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EX SITU BIOREMEDIATION - AN OVERVIEW

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ABSTRACT: Bioremediation is a process used to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. In many cases, bioremediation is less expensive and more sustainable than other remediation alternatives. There are two options for where bioremediation can take place. If we have a contaminated environment and leave everything in place and allow bioremediation to happen, we call that *in-situ* bioremediation. If we have a contaminated environment and we remove the contaminated material (for example soil or water) from the environment and let bioremediation happen off-site, we call that *ex-situ* bioremediation. When the material is removed from the environment, it can be put into bioreactors, large vessels where the contaminated material can be monitored and conditions for bioremediation can be controlled. Biological organisms typically have conditions where they operate best. In bioreactors we can control the mixing rate, temperature, pH, and nutrient levels to suit the organisms breaking down our contaminant. Landfarming involves spreading contaminated soil into a lined bed (to prevent leaching) and periodically applying nutrients and mixing the soil to boost biological activity. Biopiling places the contaminated soil into piles that are well aerated and nutrients are added to speed up bioremediation. In all cases, the contaminant levels are monitored to verify that bioremediation is taking place and steps are taken to ensure that contaminated material stays out of contact with the environment.

KEYWORDS: Bioavailability, Surfactants, Biopile, Bioreactor, Windrows, Land farming

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1.INTRODUCTION

Any unwanted substance introduced into the environment is referred to as a ‘contaminant’. Deleterious effects or damages by the contaminants lead to ‘pollution’, a process by which a resource is rendered unfit for use, more often than not, by humans. Pollution can be either natural or man-made. Pollutants are present since time immemorial, and life on the earth as we define now has always evolved amongst them. With pollutant analogues from geothermal and volcanic activities, comets, and space dust (natural sources) which are about 100 tons of organic dust per day, the earth is forever a polluted planet [1]. Relative to the pre-industrialization era, industrialization and intensive use of chemical substances by anthropogenic use, such as petroleum oil, hydrocarbons (e.g., aliphatic, aromatic, polycyclic aromatic hydrocarbons (PAHs), BTEX (benzene, toluene, ethylbenzene, and xylenes), chlorinated hydrocarbons like polychlorinated biphenyls (PCBs), trichloroethylene (TCE), and perchloroethylene, nitroaromatic compounds, (organophosphorus compounds) solvents, pesticides, and heavy metals are contributing to environmental pollution. Large-scale pollution due to man-made chemical substances and to some extent by natural substances is of global concern now. Seepage and run-offs due to the mobile nature, and continuous cycling of volatilization and condensation of many organic chemicals such as pesticides have even led to their presence in rain, fog and snow [2]. Every year, about 1.7 to 8.8 million metric tons of oil is released into the world's water. More than 90% of this oil pollution is directly related to accidents due to human failures and activities including deliberate waste disposal [3]. PAHs are present at levels varying from 1 μg to 300 g kg−1 soil, depending on the sources of contamination like combustion of fossil fuels, gasification and liquefaction of coal, incineration of wastes, and wood treatment processes [4]. Incomplete combustion of organic substances gives out about 100 different PAHs which are the ubiquitous pollutants. Except for a few PAHs used in medicines, dyes, plastics and pesticides, they are rarely of industrial use [5]. Some PAHs and their epoxides are highly toxic, and mutagenic even to microorganisms. About six specific PAHs are listed among the top 126 priority pollutants by the US Environmental Protection Agency. PCBs, used in hydraulic fluids, plasticizers, adhesives, lubricants, flame retardants and dielectric fluids in transformers are toxic, carcinogenic, and degrade slowly. Polychlorinated dibenzodioxins and dibenzofurans are recalcitrant chemicals and some of the congeners with lateral chlorine substitutions at positions 2,3,7 and 8 are carcinogenic to humans [6]. Many solvents such as TCE and carbon tetrachloride pollute the environments due to large-scale industrial production and anthropogenic uses. Pesticides are regularly used in agricultural- and public health-programs worldwide. In many cases, the environmental effects of these chemical substances outweigh the benefits they accrue to humans and necessitate the need of their degradation after the intended uses. Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources [7]. Pollutant nature, depth and degree of pollution, type of environment, location, cost, and environmental policies are some of the selection criteria that are considered when choosing any bioremediation technique [8; 9]. Apart from selection criteria, performance criteria (oxygen and nutrient concentrations, temperature, pH, and other abiotic factors) that determine the success of bioremediation processes are also given major considerations prior to bioremediation project. Although bioremediation techniques are diverse, most studies on bioremediation are focused on hydrocarbons on account of frequent pollution of soil and ground water with this particular type of pollutant [10; 11; 12; 13]. Besides, it is possible that other remediation techniques [14], which might as well be more economical, and efficient to apply during remediation, are considered when remediation of sites polluted with pollutants aside from hydrocarbons are involved. The aim of this review is to provide a comprehensive knowledge on the ex situ bioremediation techniques with regards to site of application, highlighting their principles, advantages, limitations and possible solutions.

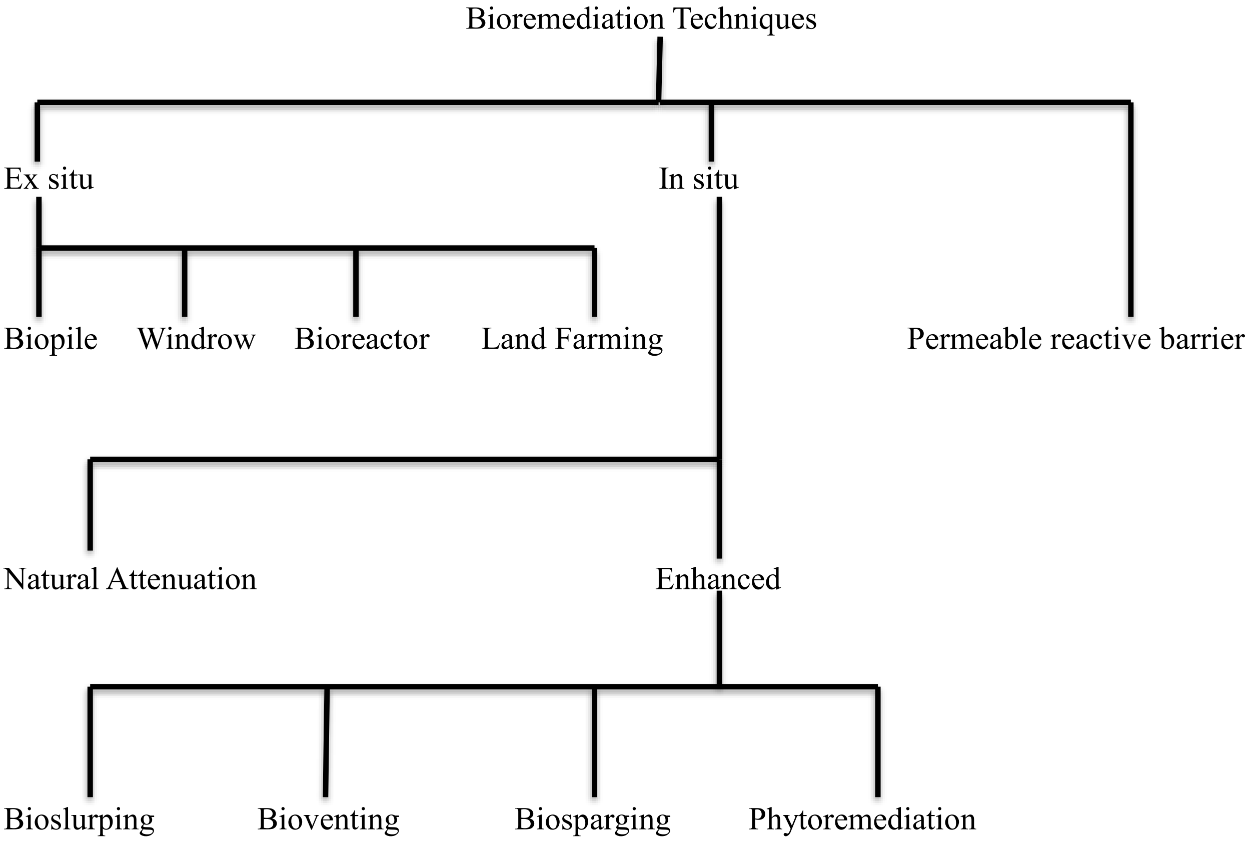

Fig. 1: Bioremediation techniques. The divergence of each technique is hypothetical. Permeable reactive barrier (PBR) is a physical remediation technique with some elements of bioremediation.

2. **Body of Paper**

Bioavailability: what fraction of pollutants is available

The process of bioremediation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule, which is dependent on both accessibility and bioavailability [25]. There is a considerable debate in the literature on “what constitutes the bioavailable fraction” and the methods of its measurements [26; 27]. Following entry into the soil environment, pollutants rapidly bind to the mineral and organic matter (solid phases) via a combination of physical and chemical processes. Sorption, complexation and precipitation constitute the pollutant–soil interaction. The ability of soils to release (desorb) pollutants determines its susceptibility to microbial degradation, thereby influencing effectiveness of the bioremediation process. In soil aggregates which are the smallest 'composite units' in the heterogeneous soil environment, bioavailability is limited by transport of the pollutant molecule to a microbial cell, i.e., diffusion of pollutant out of a soil aggregate to the cell attached to the external surface of the aggregate. Sorption which influences the bioavailability of a contaminant is a critical factor, yet a poorly understood process in bioremediation. There are two schools of thought concerning bioavailability and the consequent biodegradation of organic contaminants [28]: (i) the pre-requisite release of contaminant from sorbed phase to aqueous phase for its degradation by microorganisms [29; 30], and (ii) biodegradation of the contaminant in the sorbed phase, without being desorbed, by the enzymes [32]. The degradation of sorbed contaminants can presumably occur via microbially-mediated desorption of contaminants through production of biosurfactants and the development of a steep gradient between solid phase and interfacial contaminant [31]. Thus, these reports suggest that bioavailability is even species specific (i.e., the ability of certain species to desorb the contaminant and then degrade). The organic contaminants can also be degraded without prior desorption. Singh et al. [32] demonstrated that a soil bacterium, *Brevibacterium* sp. degraded the pesticide fenamiphos which was intercalated into the cationic-surfactant modified montmorillonite clay (CTMA–Mt–fenamiphos complex). The interlayer space is otherwise inaccessible to the bacterium due to its size of several orders lower than that of the bacteria. The scanning electron microscope analysis showed the surface attachment of bacteria to the surface of the CTMA–Mt–fenamiphos complex, suggesting the involvement of extracellular enzyme in the degradation of fenamiphos, without its prior desorption. The degradation of sorbed contaminants depends on the enrichment and isolation procedures used for obtaining the culturable bacteria. As against the conventional approach of providing the contaminant as a sole carbon source in aqueous medium, the provision of phenanthrene sorbed on a polyacrylic porous resin to the bacterial cultures led to faster degradation of phenanthrene than those isolated by the conventional technique [33;31]. Aqueous solubility, volatility or reactivity of organic pollutants varies greatly, and all of them may influence their bioavailability in water and soils. On a mass basis, no relationship exists between the chemical pollutant in soil and its biological effect. The dissolved form of contaminants in pore water is considered to be bioavailable, compared to the bound chemical which does not exert direct biological effects. This has led to the 'pore water hypothesis.' The equilibrium partitioning theory is applied to estimate the dissolved fraction of pollutant in pore water and to remove the soil to soil differences in toxicological effects [34; 35]. The basic assumption of equilibrium partitioning theory is that the partitioning of an ionic chemical between the mineral and organic matter in soil or sediment and the pore water is at equilibrium, and in each phase the chemical potential which controls its biological activity is the same. The performance of chemical extraction data of non-ionic organic chemicals can be improved by organic matter normalization in order to predict the occurrence of toxicity effects. For highly hydrophobic chemical pollutants which have higher octanol–water partition coefficient (K_{ow}) with log K_{ow} values more than 4, the measured concentration in the pore water is the sum of the free chemical and the fraction sorbed to dissolved organic matter (DOM). To account for the sorbed fraction to DOM, the separation methods for DOM are required [36]. The soil–chemical contact time determines the usefulness of pore water hypothesis in measuring bioavailability and predicting the biological effects or the fraction which can be degraded, but not immediately after contamination. There are also variations in bioavailability due to the nature of chemical pollutants, soil types, and other factors such as water content and temperature. Toxicity testing of a pollutant to microorganisms [35] or the use of extracts such as the mild hydroxypropyl-β-cyclodextrin for PAHs [37] or the matrix solid-phase microextraction for DDTs (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane and its metabolites) [38] can provide direct measures of bioavailability. Cornelissen et al. [39] demonstrated that microbial factors, not bioavailability, were responsible for the persistence of rapidly desorbing fractions of the nondegraded PAHs, and these fractions were found to be substantial (up to 55%) and remained unchanged during remediation. For the purpose of bioremediation and regulatory measures, the bioavailability in the initial rapid phase and the ensuing slow phase in the biphasic degradation profile of an organic pollutant is to be monitored. The sequestration of pollutants over time may occur due to the contact and interaction of soil with pollutant molecules. Factors such as organic matter, cation exchange capacity, micropore volume, soil texture and surface area affect the pollutant sequestration [40]. Sequestration and reduced bioavailability of phenanthrene were reported for a Gram-negative bacterial isolate (strain PS5-2) when the hydrophobic compound entered into nanopores having hydrophobic surfaces [41]. Sharer et al. [42] observed that aging caused an increase in sorption for some organic compounds (e.g., 2,4-dichlorophenoxyacetic acid) but not for others (chlorobenzene, ethylene dibromide) on a common soil type. Even a weakly sorbed and easily degraded carbamate insecticide, carbaryl, can be effectively sequestrated in soil with aging, thereby rendering it partly inaccessible to microorganisms and affecting the bioavailability [43]. Hence, the generalizations about the effects of aging on the sorption–desorption behavior of different organic chemicals are difficult to achieve. Some pertinent issues that need to be considered include: (a) bioavailability and toxicity of parent molecules and their residues in soils, (b) standardized protocols for different pollutants and their use across the sites, (c) assessment on remobilization of pollutants during the post-remediation period, and (d) determination of environmentally acceptable pollutant end-points in the bioremediated soils. The 'pollutant (or contaminant) sequestration' due to the prolonged contact between soil particles and chemical molecules, however, poses less risk and threat to the environmental health. In general, difficulties with analytical measurements for determining low levels of new organic pollutants in soils, the absence of base-line values related to their compositional, geographical and distribution patterns, and the complexities in their toxicological interactions [44] make the bioavailability measurements of organic pollutants exigent.

Surfactants: bioavailability enhancers

Application of surfactants to polluted soils has been used as one of the treatment strategies for increasing the mass transfer of hydrophobic organic contaminants [45; 46]. The surfactants are amphiphilic molecules that contain hydrophilic and hydrophobic moieties; hydrophilic groups can be anionic, cationic, zwitter ionic, and nonionic. The synthetic surfactants contain sulfate, sulfonate or carboxylate group (anionic); quaternary ammonium group (cationic); polyoxyethylene, sucrose, or polypeptide (nonionic) and the hydrophobic parts of paraffins, olefins, alkyl benzenes, alkyl phenols, or alcohols. The common chemical surfactants such as Triton X-100, Tween 80 and sodium dodecyl sulphate are petroleum derived products. The zwitter ionic surfactants (e.g., N-dodecyl betaine) which contain both anionic and cationic groups have low critical micelle concentration (CMC) values, more surface active, and high solubilization capacity. Increased desorption rates of sorbed pollutants from soils by the application of surfactants make the pollutants available for remediation [47]. Solubilization of hydrophobic contaminants is attributed to the incorporation of the molecule into the hydrophobic core of micelles in solution [48]. The salient mechanisms which are involved in the surfactant-amended remediation are: (i) lowering of interfacial tension, (ii) surfactant solubilization of hydrophobic organic compounds, and (iii) the phase transfer of organic compounds from soil-sorbed to pseudo-aqueous phase [45]. Surfactants enhance mobilization and biodegradation of PAHs in soils [49]. Enhanced rates of degradation of naphthalene and phenanthrene in the presence of some nonionic surfactants at applications below their CMC were observed by Aronstein et al. [50]. Similarly, significant solubility enhancements of DDT in Triton and Brij 35 surfactants were noticed by Kile and Chiou [51] below their CMC. Factors such as cost, effectiveness at concentrations lower than 3%, low toxicity to humans, animals and plants, low adsorption to soil, low soil dispersion, and low surface tension determine the selection of surfactants for field application [52]. Toxicities of surfactants to soil biota can prevent the biodegradation of pollutants and disturb the balanced ecological functions [53]. The food-grade surfactants (T-MAZ 28, T-MAZ 10, and T-MAZ 60) [54], the plant-based surfactants (e.g., fruit pericarp from *Sapindus mukurossi*) [55] or the natural surfactants such as humic acids [56] may be preferred to the synthetic surfactants due to high biodegradability, low toxicity, and higher public acceptance. Microorganisms also produce surfactants (surface-active amphiphilic metabolites such as glycolipids, phospholipids, lipopeptides, lipoproteins, and lipopolysaccharides). The classes of biosurfactant and microbial species which can produce them are numerous, leading to continuous search for the novel biosurfactants [57]. However, the in situ application of surfactants to enhance bioavailability of persistent organic pollutants requires careful planning and selection based on the prior information about the fate and behavior of the surfactant and the target pollutant. Caution is required to prevent groundwater contamination via leaching and consequent toxicity to microorganisms. Hence, a good strategy will be to select bacteria that are capable of not only catabolizing the target contaminant but also producing surfactant. More knowledge on the mechanisms of pollutant–surfactant interactions with regard to diffusion, in and out of the micelles, and modeling of pollutant's transport at the field site can help to design efficient remediation strategy.

Ex situ bioremediation techniques

These techniques involve excavating pollutants from polluted sites and subsequently transporting them to another site for treatment. Ex situ bioremediation techniques are usually considered based on: the cost of treatment, depth of pollution, type of pollutant, degree of pollution, geographical location and geology of the polluted site. Performance criteria, which also determine the choice of ex situ bioremediation techniques, have been described [15].

Biopile

Biopile-mediated bioremediation involves above-ground piling of excavated polluted soil, followed by nutrient amendment, and sometimes aeration to enhance bioremediation by basically increasing microbial activities. The components of this technique are: aeration, irrigation, nutrient and leachate collection systems, and a treatment bed. The use of this particular ex situ technique is increasingly being considered due to its constructive features including cost effectiveness, which enables effective biodegradation on the condition that nutrient, temperature and aeration are adequately controlled [16]. The application of biopile to polluted sites can help limit volatilization of low molecular weight (LMW) pollutants; it can also be used effectively to remediate polluted extreme environments such as the very cold regions [17; 18; 16]. The feasibility of biopiles towards bioremediation of different soil samples including clay and sandy soil has been reported [19; 20]. The flexibility of biopile allows remediation time to be shortened as heating system can be incorporated into biopile design to increase microbial activities and contaminant availability thus increasing the rate of biodegradation [21]. Furthermore, heated air can be injected into biopile design to deliver air and heat in tandem, in order to facilitate enhanced bioremediation. In another study, [22] it is reported that humidified biopile had a very low final TPH concentration compared to heated and passive biopiles as a result of optimal moisture content, reduced leaching, minimal volatilization of less degradable contaminants. In addition, it was reported that biopile could be used to treat large volume of polluted soil in a limited space. Biopile setup can easily be scaled up to a pilot system to achieve similar performance obtained during laboratory studies [19]. Important to the efficiency of biopile is sieving and aeration of contaminated soil prior to processing [23]. Bulking agents such as straw, saw dust, bark or wood chips and other organic materials have been added to enhance remediation process in a biopile construct [24]. Although biopile systems conserve space compared to other field ex situ bioremediation techniques, including land farming, robust engineering, cost of maintenance and operation, lack of power supply especially at remote sites, which would enable uniform distribution of air in contaminated piled soil via air pump are some of the limitations of biopiles. More so, excessive heating of air can lead to drying of soil undergoing bioremediation, which will result in inhibition of microbial activities, and promote volatilization rather than biodegradation [22].

Windrows

As one of ex situ bioremediation techniques, windrows rely on periodic turning of piled polluted soil to enhance bioremediation by increasing degradation activities of indigenous and/or transient hydrocarbonoclastic bacteria present in polluted soil. The periodic turning of polluted soil, together with addition of water bring about increase in aeration, uniform distribution of pollutants, nutrients and microbial degradative activities, thus speeding up the rate of bioremediation, which can be accomplished through assimilation, biotransformation and mineralization [58]. Windrow treatment when compared to biopile treatment, showed higher rate of hydrocarbon removal; however, the higher efficiency of the windrow towards hydrocarbon removal was as a result of the soil type, which was reported to be more friable [59]. Nevertheless, due to periodic turning associated with windrow treatment, it may not be the best option to adopt in remediating soil polluted with toxic volatiles. The use of windrow treatment has been implicated in CH4 (greenhouse gas) release due to development of anaerobic zone within piled polluted soil, which usually occurs following reduced aeration [60].

Bioreactor

Bioreactor, as the name implies, is a vessel in which raw materials are converted to specific product(s) following series of biological reactions. There are different operating modes of bioreactor, which include: batch, fed-batch, sequencing batch, continuous and multistage. The choice of operating mode depends mostly on market economy and capital expenditure. Conditions in a bioreactor support natural process of cells by mimicking and maintaining their natural environment to provide optimum growth conditions. Polluted samples can be fed into a bioreactor either as dry matter or slurry; in either case, the use of bioreactor in treating polluted soil has several advantages compared to other ex situ bioremediation techniques. Excellent control of bioprocess parameters (temperature, pH, agitation and aeration rates, substrate and inoculum concentrations) is one of the major advantages of bioreactor-based bioremediation. The ability to control and manipulate process parameters in a bioreactor implies that biological reactions within can be enhanced to effectively reduce bioremediation time. Importantly, controlled bioaugmentation, nutrient addition, increased pollutant bioavailability, and mass transfer (contact between pollutant and microbes), which are among the limiting factors of bioremediation process can effectively be established in a bioreactor thus making bioreactor-based bioremediation more efficient. Further, it can be used to treat soil or water polluted with volatile organic compounds (VOCs) including benzene, toluene, ethylbenzene and xylenes (BTEX). The applications of different bioreactors for bioremediation process have resulted in removal of wide range of pollutants. The flexible nature of bioreactor designs allows maximum biological degradation while minimizing abiotic losses [61]. Short or long-term operation of a bioreactor containing crude oil-polluted soil slurry allows tracking of changes in microbial population dynamics thus enabling easy characterization of core bacterial communities involved in bioremediation processes [62; 63]. Furthermore, it allows the use of different substances as biostimulant or bioaugmenting agent including sewage sludge. In addition, bioreactor being an enclosed system, genetically modified microorganism (GEM) can be used for bioaugmentation after which the organism (GEM) can be destroyed before treated soils are returned to field for landfilling. This containment of GEM in a bioreactor followed by destruction will help ensure that no foreign gene escapes into an environment after bioremediation. With bioreactor, the role of biosurfactant was found to be insignificant due to efficient mixing associated with bioreactor operations [64]. Despite that bioreactor-based bioremediation has proven to be efficient as a result of different operating parameters, which can easily be controlled, establishing best operating condition by relating all parameters using one-factor-at-atime (OFAT) approach would likely require numerous experiments, which is time-consuming. This particular challenge can be overcome by using design of experiment (DoE) tone, which provides information on optimal range of parameters using a set of independent variables (controllable and uncontrollable factors) over a specified region (level) [65]. Notwithstanding, understanding microbiological processes is of great importance when optimizing bioremediation processes. Moreover, bioreactor-based bioremediation is not a popular full-scale practice due to some reasons. Firstly, due to bioreactor being ex situ technique, the volume of polluted soil or other substances to be treated may be too large, requiring more manpower, capital and safety measures for transporting pollutant to treatment site, therefore, making this particular technique cost ineffective [15]. Secondly, due to several bioprocess parameters or variables of a bioreactor, any parameter that is not properly controlled and/or maintained at optimum, may become a limiting factor; this in turn will reduce microbial activities and will make bioreactor-based bioremediation process less effective. Lastly, pollutants are likely to respond differently to different bioreactors; the availability of the most suitable design is of paramount importance. Above all, cost of a bioreactor suitable for a laboratory or pilot-scale bioremediation makes this technique to be capitally intensive.

Land farming

Land farming is amongst the simplest bioremediation techniques owing to its low cost and less equipment requirement for operation. In most cases, it is regarded as ex situ bioremediation, while in some cases, it is regarded as in situ bioremediation technique. This debate is due to the site of treatment. Pollutant depth plays an important role as to whether land farming can be carried out ex situ or in situ. In land farming, one thing is common, polluted soils are usually excavated and/or tilled, but the site of treatment apparently determines the type of bioremediation. When excavated polluted soil is treated on-site, it can be regarded as in situ; otherwise, it is ex situ as it has more in common with other ex situ bioremediation techniques. It has been reported that when a pollutant lies <1 m below ground surface, bioremediation might proceed without excavation, while pollutant lying >1.7 m needs to be transported to ground surface for bioremediation to be effectively enhanced [66]. Generally, excavated polluted soils are carefully applied on a fixed layer support above the ground surface to allow aerobic biodegradation of pollutant by autochthonous microorganisms [15]. Tillage, which brings about aeration, addition of nutrients (nitrogen, phosphorus and potassium) and irrigation are the major operations, which stimulate activities of autochthonous microorganisms to enhance bioremediation during land farming. Nevertheless, it was reported that tillage and irrigation without nutrient addition in a soil with appropriate biological activity increased heterotrophic and diesel-degrading bacterial counts thus enhancing the rate of bioremediation; dehydrogenase activity was also observed to be a good indicator of biostimulation treatment and could be used as a biological parameter in land farming technology [67]. Similarly, in a field trial, Paudyn et al. [68] reported >80 % contaminant (diesel) removal by aeration using rototilling approach at remote Canadian Arctic location over a 3-year study period; this further demonstrates that in land farming technique, aeration plays crucial role in pollutant removal especially at cold regions. Land farming is usually used for remediation of hydrocarbon-polluted sites including polyaromatic hydrocarbons [67]; as a result, biodegradation and volatilization (weathering) are the two remediation mechanisms involved in pollutant removal. Land farming system complies with government regulations, and can be used in any climate and location. The construction of a suitable land farming design with an impermeable liner minimizes leaching of pollutant into neighbouring areas during bioremediation operation [69]. Over all, land farming bioremediation technique is very simple to design and implement, requires low capital input and can be used to treat large volume of polluted soil with minimal environmental impact and energy requirement [70]. Although the simplest bioremediation technique, land farming like other ex situ bioremediation techniques has some limitations, which include: large operating space, reduction in microbial activities due to unfavourable environmental conditions, additional cost due to excavation, and reduced efficacy in inorganic pollutant removal [70]. Moreover, it is not suitable for treating soil polluted with toxic volatiles due to its design and mechanism of pollutant removal (volatilization), especially in hot (tropical) climate regions. These limitations and several others make land farming based bioremediation time consuming and less efficient compared to other ex situ bioremediation techniques.

2. CONCLUSION

One of the major advantages of ex situ bioremediation techniques is that they do not require extensive preliminary assessment of polluted site prior to remediation; this makes the preliminary stage short, less laborious and less expensive. Due to excavation processes associated with ex situ bioremediation, pollutant inhomogeneity as a result of depth, non-uniform concentration and distribution, can easily be curbed by effectively optimizing some process parameters (temperature, pH, mixing) of any ex situ technique to enhance bioremediation process. These techniques allow modifications of biological, chemical and physicochemical conditions and parameters necessary for effective and efficient bioremediation. Importantly, the great influence of soil porosity, which governs transport processes during remediation, can be reduced when polluted soils are excavated. Ex situ bioremediation techniques are unlikely to be used in some sites such as under buildings, inner city and working sites [15]. On the other hand, the excavation features of ex situ bioremediation tend to disrupt soil structure; as a result, polluted and surrounding sites alike experience more disturbances. Moderate to extensive engineering required for any ex situ bioremediation techniques implies that more workforce and capital are required to construct any of the technique. In most cases, these techniques require large space for operation. Generally, ex situ bioremediation techniques tend to be faster, easier to control and can be used to treat wide range of pollutants [71].

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CONFLICT OF INTEREST

None

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