HANDBOOK OF ENVIRONMENTAL ENGINEERING™ VOLUME 10

Environmental Biotechnology

Edited by

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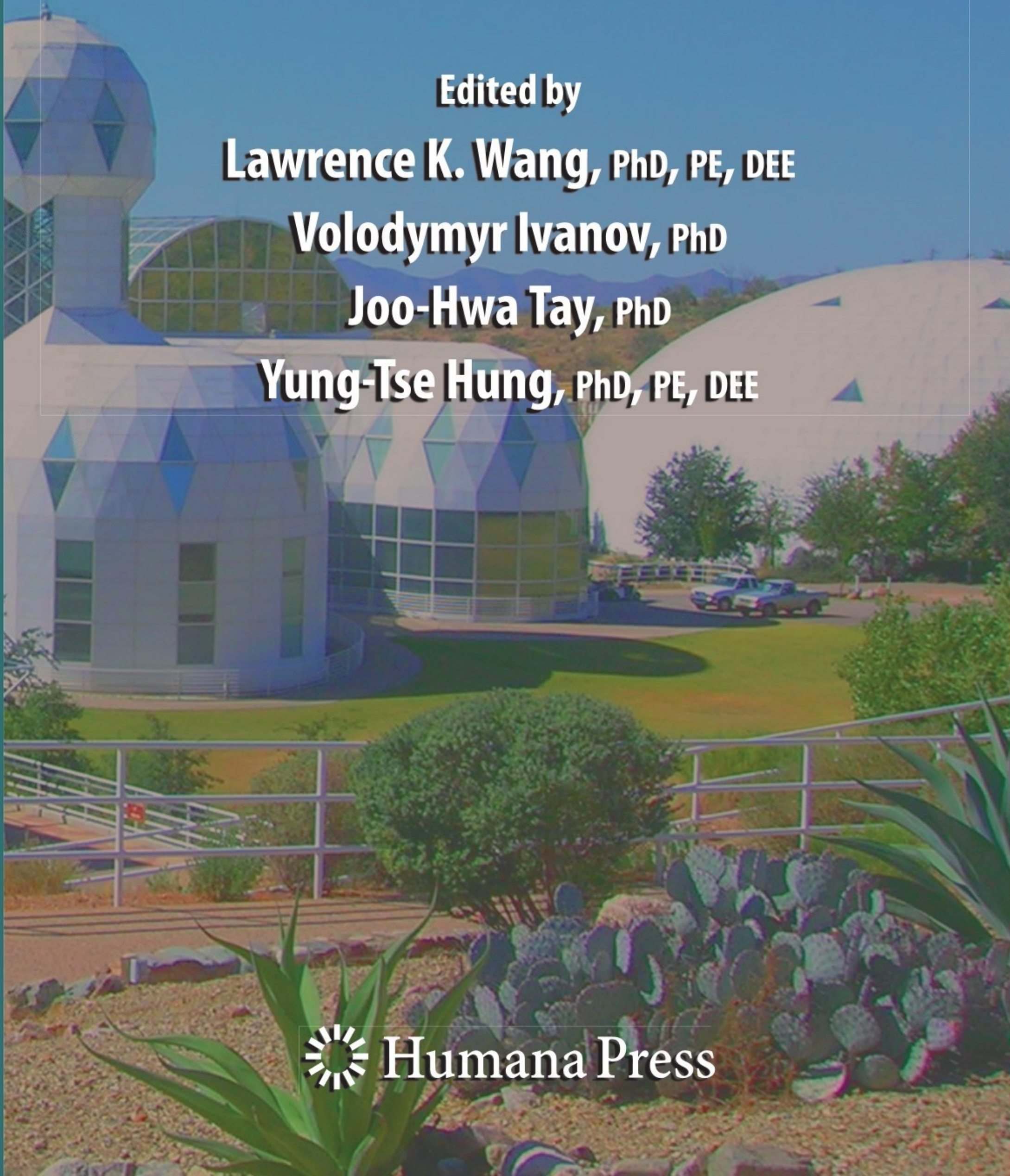

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Environmental Biotechnology

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Dedications

The Editors of the Handbook of Environmental Engineering series dedicate this volume to late Thomas L. Lanigan (1938–2006), the founder and former president of Humana Press, who encouraged and vigorously supported the editors and many contributors around the world to embark on this ambitious, life-long handbook project (1978 to present) for the sole purpose of protecting our environment, in turn, benefiting our entire mankind.

The Editors of this Handbook series also would like to dedicate this volume to Dr. Jao Fan Kao (1923–2008) of National Cheng Kung University (NCKU), Tainan, Taiwan, ROC. Dr. Kao was the founder and former Professor of the University's Department of Environmental Engineering. He educated over 1,500 environmental and civil engineers to serve the planet of earth. Both Dr. Lawrence K. Wang, Chief Editor, and Dr. Yung-Tse Hung, Co-editor, were Dr. Kao's students at National Cheng Kung University.

Preface

The past 30 years have seen the emergence of a growing desire worldwide that positive actions be taken to restore and protect the environment from the degrading effects of all forms of pollution – air, water, soil, and noise. Since pollution is a direct or indirect consequence of waste production, the seemingly idealistic demand for "zero discharge" can be construed as an unrealistic demand for zero waste. However, as long as waste continues to exist, we can only attempt to abate the subsequent pollution by converting it to a less noxious form. Three major questions usually arise when a particular type of pollution has been identified: (1) How serious is the pollution? (2) Is the technology to abate it available? and (3) Do the costs of abatement justify the degree of abatement achieved? This book is one of the volumes of the *Handbook of Environmental Engineering* series. The principal intention of this series is to help readers formulate answers to the last two questions above.

The traditional approach of applying tried-and-true solutions to specific pollution problems has been a major contributing factor to the success of environmental engineering, and has accounted in large measure for the establishment of a "methodology of pollution control." However, the realization of the ever-increasing complexity and interrelated nature of current environmental problems renders it imperative that intelligent planning of pollution abatement systems be undertaken. Prerequisite to such planning is an understanding of the performance, potential, and limitations of the various methods of pollution abatement available for environmental scientists and engineers. In this series of handbooks, we will review at a tutorial level a broad spectrum of engineering systems (processes, operations, and methods) currently being utilized, or of potential utility, for pollution abatement. We believe that the unified interdisciplinary approach presented in these handbooks is a logical step in the evolution of environmental engineering.

Treatment of the various engineering systems presented will show how an engineering formulation of the subject flows naturally from the fundamental principles and theories of chemistry, microbiology, physics, and mathematics. This emphasis on fundamental science recognizes that engineering practice has in recent years become more firmly based on scientific principles rather than on its earlier dependency on empirical accumulation of facts. It is not intended, though, to neglect empiricism where such data lead quickly to the most economic design; certain engineering systems are not readily amenable to fundamental scientific analysis, and in these instances we have resorted to less science in favor of more art and empiricism.

Since an environmental engineer must understand science within the context of application, we first present the development of the scientific basis of a particular subject, followed by exposition of the pertinent design concepts and operations, and detailed explanations of their applications to environmental quality control or remediation. Throughout the series, methods of practical design and calculation are illustrated by numerical examples. These examples clearly demonstrate how organized, analytical reasoning leads to the most direct and clear solutions. Wherever possible, pertinent cost data have been provided.

Our treatment of pollution-abatement engineering is offered in the belief that the trained engineer should more firmly understand fundamental principles, be more aware of the similarities and/or differences among many of the engineering systems, and exhibit greater flexibility and originality in the definition and innovative solution of environmental pollution problems. In short, the environmental engineer should by conviction and practice be more readily adaptable to change and progress.

Coverage of the unusually broad field of environmental engineering has demanded an expertise that could only be provided through multiple authorships. Each author (or group of authors) was permitted to employ, within reasonable limits, the customary personal style in organizing and presenting a particular subject area; consequently, it has been difficult to treat all subject material in a homogeneous manner. Moreover, owing to limitations of space, some of the authors' favored topics could not be treated in great detail, and many less important topics had to be merely mentioned or commented on briefly. All authors have provided an excellent list of references at the end of each chapter for the benefit of interested readers. As each chapter is meant to be self-contained, some mild repetition among the various texts was unavoidable. In each case, all omissions or repetitions are the responsibility of the editors and not the individual authors. With the current trend toward metrication, the question of using a consistent system of units has been a problem. Wherever possible, the authors have used the British system (fps) along with the metric equivalent (mks, cgs, or SIU) or vice versa. The editors sincerely hope that this duplicity of units' usage will prove to be useful rather than being disruptive to the readers.

The goals of the *Handbook of Environmental Engineering* series are: (1) to cover entire environmental fields, including air and noise pollution control, solid waste processing and resource recovery, physicochemical treatment processes, biological treatment processes, biosolids management, water resources, natural control processes, radioactive waste disposal, and thermal pollution control; and (2) to employ a multimedia approach to environmental pollution control since air, water, soil, and energy are all interrelated.

As can be seen from the above handbook coverage, no consideration is given to pollution by the type of industry, or to the abatement of specific pollutants. Rather, the organization of the handbook series has been based on the three basic forms in which pollutants and waste are manifested: gas, solid, and liquid. In addition, noise pollution control is included in the handbook series.

This particular book, Vol. 10, *Environmental Biotechnology*, mainly deals with theories and principles of biotechnologies, and is a sister book to Vol. 11, *Environmental Bioengineering*, which mainly deals with environmental applications of microbiological processes and technologies.

Specifically this book, Vol. 10, *Environmental Biotechnology*, introduces the mechanisms of environmental biotechnology processes, different microbiological classifications useful for environmental engineers, microbiology, metabolism, and microbial ecology of natural and environmental engineering systems, microbial ecology and bioengineering of isolated life support systems, classification and design of solid-state processes and reactors, value-added biotechnological products from organic wastes, design of anaerobic suspended bioprocesses and reactors, selection and design of membrane bioreactors, natural environmental biotechnologies systems, aerobic and anoxic suspended-growth systems, aerobic and anaerobic attached-growth systems, and sequencing batch reactors.

This book's sister book, *Environmental Bioengineering*, Vol. 11, however, introduces various environmental applications, such as land disposal of biosolids, heavy metal removal by crops, pretreatment of sludge for sludge digestion, biotreatment of sludge, fermentaion of kitchen garbage, phytoremediation for sludge treatment, phyotoremediation for heavy metal removal from contaminated soils, vetiver grass bioremediatioon, wetland treatment, biosorption of heavy metals, rotating biological contactors (RBC) for carbon and nitrogen removal, anaerobic biofilm reactor, biological phosphorus removal, black and grey water treatment, milk wastewater treatment, tomato wastewater treatment, gelatine and animal glue production from skin wastes, fungal biomass protein production, algae harvest energy conversion, and living machine for wastewater treatment.

Both books together (Vols. 10 and 11) have been designed to serve as comprehensive biotechnology textbooks as well as wide-ranging reference books. We hope and expect they will prove of equal high value to advanced undergraduate and graduate students, to designers of water and wastewater treatment systems, and to scientists and researchers. The editors welcome comments from readers in all of these categories.

The editors are pleased to acknowledge the encouragement and support received from their colleagues and the publisher during the conceptual stages of this endeavor. We wish to thank the contributing authors for their time and effort, and for having patiently borne our reviews and numerous queries and comments. We are very grateful to our respective families for their patience and understanding during some rather trying times.

Lawrence K. Wang, Lenox, Massachusetts
Volodymyr Ivanov, Singapore
Tay Joo Hwa, Singapore
Yung-Tse Hung, Cleveland, Ohio

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1
Applications of Environmental Biotechnology

Volodymyr Ivanov and Yung-Tse Hung

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Abstract Environmental biotechnology is a system of scientific and engineering knowledge related to the use of microorganisms and their products in the prevention of environmental pollution through biotreatment of solid, liquid, and gaseous wastes, bioremediation of polluted environments, and biomonitoring of environment and treatment processes. The advantages of biotechnological treatment of wastes are as follows: biodegradation or detoxication of a wide spectrum of hazardous substances by natural microorganisms; availability of a wide range of biotechnological methods for complete destruction of hazardous wastes; and diversity of the conditions suitable for biodegradation. The main considerations for application of biotechnology in waste treatment are technically and economically reasonable rate of biodegradability or detoxication of substances during biotechnological treatment, big volume of treated wastes, and ability of natural microorganisms to degrade substances. Type of biotreatment is based on physiological type of applied microorganisms, such as fermenting anaerobic, anaerobically respiring (anoxic), microaerophilic, and aerobically respiring microorganisms. All types of biotechnological treatment of wastes can be enhanced using optimal environmental factors, better availability of contaminants and nutrients, or addition of selected strain(s) biomass. Bioaugmentation can accelerate start-up or biotreatment process in case microorganisms, which are necessary for hazardous waste treatment, are absent or their concentration is low in the waste; if the rate of bioremediation performed by indigenous microorganisms

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is not sufficient to achieve the treatment goal within the prescribed duration; when it is necessary to direct the biodegradation to the best pathway of many possible pathways; and to prevent growth and dispersion in waste treatment system of unwanted or nondetermined microbial strain which may be pathogenic or opportunistic one. Biosensors are essential tools in biomonitoring of environment and treatment processes. Combinations of biosensors in array can be used to measure concentration or toxicity of a set of hazardous substances. Microarrays for simultaneous qualitative or quantitative detection of different microorganisms or specific genes in the environmental sample are also useful in the monitoring of environment.

Key Words Environmental biotechnology • wastes • biotreatment • biodegradation • bioaugmentation • biosensors • biomonitoring.

1. INTRODUCTION

Environmental biotechnology is a system of sciences and engineering knowledge related to the use of microorganisms and their products in the prevention, treatment, and monitoring of environmental pollution through solid, liquid, and gaseous wastes biotreatment, bioremediation of polluted environments, and biomonitoring of environmental and treatment processes.

Biotechnological agents used in environmental biotechnology include Bacteria and Archaea, Fungi, Algae, and Protozoa. Bacteria and Archaea are prokaryotic microorganisms. Prokaryotes are the most active organisms participating in the biodegradation of organic matter and are used in all areas of environmental biotechnology. Fungi are eukaryotic organisms that assimilate organic substances. Fungi are important degraders of biopolymers and are used in solid waste treatment, especially in composting, or in soil bioremediation. Fungal biomass can also be used as an adsorbent of heavy metals. Algae are eukaryotic microorganisms that assimilate light energy and are used in environmental biotechnology for the removal of organic matter and nutrients from water exposed to light. Protozoa are unicellular animals that absorb and digest organic food. Protozoa play an important role in the treatment of industrial hazardous solid, liquid, and gas wastes by grazing on bacterial cells, thus maintaining adequate bacterial biomass levels in the treatment systems and helping to reduce cell concentrations in the waste effluents.

The main application of environmental biotechnology is the biodegradation of organic matter of municipal wastewater and biodegradation/detoxication of hazardous substances in industrial wastewater. It is known that approximately two-thirds of the hazardous substances of oil polluted soil and sludges, sulfur-containing wastes, paint sludges, halogenated organic solvents, non-halogenated organic solvents, galvanic wastes, salt sludges, pesticide-containing wastes, explosives, chemical industry wastewaters, and gas emissions can be treated by different biotechnological methods. Organic substances, synthesized in the chemical industry, are often difficult to biodegrade. Substances that are not produced naturally and are slowly/partially biodegradable are called xenobiotics. The biodegradability of xenobiotics can be characterized by biodegradability tests such as rate of CO_2 formation (mineralization rate), rate of oxygen consumption (respirometry test), ratio of BOD to COD (oxygen used for biological or chemical oxidation), and the spectrum of intermediate products of biodegradation.

Other applications of environmental biotechnology are the prevention of pollution and restoration of water quality in reservoirs, lakes and rivers, coastal area, in aquifers of groundwater, and treatment of potable water.

Areas of environmental biotechnology also include tests of toxicity and pathogenicity, biosensors, and biochips to monitor quality of environment, prevent hazardous waste production using biotechnological analogs, develop biodegradable materials for environmental sustainability, produce fuels from biomass and organic wastes, and reduce toxicity by bioimmobilization of hazardous substances.

2. COMPARISON OF BIOTECHNOLOGICAL TREATMENT AND OTHER METHODS

The pollution of water, soil, solid wastes, and air can be prevented or removed by physical, chemical, physicochemical, or biological (biotechnological) methods. The advantages of biotechnological treatment of wastes are as follows:

1. Biodegradation or detoxication of a wide spectrum of hazardous substances by natural microorganisms
2. Availability of a wide range of biotechnological methods for complete destruction of hazardous wastes
3. A diverse set of conditions that are suitable for biotechnological methods

However, there are also many disadvantages of biotechnological methods for the prevention of pollution and treatment of environment and wastes:

1. Nutrients and electron acceptors must be added to intensify the biotreatment
2. Optimal conditions must be maintained in the treatment system
3. There may be unexpected or negative effects of applied microorganisms, such as emission of cells, odors or toxic gases during the biotreatment, presence or release of pathogenic, toxigenic, opportunistic microorganisms into the environment
4. There may be unexpected problems in the management of the biotechnological system because of the complexity and high sensitivity of the biological processes

The main considerations for application of biotechnology in waste treatment are as follows:

1. Technically and economically reasonable rate of biodegradability or detoxication of waste substances during biotechnological treatment
2. Large volume of treated wastes
3. A low concentration of pollutant in water or waste is preferred
4. The ability of natural microorganisms to degrade waste substances
5. Better public acceptance of biotechnological treatment

The efficiency of actual biotechnological application depends on its design, process optimization, and cost minimization. Many failures have been reported on the way from bench laboratory scale to field full-scale biotechnological treatment because of the instability and diversity of both microbial properties and conditions in the treatment system (1).

In some cases, a combination of biotechnological and chemical treatments may be more efficient than one type of treatment (2, 3). Efficient pre-treatment schemes, used prior to biotechnological treatment, include homogenization of the particles of solid or undissolved wastes in water, chemical oxidation of hydrocarbons by H_2O_2, ozone, or Fenton's reagent, photochemical oxidation, and preliminary washing of wastes using surfactants.

3. AEROBIC TREATMENT OF WASTES

Aerobic microorganisms require oxygen as a terminal acceptor of electrons donated by organic or inorganic substances. The transfer of electrons from donor to acceptor is a source of biologically available energy. Xenobiotics such as aliphatic hydrocarbons and derivatives, chlorinated aliphatic compounds (methyl-, ethyl, methylene and ethylene chlorides), aromatic hydrocarbons and derivatives (benzene, toluene, phthalate, ethylbenzene, xylenes and phenol), polycyclic aromatic hydrocarbons, halogenated aromatic compounds (chlorophenols, polychlorinated biphenyls, dioxins and relatives, DDT and relatives), AZO dyes, compounds with nitrogroups (explosive-contaminated waste and herbicides), and organophosphate wastes can be treated effectively by aerobic microorganisms.

3.1. Aerobic Treatment of Solid Wastes

Composting is the simplest way to treat solid waste aerobically. Composting converts biologically unstable organic matter into a more stable humus-like product that can be used as a soil conditioner or organic fertilizer. Additional benefits of composting of organic wastes include the prevention of odors from rotting wastes, destruction of pathogens and parasites (especially in thermophilic composting), and the retention of nutrients in the end products. There are three main types of composting technology: windrow system, static pile system, and in-vessel system. Composting in windrow systems involves mixing an organic waste with inexpensive bulking agents (wood chips, leaves, corncobs, bark, peanut, and rice husks) to create a structurally rigid matrix, to diminish heat transfer from the matrix to the ambient environment, to increase the treatment temperature and to increase the oxygen transfer rate. The mixed matter is stacked in rows 1–2 m high called windrows. The mixtures are turned over periodically (two to three times a week) by mechanical means to expose the organic matter to ambient oxygen. Aerobic and partially anaerobic microorganisms, which are present in the waste or were added from previously produced compost, will grow in the organic waste. Due to biooxidation and release of energy, the temperature in the pile will rise. This is accompanied by successive changes in the dominant microbial communities, from less thermoresistant to more thermophilic ones. This composting process ranges from 30 to 60 days in duration.

The static pile system is an intensive biotreatment because the pile of organic waste and bulking agent is intensively aerated using blowers and air diffusers. The pile is usually covered with compost to remove odors and to maintain high internal temperatures. The aerated static pile process typically takes 21 days, after which the compost is cured for another 30 days, dried, and screened to recycle the bulking agent.

In-vessel composting results in the most intensive biotransformation of organic wastes. In-vessel composting is performed in partially or completely enclosed containers in which moisture content, temperature, oxygen content in gas can be controlled. This process requires little space and takes some days for treatment, but its cost is higher than that of open systems. To intensify the composting of solid waste, the following pre-treatments can be used:

1. Mechanical disintegration and separation or screening to improve bioavailability of substances
2. Thermal treatment
3. Washing of waste using water or solution of surfactants to diminish toxic substances in waste
4. Chemical pre-treatment by H_2O_2, ozone, or Fenton's reagent to oxidize and cleave aromatic rings of hydrocarbons

Soil bioremediation is used in or on the sites of post-accidental wastes. There are many options in the process design described in the literature (4–6). The main options tested in the field are as follows:

1. In situ bioremediation (in-place treatment of a contaminated site)
2. On-site bioremediation (the treatment of a percolating liquid or eliminated gas in reactors placed on the surface of the contaminated site). The reactors used for this treatment are suspended biomass stirred-tank bioreactors, plug-flow bioreactors, rotating-disk contactors, packed-bed fixed biofilm reactors (biofilter), fluidized bed reactors, diffused aeration tanks, airlift bioreactors, jet bioreactors, membrane bioreactors, and upflow bed reactors (7)
3. *Ex situ* bioremediation (the treatment of contaminated soil or water that is removed from a contaminated site)

The first option is used when the pollution is weak, treatment time is not a limiting factor, and there is no groundwater pollution. The second option is usually used when the pollution level is high and there is secondary pollution of groundwater. The third option is usually used when the pollution level is so high that it diminishes the biodegradation rate due to the toxicity of substances or a low mass transfer rate. Another reason for using this option might be that the conditions in situ or on site (pH, salinity, dense texture or high permeability of soil, high toxicity of substance, and safe distance from public place) are not favorable for biodegradation.

Preventing hazardous substances from dispersing from the accident site into the environment is an important task of environmental biotechnology. This goal can be achieved by creating physical barriers in the migration pathway with microorganisms capable of biotransformation of intercepted hazardous substances, e.g., in polysaccharide (slime) viscous barriers in the contaminated subsurface. Another approach, which can be used to immobilize heavy metals in soil after pollution accidents, is the creation of biogeochemical barriers. These biogeochemical barriers could comprise gradients of H_2S, H_2, or Fe^{2+} concentrations, created by anaerobic sulfate-reducing bacteria (in absence of oxygen and presence of sulfate and organic matter), fermenting bacteria (after addition of organic matter and in absence of oxygen), or iron-reducing bacteria (in presence of Fe(III) and organic matter), respectively. Other bacteria can form a geochemical barrier for the migration of heavy metals at the boundary between aerobic and anaerobic zones. For example, iron-oxidizing bacteria oxidize Fe^{2+} and its chelates with humic acids in this barrier and produce iron hydroxides that can diminish the penetration of ammonia, phosphate, organic acids, cyanides, phenols, heavy metals, and radionuclides through the barrier.

3.2. Aerobic Treatment of Liquid Wastes

Wastewater can be treated aerobically in suspended biomass stirred-tank bioreactors, plug-flow bioreactors, rotating-disk contactors, packed-bed fixed biofilm reactors (or biofilters), fluidized bed reactors, diffused aeration tanks, airlift bioreactors, jet bioreactors, membrane bioreactors, and upflow bed reactors (4, 7). Secondary wastes include polluted air and sediments produced in the bioreactor. Wastewater with low concentrations of hazardous substances may reasonably be treated using biotechnologies such as granular activated carbon (GAC) fluidized-bed reactors or co-metabolism. GAC or other adsorbents ensure sorption of hydrophobic hazardous substances on the surface of GAC or other adsorbent particles. Microbial biofilms can also be concentrated on the surface of these particles and can biodegrade hazardous substances with higher rates compared to situations when both substrate and microbial biomass are suspended in the wastewater.

Cometabolism refers to the simultaneous biodegradation of hazardous organic substances (which are not used as a source of energy) and stereochemically similar substrates, which serve as a source of carbon and energy for microbial cells. Biooxidation of the hazardous substance is performed by the microbial enzymes due to stereochemical similarity between the hazardous substance and the substrate. The best-known applications of cometabolism are the biodegradation/detoxication of chloromethanes, chloroethanes, chloromethylene, and chloroethylenes by enzyme systems of bacteria for the oxidization of methane or ammonia as a main source of energy. In practice, bioremediation is achieved by adding methane or ammonia, oxygen (air), and biomass of methanotrophic or nitrifying bacteria to soil and groundwater polluted by toxic chlorinated substances.

To intensify the biotreatment of liquid waste, the following pre-treatments can be used:

1. Mechanical disintegration/suspension of the particles and hydrophobic substances to improve the reacting surface in the suspension and increase the rate of biodegradation
2. Removal from wastewater or concentration of hazardous substances by sedimentation, centrifugation, filtration, flotation, adsorption, extraction, ion exchange, evaporation, distillation, freezing, and separation
3. Preliminary oxidation by H_2O_2, ozone, or Fenton's reagent to produce active oxygen radicals; preliminary photo-oxidation by UV and electrochemical oxidation of hazardous substances

3.3. Aerobic Treatment of Gaseous Wastes

The main applications of biotechnology for the treatment of gaseous wastes include the bioremoval of biodegradable organic solvents, odors, and toxic gases, such as hydrogen sulfide and other sulfur-containing gases from the exhaust ventilation air in industry and farming. Industrial ventilated air containing formaldehyde, ammonia, and other low molecular weight substances can also be effectively treated in a bioscrubber or biofilter. Gaseous xenobiotics, which can be treated biotechnologically, include the following: chloroform, trichloroethylene, 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, carbon tetrachloride, xylenes, dibromochloropropane, toluene, methane, methylene chloride, 1,1-dichloroethene, bis(2-chloroethyl) ether, 1,2-dichloroethane, chlorine, 1,1-trichloroethane, ethylbenzene, 1,1,2,2-tetrachloroethane, bromine, methylmercury, trichlorofluoroethane, 1,1-dichloroethane, 1,1,2-trichloroethane, ammonia, trichloroethane, 1,2-dichloroethene, carbon disulfide, chloroethane, p-xylene, hydrogen sulfide, chloromethane, 2-butanone, bromoform, acrolein, bromodichloroethane, nitrogen dioxide, ozone, formaldehyde, chlorodibromomethane, ethyl ether, and 1,2-dichloropropane.

Gaseous pollutants of gas or air streams must pass through bioscrubbers containing suspensions of biodegrading microorganisms or through a biofilter packed with porous carriers covered by biofilms of degrading microorganisms. Depending on the nature and volume of polluted gas, the biofilm carriers may be cheap porous substrates, such as peat, wood chips, compost, or regular artificial carriers, such as plastic or metal rings, porous cylinders and spheres, fibers, and fiber nets. The bioscrubber's contents must be stirred to ensure a high mass transfer between gas and microbial suspension. The liquid that has interacted with the polluted gas is collected at the bottom of the biofilter and recycled to the top part of the biofilter to ensure adequate contact of polluted gas and liquid and optimal humidity of biofilter. The addition of nutrients and fresh water to a bioscrubber or biofilter must be made regularly or continuously. Fresh water can be used to replace water that has evaporated in the bioreactor. If the mass transfer rate is higher than the biodegradation rate, the absorbed pollutants must be biodegraded in an additional suspended bioreactor or biofilter connected in series to the bioscrubber or absorbing biofilter.

4. ANAEROBIC TREATMENT OF WASTES

There are anaerobic (living without oxygen), facultative anaerobic (living under anaerobic or aerobic conditions), microaerophilic (preferring to live under low concentrations of dissolved oxygen) and obligate aerobic (living only in the presence of oxygen), microorganisms. Some anaerobic microorganisms, called tolerant anaerobes, have mechanisms protecting them from exposure to oxygen. Others, called obligate anaerobes, have no such mechanisms and may die after several seconds of exposure to aerobic conditions. Obligate anaerobes produce energy from: a) fermentation (destruction of organic substances without external acceptor of electrons); b) anaerobic respiration using electron acceptors such as CO_2, $NO_3^-NO_2^-$, Fe^{3+}, SO_4^{2-}; 3) anoxygenic ($H_2S \rightarrow S$) or oxygenic ($H_2O \rightarrow O_2$) photosynthesis. The advantage of anaerobic treatment is that there is no need to supply oxygen in the treatment system. This is useful in cases such as bioremediation of clay soil or high-strength organic waste. However, anaerobic treatment may be slower than aerobic treatment, and there may be significant outputs of dissolved organic products of fermentation or anaerobic respiration.

The following sequence arranges respiratory processes according to increasing energetic efficiency of biodegradation (per mole of transferred electrons): fermentation $\rightarrow$ CO_2 respiration ("methanogenic fermentation") $\rightarrow$ dissimilative sulfate-reduction $\rightarrow$ dissimilative iron reduction ("iron respiration") $\rightarrow$ nitrate respiration ("denitrification") $\rightarrow$ aerobic respiration.

Facultative anaerobes can produce energy from these reactions or from the aerobic oxidation of organic matter and may be useful when integrated together with aerobic and anaerobic microorganisms in microbial aggregates. However, this function is still not well studied. One interesting and useful feature in this physiological group is the ability in some representatives (e.g., *Escherichia coli*) to produce an active oxidant, hydrogen peroxide, during normal aerobic metabolism (8).

Anaerobic respiration is more effective in terms of output of energy per mole of transferred electrons than fermentation. Anaerobic respiration can be performed by different groups of prokaryotes with such electron acceptors as NO_3^-, NO_2^-, Fe^{3+}, SO_4^{2-}, and CO_2. Therefore, if the concentration of one such acceptor in the hazardous waste is sufficient for the anaerobic respiration and oxidation of the pollutants, the activity of the related bacterial group can be used for the treatment. CO_2-respiring prokaryotes (methanogens) are used for methanogenic biodegradation of organic hazardous wastes. Sulfate-reducing bacteria can be used for anaerobic biodegradation of organic matter or for the precipitation/immobilization of heavy metals of sulfate-containing hazardous wastes. Iron-reducing bacteria can produce dissolved Fe^{2+} ions from insoluble Fe(III) minerals. Anaerobic biodegradation of organic matter and detoxication of hazardous wastes can be significantly enhanced as a result of precipitation of toxic organics, acids, phenols, or cyanide by Fe(II). Nitrate-respiring bacteria can be used in denitrification, i.e., reduction of nitrate to gaseous N_2. Nitrate can be added to the hazardous waste to initiate the biodegradation of different types of organic substances, for example polycyclic aromatic hydrocarbons (9). Nitrogroups of hazardous substances can be reduced by similar pathway to related amines.

Anaerobic fermenting bacteria (e.g., from genus *Clostridium*) perform two important functions in the biodegradation of hazardous organics: (a) they hydrolyze different natural polymers and (b) ferment monomers with production of alcohols, organic acids, and CO_2. Many hazardous substances, for example chlorinated solvents, phthalates, phenols, ethyleneglycol, and polyethylene glycols can be degraded by anaerobic microorganisms (4, 10–12). Fermenting bacteria perform reductive anaerobic dechlorination, thus enhancing further biodegradation of xenobiotics. Different biotechnological systems perform anaerobic biotreatment of wastewater: biotreatment by suspended microorganisms, anaerobic biofiltration, and biotreatment in upflow anaerobic sludge blanket (UASB) reactors (4, 5).

Organic and inorganic wastes can be slowly transformed by anaerobic microorganisms in landfills (13). Organic matter is hydrolyzed by bacteria and fungi. Amino acids are degraded using ammonification with formation of toxic organic amines and ammonia. Amino acids, nucleotides, and carbohydrates are fermented or anaerobically oxidized with formation of organic acids, CO_2, and CH_4. Xenobiotics and heavy metals may be reduced, and subsequently dissolved or immobilized. These bioprocesses may result in the formation of toxic landfill leachate, which can be detoxicated by aerobic biotechnological treatment to oxidize organic hazards and to immobilize dissolved heavy metals.

A combined anaerobic/aerobic biotreatment can be more effective than aerobic or anaerobic treatment alone. The simplest approach for this type of treatment is the use of aerated stabilization ponds, aerated and non-aerated lagoons, and natural and artificial wetland systems, whereby aerobic treatment occurs in the upper part of these systems and anaerobic treatment occurs at the bottom end. A typical organic loading is 0.01 kg BOD/m^3 day and the retention time varies from a few days to 100 days (7). A more intensive form of biodegradation can be achieved by combining aerobic and anaerobic reactors with controlled conditions, or by