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IN SITU BIOREMEDIATION - AN OVERVIEW

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ABSTRACT: The treatment of contaminated soil in the location where it was found can be considered more convenient compared to *ex-situ* bioremediation. This is called *In situ* bioremediation. This is because equipment is not needed to unearth the contaminated soil leading it to be less expensive, and cleaner since it does not send dust and contaminants into the surrounding area. Some disadvantages to this method of bioremediation is that it may take longer to decontaminate, it is less manageable and it is mostly effective in loose soil. There are numerous ways to bioremediate a site in situ. These include Natural Attenuation, Composting, Bioslurping, Bioventing, Biosparging and microbe assisted Phytoremediation to name a few. All these techniques follow any one or more of the three main strategies used in *In situ* bioremediation namely Bioattenuation, Biostimulation and Bioaugmentation. This review sheds a brief light onto each of the aforementioned topics in its current form.

KEYWORDS: Bioattenuation, Biostimulation, Bioaugmentation, Composting, Bioslurping, Bioventing, Biosparging , microbe assisted Phytoremediation.

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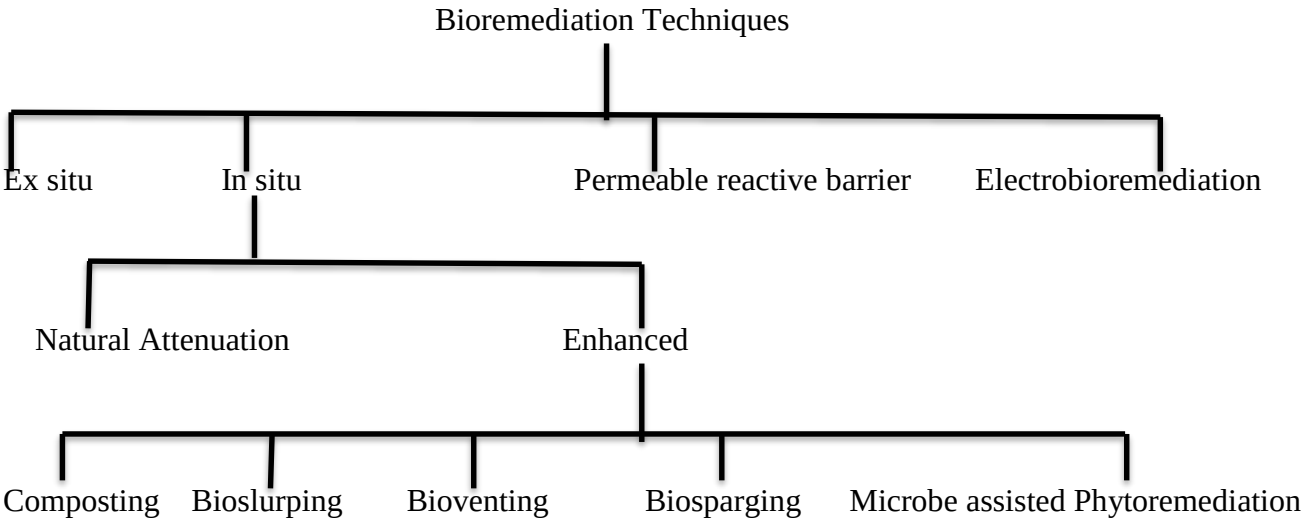
1.INTRODUCTION

In the past two decades, there have been recent advances in bioremediation techniques with the ultimate goal being to effectively restore polluted environments in an eco-friendly approach, and at a very low cost. Researchers have developed and modelled different bioremediation techniques; however, due to nature and/or type of pollutant, there is no single bioremediation technique that serves as a 'silver bullet' to restore polluted environments. Autochthonous (indigenous)

microorganisms present in polluted environments hold the key to solving most of the challenges associated with biodegradation and bioremediation of polluting substances [1] provided that environmental conditions are suitable for their growth and metabolism. Environmentally friendly and cost saving features are amongst the major advantages of bioremediation compared to both chemical and physical methods of remediation. Thus far, several good definitions have been given to bioremediation, with particular emphasis on one of the processes (degradation). Nevertheless, in some instances, the term biodegradation is used interchangeably with bioremediation; the former is a term, which applies to a process under the latter. In this review, bioremediation is defined as a process, which relies on biological mechanisms to reduce (degrade, detoxify, mineralize or transform) concentration of pollutants to an innocuous state. The process of pollutant removal depends primarily on the nature of the pollutant, which may include: agrochemicals, chlorinated compounds, dyes, greenhouse gases, heavy metals, hydrocarbons, nuclear waste, plastics, and sewage. Apparently, taking into consideration site of application, bioremediation techniques can be categorized as: *ex situ* or *in situ*. A contaminated site may be relatively stable but may pose a future threat if not remediated. If cleaning of such a site is attempted by excavation followed by, for example, mixing with a suitable matrix material and nutrients for composting, there is a risk of mobilizing the contaminant by volatilization or flushing. Therefore, remediation *in situ* by improving the conditions and/or the degradation potential in the contaminated soil layer should be preferred.



2. **BODY OF PAPER**

Factors limiting efficiency of in situ bioremediation

Soil contaminated with various organic recalcitrant compounds is a very widespread problem throughout the world, particularly in industrialized areas. There are many reasons for organic compounds being degraded very slowly or not at all in the soil environment, even though they are per se biodegradable. Among those are:

1. Low temperature. In soil, particularly in northern industrialized countries in Europe and North America, the soil temperature during a large part of the year is too low for efficient microbial degradation of soil contaminants. The same may be true also for deeper soil layers in other parts of the world.
2. Anaerobic conditions. Degradation in anaerobic conditions is slow; some compounds are not degraded anaerobically and some are degraded only partly and may give rise to toxic compounds.
3. Low levels of nutrients and co-substrates. A contaminated site usually has a sub-optimal nutrient balance. If the contaminant is a hydrocarbon, e.g. oil contamination, there is likely to be a shortage of at least nitrogen, but each site has to be evaluated case by case, taking into account also matters such as solubility of the contaminant in order not to over-fertilize.
4. Bioavaliability. Spatial distribution of contaminants in relation to degrading organisms and solubility of the contaminant; these variables are in part interrelated and are both separately and in combination major factors affecting degradation velocity.
5. Absence of degradation potential. A biological degradation pathway for synthetic, xenobiotic compounds may not exist precluding biodegradation, or genes encoding enzymes that may be active on the compound are not induced by the contaminant. Suitable pathways are, however, likely to evolve, either naturally or accelerated in laboratory conditions.

Evolution of degradation capacity in contaminated soil

Given sufficient time and sufficiently favourable conditions the capacity to degrade any organic compound is likely to evolve or immigrate to a contaminated site, even if the compound(s) are completely synthetic/xenobiotic, and no natural degradation pathway originally would exist. On the other hand, bacterial cells/clones tend to limit the amount of genetic coding capacity to only what it presently needs, but as a population the genetic capacity of a certain bacterium is wider so that a trait that gives a selective advantage in changed conditions rapidly becomes common in the population, either by giving the carrier bacterium better growth velocity or by being transferred, e.g. as a conjugative plasmid. Particularly the latter phenomenon, which has been documented to be efficient on plant root and leaf surfaces [2; 3], greatly increases the plasticity of the plant-associated soil microbial flora compared to the bulk soil microbes. There are at least four principally different routes that result in bacteria (or other microbes) capable of degradation of a certain compound or group of compounds at a certain site.

1. The indigenous microbial flora has been exposed to the xenobiotic contaminant long enough for genetic evolution to create a capacity to degrade the compound(s). This type of evolution takes place constantly, but is relatively slow. As a consequence the microbial community possesses the degradative pathways, but degradation may be inefficient because of low cell number or low activity level.

2. The indigenous microbial flora, which is adapted to the local conditions, is exposed to a contaminating xenobiotic compound(s). The bacteria acquire genes and degradation pathways from bacterial cells immigrating from elsewhere [4]. Transfer of genetic material can take place through conjugation, transduction or transformation; and all these have been shown to take place in environmental conditions [5; 6; 2]. From a bioremediation point of view, this type of evolution is also relatively slow, but can be enhanced.
3. As point 2, but the indigenous well-adapted microbial flora is artificially supplied with the required degradative capacity. Once the contaminant is known, gene-clusters (e.g. in a conjugative broad host range plasmid) may be supplied. If no natural gene clusters are available, these may be constructed. `Laboratory strains' can be used as donors, either to transfer the capacity to `wild type' strains newly isolated from the site, or by introducing the donors into the site and letting gene transfer occur. The presence of suitable recipients can be tested.
4. A bacterium that is thought to be competitive at the contaminated site is chosen. This may be a strain that is known to degrade the contaminating compound, or one that is specifically constructed for this purpose. If genetic engineering is involved, special considerations apply. Thus, if containment of the modified genes is required, suicide functions may be inserted [7]. In this case the strain has to be constructed to be as stable as possible, precluding any type of genetic exchange. Therefore, the strain itself needs to be able to compete with the indigenous flora of the site to be remediated.

Genetic engineering of microorganisms

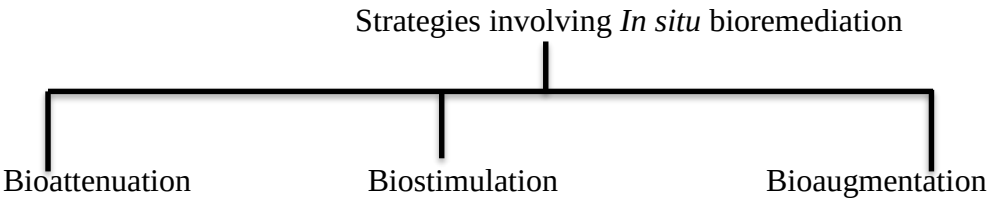
Microorganisms respond differently to various kinds of stresses and gain fitness in the polluted environment. This process can be accelerated by applying genetic engineering techniques. The recombinant DNA and other molecular biological techniques have enabled (i) amplification, disruption, and/or modification of the targeted genes that encode the enzymes in the metabolic pathways, (ii) minimization of pathway bottlenecks, (iii) enhancement of redox and energy generation, and (iv) recruiting heterologous genes to give new characteristics [8; 9; 10]. Various genetic approaches have been developed and used to optimize the enzymes, metabolic pathways and organisms relevant for biodegradation [11]. New information on the metabolic routes and bottlenecks of degradation is still accumulating, requiring the need to reinforce the available molecular toolbox [12]. Nevertheless, the introduced genes or enzymes, even in a single modified organism, need to be integrated within the regulatory and metabolic network for proper expression [13]. There are some drawbacks with the field release of genetically engineered microorganisms (GEMs), which include the decreased levels of fitness and the extra energy demands imposed by the presence of foreign genetic material in the cells [14; 15]. More importantly, there remains a great risk of mobile genetic elements entering the environment and being acquired by undesirable organisms. The biotechnological innovations for making GEMs are numerous. According to Pandey

et al. [16], the advances such as the programmed cell death based on the principle of killer–anti-killer gene(s) after detoxification can help to develop 'suicidal genetically engineered microorganisms' (S-GEMs) that can lead to safe and efficient bioremediation. Few GEMs have been used for field application because of strict regulations for the release of GEMs into the environment [17]. The only GEM approved for field testing in the USA for bioremediation was *Pseudomonas fluorescens* HK44, possessing a naphthalene catabolic plasmid (pUTK21), mutagenized by transposon insertion of *lux* genes [18]. The transition of genetically engineered microorganisms from the laboratory to the field environments is hampered due to the lack of information on the population dynamics of introduced genetically engineered microorganisms in the field and poor physiological control of catabolic gene expression in the engineered organisms under nutrient and other stresses [13]. The bioengineering and environmental release of those engineered microorganisms has to overcome several obstacles which include inconsistencies in risk assessment procedures and public health concerns before their effective application in the field. Selecting an indigenous bacterium able to grow rapidly and withstand the local stressful conditions for genetic engineering to enhance the biodegradation capabilities will be more advantageous over other bacterial strains. We hope, in 5 to 10 years from now, research into the field release of GEMs will help in designing them for alleviation or prevention of any perceived risks and eventually gaining public and regulatory acceptance in bioremediation of contaminated sites.

Strategies of *In situ* bioremediation

Bioremediation approaches are generally classified as in situ or ex situ. In situ bioremediation involves treating the polluted material at the site while ex situ involves the removal of the polluted material to be treated elsewhere [19]. In situ bioremediation can be described as the process whereby organic pollutants are biologically degraded under natural conditions to either carbon dioxide and water or an attenuated transformation product. It is a low-cost, low maintenance, environment-friendly and sustainable approach for the clean-up of polluted sites. With the need for excavation of the contaminated samples for treatment, the cost of ex situ bioremediation approaches can be high, relative to in situ methods. In addition, the rate of biodegradation and the consistency of the process outcome differ between the in situ- and ex situ bioremediation methods. While the methods of both in situ and ex situ remediation depend essentially on microbial metabolism, the in situ bioremediation methods are preferred to those of ex situ for ecological restoration of contaminated soil and water environments [20]. Three different types of in situ bioremediation process are (i) bioattenuation/Natural Attenuation, which depends on the natural process of degradation, (ii) biostimulation where intentional stimulation of degradation of chemicals is achieved by addition of water, nutrient, electron donors or acceptors, and (iii) bioaugmentation chemicals is achieved by addition of water, nutrient, electron donors or acceptors, and (iii) bioaugmentation where the

microbial members with proven capabilities of degrading or transforming the chemical pollutants are added [21].



The suitability of a particular bioremediation technology is determined by several factors, such as site conditions, indigenous population of microorganism, and the type, quantity and toxicity of pollutant chemical species present.

Bioattenuation: The natural way

During bioattenuation (natural attenuation / intrinsic bioremediation), the pollutants are transformed to less harmful forms or immobilized. Such transformation and immobilization processes are largely due to biodegradation by microorganisms [22], and to some extent by the reactions with naturally-occurring chemicals and sorption on the geologic media. The natural attenuation processes are contaminant specific, accepted as methods for treating fuel components (e.g., BTEX) [23], but not for many other classes. The time required for natural attenuation varies considerably with site conditions. Many polluted sites may not require an aggressive approach to remediation, and bioattenuation is efficient and cost effective [24; 25]. In fact, a variety of bioremediation techniques have been successfully employed at over 400 clean-up sites throughout the USA, at costs which are approximately 80–90% lower than other clean-up technologies, based on the physical and chemical principles. With minimal site disturbance, the post-clean up costs are also substantially reduced. Industrial and environmental biotechnologies also prefer newer paths, resulting in processes with ‘clean technologies’, with maximum production and fewer residues. Bioattenuation alone becomes inadequate and protracted in many cases since many soils are oligotrophic in nature or lack appropriate microorganisms.

Biostimulation: Importance of correct nutrient ratios

The acceleration of microbial turnover of chemical pollutants generally depends on the supply of carbon, nutrients such as N and P, temperature, available oxygen, soil pH, redox potential, and the type and concentration of organic pollutant itself [26]. To stimulate microbial degradation, nutrients in the form of fertilizers {water soluble (e.g., KNO_3, $NaNO_3$, NH_3NO_3, K_2HPO_4 and $MgNH_4PO_4$), slow release (e.g., customblen, IBDU, max-bac), and oleophilic (e.g., Inipol EAP22, F1, MM80, S200)} are added [27]. As a thumb rule for oil spill remediation, around 1–5% N by weight of oil with a ratio of N:P between 5 and 10:1 is applied [28]. These additions may be insufficient or inaccurate for polluted sites with different types of pollutants. Formulation of nutrient-treatment strategies and maintenance of control on the degradation rates and the outcomes of degradation need

to be tailored to specific site/pollutant combinations. Limitations of nutrients such as nitrogen and phosphorus on microbial decomposition of organic matter and the possible ecological implications of these effects for carbon flow through natural ecosystems are well known [29]. Wolicka et al. [30] optimized the C: N: P ratio (at the level of 100:9:2, 100:10:1 or 250:10:3) before commencing in situ remediation of BTEX. The 'ecological stoichiometry' is concerned with the supplies of nutrients, and their elemental stoichiometry relative to the nutritional demands of the cell's innate physiology. It also exemplifies the effects of resource (nutrient) supply rates and supply ratios on the structure and function of microbial communities [31]. Smith et al. [32] applied the resource-ratio theory to hydrocarbon degradation and demonstrated that the changes in nitrogen and phosphorus supply ratios not only altered the biodegradation rates of hydrocarbons (hexadecane and phenanthrene) but also the microbial community composition significantly. In addition, the changes in absolute nutrient supply levels, at constant supply ratio, were found to alter total hydrocarbon degrader biomass, with altered rates of hydrocarbon degradation. The 'resource-ratio approach' to gain information on the ecophysiological status of pollutant-degrading microorganisms has many practical implications. Basically, it provides the theoretical framework for optimizing nutrient formulation and application in biostimulation approaches. Moreover the concept of Biostimulation can also be applied to *Ex situ* bioremediation techniques.

Bioaugmentation: When the locals aren't up to the task?

Often, the biological response lags behind, up to weeks or months, in the polluted sites with no exposure history. The 'soil activation,' a concept which is based on the cultivation of biomass from a fraction of a contaminated soil and the subsequent use as an inoculum for bioaugmentation for the same soil was attempted by Otte et al. [33] for degradation of PCP and PAHs. The soils with microbiota, adapted by prior exposure to degradation of organic pollutants such as hydrocarbons can be a source of microorganisms for remediating soils freshly contaminated with hydrocarbons. Priming with 2% bioremediated soil was found to increase biodegradation of PAH constituents of a fuel oil-treated soil [34]. Similar priming effect of exhaustively bioremediated soils for hydrocarbon degradation was observed by Greenwood et al. [35]. Exposure history and adaptive status of microbial degraders thus determine the lag period of degradation. In addition, ascertaining the history of exposure of chemical pollutants in the contaminated sites has even become significant in the environmental forensics such as the 1989 Exxon Valdez oil spill case [36] and for ecological engineering such as the 2010 Gulf of Mexico oil spill case [37]. Pre-adaptation of catabolic bacteria to the target environment, prior to inoculation, improves survival, persistence and degradative activities, leading to enhanced remediation of the polluted soil [38]. *Sphingomonas* sp. RW1 which contained a mini transposon Tn-5 lacZ was pre-adapted to soil by growing in the soil extract medium. The pre-adapted bacterium exhibited better survival, ,and efficient degradation of and and efficient degradation of dibenzo-p-dioxin and dibenzofuran in the polluted soil, compared to the

unadapted bacterium, grown only in the nutrient-rich medium. Sudden exposure to stresses in soil (oligotrophic conditions that generally exist in soils, starvation or susceptibility/resistance, etc.) determines the physiological response of bacteria and their subsequent survival and activities. Pre-exposure and subsequent re-exposure of a chemical pollutant enhances the metabolic potential of microorganisms [39]. The phenomenon of retaining specific metabolic capacity after pre-exposure over long periods of time is referred to as 'soil memory.' The soil memory makes a contribution to the subsequent natural attenuation. Now, in a typical bioaugmentation approach, microorganisms are amended to a polluted site to hasten detoxification and/or degradation. There are many reports on bioaugmentation for treatment of soils containing organic pollutants [40]. Gilbert and Crowley [41] found that the repeated application of carvone-induced bacteria enhanced biodegradation of PCBs in soil. To improve efficiency of bioaugmentation, microorganisms of different physiological groups and of different divisions can also be brought together. Bender and Phillips [42] suggested the use of microbial mats which occur in nature as stratified communities of cyanobacteria and bacteria to remediate organic contaminants by degrading and completely mineralizing the contaminants. Wolicka et al. [30] applied aerobic microbial communities, selected from those adapted to utilize one type of BTEX compound, for bioremediation of soil contaminated with BTEX. A successful strategy for in situ bioremediation can be the combination, in a single bacterial strain or in a syntrophic bacterial consortium, of different degrading abilities with genetic traits that provide selective advantages in a given environment [43]. The present strain selection procedures dwell on isolating 'superbugs' with high resilience to environmental stresses, those harboring catabolically superior enzymes, and those species that are not human pathogens [44]. Most laboratory strains which are capable of degrading organic pollutants constitute a fraction of culturable microorganisms, making only small contributions to bioaugmentation [45]. Paul et al. [46] also pointed out that only a fraction of total microbial diversity has been harnessed so far while the genetic resource for degradation of recalcitrant and xenobiotic pollutants is vast. Bioaugmentation efforts are met with failures more often due to lesser efficiency, competitiveness and adaptability, relative to the indigenous members of natural communities. For example, the well known bacteria capable of degrading PCBs in laboratory culture media survived poorly in natural soils, and when these strains were inoculated to remediate PCB-contaminated soils, the resultant was the failure of bioaugmentation [47]. Further investigations revealed that formation of an antibiotic compound, protoanemonin, from 4-chlorocatechol via the classical 3-oxoadipate pathway by the native microorganisms was the reason for poor survival of the introduced specialist PCB-degrading strains [47; 48]. Indeed, bioaugmentation itself is undesirable in all the environmentally sensitive locations, especially those protected from the introduction of exotic flora or fauna. Scott et al. [49] proposed a new strategy of using a free enzyme-based product to remediate water bodies contaminated with atrazine. The ecological or environmental issues associated with degrading

organisms can be circumvented by this strategy. The soils do have exoenzymes (cell-free enzymes) which include proteases, and the presence of proteases along with other inhibitors may limit the longevity of free enzymes applied for bioremediation. The cell-free approach can only be used for viable and efficient enzymes that are not dependent on diffusible co-factors such as NAD (particularly hydrolases), and cannot be applied in cases where the enzyme activity (e.g., most oxygenases) is lost when the cells are broken [50]. Orica Watercare (Australia) has commercialized for the first time a free-enzyme for phosphotriester insecticides under the trade name LandGuard™ which was proven to be successful and cost effective. Nevertheless, the technical feasibility of such strategy needs careful evaluation for many contaminants or their mixtures. Immobilizing enzymes on suitable carriers will make them more stable and resistant to changes in pH, temperature and substrate concentrations [51; 52]. Other limitations for enzymes include: (a) expensive production costs for pure enzymes, (b) reduced activity due to sorption in soils requiring repeated doses, and (c) the issues with delivery of enzymes, immobilized enzymes in particular, to come in contact with the pollutant in the contaminated site. Selection of suitable carrier materials for immobilizing enzymes will not only help to increase their longevity but also allow their re-use thus making them more cost-effective. Further research into cheap nutrient sources for growing microorganisms may lower production costs of pure enzymes. Also, more research is required into the mechanisms of delivery of enzymes for their in situ application. Like biostimulation, bioaugmentation can also be used for ex situ bioremediation techniques.

In situ bioremediation techniques

These techniques involve treating polluted substances at the site of pollution. It does not require any excavation; therefore, it is accompanied by little or no disturbance to soil structure. Ideally, these techniques ought to be less expensive compared to ex situ bioremediation techniques, due to no extra cost required for excavation processes; nonetheless, cost of design and on-site installation of some sophisticated equipment to improve microbial activities during bioremediation is of major concern. Some in situ bioremediation techniques might be enhanced (bioventing, biosparging and microbe assisted phytoremediation), while others might proceed without any form of enhancement (intrinsic bioremediation or natural attenuation). In situ bioremediation techniques have been successfully used to treat chlorinated solvents, dyes, heavy metals, and hydrocarbons polluted sites [53; 54; 55; 56]. Notably, the status of electron acceptor, moisture content, nutrient availability, pH and temperature are amongst the important environmental conditions that need to be suitable for a successful in situ bioremediation to be achieved [57]. Unlike ex situ bioremediation techniques, soil porosity strongly influences the application of in situ bioremediation to any polluted site.

Enhanced in situ bioremediation

Composting and addition of composted material

Traditionally, the practice of composting is intended to reduce volume and water content of vegetable wastes, to destroy pathogens, and to remove odor-producing compounds. This technology is now applied for handling polluted soil or sediments by two chief ways: (i) composting of polluted soils for efficient degradation, and (ii) addition of composted materials. Additions of composted material were found to improve degradation of two herbicides, benthiocarb (S-4-chlorobenzyl diethylthiocarbamate) and MCPA (4-chloro-2-methylphenoxyacetic acid) in soil [58]. Van Gestel et al. [59] reported that the impact of diesel on the composting process was negligible when soil was spiked with diesel oil and mixed with biowaste (vegetable, fruit and garden waste) at a 1:10 ratio (fresh weight) and composted in a monitored composting bin system. The spent mushroom waste from *Pleurotus ostreatus* was found to degrade and mineralize DDT in soil [60]. On the contrary, Alvey and Crowley [61] observed that additions of compost suppressed soil mineralization of atrazine relative to rates in unamended soils or in soils amended with starch or rice hulls, probably due to the high nitrogen content of the compost. The critical parameters for composting depend on the type of contaminants and waste materials to be used for composting. The composting efficiency essentially depends on temperature and soil/ waste amendment ratio as the two important operating parameters for bioremediation [62]. According to Baheri and Meysami [63], the increase in the bulking agents such as peat moss, pine wood shavings, bran flakes, or a mixture of these agents from 6 to 12% led to an increase of 4–5% in the biodegradation of total petroleum hydrocarbons. In another study, the soil amendment with sludge-only or compost-only in a ratio of 1:0.1, 1:0.3, 1:0.5, and 1:1 (soil/amendment, wet weight basis) increased the rates, but higher mix ratios did not increase the degradation rates of total petroleum hydrocarbons correspondingly [64]. For the optimum removal of aged PAH during composting, Guerin [65] recommended to keep moisture and amendment ratio constant. During the composting-bioremediation, not only the contaminant but also the waste amendment and the operating conditions will determine the rate of biodegradation. Organic pollutants can be degraded during the first phase of rapid decomposition during composting. Heat which is generated by microbial metabolism is trapped in the compost matrix and most of the microbial decomposition and biomass formation occur during the thermophilic stage of composting. The mixing of remediated soil with contaminated soil can increase the effectiveness of composting because the remediated soil with acclimated microorganisms significantly influences pollutant degradation in the composting process [66]. The mineralization may be only a small fraction of pollutant degradation, with other prominent fates being partial degradation to secondary compounds, volatilization, and adsorption to compost [67]. In the composting matrices, secondary compounds, volatilization, and adsorption to compost [67]. In the composting matrices, microorganisms can degrade pollutants into innocuous compounds, transform pollutants into less

toxic substances and/or aid in locking up the chemical pollutants within the organic matrix, thereby reducing pollutant bioavailability. Even in the compost remediation strategy, the bioavailability and biodegradability of pollutants are the two most important factors which determine the degradation efficiency [68]. Cai et al. [69] showed that the efficiency of composting processes differed among the manually turned compost, inoculated manually turned compost, continuously aerated compost and intermittently aerated compost for bioremediating sewage sludge contaminated with PAHs, with the intermittently aerated compost treatment showing higher removal rate of high molecular weight PAHs. Composting or the use of composted materials can be applied to the bioremediation of polluted soils. However, the nature of waste or soil organic matter that consists of humic materials play an important role in binding of the contaminants such as PAHs and making them accessible to microbes for degradation. Plaza et al. [70] reported that composting will induce significant modifications to the structural and chemical properties of the humic material fraction including loss of aliphatic materials, an increased polarity and aromatic polycondensation resulting in a decrease in PAH-binding. Recently, Sayara et al. [71] demonstrated that stable composts in municipal solid wastes enhanced biodegradation of PAH particularly during the initial phase of composting. Humic material which accumulates with an increase in stability of the compost is known to act like a surfactant and plays an important role in releasing PAHs sorbed to the soil. PAH degradation mostly occurs during mesophilic stage of composting, while thermophilic stage is inhibitory for biodegradation [62; 71]. Similar to any other technology, composting has both advantages and limitations. Addition of compost to contaminated soil for bioremediation makes it a sustainable technology since the biodegradable organic waste in the compost is being utilized for beneficial activity. Also, composting improves the soil structure, nutrient status and microbial activity. During composting the contaminant can disappear via different mechanisms such as mineralization by microbial activity, transformation to products, volatilization, and formation of nonextractable bound residues with organic matter. The fate of nonextractable bound residues of contaminants in composting is another area of interest that requires more research into their release, behavior and risk. One of the critical knowledge gaps of composting is lack of sufficient knowledge about microorganisms involved during various stages of composting, the thermophilic stage in particular, which is almost like a black box. In fact, there are conflicting views about the role of the thermophilic stage of composting in bioremediation of contaminants. Added to this complexity is the fate of bound residues and whether or not they pose a risk in the future. Knowledge about (a) the nature and activity of microorganisms involved in various stages of composting, and (b) the degree of stability of compost and its humic matter content will greatly assist in better designing of composting as a bioremediation strategy for contaminated soils.

Bioventing

This technique involve controlled stimulation of airflow by delivering oxygen to unsaturated (vadose) zone in order to increase bioremediation, by increasing activities of indigenous microbes. In bioventing, amendments are made by adding nutrients and moisture to enhance bioremediation with the ultimate goal being to achieve microbial transformation of pollutants to a harmless state [57]. This technique has gained popularity among other in situ bioremediation techniques especially in restoring sites polluted with light spilled petroleum products [72]. A study by Sui and Li [73] modelled the effect of air injection rate on volatilization, biodegradation and biotransformation of toluene-contaminated site by bioventing. It was observed that at two different air injection rates (81.504 and 407.52 m^3/d), no significant difference in contaminant (toluene) removal was observed at the end of the study period (200 days). However, at the earlier stage of the study (day 100), it was observed that high air injection rate resulted in enhanced toluene removal by volatilization compared to low air injection rate. In other words, high airflow rate does not bring about increase in biodegradation rate nor make pollutant biotransformation more effective. This is due to early saturation of air (by high or low air injection rate) in the subsurface for oxygen demand during biodegradation. Nonetheless, low air injection rate resulted in a significant increase in biodegradation. It thus demonstrates that in bioventing, air injection rate is among the basic parameters for pollutant dispersal, redistribution and surface loss. Similarly, Frutos et al. [74] reported the effectiveness of bioventing treatment in remediation of phenanthrene-contaminated soil and recorded > 93 % contaminant removal after 7 months. Airflow intensities and airflow intervals resulted in no significant difference in diesel removal from clayey soil, implying that longer air injection interval and low air injection rate might be more economical for bioventing in diesel-polluted clayey soil [75]. Interestingly, Rayner et al. [76] observed that in a sub-Antarctic hydrocarbon-polluted site, single-well bioventing was ineffective towards hydrocarbon removal ascribable to shallow water table and thin soil cover, which led to channel development; whereas, when a microbioventing using nine small injection rods (0.5 m apart) was carried out on the same site, under identical conditions, a considerable amount of hydrocarbons were removed due to more uniform distribution of oxygen thus resulting in increased biodegradation. It becomes apparent that though airflow rates and air intervals are amongst the basic parameters of bioventing, the success of bioventing based bioremediation relies on the number of air injection points, which helps to achieve uniform distribution of air. Despite the fact that bioventing design is to encourage aeration in unsaturated zone, it can be used for anaerobic bioremediation process especially in treating vadose zone polluted with chlorinated compounds, which are recalcitrant under aerobic conditions. In this latter process, in lieu of air or or pure oxygen, mixture of nitrogen together with low concentrations of pure oxygen, mixture of nitrogen together with low concentrations of carbon dioxide and hydrogen can also be injected to bring about reduction of chlorinated vapour, with hydrogen acting

as electron donor [77]. In a soil with low-permeability, injection of pure oxygen might lead to higher oxygen concentration compared to air injection. Furthermore, ozonation might be useful for partial oxidation of recalcitrant compounds in order to accelerate biodegradation [57]. Unlike bioventing that relies on enhancing microbial degradation process at the vadose zone by moderate air injection, soil vapour extraction (SVE) maximizes volatile organic compound volatilization via vapour extraction [78]. Although both techniques use identical hardware, the configuration, philosophical design and operation differ significantly [79]. Airflow rate in SVE is higher compared to that of bioventing. SVE may be regarded as physical method of remediation due to its mechanism of pollutant removal, however, the mechanism involved in pollutant removal for both techniques are not mutually exclusive. During on-site field trials, achieving similar results obtained during laboratory studies is not always attainable due to other environmental factors and different characteristics of the unsaturated zone to which air is injected; as a result, with bioventing, treatment time may be prolonged. Apparently, high airflow rate leads to transfer of volatile organic compounds to the soil vapour phase, which requires off-gas treatment of the resulting gases prior to release into the atmosphere [80]. This particular challenge can be resolved by combining bioventing and biotrickling filter techniques to reduce both contaminant and outlet gas emission levels; thus reducing the extended treatment time associated with bioventing alone [78].

Bioslurping

This technique combines vacuum-enhanced pumping, soil vapour extraction and bioventing to achieve soil and groundwater remediation by indirect provision of oxygen and stimulation of contaminant biodegradation [81]. The technique is designed for free products recovery such as light non-aqueous phase liquids (LNAPLs), thus remediating capillary, unsaturated and saturated zones. It can also be used to remediate soils contaminated with volatile and semi-volatile organic compounds. The system uses a ‘‘slurp’’ that extends into the free product layer, which draws up liquids (free products and soil gas) from this layer in a manner similar to that of how a straw draws liquid from any vessel. The pumping mechanism brings about upward movement of LNAPLs to the surface, where it becomes separated from water and air. Following complete free products removal, the system can easily be made to operate as a conventional bioventing system to complete remediation process [54]. In this technique, excessive soil moisture limits air permeability and decreases oxygen transfer rate, in turn reducing microbial activities. Although the technique is not suitable for remediating soil with low permeability, it saves cost due to less amount of groundwater resulting from the operation thus minimizes storage, treatment and disposal costs [57]. Establishing a vacuum on a deep high permeable site and fluctuating water table, which could create saturated soil lenses that are difficult to aerate are amongst the major concerns of this particular in situ technique.

Biosparging

This technique is very similar to bioventing in that air is injected into soil subsurface to stimulate microbial activities in order to promote pollutant removal from polluted sites. However, unlike bioventing, air is injected at the saturated zone, which can cause upward movement of volatile organic compounds to the unsaturated zone to promote biodegradation. The effectiveness of biosparging depends on two major factors namely: soil permeability, which determines pollutant bioavailability to microorganisms, and pollutant biodegradability [57]. As with bioventing and soil vapour extraction (SVE), biosparing is similar in operation with a closely related technique known as in situ air sparging (IAS), which relies on high airflow rates to achieve pollutant volatilization, whereas biosparging promotes biodegradation. Similarly, both mechanisms of pollutant removal are not mutually exclusive for both techniques. Biosparging has been widely used in treating aquifers contaminated with petroleum products, especially diesel and kerosene. Kao et al. [82] reported that biosparging of benzene, toluene, ethylbenzene and xylene (BTEX)-contaminated aquifer plume resulted in a shift from anaerobic to aerobic conditions; this was evidenced by increased dissolved oxygen, redox potentials, nitrate, sulphate and total culturable heterotrophs with a corresponding decrease in dissolved ferrous iron, sulphide, methane and total anaerobes and methanogens. The over all decrease in BTEX reduction (>70 %) further indicates that biosparging can be used to remediate BTEX contaminated ground water. The major limitation however, is predicting the direction of airflow.

Microbe assisted Phytoremediation

Pollutant effects on plant growth are concentration-dependent and different plant species respond differently. Low doses of pollutant can increase plant weight while high doses can inhibit, a phenomenon referred to as ‘hormesis’ [83]. In general, plants can promote dissipation of organic pollutants by immobilization, removal, and promotion of microbial degradation. Some organic compounds are transported across plant membranes, released through leaves via evapotranspiration (phytovolatilization) or extracted, transported and accumulated in plant tissues (phytoextraction) or degraded via enzymatic processes (phytodegradation). Some of the non-volatile compounds are sequestered in planta and are less bioavailable (phytostabilization). Several limitations of bioremediation such as the inability of degrading microorganisms to compete with indigenous microflora, insufficient microbial activities at subsurface, poor support of native as well as pollutant-degrading microflora by available or limiting nutrients, heterogeneity of bioavailable contaminants, and toxic or inhibitory compounds in the pollutant mixture requires the union of phytoremediation and other bioremediation strategies [84]. Plants have several miles of roots per acre, suggesting the potential of pollutant degradation in the rhizosphere [85]. Sugars, organic acids, and larger organic compounds which constitute about 10–50% of plant's photosynthate are deposited in soils [86], and the carbon cycling from CO_2 assimilation by plants to root exudation to incorporation to microbial

biomass to microbial respiration takes about just 5 h [87]. In the rhizosphere which is dependent on morphology, proportion of fine roots, water and nutrient conditions, root exudation, and associated microbial communities, there may be either promotion or competition between the pollutant degraders and other microbial members. Ma et al. [88] suggested from a meta-analysis that the activity of PAH decomposers in soil is more likely to be enhanced by root activities than to be inhibited by other microorganisms in the rhizosphere, despite the variations due to species, habitats, contamination types and doses. The complex aromatic compounds such as flavonoids and coumarins which aid microbial colonization of roots are structurally similar to PCBs, PAHs and PHC, providing opportunities as the analogue-enrichment for stimulating degradative pathways in microorganisms [89]. Rhizoremediation, an integral component of phytoremediation can occur naturally or can be triggered by introducing specific pollutant degrading microbes or plant growth promoting microorganisms [84]. Since the root depth of herbaceous plants varies from plant to plant, from soil to soil, and season to season, the presence of contaminants in soils which is deeper than the root zone of plants requires excavation, other agronomic practices or selection of trees with deeper roots. Nevertheless, most of the recalcitrant organic contaminants are typically found in the top few cm of the soil. Dendroremediation, which is a type of phytoremediation using trees may be useful in attenuating certain pollutants such as 2,4,6-trinitrotoluene and trichloroethylene from soil and groundwater [90]. Plants produce many secondary plant metabolites (SPMEs) which include allelopathic chemicals, root exudates, phytohormones/ phytoalexins, phytosiderophores, and phytoanticipins and are derived from isoprenoid, phenylpropanoid, alkaloid or fatty acid/polyketide pathways [91]. Singer et al. [44] argued that SPMEs are pollutant analogues within the network of suprametabolism, having implications for predicting the fate of pollutants. Gilbert and Crowley [41], and Kim et al. [54] showed that SPMEs such as limonene, cymene, carvone and pinene enhanced degradation of PCBs. *Pseudomonas putida* PCL1444, isolated from the rhizosphere of *Lolium multiflorum* cv. Barmultra when grown in PAH-polluted soil degraded the PAHs and protected the plant from the pollutant, by efficient utilization of root exudates for growth and high transcription of naphthalene catabolic genes [92]. Narasimhan et al. [93] applied the rhizosphere metabolomics-driven approach, which has been referred to profiling of root exudates for identification of targeted compounds for creating the nutritional bias, to degrade PCBs (2Cl-biphenyl, 4Cl-biphenyl and Aroclor 1254 at 53 μM) in the rhizosphere of *Arabidopsis*. The growth of gfp-tagged *Pseudomonas putida* PML2 was increased due to the exudation of SPMEs such as phenylpropanoids and consequently PCB degradation was enhanced. The rhizosphere metabolomics-driven approach will become an important tool for engineering phytoremediation phytoremediation systems. The activity and the numbers of the pollutant-degrading endophytes are both plant- and contaminant-dependent [94]. Contaminants such as TCE and methyl tert-butyl ether which are routinely assimilated in the transpiration pathways of plants may be degraded effectively by the pollutant-degrading

endophytes. *Methylobacterium* sp. strain BJ001, a phytosymbiotic bacterium isolated from tissue culture plantlets of *Populus deltoides×nigra* DN34 was found to transform 2,4,6-trinitrotoluene and mineralize hexahydro-1,3,5-trinitro-1,3,5-triazine and octahydro-1,3,5,7-tetranitro- 1,3,5-tetrazocine to CO_2 [95]. Barac et al. [96] demonstrated that the engineered endophyte (*Burkholderia cepacia* strain L.S.2.4 containing the toluene-degrading plasmid, pTOM), when applied to surface-sterilized yellow lupine seeds led not only to the protection against the phytotoxic effects of toluene but also decreased emissions from the transpiration stream of its host. The pollutant-degrading endophytes are relatively free from the competition for nutrients and water among the colonizers in the rhizosphere. Greater opportunities for employing the endophyte assisted phytoremediation, either through naturally-occurring or engineered endophytes exist, especially for the mobile pollutants. Phytostimulation of pollutant degradation by microorganisms in the rhizosphere or inside the plants can offer many economic and environmental advantages compared to the conventional strategies employed in biostimulation. But, the disadvantages include hydrophobicity and chemical stability of pollutants that influence the phytostabilization and the rates of degradation by the associated microorganisms [95], and plant root exudation which modifies the structure and activities of pollutant-degrading microorganisms [97]. Besides, phytoremediation in the field is also challenged by many obstacles which include the inability to mitigate plant stress factors and non-availability of suitable methods for the assessment of phytoremediation [84].

2. CONCLUSION

The foremost step to a successful bioremediation is site characterization, which helps establish the most suitable and feasible bioremediation technique (ex situ or in situ). Ex situ bioremediation techniques tend to be more expensive due to additional costs attributed to excavation and transportation. Nonetheless, they can be used to treat wide range of pollutants in a controlled manner. In contrast, in situ techniques have no additional cost attributed to excavation; however, cost of on-site installation of equipment, coupled with inability to effectively visualize and control the subsurface of polluted site may render some in situ bioremediation techniques inefficient. Consequently, cost of remediation apparently is not the major factor that should determine the bioremediation technique to be applied to any polluted site. Geological characteristics of polluted site(s) including soil type, pollutant depth and type, site location relative to human habitation and performance characteristics of each bioremediation technique should be incorporated in deciding the most suitable and efficient method to effectively treat polluted sites.

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CONFLICT OF INTEREST

None

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