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SUITABILITY OF BACTERIA IN BIOREMEDIATION TECHNIQUES COMMON FOR PETROLEUM-RELATED POLLUTIONS

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ABSTRACT

Petroleum hydrocarbon is an energy source that drives our modern society and at the same time impacts the environment. The consequences of hydrocarbon pollution range from microbial diversity distortion to cancer scourge in humans. To reverse these negative trends imposed by the contaminated environment, deliberate remediation steps, need to be employed, which depend on physical, chemical, and biological mechanistic principles. The physicochemical approach is quick-oriented but is more expensive relative to the biological option. The latter uses microorganisms, their parts, or enzymes to decontaminate and detoxify hazardous fractions of hydrocarbons into benign products. This biotechnology is referred to as bioremediation. Bioremediation effectiveness is achieved through the implementation of various techniques that are carried out under aerobic or anaerobic conditions or in ex-situ or in-situ. However, the aeration-related condition is the most deciding factor for microbial adaptation and survival. In aerobic conditions, fungi, bacteria, and algae contribute actively in the biotransformation and detoxification process, thus give the best result in such circumstances. However, in an anoxic environment, the prominence of bacteria comes into play (due to their ability to thrive in extreme environments) in degrading the contaminants into less harmful compounds. Thus, bacteria stand the chance of been used as the most resourceful biological tool for petroleum biotechnology including environmental remediation of extreme environments due to their high adaptive index value. Moreover, the hydrocarbon impacted environment is often characterized by high salinity, extreme temperatures, high pressure, and extreme pH.

Contribution/Originality: This review contributes to existing literature by indicating the utility of bacteria in bioremediation techniques.

1. INTRODUCTION

Hydrocarbon pollution is been treated as a serious global concern because of the extent of contamination recorded around the globe. The impacts they cause are enormous, including but not limited to habitat alteration, biodiversity alteration and diseases like cancer in humans (Kalantary et al., 2014). To reverse these adverse affects chemical, physical and biological treatments are used. However, the physical and chemical approaches are not sustainable, hence the need for the biological methods called bioremediation. The latter is an alternative remediation approach currently in vogue. The reasons for this is that the technology is cheaper, simpler to implement, sustainable, and the resources used are mostly renewable, amongst other reasons. Its popularity is limiting the available space for the physicochemical techniques used in the remediation of contaminated environmental media. The remediation of the polluted environment is defined by the detoxification of the harmful compounds to less harmful products and it is attributed to organisms, their parts or their enzymes effective interaction with the pollutants (Ahmad et al., 2018). However, human intervention is needed in providing a system in which biotic factors, favourable environmental factors and the compound of interest interact to produce benign compounds for the restoration of the affected habitat. This system represents various remediation techniques suitable for air, water and land remediation. Bioremediation process can take place in an aerobic environment or anaerobic environment. Each of these processes has their limitation, thus the aerobic and anaerobic process can be coupled to reduce limit that may be imposed by each of them. Implementation of bioremediation could be done in situ or ex-situ (Tomei & Daugulis, 2013).

In-situ bioremediation techniques are applied to groundwater and soil at the site to establish remediation without removal of soils or water from the contaminated site it is applied in saturated soils and groundwater with a limited release of the pollutants (Mark, Kirsten, Hung, & Richard, 2002). The common variants of in situ techniques are monitored natural attenuation, which relies on monitoring and verification of mixed (biotic and abiotic) natural remediation process (Khan et al., 2001) bioventing characterized with an inlet of oxygen to an unsaturated zone of subsurface soil to stimulate microbial growth and metabolism mineralization (Salleh, Ghazali, Abd Rahman, & Basri, 2003) and phytoremediation, which employs adapted plants to remove or transform contaminants (Yan et al., 2020). Ex-situ remediation techniques are defined through biopiles, land farming, composting and slurry bioreactors (Cristorean, Micle, & Sur, 2016; Kumar, Ashok, & Rajesh, 2010; Sharma, 2012). These remediation techniques and methods have been used to detoxify and remove different species of hydrocarbons, including but not exclusive to BTEX, PAHs, PCB, diesel, lubricating oil wastes (Balseiro-Romero et al., 2019; Piccini, Raikova, Allen, & Chuck, 2019).

The detoxification and removal of contaminants from an impacted environment are usually carried out by different taxa of microorganisms either indigenous or alien to the affected habitat. The most dominant process in bioremediation is biotransformation/biodegradation. The adapted microorganisms metabolize the pollutants as their source of energy and carbons. Other bioremediation process driven by microorganisms, their parts and enzymes are bioextraction, bioaccumulation, biofiltration etc. The microorganism that is most adapted to variations inherent in the environment, techniques and pollutants is bacteria amid other microorganisms such as fungi, algae and yeasts. This mini-review thus focused on bacteria and other microbiota that features in different bioremediation techniques.

1.1. Impact of Hydrocarbons

Crude oil deposits because of its strategic relevance in modern society are extracted, refine and distributed across the globe. During these processes, they are elicited into the environment thereby causing an ecological impact that draws global and regional attention. All the fractions of crude: saturated, aromatic, resins and asphaltenes, negatively affect the ecosystem when released.

The impacts are reflected in diverse ways including but not exclusive to changes in the physicochemical characteristic, Habitat alteration, biodiversity depletion, groundwater contamination, bioaccumulation in ecological receptors, and cancer in humans (Kalantary et al., 2014). To reverse these adverse effects, different treatment mechanisms are applied to habitat or environmental media.

Chemical, physical and biological treatments are available for remediation purposes for environmental safety, removal of hazardous compounds and sustainable development (Tomei & Daugulis, 2013). However, the physical and chemical approaches are better oriented for emergencies but are not sustainable apart from not being cost-effective in comparison to the biological methods, bioremediation.

1.2. Bioremediation

This biotechnology is a relatively new concept and currently serve as an alternative to pre-existing pollution remediation techniques referred to as physicochemical methods (Table 1). Bioremediation is any process that makes use of organisms, their parts and enzymes to detoxify and restore contaminated environmental media to its former safe condition (Ahmad et al., 2018). As a biotechnology approach, bioremediation may be employed to decontaminate specific contaminants (Table 2) such as pesticides, organic liquids, oils and organic sludge or solids (Ke et al., 2021). Some contaminants especially oil spills can be biodegraded using multiple techniques including application of biosurfactants and nutrients. According to Rockne and Reddy (2003) processes of bioremediation may be directed toward accomplishing:

1. Mineralization.
2. Biotransformation.
3. Reduction of highly electrophilic halo-and nitro-groups.

Bioremediation may be either aerobic or anaerobic (Wang, Yang, Song, Tang, & Yu, 2019). To reduce the limitation inherent in each of these methods to treat pollutants of concern, at times sequential anaerobic-aerobic processes of bioremediation are employed to treat contaminated sites (Master, Lai, Kuipers, Cullen, & Mohn, 2002). Based on the application location and the polluted soil to be handled, bioremediation can be grouped into in situ and ex situ technology (Tomei & Daugulis, 2013).

1.3. In-Situ Bioremediation Technologies

Juwarkar, Misra, and Sharma (2014) defined in situ bioremediation techniques as methods applied to soil and groundwater at the site to establish remediation. It requires no removal of soils or water from the polluted site. In-situ bioremediation are often incorporated with oxygen and nutrient supply to the impacted site to stimulate indigenous microorganisms in the site to degrade the contaminants (Kumar, Bisht, Joshi, & Dhewa, 2011). In most cases, in situ bioremediation is employed to degrade pollutants of interest in saturated soils and groundwater with a limited release of the pollutants into the environment. In situ technology is often accompanied in conjunction with a water pumping and soil-flushing system to circulate nutrients and oxygen through contaminated aquifers and associated soils (Mark et al., 2002).

Table-1. Physicochemical techniques

Methods	Principle	Variation of Technique	Comments
Thermal	Evaporation/destruction of hydrocarbons	Rotating tube furnace incinerators, fluidized bed furnace	Excavation needed, topsoil only, off-gas must be treated, it is expensive
Extraction	Removal of hydrocarbons in soluble form	Scrubbing towers, percolating towers, fluidized beds. Solvent extraction (Terra-Kleen) technique	As above, efficiency unknown, little data
Steam stripping	Removal of volatiles	Rotating drum; *in situ*	Volatiles only, good for subsoils, steam, little data
Hot-air stripping	Removal of volatile	Rotating drum; *in situ*	Volatiles only, good for subsoils, steam, little data
Chemical oxidation	Alteration of contaminant to ease removal	Reaction chamber *in situ*	No information for hydrocarbons
Immobilization	Binding hydrocarbons *in situ*	Chemical bonding; soil solidification	Not widely tested, does not remove pollutants
Adsorption	Groundwater pumped via activated carbon		Expensive, waste requires disposal, efficiency unknown
Detergent extraction	Soil is flushed with surface soil		Efficiency unknown, expensive

Source: Salleh et al. (2003).

Table-2. Application of soil *in situ* bioremediation processes.

Contaminant	Bioremediation	References
Diesel	Bioventing with biostimulation	Juwarkar et al. (2014)
Petroleum hydrocarbons	Bioventing with an addition of nitrogen source	Shewfelt, Lee, and Zytner (2005)
Gasoline-contaminated soil	Bioventing and biostimulation	Mark et al. (2002)
Mining soils contaminated with hydrocarbons	Biostimulation	Salinas-Martínez et al. (2008)
Polychlorinated biphenyls	Biostimulation by adding ferrous sulphate	Meckenstock, Safinowski, and Griebler (2004)
Kerosene contaminated soil	Biostimulation	Agarry and Oghenejoboh (2015)
PAHs	Biostimulation and bioaugmentation	Atagana (2006)
Heavy metals	Phytoremediation	Ebbs, Hatfield, Nagarajan, and Blaylock (2009)

Source: Juwarkar et al. (2014).

1.4. Types of in Situ Bioremediation

1.4.1. Monitored Natural Attenuation

This is proactive in-situ bioremediation approach that relies on consistent monitoring and verification of natural bioremediation process (Khan et al., 2001). Monitored natural attenuation is also known as passive remediation (Juwarkar et al., 2014). Along with reliance on microorganisms to degrade the organic pollutants, abiotic factors do also contribute to the reduction of the pollutants (Wang, Zhang, Li, & Klassen, 2011). In this approach, the subterranean soil matrix behaves as a natural bioreactor for the biodegradation process to occur (Kumar et al., 2011). The applicability of monitored attenuation rests on the biogeochemical properties, contaminant properties, no-spreading potential of the pollutant plume and zero-risk for living organisms (Tomei & Daugulis, 2013). Target contaminants for monitored natural attenuation include fuels, pesticides, herbicides, semi-volatile and non-halogenated volatile organic compounds (Juwarkar et al., 2014). Also, HMW PAHs that tend to bind to soil particles so tightly and at the same time have very low mobility can be remediated through this means, only that it requires longer treatment time (Salleh et al., 2003). Fungi, bacteria and algae play significant roles. Fungi and algae are more adapted to aerobic degradation of high molecular weights of hydrocarbons especially polyaromatic hydrocarbons (PAHs). In most cases, the degradation of hydrocarbons and PAHs are driven through cometabolism (Alexis & Musa, 2020). Bacteria are both amenable to aerobic and anaerobic degradation. Both bacteria and fungi (especially yeasts) produce biosurfactants in the presence of hydrocarbons, which aid the bioavailabilty of the hydrophobic compounds and bioaccessibility of the hydrocarbons to adapted microbes in the environment (Ławniczak, Woźniak-Karczewska, Loibner, Heipieper, & Chrzanowski, 2020). Conclusively, the hydrocarbons and associated heavy metals are either transformed or mineralized. It is important to note that metals cannot be mineralized (Rahman & Singh, 2020).

1.5. Bioventing

This is a technique through which oxygen is pumped to the unsaturated zone. This technique is similar in principle to biosparging and soil vapour extraction (Shukla, Singh, & Sharma, 2010). Soil vapour extraction is aimed to maximize volatilization of contaminant while bioventing is designed to maximize biostimulation of indigenous microbes around the zone that is unsaturated (saturated zone for biosparging) to enhance contaminant mineralization (Salleh et al., 2003). The supplied oxygen is delivered through an aeration pipe network through the contaminant zone. Though soil permeability is the most critical factor, moisture and nutrient availability affect the effectiveness of this technique, thus it is often used to deliver nutrient to the impacted site (Juwarkar et al., 2014). Bioventing employs low airflow rates to make available just enough oxygen to stimulate microbial activity. Biodegradation is maximized by optimal flow rates as vapours move slowly and interact with biologically active soil

(Shukla et al., 2010). Bioventing had shown to be effective in remediation of petroleum hydrocarbons, hydraulic oils and aromatic hydrocarbons. Bacteria is more amenable to this technique because the environment is a bit inauspicious. Bacteria are known to withstand the extreme environment in comparison to other microbiota. The adapted few indigenous bacteria degrade the hydrocarbons using pathways suited for them. Factors that may enhance the degradation are biosurfactants, availability of nutrients, oxygen and functional genes. Frutos, Escolano, García, Babín, and Fernández (2010) recorded a 93% reduction of phenanthrene soil pollution in seven months. Earlier, Lee and Swindoll (1993) had shown through a laboratory treatability study that bioventing can be used successfully to remediate diesel, fuel oils and semivolatile organic compounds.

1.6. Phytoremediation

Phytoremediation is emerging biotechnology that employs plants to extract, transfer, stabilize and/or destroy organic/inorganic pollutants (Yan et al., 2020). Plants that are useful for environmental remediation has four basic attributes, which are accumulation property of shoots, wild root system, rapid growth and easily harvestable (Nedjimi, 2021).

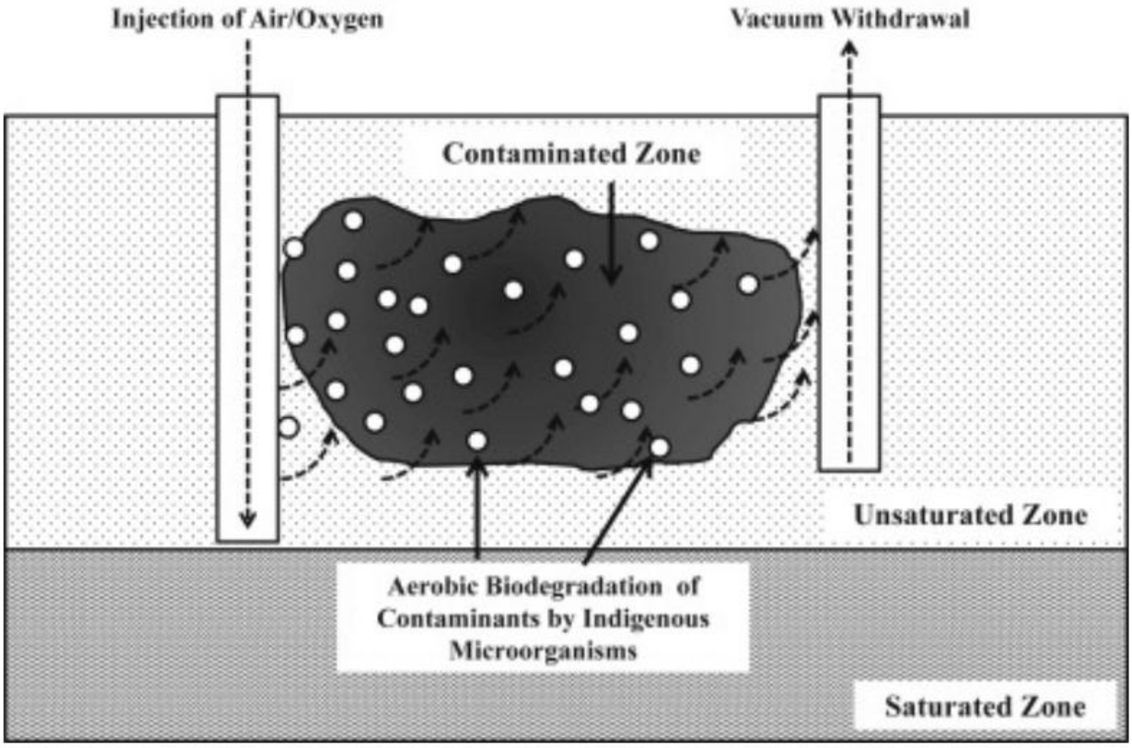

Figure-1. A typical model of bioventing system.

Source: Brown and Cologgi (2015).

Different strategies of phytoremediation that exist are:

1. Phytoextraction; Removes contaminants from soil or water at relatively low concentration. Ornamental plants represent the most viable option for this type of phytoremediation because of their efficiency, esthetic value and are not involved in a food chain (Kumar, Shahi, & Singh, 2018; Lajayer, Moghadam, Maghsoodi, Ghorbanpour, & Kariman, 2019).
2. Rhizofiltration: This involves the adsorption of contaminants-in-solution onto plant roots. Sunflower, rye and Indian mustard are common plants used for phytofiltration (Singh & Prasad, 2015).
3. Phytotransformation: This refers to the degradation or transformation of contaminants through the intervention of plants. Plants commonly used in phytotransformation are Elodeia, Grass, Pondweed, Arrowroot etc. (Kushwaha, Rani, Kumar, & Gautam, 2015; Saber et al., 2016).

4. Phytovolatilization: Refers to a process in which contaminants are transformed into various volatile compounds (Ali et al., 2020).
5. Phytostabilization: The use of plants to fix contaminant which results in reduxed bioavailability in soil.

Phytoremediation has been applied in the remediation of heavy metals, radionuclides, chlorinated solvents, ammunition wastes, excess nutrients, aromatic (including PAHs) and non-aromatic petroleum hydrocarbons in soil and aquifers (Doty et al., 2017; Hussain et al., 2018; Li, Ji, & Luo, 2019; Lu, Wang, Li, & Zhu, 2019; Yan. et al., 2021). Microorganism interacts with plants, especially in the rhizosphere. Such plant-microbe interactions shape the rhizosphere microbiome due to substrates secreted by the plants and vary between species (Zhalnina et al., 2018). Actinobacteria, bacteria, algae, protozoa in the rhizosphere influence bioavailability and mobility of contaminants to the plants by releasing chelating agents, nitrate/phosphates solubilization, and acidification (Mishra, Mishra, Arshi, Agarwal, & Dwivedi, 2020). In return, the microorganisms benefit from the exudates and redox changes derived from the plants. Some of these microorganisms are biofertilizers (Ajeng et al., 2020) hence serve as biological plant growth-promoting agent, which enhances the rapid growth of plants suited for phytoremediation. Ruley et al. (2019) achieved a 50% reduction of TPH using *Gossypium barbadense*, *Hyparrhenia rufa*, and *Sorghum arundinaceum*. Further, phytoremediation technique was used to record maximum reduce Ni (23%), Pb (34%), Cd (46%) and TPH (60%) (Steliga & Kluk, 2020).

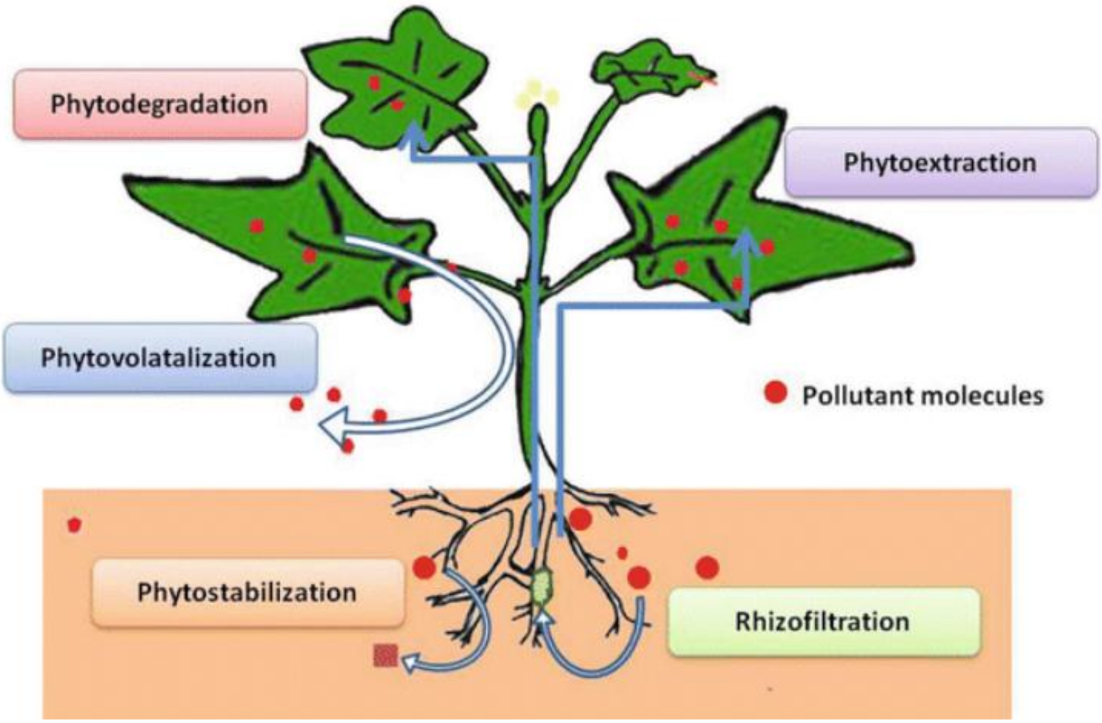

Figure-2. Schematic presentation of various phytoremediation strategies involved in the remediation of pollutants from a contaminated environment.
Source: Herath and Vithanage (2015).

1.7. Reasons for in-Situ Remediation

In-situ technique is usually selected on sites where the treatment undertaken by traditional methods would lead to extensive cost. The technology is often the only solution on sites where excavation would be technically difficult or impossible. Such areas may be found under or near buildings, under hard surface materials, around sewers, cables or pipelines, at great depths and in areas of widespread contamination. In situ method is mainly suitable for soils with sufficient hydraulic conductivity and low to medium contamination concentration (Salinas-Martínez et al., 2008).

1.8. Advantages of in-Situ Remediation, in Addition to Other Reasons, Are:

1. Bioremediation is implemented without causing major disruption to normal activities.
2. Eliminates the cost of transportation of impacted environmental media for treatment.
3. Drastically reduce future liability associated with products released since carbon dioxide and water are the major end products of bioremediation.
4. Contaminant spreading is completely avoided since transportation is avoided.
5. It is relatively simple to adopt and needs minimal equipment for the addition of air, nutrient and/or microorganisms and water as the case may be.

1.9. Limitation of in-Situ Remediation Include:

1. Increase in soil's depth reduces the availability of oxygen, water or nutrient.
2. Uncontrolled nature of the process makes it difficult to ascertain the extent of remediation of the polluted soil.
3. Intensive monitoring responsibility involved makes it more challenging, at times reflecting in cost, even though the overall cost is cost-effective.

1.10. Ex-Situ Bioremediation Technologies

Ex-situ bioremediation requires contaminated site excavation followed by treatment in a bioreactor or a treatment plant (Rockne & Reddy, 2003) located on the site or far away from the site. Based on the state of the contaminant to be eliminated or treated, ex-situ treatment can be implemented as either solid-phase system or slurry-phase system (Kheirkhah, Hejazi, & Rahimi, 2020; Partovinia & Naeimpoor, 2018). Where predictable and efficient removal of contaminants of soil is required, ex-situ technique (Table 3) is most likely to be employed provided the polluted site allows for excavation and the pollutant mostly concentrated in the upper layer of the soil (Tomei & Daugulis, 2013). According to the later authors, ex-situ technology has conventional and innovative methods.

1.11. Conventional Ex-Situ Bioremediation Techniques

This includes contained solid-phase bioremediation, composting and land farming which are all examples and types of solid-phase system (Rockne & Reddy, 2003). In solid-phase system, soil is treated above ground with collection systems to avoid the spread of pollutants (Sharma, 2012). According to Wang et al. (2011) solid-phase systems are relatively simple to operate, but requires a large amount of space, with certain parameters (moisture, temperature, nutrients or oxygen) that should be controlled for maximum result. The slurry bioreactor is an example of the slurry-phase bioremediation technology. It is also a conventional approach employed under ex-situ technology.

1.12. Biopile (Contained Solid Phase)

In this technique (see Figure 3), the contaminated soils are blended to achieve homogenous texture. This is followed by amendment of the soil with nutrient, water for moisture control, pH modifiers, the addition of microbes and occasionally bulk amendment are carried out (Piccini et al., 2019). The soil is then placed in an enclosed building, tank or vessel. For optimal result, the temperature and moisture condition including aeration is controlled to facilitate the degradation of the contaminants by the indigenous microbes (Atagana, 2006). In most cases, microorganisms that have been confirmed as hydrocarbon-degraders are seed into the vessel. Bacteria and fungi species find usefulness in this technique. Genetically modified microorganisms can also be employed in this regard. Granted that rainfall and runoff are eliminated and volatile organic compounds are under control due to the enclosed nature of the soil mass, there is need for adopting mechanisms to control explosive atmospheres and equipment for constant aeration and blending (Sharma, 2012). Gomez and Sartaj (2014) in a field-scale biopiles recorded 74-82% TPHs removal in a space of 94 days with the aid of microbial consortia and mature compost. Kim, Lee, and Chang (2018) used a pilot-scale biopile field remediation in a cold climate to achieve a 58% TPH reduction.

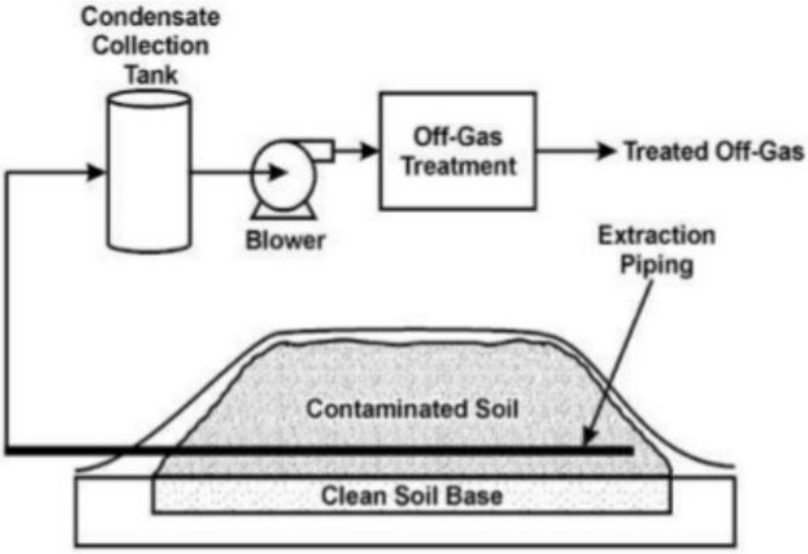

Figure-3. A biopile process.

Source: https://frtr.gov/matrix/Biopiles.

1.13. Land Farming

In this simple method (Figure 4), the excavated polluted soil is spread over a prepared field/treatment beds. The soil is spread in a layer not thicker than 40 cm (Micle & Neag, 2009). The spreading of the soil can be done using conventional construction or farm equipment. The goal of this method is to stimulate autochthonous microbes and facilitate the degradation (aerobic) of the contaminants (Kumar et al., 2010). Thus optimal pH, nutrient application, moisture content and constant tilling for aeration and blending are a requirement (Rubinos, Villasuso, Muniategui, Barral, & Díaz-Fierros, 2007). To avoid the spread of contamination, plastic liners can be planted in the treatment area before the spreading of the contaminated soil. Leachate can be collected and treated as a result. Land farming has often been used to treat soil polluted with PAHs, petroleum hydrocarbons, refinery waste and drill wastes (Nkeng, Nkwelang, & Mattew, 2012). With the application of inorganic fertilizer, Brown and Cologgi (2015) recorded 64% TPH removal in 110 days using the land farming technique.

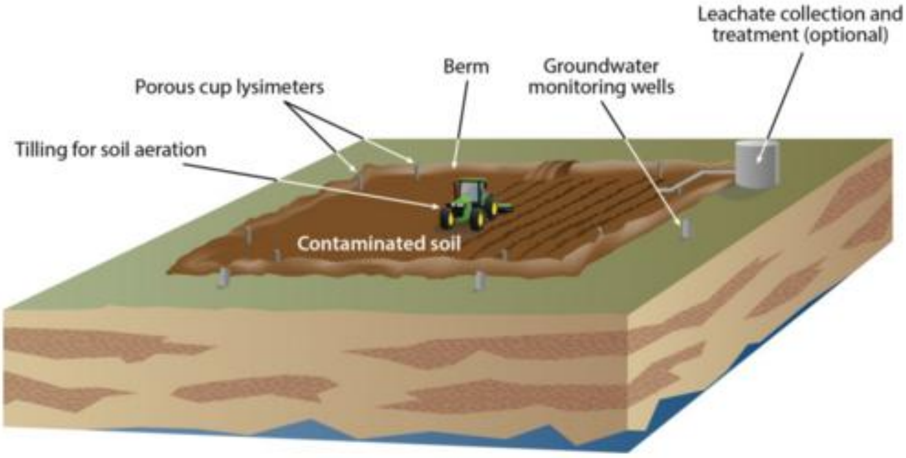

Figure-4. A Land farming system.

Source: U.S. Environmental Protection Agency (USEPA) (1993).

1.14. Composting

Compositing is a soil treatment technique which involves combining excavated contaminated soil with manure or agricultural wastes to improve the soil's handling characteristics (Rockne & Reddy, 2003) provide elevated temperature (Kumar et al., 2011) and to encourage the development of pollutant degrading microbial population. Moisture is usually established and aeration provided by tilling. Hence, composting is technically the conversion of wastes into useful organic soil amendment to crops. Three designs are recognized in composite technology: windrow method, static pile method and mechanically agitated in-vessel method (Cristorean et al., 2016). The windrow method is the most effective and it is characterized as long, narrow, low piles that are periodically mixed with mobile (or agrotechnical) equipment for aeration and porosity. In a static pile, forced aeration system, made up of perforated pipes, provides the aeration (Antízar-Ladislao, Lopez-Real, & Beck, 2006). The mechanically agitated in-vessel method combines the first two methods (Cristorean et al., 2016). Composting is less controlled and subject to the changes of the natural environmental phenomena, such as rainfall and temperature fluctuations (Thapa, Kc, & Ghimire, 2012). Composting has been applied successfully in the removal of PAHs with the use of amendment agents like soot waste, spent mushroom waste, alfalfa and maple leaves (Haderlein, Legros, & Ramsay, 2006).

1.15. Slurry Bioreactors

Slurry bioreactor (Figure 5) requires the mixing of the contaminated soil with water to create a slurry of predetermined consistency (Balseiro-Romero et al., 2019). The slurry requires aeration through an effective mixing device to effectively aid aerobic biodegradation of the contaminants. The vessel or tank in which this bioremediation takes place is called a bioreactor. However, the process can also take place in a lagoon (Rockne & Reddy, 2003). Operationally, polluted soil is excavated, screened to remove large particles and debris, the specified weight of the polluted soil is mixed with a specified volume of water (to yield a predetermined percentage weight per volume), nutrient and competent microorganisms (Juwarkar et al., 2014). The slurry pH may be adjusted and treatment is carried out until the desired level of decontamination is achieved. Mixing and aeration (provided by compressors or air spargers) are requirements if the effective result is anticipated with simultaneous adjustment of nutrient, pH, consistency and temperature and maintained at levels suitable for optimal growth of the degraders and their catabolic activities.

Although the aerobic condition is more common, Tomei and Daugulis (2013) noted that the system can also be operated anaerobically and in different feed modes such as continuous, semi-continuous and batch operations. The batch operation is the commonest mode for soils and sediments treatment. Sequencing batch reactors (a modified form of the batch operation) is characterized with a feed phase (addition of water and nutrient to soil), reaction phase (mixing and aeration until the desired level of contamination is achieved), setting and extraction of the supernatant plus the decontaminated soil ready to be disposed of. The treated and dewatered soil is backfill into the excavation. The wastewater is treated and disposed of or recycled and the second volume of soil is treated (Chowdhury, Bala, & Dhauria, 2012). Balseiro-Romero et al. (2019) modelling experiment using a slurry bioreactor recorded 90% diesel removal in 22 days.

1.16. Advantages of Ex-Situ Remediation

1. Treatment process is subject to control and predictability.
2. Kinetics of biodegradation can possibly be increased.
3. Pre-requisite pretreatment is possible to enhance process efficiency.
4. Operating factors can be optimized for efficient result.
5. It can be done on site to eliminate cost of transportation and reduce contamination of healthy soil.

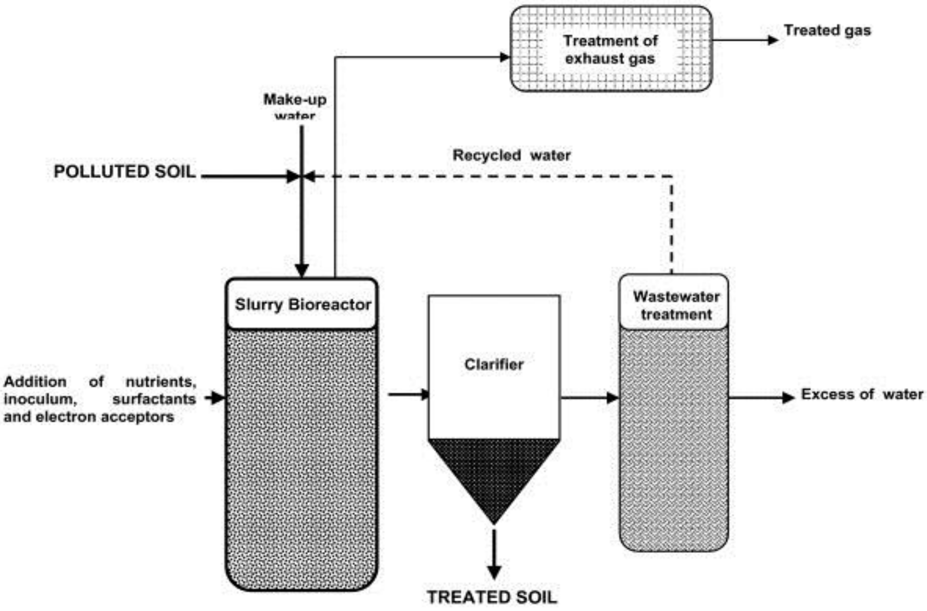

Figure-5. Slurry bioreactor.
Source: Robles-González, Fava, and Poggi-Varaldo (2008).

1.17. Limitation of Ex-Situ Remediation

1. *Ex situ* remediation is associated with extra cost (due to transportation cost).
2. It increases risk exposure to environmental receptors due to possible dispersal of contamination during excavation and transportation.

Requires technological equipment to drive the process to required decontamination level.

1.17.1. Requirement for Bioremediation Application

Bioremediation in its right is a technology that takes advantage of the mechanism of the science of microbial degradation to achieve its prime goal of restoring impacted site. Bioremediation as a technology is unique, not because it is a relatively new concept but that it is a technology created from judicious exchange of skills from various professional disciplines such as microbiologist, ecologist, genetic engineer, environmental scientist and chemical engineer. Despite the involvement of multi-disciplinary professionals in the technology, there are still some limitations associated with bioremediation as reflected in Table 4.

Table-3. Features of ex-situ technology used in remediating soil

	Land farming	Composting	Biopile	Slurry reactor
Applicability	TPH, phenolic compounds, PAHs, lubricating oils	BTEX, diesel, PAHs, polychlorinated biphenyl, chlorobenzene	Petroleum hydrocarbons of various kinds, pesticides, halogenated VOCs	Aromatic hydrocarbons, explosives, PAHs,
Optimum parameter	C-N ratio: 9:1 Temp.: 20 – 40 ℃ O_2: 10 – 15%, pH: 6.5 – 7.5 Moisture: 12-30% by weight	C-N ratio: 25:1 – 35:1 Temp.: 54 – 65 ℃ O_2: 10 – 15%, pH: 6 – 9 Moisture: 50-55% Porosity: 1 – 5 cm	Relies mostly on the prevailing environmental condition	Relies mostly on the prevailing environmental condition, the contaminated soil and the indigenous microbes
Efficiency	Up to 90%	Up to 95%	>90%	>90%
Limiting factor	Large treatment area	Kinetics of degradation	Cost	Cost
Treatment time	3 – 24 months	2 -12 months	Weeks – few months	Weeks
Costs	\$75 per ton	\$33 per ton	\$90 per ton	\$280 per ton
Advantages	Simplicity and operability	Simple and sustainable	Smaller treatment area	Suitable for clay and rapid
Disadvantages	Secondary pollution (air)	Slower and less efficient	Complex technology	Pretreatment and technology cost

Source: Cristorean et al. (2016)

From the information provided in Table 4, it becomes obvious that bioremediation implementation requires not less than three fundamental objectives:

1. To enhance the extent and rate of biodegradation of the target contaminant.
2. To develop competent microorganisms that can survive the toxicity of the pollutant(s).
3. To take advantage of the plasticity of microorganisms such that no toxic product is produced.

To proffer solutions to these fundamental objectives outlined above demand site characterization, optimization of biotreatment techniques and monitoring, all of which demand practical implementation of bioremediation either at the bench scale, pilot scale or full scale. Full-scale bioremediation usually stems from laboratory treatability studies. Laboratory treatability studies provide information to address site-specific questions, which among other things include:

1. Will the pollutants biodegrade under prevailing site conditions?
2. Will amendments such as nutrients, electron donor, bioaugmentation and pH adjustment increase extent and rate of biodegradation?
3. Will the contaminant degrade better under anaerobic or aerobic conditions?
4. What are the biodegradation intermediates and end-products?
5. What are the biodegradation synergies/inflators and lots more

Table-4. Disadvantages and limitations of bioremediation.

Advantages	Limitation
Bioremediation is ecofriendly and has gained/gaining public acceptance	Bioremediation takes a long time to achieve the expected end.
Less toxic or innocuous by-products (CO_2, H_2O and biomass) are generated	Bioremediation is limited to biologically degradable compounds
Eliminates future/long-term liability, since hazardous chemicals are transformed or degraded to harmless products.	Bioremediation is sensitive to site-specific factor(s), thus can be affected adversely in an unfavourable environmental condition(s)
Bioremediation are implemented with minimal site disruption and reduces to the barest minimum trans-contamination.	At times by-products produced are more hazardous than the compound(s) of focus
Bioremediation is cost-effective compared physicochemical processes.	Bioremediation treatment has no regulatory acceptable end-points
It is coupled with other treatment technologies-treatment train	Laboratory treatability studies are expensive and result from such bench studies are difficult to replicate in full-scale field operations.

Source: Iosob, Prisecaru, Stoica, Călin, and Cristea (2016).

1.18. Strategies that Improve Bioremediation Performance

One unique feature of bioremediation is that site factors can be manipulated and controlled (Jam et al., 2011). If the control of a particular factor or factors can boost biological activity and degradation of the pollutant of interest, then bioremediation performance is improved. Where such performance reaches its peak then we can speak of optimized state (Scherr, Hasinger, Mayer, & Loibner, 2009). Various studies carried out have shown that calculated control of environmental factors, stimulation of indigenous populations, the introduction of competent microbes, manipulating the availability of the contaminant to the degraders and enzyme application at critical condition all have the effect of improving bioremediation process (Agarry & Oghenejoboh, 2015; Hung, Cam, & Dung, 2013; Rocchetti, Beolchini, Ciani, & Anno, 2011). A brief discussion on each of these controllable factors is considered below.

1.19. Optimizing Abiotic Factors

Temperature, pH value, moisture, nutrients, oxygen and redox potential influences bioremediation (Sharma, 2012) and when optimized, yield best bioremediation performance. Table 5 shows the optimal conditions of these abiotic factors.

1.20. Biostimulation

This involves the addition of limiting nutrients (like nitrogen and phosphorus), electron acceptor, (especially oxygen), easily biodegradable substrates and/or pH modifiers to provide the indigenous microorganisms favourable biochemical environment to break down the contaminant much faster. Biostimulation is driven by nutrients and oxygen (Xu, 2012). Nitrogen and phosphorus are commonly provided by three strategies (Xu & Obbard, 2003) of application: addition of soluble mineral nutrients (N.P.K 20:10:10), the addition of oleophilic fertilizer (Inipol EAP.22) and/or through the addition of slow-release inorganic fertilizer (OsmocoteTM). The slow-release inorganic fertilizers have the advantage of been controlled, durable, constantly supply nutrient, and it is cheap (Gertler, Gerdts, Timmis, & Golyshin, 2009). Ureaform is a modified slow-release of organic fertilizer (Agamuthu, Tan, & Fauziah, 2013; Xu & Obbard, 2003). Concerning soil, oxygen can be supplied in different ways: through wells (can also transmit nutrients) in subsurface soils, and moderate tilling. Also, oxygen releasing compounds such as calcium peroxides: $CaO_2$and Ca_2O_4 (Wang et al., 2011).

1.21. Bioaugmentation

Bioaugmentation is a practice of inoculating or seeding the impacted site with competent and acclimatized microorganisms to carry out biodegradation. This is usually the case where indigenous microflora is so few and un-acclimatized. Studies have shown that seeded microbes are mostly outcompeted by the autochthonous microflora (Nwinyi, Picardal, An, & Amund, 2013) for nutrients and substrates. Also, foreign microbes prey on already existing protozoa and bacteriophages. In such circumstance, the only survival strategy for allochthonous microbes to survive competitively in the presence of indigenous microflora is to be encapsulated or applied through biofilm or microbial mats (Wang et al., 2011). Another strategy is to seed into the impacted medium genome modified organisms.

Table-5. Abiotic factors that influence bioremediation.

Environmental Factor	**The condition required for microbial activity**	**Optimum values**
Moisture (soil)	25-30 % of water holding capacity	25-85% water holding capacity
Nutrients	Nitrogen and phosphorus for microbial growth	Molar ratio of C:N:P = 120:10:1
pH	5.5-8.5	6.5 -8.0
Temperature	15-45°C	20-30°C (mesophilic microbes)
Redox potential		Eh > 50 millivolts (aerobes) Eh < 50 millivolts (anaerobes)
Contaminants	Not too toxic	Hydrocarbons (5-10%) of dry weight of soil

Source: U.S. Environmental Protection Agency (USEPA) (1993).

1.22. Bioavailability/Bioaccessiblity

High molecular weight (HNW) hydrocarbons, due to their low octanol-water partition coefficient ratio constant, sorb to soil particles thus remain unavailable for the microflora to degrade the contaminants. Some microbes, bacteria and fungi inclusive, produce biosurfactants that solubilize the HMW hydrocarbons thus making them available for biodegradation (Hung et al., 2013). Certain organic solvents are added to hydrocarbon polluted soil which increases the rate of transfer of oleophilic substances in soil (Ossai, Ahmed, Hassan, & Hamid, 2020) For instance, 2-propanol solvent has been investigated to have a positive result in PAH degradation in soils (Ravanipour et al., 2015). Scherr et al. (2009) use canola oil in their bioaccessibility study on PAHs in a historically contaminated soil.

1.23. Enzymatic Remediation

Enzymatic remediation is an active bioremediation approach which uses extracellular enzymes (Peoxoto, Vermelho, & Rosado, 2011). This unique method possesses the potential to be less expensive, less invasive and much eco-friendlier when compared to physical and chemical remediation options (Ruggaber & Talley, 2006). Extracellular enzymes according to Bhargava, Wenger, and Marten (2003) refer to catalytic proteins that are either secreted by microorganisms or ones that enter the aqueous phase during a process of fermentation. Some of the enzymes have a specific function such as reducing the activation energy to break intermolecular bond while some have a wide range of specificity against substrates. Enzymes with a wide range of target comounds are the most required and useful for bioremediation (Gianfreda & Rao, 2004). In most cases, more than one enhancement strategy is employed to have a good result.

2. CONCLUSION

Different bioremediation techniques are applied in an impacted environment to remediate environments back to their original healthy state. The detoxification and elimination process is usually done by different taxa of microorganisms either alien or indigenous to the affected habitat. Bacteria, fungi, algae are the common bioresource used in achieving remediation. However, bacteria is the most outstanding because it is adaptable to environmental conditions inauspicious to other microorganisms, including fungi and algae. In this sense, bacteria can be relied upon as the most valuable bio-tool in addressing environmental pollution. Using bacteria in bioremediation techniques and processes would achieve greater success in the future with less constrains.

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