Elements of Sustainability and Bioremediation

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Abstract
Bioremediation is becoming an increasingly popular and sustainable alternative to conventional methods for treating wastes and contaminated media in view to degrade and ultimately stabilize the contaminants using microbial activity. Many studies on bioremediation have been reported and the scientific literature has been accordingly enriched with an emergence of various bioremediation techniques. In this introductory chapter, the essential features of sustainability and the various in situ and ex situ bioremediation techniques have been outlined. This chapter also provides groundwork for the subsequent chapters which give a more detailed account of the anaerobic digestion technology, phytoremediation, vermicomposting and biosorption in their applicability and effectiveness for the biological treatment, stabilization and eventually remediation of contaminated environments.

Keywords: Sustainability, Biotechnology, Environmental contaminants, Bioremediation techniques

The Sustainability, Remediation and Biotechnology Link

Most segments of industrialized society are rethinking how behavior, reliance on technology, and consumption of energy impact the environment. Society is looking for ways to minimize these
impacts, or avoid them altogether, so that human activity can become more sustainable. In point of fact, sustainability is a concept that coincides with many of the principles of Green Chemistry. Green chemistry, in first instance, is an essential part of green engineering. The definitions of green chemistry and green engineering share many commonalities, and the application of both chemistry and engineering principles is needed to advance the goals of environmental sustainability [1]. A working definition of green engineering proposed by Kirchhoff [1] is the design, commercialization, and use of processes and products that are feasible and economical while minimizing pollution at the source and risk to human health and the environment. The link between green chemistry and green engineering is strong in ensuring that inputs and outputs, both for materials and energy flows and budgeting, are as inherently safe as possible. Whilst Green Chemistry focuses on the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances, it also lays down the ground plan for the design of the green engineering technologies needed to implement sustainable products, processes, and systems [1].

Features and Concepts in Sustainability

At a conference related to the environment in Rio in 2002, it is impossible to forget that 10 years earlier in June 1992 Rio hosted one of the most important international summits of the modern era, The Rio Earth Summit. Boutros Boutrous-Ghali the then United Nations Secretary-General in his opening address to the United Nations General Assembly in September 1992 commented that ‘The Earth Summit held in Rio in June marked an important milestone in awakening the world to the need for a development process that does not jeopardise future generations’. The 1992 Rio Conference achieved consensus in three important areas, namely,

i. it secured a set of agreements between governments which marked a significant advance in international cooperation on development and environment issues;

ii. it marshalled political commitment to these arrangements at the highest level and placed the issue of sustainable development at the heart of the international agenda, and
iii. it opened new paths for communication and cooperation between official and non-official organisations working towards developmental and environmental goals.

The developments leading up to the Rio Summit and which have flowed from it have made sustainability a serious issue for all developed societies and all sectors of business. The remediation of contaminated land offers a chance to demonstrate the concepts of sustainability but it must be recognised that we are still struggling to balance the many components of sustainability, despite for example, Agenda 21, Chapter 8(D) which was entitled ‘Establishing systems for integrated environmental and economic accounting’. Much has been done in this area but the systems are still complex and the concepts in need of further development. On the other hand, the Bruntland statement on sustainability is perhaps the most often cited definition of sustainability: Humanity has the ability to make development sustainable – to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs. Sustainability is not a panacea but a tool, under development, which still requires much honing. The main components of sustainability are typically expressed in terms of three main criteria which can be widened into many sub-criteria. These are:

- Technical/Environmental: Human health, Ecology, Resources, Climate
- Economic: Macro and Micro
- Social: Political and Legal

**Sustainability and Scale of Environmental Pollution Clean-up**

It is perhaps the accepted wisdom that contaminated land and waters are not a big enough issue to require detailed assessment as to sustainability. The common assumption is that on a national scale clean-up is a sustainable industry. The technologies for remediation of contaminated land and waters are beneficial as they reduce the risk from contamination and are unlikely to do much other harm. A little ponder on this issue shows that this is manifestly wrong since in many a way, little normalisation of the life cycle impacts of contaminated land and waters remediation has been carried out, even for simple situations such as dig and dump.
In this chapter, sustainable remediation is broadly defined as a remedy or combination of remedies whose net benefit on human health and the environment is maximized through the careful use of limited resources. To accomplish this, it becomes an instinctive need to embrace sustainable approaches to remediation that provide a net benefit to the environment. To the extent possible, approaches consist in minimizing or eliminating energy consumption or the consumption of other natural resources; reducing or eliminating releases to the environment, especially to the air; harnessing or mimicking a natural process; reusing or recycling of land or otherwise undesirable materials; and/or encouraging the use of remedial technologies that permanently destroy contaminants. It is also important to portray sustainable remediation practices not only as those practices that reduce global impacts (e.g., greenhouse gases), but also as those that reduce local atmospheric effects, potential impacts on worker and community safety, and/or the consumption of natural energy resources that might be attributable to remediation activities.

Conventionally, the selection of a remediation technology is based on factors such as the effectiveness of the remedy, implementability, cost considerations, and time constraints. Protection of the public via interception of contaminants, reduction of source(s), and mitigation of exposure pathways are prerequisites of remedy selection. Although these considerations are critical components in a traditional evaluation of remediation options, they do not evaluate and balance fully the external environmental, social, and economic impacts of a project. In other terms, the conventional approach generally focuses on the "internalities" of a remediation project and gives very little attention to its "externalities" where internalities lump the remedial objectives, system performance, environmental impacts local to the remediation site such as waste generation, water discharge, and air emissions (generally required by permit) and the externalities encompass the environmental impacts at the community, regional, and global levels.

**Biotechnology and Bioremediation**

Among the major new technologies that have appeared since the 1960s, biotechnology has attracted a great deal of attention and interest. Biotechnology has proved capable of generating enormous wealth and influencing every significant sector of the economy. Biotechnology has already substantially affected healthcare; production and processing of food; agriculture and forestry; environmental
protection; and production of materials and chemicals [2]. The treatment of municipal wastewater by activated sludge method was perhaps the first major use of biotechnology in bioremediation applications. Activated sludge treatment remains a workhorse technology for controlling pollution of aquatic environment. Similarly, aerobic stabilization of solid organic waste through composting has a long history of use. Both these technologies have undergone considerable improvement [3].

The application of microbial metabolic potential (bioremediation) is accepted as an environmentally benign and economical measure for decontamination of polluted environments. Bioremediation methods are generally categorized into ex situ and in situ bioremediation. Although in situ bioremediation methods have been in use for two to three decades, they have not yet yielded the expected results. Their limited success has been attributed to reduced ecological sustainability under environmental conditions. An important determinant of sustainability of in situ bioremediation is pollutant bioavailability. Microbial chemotaxis is then postulated to improve pollutant bioavailability significantly; consequently, the application of chemotactic microorganisms can considerably enhance the performance of in situ degradation [2]. Therefore, in view of improving the potentialities of bioremediation, microorganisms have been isolated, selected, mutated and genetically engineered for effective bioremediation capabilities including the ability to degrade recalcitrant pollutants, achieve enhanced rates of degradation of target compounds, and assure better survival and colonization in target polluted niches [3]. More recently, enzymes have been successfully used in diverse bioremediation applications. Effective and controlled bioremoval of nitrate and phosphate contamination from wastewater has become possible. Hence, biotechnology is already playing a major role in maintaining a clean environment and this role will expand substantially as new bioremediation methods are developed and deployed for bioremediation of all kinds of industrial effluents and contaminated media.

Environmental Pollution and Biotreatment

Variants

The problems of environment can be classified into the following subheads as most of the problems can be traced to one or more of the following either directly or indirectly: Waste generation
(sewage, wastewater, kitchen waste, industrial waste, effluents, agricultural waste, food waste) and use of chemicals for various purposes in the form of insecticides, pesticides, chemical fertilizers, toxic products and by-products from chemical industries. Waste generation is a side effect of consumption and production activities and tends to increase with economic advance. The more serious concern is the increased presence of toxic chemicals such as halogen aliphatics, aromatics, polychlorinated biphenyls and other organic and inorganic pollutants which may reach air, water or soil and affect the environment in several ways, ultimately threatening the self-regulating capacity of the biosphere [4]. They may be present in high levels at the points of discharge or may remain low but can be highly toxic for the receiving bodies. The underground water sources are increasingly becoming contaminated. Zhang et al. [5] have recently detected legacy pollutants, polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane and its metabolites (DDTs), and some emerging organohalogen pollutants, such as polybrominated diphenyl ethers (PBDEs), hexabromobenzene (HBB), pentabromotoluene (PBT), 2,3,4,5,6-pentabromoethyl benzene (PBE), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), and dechlorane plus (DP) in an aquatic food chain (invertebrates and fish) from an E–waste recycling region in South China. Polychlorinated biphenyls, DDTs, PBDEs, and HBB were detected in more than 90% of the samples, with respective concentrations ranging from not detected (ND)–32,000 ng/g lipid weight, ND–850 ng/g lipid weight, 8 to 1,300 ng/g lipid weight, and 0.28 to 240 ng/g lipid weight. Pentabromotoluene, PBE, BTBPE, and DP were also quantifiable in collected samples with a concentration range of ND–40 ng/g lipid weight.

There are three main approaches in dealing with contaminated sites: Identification of the problem, assessment of the nature and degree of the hazard, and the best choice of remedial action. The need to remediate these sites has led to the development of new technologies that emphasize the detoxification and destruction of the contaminants [6,7] rather than the conventional approach of disposal. Wang and Chen [8] recently developed a novel system of phytoremediation *ex planta* based on the overexpression of a secretory laccase [9] that catalyzes the oxidation of various aromatic compounds, including 2,4,6-trichlorophenol. All the more, rapid developments in understanding activated sludge processes and wastewater remediation warrant exploitation of different
strategies for studying their degradation and some of the biological remediation terminologies such as bioleaching, biosorption, bioaugmentation, biostimulation, biopulping, biodeterioration, biobleaching, bioaccumulation, biotransformation and bioattenuation are being actively researched on [10]. Enzyme technology has equally been receiving increased attention.

**Main Features of Bioremediation**

Bioremediation is a fast growing and promising set of remediation techniques increasingly being studied and applied in practical use for pollutant clean-up. Vidali [11] has proposed the following classification of microorganisms involved in bioremediation processes: *Aerobic* microbes which bring about biodegradation in the presence of oxygen with *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus* and *Mycobacterium* being the aerobic bacteria recognized for their degradative abilities; *Anaerobic* bacteria which cause degradation in the absence of oxygen; *Ligninolytic fungi* are fungi such as the white rot fungus *Phanerochaete chrysosporium* and have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants; and *Methylotrophs* which are aerobic bacteria that grow utilizing methane for carbon and energy.

Advances in the application of bioremediation harness molecular, genetic, microbiology, and protein engineering tools and rely on the identification of novel metal-sequestering peptides, rational and irrational pathway engineering, and enzyme design [12]. In the sections to follow, several *in situ* and *ex situ* bioremediation techniques namely anaerobic digestion technology, phytoremediation, composting, bioaugmentation, biostimulation and biosorption have been described and discussed for their effectiveness in the biotreatment, stabilization and eventually overall remediation of contaminated strata and environments. Selected case studies on biosorption of heavy metals, anaerobic digestion, phytoremediation and vermicomposting of pollutants have also been appraised.

**Advantages of Bioremediation**

Bioremediation offers several advantages over the conventional remediation techniques such as landfilling. Often, bioremediation can be done on site, thereby eliminating transportation costs and
liabilities. In many instances, manufacturing and industrial use of the site can continue while the bioremediation process is being implemented. Bioremediation results in the decomposition of the waste and the long-term liability associated with non-destructive treatment methods. Finally, bioremediation can be coupled (i.e. integrated) with other treatment technologies into a treatment chain allowing for the treatment of mixed and complex wastes [13,14]. The use of renewable (waste) materials has also boosted the bioremediation of waste streams [15]. Residues such as cereals straw, corn cobs, cotton stalks, various grasses and reed stems, maize and sorghum stover, vine prunings, sugarcane and tequila bagasse, coconut and banana residues, corn husks, coffee pulp and coffee husk, cottonseed and sunflower seed hulls, peanut shells, rice husks, sunflower seed hulls, waste paper, wood sawdust and chips, are some examples of residues and by-products that can be recovered and upgraded to higher value and useful products by chemical or biological processes [16,17]. In fact, the chemical properties of such lignocellulosic agricultural residues make them a substrate of enormous biotechnological value. They can be converted by solid state fermentation (SSF) into various different value-added products including mushrooms, animal feed enriched with microbial biomass, compost to be used as biofertilizer or biopesticide, enzymes, organic acids, ethanol, flavours, biologically active secondary metabolites and also for bioremediation of hazardous compounds, biological detoxification of agro-industrial residues and biopulping [18].

Disadvantages of Bioremediation

Bioremediation also has its limitations and disadvantages. Some chemicals such as highly chlorinated compounds and heavy metals, are not readily amenable to biological degradation and stabilization. In addition, for some chemicals, microbial degradation may lead to the production of more toxic or mobile substances than the parent compound(s). For example, under anaerobic conditions, trichloroethenyes (TCE) undergo a series of microbiologically mediated reactions resulting in the sequential removal of chlorine atoms from the molecule. This process is called reductive dehalogenation. The end product of this series of reactions is vinyl chloride, a known carcinogen. Thus, if bioremediation is applied without a through understanding of the microbial processes involved and the
metabolic and chemical pathways, it could actually lead to a worse situation than already exists in some cases. Hence, bioremediation is a scientifically intensive procedure, which must be tailored to the site-specific conditions to minimize the effects of environmental and kinetic constraints [19,20]. Therefore, initial costs for site assessment, characterization and feasibility evaluation for bioremediation may be higher than the costs associated with more conventional technologies such as air stripping. As with remediation technologies, there is also the need for extensive monitoring of the site during implementation of the project to assess the effectiveness of the bioremediation technique in its clean-up performance [21]. Monitoring requirements may include some form of microbiological monitoring in addition to the chemical monitoring associated with physical/chemical remediation techniques. Finally, there are regulatory constraints that impact on the implementation of bioremediation [22].

General Approach to Bioremediation

Bioremediation technologies can be broadly classified as \textit{ex situ} or \textit{in situ} [22]. \textit{Ex situ} bioremediation technologies are those which involve the physical removal of the contaminated to another area (possibly within the site) for treatment. Bioreactors, landfarming, anaerobic digestion, composting, biosorption and some forms of solid-phase treatment are all examples of \textit{ex situ} treatment techniques. In contrast, \textit{in situ} bioremediation involves treatment of the contaminated material in place. Bioventing for the treatment of the contaminated soil and biostimulation of indigenous aquifer microorganisms are examples of these treatment techniques. Although some sites may be more easily controlled and maintained with \textit{ex situ} configurations [22], others are more effective with \textit{in situ} treatment.

The successful implementation of bioengineered remediation techniques will involve a multidisciplinary approach requiring input from individuals with expertise in microbiology, chemistry, geology, soil science, environmental engineering and chemical engineering. In order to use bioengineering successfully for the remediation of environmental contamination problems, the first step is to obtain a through understanding of the matrix characteristics of the media to be treated and the properties (physical, chemical and microbiological) of the contaminant(s). Lai \textit{et al.} [23] stress that the
performance monitoring of applied remediation technologies is an important part of site remediation. It involves periodic measurement of site parameters to evaluate whether the remediation technologies perform as expected or to determine the termination date of remediation projects. According to Lai et al. [23], performance monitoring can be a difficult undertaking if there are no well-defined and measurable remediation objectives, such as a reduction in mass discharge rate from a contaminant source. The monitoring requirements for a bioremediation treatment system shall most reasonably comprise the following analyses and inspections: a daily inspection of the system components, i.e., piping, pumps and valves; monitoring of pH, dissolved oxygen, temperature, and mineral nutrient levels within the treatment system, and monitoring flow rates and pumping rates; and a monthly monitoring the parameters within the treatment system and in the off-site monitoring wells such as contaminant concentration, aerobic heterotrophic bacterial population density, pH, dissolved oxygen, temperature and available mineral nutrient concentrations.

**In Situ Bioremediation Technologies**

**Bioventing**

In bioventing, the aerobic biodegradation of soil contamination is stimulated by delivery of oxygen to the subsurface. This is accomplished by injecting or extracting air through unsaturated soil in a passive system. This technology is designed primarily to treat soil contamination by fuels, non-halogenated volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs), pesticides and herbicides. The process may be applied to halogenated organics, but is less effective. Bioventing typically costs around $15 per cubic yard of soil and uses simple, inexpensive, low-maintenance equipment that can be left unattended for long periods of time.

The technology requires the presence of indigenous organisms capable of degrading the contaminants of interest, as well as nutrients necessary for growth. Also, it is necessary that the contaminants be available to the organisms, and not tightly sorbed to soil particles. Bioventing is not as effective in treating areas where the water table is high, and soils with very low moisture content.
Lastly, the technology is not applicable in sites where high concentrations of inorganic salts, heavy metals, or organic compounds are present, as these hinder microbial growth. However, some studies have demonstrated the merits of bioventing as a bioremediation technique. Shewfelt et al. [24] have conducted experiments using small-scale respirometers containing gasoline–contaminated soil from an active remediation site to determine the effects of soil water content, nitrogen content, nitrogen form, and the composition of the microbial population on the gasoline biodegradation rate. Results indicated that optimum bioventing conditions were 18 wt.% soil water content, C:N ratio of 10, using NH$_4^+$–N. Sui et al. [25] have studied the cometabolic bioventing for removal of TCE in the unsaturated zone in a soil column study using methane as growth substrate, and the experimental data showed that a total TCE remediation efficiency of over 95% was obtained with a volatilization–to–biodegradation ratio of TCE being about 7:1.

**Biostimulation**

Biodegradation in the subsurface may be stimulated by addition of water–based solutions carrying nutrients, electron acceptor or other amendments. These technologies are designed primarily to treat soil and groundwater contamination by fuels, non–halogenated VOCs, SVOCs, pesticides, and herbicides. These processes may be applied to halogenated organics, but are sometimes less effective. Although the costs of biostimulation technologies vary tremendously from site to site, these technologies tend to be amongst the cheapest alternatives when applicable. The technology requires the presence of indigenous organisms capable of degrading the contaminants of interest. Also, it is necessary that the contaminants be available to the organisms, and not tightly sorbed to soil particles. Biostimulation is not applicable in sites where high concentrations of inorganic salts, heavy metals, or organic compounds are present, as these hinder microbial growth. Lastly, the calculation of water–based solutions through the soil may increase contaminant mobility and necessitate treatment of underlying groundwater. Preferential colonization by microbes may occur causing clogging of nutrient and water injection wells.

Recent studies on the application of biostimulation for degrading a variety of contaminants unanimously advocate the merit of this technique. Krishnani et al. [26] have used molecular methods
based on sequencing of clone libraries to provide sequence and the phylogenetic information of ammonia oxidizing bacteria (AOB). *Ammonia monooxygenase* (amoA) gene, which catalyzed the oxidation of ammonia to hydroxyl amine in the initial rate-determining step of nitrification was targeted for detection and characterization of AOB using gene-specific primers. The use of a matrix prepared from abundantly available lignocellulosic agrowaste–bagasse has successfully been demonstrated for biostimulation of AOB in aquaculture environment by supplementing nutritional requirement facilitating the biofilm mode of growth of the autotrophic consortia, the application of the results of this study could be useful in enhanced predictability and reliability of the treatment of ammonia in brackishwater aquaculture. Dafale et al. [27] have identified the strains viz. *Pseudomonas aeruginosa* and *Bacillus circulans* and other unidentified laboratory isolates (NAD1 and NAD6) to be predominantly present in a microbial consortium acclimatized from activated sludge from a textile effluent treatment plant to effectively decolorize RB5 dye solutions. The optimum inoculum concentration for maximum decolorization were found to be 1–5 mL of 1800 mg/L MLSS and 37°C, respectively. Overall, the effectiveness of the acclimatized biomass under optimized conditions towards decolorization of two types of synthetic dye bath wastewaters that were prepared using chosen azo dyes has been demonstrated. Earlier, Hamdi et al. [28] had studied the degradation of spiked anthracene (ANT), pyrene (PYR) and benz[a]pyrene (Bl[a]P) in soil (3000 mg Σ 3 PAHs/kg dry soil) in aerobically incubated microcosms for 120 days. The applied treatments aimed at enhancing PAH removal from the heavily contaminated soils were: (i) bioaugmentation by adding aged PAH–contaminated soil (ACS) containing activated indigenous degraders; and (ii) combined bio-augmentation/biostimulation by incorporating sewage sludge compost (SSC) and decaying rice straw (DRS). Hamdi et al. [28] reported that the adopted treatments produced higher PAH dissipation rates than those observed in unamended PAH–spiked soils, especially for ANT and PYR in the presence of DRS or ACS (>96%).

**Air-sparging**

Air-sparging stimulates aerobic biodegradation of contaminated groundwater by delivery of oxygen to the subsurface [29]. This is accomplished by injecting air below the water table. This technology
is designed primarily to treat groundwater contamination by fuels, non-halogenated VOCs, SVOCs, pesticides, organics, and herbicides. Air sparging has also been demonstrated to be an innovative groundwater remediation technology capable of restoring aquifers that have been polluted by volatile and (or) biodegradable contaminants, such as petroleum hydrocarbons [30]. The process may be applied to halogenated organics, but is less effective. Air-sparging can cost less than $1 per 1000 liters in favorable situations and tends to be among the cheapest remedial alternatives when applicable. The technology uses simple, inexpensive, low-maintenance equipment that can be left unattended for long periods of time. Also, the technology tends to enjoy good public acceptance. The technology requires the presence of indigenous organisms capable of degrading the contaminants of interest, as well as nutrients necessary for growth. Also, it is necessary that the contaminants be available to the organisms, and not tightly sorbed to soil particles. Air sparging is not applicable in sites where high concentrations of inorganic salts, heavy metals, or organic compounds are present, as hinder microbial growth.

**Natural Attenuation**

Natural attenuation is a proactive approach that focuses on the verification and monitoring of natural remediation processes [31]. Also known as passive remediation, *in situ* bioremediation, intrinsic remediation, bioattenuation or intrinsic bioremediation, natural attenuation is an *in situ* treatment method that uses natural processes to contain the spread of contamination from chemical spills and to reduce that concentration and amount of pollutants at contaminated sites [31,32]. This means the environmental contaminants are undisturbed while natural attenuation works on them. Natural attenuation processes are often categorized as destructive or non-destructive [33]. Destructive processes destroy the contaminant, while non-destructive processes cause a reduction in contaminant concentrations [31].

Target contaminants for natural attenuation include fuels, non-halogenated VOCs, SVOCs, pesticides and herbicides. The process may be applied to halogenated organics, but it requires longer treatment times. Also, the technology is applicable to especially hydrophobic contaminants such as high molecular weight PAHs that tend to sorb very tightly to soil particles and have very low rates of
migration. Often, communities of adopted degraders will mineralize such contaminants quickly after they desorb from soil particles. The costs of natural attenuation are associated with modeling contaminant migration, degradation rates to determine its feasibility, and evaluation and monitoring costs to confirm adequate performance. Although these costs tend to be low compared with other remedial alternatives, the public is often suspicious of natural attenuation due to the impression that nothing is being done. Some very important observations related to the performance of natural attenuation technology are: it is a relatively simple technology compared to other remediation technologies; it can be carried out with little or no site disruption; it often requires more time to achieve clean-up goals than other conventional remediation methods; it requires a long-term monitoring program and the program duration affects the cost; if natural attenuation rates are too slow, the pollution/contaminant plume could migrate; and it is difficult to predict with high reliability the performance of natural attenuation [31].

Landfarming

This technology involves the application of contaminated material that has been excavated onto the soil surface and periodically tilled to mix and aerate the material [34]. The contaminants are degraded, transformed and immobilized by means of biotic and abiotic reactions [35]. Sometimes, in cases of very shallow contamination, the top layer of the site may simply be tilled without requiring any excavation. Liners or other methods may be used to control leachate. This technology is designed primarily to treat soil contamination by fuels, PAHs, non-halogenated VOCs, SVOCs, pesticides, and herbicides. The process may be applied to halogenated organics, but is less effective. Although the technology is very simple and inexpensive, it does require large space, and reduction in contaminant concentrations may sometimes be due to volatilization rather than biodegradation [36]. Marin et al. [37] assessed the ability landfarming to reduce the total hydrocarbon content added to soil with refinery sludge in low rain and high temperature conditions. It was seen that 80% of the hydrocarbons were eliminated in 11 months, half of this reduction taking place during the first 3 months. Rubinos et al. [35] treated a soil heavily contaminated (>5000 mg/kg) with hexachlorocyclohexane (HCH) isomers derived from lindane production using the landfarming technique and observed
significant decreases of the $\alpha$- and $\gamma$–HCH isomers with up to 89% and 82% of the initial concentration, respectively, at the end of the 11 months. In this respect, the aerobic landfarming appeared to be a viable and cost-effective bioremediation treatment technology for soils contaminated with $\alpha$- and $\gamma$–HCH isomers on large scales.

**Phytoremediation**

Plants facilitate remediation of pollutants via the following mechanisms:

- Direct uptake, and incorporation of contaminants into plant biomass
- Immobilization, or phytostabilization of contaminants in the subsurface
- Release plant enzymes into the rhizosphere that act directly on the contaminants
- Stimulation of microbiologically mediated degradation in the rhizosphere

Using plants in soil and groundwater remediation (i.e. phytoremediation) is a relatively new concept and the technology has yet to be extensively proven in the marketplace. Because of this, most information about phytoremediation comes mainly from field and laboratory research. However, the potential of phytoremediation for cheap, simple and effective soil and groundwater remediation is generating considerable interest.

Phytoremediation may be used for remediation of soil and groundwater contaminated with toxic heavy metals, radionuclides, organic contaminants such as chlorinated solvents, BTEX compounds, non-aromatic petroleum hydrocarbons, nitrotoluene ammunition wastes, and excess nutrients [38]. Botanists first recognized the metal–accumulating ability of the genus *Thlaspi* over a century ago. Some have suggested this ability may have evolved as a defense against herbivores [39]. Baker and co-workers were able to grow some individual *Thlaspi caerulscens* plants that accumulated more than 30,000 mg of Zn and over 1000 mg Pb per gram dried biomass [39]. They also showed that the five isolated populations of the plant were adapted in tolerating and accumulating metals not present in the parent soil, which suggested the mechanisms of tolerance and accumulation may be similar for different metals.
Dushenkov and co-workers have evaluated the heavy-metal accumulating abilities of several high biomass crop species such as Brassica juncea (Indian mustard) [40]. Although T. caerulescens has a higher tolerance for the heavy metals tested and demonstrated a higher ratio of metal accumulation to plant mass. B. juncea produces 20 times more biomass. They found B. juncea to be especially adapted in accumulating lead. One strain was able to accumulate Pb at up to 3.5% dry weight in the shoots suggesting that a crop of such plants could extract 630 kg/ha of Pb in above ground biomass with a single harvest, more if some root material was harvested as well [40]. It is important to note however, that these experiments were done hydroponically.

In soil, the property of Pb to bind to clay soil particles and organic matter, and its inclusion in insoluble precipitates significantly reduces the bioavailability of Pb to the plant [40]. This is true of other metals as well. For this reason, biological mechanisms that enhance metal bioavailability are being investigated. For instance, in response to nutrient deficiencies, plants can secrete metal–chelating molecules (phytoserophores) that chelate and solubilize soil–bound metals such as Fe, Mn, Cu, and Zn [41]. Also, plants can reduce soil bound metal ions by specific plasma membrane–bind metal reductases; and they can adjust soil pH, which decreases metal adsorption, by exuding protons [41]. Lastly, the presence of some microorganisms in the rhizosphere has been shown to enhance metal availability.

**Ex Situ Bioremediation**

**Composting**

Composting is the biochemical degradation of organic materials to a sanitary, nuisance–free, humus–like material [42]. Composting has been defined as a controlled microbial aerobic decomposition process with the formation of stabilized organic materials that may be used as soil conditioner [43]. The main factors in the control of a composting process include environmental parameters (temperature, moisture content, pH and aeration) and substrate nature parameters (C/N ratio, particle size, and nutrient content). Aerobic composting is the decomposition of organic substrates in the presence of sufficient oxygen [44]. The main products of the biological metabolism
are carbon dioxide, water and considerable amounts of heat [45]. Various factors correlate with each other physically, chemically and biologically in complicated composting processes [44]. Under optimal conditions, composting proceeds from the psychrophilic state through three phases: (a) the mesophilic or moderate-temperature phase, (b) the thermophilic or high temperature phase, and (c) the cooling and maturation phase which lasts for several months [46]. The first, second and third phases are referred to as the active stage in which heat is produced [45]. This active stage is governed by the basic principles of heat and mass transfer and by the biological constraints of living microorganisms [47,48].

Vermicomposting is the term given to the process of conversion of biodegradable matter by earthworms into vermicompost [49,50]. In the process, a major fraction of the nutrients contained in the organic matter is converted to more bioavailable forms. The first step in vermicomposting occurs when earthworms break the substrate down to small fragments prior to ingesting the substrate [51]. This increases the surface area of the substrate, facilitating microbial and enzymatic actions. The substrate is then ingested and goes through a process of "enzymatic digestion" brought about by numerous species of bacteria and enzymes present in the worms' gut [51]. Earthworms have been shown to aerate and bioturbate soils and thence improve their nutritional status and fertility, which are normally variables known to limit bioremediation rates [52]. Earthworms also hinder processes during which organic contaminants bind to soils, and thus promote the dispersion and bioavailability of organic contaminants to the degrading microorganisms [52]. Earthworms in general are tolerant to many chemical contaminants including heavy metals and organic pollutants in soil and can bio-accumulate them in their tissues [53]. Earthworms species like Eisenia fetida, Eisenia fetida, Lumbricus terrestris, Lumbricus rubellus and Allobophora chlorotica have been found to remove heavy metals (Cd, Pb, Cu and Hg) pesticides and lipophilic organic micropollutants like the PAH from the soil [53]. Therefore, by using these excellent properties of earthworms, the vermicomposting process has been employed to degrade organic pollutants like PAHs, PCBs [54], atrazine and metamitron [55].

Polycyclic aromatic hydrocarbons are a class of organic compounds that have accumulated in the natural environment mainly as a result of anthropogenic activities such as the combustion of fossil fuels [56]. Antizar-Ladislao et al. [57] investigated the
biodegradation of 16 United States Environmental Protection Agency (USEPA)–listed PAHs present in contaminated soil from a manufactured gas plant site using laboratory-scale in-vessel composting–bioremediation reactors over 8 weeks. Antizar-Ladislao et al. [57] found that temperature and amendment ratio were important operating parameters for PAH removal for in-vessel composting–bioremediation of aged coal tar–contaminated soil and thereafter recommended that when conventional composting processes using temperature profiles to meet regulatory requirements for pathogen control need to be used, these should be preferably started with a prolonged mesophilic stage followed by thermophilic, cooling, and maturation stages. More recent studies on the application of composting to degrade PAHs have been conclusive and in concert with the findings of earlier studies. Mihial et al. [58] determined that bioremediation by composting was a suitable alternative for the remediation of soil in and around a pit contaminated with petroleum waste comprising used oil, gasoline, diesel fuel and paint thinners. Mihial et al. [58] conducted a bench scale treatability study to assess the potential for successful bioremediation of the site using composting. They set up two reactors each with ammonium phosphate fertilizer as the nutrient amendment using a mixture of grass clippings and sheep manure in one reactor to determine if the composting process could be accelerated by the addition of these abundantly available waste materials. Based on the results of the treatability study, the half-life of the petroleum hydrocarbons at the subject site was estimated to be 36.3 and 121.6 days with and without the addition of grass clippings and sheep manure, respectively. It was estimated that it would take approximately 192 and 643 days to remediate the soil and lower reduce the TPH to 1,000 mg/L using the amendments of the reactors, respectively.

Phenol is both a synthetically and naturally produced aromatic compound. Microorganisms capable of degrading phenol are common and include both aerobes and anaerobes [59]. Many aerobic phenol–degrading microorganisms have been isolated and the pathways for the aerobic degradation of phenol are now established [59]. The first steps include oxygenation of phenol by phenol hydroxylase enzymes to form catechol, followed by ring cleavage adjacent to or in between the two hydroxyl groups of catechol. Recently, Das and Xia [60] characterized the transformation kinetics of 4–NP and its isomers during biosolids composting. Five distinctive
4-NP isomer groups with structures relative to α- and β-carbons of the alkyl chain were identified in biosolids. Composting biosolids mixed with wood shaving at a dry weight percentage ratio of 43:57 (C:N ratio of 65:1) removed 80% of the total 4-NP within two weeks of the composting experiments. Das and Xia [60] have also found that isomers with α-methyl-α-propyl structure transformed significantly slower than those with less branched tertiary α-carbon and those with secondary α-carbon, suggesting isomer-specific degradation of 4-NP during biosolids composting.

Only one research has been reported in the literature where composting has been applied for bioremediating PCBs. Michel Jr. et al. [61] determined the effects of soil to amendment ratio on PCB degradation when a PCB-contaminated soil from a former paper mill was mixed with a yard trimmings amendment and composted in field scale piles. Temperature, oxygen concentrations, and a number of other environmental parameters that usually influence microbial activity during composting were monitored. The PCBs in the contaminated soil had a concentration of 16 mg/kg dry weight and an average of 4 chlorines per biphenyl. The soil was composted with five levels of yard trimmings amendment (14 to 82% by weight) in pilot scale compost piles of volume 25 m³ and turned once every month. Michel Jr. et al. [61] observed that up to a 40% loss of PCBs with amendment levels of 60% and 82%. Also, congener specific PCB analysis indicated that less chlorinated PCB congeners (1–3 chlorines per biphenyl) were preferentially degraded during the composting process. On the other hand, bench-scale studies indicated that less than 1% of the PCBs in the contaminated soil were volatilized from composts during incubation with forced aeration at 55°C. In conclusion, Michel Jr. et al. [61] observed PCB loss during the composting of the PCB-contaminated soil and this appeared to be for the most part due to biodegradation, rather than volatilization.

Ghaly et al. [62] have evaluated the effectiveness of in-vessel thermophilic composting on the destruction of pirimiphos-methyl (O-(2-diethylamino-6-methylpyrimidin-4-yl) O,O-dimethyl phosphorothioate). Pirimiphos-methyl is an insecticide with both contact and fumigant action and shows activity against a wide variety of insects including ants, beetles, caterpillars, cockroaches, fleas, flies, mites, mosquitoes and moths. With a half-life of 117 days in water, 180–270 days on greens and seeds, pirimiphos-methyl has been reported to cause cholinesterase inhibition in humans which
at high dose rates results in nausea, dizziness, and confusion and at high exposure due to accidents and major spills results in respiratory paralysis and death. The bioreactor for the composting process studied by Ghaly et al. [62] was operated on a mixture of tomato plant residues, wood shavings and municipal solid compost. Ghaly et al. [62] found that the composting process successfully destroyed 81–89% of pirimiphos–methyl within the first 54 hours of the composting process, while the complete destruction of the pesticide required approximately 440 hours. Ghaly et al. [62] also inferred that a number of physical, chemical and biological mechanisms contribute to the degradation of pirimiphos–methyl in the environment and these consist of mineralization, abiotic transformations, adsorption, leaching, humification, and volatization. During composting of greenhouse wastes, in particular, the degradation of pirimiphos–methyl is accelerated by high temperatures developed during the thermophilic stage of the process, OM content, moisture of the compost matrix and level of biological activity. Delgado–Moreno and Peña [63] amended a typical calcareous agricultural soil of the Mediterranean area contaminated with four triazine herbicides with olive cake, compost and vermicompost of olive cake at rates four times higher than the agronomic dose in order to stimulate the biodegradation of simazine, terbuthylazine, cyanazine and prometryn, and thereafter observed that the residual herbicide concentrations at the end of the degradation assay showed no significant differences between non amended and amended soil. However, interestingly, Delgado–Moreno and Peña [63] found that the addition of compost and vermicompost had enhanced the biological degradation rate of triazines during the first week of incubation, with half–lives ranging from 5 to 18 days for the amended soils.

**Controlled Solid Phase Biotreatment**

Controlled solid phase processes include prepared treatment beds, biotreatment cells, soil piles, biopiles or composting matrices. Moisture, heat, nutrients, oxygen, and pH can be controlled to enhance biodegradation. These technologies differ from landfarming in that treatment processes are often enclosed to control off–gases. Typically, excavated material is mixed with soil amendments and placed on a treatment area that includes leachate collection systems and some of aeration. The costs of these techniques vary widely, but are among the expensive ones when applicable. Some prepared bed bioremediation
techniques involved the continuous spray application of a nutrient solution into the soil and collection and recycle of the drainage from the soil pile. The drainage itself may be treated in a slurry-phase bioreactor before recycling. Vendors have developed proprietary nutrient and additive formulations and methods for incorporating the formulation into the soil to stimulate biodegradation. Target contaminants include non-halogenated VOCs and SVOCs.

Pesticides also can be treated, but the process may be less effective and may be applicable only to some compounds within these contaminant groups. Like landfarming, these technologies require a lot of space, and excavation of contaminated material is required. One advantage, however, of contained ex situ methods is that toxic byproducts or metabolites formed during the biodegradation process (e.g., vinyl chloride from TCE) are contained.

**Slurry Phase Bioremediation**

These technologies involve the treatment of excavated contaminated soils in the controlled environment of a bioreactor. Excavated soil is processed to separate stones and rubble, then mixed with water to a predetermined concentration dependent upon the concentration of the contaminants, the rate of biodegradation, and the physical nature of the soils. Usually slurries contain from 10 to 40% solids. Electron acceptors and nutrients are added to the reactor, and parameters such as pH and temperature are controlled to optimize biological processes. Also, the reactor may be inoculated with specialized organisms if a suitable population is not present. Both aerobic and anaerobic reaction environments may be used. Target contaminants include petrochemicals, solvents, pesticides, wood preservatives, explosives, petroleum hydrocarbons and other organic chemicals. Bioreactors are favored over in situ biological techniques for heterogeneous soils, low permeability soils, areas where underlying groundwater would be difficult to capture, or when faster treatment times are required.

**Anaerobic Digestion Processes**

The anaerobic biotechnology is becoming widely popular due to its potential to produce renewable biofuels and value-added products from low-value feedstock such as waste streams [64]. In addition,
it provides an opportunity for the removal of pollutants from liquid and solid wastes more economically than the aerobic processes [64,65]. The merits of anaerobic digestion technology are a recovery of bioenergy and biofuels, recovery of value-added products and waste treatment. Although the anaerobic process has many inherent benefits, it is not a panacea for the treatment of all types of wastewaters and sludges [64]. Some of the limitations of anaerobic treatment system are: long start-up time, long recovery time, specific nutrients and trace metal requirements, more susceptible to changes in environmental conditions, treatment of high-sulphate wastewater and constant meticulous operational attention.

**Principles of Anaerobic Digestion Processes**

Anaerobic processes are defined as biological processes in which organic matter is metabolized in an environment free of dissolved oxygen or its precursors [64]. The anaerobic process is classified as either anaerobic fermentation [66] or anaerobic respiration [67] depending on the type of electron acceptors [64]. In an anaerobic fermentation, organic matter is catabolized in the absence of an external electron acceptor by facultative anaerobes through internally balanced oxidation-reduction reactions under dark conditions [64,68]. The product generated during the process accepts the electrons released during the breakdown of organic matter. Thus, organic matter acts as both electron donor and acceptor. During the fermentation reactions, the substrate is only partially oxidized, and therefore, only a small amount of the energy stored in the substrate is conserved [64]. The major portion of the adenosine triphosphate (ATP) or energy is generated by substrate-level phosphorylation [69,70]. Anaerobic respiration on the other hand requires external electron acceptors for the disposal of electrons released during the degradation of organic matter. The electron acceptors in this case could be CO₂, SO₄²⁻ or NO₃⁻. Both substrate-level phosphorylation and oxidative phosphorylation generate energy (or ATP) [64]. The energy released under such a condition is much greater than anaerobic fermentation [71]. Skoog et al. [71] have reported that at in situ geochemical conditions where large numbers of heterotrophic microorganisms inhabit hydrothermal systems, for aldose being reacted upon by these microbial populations, fermentation yields 220–420 kJ/mol of energy while anaerobic respiration releases 500–2,400 kJ/mol.
The anaerobic digestion process is characterized by a series of biochemical transformations brought on by different consortia of bacteria [72]. The anaerobic digestion of organic matter basically follows the following stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis [72–74]. Despite the successive steps, hydrolysis is generally considered as rate limiting [74] and the rate of hydrolysis depends on the pH, temperature, composition and concentration of intermediate compounds [72]. The hydrolysis step degrades both insoluble organic material and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids, into soluble organic substances (e.g. amino acids and fatty acids) [74] by extracellular hydrolytic enzymes produced by hydrolytic bacteria and then dissolved into solution. The components formed during hydrolysis are further split during acidogenesis, the second step. Volatile fatty acids, alcohols [72] are produced by acidogenic bacteria along with ammonia, carbon dioxide, hydrogen sulphide and other by-products [75]. This phase is accompanied by decrease of pH due to production of acids and protonic acidification. If the reactor is overloaded, low pH value may inhibit the process. The main species identified as responsible for the biological hydrogen production during the acidogenesis of the carbohydrates are Enterobacter, Bacillus and Clostridium [76]. The third stage in anaerobic digestion is acetogenesis, where the higher organic acids and alcohols produced by acidogenesis [77] are further digested by acetogens to produce mainly acetic acid as well as CO₂ and H₂. This conversion is controlled to a large extent by the partial pressure of H₂ in the mixture [74]. The final stage of methanogenesis produces methane [78] by two groups of methanogenic bacteria [79]: the first group splits acetate into methane and carbon dioxide and the second group uses hydrogen as electron donor and carbon dioxide as acceptor to produce methane. The bacteria involved in the methanogenesis stage are sensitive to low as well as to high pH, which must be kept within a range of 6.5–8.

Pollutant Remediation by Anaerobic Processes

During the last recent years, research interest has also been growing in the study and application of anaerobic digestion for the degradation and elimination of pollutants such as dyes [80], PAHs [81], highly chlorinated hydrocarbons and xenobiotics [82], adsorbable organic halides [83] and pesticides [84].
Very recently, Baczynski and Pleissner [85] have used methanogenic granular sludge and wastewater fermented sludge as inocula for batch tests of anaerobic bioremediation of chlorinated pesticide contaminated soil, and their results obtained for both types of biomass were similar with 80 to over 90% of γ-hexachlorocyclohexane (γ-HCH), 1,1,1-trichloro-2,2-bis-(4-methoxyphenyl)ethane (methoxychlor) and 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane (DDT) removed in 4–6 weeks. Bernal-Martinez et al. [86] have assessed the removal of PAH naturally present in sludge by continuous anaerobic digestion with recirculation of ozonated sludge. Recirculation of ozonated digested sludge allowed enhancing PAH removals with the highest efficiency obtained with the highest ozone dose (0.11 g O₃/g TS). In their study, Fuchedzhiieva et al. [87] have tested Bacillus cereus isolated from municipal wastewater treatment plant to assess the efficiency of two anionic surfactants, a chemical surfactant and a biosurfactant during fluoranthene biodegradation under anaerobic methanogenic conditions (linear alkyl benzene sulphonates and rhamnolipid–biosurfactant complex from Pseudomonas sp. PS-17, respectively). No change in the fluoranthene concentration was registered after 7th day. While it was reported that the rhamnolipid–biosurfactant had inhibited the cell growth and had no effect on the biodegradation rate, LAS enhanced the cell growth as well as the fluoranthene biodegradation, thereby demonstrating the latter surfactant’s promise as an agent for facilitating the process of anaerobic PAH biodegradation under methanogenic conditions.

Biosorption of Heavy Metals

Most of the organic pollutants are degraded or detoxified by physical, chemical and biological treatments before released into the environment. Although the biological treatments are a removal process for some organic compounds, their products of biodegradation may also be hazardous. Moreover, some non-degradable compounds like the heavy metals ions discharged into the environment along with the treated compounds can cause problems due to non-degradability, bioaccumulation, biomagnification and transport to long distances. As a result, some organic molecules and the heavy metals ions are not biodegradable and persist in the environment.
Conventional methods for the removal of the heavy metals ions from wastewaters include chemical precipitation, electroflotation, ion exchange, reverse osmosis and adsorption onto activated carbon [88]. But due to operational demerits, high cost of the treatment and the generation of toxic chemical sludges, some new technologies have been tried for a long time [89]. Among them less expensive non-conventional adsorbents (referred to as biosorbents) like apple waste [90], peanut hull carbon [91], agricultural wastes [92] and red mud [93] are being investigated for the removal of ions like the Cd and Ni ions. Sud et al. [94] propose the use of agricultural waste materials as biosorbents of heavy metals as a low cost and highly efficient technology, because the functional groups present in agricultural waste biomass (acetamido, alcoholic, carbonyl, phenolic, amido, amino and sulphhydryl groups) have affinity for heavy metals ions to form metal complexes or chelates that immobilize the contaminants through reactions of chemisorption, complexation, adsorption on surface, diffusion through pores and ion exchange. As a result, researchers and engineers, all alike, have been oriented toward the practical use of adsorbents for the treatment of wastewater polluted by heavy metals [95].

Science of Biosorption

Several important aspects of biosorption for heavy metal removal need to be considered when exploring this emerging bioremediation technique for optimization purposes. The underlying principles of biosorption for removal of metal ions, the kinetics of mass transfer during the process of biosorption of metal ions, the theory and models that can be used to describe the mass transfer process and the thermodynamics of biosorption of heavy metals onto biomass and the models which can be used to quantify metal–biomass interactions at equilibrium, all are key knowledge areas in biosorption science which have been hence so far relatively well presented, discussed and reviewed in the literature.

A biosorption process can be performed via several modes [96]; of which, batch and continuous modes of operation are frequently employed to conduct laboratory scale biosorption processes. Although most industrial applications prefer a continuous mode of operation, batch experiments have to be used to evaluate the required fundamental information, such as biosorbent efficiency,
optimum experimental conditions, biosorption rate and possibility of biomass regeneration. The factors influencing bacterial batch biosorption are solution pH, temperature, ionic strength, biosorbent dosage, biosorbent size, initial solute concentration and agitation rate [96]. Different metal-binding mechanisms have been postulated to be active in biosorption metal uptake such as chemisorption by ion-exchange, complexation, coordination, chelation; physical adsorption and microprecipitation [97]. There are also possible oxidation-reduction reactions taking place in the biosorbent. Due to the complexity of biomaterials and biosorbents, it is also plausible that at least some of these mechanisms are acting simultaneously to varying extents depending on the biosorbent composition, surface properties and functional chemical groups, and the solution environment [97]. Biomass materials offer several molecular groups that are known to offer ion exchange sites, carboxyl, sulphate, phosphate, and amine, could be the main ones [97]. Ion-exchange is an important concept in biosorption, because it explains many of the observations made during heavy metal uptake experiments [98]. It should be pointed out that the term ion-exchange does not explicitly identify the binding mechanism, rather it is used here as an umbrella term to describe the experimental observations [98]. The precise binding mechanism(s) may range from physical (i.e. electrostatic or London-van der Waals forces) to chemical binding (i.e. ionic and covalent).

Within the literature, the Langmuir and Freundlich models (two-parameter models) have been used to describe biosorption isotherm. The models are simple, well-established and have physical meaning and are easily interpretable, which are some of the important reasons for their frequent and extensive use [96]. Some other two-parameter models widely used for describing biosorption isotherms include the Temkin isotherm, the Dubinin-Radushkevich model, the Redlich-Peterson model, the Sips model, the Khan model, the Radke-Prausnitz model and the Toth model. Of these three-parameter models, the Redlich-Peterson and Sips models have been used with most success. Besides the Langmuir, Freundlich and Redlich-Peterson adsorption isotherm models which fitted accurately with the experimental data, the Thomas model, Adams-Bohart and Wolborska models were also used to represent the dynamic sorption of chromium using immobilized beads.
Biosorption and Heavy Metal Removal

Biosorption is the binding and concentration of adsorbate(s) from aqueous solutions (even very dilute ones) by certain types of inactive, dead, microbial biomass. The major advantages of biosorption over conventional treatment methods include: low cost, high efficiency, minimization of chemical or biological sludge, regeneration of biosorbents and possibility of metal recovery [94]. Hence, research on biosorption of heavy metals, intrinsically guided by Green Chemistry, has led to the identification of a number of microbial biomass types that are extremely effective in concentrating metals. Some types of biomass are waste byproducts of large-scale industrial fermentations while other metal-binding biomass types can be readily harvested from the oceans. These biomass types can accumulate in excess of 25% of their dry weight in deposited heavy metals: Pb, Cd, U, Cu, Zn, Cr and others. Some biosorbents can bind and collect a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals. When choosing the biomass for metal biosorption experiments, its origin is a major factor to be considered. In general terms, biomass can come from industrial wastes which should be obtained free of charge, organisms that can be obtained easily in large amounts in nature (e.g., bacteria, yeast and algae) or fast-growing organisms that are specifically cultivated or propagated for biosorption purposes (crab shells, seaweeds). Research on is revealing that it is sometimes a complex phenomenon where the metallic species could be deposited in the solid biosorbent through various sorption processes, such as ion exchange, complexation, chelation, microprecipitation and oxidation/reduction.

Factors Influencing Bioremediation Rates

The microbial population follows a growth cycle comprising the three distinct phases namely the lag phase, exponential phase, stationary phase, and death phase [99]. In the lag phase there is a delay in the microbial population growth until the microbes have become acclimatized to the substrate(s) [100], which in many instances are the contaminants/pollutants under remediation, and surrounding conditions. The microbes cannot consume the food source until they have developed the required enzymes and metabolites necessary to
break down the contaminant [22]. After the necessary enzymes and metabolites have been produced, the microbes enter the exponential phase of growth [101]. The rate of exponential growth is influenced by environmental conditions as well as by characteristics of the organism itself. However, exponential growth cannot occur indefinitely. Generally, either an essential nutrient for growth is used up or some waste product of the organism builds up to such a level that the exponential growth is inhibited and ultimately ceases [102]. At this point the microbial population reached the stationary phase, where there is no net increase or decrease in microbial cell populations. Dependent on the possible build up of environmental toxins or depletion of bioavailable substrates [103], the microbial population may enter the death phase and the viable number of microbes will decrease. Based on this growth cycle, almost all organic compounds are degradable given the proper environmental, physicochemical and time conditions [22]. However, a range of other physical, chemical and biochemical conditions or materials can interfere with bioremediation rates. The most important factors are discussed below.

**pH**

pH values < 3 and > 9 or 10 as well as sudden changes in the pH of the waste/treatment system matrix can significantly inhibit microbial growth by interfering with the microbial metabolism, gas solubility in soil water, nutrients availability and bioavailability in soil water, and heavy metal solubilities [104]. Most natural environments have values of pH between 5.0 and 9.0, and as a result this range is optimal for microbial enhanced biodegradation of waste contamination. This pH range is maintained by a natural buffering capacity that exists in most fertile native soils due to the presence of carbonates and other minerals [105].

**Temperature**

Temperature affects (a) the bacterial metabolism (b) microbial growth rates (c) the soil matrix and (d) physic-chemical state of the contaminants. Generally in situ bioremediation is carried under mesophilic condition (20°C to 40°C). Even for laboratory studies, bacteria with potential remediation value have focused on mesophilic species because of the species, of cultivation and relatively
short doubling times [106]. The rate of biochemical reactions in cells increases with temperature up to a maximum, above which the rate of activity declines as enzyme denaturation occurs and organisms either die or become less active [107]. Low temperatures seldom kill the microbes and with warming the microbes typically recover. Temperature also affects gas solubilities and must be taken into account when designing a remediation system. In compost heaps or biopiles, the temperature in the center of the soil/sediment may reach 70°C or higher during the initial active phase and thermophilic bacteria can be seen performing under such conditions [108].

**Metals**

Metals can inhibit various cellular processes and their effects are often concentration-dependent [109]. Metal toxicity for microbes will usually involve specific chemical reactivity. Metals such as copper, silver, and mercury are typically very toxic particularly as ions, while metals such as lead, barium and iron are usually benign to the microbes at levels typically encountered. The nutrient metals are usually found naturally in the necessary amounts for plants and microbes in fertile soils [110]. The principal inorganic nutrients are nitrogen and phosphorus; however, trace amounts of potassium, calcium, sulphur, magnesium, iron, and manganese are also required for optimum biological growth. The availability and/or toxicity of these metals to the microbes is usually dependent on pH, with the metals becoming more mobile/available at higher values of pH.

**Toxic Compounds**

At high concentrations, some chemicals are toxic to microbes, even if the same chemical is readily degraded at lower concentrations [111]. Toxicity prevents or slows metabolic reactions and often prevents the growth of new biomass needed to stimulate rapid contaminant removal [104]. The degree and mechanisms of toxicity vary with specific toxicants, their concentration, and the exposed microorganisms. Microbial cells cease to function when at least one of the essential steps in their numerous physiological processes is blocked. The blockage may result from gross physical disruption of the cell structure or competitive binding of a single enzyme essential for metabolizing the toxicant [22,104]. By design,
some organic compounds are toxic to targeted life forms such as insects and plants, and may also be toxic to microbes. These compounds include herbicides, pesticides, rodenticides, fungicides and insecticides.

In addition, some classes of inorganic compounds such as cyanides and azides are toxic to many microbes [22]; however, these compounds may be degraded following a period of microbial adaption [112]. In this respect, certain studies have indeed shown the fungal biodegradation of cyanide and microbial adaption to such toxic compounds. Gurbuz et al. [113] have examined cyanide effluent degradation by Scenedesmus obliquus. Gold mill effluents containing cyanide concentration of 77.9 mg/L was fed to batch unit to examine the ability of S. obliquus for degrading cyanide. Cyanide was reduced down to 6 mg/L in 77 h. Gurbuz et al. [113] reported that the cells had well adapted to high pH and the effluent contained cyanide and the metals. All the more, chlorinated aromatic compounds are biorecalcitrant, and in particular, 2,4,5-trichlorophenol demonstrates greater resistance to biodegradation than other trichlorophenols and is also a known uncoupler of the electron transport chain [112]. In this respect, Marsolek et al. [112] have investigated the biorecalcitrance, inhibition, and more specifically the adaptation to 2,4,5-trichlorophenol by aerobic mixed microbial communities. Although it was initially observed that 2,4,5-trichlorophenol was strongly resistant to biodegradation at concentrations greater than 40 μmol/L, and inhibited to respiration in direct proportion to 2,4,5-trichlorophenol concentration, the microbial communities later showed consistent adaptation patterns to 2,4,5-trichlorophenol at concentrations of 10 μmol/L and 20 μmol/L.

Water Content

Water content, and especially water availability, influences bioremediation rates [114]. Li et al. [15] have reported that a lack in the effect from bioremediation could be attributed to poor soil water sorption, which was negatively influenced by hydrocarbon residuals. This study hence supported that the soil–water relation is one of the most important factors in assessing endpoint of bioremediated soils for plant growth. Water in soils or sediments may not be available to microorganisms because it is absorbed by solid substances or tied up as water of hydration to dissolved solutes. This can be solved by irrigating the contaminated soils, compost heaps and/or biopiles.
Vinãs et al. [116] maintained the moisture content and aeration were the key factors associated with PAH bioremediation.

**Nutrient Availability**

Nutrients are generally supplemented in both *in situ* and *ex situ* bioremediation of soils, sediments, ground and surface waters for the promoting the bioremediation rates [117,118]. Nutrient requirement depends on the nature of contaminants and the extent to which the polluted site has been subjected to agricultural use. Zhou et al. [119] have investigated the effect of phosphorus concentration on PAH dissipation in the rhizosphere of mycorrhizal plants in a pot experiment using two plant species, alfalfa (*Medicago sativa*) and tall fescue (*Festuca arundinacea*). The major finding was the significant positive impact of mycorrhizal plants on the dissipation of high molecular weight PAH in high–water low–phosphorus treatment. Earlier, El–Bestawy and Albrechtsen [120] investigated the mineralization and/or degradation of the phenoxy herbicide mecoprop (MCPP) by a group of soil bacteria under the effects of nutrient amendments. Five different species of *Pseudomonas* (*P. paucimobilis, P. aeruginosa, P. mallei, P. pseudomallei*, and *P. pickettii*) were isolated for the MCPP mineralization and/or removal. Significant variations in the removal percentages of MCPP by either mineralization or biodegradation were observed. Also, the highest MCPP mineralization and degradation by the selected *Pseudomonas* spp. were achieved by their inactive (dead) followed by active–rich cultures with both inoculated in nutrient–rich cultures and sterilization on MCPP decontamination.

**Bioavailability of Pollutants**

Biology in regards to bioremediation refers to the intrinsic ability of the biota to assimilate and metabolize the contaminant [121], and matrix effects include the ways in which the biodegradation is influenced by the interactions of the soil with the biota and the contaminants. Bacteria in soils are predominantly attached to soil particles, and so will be constrained by this attachment and by the physico–chemical properties of the surface [121]. Contaminants interact with soils in complex ways through sorption and mass transfer resistance that generally impede their availability to
organisms. For example, anthropogenic organic polymers such as polystyrene and polyvinyl chloride are highly recalcitrant because of their insolubility and the lack of extracellular microbial enzymes capable of catalyzing depolymerization. However, non-polymer degrading bacteria and actinomycetes are able to degrade oligomeric polystyrene fragments and low molecular weight fragments of lignin resulting from fungal attacks on the lignin polymer.

Recently, Trinh Tan et al. [122] have investigated the aerobic biological degradation of the synthetic aliphatic–aromatic co–polyester Ecoflex™ (BASF) by 29 strains of enzyme–producing soil bacteria, fungi and yeasts at moderate environmental conditions. It was found that the aliphatic–aromatic co–polyester could be degraded by a number of different microorganisms and the bacteria studied preferentially degraded the bonds between aliphatic components of the copolymer and the rate of biodegradation of oligomers was appreciably faster than that for the polymer chains. Using GC–MS techniques, Trinh Tan et al. [122] identified the degradation intermediates as the monomers of the co–polyester, and gel permeation chromatography was able to suggest exo–enzyme type degradation, whereby the microbes had hydrolysed the ester bonds at the termini of the polymeric chains preferentially. All the more, low water solubility and a tendency to adsorb to particulate matter in soils and sediments are factors that can severely limit in situ biodegradation of many sediments contaminated with organic contaminants, polychlorinated and polycyclic aromatic hydrocarbons.

Biosurfactants produced by microorganisms within soils and sediments have been shown to enhance biodegradation rates. In a laboratory study to assess the effect of adding either Pseudomonas aeruginosa UG2 cells or the biosurfactants produced by this microorganism on the biodegradation of a hydrocarbon mixture in soil at 20°C over a 2–month incubation period, Jain et al. [123] had observed that the addition of 100 µg of UG2 biosurfactants g⁻¹ soil significantly enhanced the degradation of tetradeane, hexadecane and pristane but not 2–methyl naphthalene, the most water–soluble of the hydrocarbons. Lately, Whang et al. [124] have investigated the potential application of two biosurfactants, surfactin (SF) and rhamnolipid (RL), for the biodegradation of diesel–contaminated water and soil with a series of bench–scale experiments. The rhamnolipid used in this study was a commonly isolated glycolipid biosurfactant produced by Pseudomonas aeruginosa J4, while the surfactin was a lipoprotein type biosurfactant produced by Bacillus subtilis
ATCC 21332. It was deduced that both biosurfactants had been able in increasing the diesel solubility with increased biosurfactant addition. In the diesel/water batch experiments, an addition of 40 mg/L of surfactin significantly enhanced biomass growth (2500 mg VSS/L) as well as increased the diesel biodegradation percentage to 94%, compared to batch experiments with no surfactin addition (1000 mg VSS/L and 40% biodegradation). The addition of rhamnolipid to diesel/water systems from 0 to 80 mg/L substantially increased biomass growth and diesel biodegradation percentage from 1000 to 2500 mg VSS/L and 40 to 100%, respectively.

Co-metabolism

Co-metabolism is a process whereby microorganisms involved in the metabolism of a growth promoting substrate also transform other organic contaminants which can be called as co-substrates [125]. The latter are however not growth supporting if they are provided as the only sources of carbon and energy. This is one of the most important mechanisms involved in the transformation of chlorinated organic aliphatic and aromatic contaminants by microbes. Such co-metabolic transformation of organic pollutants is an important process in both aerobic and anaerobic environments namely bacterial transformation of dichlorodiphenyl trichloroethane DDT, PCBs [126] and trichloroethylene. Ziaogova et al. [127] have recently reported a comparison of the ability of Staphylococcus xylosus to degrade 2,4-dichlorophenol and 4-Cl-m-cresol in separate cultures. In this study, bacterial adaptation and the continuous presence of glucose, as a conventional carbon source, were found to stimulate the degrading efficiency of S. xylosus. All the more, microbes can sequentially remove halogen atoms from polluting halogenated compounds.

Bioaugmentation

Where degradative microbes do not exit or where the process is too slow, microbial inoculates may be added to enhance bioremediation rates. This technique is known as bioaugmentation [128] and may involve (a) an addition of natural isolates of bacteria or (b) genetically engineered organisms (GEMs). There are rigid rules governing the release of GEMs as there is concern about their potential negative impacts on the environment. The genetic
patterns have evolved over several decades and they are relatively stable. It is believed that altered genomes have greater instability and increase the chances of mutations, some of which may not be safe. Bioaugmentation has met with varying degrees of success. Gertler et al. [129] have applied an experimental prototype oil boom including oil sorbents, slow-release fertilizers and biomass of the Marine oil-degrading bacterium, *Alcanivorax borkumensis*, for sorption and degradation of heavy fuel oil in a 500-L mesocosm experiment, and it was found that growth of this obligate oil-degrading bacterium on immobilized oil coincided with a 30-fold increase in total respiration. Earlier, Bento et al. [130] evaluated the effect of bioaugmentation on the degradation of TPH in soil. It was reported that bioaugmentation of the contaminated soil showed the greatest degradation in the light (72.7%) and heavy (75.2%) fractions of TPH since the greatest microbial activity (dehydrogenase activity) had occurred with bioaugmentation up to 3.3-fold. More recently, Teng et al. [131] have conducted a microcosm study to test the bioremediation potential of *Paracoccus* sp. strain HPD–2 on an aged PAH-contaminated soil. The bioaugmented microcosms showed (a) a 23.2% decrease in soil total PAH concentrations after 28 days, with a decline in average concentration from 9,942 to 7,636 μg/kg dry soil, and (b) higher counts of culturable PAH-degrading bacteria, microbial biomass and enzyme activities were observed in bioaugmented soil.

**Trends in Bioremediation Research**

Bioremediation has considerable public support because it aims to enhance natural processes and it is generally seen as "environmentally appropriate." However, bioremediation rates are often considerably slower than physical methods such as removing the contaminated material to a secure landfill [125]. In this respect, and in pursuit to improve the performance of bioremediation processes, there have been a number of different biotechnological procedures that have been tested with a view to improve reliability, cost efficiency and bioremediation rates.

Modern biotechnological techniques including genetic engineering; culture of recombinant microorganisms, cells of animals and plants; metabolic engineering; hybridoma technology; bioelectronics; nanobiotechnology; protein engineering; transgenic animals
and plants; tissue and organ engineering; immunological assays; genomics and proteomics; bioseparations and bioreactor technologies [3] have been gaining thrust in research and show much scope to improve bioremediation rates. Strategies for improving bioremediation efficiency using genetic engineering consist in improving strains and chemotactic ability, the use of mixed population biofilms and optimization of physico-chemical conditions. Gene expression in biofilm cells differs from planktonic stage expression and these differentially expressed genes regulate biofilm formation and development. Biofilm systems have been shown to be especially suitable for the treatment of recalcitrant compounds because of their high microbial biomass and ability to immobilize compounds [132].

Concluding Note

A number of organic environmental pollutants, such as PAHs, PCBs and pesticides, and inorganic pollutants (heavy metals like arsenic, cadmium, chromium, lead and zinc) are resistant to degradation and represent an ongoing toxicological threat to both wildlife and human beings. Bioremediation has grown into a green, attractive and promising alternative to traditional physico-chemical techniques for the remediation of these POPs at a contaminated site, as it can be more cost-effective and it can selectively degrade the pollutants without damaging the site or its indigenous flora and fauna. However, bioremediation technologies have had limited applications due to the constraints imposed by substrate and environmental variability, and the limited biodegradative potential and viability of naturally occurring microorganisms. This chapter revisited the essentials aspects of bioremediation and demonstrated that the application of biotreatment is growing rapidly due to its merits which outweigh the demerits. The application of the diverse bioremediation technologies must be based on sound and reliable scientific data obtained from both fundamental and applied research studies. Hence, the success of bioremediation depends in continually expanding the scientific and engineering work that provide the real bases for both the technology and its evaluation; and simultaneously in explaining and justifying the reasons which allow scientists and engineers to actually use these technologies to uphold environment stewardship and protection.
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