Chapter 2
Microbes and Processes in Bioremediation of Soil

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Abstract Environmental pollution has been increasing at an alarming rate since the beginning of the twenty-first century. There is an enormous increase in the production and use of xenobiotic compounds that have created new sites of environmental contamination and problem worsens as many of such xenobiotic compounds are either persistent or recalcitrant to microbial breakdown. The presence of anthropogenic organic compounds/chemicals in the environment is a matter of significant concern because of their potential toxicity, mutagenicity, and bioconcentration (biomagnification) in higher organisms. This is of immense concern and hence provides impetus to the development of certain remediation techniques. Various microorganisms play a key role in the bioremediation of soil and may range from bacteria majorly to a few actinomycetes and fungi. Bioremediation can be carried out *via* two main approaches, ex situ and in situ, and choice of method depends largely on site characteristics, concentration, and type of pollutants present. To enhance the remediation process, a more recent approach called bioaugmentation is also practiced. Bioaugmentation trials have met varying degrees of success. This chapter will largely focus on various microorganisms which are potent biomediators and also the processes involved in the same.

Keywords Bioremediation · Xenobiotic compounds · Bioconcentration · Bioaugmentation

2.1 Introduction

Increasing the standard of living and urbanization has posed a great degradative threat to the environment and ecosystem. A typical example is the monstrous sized heaps of waste dumped daily into the dumping yards of cities. Also, the

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advancements in science, technology, and industrial sector have led to the production of waste ranging from municipal sewage to nuclear waste, and also this has rendered our ecosystem unfit for the survival of life forms on earth (Lin et al. 2018; Ontanon et al. 2018; Parewa et al. 2018). Previously, the use of conventional techniques was practiced such as waste disposal by landfilling, dumping in open grounds, etc. With rapid and ever-growing waste disposal problems, conventional methods failed to cope up with this issue. New methods like incineration and chemical decomposition are being developed, but the use of such methods is either uneconomical or not environment-friendly. Such problems lead to the development of newer and better technologies which may better solve the purpose. Modern-day bioremediation is one such method (Karigar and Rao 2011).

The literal meaning of "bioremediation" is "biological treatment". So, bioremediation by definition means the use of biological agents such as microorganisms (majorly bacterial and fungi) and/or plants (in case of phytoremediation) for the treatment of contaminated soil and water, so as to make it fit for reuse by all the biological entities. Some of the remediation processes used for the treatment of contaminated area include natural attenuation, composting, biopiling, bioventing, landfarming, thermal desorption, landfilling, soil washing, and incineration (USEPA 2014). Till now, majorly the success to bioremediation and biodegradation has been provided by the indigenous microbes thriving in that very environment, and this is highly dependent upon the growth characteristics and nutritional requirements of the microbes used for the purpose (Verma and Jaiswal 2016). There are several factors that define the choice of bioremediation techniques, e.g., nature of pollutant, degree of pollution, geographical location, the cost involved in the process, etc. (Frutos et al. 2012; Smith et al. 2015). Biological treatment of soil using various biological agents primarily plants and microorganisms is considered as one of the cheapest and safest methods to remove the hazardous contaminants from the soil. Plants have the capability to neutralize various types of harmful chemicals in the soil by direct utilization, followed by the biotransformation of such compounds into nontoxic products which are harmful neither to the environment nor to any other form of life (Macek et al. 2008).

The major focus of this chapter is the microbes which play a vital role in effective bioremediation of soil and also on processes involved in the biodegradation. Microorganisms have this inherent capability to catalyze the degradation and mineralization of various contaminating xenobiotic compounds, thus converting them into nontoxic by-products (Seshadri and Heidelberg 2005; Head et al. 2006; Gomez et al. 2017). Such a conversion process is often a result of consortia of microorganisms. Recently, biodegradation of total petroleum hydrocarbons was investigated in slurry phase bioreactor using aged refuse (Fu Chen et al. 2019). Bioremediation can only be effective when the environmental conditions permit the microbial growth and activity, or conversely, there is a need to manipulate certain environmental parameters to allow the growth of microbes so that degradation could proceed at a faster pace (Vidali 2001). Most of the bioremediation procedures run under complete aerobic environment, but to treat certain recalcitrant molecules, the system may run under anaerobic environment (Colberg and Young 1995).

2.2 The Basic Approach to Bioremediation

Mostly bioremediation proceeds through a process of oxidation-reduction reactions (redox), whereby a chemical species donates an electron to a different species that accepts an electron. Bioremediation procedures can be broadly classified as aerobic and anaerobic bioremediation.

2.2.1 Aerobic Bioremediation

Aerobic bioremediation is the most practiced and most prevalent form of oxidative bioremediation. As the name suggests here oxygen acts as the terminal electron acceptor for the oxidation of various contaminants such as polyaromatic hydrocarbons (PAHs), phenols, petroleum, etc. Preference of oxygen points toward higher oxidative potential of oxygen and its requirement by some enzyme systems to initiate the degradation process.

Under ideal conditions, the biodegradation rates of aliphatic, alicyclic, and aromatic compounds (low to moderate molecular weight) can be very high. With an increase in the molecular weight of the compound, its resistance toward biodegradation increases (Norris 1993).

There are several physical methods for aerating the soil above water table, e.g., landfarming, composting, and bioventing (Frutos and Fernandez 2010). Approaches for aeration of soil below water table include flushing aerated water through treatment zone, air sparging, and the addition of molecular oxygen or peroxide.

2.2.2 Anaerobic Bioremediation

This technique can be employed to remediate a broad range of already oxidized contaminating pollutants including ethenes (PCE, TCE, DCE, VC), chlorinated ethanes (TCA, DCA), chloromethanes (CT, CF), chlorinated cyclic hydrocarbons, various energetics (e.g., perchlorate, RDX, TNT), and nitrate.

Anaerobic bioremediation occurs in two steps:

1. Depletion of background electron acceptors like oxygen (O), nitrate (NO_3^-), ferric ion (Fe^{3+}), etc.
2. Stimulation of biochemical reduction of oxidized contaminating pollutants.

2.2.3 The Fate of Organic Contaminating Pollutants

The knowledge of various catabolic pathways involved in the degradation of contaminating pollutants of both aerobic and anaerobic microorganisms has a major beneficial impact in the development of *in situ* and *ex situ* bioremediation protocols. The intrinsic chemical consideration that limits the biodegradability of aromatic pollutants in both aerobic and anaerobic environments was reviewed by Field et al. 1995.

The aerobic microorganisms make use of oxygenase enzyme to initiate the electrophilic attack on aromatic molecules. The process is greatly suppressed by the presence of electron withdrawing groups such as chloro, azo, and nitro (Dorb and Knackmuss 1978; Knackmuss 1981). The microorganisms involved in the aerobic degradation are *Candida*, *Anabaena*, *Nostoc*, *Chlamydomonas*, *Microcoleus*, *Oscillatoria*, *Saccharomyces*, *Chlorella*, and *Phormidium* (DorotaWolicka et al. 2009). On the other hand, anaerobic microorganisms proceed with the degradation of aromatic pollutants in a completely reciprocal manner, i.e., under anaerobic conditions, the microorganisms make use of enzymes to initiate an electrophilic attack on the aromatic molecules. So here, the presence of electron withdrawing group will enhance the initial reductive attack on aromatic contaminants (Knackmuss 1992, Dolfing and Harrison 1993, Ann-Kathrin Ghattas et al. 2017). Conversely, electron donating groups will hinder the anaerobic transformation of aromatic compounds but will favor the aerobic biotransformation process (Field et al. 1995). However, the complete absence of electron withdrawing as well as electron donating group will enhance the recalcitrance of hydrocarbon in the anaerobic environment (Schink 1985; Schink 1988). It has also been noted that the resulting products of anaerobic biodegradation of complex molecules such as polychlorinated and polynitroaromatic compounds are appropriate products for aerobic mineralization but they resist further anaerobic biodegradation (Zitomer and Speece 1993).

2.3 Microbes Involved in Bioremediation of Soil

The contamination of soil, sediment, and water from industrial and other human inputs is widespread and poses a threat to human and ecological health. Bioremediation is the use of microbes for the beneficial removal of contaminants of concern. The microbial processes involved in bioremediation are normally natural components of respiration or adaptation, often a component of carbon cycling or metal redox cycling. Thus, bioremediation often occurs without direct intervention; however, biostimulation (the addition of nutrients or adjustment of conditions) and bioaugmentation (the addition of microbes capable of bioremediation) are however important for the complete removal of contaminants within an economical timeframe. Various microorganisms involved in bioremediation of various contaminating pollutants are listed in Table 2.1.

Table 2.1 Microbes involved in the bioremediation of contaminants

Contaminants	Microorganisms	References
Monocyclic aromatic hydrocarbons	*Penicillium chrysogenum*	Abdulsalam et al. (2013) and Pedro et al. (2014)
BTEX	*P. chrysogenum*	
Phenols	*Bacillus subtilis*, *Penicillium chrysogenum*, *Corynebacterium propinquum*, *Alcaligenes odorans*, *Pseudomonas aeruginosa*	Singh et al. (2013)
Petrol and diesel oil	*Penicillium alcaligenes*, *P. putida*, *P. veronii*, *P. mendocina*, *Achromobacter*, *Flavobacterium*, *Acinetobacter*	Safiyanu et al. (2015) and Sani et al. (2015)
PAHs	*Pseudomonas putida*, *Pseudomonas* sp., *Coprinellus radians*, *Ralstonia* sp., *Microbacterium* sp.	Sarang et al. (2013), AI-Jawhari (2014) and Safiyanu et al. (2015)
Biphenyls and triphenylmethanes	*Phanerochaete chrysosporium*	Erika et al. (2013)
Hydrocarbons	*Aspergillus niger*, *A. fumigatus*, *F. solani*, *P. funiculosum*, *Tyromyces palustris*, *Gloeophyllum trabeum*, *Trametes*, *Versicolor*	Karigar and Rao (2011), AI-Jawhari (2014) and Xu et al. (2017)
Methylnaphthalene and dibenzofurans	*Coprinellus radians*	Aranda et al. (2010)
Phenanthrene and benzopyrene	*Candida viswanathii*	Hesham et al. (2012)
Oil	*Alcaligenes odorans*, *Bacillus subtilis*, *Fusarium* sp., *Corynebacterium propinquum*, *Penicillium chrysogenum*, *Pseudomonas aeruginosa*	Hidayat and Tachibana (2012) and Singh et al. (2013)
Crude oil	*Aspergillus niger*, *Saccharomyces cerevisiae*, *Candida glabrata*, *Candida krusei*, *B. brevis*, *P. aeruginosa* KH6, *B. licheniformis*, *B. sphaericus*	Aliaa et al. (2016) and Burghal et al. (2016)
Paints (oil based)	*B. subtilis* strain NAP2, NAP1, NAP4	Phulpoto et al. (2016)
Industrial dyes	*Myrothecium roridum* IM 6482, *Pycnoporus sanguineus*, *Phanerochaete chrysosporium*, *Trametes trogii*, *Penicillium ochrochloron*	Shedbalkar and Jadhav (2011) and Hassan et al. (2013)
Textile dyes	*Micrococcus luteus*, *Nocardia atlantica*, *Bacillus* spp. ETL-2012, *Pseudomonas aeruginosa*, *Bacillus pumilus* HKG212, *Listeria denitrificans*	Hassan et al. (2013), Maulin et al. (2013), Das et al. (2015) and Yogesh and Akshaya (2016)
Black liquor	*Bacillus firmus*, *Staphylococcus aureus*, *Bacillus macerans*, *Klebsiella oxytoca*	Adebajo et al. (2017)
Lead, nickel, and mercury	*Saccharomyces cerevisiae*	Chen and Wang (2015), Infante et al. (2014)

(continued)

Table 2.1 (continued)

Contaminants	Microorganisms	References
Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Cu^{2+}	*Pseudomonas fluorescence*, *P. aeruginosa*	Paranthaman and Karthikeyan (2015)
Co, Cu, Cr, and Pb	*Lysinibacillus sphaericus* CBAM-5	Peña-Montenegro et al. (2015)
Cadmium	*Aspergillus versicolor*, *Trichoderma* sp., *A. fumigatus*, *Paecilomyces* sp., *Microsporum* sp., *Cladosporium* sp.	Priyalaxmi et al. (2014) and Soleimani et al. (2015)
Endosulfan	*Bacillus*, *Staphylococcus*	Mohamed et al. (2011)
Chlorpyrifos	*Enterobacter*	Niti et al. (2013)
Ridomil MZ 68MG, Fitoraz WP76, Decis 2.5EC, Malation	*Pseudomonas putida*, *Arthrobacter* sp., *Acinetobacter* sp.	Hussaini et al. (2013) and Mónica et al. (2016)
Chlorpyrifos and methyl parathion	*Acinetobacter* sp., *Photobacterium* sp., *Pseudomonas* sp., *Enterobacter* sp.	Ravi et al. (2015)

2.3.1 Bioaugmentation

Bioaugmentation is the process of enhancing/stimulating the rate of bioremediation by addition of single strain or consortia of microorganisms as to mimic the competitiveness among the indigenous microflora and also to remove/decrease adaptation/acclimatization time (Bourier and Zeahnder 1993; Liu and Suflita 1993; Singleton 1994). This technique may involve single strain or consortia of microorganisms but also involve genetically engineered microorganisms (GEMs) within certain strict international rules and regulations. Although GEMs are very efficient in such processes, their accidental release into the environment may pose a serious threat to mankind. Keeping in mind such negative impacts, the use of GEMs has been limited to laboratory-based bioreactor applications.

Bioaugmentation strategy used as per model proposed by Forsyth et al. (1995) for soil is:

1. Where the number of degrading microorganisms is low or sub-detectable.
2. Contaminating pollutants which require a multitude of processes to degrade contaminants.
3. Small-scale contaminated site where non-biological treatment processes are not economical.

2.3.1.1 Factors Affecting Bioaugmentation

Although bioaugmentation has solved a number of issues pertaining to bioremediation of contaminants which are aromatic in nature primarily, still there are a number of ecological constraints which hamper its effectiveness and have kept it to a minimal level. One of the major difficulties that arises during the process is the survival of non-native microbial species which are introduced to the contaminated site. Studies have revealed that the number of exogenous microorganisms has reduced shortly after the inoculation of soil. Hence both abiotic and biotic factors are shown to cause such decrease (Cho et al. 2000; Bento et al. 2005; Wolski et al. 2006). Various abiotic factors include temperature, moisture, pH, and organic content of the soil, and biotic factors include aeration, amount of nutrients, and type of soil.

There are various studies and examples which may prove above mentioned points.

The effect of moisture content in the soil on the survival of *Achromobacter piechaudii* TBPZ and degradation of tribromophenol (TBP) indicating minimum 25% water content was required for rapid degradation, whereas soils with 10% moisture content show limited activity (Ronen et al. 2000). Low moisture content in the soil decreases the efficiency with which microorganisms perform the degradation of contaminants, such effect can be attributed to the fact that the decreased bacterial activity is due to the diffusional limitation of substrate supply and adverse physiological effects associated with cell dehydration (Mashregi and Prosser 2006).

Other most crucial factors influencing the efficiency of bioaugmentation is the organic content of the soil. It plays an important role in the bioavailability of contaminants and hence impairs the survival of inoculated strains and ultimately their availability to degrade contaminants, e.g., the rate of 2,4-D degradation was lower in the soil with high organic content but was considerably higher in soils with lower organic content (Greer and Shelton 1992). Conversely, when the soil was combusted to remove the organic content, microbes completely lost their degradative activity. This indicated that there is presence of some components of insoluble organic matter that is nutritionally beneficial for microorganisms involved in BTEX degradation (Kim et al. 2008).

Other factors including competition primarily between indigenous and exogenous microorganisms for limited C-sources and also antagonistic interaction and predation by protozoa and bacteriophages also play an essential role in the final results of bioaugmentation. All these interactions greatly decrease the number of inoculated cells (England et al. 1993; Sorensen et al. 1999).

2.3.1.2 Microbes in Bioaugmentation

Before performing augmentation in the soil for the purpose of enhanced biodegradation, one should fully know the type and level of contaminants and about the strains of microorganisms and their consortia which play active role in the process. The following features should be kept in mind before augmenting soil:

1. The organism should be easily cultivable.
2. The organism used for the purpose should be able to grow fast under given environmental and nutritional conditions.
3. The organism should be able to withstand a high concentration of contaminants and also should be able to survive in varying environmental conditions.

In case of contaminants such as PAHs, it is especially necessary to use organisms which are capable of producing surfactants, so that these contaminants are more accessible and the process becomes more feasible (Forsyth et al. 1995; van der Gast et al. 2003; Gentry et al. 2004).

Several approaches can be followed to select for the microorganisms useful in bioaugmentation. First being, isolating microorganisms from a contaminated site in question and then growing it under laboratory conditions. Finally, this pre-adapted pure culture is returned to the contaminated site. The process is called reinoculation and involves the use of indigenous microflora. The second approach involves the use of microorganisms from the contaminated site having similar kind of contamination. Various studies revealed that microbial consortia for degradation of aromatic contaminants are effective as compared to selected single strains (Goux et al. 2003; Ghazali et al. 2004).

Both Gram-positive and Gram-negative bacteria play a major role in the bioaugmentation. Experiments pertaining to bioaugmentation were done using both the organisms belonging to genera *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Achromobacter*, and *Sphingobium* (Gram-negative bacteria) and *Mycobacterium*, *Bacillus*, and *Rhodococcus* (Gram-positive). Potentially useful fungi in bioaugmentation are represented by genera *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Acremonium*, and *Verticillium.*

2.3.1.3 Delivery of Inoculum

The efficiency of bioaugmentation entirely depends upon the number of microorganisms and total biomass introduced in the soil. The delivery of microbes is also another important factor responsible for efficient bioaugmentation. The conventional delivery mechanisms make use of liquid culture for the introduction of microorganisms into the contaminated site. But nowadays various modifications to such systems have been made. The basic idea of such modifications aimed at maintaining optimum activity of inoculum over an extended period of release which was significantly hampered in case of liquid culture introduction methods. Various modifications include the use of certain carrier material which enhances the activity of microbes and also provides nutrition to the growing microbial population (van Veen et al. 1997). Example of carrier materials includes charcoal-amended soil (Beck 1991), chitin or chitosan (Gentili et al. 2006; Chen et al. 2007), nylon (Heitkamp and Steward 1995), zeolite (Liang et al. 2009), and clay (Omar et al. 1990). A study on activated carbon and zeolite in the treatment of site contaminated with crude oil showed that these materials increased microbial growth and enhanced hydrocarbon degradation (Liang et al. 2009). This revealed that dehydrogenase activity was three times higher in activated carbon than in zeolite. Such an increase in overall activity can be attributed to biocarriers as they improve the diffusion of oxygen, nutrient uptake, and water retention capacity.

Other entirely different approaches primarily used for biodegradation of aromatic compound make use of immobilized cells. This method offers a protective environment to the inoculated microorganisms and provide protection from environmental conditions not suited for their growth (improper pH and presence of toxic contaminants) and also eliminates competition with indigenous microflora (Lin and Wang 1991). Moreover, immobilization is known to increase the stability of cells (DNA, plasmids) (Cassidy et al. 1992). Immobilization is usually performed using both synthetic (poly(carbamoyl) sulfate, polyacrylamide, and polyvinyl alcohol) and natural materials (Dextran, agar, agarose, k-carrageenan, alginate) (Cassidy et al. 1992; Jen et al. 1996; Gardin and Pauss 2001).

2.4 Processes Involved in Bioremediation

The basic ideology of bioremediation is to treat or inactivate the toxic-contaminating pollutants to less toxic or completely nontoxic products which will not cause any deteriorating effect on the environment but in turn may have a positive effect. Bioremediation is generally done by consortia of microorganisms which generally reside in that very habitat which requires treatment. This is an example of in situ bioremediation. This enables the microorganisms to work efficiently because the local environment in which the microorganisms are already growing proves beneficial to their growth and also no adaptory phase is required. Ex situ bioremediation techniques, on the other hand, are primarily based upon physical manipulation of the contaminants, and in this case, there is no direct involvement of microorganisms for remediation procedures.

2.4.1 In Situ Bioremediation Procedures

Over the last few decades, the major thrust area in the field of bioremediation is to understand various nutritional requirements of microorganisms, their biosynthetic and degradative pathways, and their enzymatic machinery. This has led to an increased interest of many scientists, and now many people are working on how to enhance the rate of bioremediation. This is because the waste is generated on a daily basis throughout the world at an alarming rate. So now various strategies are being opted to enhance the biodegradation. Such enhanced *in situ* bioremediation methods have proved beneficial, and various processes have been designed as explained in the following sections.

As the conventional methods of *in situ* bioremediation cannot treat such a heavy load of contaminants of today's world, so now enhanced bioremediation techniques are being developed so that the biodegradation can be carried out at an enhanced rate. Enhanced *in situ* bioremediation of organic contaminants requires the stimulation of biodegradative activities of microbial population thriving in that particular environment by the involvement of certain nutrients or external electron acceptors. For this purpose, the microorganisms are provided with some combination of oxygen, nutrients, and moisture and controlling temperature and pH. There are various procedures through which this can be achieved and are as follows:

2.4.1.1 Bioventing

This is the cheapest mode of *in situ* bioremediation. As the name suggests, bioventing involves supplying the oxygen-rich air into the soil so as to increase the rate of degradation of contaminating organic pollutants in the soil. As already mentioned in the previous section, oxygen acts as the terminal electron acceptor for the oxidation of various organic contaminants. This technology is a choice for the treatment of petroleum waste and another similar kind of recalcitrant toxins (McCauly 1999). There are several kinds of bioventing remediation technologies, one of which includes air sparging, which includes forcing the compressed air into saturated soil, whereas venting technology uses low-pressure air and is more focused toward the deeper unsaturated zone of the soil. The simple bioventing setup consists of a blower or air compressor which is connected to air supply wells and soil-gas monitoring wells which are connected in series (Sellers 1999). Bioventing is the gentlest and stripped up a form of bioremediation because it occurs without intervention into the natural environment of microorganisms. But at the same time, the process of degradation is enhanced by the addition of oxygen (Leahy and Erickson 1995; US EPA 1995b).

2.4.1.1.1 Methods Involved in the Process

a. Injection system: These systems are generally economically feasible to install and simple to operate primarily because this system requires limited/no treatment of off-gas. Injection systems are set up at those locations which are away from various installments such as buildings/property boundary because they, upon injection of air, may push the contaminants deep or away from the actual site. This actually is the beneficial property of the injection system that when the contaminants are moved away, their concentration decreases per unit area, also increasing the contact with layer area of soil with microorganisms. This will result in enhanced biodegradation (US EPA 1995b; Sellers 1999).

b. Extraction system: This system works in a completely opposite way. Extraction system actually sucks out the contaminants. This system is installed in densely populated areas, but there are various side effects, few of which include that it causes the water table to rise and may cause contamination and also it requires treatment of off-gas (Fig. 2.1).

2.4.1.1.2 Applications

The principal compound of crude petroleum is the hydrocarbons; because of this reason, they have become a significant substrate for microbial oxidation (Rosenberg and Ron 1996). Hence, bioventing is preferably used for the treatment of oil spills and has proved to be an excellent option for petrochemical contaminants.

Bioventing is mostly preferred with hydrocarbons whose volatility is very low. Because of effective bioremediation of these petrochemicals, the rate of volatilization

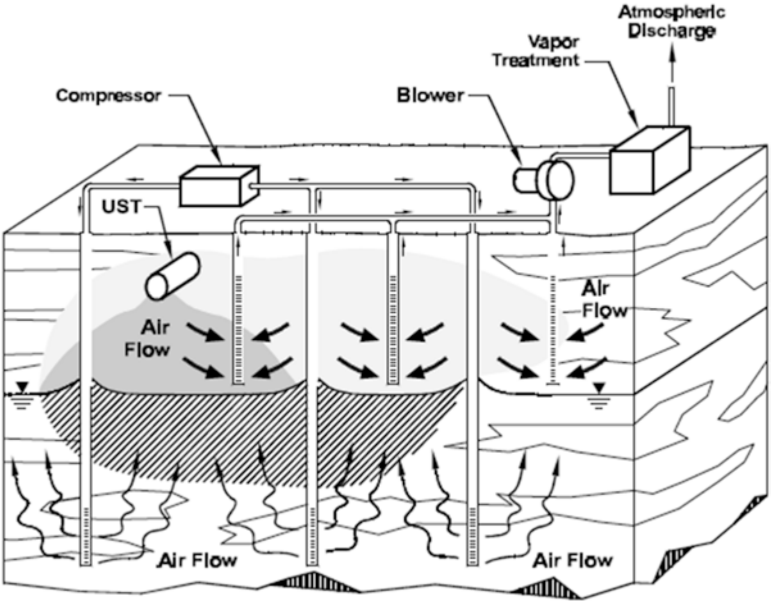

Fig. 2.1 Bioventing showing injection and extraction system (NMED 2010)

should be maintained at optimal level which should be lower than the rate of biodegradation. Low volatility also reduces the chances of degradation because air injection by the process of bioventing will push contaminants into the surrounding environment.

Majorly, gasoline, fuel oil, and bitumen are efficiently reduced by bioventing. Also, bioventing has shown to effectively reduce toluene, benzene, ethylbenzene, and xylenes to the levels below the detection within 1 year (US EPA 1995a). A laboratory test showed that bioventing is quite superior in remediating toluene and decane than other methods of bioremediation (Malina et al. 1998).

2.4.1.2 Biosparging

Biosparging/air sparging is the process of blowing compressed air (composed primarily of oxygen) directly into the saturated subsurface. As a result of this, the bubbles thus formed result in the physical separation of contaminants from groundwater (i.e., stripping) and are thus carried up into the unsaturated zone, where the contaminants are biodegraded by the process of *in situ* bioremediation. This process is further stimulated because of the addition of oxygen-rich air.

A conventional biosparging unit consists of air injection well, an air compressor, monitoring points and wells, and a vapor extraction system which is optional (Fig. 2.2).

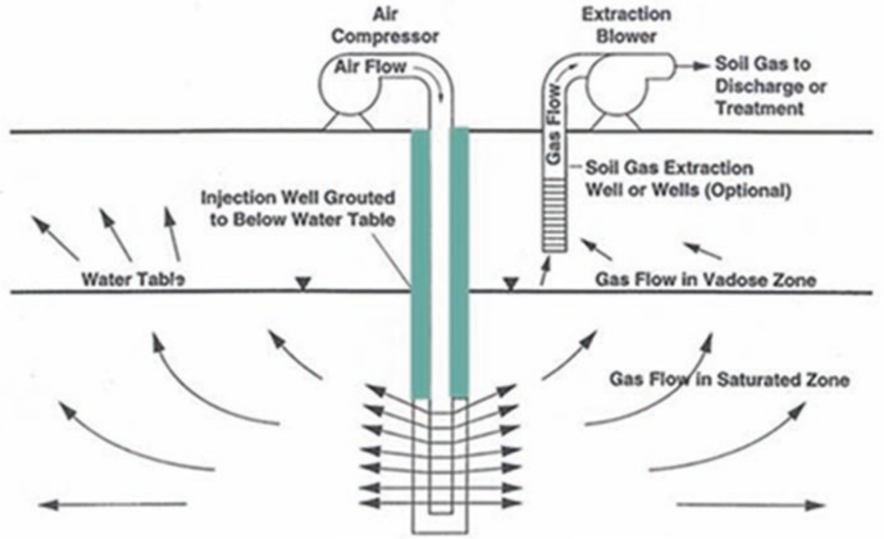

Fig. 2.2 A biosparging unit showing air injection well, monitoring points, deeper extraction system, and air compressor

The air injection wells are generally vertical and are dug to the depths below groundwater table to prevent further mixing of groundwater and contaminants. When compressed air is sparged below the groundwater level, this results in the rising up of gas bubbles thus formed. If the medium is homogeneous (i.e., soil particles are sandy), this may result in the homogeneous flow of air, this is rarely seen as there exists some kind of heterogeneity as non-uniform airflow is quite common.

The compressed system is used to supply compressed air into the injection well, and the choice of the compressor system depends entirely on the nature of the bed below the groundwater (e.g., clay, sand, etc.) and also the pressure required. Biosparging is most effective against contaminants which have higher Henry's law constant, such as benzene, toluene, ethylbenzene and xylenes, and TCE and PCE. However, it can be used to target less volatile compounds by enhancing the biodegradation of compounds like diesel fuel and waste oils (Anderson and Ward 1995; Miller 1995).

2.4.1.3 Anaerobic Bioremediation

Maximum bioremediation technologies focused on the addition of oxygen which acts as a terminal electron acceptor and hence enhances the process of bioremediation. But as this process of delivery of oxygen to subsurface contaminated sites is difficult and also the solubility of oxygen in water is also very low, alternate terminal electron acceptors are required to solve this purpose. A number of oxy-anions substituting oxygen can act as terminal electron acceptors and solve the purpose of microbial degradation of organic compounds. These include salts of iron III, sulfate, and nitrate. Also, there exist wide consortia of anaerobic bacteria which can use these electron acceptors to degrade the organic contaminants (Anderson and Ward 1995; Qencrantz et al. 1995).

Nitrates are highly soluble in water, are less reactive, and are much more mobile than oxygen. Such properties of nitrates make it suitable electron acceptor for anaerobic bioremediation. Sulfate is also highly soluble in water, and in comparison to its mass, it is having higher electron accepting capacity. Its inexpensiveness and nontoxicity to microorganisms make it highly suitable for use in anaerobic bioremediation (Freedman et al. 1995; Sherwood et al. 1995).

2.4.1.4 Phytoremediation

The basic concept of phytoremediation is that the plants are grown in the contaminated site and, in turn, when they grow to extract various contaminating pollutants from the site and concentrate them in the biomass (bioextraction) (Fig. 2.3). Such plants can further be burnt to produce energy. This way it is also possible to extract some metals from plant biomass (phytomining) (Meager 2000). Not only plants are able to extract some of the toxic minerals but also are able to accumulate a variety of organic contaminants, e.g., PCB (polychlorinated biphenyls), ammunition wastes (TNT, GTN), halogenated hydrocarbons, etc. These organic toxins then further

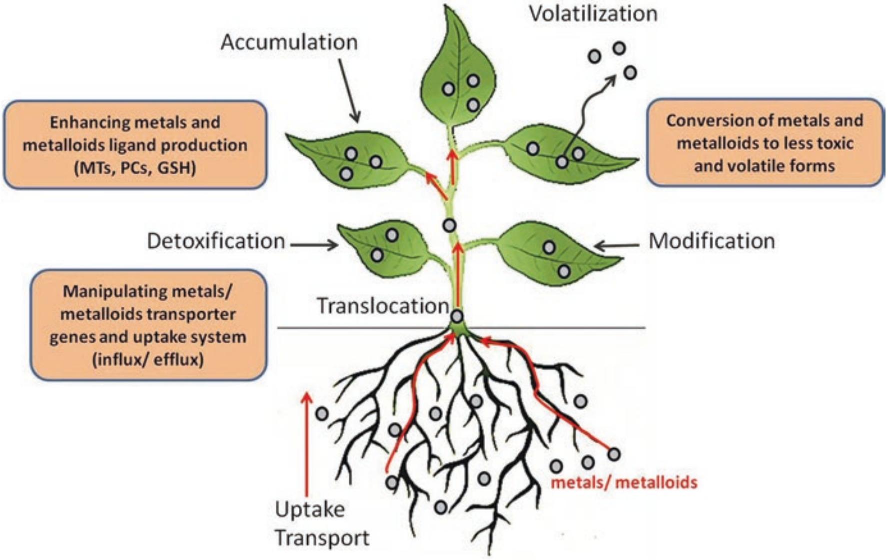

Fig. 2.3 Outline of phytoremediation showing transport, accumulation, volatilization, and detoxification of contaminants. (Adapted from Dhankher et al. (2011))

undergo metabolism in plant body and are converted into less toxic or nontoxic by-products (Salt et al. 1998; Meager 2000; Dietz and Schnoor 2001).

Rarely, there are some plants in kingdom Plantae which are known to accumulate large concentration of metals (Pajak et al. 2017; Pajak et al. 2018). Some of the hyperaccumulating or metal-resistant species are *Silene vulgaris*, *Arabidopsis halleri*, *Alyssum lesbiacum*, and *Brassica* spp. (Clemens et al. 2002; Kramer 2003). These species are known to accumulate high concentrations of essential as well as non-essential metals such as copper (Cu), iron (Fe), zinc (Zn), selenium (Se), cadmium (Cd), mercury (Hg), lead (Pb), aluminum (Al), and arsenic (As) (Salt et al. 1998, Meagher 2000, Clemens 2001,Guerrinot and Salt 2001, Clemens et al. 2002, Hall 2002, McGrath 2003).

2.4.1.4.1 Mechanism of Phytoremediation

The first step in the process is the uptake of metal ions from the root to the root cells. This process is primarily performed by organic acids in the plant system such as citrate, oxalate, formate, etc. (Michael and Christopher 2007). But at the same time there are certain examples of organic acids only which are known to cause a strong inhibition to this process (Guerra et al. 2011), e.g., avenic acids and mugineic acids are released by certain species of plants to increase the bioavailability of heavy metals for root uptake as is reported in some species of family Gramineae (Jakagi et al. 1984). On the other hand, zinc, copper, and aluminum uptake is inhibited by the formate, oxalate, and malate collectively (Dehaize et al. 1993; Kochian et al. 2007; Qin et al. 2007). The next step after root uptake is the vacuolar sequestration inside the plant cell. The first step includes the transport of heavy metals inside the cytosol, and this is mediated by ZIP (zinc−/iron-regulated transporter) proteins. This further stimulates phytochelatin synthetase for the production of phytochelatin from glutathione. This results in the formation of the heavy metal-phytochelatin complex which actually is transported inside the vacuole of a plant cell.

2.4.1.4.2 Translocation of Heavy Metals from Roots to Shoots and Shoot Metabolism

Heavy metals follow the path from roots to epidermal tissue, to pericycle, and finally to xylem parenchyma; from xylem parenchyma it is transported by transmembrane channels to xylems (Palmer and Guerinot 2009). In *Arabidopsis* species, ATPases HMA2 and HMA4 proteins are responsible for transportation and accumulation of zinc from roots to shoots (Hanikenne et al. 2008; Wong and Cobbett 2009). Some amino acids such as histidine (hyperaccumulator) and some organic acids such as citrate and malate play an active role in the translocation of metals such as zinc, copper, and cadmium (Pilon et al. 2009).

The excessive accumulation of heavy metals (redox active and non-redox active) inside the plant cells cause a huge amount of oxidative damage and stress by replacement of metal ions in pigments and another essential molecule such as chlorophyll. To counteract this, plants have an inbuilt anti-oxidative defense mechanism based upon the enzymes and reducing metabolites (GSH) which regulate redox status. GSH binds to metals and metalloids and eliminates reactive oxygen species (ROS) whose production is stimulated by heavy metals and thus maintains homeostasis for metabolism (Foyer and Noctor 2005).

2.4.2 *Ex Situ Bioremediation*

Ex situ bioremediation technology makes use of aerobic treatment of soil to make it free from contaminants. The major difference between *in situ* and *ex situ* bioremediation technique is that *in situ* remediation involves the treatment of contaminated soil on site, whereas *ex situ* methods involve physical extraction of media/soil from a contaminated site and move it to another location for efficient and controlled treatment. One of the major advantages of this method over the *in situ* method is its efficiency and certainty of control treatment due to the ability to uniformly screen and homogenize the soil mixture. However, the *ex situ* method involves the complete removal of contaminated soil and its transport to a different new location for treatment which makes this treatment method less cost-effective.

2.4.2.1 Nonbiological Methods of *Ex Situ* Bioremediation

2.4.2.1.1 Dig and Dump (Landfilling)

This is the most conventional method for ex situ bioremediation for the purpose of the following: first, a target land is engineered and prepared to receive the dumped waste. The site is so engineered so that it is able to receive inert, solid, and hazardous waste with a degradation rate between 5000 and 230,000 metric ton per year and also no leachate should leach into the dumping ground and contaminate groundwater table. To prevent the problem of leaching, covering and capping are usually preferred, but now newer technology makes use of leachate system which is an underground pipe network to collect leachate. One upgraded form of landfilling is called landfill bioreactor. This type of landfill makes use of microorganisms for a quick breakdown of all the contaminants present in the waste disposed into it. They may be aerobic or anaerobic and can also be hybrid (aerobic-anaerobic). Advantages of this type of landfilling include:

(a) Reduce greenhouse gas emission.
(b) The end product produced during the process does not require further landfilling.
(c) Leachate treatment cost is decreased exponentially.
(d) The decrease in overall landfilling cost over long periods of treatment.

Several disadvantages include:

(a) Open landfills emit certain kind of greenhouse gases, which may indirectly pose a serious threat to the environment (Gong et al. 2018).
(b) Excavation of landfills is very dangerous as the waste dumped into it is not pretreated.
(c) The cost of excavated material transfer to the final destination site is very huge and hence makes this process less economical.
(d) Finally, the landfill gas although have the advantage of being used as a biofuel but, if released into the open environment, proves harmful for local flora and fauna.

2.4.2.1.2 Incineration

Incineration is the method of treatment of solid waste by thermal energy. Actually, it involves the combustion of organic matter in waste material (Silva-Castro et al. 2012). Incineration is also called as thermal treatment, as direct heat is involved in the process. The result of incineration is the production of three major components, i.e., ash, flue gas, and heat.

(a) Ash mainly constitute inorganic matter from the waste which is left after burning. It forms solid clumps and is carried away by flue gas.
(b) Flue gas actually is the gas which is emitted after combustion of waste, and its composition depends entirely upon the constituents of the solid waste.
(c) There is a considerable amount of heat produced when the waste is combusted, and the calorific value here also depends upon the constituents of the solid waste. This heat is nowadays channelized into various fields with newer advanced technologies.

The method utilizes very high temperature (750–1200 °C) for disposal of solid waste. There are various incinerator systems which are infrared combustors using silicon carbide rods; fluidize bed combustors (high-velocity air with infrared heat source is used to attain temperature of 850 °C); circulating bed combustors (utilizes the kinetic energy of high-velocity air to create high turbulent combustion zone to attain temperature of 850 °C); and rotary kilns (consist of inclined rotating cylinders with an afterburner that burns at 980 °C) (FRTR 2012).

2.4.2.1.3 Soil Washing

Mechanical scrubbing, physical separation, soil scrubbing, and attrition scrubbing are some of the other names used to refer to soil washing. As the name suggests, soil washing involves physical separation of contaminants from the soil particles via various methods. This technology uses aqueous-based separation and physical separation units which are used to minimize the toxic levels of contaminated site (age-prone) to site-specific objectives. It is worth noting that there is no detoxification of pollutants, but by various means, it involves a concentration of hazardous material into smaller soil fraction or transfers the contaminants from the soil into washing fluids for further treatments (Dermont et al. 2008).

Soil washing involves the following procedures:

1. Mechanical screening (sorting, crushing, physical processing which includes soaking, spraying and tumbling attrition scrubbing).
2. Treatment of coarse- and fine-grained soil portions (includes leaching and physical separations).
3. Further treatment of generated toxins.

Hence, this is considered a stand-alone approach (Fig. 2.4).

Soil washing can be used for a wide variety of contaminants including heavy metals, PCBs, SVOCs, PAHs, petroleum, as well as fuel residues and pesticides. Soil washing is considered one of the cheapest methods of *ex situ* bioremediation as it limits the final fraction of soil which requires further treatment which in turn minimizes the post-remedial expenditures (Urum et al. 2003).

One of the major limitations is that this technique is highly unsuitable for soils containing more than 40% silt and clay. This is because homogenization is not feasible for such soils. Also, multicomponent mixture hampers the effectiveness of the method.

2.4.2.2 Biological Methods of *Ex Situ* Bioremediation

2.4.2.2.1 Solid Phase Bioremediation Technologies

Land farming

It is one of the most widely used conventional remediation technologies because of two main reasons: one being low technological input and the other being its

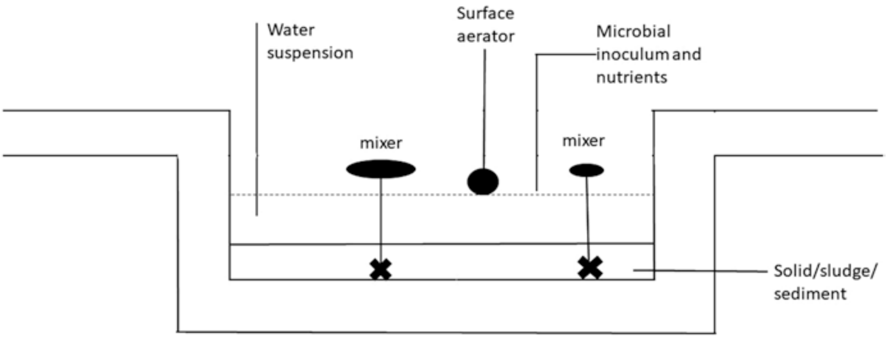

Fig. 2.4 Typical soil washing procedure

cost-effectiveness. It involves the excavation of a contaminated site and places it in the form of a thin bed of about 0.46 m which are lined by high-density polyethylene (HDPE) sheets or other such impermeable material. These are used to prevent leachate from coming in contact with groundwater through infiltration. Landfarming is based upon the principle that the indigenous microflora is used to aerobically degrade the contaminating pollutants present in the soil under the treatment process. For this purpose, the beds are provided with sufficient aeration by periodically tilling the bed and turning over until the bioremediation process is complete. The chemistry behind the chemical breakdown remains the same as discussed under aerobic bioremediation.

The optimized parameters for landfarming include moisture content (40–85%), aeration (periodic tilling), pH (6–8), temperature (20–40 °C), and C:N ratio (9:1) (Khan et al. 2004). There exist a plethora of contaminants which can be treated via landfarming and includes aliphatic and aromatic hydrocarbons, oily sludge from refineries, pesticides, etc. To enhance the rate of biodegradation, various strategies can be applied which include the regular addition of soils contaminated with hydrocarbons primarily to replenish the supply of hydrocarbons, co-substrates, and bulky agents can also be added to stimulate microbial metabolism (Straube et al. 2003; Maila et al. 2005). Silva-Castro et al. (2012) reported that a consortium of four bacteria (*Bacillus pumilus*, *Alcaligenes faecalis*, *Micrococcus luteus*, and *Enterobacter* sp.) can remediate 100% of PAH in 7 months from PAH-contaminated soil with organic fertilizers. The use of additive (kitchen waste compost), activated sludge, bully agent (rusk-husk), and petroleum-degrading bacteria removes 92.4% total petroleum hydrocarbons (TPH) in 25 days (Kuo et al. 2012).

2.4.2.2.2 Composting

It is the most primitive technology for the treatment of agricultural, municipal solid waste, and sewage sludge. The principle of operation consists of mixing of contaminated soil with nontoxic organic waste, another agricultural waste, manure, etc. This mix encourages the growth of aerobic microorganisms and hence biodegrades the toxic contaminants into nontoxic end products. The biodegradation occurs via co-metabolic pathways. The process is purely aerobic in nature and makes use of heat generated during the oxidative exothermic reaction to speed up the process. So this can be considered as an autonomously driven process. The positive characteristic of composting is that the product, i.e., mature compost, can be used as fertilizers in the field and also may be used for land restoration purposes (Antizar-Ladislao et al. 2006).

For the purpose of composting, there are various approaches, but the cheapest one makes use of windrows which are actually long mounds in which the entire composting mixture is kept for bioremediation. The optimal size for windrows is (3 l × 4w × 1.5 h)m^3. Other approaches include vessels and engineered windrows which are also used for biopiling. Engineered vessels can also be called as solid phase bioreactors in which all the physical and chemical parameters can be controlled. But such installations require high capital input.

Thermophilic composting is capable of reducing levels of monoaromatics (BTEX), phenols, PAHs, petroleum hydrocarbons, PCB, PCP, etc. (Nadeef et al. 2012). Metal-contaminated soil can also be treated by composting methods, e.g., van Herwizen et al. (2007) remediated 80% metal polluted soil with mineral-amended composts.

Limiting factors include the requirement of space and post-excavation treatment of contaminated soil. Management of order and the problem of leachate pose a major problem during composting.

2.4.2.2.3 Biopiling

Biopiling is a combination of two techniques (landfarming and composting) that provides a favorable environment for indigenous aerobic and anaerobic microorganisms and also controls physical losses of contaminants by leaching and volatilization (Kumar et al. 2011; Mani and Kumar 2014). There are various other names for biopile such as bioheaps, biomounds, biocells, compost cells, etc. A wide range of petrochemical contaminants in soils and sediments have been remediated by extensive use of biopiles (Germaine et al. 2014). In this method, contaminated soils are piled up or heaped, and then the microbiological activity is stimulated by aeration along with the addition of water and nutrients.

Its similarity with landfarming can be stated as it also involves the remediation of soil above the ground, and moreover, this system utilizes the aerobic environment to stimulate microbial activity. It differs from landfarming with respect to the control it provides over different physical as well as chemical parameters so that rapid biodegradation may occur (McCarthy et al. 2004). In comparison to both landfarming and composting, the mass transfer efficiency of nutrients, water, and air in biopiles offers a better potential for treatment of contaminating pollutants.

Biopiles are generally operated up to a height of 0.9–3.1 m, and also various strategies can be used to prevent volatilization, runoff, and evaporation, which includes covering the biopile with an impervious lining, which also promotes thermal heating up of biopile and enhances the microbial activity.

Biopiles are capable of degrading various contaminants such as pesticides, halogenated VOCs/SVOCs, and non-halogenated VOCs. Lighter petroleum products like gasoline are removed at the time of air injection or pile turning. Heavier petroleum products generally take more time to biodegrade than lighter petroleum products. Soil characteristics, climatic conditions, and contaminant characteristics are deciding factors for the efficiency of biopile (Giasi and Morelli 2003).

2.4.2.2.4 Slurry-Based Bioremediation (Bioreactors)

This is currently the most advanced system for the treatment of soil. The main advantage of this approach is the fine control of various physical and chemical parameters. The slurry bioreactors are so designed so as to provide a very efficient

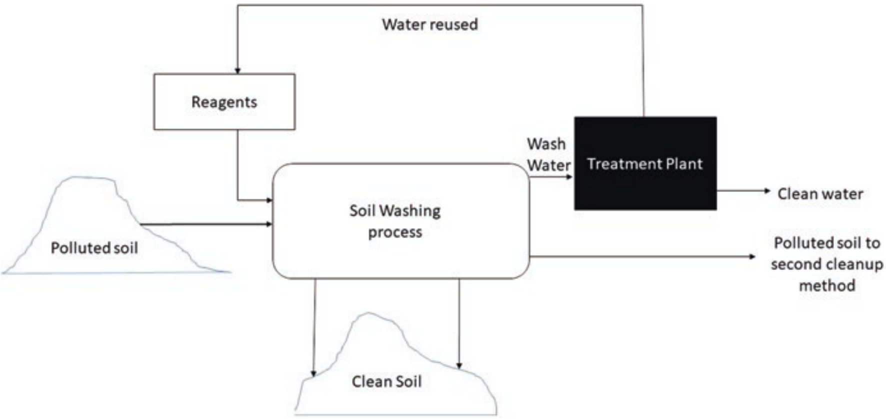

Fig. 2.5 Slurry-phase bioreactor (a typical open system)

system for optimizing and controlling critical operating parameters. The major disadvantage being the high cost of bioreactor as well as the operating cost which needs justification for each particular application.

The slurry-based bioreactor system is a stand-alone approach (Fig. 2.5) which is able to remediate the soil completely without the intervention or use of other approaches. The working principle includes first the mixing of contaminated soil with water/wastewater and other additives in the bioreactor vessel. Next, various critical operating parameters are set (Latha and Reddy 2013; Bhardwaj and Kapley 2015). Slurry-based soil treatment requires mechanical mixing, grinding, and volumetric classification before initializing biological treatment process for degradation of pollutants (Volf 2007). The formation of slurry depends upon how much soil is mixed with a specific amount of water which in turn depends upon the concentration of pollutants, soil type, and rate of biodegradation (Pavel and Gavrileseu 2008). Normally, a slurry is 10–30% solids by weight. The slurry is maintained under optimum conditions by providing oxygen via the aeration facility of bioreactor. Nutrients are also added depending upon the total amount of pollutants present in the soil and thus to maintain the optimal ratio between carbon, phosphorus, and nitrogen. After the bioremediation process is over, the slurry is drained via various downstream processing approaches (filtration (vacuum/pressure), centrifugation, etc.).

As bioreactors are closed systems in which all the environmentally critical parameters are maintained at an optimal level, hence such bioreactors provide accelerated and enhanced treatment rates (Bhardwaj and Kapley 2015; Kuppusamy et al. 2016). Slurry phase bioreactors are normally operated under batch, continuous, or fed-batch modes, and they may be operated under aerobic, anaerobic, or anoxic conditions depending upon the type of contaminants being treated.

Slurry bioreactors are efficient in the treatment of aerobically degradable compounds such as SVOCs, recalcitrant pesticides, explosive substances, aromatic hydrocarbons, chlorinated organic compounds, and PAHs. This technique is able to treat soils which are otherwise difficult to treat via other processes for, e.g., soils with high clay content (>40%).

2.5 Conclusion

The degradative potential of microorganisms has been used to a much greater extent, and this fundamental principle forms the basis of bioremediation of organic toxic-contaminated pollutants. For a successful bioremediation procedure, one must be equipped with the knowledge of physiological characteristics, biochemical capabilities, ecology, and genetic plasticity of the microorganism or consortia being utilized in the process. Also, the knowledge of contaminants with which the site is contaminated also plays a vital role in the efficient bioremediation of the soil. However, a complete and efficient process development requires a multi-disciplinary approach involving from chemical sciences through physical sciences to biological sciences. The increase in interest of various scientific communities towards this field has promised a very bright and promising future.

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