
	Scenedesmus obliquus
	Chlamydomonas reinhardtii
Zr(II)	*Chlorella emersonii*
	Scenedesmus obliquus
	Chlamydomonas sp.
Hg(II)	*Chlorella* sp.
Al(III)	*Euglena* sp.

Table 7 (Cont.)

Metal	Organism
Macroalgal biomass	
Cd(II)	*Sargassum natans*
	Ascophyllum nodosum
	Halimeda opuntia
	Fucus vesiculosus
Pb(II)	*Sargassum natans*
	Sargassum fluitans
	Sargassum vulgaris
	Ascophyllum nodosum
	Palmaria palmate
	Chondrus Crispus
	Fucus vesiculosus
	Padina gymnospora
	Codium taylori
Au(I)	*Sargassum natans*
	Ascophyllum nodosum
	Palmaria palmate
	Chondrus Crispus
	Porphyra palmata
Ag(I)	*Sargassum natans*
U(II)	*Sargassum natans*
Zn(II)	*Sargassum natans*
Cu(I)	*Sargassum natans*
	Vaucheria
Co(II)	*Sargassum natans*
	Ascophyllum nodosum
	Chondrus Crispus
	Porphyra palmata
	Halimeda opuntia
Sr(II)	*Vaucheria*

Soil bioremediation

Soil biotreatment technologies use living organisms to degrade soil contaminants, either *ex situ* (*i.e.*, above ground, in another place) or *in situ* (*i.e.*, in place, in ground), and include biotreatment cells, soil piles, and prepared treatment beds (Trejo and Quintero 1999; Khan *et al.* 2004; Gavrilescu 2006).

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. Since bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. **Table 2** reviews some environmental conditions for degradation of contaminants (Vidali 2001).

Oil bioremediation is typically based on the principles of soil composting that means controlled decomposition of matter by bacteria and fungi into a humus-like product. This process can be performed in an *ex situ* system, when contaminated soils are excavated, mixed with additional soil and/or bacteria to enhance the rate of degradation, and placed in aboveground areas or treatment compartments. Another type of soil biotreatment consists of an *in situ* process, when a carbon source such as manure is added, in an active or passive procedure depending upon whether the carbon source is applied directly to the undisturbed soil surface (*i.e.*, passive) or physically mixed into the soil surface layer (*i.e.*, active).

Table 8 summarizes some of the advantages and disadvantages of soil bioremediation techniques (Vidali 2001; Gavrilescu 2006; Gavrilescu *et al.* 2008; Pavel and Gavrilescu 2008).

Both *in situ* and *ex situ* methods are commercially exploited for the cleanup of soil and the associated groundwater (Langwaldt and Puhakka 2000). The effectiveness of both alternatives is dependent upon careful monitoring and control of environmental factors such as moisture, temperature, oxygen, and pH, and the availability of a food source for the bacteria to consume (Saval 1999).

Bioremediation of land (biorestoration) is often cheaper than physical methods and its products are harmless if complete mineralization takes place. Its action can, however, be time-consuming, tying up capital and land.

Bioremediation using plants, identified as phytoremediation (**Fig. 5**) is presently used to remove metals from contaminated soils and groundwater and is being further explored for the remediation of other pollutants. Certain plants have also been found to absorb toxic metals such as mercury, lead and arsenic from polluted soils and water, and scientists are hopeful that they can be used to treat industrial waste.

Vidali (2001) described five types of phytoremediation techniques, classified based on the contaminant fate: phytoextraction, phytotransformation, phytostabilization, phyto-

Table 8 Summary of some bioremediation strategies.

Technology	Examples	Benefits	Limitations	Factors to consider
In situ	In situ bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constrains Extended treatment time Monitoring difficulties	Biodegradative abilities of indigenous microorganisms Presence of metals amd other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Landfarming Composting Biopiles	Cost efficient Low cost Can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation	See above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relatively high cost capital Relatively high operating cost	See above Bioaugmentation Toxicity of amendments Toxic concentration of contaminants
Biopiles	*ex-situ* method	sited under covered structures, bunded to manage leachate generation	the physical characteristics of biopiles are difficult to engineer	using various methods to enhance the growth and viability of the microbes
Windrows	*ex-situ* method	piles of contaminated solids, fashioned to maximise oxygen availability, covered with readily-removable structures, and bunded to manage leachate generation	the method is often preferred since ease of engineering ensures the microorganisms are in direct contact with contaminants	moisture content, nutrient levels, pH adjustment, and biological material maintenance is facilitated by recirculation of generated leachate, with any necessary supplements

Table 9 Overview of phytoremediation applications.

Technique	Plant mechanism	Surface medium
Phytoextraction	Uptake and concentration of metal via direct uptake into the plant tissue with subsequent removal of the plants	Soils
Phytotransformation	Plant uptake and degradation of organic compounds	Surface water, groundwater
Phytostabilization	Root exudates cause metal to precipitate and become less available	Soils, groundwater, mine tailing
Phytodegradation	Enhances microbial degradation in rhizosphere	Soils, groundwater within rhizosphere
Rhizofiltration	Uptake of metals into plant roots	Surface water and water pumped
Phytovolatilization	Plants evapotranspirate selenium, mercury, and volatile hydrocarbons	Soils and groundwater
Vegetative cap	Rainwater is evapotranspirated by plants to prevent leaching contaminants from disposal sites	Soils

degradation, rhizofiltration, and summarizes some phytoremediation mechanisms and applications (**Table 9**).

Together with other near-natural processes and the monitored natural attenuation procedures, sustainable strategies have to be developed to overcome the complex problems of contaminated sites (Gallert and Winter 2005).

Solid waste biotreatment

The implementation of increasingly stringent standards for the discharge of wastes into the environment, as well as the increase in cost of habitual disposal or treatment options, has motivated the development of different processes for the production of goods and for the treatment and disposal of wastes (Nicell 2003; Hamer *et al.* 2007; Mazzanti and Zoboli 2008). These processes are developed to meet one or more of the following objectives (Evans and Furlong 2003; Gavrilescu *et al.* 2005, Banks and Stentiford 2007): (1) to improve the efficiency of utilization of raw materials, thereby conserving resources and reducing costs; (2) to recycle waste streams within a given facility and to minimize the need for effluent disposal; (3) to reduce the quantity and maximize the quality of effluent waste streams that are created during production of goods; and (4) to transform wastes into marketable products.

The multitudes of ways in which the transformation of wastes and pollutants can be carried out can be classified as being chemical or biological in nature. Biotreatment can be used to detoxify process waste streams at the source – before they contaminate the environment – rather than at the point of disposal. In fact, waste represents one of the key intervention points of the potential use of environmental biotechnology (Evans and Furlong 2003).

Biowaste is generated from various anthropogenic activities (households, agriculture, horticulture, forestry, wastewater treatment plants), and can be categorized as: manures, raw plant matter, process waste. For example, in Europe, 40–60% of municipal solid wastes (MSW) consist of biowaste, most of it collected separately and used for many applications such as aerobic degradation or composting, which can provide (through anaerobic degradation or fermentation) nutrients and humus compounds for improving the soil structure and compost quality for agriculture uses provides nutrients in soil and compost for agriculture uses. The energy output is biogas, which can be used as energy source e.g. to generate electricity and heat (Fischer 2008). The potential for nutrient and humus recycling from biowaste back into the soil, via composted, digested or otherwise biologically treated material was often mentioned.

This approach involves carefully selecting organisms, known as biocatalysts, which are enzymes that degrade specific compounds, and define the conditions that accelerate the degradation process.

Biological waste treatment aims to the decomposition of biowaste by organisms in more stable, bulk-reduced material, which contributes to:

- reducing the potential for adverse effects to the environment or human health
- reclaiming valuable minerals for reuse
- generating a useful end product

Advantages of the biological treatment include: stabilization of the waste, reduced volume in the waste material, destruction of pathogens in the waste material, and production of biogas for energy use. The end products of the biological treatment can, depending on its quality, be recycled as fertilizer and soil amendment, or be disposed.

Solid waste can be treated by biochemical means, either *in situ* or *ex situ* (Doble *et al.* 2004). The treatments could be performed as *aerobic* or *anaerobic* depending on whether the process requires oxygen or not.

1. Anaerobic digestion

Anaerobic digestion of organic waste accelerates the natural decomposition of organic material without oxygen by maintaining the temperature, moisture content and pH close to their optimum values. Generated CH_4 can be used to produce heat and/or electricity (Mata-Alvarez *et al.* 2000; Salminen and Rintala 2002).

The most common applications solid-waste biotreatment include (TBV GmbH 2000):

- the anaerobic treatment of biogenic waste from human settlements
- the co-fermentation of separately collected biodegradable waste with agricultural and/or industrial solid and liquid waste
- co-fermentation of separately collected biodegradeble waste in the digesting towers of municipal waste treatment facilities
- fermentation of the residual mixed waste fraction within the scope of a mechanical-biological waste-treatment concept

Anaerobic processes consume less energy, produce low excess sludge, and maintain enclosure of odor over conventional aerobic process. This technique is also suitable when the organic content of the liquid effluent is high. The activity of anaerobic microbes can be technologically exploited under different sets of conditions and in different kinds of processes, all of which, however, rely on the exclusion of oxygen (TBV GmbH 2000).

Important characteristics and requisite specifications for classifying the various fermentation processes and essential steps in the treatment of organic waste were presented in **Table 10** (TBV GmbH 2000).

2. Composting

The biological decomposition of the organic compounds of wastes under controlled aerobic conditions by composting is largely applied for waste biotreatment.

The effective recycling of biowaste through composting or digestion can transform a potentially problematic ‘waste’ into a valuable ‘product’: *compost.* Almost any organic waste can be treated by this method (Haug 1993; Krogmann and Körner 2000; Kutzner 2000; Schuchardt 2005), which results in end products as biologically stable humus-like product for use as a soil conditioner, fertilizer, biofilter material, or fuel. Degradation of the organic compounds in waste during composting is initiated predominately by a very dissimilar community of microorganisms: bacteria, actinomyctes, and fungi.

An additional inoculum for the composting process is

Table 10 Systematic overview of fermentation processes and essential steps in the treatment of organic waste (TBV GmbH 2000).

1. Requirements concerning the composition of the input material(s)

i.e.: limits, e.g., TS content, fiber content and length, particle size, viscosity, foreign-substance content

2. Pretreatment for reducing the pollutant and inert-material contents

e.g.: manual sorting, mechanical/magnetic separation, wet processing

3. Pretreatment required for the process

e.g.: size reduction and substance exclusion: mechanical, chemical, enzymatic, thermal, bacteriological [methods, employed process additives]
TS-content range: admixture of process water
[dry/wet fermentation processes], monocharges requiring admixture of other fermentable starting materials

4. Processes

a1) Single-phase fermentation		*a2) Two-phase fermentation*			
Single-stage process	Multiple-stage process	Stationary solid phase/mobile liquid phase	Mobile solid phase/ Stationary liquid phase	Upgrading (concentration)	Downgrading (deconcentration)

b) Fermentation temperature range(s) (mesophilic/thermophilic)

c) Stirring/mixing- stirring/mixing system

d) Interstage conveyance [e.g., pump, gravimetric]

e) In-process separation of sediments/floating matter

f) Retention time(s)

g) Equipment for controlling the process milieu

h) Phase separation at the end of fermentation

5. Post-treatment processes

Secondary fermentation (e.g., time span for degree of fermentation V, time history of temperature during secondary fermentation), drying, disinfection, reduction of (nutrient) salinity, wastewater treatment

6. End product(s)

i.e.: specification according to recognized criteria
e.g., degree of fermentation, degree of hygienization, nitrate/salt content

not generally necessary, because of the high number of microorganisms in the waste itself and their short generation time. A large fraction of the degradable organic carbon (DOC) in the waste material is converted into carbon dioxide (CO_2). CH_4 is formed in anaerobic sections of the compost, but it is oxidized to a large extent in the aerobic sections of the compost. The estimated CH_4 released into the atmosphere ranges from less than 1% to a few per cent of the initial carbon content in the material (Beck-Friis 2001).

Composting can lead to waste stabilization, volume and mass reduction, drying, elimination of phytotoxic substances and undesired seeds and plant parts, and sanitation. Composting is also a method for restoration of contaminated soils.

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. In many respects, composting and digestion differ from other waste management processes in that they can be carried out at varying scales of size and complexity. Therefore, this enables regions to implement a range of different solutions: large and small-scale systems, a centralized or decentralized approach (Gilbert *et al* 2006).

3. Mechanical-biological treatment

Mechanical-biological (MB) treatment of waste is becoming popular in Europe (Steiner 2005). In MB treatment, the waste material undergoes a series of mechanical and biological operations that aim to reduce the volume of the waste as well as stabilize it to reduce emissions from final disposal.

Biotreatment of gaseous streams

In the waste gas treatments (odours and volatile organic compounds, VOC) biotechnology has been applied to find green and low cost environmental processes (Devinny *et al.* 1999; Penciu and Gavrilescu 2003; Le Cloirec *et al.* 2005).

Odorous emissions represent a serious problem related to biowaste treatment facilities as they may be a trouble to the local residents since they may result in complaints and a lack of acceptance of the facility because odours may be carried away several kilometers, depending on weather and topographical conditions (Héroux *et al.* 2004).

Table 11 shows the substances analyzed in the exhaust air of an enclosed composting facility. As can be seen from **Table 11** the exhaust air mainly contains alcohols, esters, ketones and aldehydes, as well as terpenes (Schlegelmilch *et al.* 2005). Most of them are products of biological degradation, with alcohols, esters, ketones, holding the main por-

Table 11 Chemical composition of waste gas of composting plant (Herold *et al.* 2002).

Alcohols	**Esters**	**Ketones/aldehydes**	**Terpenes**	**Others**
Ethanol	Ethylacetate	Acetone	α-Pinene	Acetic acid
Butanol(2)	Ethylpropionate	Butanone	Camphene	2-Ethylfurane
2-Me-propanol	Propylacetate	3-Me-butanal	β-Phellandrene	Toulene
n-Butanol	Ethylbutyrate	3-Me-butanone(2)	β-Pinene	Xylene
Cyclopentanol	i-Butylacetate	Pentanone(2)	β-Myrcene	Dibutylphthalate
3-Me-butanol(1)	Methylbutyrate	Me-isobutylketone	3-Carene	Bis-2-Ethylhexyl-adipinate
2-Me-butanol(1)	Propylpropionate	Hexanone(2)	Limonene	
n-Pentanol	Methylpentoate	5-Me-Hexanone(2)	Thujone	
n-Hexanol	Et-2-Me-butyrate	Benzaldehyde	Camphor	
	Propylbutyrate	Nonanal	Thymol	
	Ethylpentanoate	Decanal	Thujoprene	
	Methylhexanoate		Bornylacetate	
	Ethylhexanoate			
	Propylhexaonate			
	Ethylheptanoate			

tion (Herold *et al.* 2002; Schlegelmilch *et al.* 2005).

Biofilters are one of the main biological systems used, which work at normal operating conditions of temperature and pressure. Therefore they are relatively cheap, with high efficiencies when the waste gas is characterized by high flow and low pollutant concentration (Gavrilescu *et al.* 2005; Andres *et al.* 2006). Biological waste air treatment using biofilters and biotrickling filters was developed as a reliable and cost-effective technology for treatment of polluted air streams (Cohen 2001; Cox *et al.* 2001; Iranpour *et al.* 2002; Penciu *et al.* 2004). The biodegradation of pollutants by microorganisms leads to harmless end-products (Kennes and Thalasso 1998; Penciu and Gavrilescu 2004). Because microbial populations in biofilters and biotrickling filters generally are very diverse, these types of reactors can simultaneously remove complex mixtures of pollutants, which would otherwise require a series of alternative technologies (Deshusses 1997; Cox and Deshusses 1998; Cox and Deshusses 2001; Kennes and Veiga 2001; Shareefdeen *et al.* 2005).

Bioscrubber/biofilter combinations also proved to be an efficient system to treat odorous off-gases from composting processes. Results revealed that the main part of the odour load was degraded within the biofilter (Schlegelmilch *et al.* 2005).

Biodegradation of hydrocarbons

Hydrocarbons can generate significant pollution because they are among the most common contaminants of groundwater, soil and sea when oil is spilled (Mohn 1997; Stapleton *et al.* 1998). The damage caused by oil spills in marine or freshwater systems is usually caused by the water-in-oil emulsion.

Various types of microorganisms can degrade hydrocarbons: bacteria, yeasts, filamentous fungi, but none of them degrade all of the possible hydrocarbon molecules at the same rate. Each organism may have a different spectrum of activity and a definite preferential use of certain chain lengths hydrocarbon structures.

Almost all petroleum hydrocarbons can be oxidized to mainly water and carbon dioxide, but the rate at which the process takes place is dependent on their nature, amount and the physical and chemical properties that influence their persistence and biodegradability (Atlas 1981; Leahy and Colwell 1990; EIBE 2000; Baheri and Meysami 2002; Torkian *et al.* 2003). Hydrocarbons are subject to both aerobic and anaerobic oxidation. Usually, the first stage of biodegradation of insoluble hydrocarbons is predominantly aerobic, while the organic carbon content is reduced by the action of anaerobic organisms. **Table 12** presents some groups of microorganisms that can degrade various hydrocarbons, while in **Table 13** the adequacy of aerobic or anaerobic degradation is done according to various types of contaminants from petroleum derivatives.

The prevailing environmental factors and the types, numbers and capabilities of the microorganisms present affect the biodegradation occurrence and rate. Factors affecting hydrocarbon biodegradation in contaminated soils can be: the occurrence of optimal environmental conditions to stimulate biodegradative activity; the predominant hydrocarbon types in the contaminated matrix; the bioavailability of the contaminants to microorganisms; dispersion and emulsification enhancing rates in aquatic systems and absorption by soil particulates (Leahy and Colwell 1990; Kastner *et al.* 1998; Marques-Rocha *et al.* 2000).

Hydrocarbons have different solubility in water where they are only degraded. Due to different hydrophobicity and low solubility in water of the hydrocarbons, the process should be intensified by enhancing physical contact between microorganisms and oil by adding adjuvants to improve the contact areas or by injecting of mixtures of microorganisms, during the so-called *bioaugmentation* (Baheri and Meysami 2002; Baptista *et al*. 2006; Malina and Zawierucha 2007).

It is also known that the activity of bacteria and fungi able to oxidize hydrocarbons could be improved by supplementation with various nutrients (sources of nitrogen and phosphorous). Different organisms need different types of nutrients. *Bioenhancement* is applied to stimulate the activity of bacteria already present in the soil at a waste site by adding different nutrients (Baheri and Meysami 2002; Gupta and Seagren 2005).

Biosorption

Biosorption is a fast and reversible process for the removal of toxic metal ions from wastewater by live or dried biomass, which resembles adsorption and in some cases ion exchange (Volesky 1990; Volesky *et al.* 1993; Seidel *et al.* 2002; Gavrilescu 2004a). The biosorption offers an alternative to the remediation of industrial effluents as well as the recovery of metals contained in other media.

Biosorbents are prepared from naturally abundant and/or waste biomass. Due to the high uptake capacity and very cost-effective source of the raw material, biosorption is a progression towards a perspective method. It has been demonstrated that both living and non-living biomass may be utilized in biosorptive processes, as they often exhibit a marked tolerance towards metals and other adverse conditions (Brinza and Gavrilescu 2003; Gavrilescu 2004a, 2005;

Table 12 Degradation of petroleum compounds and fuel components by different groups of microorganisms (Riser-Roberts 1998).

Microorganism	Compound
Yeasts	
Thrichosporon, *Pichia rhodosporidium*, *Rhodotorula*, *Debraryomyces*, *Endomycopsis*, *Candida parapsilasis*, *C. tropicalis*, *C. guilliermondii*, *C. lipolytica*, *C. maltosa*, *Debaramyces hansenii*, *Trichosporon* sp., *Rhodosporium taruloidles*	Hexadecane and kerosene (naphthalene, biphenyl, benzo(a)pyrene)
Actinomycetes	
Nocardia spp.	*n*-Paraffins: pentane, hexane, heptane, octane, 2-methylbutane, 2-methylpentane, 3-methylpentane, 2,2,4-trimethylpentane, ethylbenzene, hexadecane, kerosene
Algae	
Selanastrum capricornatum	Benzene, toluene, naphthalene, phenanthrene, pyrene
Cyanobacteria (blue-green algae)	Benzene, toluene, naphthalene, phenanthrene, pyrene
Microcystis aeruginosa	
Mixed cultures (yeasts, molds, protozoa, bacteria; activated sludge)	Acrylonitrile
Activated sludge	Dibenzanthracene
Sewage sludge	Fluoranthene
Acinetobacter calcoaceticus	Petroleum derivates
Strains of *Pseudomonas putida*	Phenol cresols
Trichosporon pullulans	Paraffins
Aeromonium sp.	Total petroleum hydrocarbons
Mycobacterium sp.	*n*-Undecane

Table 13 Some contaminants as petroleum derivatives removable through bioremediation (Vidali 2001).

Contaminants		Biotreatment		Potential sources
Class	**Examples**	**Aerobic**	**Anaerobic**	
Chlorinated solvents	Trichloroethylene Perchloroethylene		in situ bioremediation - reductive dechloration with fresh cheese whey as a substrate	Drycleaners Chemical manufacture
Polychlorinated biphenyls	4-Chlorobiphenyl 4,4-Dichlorobiphenyl		yes	Electrical manufacturing Power station Railway yards
Chlorinated phenols	Pentachlorophenol Trichlorophenol Tetrachlorophenol		yes	Timber treatment Landfills
BTEX	Benzene Toluene Ethylbenzene Xylene	*in situ* aerobic biodegradation - indigenous soil bacteria respiration activity stimulated with air input (venting, air sparging) and nutirent solution delivery in-situ bioremediation (i.e. aerobic enhancement by fertilizer and nutrient addition plus application of chosen allochthonous bacterial strains)	yes	Oil production and storage Gas work sites Airports Paint manufacture Port facilities Railway yards Chemical manufacture
Polyaromatic hydrocarbons (PAHs)	Naphthalene Antracene Fluorene Pyrene Benzo(a)pyrene	yes		Oil production and storage Gas work sites Coke plants Engine works Landfills Tar production and storage Boiler ash dump sites Power stations

Kicsi *et al.* 2006a, 2006b; Brinza *et al.* 2007).

Metal ions can bind to cells by different physiochemical mechanisms, depending on the bacterial strain and environmental conditions (**Fig. 7**). Because of this variability, current knowledge of these processes is incomplete. In general, bacterial cell walls are polyelectrolytes and interact with ions in solution so as to maintain electroneutrality. The mechanisms by which metal ions bind onto the cell surface most likely include electrostatic interactions, van der Waals forces, covalent bonding, redox interactions, and extracellular precipitation, or some combination of these processes (Blanco 2000; Gavrilescu 2004a).

Biosorption of heavy metals by algal biomass is an advantageous alternative, an appropriate and economically feasible method used for wastewater and waste clean up, because it uses algal biomass sometimes considered waste from some biotechnological processes (Sandau *et al.* 1996; Feng and Aldrich, 2004; Vilar *et al.* 2007) or simply its high availability in costal areas makes it suitable for developing new by-products for wastewater treatment plants (Sandau *et al.* 1996; Brinza *et al.* 2005a, 2005b; Brinza *et al.* 2007).

Biodegradation of refractory pollutants and waste

The biodegradability of refractory pollutants was investigated and applied by numerous researchers, since this becomes more and more a stringent problem of the environment because of previous or current pollution.

1. Cyanide removal

Effluents containing cyanide from various industries must be treated before discharging into the environment. The conventional physico-chemical processes for removal of cyanides from wastewater proved to present advantages, but also disadvantages burdened with high reagent and liability costs. Bioremoval/biotreatment was seen as an environmentally friendly alternative treatment process able to achieve high degradation efficiency at low costs (Campos *et al.* 2006; Dash *et al.* 2008; Chen *et al.* 2008; Dash *et al.* 2009). In biological treatment of cyanide, bacteria convert free and metal-complex cyanides to bicarbonate and ammonia. The free metals are further adsorbed or precipitated from solution. The microorganisms responsible for cyanide degradation could be bacteria or fungi, which use cyanide as a source of nitrogen and carbon (**Table 14**).

2. Distillery spent wash

This is a liquid waste generated during alcohol production, which confers unpleasant odors for wastewater, posing a serious threat to water quality. Disposal of distillery spent wash on land is moreover hazardous to the vegetation, since it reduces soil alkalinity and manganese availability, thus inhibiting seed regeneration (Kumar *et al.* 1997; Mohana *et al.* 2009).

A number of cleanup technologies are used to process this effluent efficiently and economically and novel bioremediation approaches for treatment of distillery spent wash are being worked out (**Table 14**).

3. Radionuclides

Radionuclide like uranium or thorium are of particular concern in environmental impact and remediation researches due to their high toxicity and long half-lives, thus they are considered severe ecological and public health hazards (Gavrilescu *et al.* 2008; Kazi *et al.* 2008) (**Table 14**).

Biosorptive accumulation of uranium and other radionuclides is of great interest for the development of microbe-based bioremediation strategies (Kazi *et al.* 2008).

4. Heavy metals

The application of biotechnological processes for the effective removal of heavy metals from contaminated wastewaters has emerged as an alternative to conventional remediation techniques. Heavy metal pollution is usually generated from electroplating, plastics manufacturing, fertilizers, pigments, mining, and metalurgical processes (Gavrilescu 2004b; Zamboulis *et al.* 2004).

The application of conventional treatments is sometimes restricted due to technological and economical constraints.

Metal accumulation on biomass can be passive (biosorptive), when non-living biomass is used as biosorbent, or

Table 14 Removal methods for some refractory pollutants and waste.

Compounds	**Removal method**	**Advantages**	**Disadvantages**	**References**
Cyanide	Biological oxidation/ biodegradation - hydrolytic reactions - oxidative reactions - reductive reactions - substitution/transfer reactions	Natural approach, received well by public and by regulators Use heaps as reactors, reducing total washed volume, and possible reach low flow areas of the heap more effectively Relatively inexpensive No chemical handling equipment or expensive control needed Biomass can be activated by aeration No toxic by-products Can treat cyanides without generating another waste stream	Innovative technology not well established Tends to be very site specific with specific evaluation and study required for each type of compound and site Cannot treat high concentration	Patil and Pakniar 2000 Campos *et al.* 2006 Chen et al. 2008 Dash *et al.* 2008 Dash *et al.* 2009
Distillery spent wash	Biodegradation: - Anaerobic systems • single phase, biphasic system • anaerobic lagoons • high rate anaerobic reactors - Aerobic systems (may follow the anaerobic treatment) • fungal systems • bacterial systems • cyanobacterial/algal systems • phytoremediation/constructed wetlands	Biomethanation of distillery spent wash is a well established technology Biological aerobic treatment employing fungi and bacteria is very effective for the decolorization of distillery spent wash	Research on advanced anaerobic treatment technologies are further necessary to bring into practice outstanding technologies for ecological restoration Aerobic treatment needs to be implemented with additional nutrients as well as diluting the effluent for obtaining optimal microbial activity Needs to be sometimes combined sequentially with physico-chemical treatment	Kumar *et al.* 1997 Fitzgibbon *et* al. 2007 Kumar *et al.* 2007 Mohana *et al.* 2009 Satyawali and Balakrishanan 2008 Mohana *et al.* 2009
Radionuclides (Uranium, Thorium)	Biosorption/microbe based immobilization-sequestration		Innovative/emerging technology, still to be studied in more details	Gavrilescu *et al.* 2008
Heavy metals	Biosorption using biomaterials, bacteria, fungi, yeasts, algae, natural materials, industrial and agricultural waste	Cost-effective biotechnology for the treatment of high volume and low concentration complex wastewaters (1-100 mg/L) Microorganisms provide a large contact area that can interact with metal	Biosorption is basically at lab scale in spite of its development for years The mechanism is not fully understood and shortcomings of biosorption technology limit application	Beolcini 1977 Gavrilescu 2004 Zouboulis *et al.* 2004 Wang and Chen 2006
Gasoline, ethers, benzene, toluene, n-hexane, methyl-cyclopentane, mtthyl tert-butyl ether (MTBE)	Anaerobic biodegradation using electron acceptors (nitrate, FeIII, sulfate, bicarbonate) Aerobic biodegradation of MTBE combined with another carbon source (tertiary buthanol, buthyl formate, isopropanol, acetone, pyruvate) (mixed and pure cultures)	Cost effective and feasible Environmentally friendly process Simpler, less expensive alternative to chemical and physical processes	Aerobic biodegradation of MTBE is still a rare occurrence because pf the difficulty of organisms to biodegrade MTBE Culture composition and reactor configuration are key factors	Fayolle *et al.* 2003 Lin *et al.* 2007 Raynal and Pruden 2008 Waul *et al.* 2009
Polychlorinated biphenyls	Aerobic biofilm developed using mixed microbial culture isolated from PCB-contaminated soil, acclimatized to PCBs by feeding the reactor alternately with biphenyl and PCBs		Accumulation of chlorobenzoic acids and chlorophenylglyoxylic acid in the environment	Sayler *et al.* 1982 Borja *et al.* 2006
Trichloroethylene (TCE)	Anaerobically (TCE acts as an electron acceptor in reductive dehalogenation by methanotropic organisms) Aerobic biodegradation using inducers for cometabolism and enzyme production (as toluene) and electron acceptors (hydrogen peroxide)	Anaerobic bioremediation where electron acceptors, others than oxygen are needed to be used is a potential advantage Degradation efficiency higher than 80% for TCE concentrations up to 700 mg/L Mixed cultures are generally preferred	The rates of TCE removal depend on the conditions, reactors, electron acceptors The effect of biostimulation of multiple groups of bacteria on TCE metabolism not entirely known	Wilson and Wilson 1985 Lee *et al.* 1998 Lyew and Guiot 2003 Cutright and Meza 2007 Shukla *et al.* 2009
Textile azodyes	Anaerobic treatment (white rot fungi, due to extracellular enzymes they produce) Aerobically, by using bacterial consortia, actinomycetes, fungi, algae	Inexpensive, eco-friendly, produces less amount of sludge comparative to physico-chemical methods Aerobic treatment is safer because toxic intermediates do not appear	The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms Individual bacteria strain usually cannot degrade azo dyes completely and the intermediate products are often carcinogenic and mutagenic aromate amines The decolorization rate depends on the oxidation potential of the azo dyes	Lopez *et al.* 2004 Senan and Abraham 2004 Steffan *et al.* 2005 Joshi *et al.* 2008 Saratale *et al.* 2009

bioaccumulative, by applying living cells (Veglio *et al.* 1996; Zamboulis *et al.* 2002; Zamboulis *et al.* 2004) (**Table 14**).

5. Gasoline ethers, methyl tert-butyl ether (MTBE)

The contamination of methyl tert-butyl ether (MTBE) in water and especially in underground water has become a problem of great concern all over the world (Fiorenza and Rifai 2003; Lin *et al.* 2007; Zhong *et al.* 2007). The massive production of MTBE, a primary constituent of reformulated gasoline, combined with its mobility, persistence and toxicity, makes it an important pollutant.

Some studies of MTBE natural attenuation have attributed mass loss to biodegradation, while others attributed mass loss to dilution and dispersion (Fiorenza and Rifai 2003). MTBE degradation is known to be difficult in natural environments (Martienssen *et al.* 2006). Currently, there are few reports in the literature which have documented anaerobic degradation of gasoline oxygenates (Fiorenza and Rifai 2003; Waul *et al.* 2009). In parallel, aerobic degradation of MTBE and similar compunds was also demonstrated with both mixed and pure cultures (Zanardini *et al.* 2002; Fiorenze and Rifai 2003; Zhong *et al.* 2007) (**Table 14**). It was demonstrated that mixed cultures are generally more effective than pure cultures. Supplements with readily metabolizable organic substrates were investigated to increase the biomass and enhance degradation of MTBE (Martienssen *et al.* 2006; Zhong *et al.* 2007) (**Table 14**).

6. Trichloroethylene (TCE)

Pollutants including haloalkenes (as trichloroethylene) enter into the biosphere and contaminate the soil and groundwaters. Trichloroethylene is one of the most important volatile chlorinated organic compounds used as solvent in various industries (Lyew and Goniat 2003; Shukla *et al.* 2009).

It is generally resistant to biodegradation, as microorganisms do not use it as a carbon and energy source (Wilson and Wilson 1985; Shukla *et al.* 2009).

Aerobic bacterial cultures that utilize various carbon and energy sources can be used (Ferhan 2003). Also, anaerobic bioremediation can be applied for TCE biodegradetion at higher TCE metabolic rates under mixed electron acceptor conditions (Boopathy and Peters 2001). The mixed population of microorganisms with the ability to degrade various organic compounds such as TCE may follow diverse metabolic ways and physiological characteristics depending on working conditions (Cutrught and Meza 2007).

7. Textile azo dyes

Azo dyes are used for numerous textile dyestuff, produced because of their cost-effective synthesis and their stability and variety of colors compared to natural dyes. Also, azo dyes are used in paper, food, leather, cosmetics, pharmaceutical industries (Chang *et al.* 2001; Saratale *et al.* 2009).

Bacteria, fungi, yeasts, actinomycetes, algae are able to degrade azo dyes, by a mechanism which involves the reductive breaking of azo bonds. The process can be carried out in anaerobic conditions with the help of azoreductaze. The resulting intermediate metabolites can be further degraded aerobically or anaerobically (Chang *et al.* 2000; Rarshetti *et al.* 2007; Saratale *et al.* 2009). Microbial degradation of azo dyes usually starts in anaerobic conditions with a reductive cleavage of the azo bond, followed by an aerobic step necessary for the degradation of the aromatic amines formed (Steffan *et al.* 2005; Joshi *et al.* 2008; Saratale *et al.* 2009) (**Table 14**).

ENVIRONMENTAL BIOTECHNOLOGY IN POLLUTION DETECTION AND MONITORING

Environmental monitoring deals with the assessment of environmental quality, essentially by measuring a set of selected parameters on a regular basis. In general, two methods – physicochemical and biological – are available for measuring and quantifying the extent of pollution (Jamil 2001; Lam and Gray 2003; Hagger *et al.* 2006; Hart and Martínez 2006; Conti 2007).

In the past decades environmental monitoring programmes concentrated on the measurement of physical and chemical variables, while biological variables were occasionally incorporated. Physicochemical methods involve the use of analytical equipment, having as limitations their cost (because of the complexity of the samples and the expertise of the operators needed to conduct the analysis) and the lack of hazard and toxicological information (Cannons and Harwood 2004; Gu *et al.* 2004).

Environmental monitoring is of great importance for its protection. The harmful effect of toxic chemicals on natural ecosystems has led to an increasing demand for early-warning systems to detect those toxicants at very low concentrations levels (Durrieu *et al.* 2006).

Typically contaminant monitoring involves the regular and frequent measurement of various chemicals in water, soil, sediment and air over a fixed time period, e.g., a year.

Integration of environmental biotechnology with information technology has revolutioned the capacity to monitor and control processes at molecular levels “in order to achieve real-time information and computational analysis in complex environmental systems” (Hasim and Ujang 2004).

Bioindicators/biomarkers

More recently, environmental monitoring programmes have, apart from chemical measurements in physical compartments, included the determination of contaminant levels in biota, as well as the assessment of various responses/parameters of biological/ecological systems. Nowadays, temporal and spatial changes in selected biological systems/parameters can and are used to reflect changes in environmental quality/conditions through *biomonitoring* (Market *et al.* 2003; Conti 2007; Lam 2009).

In this context, some organisms or communities may react to an environmental effect by changing a measurable biological function and/or their chemical composition. This way it is possible to infer significant environmental change and their responses are referred to as *bioindicators/biomarkers* (NRC 1987; Jamil 2001; Market *et al.* 2003; Conti 2007). Biomarkers are thus used in biomonitoring programmes to give biological information, i.e. the effects of pollutants on living organisms. Three main types of indications can be obtained: on exposure, effect, and susceptibility.

Biomarkers that have potential for use in biomonitoring are:

- *molecular* (gene expression, DNA integrity)
- *biochemical* (enzymatic, specific proteins or indicator compounds)
- *histo-cytopathological* (cytological, histopathological)
- *physiological*
- *behavioural*

Unfortunately, field application of biomarkers is subject to various constraints (e.g., the availability of living material) that can limit data acquisition and prevent the use of multivariate methods during statistical analysis. Besides, they should have the following attributes: be sensitive (so that it can act as an early-warning), specific (either to a single compound or a class of compounds), broad applicable, easy to use, reliable and robust, good for quality control, able to be readily taught to the personnel, provide the data and information necessary (Beliaeff and Burgeot 2002; Lam 2009).

Biosensors for environmental monitoring

Research on biosensing techniques and devices for environment, together with that in genetic engineering for sensor cell development have expanded in the latest time.

Environmental biosensors are analytical devices composed of a biological sensing element or biomarker (enzyme, receptor antibody or DNA) in intimate contact with a physical transducer (optical, mass or electrochemical), which together relate the concentration of an analyte to a measurable electrical signal (Reis and Hartmeier 1999; Rodríguez-Mozaz *et al.* 2004).

The biosensors exploit biological specificity to produce signals that can be used to measure pollution levels. Generally speaking, *biosensor* is a broad term that refers to any system that detects the presence of a substrate by use of a biological component which then provides a signal that can be quantified. The signal may be electrical (**Fig. 16**), or in the form of a dye that changes colour. They comprise a biological recognition element such as an enzyme, antibody or cell that will react with the material to be detected.

Biosensors based on a combination of a biological sensing element and an electronic signal-transducing element that offer high selectivity, high sensitivity, short-response time, portability and low cost, are ideal for monitoring pollutants in environment (Lam and Gray 2003; Rodríguez-Mozaz *et al.* 2006). As it can be seen from **Table 15**, various biological reactions can be used for pollutant detection. Biosensors use both protein (enzyme, metal-binding protein and antibody)-based and whole-cell (natural and genetically engineered microorganisms)-based approaches **Table 15**, In fact, biosensors represent a synergistic combination of biotechnology and microelectronics (Verma and Singh 2005).

They have found a place in monitoring for evaluation of a sample and its ecological toxicity. The sensing element can be enzymes, antibodies (as in immunosensors), DNA, or microorganisms; and the transducer may be electrochemical, optical, or acoustic (Biotech, 2000) (**Fig. 17**).

Use of biosensors enables repeated measurements with the same recognition element and can be applied to a wide range of environmental pollutants as well as biological products (**Fig. 16**). The biocatalyst (3) converts the substrate to product. This reaction is determined by the transducer (5) which converts it to an electrical signal. The output from the transducer is amplified (6), processed (7) and displayed (8).

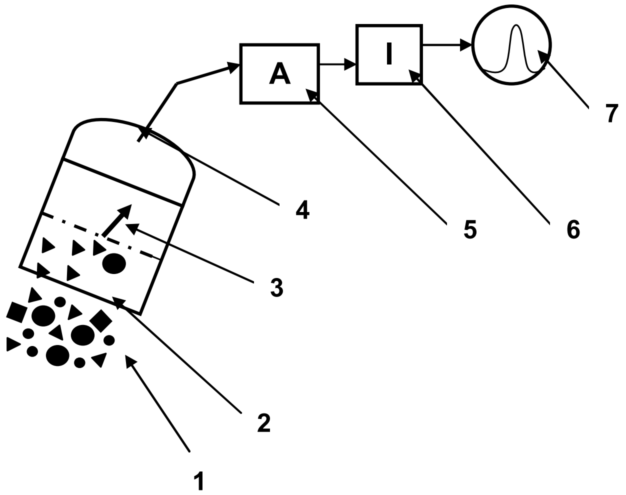


Fig. 16 Detection chain for a biosensor (a biological sensing element and an electronical signal-transducing element). 1 – substrate; 2 – membrane; 3 – immobilized biodetector for recognition of a system of biological origin like enzymes, antibodies, microorganisms; 4 – product resulted from the reaction of substrate with the biodetector; 5 – transducer (detects the product and converts it in an electrical signal); 6 – amplifier; 7 – interface for signal processing; 8 – displayer of output signal. (Adapted from Mulchandani and Rogers 1998).

Whole-cell biosensors based either on chlorophyll fluorescence or enzyme (phosphatase and esterase) inhibition are constructed for real-time detection and on-line monitoring.

A genetically modified yeast was used as biosensor to detect endocrine disruptors such as oestrogen or 17β-oestradiol. Although it was initially developed for use in human therapeutics, there is the potential use in pollution detection (Tucker and Fields 2001; Evans and Furlong 2003).

A variety of whole-cell-based biosensors has been developed using numerous native and recombinant biosensing cells. These biosensors utilizing microorganisms address and overcome many of the concerns which arose with other conventional methods, because they are usually cheap and

Table 15 Some biosensors for detection of environmental pollution.

Principle mode of detection	Pollutants detected	References
Hydrothermally grown ZnO nanorod/nanotube and metal binding peptide	Heavy metals	Jia *et al.* 2007
Protein based: Synthetic phytochelatins	Heavy metals (Hg^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+})	Bontidean *et al.* 2003
Chloroplast D1 protein	Herbicide	Piletska *et al.* 2006
Enzymes immobilized by electropolymer*i*zation	Heavy metals (Hg^{2+}: an established glucose biosensor based on glucose oxidase immobilized in poly-*o*-phenylendiamine)	Maliteste and Guasceto 2005
Enzymatic reaction or microbial metabolism	Pesticides, phenols, halogenated hydrocarbons	Riedel *et al.* 1991 Rogers 1995
Recombinant bioluminescent bacteria	Organic compounds (in air, water, soil), heavy metals	Hyun *et al.* 1993 Tescione and Belfort 1993 Gu 2005
Enzyme inhibition	Pesticides, heavy metals, herbicides	Marti *et al.* 1993 Botrè *et al.* 2000 Kuswandi and Mascini 2005
Photosynthetic activity	Herbicides	Durrieu *et al.* 2006 Giardi *et al.* 2007 Wang *et al.* 2007 Campàs *et al.* 2008
Molecularly imprinted membranes	Pesticides	Scheller *et al.* 1997 Haupt and Mosbach 2000 Uludağ *et al.* 2007 Vo-Dinh 2007
Immunochemistry	Organic compounds, pesticides, herbicides, PCBs	Chemnitius *et al.* 1996 Marty *et al.* 1998 Ashley *et al.* 2008

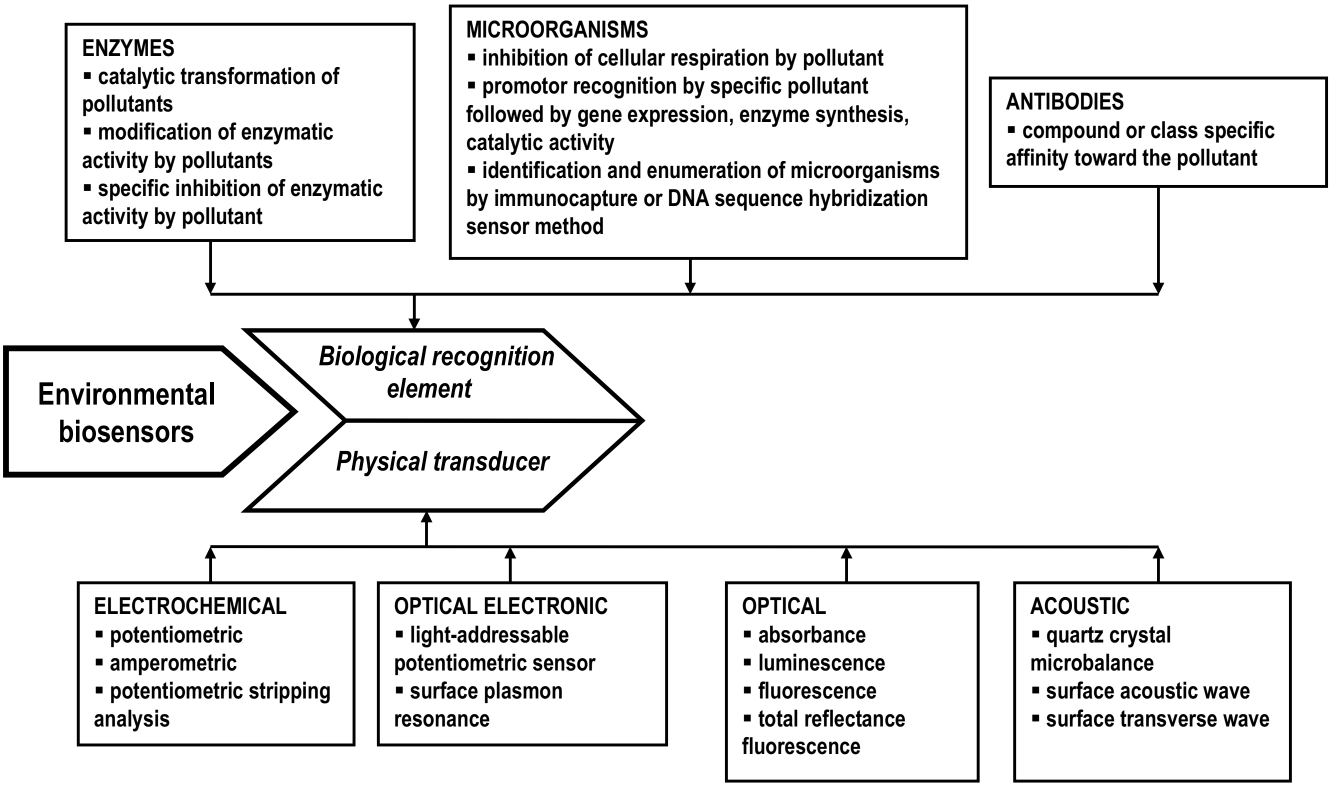


Fig. 17 Structure of environmental biosensors. (Adapted from Mulchandani and Rogers 1998; Rodriguez-Mozaz *et al.* 2004, 2006).

easy to maintain while offering a sensitive response to the toxicity of a sample (Gu *et al.* 2004). Results show that these devices are sensitive to heavy metals and pesticides (Durrieu *et al.* 2006; Mauritz *et al.* 2006).

A very high selective and sensitive sensor was developed as a "microchip" by combining biological activity with nanowire electronics (Cui *et al.* 2001), which is able to detect an electric current equivalent to the binding of a single molecule (Evans and Furlong 2003).

Plants are also used as biological indicators, namely sensitive and resistant white clover (*Trifolium repens*) clones (as descriptors of biomass reduction in crops species) and *Centaurea jacea* (brown knapweed) as a model species, the leaves of *Brassica oleracea* var. *acephala*, used as biosampler, common species of trees (wild olive, holm oak, white poplar) (Bargagli 1998; Mertens *et al.* 2005; Madejon *et al.* 2006; Nali *et al.* 2006; Zelano *et al.* 2006).

Invertebrate species (target and non-target insects), crustaceans can be also used for biomonitoring (Lagadic *et al.* 2004; Raeymaekers 2006).

Biosensors can be applied for:

- *toxicity screening of samples* using bioluminescence or fluorescence (Rabbow *et al.* 2002; Weitz *et al.* 2002; Gu *et al.* 2004; Rodriguez-Mozaz *et al.* 2004)
- *water quality monitoring* (Ramsden 1999; Ashbolt *et al.* 2001; Cannons and Harwood 2004; Starodub *et al.* 2005; Mauritz *et al.* 2006; Mwinyihija *et al.* 2006)
- *atmospheric quality biomonitoring* (Nali *et al.* 2006; Zelano *et al.* 2006)
- *soil-contamination biomonitoring* (Doran and Parkin 1994; Tom-Petersen *et al.* 2003; Gu *et al.* 2004; Ahn *et al.* 2005; Tarazona *et al.* 2005).

ENVIRONMENTAL BIOTECHNOLOGY FOR POLLUTION PREVENTION AND CLEANER PRODUCTION

Role of biotechnology in integrated environmental protection approach

Biotechnology is regarded as the motor for integrated environmental protection. Complementary to pollution control which struggles for the tail end of the processes and manages pollution once it has been generated, pollution prevention works to stop pollution at its source by applying a number of practices, such as:

- using more efficient raw materials
- substituting less harmful substances for hazardous materials
- eliminating toxic substances from production process
- changing processes
- others

The strengthening of concerns for the global environment is resulting in increased pressure for economical branches (industry, agriculture, transport, market) to focus on pollution prevention rather than end-of-pipe cleanup. From an overall material consumption perspective, excessive quantities of waste in society result from inefficient production processes (on the industrial side), and unsustainable consumption patterns combined with low sustainability of goods (on the consumer side) (Cheremisinoff 2003; Gavrilescu 2004b; Gavrilescu and Nicu 2005). Modern environmental protection starts with the prevention of harmful substances prior to and during industrial production processes. Doble and Kruthiventi (2007) have characterized an ideal process as follows: *an ideal process is simple, requires one step, is safe, uses renewable resources, is environmentally acceptable, has total yield, produces zero waste, is atom-efficient, and consists of simple separation steps* (**Fig. 18**).

Since biotechnology can contribute to the elimination of hazardous pollutants at their source before they enter the environment, industrial and environmental biotechnology - biotech's *third wave* - uses biological processes to make industrially useful products in a more efficient, environmentally friendly way, by cutting waste byproducts, air emissions, energy consumption and toxic chemicals in several industries (Bull 1995; Olguin 1999; Gavrilescu and Chisti 2005).

Although environmental biotechnology has primarily focused on the development of technologies to treat aqueous, solid and gaseous wastes at present, the basic information on how "biotechnology can handle these wastes has

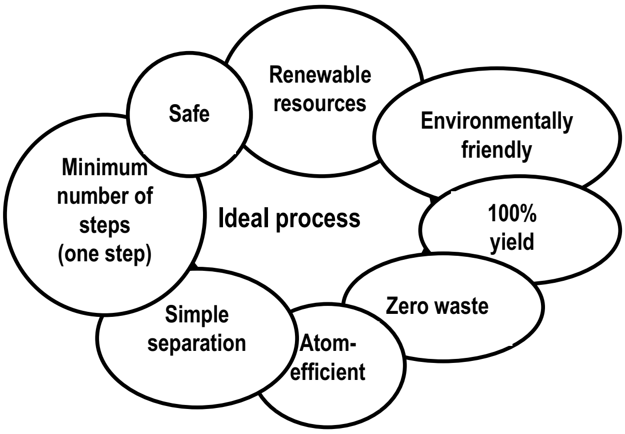


Fig. 18 Criteria for an ideal production process.

been gained and the focal point is now on the implementation of these processes as Best Available Technology Not Entailing Excessive Costs (BATNEEC) in the framework of strict and transparent environmental legislation" (Grommen and Verstraete 2002).

The application of biotechnology as an environmentally friendly alternative in conventional manufacturing proves to be very useful for pollution prevention through source reduction, waste minimization, recycling and reuse. In most cases, this results in lower production costs, less pollution and resource conservation and may be considered as task force of biotechnology for sustainability in industrial development. The main areas in which biotechnology contribution may be relevant fall into three broad categories (Evans and Furlong 2003): process changes, biological control, biosubstitutions.

Because biotechnological processes, once set up are considered cheaper than traditional methods, changes in production processes will not only contribute to environmental protection, but also help companies save money and continuously improve their public image (Olguin 1999; Evans and Furlong 2003; Gavrilescu and Nicu 2005; Willke *et al.* 2006).

In the context of pollution prevention practices, biotechnology can contribute to substitute multistep chemical processes with a one-step biological process using genetically modified organisms (GMOs) as well (Reis *et al.* 2006). This action should have other beneficial results because land disposal of hazardous waste, wastewater loadings, air emissions and production costs are greatly reduced. Also, prevention practices assisted by environmental biotechnology may prove instrumental in permitting procedural changes.

Process modification and product innovation

The techniques of modern molecular biology are applied in the industry and environment to improve efficiency and diminish the environmental impact. Process innovation, the development of new biological processes, and the modification or replacement of existing processes by the introduction of biological steps based on microbial or enzyme action are increasingly being used in industrial operations as an important potential area of primary pollution prevention (Olguin 1999; Gavrilescu 2004b; Gavrilescu and Nicu 2005) (**Table 16**). Similarly, the use of new biofuels and biomaterials that have little or no environmental impact is expanding rapidly.

Biodegradation, biotransformation and biocatalysis are three processes that occur as a result of microbial metabolism. A manufacturer using microbial metabolism is said to be conducting a biotransformation or to be using biocatalysis. In some cases, these interests can overlap (**Fig. 19**).

Biotransformation involves modifications of organic molecules into products of defined structure, in the presence of microbe, plant or animal cells or enzymes.

Biotransformations by microbes furnish both regio- and stereospecific products, the reactions can be run under gentle and controlled conditions and new products can be biosynthesized.

A survey carried out by the Fraunhofer Institute for Systems and Innovation Research in Karlsruhe on behalf of the Ministry of the Environment in Stuttgart revealed that the potential of product-integrated environmental biotechnology is enormous: reduced environmental pollution (70%), reduced process costs (64%) and improved product quality (22%).

In its specific use in production and product processing, biotechnology helps save energy and raw materials in the production of textiles, food, washing detergents, pharmaceuticals, by means of genetically modified enzymes. They also help avoid undesired waste products during production.

Biotechnological processes generally operate under gentle conditions, use biodegradable raw materials and intermediates and water is usually the solvent. As a result of high enzymatic specificity, biological synthesis can lead to increased yields and less by-products, thus saving additional

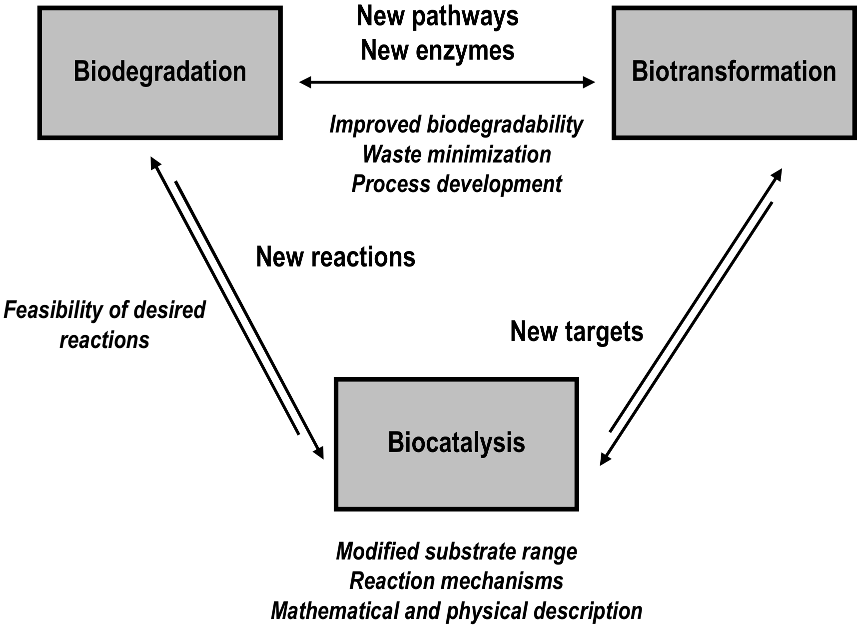


Fig. 19 Interdependence of the three main application areas of enzyme catalysis. (Parales *et al.* 2002).

Table 16 Industrial processes or products changed by establishing biotechnological steps.

Process or product	Conventional manufacturing process	New industrial biotech process	Costs and environmental benefits
Detergent	Phosphates added as a brightening and cleaning agents	Genetically enhanced microbes or fungi engineered to make enzymes Addition of biotechnology enzymes as brightening and cleaning agents: Proteases remove protein stains Lipases remove grease stains Amylases remove starch stains	Elimination of water pollution from phosphates Brighter, cleaner clothes with lower temperature wash water Energy savings
Bread	Potassium bromate, a suspected cancer-causing agent at certain levels, added as a preservative and a dough strengthening agent	Microorganisms genetically enhanced to produce baking enzymes (directed evolution and recombinant DNA) Addition of biotechnology enzymes to: enhance rising strengthen dough prolong freshness	High-quality bread Longer shelf life No potassium bromate
Polyester bedding	Polyester produced chemically from petroleum feedstock	Existing bacillus microbe used to ferment corn sugar to lactic acid; lactic acid converted to a biodegradable polymer by heating; polymer made into plastic products and polyester Biotech polyester (PLA) produced from corn starch feedstock	PLA polyester does not harbor body odor like other fibers Biodegradable Not made from petroleum Does not give off toxic smoke if burned
Plastics	Petroleum is used as feedstock, cracked in monomers Polymerization include several steps, polymers are processed further into plastics	Use plant sugars, lignocellulosic biomass, straw or corn residues The process harnesses carbon stored in plants to create the PLA polymer	PLA plastics are biodegradable Up to 80% reduction in petroleum usage
Antibiotics	Chlorinated solvents and hazardous chemicals used to produce antibiotics through chemical synthesis	Genetically enhanced organism developed to produce the key intermediate of certain antibiotics (recombinant DNA) One-step biological process uses direct fermentation to produce antibiotic intermediate	65% reduction in energy consumption Overall cost savings Reduced environmental impact Reduces green house gas emissions
Vitamin B2	Production starts with glucose followed by six chemical steps using hazardous chemicals and generating hazardous waste Toxic chemicals, such as aniline, used in chemical synthesis process	Genetically enhanced microbe developed to produce vitamin B2 (directed evolution) One-step fermentation process uses vegetable oil and glucose as a feedstock Crude riboflavin is produced directly from glucose with a genetically modified strain of *Bacillus subtilis* (a gram-positive bacterium) A 10-step chemical process was replaced by a single fermentation process, eliminating the use of numerous toxic chemicals and reducing the acidity of the wastewater produced	Biologically produced without chemicals Less chemically intensive Based of the use on a renewable raw material (glucose) Reduced land disposal of hazardous waste, waste-to-water discharge by 66%, air emissions by 50%, and costs by 50%
Textile finishing Stonewashed Blue Jeans	Textile bleaching by using hydrogen peroxide Chemical treatment using hot sodium hydroxide to remove impurities Open-pit mining of pumice fabric washed with crushed pumice stone and/or acid to scuff it	Textile enzymes produced by genetically enhanced microbe (extremophiles and recombinant DNA) Enzymes used in highly specialized textile finishing process Fabric washed with biotechnology enzyme (cellulase) to fade and soften jeans or khakis (biostoning)	Less mining Softer fabric Superior products such as more durable carpeting, lightweight bulletproof material, stronger silk Up to 18% reduction of the amount of bleaching agents and water Reduced energy consumption Lower cost Reduced environmental impact
Paper bleaching De-inking recycled paper	Wood chips boiled in a harsh chemical solution then bleached with chlorine to yield pulp for paper making	Wood-bleaching enzymes produced by genetically enhanced microbes (recombinant DNA) Enzymes selectively degrade lignin and break down wood cell walls during pulping	Reduces use of chlorine bleach and reduces toxic dioxin in the environment Up to 15% reduction of chlorine in wastewater Up to 40% reduction of energy usage Cost savings due to lower energy and chemical costs
Fuel based on ethanol	Food and feed grains fermented into ethanol (a technology that is thousands of years old)	Genetically enhanced organism developed to produce enzymes that convert agricultural wastes into fermentable sugars (directed evolution, gene shuffling) Cellulase enzyme technology can convert cellulose to its constituent sugars, which are then fermented and distilled to make bioethanol (and other chemicals and products if desired) Cellulase enzyme technology allows conversion of crop residues (stems, leaves, straw, and hulls) to sugars that are then converted to ethanol	Renewable feedstock Increases domestic energy production Reduces green house gas emissions The use of crop residue rather than the grain crop itself allows for significant reductions in energy inputs and pollution related to bioethanol production Bioethanol from cellulose generates 8 to 10 times as much net energy as is required for its production
Cosmetics	Isopropyl myristale production, as moisturing agent; Large energy requirement process (high temperature and pressure); The products needs further refinement	Enzyme-based esterification process	Reducing the environmental impact by deriving a cleaner, odorfree product High yields Lower energy requirement Less waste for disposal

costs for further purification. Biotechnological and genetic engineering methods are also able to reduce the environmental load in the field of renewable raw materials ("metabolic design").

The practice has demonstrated that biotechnology cannot solve all the problems associated with pollution prevention and cleaner production, but it has proven itself to be a powerful and flexible means in a range of industry sectors (pulp and paper, fine chemicals, plastics, mining, energy) (**Table 16**).

Biotechnological processes can contribute to sustainability, provided they replace chemical production methods.

Pulp and paper industry

Pulp and paper industry has achieved an impressive record in becoming an environmentally cleaner industry. A long term objective refers to the genetic engineering that can exploit its ability to revolutionize the forests so that trees with fibers having optimal papermaking properties will grow (Pullman *et al.* 1998). Fungi are used for lignin degradation during biopulping, the treatment of wood chips and other lignocellulosic materials prior to thermomechanical pulping. This is a way to reduce the requirements for chemicals and energy, which would also decrease the environmental impact of pulping process. In 2004, two industries sponsored consortia and 22 pulp and paper and related companies of U.S.A have reported the technical and economic feasibility of biopulping (Shukla *et al.* 2004). Also, the biobleaching of pulp with enzymes (laccase/mediator, xylanases, manganese peroxidase, lignolytic enzymes) has gained significant interest because of its selectivity and the possibility to save up to 25% of chlorine containing bleaching chemicals or to establish a chlorine-free bleaching process (Lema *et al.* 1999; Balakshin *et al.* 2001; Sasaki *et al.* 2001; Chakar and Ragauskas 2004; Shukla *et al.* 2004). Also, paper recycling tries to change from the chemical-based deinking process that currently uses sodium hydroxide and a variety of flocculants, dispersants, and surfactants toward an alternative which is based on microbial enzymes. Aside from that, the in-plant wastewater biotreatment could remove dissolved and colloidal organic material and metal ions in order to prevent deposit and slime problems (Ah-You *et al.* 2000; Gavrilescu *et al.* 2008).

Enzymes have found wide applications in the *textile industry* for improving production methods and fabric finishing, for example to remove lubricants, which are introduced in natural fibers production to prevent snagging and reduce thread breakage during spinning (Novozymes 2001; Evans and Furlong 2003). The process of bioscouring for wool and cotton which uses enzymes tends to replace the traditional chemical treatment. Technical support was offered to an Indian textile mill in order to apply a biological scouring process for removal of non-cellulosic components and other impurities found in native cotton, which led to a 90% reduction of chemicals (Novozymes 2001). Biopolishing involves enzymes in shearing off cotton microfibres to improve material softness.

A current application of biotechnology is the bleaching of denim fabrics. The use of biotechnological procedures employing enzymes reduces energy consumption, as well as wastewater pollution, because enzymes remove the residual bleach from textiles.

In the *leather industry*, the use of enzymes not only leads to more consistent quality, better final color, but also considerably reduces VOC and surfactants.

Microbial *desulphurization of coal and oil* is an important sector where environmental biotechnology is involved. The use of microorganisms may increase the sulphur oxidation rate in a certain bioreactor configuration. The development of biocatalytic desulphurization process and bioreactors is an important advance in environmental friendly biotechnological processes (Monticello 2000; Li *et al.* 2005; Killbane 2006).

Biofuels

Production of bioethanol, biodiesel, biogas using agricultural substrates, wastes (forestry, landfill, municipal, industrial, farming) vegetable oils (soybean, canola, sunflower) by enzymatic conversion or digestion is already in force as a result of excellent research and development capacities in industry, universities and other laboratories interested in application of biotechnology for energy saving, resource conservation, waste management and environmental protection (Ah-You *et al.* 2000; Dale and Kim 2006; Willke *et al.* 2006).

A number of different applications have developed the idea of anaerobic digestion for methane production, notably in the waste management, sewage treatment, agricultural and food processing industries. *Biogas* is a methane-rich gas resulting from the activities of anaerobic bacteria, responsible for the breakdown of complex organic molecules, as shown in **Fig. 20**. It is combustible, with an energy value typically in the range of 21–28MJ/m^3 (Doble *et al.* 2004).

Chemicals

Bulk chemical synthesis from renewable resources is still limited, but it is confirmed that the bioconversion of renewable biomass feedstock such as agricultural and wood wastes into ethanol or other fuels can lead to major environmental and economic benefits (Gavrilescu and Chisti 2005; Willke *et al.* 2006; Chisti 2007). The company DuPont intends to produce an important volume of its products (e.g. plastics) from renewable resources, starting with 2010 (Willke *et al.* 2006).

Currently, traditional methods are still used in fine chemical industries, which continue to generate severe environmental problems.

An Eco-Efficiency Analysis, performed by Saling (2005) with the aim to harmonize economical and ecological features of vitamin B2 fabrication demonstrated which vitamin B2 production process (biotechnological and chemical) is the most eco-efficient. The biotechnological process was more eco-efficient, since it had the lower overall environmental impact and the lower cost.

Progress in bio- and genetic engineering has shown that vitamin B2 (riboflavin) can be produced using biotechnological tools, at costs reduced by 50%, and also in more environmentally-sound ways (BIO–PRO 2008). A one step, purely fermentative process replaced the traditional method, in six steps.

The remarkable potential of microbes in the transformation of steroids through hydroxylation led to the development of antiarthritic steroids. Various strains were tested, such as: *Rhizopus arrhizus* (Dutta and Samantha 1997),

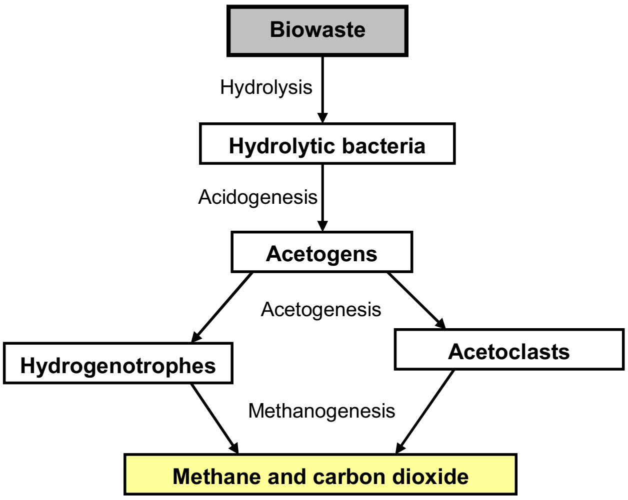


Fig. 20 Schematic representation of the reaction pathways for biowaste methanisation. (Adapted form Blonskaja and Vaalu 2006).

Syncephalastrum racemosum (Sen and Samantha 1981).

New semisynthetic penicillins were produced and used in chemotherapy, 6-aminopenicillanic acid (6-APA) being the key intermediate used for the synthesis of these penicillins. The biological synthesis of 6-APA is 20% cheaper than chemical synthesis. In addition it meets some criteria for an ideal process shown in **Fig. 18**.

Detergent enzymes

Enzymes have been used in detergents since the 1960s. The use of enzymes in detergents provides consumers with well proven benefits. Detergent enzymes present no risk to consumers, or to employees in enzyme production.

Enzymes can reduce the environmental load of detergent products since they meet the following criteria (**Fig. 18**):

- Save energy by enabling a lower wash temperature
- Partly replace other, often less desirable, chemicals in detergents
- Are biodegradable, leaving no harmful residues
- Have no negative environmental impact on sewage treatment processes
- Do not present a risk to aquatic life

The use of enzymes, together with developments in detergents, has reduced washing temperatures to 30-40 degrees, temperatures which are expected to be reduced even further. Scarcity of water and increasing oil and water prices are expected to further the development. Calculations show that in Denmark with five million inhabitants, a reduction of wash temperature from 60 to 40°C would lead to an energy saving equivalent to approx. 40,000 tonnes of coal a year. By comparison, less than 300 tonnes of coal a year would be needed to produce the enzymes that enable lower wash temperature.

Although their biotechnological production is material and energy consuming, the results in cleanliness obtained with enzyme-containing detergents are far superior to those obtained with traditional phosphate-containing washing detergents. Also, due to their specific cleansing effect, enzymes reduce the amount of washing detergents and additives, the washing temperature and energy consumption.

Some companies used wild-type and natural enzymes, but also genetically modified enzymes as components of washing detergents.

Bioplastics

Plastics production from synthetic polymers consumes vast quantities of non-renewable resources, while they represent a major environmental problem as they are non-biodegradable (Stevens 2002; Chiellini *et al.* 2003; Reddy *et al.* 2003). The production of new biomaterials like bioplastics based on sugars, oils, proteins, fibers and other natural substances extracted from plants avoids the use of non-renewable resources like fossil fuels, with less energy, fewer resources, and reducing global greenhouse-gases emissions. Microbes can be induced to produce enzymes needed to convert plant and vegetable materials into building blocks for biodegradable plastics (Luengo *et al.* 2003; Reddy *et al.* 2003; Moldes *et al.* 2004).

Both bioplastic production from organic waste material and plastic reduction with the contribution of enzymes have attained two environmental objectives:

- the release of plastic production from fossil fuels
- biodegradation of the plastic material to reduce waste, especially in food packaging and field-covering plastic

The report released by OECD (2001) assessed the widespreading of industrial biotechnology based on 21 companies case study data, including pharmaceutical, chemical, paper, textiles and energy sectors. This report has shown that industrial biotechnology led to cleaner production and products, having an environmentally sound profound character.

Reducing the environmental impact of agricultural pesticides

The excessive use of chemical herbicides, pesticides, fungicides and fertilizers as an integral part of intensive agriculture caused environmental hazards as a result of low biodegradability.

The use of genetically modified plant varieties which are resistant to insects and/or diseases may considerably diminish the use of pesticides.

Biopesticides (also known as biological pesticides) are derived from natural materials (animals, plants, bacteria, minerals) and are considered less toxic than conventional pesticides. USEPA (2008) indicates that at the end of 2001 there were approximately 195 registered biopesticide active ingredients and 780 products (Menn and Hall 1999).

They can be classified as (Fraser 2005; USEPA 2008):

- microbial pesticides, containing a microorganism (bacterium, fungus, virus or protozoa) as active ingredients (**Table 17**).
- plant-incorporated protectants, which means that the active pesticide is produced by plants from genetic materials added to the plant.
- biochemical pesticides, include substances which

Table 17 Organism generating biopesticides and their control targets (MCD 2008).

Target	Organism	Example
Insects	Bacteria	*Bacillus thuringiensis*
		Bacillus sphaericus
		Paenibacillus popillae
		Serratia entomophila
	Viruses	nuclear polyhedrosis viruses
		granulosis viruses
		non-occluded baculoviruses
	Fungi	*Beauveria* spp.
		Metarhizium
		Entomophaga
		Zoopthora
		Paecilomyces fumosoroseus
		Nornuraea
		Lecanicillium lecanii
	Protozoa	*Nosema*
		Thelohania
		Vairimorpha
	Entomopathogenic nematodes	*Steinernema* spp.
		Heterorhabditid spp.
	Others	pheromones
		parasitoids
		predators
		microbial byproducts
Weed control	Fungi	*Colletotrichum gloeosporioides*
		Chondrostereum purpureum
		Cylindrobasidium laeve
		Xanthomonas campestris
Plant disease control	Fungi	*Ampelomyces quisqualis*
		Candida spp.
		Clonostachys rosea
	Competitive innoculants	*Coniothyrium minitans*
		Pseudozyma flocculosa
		Trichoderma spp.
	Composts, soil innoculants	*Bacillum pumilus*
		Bacillus subtilis
		Pseudomonas spp.
		Streptomyces griseoviridis
		Burkholderia cepacia
Nematicides	Nematode trapping fungi	*Myrothecium verrucaria*
		Paecilomyces lilacinus
	Bacteria	*Bacillus firmus*
		Pasteruria penetrans
	Mollusc panasitic nematode	*Phasmarhabitis hermaphrodita*

control pests by nontoxic mechanisms

Biopesticides are often effective in very small quantities and often decompose quickly, and the exposure is low (Boyetchko *et al.* 1999), so that their use could result in reduced risk to human health and the environment. Biopesticides exhibit one or more of the following characteristics (Fraser 2005): low toxicity to nontarget organisms, low potential to contaminate environmental components and resources, low risk to human health. Examples of biopesticides and their targets are given in **Table 17** (MCD 2008).

The use of genetically modified plant varieties that are resistant to insects and/or diseases may considerably diminish the use of pesticides. Insect-protected crops allow for less potential exposure of farmers and groundwater to chemical residues.

Integration of nanotechnology with environmental biotechnology

The nanoscale bioscience and biotechnology integration leads to potential and actual breakthroughs in areas such as materials and manufacturing, medicine, healthcare, energy, environment, chemicals, agriculture, information technology etc. (Hasim and Ujiang 2004). The emergence of nanobiotechnology and the incorporation of living microorganisms in biomicroelectronic devices are revolutionizing interdisciplinary opportunities for microbiologists and biotechnologists to participate in understanding microbial processes in and from the environment. Moreover, it offers revolutionary perspectives to develop and exploit these processes in completely new ways.

"Biomedical and biotechnological applications of nanoparticles have been of special recent research and development interest, with potential applications that include use of nanoparticles as drug (or DNA) delivery vehicles, and as components in medical diagnostic kits, biosensors and membranes for bioseparations" (Kohli and Martin 2005).

Carbon nanotubes, another exciting area of research and development in the *nano- world*, can be coated with reaction specific biocatalysts and other proteins for specialized applications, making them even more environmentally friendly and economically attractive. Scientists have developed versatile methods for targeting carbon nanotubes to specific types of cells that could spur the development of new anticancer agents that rely on the unique physical characteristics of carbon nanotubes. Such bio-nano-systems lead to a new generation of integrated systems that combine unique properties of the carbon nanotube (CNT) with biological recognition capabilities (Alivisatos 2004; Gao and Kong 2004; Wong Shi Kam *et al.* 2005).

Though, high operative costs, expenditure for research and development as well as investment still limit the establishment of biotechnological processes.

Bioenergy from biomass

Using biomass to generate energy has positive environmental implications and creates a great potential to contribute considerably more to the renewable energy sector, particularly when converted to modern energy carriers such as electricity and liquid and gaseous fuels (IBEP 2006; Gavrilescu 2008).

By the year 2120, 3.6% of electric power and 6-7% of the total energy will come from renewable resources (Lako *et al.* 2008).

Biorefining

The biorefining concept is an analogue of today's petroleum refineries producing multiple fuels and prodcuts from petroleum. By combining chemistry, biotechnology, engineering and system approach, biorefinery could produce food, fertilizers, industrial chemicals, fuels, power from biomass (Gravitis *et al.* 1998; Kamm and Kamm 2004).

ENVIRONMENTAL BIOTECHNOLOGY AND ECO-EFFICIENCY

Eco-efficiency analysis can offer comprehensible information for a large number of applications concerning multifactorial problems within relatively short times and at relatively low cost, since it was discerned as an important assessment method for research and development, production and marketing (Saling 2005).

There is no doubt that environmental biotechnology has a great potential to be an ecologically beneficial and at the same time economically profitable in many areas. Environmental challenges increasingly affect the competitiveness, not only in terms of clean-up and pollution-control costs but also in the marketplace.

World Business Council for Sustainable Development (WBCSD) developed eco-efficiency as a way for an operational sustainable development driving force from a business perspective (WBCDS 2000). Eco-efficiency is more and more becoming the heart of success in the economic world as a way to maximize efficiency, while minimizing the impact on the environment. It is achieved in practice by means of three key objectives that regard increasing product or service value, optimizing the use of resources, reducing environmental impact (Gabriel and Braune 2005; Gavrilescu and Chisti 2005; Bidoki 2006). Because of the opportunity for cost savings associated with each of these objectives, eco-efficient technologies and practices demonstrate that eco-efficiency stimulates productivity and innovation, increases competitiveness and improves environmental performance that means creating more value with less impact (Bidoki 2006). Biotechnology – in general, and environmental biotechnology – in particular can be considered one of the most useful means to attain eco-efficiency and for decision-making because offers a number of practical benefits, illustrated in (**Table 18**) (Wall-Markowski *et al.* 2004; Saling 2005). For example, minimization of pesticide use is one of the main practices for sustainable farming, but also a proactive consideration for the future of an eco-efficient agriculture, as an illustration for one element of eco-efficiency: reduce toxic dispersion. Also, eco-efficiency goes hand-in-hand with pollution prevention and eco-design practices that essentially involve reduction in the material and energy flow intensity, improved recyclability, maximum use of renewable resources in order to ensure sustainable production and consumption (Olguin 1999; WBCSD 2000; Gavrilescu 2004b; Gavrilescu and Nicu 2005).

A study of OECD emphasizes that great industrial companies are becoming aware of the importance of sustainable development and of the great potential of biotechnology that can help them improve the environmental friendliness of industrial activities and lower both capital expenditure and operating costs, operating as an environmentally-sound basis for economy and society (OECD 2001).

Some case studies presented by EuropaBio as a result of

Table 18 Some of the practical benefits of the eco-efficiency by biotechnology.

Eco-efficiency practical benefit	Means to achieve
reduced costs	through more efficient use of energy and materials
reduced risk and liability	by designing out the need for toxic substances
increased revenue	by developing innovative products and increasing market share
enhanced brand image	through marketing and communicating the improvement efforts
increased productivity and employee confidence	through closer alignment of company values with the personal values of the employees
improved environmental performance	by reducing toxic emissions, and increasing the recovery and reuse of waste material

Table 19 Illustration of economic and environmental impacts of various products/processes based on white biotechnology (Saling 2005).

Product/process	**Environmental impact**			**Economic impact/ Production costs**
	Energy efficiency	**Raw materials consumption**	**CO_2 emissions**	
Vitamin B2 (BASF)	+	++	+	+
Cephalexin (DSM)	++	++	+	+
Scouring enzyme (Novozymes)	+	+	0	+
Biopolimers (Cargill Dow)	+	++	++	0
Biopolymers (Du Pont)	+	++	+	+
Ethylene from biomass (under research)	0	++	++	--

Eco-Efficiency analyses showed that there is some potential for biobased materials and white biotechnology, and that the greatest impact of white biotechnology may be in the fine chemicals segment, where up to 60% of products may use biotechnology (EuropaBio 2004; Saling 2005). In addition, the economic and environmental impacts are favourable (**Table 19**) (Saling 2005).

CONCLUDING REMARKS - ENVIRONMENTAL BIOTECHNOLOGY CHALLENGES AND PERSPECTIVES

New environmental challenges continue to evolve and new technologies for environmental protection and control are currently under development. Also, new approaches continue to gain more and more ground in practice, harnessing the potential of microorganisms and plants as eco-efficient and robust cleanup agents in a variety of practical situations such as (Urbain *et al.* 1996; van Wyk 2001; Grommen and Verstraete 2002; Cicek 2003; Kohli and Martin 2005):

- enzyme engineering for improved biodegradation
- evolutionary and genomic approaches to biodegradation
- designing strains for enhanced biodegradation
- process engineering for improved biodegradation
- re-use of treated wastewater
- biomembrane reactor technology
- design wastewater treatment based on decentralized sanitation and reuse
- implementation of anaerobic digestion to treat biowaste
- biodevelopment of biowaste as an alternative and renewable energy resource
- emerging and growing-up technological applications of soil remediation and cleanup of contaminated sites

Along with a wide group of technologies with the potential to accomplish the objectives of sustainability, biotechnology will continue to play an important role in the fields of food production, renewable raw materials and energy, pollution prevention, bioremediation.

Since environmental biotechnology proved to have a large potential to contribute to the prevention, detection and remediation of environmental pollution and degradation, it is a sustainable way to develop clean processes and products, less harmful, with reduced environmental impact than their forerunners, and this role is illustrated with reference to clean technology options in the industrial, agro forestry, food, raw materials, and minerals sectors.

Since some new techniques make use of genetically modified organisms, regulation to guarantee safe application of new or modified organisms in the environment is important.

A wide range of biological methods are already in use to detect pollution incidents and for the continuous monitoring of pollutants, but new developments are expected.

Environmental and economic benefits that biotechnology can offer in manufacturing, monitoring and waste management are in balance with technical and economic problems which still need to be solved. All this is being achieved with reduced environmental impact and enhanced sustainability.

An evaluation of the consequences, opportunities and challenges of modern biotechnology is important both for policy makers and the industry.

ACKNOWLEDGEMENTS

This work was supported by the Program IDEI, Grant ID_595, Contract No. 132/2007, in the frame of the National Program for Research, Development and Innovation II—Ministry of Education and Research, Romania.

REFERENCES

Ahn Y, Jung H, Tatavarty R, Choi H, Yang J, Kim IS (2005) Monitoring of petroleum hydrocarbon degradative potential of indigenous microorganisms in ozonated soil. *Biodegradation* **16**, 45-56

Ah-You K, Suleiman M, Jaworski J (2000) Biotechnology and Cleaner Production in Canada. Program for Energy Research and Development (PERD), Life Sciences Branch Industry Canada, Ottawa, 96 pp

Alivisatos P (2004) The use of nanocrystals in biological detection. *Nature Biotechnology* **22**, 47-52

Alkhaddar RM, Phipps DA, Cheng C (2005) Today and Tomorrow! Research Prospects for Aerobic Biological Liquid Waste Treatment for Reduction of Carbon Load. *E-Water*, Official Publication of the European Water Association (EWA), pp 1-18

Andres Y, Dumont E, Le Cloirec P, Ramirez-Lopez E (2006) Wood bark as packing material in a biofilter used for air treatment. *Environmental Technology* **27**, 1297-1301

Asante-Duah DK (1996) *Managing Contaminated Sites: Problem Diagnosis and Development of Site Restoration*, Wiley, New York, 254 pp

Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J (Eds) *Water Quality: Guidelines, Standards and Health*, IWA Publishing, London, pp 289-316

Ashley K, Biagimi R, Smith JP, Sammons DL, Mackenzie BA, Striley CAF, Robertson SK, Snawder JE (2008) The use of immunochemical and biosensor methods for occupational and environmental monitoring. Part I: Introduction to immunoassays. *Journal of Occupational and Environmental Hygiene* **5**, D25-D32

Atlas RM (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological Reviews* **45**, 180-209

Arai K (2006) Toward biotechnology: The mission of IUBMB in the 21st century. *IUBMB Life* **58**, 267-268

Baheri H, Meysami P (2002) Feasibility of fungi bioaugmentation in composting a flare pit soil. *Journal of Hazardous Materials* **89**, 279-286

Bajpai P (2000) *Treatment of Pulp and Paper Mill Effluents with Anaerobic Technology*, Pira International, Leatherhead, UK, 129 pp

Balakshin M, Capanema E, Chen CL, Gratzl J, Kirkman A, Gracz H (2001) Biobleaching of pulp with dioxygen in the laccase-mediator system - reaction mechanisms for degradation of residual lignin. *Journal of Molecular Catalysis B: Enzymatic* **13**, 1-16

Balkema AJ, Preisig HA, Otterpohl R, Lambert F (2002) Indicators for the sustainability assessment of wastewater treatment systems. *Urban Water* **4**, 153-161

Banks CJ, Stentiford EI (2007) Biodegradable municipal solid waste: biotreatment options. *Waste and Resource Management* **160**, 11-18

Baptista SJ, Camporese EFS, Freire DDC (2006) Evaluation of biostimulation, bioaugmentation and use of biosurfactant as treatment technique of clay soil contaminated with diesel oil, *Environmental Engineering and Management Journal* **6**, 1325-1332

Bargagli R (1998) *Trace Elements in Terrestrial Plants: An Ecophysiological Approach to Biomonitoring and Biorecovery*, Springer-Verlag, Berlin, 324 pp

Beaudette LA, Cassidy MB, England L, Kirk JL, Habash M, Lee H, Trevors JT (2002) Bioremediation of soils. In: Bitton G (Ed) *Encyclopedia of Environmental Microbiology*, Wiley-Interscience, New York, pp 722-737

Beck-Friis BG (2001) Emissions of ammonia, nitrous oxide and methane during composting of organic household waste. PhD thesis, Swedish University of Agricultural Sciences, Uppsala, 331 pp

Beliaeff B, Burgeot T (2002) Integrated biomarker response: a useful tool for ecological risk assessment. *Environmental Toxicology and Chemistry* **21**, 1316-1322

Ben Aim RM, Semmens MJ (2003) Membrane bioreactors for wastewater treatment and reuse: a success story. *Water Science and Technology* **47**, 1-5

Benz M, Brune A, Schink B (1998) Anaerobic and aerobic oxidation of ferrous iron at neutral pH by chemoheterotrophic nitrate-reducing bacteria.

Archives of Microbiology **169**, 159-165

Betianu C, Gavrilescu M (2006a) Environmental behavior and assessment of persistent organic pollutants. *Environmental Engineering and Management Journal* **5**, 213-241

Betianu C, Gavrilescu M (2006b) Persistent organic pollutants in environment: Inventory procedures and management in the context of the Stockholm Convention. *Environmental Engineering and Management Journal* **5**, 1011-1028

Bidoki SM, Wittlinger R, Alamdar AA, Burger J (2006) Eco-efficiency analysis of textile coating materials. *Journal of the Iranian Chemical Society* **3**, 351-359

BIO-PRO (2008) *Biotechnology in the Chemical Industry*: *The Long Road from Exception to Standard (II)*, The Biotech/Life Science Portal Baden-Württemberg. Online at: http://bio-pro.de/en/region/ulm/magazin/00698/index-html

Biotech (2000) *Environmental Biotechnology*, *The Welcome Trust*. Online at: http://www.biochemistry.org/education/pdfs/enviro-card.pdf

Bitton G (2005) *Wastewater Microbiology*, Wiley-Liss, John Wiley and Sons, New Jersey, USA, 766 pp

Blanco A (2000) Immobilization of non-viable cyanobacteria and their use for heavy metal adsorption from water. In: Olguin EJ, Sánchez G, Hernández E (Eds) *Environmental Biotechnology*, Philadelphia, pp 135-151

Blonskaya V, Vaalu T (2006) Investigation of different schemes for anaerobic treatment of food industry wastes in Estonia. *Proceedings of the Estonian Academy of Sciences: Chemistry* **55**, 14-28

Bontidean I, Ahlqvist J, Mulchandani A, Chen W, Bae W, Mehra R, Mortari A, Csöregi E (2003) Novel synthetic phytochelatin – based capacitive biosensor for heavy metal in detection. *Biosensors and Bioelectronics* **18**, 447-553

Boopathy R (2000) Factors limiting bioremediation technologies. *Bioresource Technology* **74**, 63-67

Boopathy R, Peters R (2001) Enhanced transformation of trichloroethylene under mixed electron acceptor conditions. *Current Microbiology* **42**, 134-138

Borja JQ, Aureseniaa JL, Gallardoa SM (2006) Biodegradation of polychlorinated biphenyls using biofilm grown with biphenyl as carbon source in fluidized bed reactor. *Chemosphere* **64**, 555-559

Botrè C, Botrè F, Mazzei F, Podesta E (2000) Inhibition-based biosensors for the detection of environmental contaminants determination of 2,4-dichlorophenoxyacetic acid. *Environmental Toxicology and Chemistry* **19**, 2876-2881

Boyetcho SM, Pedersen E, Punja ZK, Reddy MS (1999) Formulations of biopesticides. In: Hall FR, Menn JJ (Eds) *Biopesticides. Use and Delivery*, Humana Press, Totowa, New Jersey, USA, pp 487-508

Bremer JP, Geesey GG (2001) Laboratory-based model of microbiologically induced corrosion of cooper. *Applied and Environmental Microbiology* **57**, 1956-1962

Brinza L, Gavrilescu M (2003) pH effect on the biosorption of Cu^{2+} from aqueous solution by *Saccharomyces cerevisiae*. *Environmental Engineering and Management Journal* **2**, 243-254

Brinza L, Dring M, Gavrilescu M (2005a) Ability of different algal species to take up heavy metals from wastewater: A review. *The Phycologist* **68**, 30-31

Brinza L, Dring M, Gavrilescu M (2005b) Biosorption of Cu^{2+} ions from aqueous solution by *Enteromorpha* sp. *Environmental Engineering and Management Journal* **4**, 41-50

Brinza L, Dring MJ, Gavrilescu M (2005) Biosorption of Cu^{2+} ions from aqueous solution by *Enteromorpha* sp. *Environmental Engineering and Management Journal* **4**, 29-46

Brinza L, Dring MJ, Gavrilescu M (2007) Marine micro and macro algal species as biosorbents for heavy metals. *Environmental Engineering and Management Journal* **6**, 237-251

Brinza L, Gavrilescu M (2003) pH effect on the biosorption of Cu^{2+} from aqueous solution by *Saccharomyces cerevisiae*. *Environmental Engineering and Management Journal* **3**, 243-254

Bull AT (1995) Biotechnology for environmental quality: closing the circles. *Biodiversity and Conservation* **5**, 1-25

Burton FL, Stensel HD, Tchobanoglous G (2002) *Wastewater Engineering: Treatment and Reuse*, Metcalf and Eddy Inc., McGraw-Hill Professional, 1848 pp

Campos MG, Pereira P, Roseora JC (2006) Packed bed reactor for the integrated biodegradation of cyanide and formamide by immobilized *Fusarium oxysporium* CCMI 876 and *Methylabacterium sp.* RXM CCMI 908. *Enzyme and Microbial Technology* **38**, 848-854

Campàs M, Carpentier R, Rouillon R (2008) Plant tissue-and photosynthesis-based biosensors. *Biotechnology Advances* **26**, 370-378

Cannons AC, Harwood VJ (2004) *Sensor Technology for Water Quality Monitoring: Fiber-Optic Biosensor (WERF Report)*, IWA Publishing, London, 72 pp

Cantor CR (2000) Biotechnology in the 21st century. *Trends in Biotechnology* **18**, 6-7

Chakar FS, Ragauskas AJ (2004) Biobleaching chemistry of laccase-mediator systems on high-lignin-content kraft pulps. *Canadian Journal of Chemistry* **82**, 344-352

Chandler D, Davison G, Grant WP, Greaves J, Tatchell GM (2008) Microbial pesticides for integrated crop management: an assessment of environmental and regulatory sustainability. *Trends in Food Science and Technology* **19**, 275-283

Chang JS, Chen BY, Lin YS (2004) Stimulatori of bacterial decolorization of azo dye by extracelular metabolites from *Escherichia coli* strain N03. *Bioresource Technology* **91**, 243-248

Chang JS, Kuo TS, Chao YP, Ho YS, Lin PJ (2000) Azo dye decolorization with a mutant *Escherichia coli* strain. *Biotechnology Letters* **22**, 807-812

Chavan A, Mukherji S (2010) Response of an algal consortium to diesel under varying culture conditions. *Applied Biochemistry and Biotechnology* **160 (3)**, 719-729

Chemnitius G, Messel M, Zaborosch C, Knoll M, Spener F, Cammann K (1996) Highly sensitive electrochemical biosensors for water monitoring. *Food Technology and Biotechnology* **34**, 23-29

Chen W, Mulchandani A, Deshusses MA (2005) Environmental biotechnology: challenges and opportunities for chemical engineers. *AIChEJ* **51**, 690-695

Chen CY, Kao CM, Chen SC (2008) Application of *Klebsiella oxyteca* immobilized cells on the treatment of cyamide wastewater. *Chemosphere* **71**, 133-139

Cheremisinoff NP (1996) *Biotechnology for Waste and Wastewater Treatment*, Noyes Publications, Westwood, New Jersey, 231 pp

Cheremisinoff NP (2003) *Handbook of Solid Waste Management and Waste Minimization Technologies*, Butterworth-Heinemann/Elsevier Science, Amsterdam, 477 pp

Chiellini E, Chiellini F, Cinelli P (2003) Polymers from renewable resources. In: Scott G (Ed) *Degradable Polymers: Principles and Applications*, Kluwer Academic Publishers, Dordrecht, pp 163-264

Chisti Y (2007) Biodiesel from microalgae. *Biotechnology Advances* **25**, 294-306

Chisti Y (2008) Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology* **26**, 126-131

Chisti Y, Moo-Young M (1999) Fermentation technology, bioprocessing, scale-up and manufacture. In: Moses V, Cape RE, Springham DG (Eds) *Biotechnology: The Science and the Business* (2nd Edn), Harwood Academic Publishers, New York, pp 177-222

Cicek N (2003) A review of membrane bioreactors and their potential application in the treatment of agricultural wastewater. *Canadian Biosystems Engineering* **45**, 6.37-6.49

Cohen Y (2001) Biofiltration – the treatment of fluids by microorganisms immobilized into the filter bedding material: a review. *Bioresource Technology* **77**, 257-274

Conti ME (2007) *Biological Monitoring; Theory and Applications, Bioindicators and Biomarkers for Environmental Quality and Human Exposure Assessment*, WIT Press, Southampton, UK, 228 pp

Cost 624 (2001) *Microbial Tools: Application in Wastewater Treatment Processes (WWTP)*. Cost 624 WG4 Meeting Rapport, Lisbon

Cox HH, Deshusses MA (1998) Biological waste air treatment in biotrickling filters. *Current Opinion in Biotechnology* **9**, 256-262

Cox HHJ, Deshusses MA (2001) Biotrickling filters. In: Kennes C, Veiga MC (Eds) *Bioreactors for Waste Gas Treatment*, Kluwer Academic Publishers, The Netherlands, pp 99-131

Cox HHJ, Deshusses MA, Converse BM, Schroeder ED, Patel DD, Vosooghi D, Iranpour R (2001) Odor and VOC treatment by biotrickling filters: pilot scale studies at the Hyperion Treatment Plant. *Water Environment Research* **74**, 557-563

Cutright T, Meza L (2007) Evaluation of aerobic biodegradation of trichloroethylene in response surface methodology. *Environment International* **33**, 338-345

Dale BE, Kim S (2006) Biomass refining global impact – The biobased economy of the 21st century. In: Kamm B, Gruber PR, Kamm M (Eds) *Biorefineries - Industrial Processes and Products. Status Quo and Future Directions* (Vol 1), Wiley-VCH, Weinheim, Germany, pp 41-66

Danalewich JR, Papagiannis TG, Belyea RL, Tumbleson ME, Raskin L (1998) Characterization of dairy waste streams, current treatment practices and potential for biological nutrient removal. *Water Research* **32**, 3555-3568

Das TK (2005) *Toward Zero Discharge. Innovative Methodology and Technologies for Process Pollution Prevention*, John Wiley and Sons, Hoboken, New Jersey, 744 pp

Das TK, Jain AK (2001) Pollution prevention advances in pulp and paper processing. *Environmental Progress* **20**, 87-92

Das K, Mukherjee AK (2007) Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresource Technology* **98**, 1339-1345

Dash RR, Majumder CB, Kumar A (2008) Treatment of metal cyanide bearing wastewater by simultaneous adsorption and biodegradation (SAB). *Journal of Hazardous Materials* **152**, 387-396

Dash RR, Gaur A, Balomajumder C (2009) Cyanide in industrial wastewater and its removal: A review on biotreatment. *Journal of Hazardous Materials* **163**, 1-11

Deshusses MA (1997) Biological waste air treatment in biofilters. *Current Opinion in Biotechnology* **8**, 335-339

Devinny JS, Deshusses AA, Webster TS (1999) *Biofiltration for Air Pollution Control*, CRC Press, Boca Raton, 299 pp

Doble M, Kruthiventi AK, Gaikar VG (2004) *Biotransformations and Bio-*

processes, Marcel Dekker, New York-Basel, 371 pp

Doble M, **Kumar A** (2005) *Biotreatment of Industrial Effluents*, Elsevier-Butterworth-Heinemann, 337 pp

Donkin J (1997) Bulking in aerobic biological systems treating dairy processing wastewater. *International Journal of Dairy Technology* **50**, 67-72

Doran JW, Parkin TB (1994) Defining and assessing soil quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (Eds) *Defining Soil Quality for a Sustainable Environment*, SSSA Special Publication No. 35, Madison, pp 3-21

Droste RL (1997) *Theory and Practice of Water and Wastewater Treatment*, John Wiley and Sons Inc., New Jersey, 816 pp

Dunn IJ, Heinzle E, Ingham J, Prenosil JE (2003) *Biological Reaction Engineering. Dynamic Modelling Fundamentals with Simulation Examples*, Wiley-VCH, Weinheim, 532 pp

Duncan M, Horan NJ (2003) *Handbook of Water and Wastewater Microbiology*, Elsevier, 832 pp

Durrieu C, Tran-Minh C, Chovelon JM, Barthet L, Chouteau C, Védrine C (2006) Algal biosensors for aquatic ecosystems monitoring. *The European Physical Journal - Applied Physics* **36**, 205-209

Dutta TK, Samanta TB (1997) Novel catalytic activity of immobilized spores under reduced water activity. *Bioorganic and Medicinal Chemistry Letters* **7**, 629-632

EC (2002) *Life Sciences and Biotechnology - A Strategy for Europe*, Communication from the Commission to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions. On-line at: http://ec.europa.eu/biotechnology/pdf/com2002-27_en.pdf

EFB (1995) *Environmental Biotechnology*, EFB Task Group on Public Perceptions of Biotechnology, On-line at: http://www.bioportfolio.com/efb4.htm

Eglit T (2002) Microbial degradation of pollutants at low concentrations and in the presence of alternative carbon substrates: emerging patterns. In: Agathos SN, Reineke W (Eds) *Biotechnology for the Environment: Soil Remediation*, Springer, Berlin, pp 131–139

EIBE (2000) *Biotechnology and Environment. European Initiative for Biotechnology Education.* Available on-line at: http://www.ipn.uni-kiel.de/eibe/UNIT16EN.PDF

El-Sheekh MM, Gharieb MM, Abou-El-Souod GW (2009) Biodegradation of dyes by some green algae and cyanobacteria. *International Biodeterioration and Biodegradation* **63**, 699-704

Eremektar G, Selcuk H, Meric S (2007) investigation of the relation between COD fractions and the toxicity in a textile finishing industry wastewater. Effect of preozonation. *Desalination* **211**, 314-320

EuropaBio (2003) White biotechnology: gateway to a more sustainable future, On line at: http://www.europabio.org/documents/100403/Innenseiten_final_screen.pdf

Evans GM, Furlong JC (2003) *Environmental Biotechnology. Theory and Application*, John Wiley and Sons, Chichester, 300 pp

Fane AG (2007) Sustainability and membrane processing of wastewater for reuse. *Desalination* **202**, 53-58

Fayolle F, Francois A, Gariner L, Godefroy D, Matgis H, Piveteau P, Monat F (2003) Limitations in MTBE biodegradation. *Oil and Gas Science and Technology* **58**, 497-504

Ferhan M (2003) Biodegradation of trichloroethylene (TCE) in the presence of phenolic compound. *Journal of Biological Sciences* **3**, 973-983

Feng D, Aldrich C (2004) Adsorption of heavy metals by biomaterials derived from the marine alga *Ecklonia maxima*. *Hydrometallurgy* **73**, 1-10

Fiorenza S, Rifai H (2003) Review of MTBE Biodegradation and Bioremediation. *Bioremediation Journal* **7**, 1-35

Fischer K (2008) Biological waste management and treatment in Europe. International Conference on Environmental Research and Technology (ICERT 2008) 28-30 May 2008, Penang, Malaysia

Fitzgiblon FJ, Nigam DP, Singh D, Marchant R (2007) Biological treatment of distillery waste for pollution-remediation. *Journal of Basic Microbiology* **35**, 293-301

Fraser H (2005) Reduced-risk pesticides and biopesticides. Ministry of Agriculture, Food and Rural Affairs, Ontario. Online at: http://www.omafra.gav.on.ca/english/crops/hort/news/vegnews/2005/vg1105a5.htm

FRTR (1999), Remediation technologies. Treatment Perspectives. On-line at: http://www.frtr.gov/matrix2/section3/figure3_1.html

Futrell R (2000) Politics of space and the political economy of toxic waste. *International Journal of Politics, Culture, and Society* **13**, 447-476

Gabriel R, Braune A (2005) Eco-efficiency analysis: applications and user contacts. *Journal of Industrial Ecology* **9**, 19-21

Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* **111**, 3-49

Gallert C, Winter J (1999) Bacterial metabolism in wastewater treatment system. In: Rehm H-J, Reed G (Eds) *Biotechnology* (2^nd^ Edn), Wiley-VCH Verlag, GmbH, Weinheim, pp 17-94

Gallert C, Winter J (2005) Perspectives of wastewater, waste, off-gas and soil treatment. In: Jördening H-J, Winter J (Eds) *Environmental Biotechnology. Concepts and Applications*, Wiley-VCH, Weinheim, pp 439-451

Gander M, Jefferson B, Judd S (2001) Aerobic MBRs for domestic wastewater treatment: a review with cost considerations. *Separation and Purification Technology* **18**, 119-130

Gao H, Kong Y (2004) Simulation of DNA-nanotube interactions. *Annual Review of Materials Research* **34**, 123-150

Gavrilescu M (2002) Engineering concerns in anaerobic waste-water treatment. *Clean Technology and Environmental Policy* **3**, 346-362

Gavrilescu M (2004) Cleaner production as a tool for sustainable development. *Environmental Engineering and Management Journal* **3**, 45-70

Gavrilescu M (2004) Removal of heavy metals from the environment by biosorption. *Engineering in Life Sciences* **4**, 219-232

Gavrilescu M (2005) Fate of pesticides in the environment and its bioremediation. *Engineering in Life Sciences* **5**, 497-526

Gavrilescu M (2006) Overview of *in situ* remediation technologies for sites and groundwater. *Environmental Engineering and Management Journal* **5**, 79-114

Gavrilescu M (2008) Biomass power for energy and sustainable development. *Environmental Engineering and Management Journal* **7**, 617-640

Gavrilescu M, Chisti Y (2005) Biotechnology – a sustainable alternative for chemical industry. *Biotechnology Advances* **23**, 471-499

Gavrilescu M, Macoveanu M (1999) Process engineering in biological aerobic waste-water treatment. *Acta Biotechnologica* **19**, 111-145

Gavrilescu M, Macoveanu M (2000) Attached-growth process engineering in wastewater treatment. *Bioprocess Engineering* **23**, 95-106

Gavrilescu M, Nicu M (2005) *Source Reduction and Waste Minimization* (in Romanian), second edition, EcoZONE Press, Iasi, Romania, 230 pp

Gavrilescu M, Pavel LV, Cretescu I (2008) Characterization and remediation of soils polluted with uranium. *Journal of Hazardous Materials* **163**, 475-510

Gavrilescu M, Teodosiu C, Gavrilescu D, Lupu L (2008) Strategies and practices for sustainable use of water in industrial papermaking processes. *Engineering in Life Sciences* **8**, 99-124

Gavrilescu M, Ungureanu F (2002) Modelling and simulation of an activated sludge bioreactor. *Bulletin of the Polytechnic Institute of Iasi* **52**, 89-100

Gavrilescu M, Ungureanu F, Cojocaru C, Macoveanu M (2005) *Modelling and Simulation of the Processes in Environmental Engineering* (Vol 1), EcoZone Press, Iasi, Romania, 448 pp (in Romanian)

Gavrilescu M, Ungureanu F, Cretescu I (2002b) Simulation of a biofilm reactor with suspended particles. *Bulletin of the Polytechnic Institute of Iasi* **52**, 69-81

Gavrilescu M, Ungureanu F, Robu B (2002a) Modelling and simulation of a three phase fluidized system applied to attached-growth nitrification of wastewater. *Environmental Engineering and Management Journal* **1**, 517-532

Gerardi MH (2006) *Wastewater Bacteria*, Wiley Interscience, John Wiley and Sons, 255 pp

Giardi MT, Pace E (2007) Photosystem II – Based biosensors for the detection of photosynthetic herbicides. In: Giardi MT, Piletska EV (Eds) *Biotechnological Applications of Photo-Synthetic Proteins: Biochips, Biosensors and Biodevices*, Springer, pp 147-154

Gilbert J, Barth J, Brögger B (2006) Promoting the sustainable management of biowaste across the EU: Bridging the policy gaps. Final ECN Position Paper for Biowaste Conference 31st Mai/1st June 06, pp 1-3

Grady LJR, Daigger GT, Lim HC (1999) *Biological Wastewater Treatment*, Marcel Dekker, New York, 1076 pp

Gravitis J, Abolins J, Kokarevics A (2008) Integration of biorefinery clusters toward zero emissions. *Environmental Engineering and Management Journal* **7**, 569-577

Gray NF (2004) *Biology of Wastewater Treatment* (2^nd^ Edn), World Scientific Publishing, New Jersey, 1444 pp

Grommen R, **Verstraete W** (2002) Environmental biotechnology: the ongoing quest. *Journal of Biotechnology* **98**, 113-23

Gu MB, Mitchell RJ, Kim BC (2004) Whole-cell-based biosensors for environmetal biomonitoring and application. In: Zhong JJ (Ed) *Biomanufacturing*, Scheper T (Series Ed) *Advances in Biochemical Engineering/Biotechnology,* Springer-Verlag Berlin Heidelberg New York, pp 269-305

Gu MB (2005) Environmental biosensors using bioluminescent bacteria. In: Lichtfonse E, Schwarzbaner J, Robert D (Eds) *Environmental Chemistry. Green Chemistry and Pollutants in Ecosystems*, Springer-Verlag Berlin, pp 691-698

Guest RK, Smith DW (2002) A potential new role for fungi in a wastewater MBR biological nitrogen reduction system. *Journal of Environmental Engineering and Science* **1**, 433-437

Gupta SK, Gupta SK, Hung Y-T (2004) Treatment of pharmaceutical waste. In: Wang LK, Hung Y-T, Lo HH, Yapijakis C (Eds) *Handbook of Industrial and Hazardous Wastes Treatment* (2^nd^ Edn), Marcel Dekker, pp 63-130

Gupta S, Seagren E A (2005) Comparison of bioenhancement of nonaqueous phase liquid pool dissolution with first- and zero-order biokinetics. *Journal of Environmental Engineering* **131**, 165-169

Hagger JA, Jones MB, Leonard DRP, Owen R, Galloway TS (2006) Biomarkers and integrated environmental risk assessment: Are there more questions than answers? *Integrated Environmental Assessment and Management* **2**, 312-329

Hamer G (1997) Microbial consortia for multiple pollutant biodegradation. *Pure and Applied Chemistry* **69**, 2343-2356

Hamer K, Arevalo E, Deibel I, Hakstege AL (2007) Assessment of treatment and disposal options. *Sustainable management of Sediment Resource* **2**, 133-159

Hart JK, Martínez K (2006) Environmental Sensor Networks: A revolution in the earth system science? *Earth-Science Reviews* **78**, 177-191

Hashim MA, Uijang Z (2004) Environmental biotechnology: its relevance and prospects for developing countries. In: Vjang Z, Menze M (Eds) *Environmental Biotechnology*, IWA Publishing, pp 7-12

Haug RT (1993) *Practical Handbook of Composting Engineering*, Lewis, Boca Raton, 717 pp

Haupt K, Mosbach K (2000) Molecularly imprinted polymers and their use in biomimetic sensors. *Chemical Reviews* **100**, 2495-2504

Hernández-Luna CE, Gutiérrez-Soto G, Salcedo-Martínez SM (2007) Screening for decolorizing basidiomycetes in Mexico. *World Journal of Microbiology and Biotechnology* **24**, 465-473

Herold T, Biedermann W, Schlegelmilch M, Hensel A (2002) Einfluss verschiedener Filtermaterialien auf den Wirkungsgrad von Biofiltern zur Reinigung von Rotteabluft einer Kompostierungsanlage. *Gefahrstoffe – Reinhaltung der Luft* **62**, 147-153

Héroux M, Pagé T, Gélinas C, Guy C (2004) Evaluating odour impacts from a landfilling and composting site: involving citizens in the monitoring. *Water Science and Technology* **50**, 131-137

Hettenhaus J (2006) Achieving sustainable production of agricultural biomass for biorefinery feedstock. *Industrial Biotechnology* **2**, 257-274

Hwang S, Hansen CL (1998) Characterization and bioproduction of short-chain organic acids from mixed dairy-processing wastewater. *Transactions of the ASABE* **41**, 795-802

IBEP (2006) Introducing the International Bioenergy Platform. Food and Agriculture Organization of the United Nations, Rome. On-line at:
http://esa.un.org/un-energy/pdf/FAO%20Bioenergy%20platform.pdf

Hyun C-K, Tamiya E, Takeuchi T, Karube I (1993) A novel BOD sensor based on bacterial luminescence. *Biotechnology and Bioengineering* **41**, 1107-1111

Ince O (1998) Potential energy production from anaerobic digestion of diary wastewater. *Journal* of *Environmental Science and Health* **33**, 1219-1228

Iranpour R, Deshusses MA, Cox HHJ, Schroeder ED (2002) Practical experiences with biological treatment of odor and VOCs at POTWs in USA. In: *Proceedings WEF Odors and Toxic Air Emissions 2002 Specialty Conference*, Albuquerque NM, April 28 – May 1, Water Environment Federation, pp 705-726

Jamil K (2001) *Bioindicators and Biomarkers of Environmental Pollution and Risk Assessment*, Science Publishers, New Hampshire, USA, 204 pp

Joshi T, Iyengar L, Singh K, Garg S (2008) Isolation, identification and application of novel bacterial consortium TJ-1 for the decolorization of structurally different azo dyes. *Bioresource Technology* **99**, 7115-7121

Jia W, Reitz ET, Lei Y (2007) Biosensors for heavy metal using hydrothermally grown ZnO nanorod/nanotube and metal binding peptides. *Nanotech 2007 (Vol 2) Technical Proceedings of the 2007 NSTI Nanotechnology Conference of Trade Show*, Nano Science and Technology Institute, Cambridge MA, USA, pp 508-510

Johnston DJ (2003) Biotechnology: the next wave of innovation technologies for sustainable development. In: Seralgedin I, Persley GJ (Eds) *Biotechnology and Sustainable Development*: *Voices of the South and North*, CABI Publishing, pp 67-74

Judd S (2006) *The MBR Book: Principles and Applications of Membrane Bioreactors in Water and Wastewater Treatment*, Elsevier, Amsterdam, 344 pp

Kamm B, Kamm M (2004) Principles of biorefineries. *Applied Microbiology and Biotechnology* **64**, 137-145

Kapdan IK, Alpanslan S (2005) Application of anaerobic-aerobic sequential treatment system to real textile wastewater for color and COD removal. *Enzyme and Microbial Technology* **36**, 273-279

Kastner M, Breuer-Jammali M, Mahro B (1998) Impact of inoculation protocols, salinity and pH on the degradation of PAHs and survival of PAH-degrading bacteria introduced into soil. *Applied and Environmental Microbiology* **64**, 359-362

Kazi SK, D'Souza SF, Sar R (2008) Uranium and thorium sequestration by a *Pseudomonas* sp.: Mechanism and chemical characterization. *Journal of Hazardous Materials* **163**, 65-72

Kennes C, Veiga MC (2001) Conventional biofilters. In: Kennes C, Veiga MC (Eds) *Bioreactors for Waste Gas Treatment*, Kluwer Academic Publisher, The Netherlands, pp 47-98

Kennes C, Thalasso F (1998) Waste gas biotreatment technology. *Journal of Chemical Technology and Biotechnology* **72**, 303-319

Khan FI, Husain T, Hejazi R (2004) An overview and analysis of site remediation technologies. *Journal of Environmental Management* **71**, 95-122

Khan MA, Satoh H, Katayanna H, Kurisn H, Mino T (2004) Fluorescently labeled bacteriophages as a means to identify bacteria in activated sludge process. In: Ujiang Z, Henze M (Eds) *Environmental Biotechnology Advancement in Water and Wastewater application in The Tropics*, IWA Publishing, pp 349-356

Kicsi A, Bilba D, Macoveanu M (2006b) Batch copper (II) removal from aqueous solution by sphagnum moss peat. *Environmental Engineering and Management Journal* **5**, 19-28

Kicsi A, Cojocaru C, Macoveanu M, Bilba D (2006a) Optimization of batch process variables using response surface methodology for Cu^{2+} removal from aqueous solution by peat adsorbent. *Environmental Engineering and Management Journal* **5**, 1291-1300

Kilbane JJ II (2006) Microbial biocatalyst developments to upgrade fossil fuels. *Current Opinion in Biotechnology* **17**, 305-314

Kohli P, Martin CR (2005) Smart nanotubes for biotechnology. *Current Pharmaceutical Biotechnology* **6**, 35-47

Krieg EJ (1998) The two faces of toxic waste: trends in the spread of environmental hazards. *Sociological Forum* **13**, 3-20

Krogmann U, Körner I (2000) Technology and strategies of composting. In: Rehm HJ, Reed G (Eds) *Biotechnology Vol. 11c: Environmental Processes III*, Wiley-VCH, Weinheim, pp 127-150

Kryl D (2001) Environmental and industrial biotechnology in developing countries. *Electronic Journal of Biotechnology* **4**, On line at:
http://www.ejbiotechnology.info/content/vol4/issue3/issues/03/index.html

Kuhn T, Pittel K, Schulz T (2003) Recycling for sustainability – a long run perspective? *International Journal of Global Environmental Issues* **3**, 339-s355

Kulbat E, Olanczuk-Neyman K, Quant B, Geneja M, Haustein E (2003) Heavy metals removal in the mechanical-biological wastewater treatment plant "Wschad" in Gdansk. *Polish Journal of Environmental Studies* **12**, 635-641

Kumar V, Wati L, FetzGibbon F, Nigan P, Banat IM, Singh D, Marchant R (1997) Bioremediation and decolonization of an aerobically digested distillery spent wash *Biotechnology Letters* **19**, 311-313

Kumar GS, Gupta SK, Singh G (2007) Biodegradation of distillery spent wash in anaerobic hybrid reactor. *Water Research* **41**, 721-730

Kuswandi B, Mascini M (2005) Enzyme inhibition based biosensors for environmental monitoring. *Current Enzyme Inhibition* **1**, 207-221

Kutzner HJ (2000) Microbiology of composting. In: Rehm HJ, Reed G (Eds) *Biotechnology Vol. 11c: Environmental Processes III*, Wiley-VCH, Weinheim, pp 35-100

Lagadic L, Caquet T, Ramade F (1994) The role of biomarkers in environmental assessment (5). Invertebrate populations and communities. *Ecotoxicology* **3**, 193-208

Lakó J, Hancsók J, Yuzhakova T, Marton G, Utasi A, Rédey Á (2008) Biomass – a source of chemicals and energy for sustainable development. *Environmental Engineering and Management Journal* **7**, 499-509

Lakshmi CV, Kumar M Khann S (2008) Biotransformation of chlorpyrifos and bioremediation of contaminated soil. *International Biodeterioration and Biodegradation* **62**, 204-209

Lam PKS, Gray JS (2003) The use of biomarkers in environmental monitoring. *Marine Pollution Bulletin* **46**, 182-186

Lam PKS (2009) Use of biomarkers in environmental monitoring. *Ocean and Coastal Management* **52***,* 348-354

Langwaldt JH, Puhakka JA (2000) On-site biological remediation of contaminated groundwater: a review. *Environmental Pollution* **107**, 187-197

Le Cloirec P, Andrès Y, Gérente C, Pré P (2005) Biological treatment of waste gases containing volatile organic compounds. In: Shareefdeen Z, Singh A (Eds) *Biotechnology for Odor and Air Pollution Control*, Springer, Berlin, pp 3-16

Lee MD, Odum JM, Buchanan RJ Jr. (1998) New perspective on microbial dehalogenation of chlorinated solvents: insights from the field. *Annual Review of Microbiology* **52**, 423-452

Leahy JG, Colwell RR (1990) Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews* **54**, 305-315

Lema JM, Moreira MT, Palma C, Feijoo G (1999) Clean biological bleaching processes in the pulp and paper industry. In: Olguin EJ, Sánchez G, Hernández E (Eds) *Environmental Biotechnology and Cleaner Bioprocesses,* Taylor and Francis, Boca Raton, pp 211-226

Lens P, van der Maas P, Zandvoort M, Vallero M (2004) New developments in anaerobic environmental biotechnology. In: Ujang Z, Henze M (Eds) *Environmental Biotechnology: Advancement in Water and Wastewater Application in the Tropics*, IWA Publishing, pp 13-18

Li W, Zhang Y, Wang MD, Shi Y (2005) Biodesulphurization of dibenzothiophene and other organic sulphur compounds by a newly isolated *Microbacterium* strain ZD-M2. *FEMS Microbiology Letters* **247**, 45-50

Lin C-W, Cheng Y-W, Tsai S-L (2007) Multi-substrate biodegradation kinetics of MTBE and BTEX mixture by *Pseudomonas aeruginosa*. *Process Biochemistry* **42**, 1211-1217

Lloyd JR (2002) Bioremediation of metals: The application of microorganisms that make and break minerals. *Microbiology Today* **29**, 67-69

Lopez C, Valade A-G, Combourieu B, Mielgo I, Bouchon B, Lema JM (2004) Mechanism of enzymatic degradation of the azo dye Orange II determined by *ex situ* ^{1}H nuclear magnetic resonance and electrospray ionization-ion trap mass spectrometry. *Analytical Biochemistry* **335**, 135-149

Luengo JM, García B, Sandoval A, Naharro G, Olivera ER (2003) Bioplastics from microorganisms. *Current Opinion in Microbiology* **6**, 251-260

Luo H, Liu G, Zhang R, Jin S (2009) Phenol degradation in microbial fuel cells. *Chemical Engineering Journal* **147**, 259-264

Lupasteanu D, Ungureanu F, Gavrilescu M (2004) Studies of various wastewater nitrification bioreactor types based on modelling and simulation. *Envi-*

ronmental Engineering and Management Journal **3**, 101-128

Lyew D, Guiot S (2003) Effects of aeration and organic loading rates on degradation of trichloroethylene in a methanogenic-methanothrophic coupled reactor. *Applied Microbiology Biotechnology* **61**, 206-213

Madejon P, Maranon T, Murillo JM (2006) Biomonitoring of trace elements in the leaves and fruits of wild olive and holm oak trees. *Science of the Total Environment* **355**, 187-203

Malina G, Zawierucha I (2007) Potential of bioaugmentation and biostimulation for enhancing intrinsic biodegradation in oil hydrocarbon-contaminated soil. *Bioremediation Journal* **11**, 141-147

Markert BA, Breure AM, Zechmeister HG (2003) Definitions, strategies and principles for bioindication/biomonitoring of the environment. In: Markert BA, Breure AM, Zechmeister HG (Eds) *Bioindicators and Biomonitors*, Elsevier Science, Oxford, pp 3-39

Maletesta C, Guascito MR (2005) Heavy metal determination by biosensors based on enzyme immobilized by electropolymerization. *Biosensors and Bioelectronics* **20**, 1643-1647

Marques-Rocha FJ, Hernández-Rodrigues V, Lamela MAT (2000) Biodegradation of diesel oil by microbial consortium. *Water, Soil and Air Pollution* **128**, 313-320

Martienssen M, Fabritius M, Kukla S, Balcke G, Hasselwander E, Schirmer M (2006) Determination of naturally occurring MTBE biodegradation by analyzing metabolites and biodegradation by-products. *Journal of Contaminant Hydrology* **87**, 37-53

Marty J–L, Miovetto N, Noguer T, Ortega F, Roux C (1993) Enzyme sensor for the detection of pesticides. *Biosensors and Bioelectronics* **8**, 237-280

Marty J–L, Lecea B, Naguer T (1998) Biosensors for the detection of pesticides. *Analysis* **26**, 144-148

Mata-Alvarez J, Macé S, Llabrés P (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology* **74**, 3-16

Mauriz E, Calle A, Montoya A, Lechuga LM (2006) Determination of environmental organic pollutants with a portable optical immunosensor. *Talanta* **69**, 359-364

Mazzanti M, Zoboli R (2008) Waste generation, waste disposal and policy effectiveness: Evidence on decoupling from the European Union. *Resources, Conservation and Recycling* **52**, 1221-1234

MCD (2008) Biopesticides, Microbial Control Division. On-line at: http://www.dropdata.net/Sip_micontrol/biopesticides.htm

Menn JJ, Hall FR (1999) Biopesticides: Present status and future prospects. In: Hall FR, Menn JJ (Eds) *Biopesticides, Use and Delivery*, Humana Press, Totowa, New Jersey, USA, pp 1-10

Mertens J, Luyssaert S, Verheyen K (2005) Use and abuse of trace metal concentrations in plant tissue for biomonitoring and phytoextraction. *Environmental Pollution* **138**, 1-4

Metcalf and Eddy Inc. (1991) *Wastewater Engineering: Treatment, Disposal and Reuse* (3rd Edn), McGraw-Hill, New York, 1334 pp

Mohana S, Desai Chiranyn, Madammar D (2007) Biodegradation and decolorization of anaerobically treated distillery spent wash by a novel bacterial consortium. *Bioresource Technology* **98**, 333-339

Mohana S, Archarya BK, Madammar D (2009) Distillery spent wash: treatment technologies and potential application. *Journal of Hazardous Materials* **163**, 12-25

Moharikar A, Purohit HJ, Kumar R (2005) Microbial population dynamics at effluent treatment plants. *Journal of Environmental Monitoring* **7**, 552-558

Mohn WW (1997) Indirect bioremediation: biodegradation of hydrocarbons on a commercial sorbent. *Biodegradation* **8**, 15-19

Moldes C, García P, García JL, Prieto MA (2004) *In vivo* immobilization of fusion proteins on bioplastics by the Novel Tag BioF. *Applied and Environmental Microbiology* **70**, 3205-3212

Monticello DJ (2000) Biodesulphurization and the upgrading of petroleum distillates. *Current Opinion in Biotechnology* **11**, 540-546

Mulchandani A, Rogers KR (Eds) (1998) *Enzyme and Microbial Biosensors: Techniques and Protocols*, Humana Press, Totowa, New Jersey, 284 pp

Mulligan CN (2002) *Environmental Biotreatment*, Government Institutes, Rockville, Maryland, USA, 395 pp

Murthy YS, Subbiah V, Rao DS, Reddy RC, Kumar LS, Elyas SI, Rao RKG, Gadgill JS, Deshmukh SB (1984) Treatment and disposal of wastewater from synthetic drugs plant (IDPL), hyderabad, Part I-Wastewater characteristics. *Indian Journal of Environmental Health* **26**, 7-19

Mwinyihija M, Meharg A, Dawson J, Strachan NJC, Killham K (2006) An ecotoxicological approach to assessing the impact of tanning industry effluxent on river health. *Archives of Environmental Contamination and Toxicology* **50**, 316-324

Nakajima F, Izu K, Yamamoto K (2001) Material recovery from wastewater using photosynthetic bacteria. In: Matsuo T, Hanaki K, Takizawa S, Satoh H (Eds) *Advances in Water and Wastewater Treatment Technology. Molecular Technology, Nutrient Removal, Sludge Reduction and Environmental Health*, Elsevier, Amsterdam, pp 261-269

Nali C, Francini A, Lorenzini G (2006) Biological monitoring of ozone: the twenty-year Italian experience. *Journal of Environmental Monitoring* **8**, 25-32

Nazaroff WW, Alvarez-Cohen L (2001) *Environmental Engineering Science*, John Wiley and Sons, Inc., New York, 704 pp

Nicell JA (2003) Enzymatic treatment of waters and wastes. In: Tarr MA (Ed) *Chemical Degradation Methods for Wastes and Pollutants. Environmental and Industrial Applications*, Marcel Dekker, New York, pp 423-476

Novozymes (2001) *Enzymes at work*. Novozymes A/S, Bagsvaer, Denmark, On-line at: http://www.novozymes.com/NR/rdonlyres/FB09CA43-EA35-4F0B-B55C-909C3D916B08/0/EnzymesAtWork20010026904.pdf

NRC (1987) Biological markers in environmental health research (National Research Council. Committee on biological markers). *Environmental Health Perspectives* **74**, 3-9

OECD (1994) *Biotechnology for a Clean Environment: Prevention, Detection, Remediation*, OECD Publishing, Paris, 204 pp

OECD (1998) *Biotechnology for Clean Industrial Products and Processes. Towards Industrial Sustainability*, OECD Publishing, Paris, 200 pp

OECD (2001) *The Application of Biotechnology to Industrial Sustainability*, OECD Publishing, Paris, 158 pp

Ohtake H, Hardoyo JK (1992) New biological method for detoxification and removal of hexavalent chromium. *Water Science and Technology* **25**, 395-405

Oktem YA, Ince O, Sallis P, Donnelly T, Ince BK (2007) anaerobic treatment of chemical synthesis-based pharmaceutical wastewater in a hybrid upflow anaerobic sludge blanket reactor. *Bioresource Technology* **99**, 1089-1096

Olguin EJ (1999) Cleaner bioprocesses and sustainable development. In: Olguin EJ, Sánchez G, Hernández E (Eds) *Environmental Biotechnology and Cleaner Bioprocesses*, Taylor and Francis, Boca Raton, pp 3-18

Pandey A (2004) *Concise Encyclopedia of Bioresource Technology*, Food Products Press - The Haworth Reference Press, New York, 735 pp

Parales RE, Bruce NC, Schmid A, Wackett LP (2002) Biodegradation, biotransformation, and biocatalysis (B3). *Applied and Environmental Microbiology* **68**, 4699-4709

Parshetti GK, Kalme SD, Gomare SS, Govindwar SP (2007) Biodegradation of reactive Blue-25 by *Aspergillus ochraceus* NCIM-1146. *Bioresource Technology* **98**, 3638-3642

Patel YB, Paknikar KM (2000) Development of a process for biodetoxification of metal cyanides for wastewater. *Process Biochemistry* **35**, 1139-1151

Pavel LV, Penciu OM, Gavrilescu M, Ungureanu F (2004) Modeling and simulation of three-phase systems with fixed bed applied for the treatment of gaseous effluents. *Bulletin of the Polytechnic Institute of Iasi* **54**, 115-128

Pavel LV, Gavrilescu M (2008) Overview of *ex situ* decontamination techniques for soil cleanup. *Environmental Engineering and Management Journal* **7**, 815-834

Penciu OM, Ungureanu F, Gavrilescu M (2004) Modelling and simulation of three phase bioreactors applied to the depollution of gaseous streams containing volatile organic compounds – A comparison between fixed bed and fluidized bed reactors. *Environmental Engineering and Management Journal* **3**, 177-197

Penciu OM, Gavrilescu M (2003) Survey on the treatment of gaseous streams containing volatile organic compounds. *Environmental Engineering and Management Journal* **2**, 77-160

Penciu OM, Gavrilescu M (2004) Biodegradation - Innovative technology for treating gaseous fluxes containing VOCs. *Environmental Engineering and Management Journal* **3**, 737-754

Piletska EV, Piletsky SA, Rouillon R (2006) Application of chloroplast D1 protein in biosensors for monitoring photosystems II – Inhibiting herbicides. In: Giardi MT, Piletska EU (Eds) *Biotechnological Application of Photosynthetic Proteins: Biodips, Biosensors and Biodevices*, Springer, Berlin, pp 130-146

Poindexter JS, Pujara KP, Staley JT (2000) *In situ* reproductive rate of freshwater *Caulobacter* spp. *Applied Environmental Microbiology* **66**, 4105-4111

Pokhrel D, Viraraghavan D (2004) Treatment of pulp and paper mill wastewater – a review. *Science of the Total Environment* **333**, 37-58

Pullman GS, Cairney J, Peter G (1998) Clonal forestry and genetic engineering: where we stand, future prospects and potential impacts on mill operations. *TAPPI Journal* **81**, 57-63

Rabbow E, Rettberg P, Baumstark-Khan C, Horneck G (2002) SOS-LUX and LAC-FLUORO-TEST for the quantification of genotoxic and/or cytotoxic effects of heavy metal salts. *Analytical Chimica Acta* **456**, 31-39

Raeymaekers B (2006) A prospective biomonitoring campaign with honey bees in a district of Upper-Bavaria (Germany). *Environmental Monitoring and Assessment* **116**, 233-243

Ramsden JJ (1999) A sum parameter sensor for water quality. *Water Research* **33**, 1147-1150

Raynal M, Pruden A (2008) Aerobic MTBE biodegradation in the presence of BTEX by two consortia under batch and semi-batch conditions. *Biodegradation* **19**, 427-432

Reddy CSK, Ghai R, Rashmi, Kalia VC (2003) Polyhydroxyalkanoates: an overview. *Bioresource Technology* **87**, 137-146

Riedel K, Naumov AV, Boronin AM, Golovleva LA, Stein HJ, Scheller F (1991) Microbial sensors for determination of aromatics and their chloroderivatives I: Determination of 3-chlorobenzoate using a *Pseudomonas*-containing biosensors. *Applied Microbiology and Biotechnology* **35**, 559-562

Reiss M, Hartmeier W (1999) Monitoring of environmental processes with biosensors. In: Rehm H-I, Reed G (Eds) *Biotechnology* (2nd Edn), Wiley-VCH, Weinheim, pp 125-139

Reis LFL, Van Sluys M-A, Garratt RC, Pereira HM, Teixeira MM (2006) GMOs: Building the future on the basis of past experience. *Anais da Academia Brasileira de Ciencias* **78**, 667-686

Rigaux F (1997) *Industrial Biotechnology in the Atlantic Provinces. From Emergence to Development?* Toronto: The Canadian Institute for Research on Regional Development, 139 pp

Riser-Roberts E (1998) *Remediation of Petroleum Contaminated Soils. Biological, Physical and Chemical Processes*, Lewis Publishers, CRC Press, 542 pp

Rodríguez-Mozaz S, Marco M-P, López de Alda MJ, Barceló D (2004) Biosensors for environmental applications: Future development trends. *Pure and Applied Chemistry* **76**, 723-752

Rodríguez-Mozaz S, López de Alda MJ, Barceló D (2006) Biosensors as useful tools for environmental analysis and monitoring. *Analytical and Bioanalytical Chemistry* **386**, 1025-1041

Rogers K (1995) Biosensors for environmental applications. *Biosensors Bioelectron* **10**, 533-541

Rosenwinkel K–H, Austermann–Hann U, Meyer H (1999) Industrial wastewater source and treatment strategies. In: Rehm HJ, Reed G (Eds) *Biotechnology* (2nd Edn), Wiley VCH Weinheim, pp 193-215

Russell DL (2006) *Practical Wastewater Treatment*, John Wiley & Sons, Hoboken, New Jersey, 288 pp

Saling P (2005) Eco-efficiency analysis of biotechnological processes. *Applied Microbiology and Biotechnology* **68**, 1-8

Salminen E, Rintala J (2002) Anaerobic digestion of organic solid poultry slaughterhouse waste – a review. *Bioresource Technology* **82**, 13-26

Sandau E, Sandau P, Pulz O, Zimmermann M (1996) Heavy metal sorption by marine algae and algal by-products. *Acta Biotechnologica* **16**, 103-119

Sanz JL, Kochling T (2007) Molecular biology techniques used in wastewater treatment: An overview. *Process Biochemistry* **42**, 119-133

Samkutty PJ, Gough RH (2002) Filtration treatment of dairy processing wastewater. *Journal of Environmental Science and Health* **A37**, 195-199

Saratale RG, Saratale GD, Kalyani DC, Chang JS, Govindwar SP (2009) Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium GR. *Bioresource Technology* **100**, 2493-2500

Sarkar B, Chakraharti PP, Vijaykumar A, Kale V (2006) Wastewater treatment in dairy industries-possibility of reuse. *Desalination* **195**, 141-152

Sasaki T, Kajino T, Li B, Sugiyama H, Takahashi H (2001) New pulp biobleaching system involving manganese peroxidase immobilized in a silica support with controlled pore sizes. *Applied and Environmental Microbiology* **67**, 2208-2212

Sasikumar CS, Papinazath T (2003) Environmental management: bioremediation of polluted environment. In: Bunch MJ, Suresh VM, Kumaran TV (Eds) *Proceedings of the Third International Conference on Environment and Health*, Chennai, India, 15-17 December, Chennai: Department of Geography, University of Madras and Faculty of Environmental Studies, York University, pp 465-469

Satyawali Y, Balakrishanan M (2008) Wastewater treatment in molasses based alcohol distilleries for COD and color removal: A review. *Journal of Environmental Management* **86**, 481-497

Saval S (1999) Bioremediation: Clean-up biotechnologies for soils and aquifers. In: Olguin EJ, Sánchez G, Hernández E (Eds) *Environmental Biotechnology and Cleaner Bioprocesses*, Taylor and Francis, Boca Raton, pp 155-166

Sayler GS, Shiaris MP, Beck TW, Held S (1982) Effects of polychlorinated biphenyls and environmental biotransformation products on aquatic nitrification. *Applied and Environmental Microbiology* **43**, 949-952

Scheller FW, Schubert F, Fedrowitz J (1997) *Frontiers in Biosensorics I. Fundamental Aspects*, Birkhauses, Basel, 287 pp

Schuchardt F (2005) Composting of organic waste. In: Jördening H-J, Winter J (Eds) *Environmental Biotechnology. Concepts and Application*, Wiley-VCH, Weinheim, pp 333-354

Schlegelmilch M, Streese J, Biedermann W, Herold T, Stegmann R (2005) Reducing odorous emissions from biowaste composting plants by means of biological waste gas treatment systems. Proceedings (CD-ROM) of Sardinia 2005 – 10th International Waste Management and Landfill Symposium. Cossu R, Stegmann R (Eds) Session G4: Odour analyses, Cagliari, Italy, 3-7 October 2005, *CISA,* Environmental Sanitary Engineering Centre, Italy

Seidel H, Mattusch J, Wennrich R, Morgenstern P, Ondruschka J (2002) Mobilization of arsenic and heavy metals from contaminated sediments by changing the environmental conditions. *Acta Biotechnologica* **22**, 153-160

Selcuk H (2005) Decolorization and detoxification of textile wastewater by ozonation and coagulation process. *Dyes and Pigments* **64**, 217-222

Sen R, Samanta TB (1981) C21 – steroids by *Syncephalastrum racemosum*. *Journal of Steroid Biochemistry* **14**, 307-309

Senan RC, Abraham TE (2004) Bioremediation of textile azo dyes by aerobic bacterial consortium. *Biodegradation* **15**, 275-280

Shareefdeen Z, Herner B, Singh A (2005) Biotechnology for air pollution control – an overview. In: Shareefdeen Z, Singh A (Eds) *Biotechnology for Odor and Air Pollution Control*, Springer, Berlin-Heidelberg, pp 3-16

Shukla OP, Rai UN, Subramanyam SV (2004) Biopulping and biobleaching: an energy and environment saving technology for indian pulp and paper industry. *EnviroNews* **10**. On line at: http://www.geocities.com/isebindia/01_04/04-04-3.html

Shukla AK, Vishmakarma P, Upodhyay SN, Tripathi AK, Prasana HC, Dubey SK (2009) Biodegradation of trichloroethylene (TCE) by methanotropic community. *Bioresource Technology* **100**, 2469-2474

Silver S, Misra TK, (1988) Plasmid mediated heavy metal resistance. *Annual Review of Microbiology* **42**, 717-743

Singhal PK, Shrivastava P (2004) *Challenges in Sustainable Development*, Anmol Publications Pvt. Ltd., New Delhi, India, 373 pp

Sirtori C, Zapata A, Oller I, Gernjak W, Aguera A, Malato S (2009) Decontamination industrial pharmaceutical wastewater by combining solar photo-Fenton and biological treatment. *Water Research* **43**, 661-668

Smith LA, Alleman BC, Copley-Graves L (1994) Biological treatment options. In: Means JL, Hinchee RE (Eds) *Emerging Technology for Bioremediation of Metals*, Lewis Publishers, New York, pp 1-12

Stapleton RD, Savage DC, Sayler GS, Stacey G (1998) Biodegradation of aromatic hydrocarbons in an extremely acidic environment. *Applied and Environmental Microbiology* **64**, 4180-4184

Starodub NF, Katzev AM, Starodub VM, Levkovetz IA, Goncharuk VV, Klimenko NA, Shmir'ova AN, Piven NV, Dzantijev BB (2005) Biosensors for water quality monitoring. In: Omelchenko A, Pivovarov AA, Swindall WJ (Eds) *Modern Tools and Methods of Water Treatment for Improving Living Standards*, Proceedings of the NATO Advanced Research Workshop on Modern Tools and Methods of Water Treatment for Improving Living Standards Dnepropetrovsk, Ukraine 19–22 November 2003, pp 51-70

Steffan S, Bardi L, Marzona M (2005) Azo dye biodegradation by microbial cultures immobilized in alginate beds. *Environment International* **31**, 201-205

Steiner M (2005) The status of mechanical-biological treatment of residual waste and utilization of refuse-derived fuels in Europe. Presentation to the Conference *The Future of Residual Waste Management in Europe*, 17-18 November, Luxembourg, On line at: http://www.orbit-online.net/orbit2005/vortraege/steiner-ppt.pdf

Stevens ES (2002) *Green Plastics: An Introduction to the New Science of Biodegradable Plastics*, Princeton University Press, New Jersey, USA, 272 pp

Strydom JP, Britz TJ, Moster JF (1997) Two phase anaerobic digestion of three different dairy effluents using a hybrid bioreactor. *Water SA* **23**, 151-156

Sukla LB, Panchanadikar V (1993) Bioleaching of lateric nickel ore using a heterotrophic micro-organism. *Hydrometallurgy* **32**, 373-379

Sukumaran Nair MP (2006) Environmental biotechnology for sustainable chemical processing, *wfeo* **27**. On line at: http://www.wfeo-cee.org/news/v27n10pg2.htm

Talley J (2005) Introduction to recalcitrant compounds. In: Jaffrey W, Talley J (Eds) *Bioremediation of Recalcitrant Compounds*, CRC Press, Boca Raton, pp 1-9

Tarazona JV, Fernández MD, Vega MM (2005) Regulation of contaminated soils in Spain – a new legal instrument. *Journal of Soils and Sediments* **5**, 121-124

TBV GmbH (2000) Anaerobic methods of waste treatment. *Gate. Technical Information W2e,* On line at: http://www.gate-international.org/documents/techbriefs/webdocs/pdfs/w2e_2000.pdf

Tescione L, Belfort G (1993) Construction and evaluation of a metal ion biosensor. *Biotechnology Bioengineering* **42**, 945-952

Timmis KN, Steffan RJ, Unterman R (1994) Designing microorganisms for the treatment of toxic wastes. *Annual Reviews in Microbiology* **48**, 525-557

Tom-Petersen A, Leser TD, Marsh TL, Nybroe O (2003) Effects of copper amendment on the bacterial community in agricultural soil analyzed by the T-RFLP technique. *FEMS Microbiology Ecology* **46**, 53-62

Torkian A, Dehghanzadeh D, Hakimjavadi M (2003) Biodegradation of aromatic hydrocarbons in a compost biofilter. *Journal of Chemical Technology and Biotechnology* **78**, 795-801

Trejo M, Quintero R (1999) Bioremediation of contaminated soils. In: Olguin EJ, Sánchez G, Hernández E (Eds) *Environmental Biotechnology and Cleaner Bioprocesses*, Taylor and Francis, Boca Raton, pp 179-190

Tucker CL, Fields S (2001) A yeast sensor of ligand binding. *Nature Biotechnology* **19**, 1042-1046

Tuppurainen KO, Väisänen O, Rintala JA (2002) Sulphate reducing laboratory scale high-rate anaerobic reactors for treatment of metal and sulphate containing mine wastewaters. *Environmental Technology* **23**, 599-608

Tuzen M, Sarıa A, Mendil D, Uluozlu OD, Soylak M, Dogan M (2009) Characterization of biosorption process of As(III) on green algae *Ulothrix cylindricum. Journal of Hazardous Materials* **165**, 566-572

Uludağ Y, Piletsky SA, Turner APF, Cooper MA (2007) Piezoelectric sensors based on molecular imprinted polymers for detection of low molecular mass analytes. *FEBS Journal* **274**, 5471-5480

Urbain V, Benoit R, Manem J (1996) Membrane bioreactor: A new treatment tool. *Journal American Water Works Association* **88**, 75-86

USEPA (1994) Assessment and Remediation of Contaminate Sediments (ARCS) Program. Final Summary Report, EPA-905-S-94-001, USEPA, Chicago

van Beuzekom B, Arundel A (2006) *OECD Biotechnology Statistics – 2006.* OECD, Available on-line at: http://www.oecd.org/dataoecd/51/59/36760212.pdf

van Wyk JPH (2001) Biotechnology and the utilization of biowaste as a resource for bioproduct development. *Trends in Biotechnology* **19**, 172-177

Veglio F, Beolcini F (1997) Removal of metals by biosorption: A review. *Hydrometallurgy* **44**, 301-316

Verma N, Singh M (2005) Biosensors for heavy metals. *Biometals* **18**, 121-129

Vidali M (2001) Bioremediation. An overview. *Pure and Applied Chemistry* **73**, 1163-1172

Vilar VJP, Botelho CMS, Boaventura RAR (2007) Kinetics and equilibrium modelling of lead uptake by algae *Gelidium* and algal waste from agar extraction industry. *Journal of Hazardous Materials* **143**, 396-408

Vo–Dinh T (2007) *Biosensors and biochips*. In: Ferrari M, Bashir R, Wereley S (Eds) *BioMEMS and Biomedical Nanotechnology (Vol IV) Biomolecules Sensing, Processing and Analysis*, Springer, Berlin, pp 1-20

Volesky B (1990) *Biosorption of Heavy Metals*, CRC Press, Boca Raton, Florida, 396 pp

Volesky B, May H, Holan ZR (1993) Cd(II) biosorption by *Saccharomyces cerevisiae. Biotechnology Bioengineering* **41**, 826-829

Wagner M, Amann R (1997) Molecular techniques for determining microbial community structures in activated sludge. In: Cloete TE, Muyima NYO (Eds) *Microbial Community Analysis: The Key to the Design of Biological Wastewater Treatment Systems*, University Press, Cambridge, pp 61-71

Wagner M, Loy A, Nogueira R, Purkhold U, Lee N, Daims H (2002) Microbial community composition and function in wastewater treatment plants. *Antonie van Leeuwenhoek* **81**, 665-680

Wall-Markowski CA, Kicherer A, Saling P (2005) Using eco-efficiency analysis to assess renewable-resource-based technologies. *Environmental Progress* **23**, 329-333

Wang J, Chen C (2006) Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. *Biotechnology Advances* **24**, 427-451

Wang J, Xing D, Zhang L, Jia L (2007) A new principle photosynthesis capacity biosensor based on quantitative measurement of delayed fluorescence *in vivo*. *Biosensors and bioelectronics* **22**, 2861-2868

Watson JS (1999) *Separation Methods for Waste and Environmental Applications*, Marcel Dekker, New York, 616 pp

Waul C, Arvin E, Schmidt JE (2009) Long term studies on the anaerobic biodegradability of MTBE and gasoline ethers. *Journal of Hazardous Materials* **163**, 427-432

WBCSD (2000) *Eco-efficiency. Creating More Value with Less Impact*, World Business Council for Sustainable Development, Geneva, Switzerland

Weitz HJ, Campbell CD, Killham K (2002) Development of a novel, bioluminescence-based, fungal bioassay for toxicity testing. *Environmental Microbiology* **4**, 422-429

Wiesmann U, Choi IS, Dombrowski E-M (2007) *Fundamentals of Biological Wastewater Treatment*, Wiley-VCH, Weinheim, 391 pp

Willke T, Prüsee U, Vorlop K-D (2006) Biocatalytic and catalytic routes for the production of bulk and fine chemicals from renewable resources. In: Kamm B, Gruber PR, Kamm M (Eds) *Biorefineries - Industrial Processes and Products. Status Quo and Future Directions* (Vol 1). Wiley-VCH, Weinheim, Germany, pp 385-406

Wilson JT, Wilson BH (1985) Biotransformation of thrichloroethylene in soil. *Applied and Environmental Microbiology* **49**, 242-243

Wong S-K N, O'Connell M, Wisdom JA, Dai H (2005) Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proceedings of the National Academy of Sciences USA* **102**, 11600-11605

Xu T, Chen C, Liu C, Zhang S, Wu Y, Zhang P (2009) A novel way to enhance the oil recovery ratio by *Streptococcus* sp. BT-003. *Journal of Basic Microbiology* **49 (5)**, 477-481

Yamamoto K (2001) Membrane bioreactor: an advanced wastewater treatment/reclamation technology and its function in excess-sludge minimization. In: Matsuo T, Hanaki K, Takizawa S, Satoh H (Eds) *Advances in Water and Wastewater Treatment Technology. Molecular Technology, Nutrient Removal, Sludge Reduction and Environmental Health*, Elsevier, Amsterdam, pp 229-237

Zanardini E, Pisoni C, Ranalli G, Zucchi M, Sorlini C (2002) Methyl tert-butyl ether (MTBE) bioremediation studies. *Annals of Microbiology* **52**, 207-221

Zelano V, Torazzo A, Berto S, Ginepro M, Prenesti E, Ferrari A (2006) Biomonitoring of traffic originated PAHs in the air. *International Journal of Environmental and Analytical Chemistry* **86**, 527-540

Zhong W-H, Chen J-M, Lu Z, Chen D-Z, Chen X (2007) Aerobic degradation of methyl tertbutyl ether by a proteobacteria strain in a closed cultural system. *Journal of Environmental Sciences* **19**, 18-22

Zouboulis AI, Loukidon MX, Matis KA (2004) Biosorption of toxic metals from aqueous solution by a bacteria strain isolated from metal-polluted soil. *Process Biochemistry* **39**, 909-916

Zouboulis AI, Lazaridis NK, Matias KA (2002) Removal of toxic metal ions from aqueous systems by biosorptive flotation. *Journal of Chemical Technology and Biotechnology* **77**, 958-964