An Overview of Bioremediation Process – Mechanisms and Factors Influencing It

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Abstract

With the emerging of several industries, environmental pollution has been on the rise. These pollutants could include heavy metals, nuclear wastes, pesticides, green house gases, agrowastes and hydrocarbons. This called for expedite action by using greener technology which will not unnecessarily add more toxins to the soil. Remediation of polluted sites using microbial process (bioremediation) has proven effective and reliable due to its eco-friendly features. Bioremediation can either be carried out ex situ or in situ, depending on several factors, which include but not limited to cost, site characteristics, type and concentration of pollutants. Generally, ex situ techniques apparently are more expensive compared to in situ techniques as a result of additional cost attributable to excavation. However, cost of on-site installation of equipment, and inability to effectively visualize and control the subsurface of polluted sites are of major concerns when carrying out in situ bioremediation. This paper presented a general overview of bioremediation process especially with respect to its mechanisms and factors influencing it. Several research works on the different types of bioremediation process were discussed. The mechanisms involved in bioremediation process was vividly described by giving in-depth explanation of heavy metals uptake by (microbes, plants and yeast) accompanied with cited works. Additionally, the degradation mechanism for hydrocarbons was highlighted and the different pathways (aerobically and anaerobically) were explained using equations and diagrams. The factors affecting the bioremediation process were outlined and lastly, the advantages and disadvantages of bioremediation process were enumerated.

Keywords: Bioremediation process, Bioremediation mechanisms, Bioavailability, Aerobic degradation, Anaerobic degradation

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INTRODUCTION

In man's quest for a better life in the 21st century, he has ventured on a large scale into activities in agriculture, manufacturing, mineral exploration and exploitation, construction etc. These activities have led to the production of various items which include but are not limited to: clothings and textiles, fuel, water, automobiles, household appliances, processed foods and beverages, etc. These things now seem to be necessities, and meeting these acquired needs had become a major thrust of modern industrial society. These acquired needs are usually met by items that must be processed or manufactured or refined. Since most of the activities in these areas are done mostly on land or have a bearing with the soil, the production, distribution and use of such items usually results in more complex residuals and/or wastes, many of which are not compatible with or readily assimilated by the soil or environment as a whole thereby interfering with nature's ecological balance (Bejarano and Michel, 2010). For example, in the drilling and production of crude oil, accidental crude oil spills from oil pipeline and tank leakage affect the soil's fertility adversely (Kulkarni et al. 2012; Wang et al. 2013), in agriculture, the use of pesticides may leave behind high concentration of toxic chemicals in the soil that may deplete its fertility (Riah et al. 2014); and in the processing of agricultural products, waste water is generated; and when released into the soil is potent enough to contaminate it (Slyvester and Ayobami, 2017). Some of the soil contaminants generated from these activities include but are not limited to: heavy metals, polycyclic aromatic hydrocarbons (PAH), cyanides, and radioactive material (Amodu, 2015; Deary et al., 2016; Huertas et al., 2010; Luque-Almagro et al., 2016). Since man intensifies efforts in agricultural, manufacturing and mineral exploitation activities, then it is safe to say that the generation of soil pollutants would continue to increase. Often the first response is removal of some of the contaminated soil (Okoh, 2006). Even after soil removal, it is possible that some portion of the soil contaminant will remain. To combat the residuals left in the soil after removal, remediation procedures are used. One remediation measure is bioremediation. Bioremediation by definition is the 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. The key players in bioremediation are bacteria-microscopic organisms that live virtually everywhere. Bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, biostimulation, and bioaugmentation (Agarry and Oghenejoboh (2015). Bioremediation offers numerous advantages some of which include: allowing wastes to be recycled and it does not make use of chemicals that would further harm the environment (Azubuike et al., 2016). As a result of this, many scholarly papers have been published in both local and international journals that address the subject of soil bioremediation. Most of these publications give qualitative and quantitative studies of what has been done in bioremediating contaminated soils. However, due to the elaborate nature of the studies that have been presented by diverse researchers, there is need to assess all the studies and the research findings in one piece so as to isolate what has been achieved and identify possible areas of interest where more studies should be directed. To achieve this, this paper made a chronicle of the available studies on soil bioremediation. The various research findings as presented by diverse authors are

highlighted and the future research needs on the subject are presented. This work would follow this sequence: First, the classes of bioremediation would be highlighted, the types of bioremediation would follow, then mechanism of bioremediation, factors that assist bioremediation to be effective, advantages and disadvantages of bioremediation. Thereafter, the conclusions and future perspectives would climax the study.

1. Classes of bioremediation

There are two classes of bioremediation used. Don't confuse the class type with the actual types of bioremediation available, the classes describe the general application of the organisms. The two classes are:

(a) In-situ – In situ refers to when contaminated waste is treated right at its point of origin. For example, there may be soil that is contaminated. Rather than remove the soil from its point of origin, it is treated right where it is. The benefit of in-situ treatment is that it prevents the spread of contamination during the displacement and transport of the contaminated material.

(b) **Ex-situ** – Ex-situ refers to treatment that occurs after the contaminated waste has been removed to a treatment area. To use soil as the example again, the soil may be removed and transported to an area where the bioremediation may be applied. The main advantage to this is it helps to contain and control the bioremediation products, as well as making the area that was contaminated available for use.

2. Types of bioremediation

The following are types of bioremediation that are commonly used.

(i) . **Phytoremediation**

The term 'phytoremediation' consists of the Greek prefix phyto that connotes plant and the suffix, remediation which is obtained from the Latin word remedium which means to correct, restore or remove an evil (Erakhrumen and Agbontalor, 2007). In order words, phytoremediation simply refers to using of plants to remove contaminants from either water or soil. This technology makes use of naturally occurring or genetically engineered plants to clean polluted sites (Flathman and Lanza, 1998). The plants are capable of drawing and holding contaminants in their structures, which leads to effective removal of them from soil or water. There are several uptake mechanisms employed by phytoremediation technology depending on the kind of pollutants (elemental or organic). Elemental pollutants such as harmful radionuclides and heavy metals use mechanisms such as phytoextraction, phytovolatilization, phytostabilization, and rhizofiltration (Tangahu et al., 2011). While organic pollutants are removed by phytostabilization, rhizofiltration, phytodegradation, phytovolatilization (Kuiper et al., 2004). In choosing a plant to use for the remediation process, the following factors are to be considered: the root system (taproot or fibrous root) which is based on the depth of the pollutant, the toxicity of the pollutant, the plant adapting to different climates or environmental conditions, its resistance to pests and diseases, the growth rate of the plant and the time needed to reach the required level of remediation (Lee, 2013). The

mechanism used by plants to draw away contaminants from the soil are: pollutants/heavy metals uptake by plants, translocation of it from roots to shoots by xylem flow, and its accumulation in the shoots of the plants (Miguel et al., 2013). The plants capability to restore the polluted soil can be enhanced by using plant rhizobacteria that can either be endogenous or exogenous (bioaugmentation) or biostimulation.

Reports showed that the use of plant growth promoting rhizobacteria (PGPR) has helped in increasing the biomass production as well as plants tolerance to toxic pollutants as well as heavy metals (Yancheshmeh et al., 2011; de-Bashan et al., 2012). Grobelak et al. (2015) reported rapid growth in plant height, root and stem on inoculating exogenous PGPR with Brassica napus L. subsp. Napus and Festuca ovinia L had a rapid rate of removal of total petroleum hydrocarbon (TPH) in the rhizosphere of non-inoculated Sesbania cannabina soil (Maqbool et al., 2012). Likewise, Mesa et al., (2015) used Spartina maritima and endogenous rhizobacteria to remediate estuaries contaminated with metals and they observed a high rate of metal removal and accumulation as well as larger plant biomass. Zea mays and Brachiaria mutica have also been proven to be good phytoremediators of soils polluted with heavy metals (Ijaz et al., 2015; Tiecher et al., 2016). A number of other plants with pytoremediation potentials have been listed (Tangahu et al., 2011; Omovbude and Udensi, 2016; Yavari et al., 2015; Ali et al., 2013). The advantages of phytoremediation are: environmentally friendly, low cost, could be used for a variety of pollutants, installing as well as maintaining of equipment at low price, preserving soil structure, improving soil fertility and preventing metal erosion and leaching (Mench et al., 2009; Tangahu et al., 2011; Ali et al., 2013). Its limitations include: longer remediation time, soil chemistry, the toxicity of the contaminant as a result of its level in the soil, root depth and slow growth rate of the plant. Also, there is every possibility of the toxic compounds to be transferred along food chain (Azubuike et al., 2016; Vangronsveld et al., 2009).

(ii) **Bioventing**

Bioventing is a technique that requires a considerate amount of air to be blown into the soil so as to increase the rate of oxygen in the contaminant. This method is suitable especially for chemicals/metals that may be sensitive to oxygen. It is a technique used to degrade any degradable contaminant that is aerobic in nature (Rockne and Reddy, 2003). This process uses nutrients and water to improve bioremediation with the sole aim of transforming pollutants to a less harmful form by means of microbial activities (Philip and Atlas, 2005). The texture of the soil will determine the amount of oxygen and nutrients that should be distributed in the soil. Bioventing also involves the pumping of air into contaminated soil above the water through well which sucked the air. For bioventing to be successful, the water table must be deep enough from the surface and also the area having high temperature (Garima and Singh, 2014). This technique suitable especially for light spilled hydrocarbons like gasoline, oil, petroleum and so on (Hohener and Ponsin, 2014; Eyvazi and Zytner, 2010). Sui and Li (2011) carried out a modelling work that explains how the rate of air injection can affect biodegradation, volatilization and biotransformation of toluenecontaminated site by bioventing. Based on their results, they concluded that the higher the rate of airflow the lower the biodegradation rate and the lesser the effectiveness of pollutant biotransformation, but with lower rates of airflow the higher the biodegradation rate. Thus, it shows that in bioventing, a key parameter though not the least to consider in the dispersal of contaminants, redistribution and its surface removal is the air injection rate (Azubuike et al., 2016). Bioventing was used to amend soil contaminated with phenanthrene and after 7 months over 93% of the contaminant was removed (Frutos, 2010). Thome et al., 2014 also applied bioventing technique in treating of a diesel-polluted clayey soil, and it was noticed that the intensity and interval of airflow had no drastic effect in removing diesel from the clayey soil. Thus, whether the time interval for air to be injected through the soil is high or low, has less effect on the cost. Rayner et al., (2007) also observed that using a one-well bioventing system was ineffective in a sub-Antarctic hydrocarbon-polluted soil. This is caused by the soil cover being thin and the water table being shallow. However, on using nine smaller injection rods of 0.5 m apart, under the same conditions, a large amount of hydrocarbons were removed due to increased biodegradation rate of the hydrocarbons that is caused by an even distribution of air in the soil. Conclusively, airflow rates, air intervals and points of air injection all contribute to the success of bioventing-based bioremediation. Apart from pure oxygen or air being injected, a mixture of nitrogen, hydrogen and low carbon dioxide concentrations could also be injected to help reduce chlorinated contaminant, in which hydrogen donates electron (Mihopoulos et al., 2000, 2002; Shah et al., 2001). Soil vapour extraction (SVE) can be applied in place of bioventing especially in the volatilization of volatile organic compounds by means of vapour extraction (Magalhaes et al., 2009). This is because SVE has more air flow rate than bioventing (Baker and Moore, 2000). Even though both

processes use the same hardware, their mode of operation, configuration and design differs (Diele *et al.*, 2002). The method of remediation used by SVE is a physical one because of its mechanism of removing pollutant, but both techniques have their mechanism of removing pollutant to be not mutually exclusive.

(iii) Land farming

Land farming involves excavating and/or tilling a contaminated soil. This increases the aeration of the soil thereby removing contaminants from the soil. It could also mean intentionally reducing nutrients of nitrogen in a soil so that nitrogeneous organisms can be removed. It is cheap to operate and requires less equipment and as such it is one of the simplest bioremediation techniques. Land farming could be employed either as in situ or as ex situ bioremediation depending on the depth of the pollutant. If the polluted soil is excavated and treated on site, it is called in situ, otherwise it is ex situ. Excavation is required if a pollutant is 1 m below ground surface, but if the pollutant exceeds 1.7 m, it will be transported to another treatment site (Nikolopoulo et al., 2013). Excavation and tillage of the polluted soil helps enhancing bioremediation during land farming since excavation allows aerobic biodegradation of the contaminants by microorganisms while tillage encourages aeration and adding of nutrients such as nitrogen, phosphorus and potassium (Philip and Atlas, 2005; Paudyn et al., 2008; Volpe et al., 2012; Silva-Castro et al., 2015). Land farming is commonly used to remediate soils contaminated with polyaromatic hydrocarbons (PAH) and also petroleum sludge (Bambang et al., 2010; Silva-Castro et al., 2012; Cerqueira et al., 2014). Using impermeable liner in land farming design helps prevent the washing away of the contaminants into nearby lands (da Silva et al., 2012).

Some of the advantages of using land farming as a bioremediation technique include – its compliance to governmental regulations, adaptable to any climate and location, very easy to design and implement, is not capital intensive and may be used in amending large volume of polluted soil with less impact on the environment and requires less energy to operate (Azubuike *et al.*, 2016; Besaltatpour *et al.*, 2011). Just like for other bioremediation techniques, land farming has its own limitations which are requiring large operating space, low microbial actions due to poor environmental conditions, extra cost from excavating the soil and less efficient in removing inorganic pollutant. It is also not suitable for amending soils contaminated with toxic volatiles because its design and mechanism of removing the pollutants (volatilization) is not favourable especially in hot climate areas (Maila and Colete, 2004). For these limitations and many others, land farming based bioremediation takes a lot of time and is less productive when compared to other bioremediation techniques.

(iv) **Bioreactor**

Bioreactor is a container specially designed to hold the waste while bioremediation is occurring. It could also refer to a vessel that contains raw materials that are converted to specific product(s) by means of series of biological reactions. Various types of bioreactors which exist are: batch, fedbatch, continuous and multistage reactors. To select the type or mode of bioreactor to be used, its cost must be put into consideration. The bioreactor is fed with contaminants which may either be in dry form or slurry form. Bioreactor is advantageous in that bioprocess parameters such as temperature, rates of agitation and aeration, pH, substrate and inoculum concentrations can be successfully monitored or controlled. Also, conditions in a bioreactor are such that mimicks that of the natural environment so as to achieve enhanced efficiency of contaminant removal and better growth conditions. Bioremediation time can effectively be reduced by manipulating process parameters. Controlled bioaugmentation, addition of nutrients, increased bioavailability of contaminants and improved mass transfer between pollutants and microorganisms makes bioreactor-based bioremediation process more successful. Different bioreactors were used to treat a variety of pollutants be it in the soil or water. For instance, Jua'rez-Rami'rez et al. (2015) used a packed-bed reactor (PBR) to remove a mixture of sulfonated amines; a roller slurry bioreactor was used to remove 2, 4-Dichlorophenoxyacetic acid (Mustafa et al., 2015); stir tank bioreactor for removing total petroleum and polyaromatic hydrocarbons (Chikere et al., 2016). The design of bioreactors is flexible enough to accommodate high biodegradation rate and low abiotic losses (Mohan et al., 2004). It accepts different biostimulating materials or bioaugmenting agent even sewage sludge. Bioaugmentation can successfully be done in a bioreactor especially when using genetically modified microorganism (GEM). This is made possible because the bioreactor is in an enclosed vessel and as such GEM organism might die even before the amended soils are returned for landfilling thereby ensuring no foreign gene is permitted to volatilize into an environment after bioremediation. Since bioreactors can be operated in batches or for a long time, this allows for monitoring of changes in the microbial population and easily characterizing the microbes (Zangi-Kotler et al., 2015).

Although, bioreactor-based bioremediation have proved successful in the aforementioned ways, it also has its lapses. It is time consuming in that it will require a lot of approaches that will be able to control different operating parameters for effective operating conditions. The process is capital intensive due to large volume of polluted soil or substances to be treated which will in turn call for more manpower (Philip and Atlas, 2005). Additionally, pollutants respond differently to different bioreactors, that is to say that pollutant is sensitive to the type of bioreactor used. Lastly, since various parameters of a bioreactor are considered, any of it which is incorrectly monitored and measured can reduce the microbial activity and hence the effectiveness of bioreactor-based bioremediation.

(v) **Bioaugmentation**

Bioaugmentation is the introduction of microbes isolated from the polluted site, from a site that is historic or carefully chosen and genetically engineered to enhance the remediation of sites polluted with petroleum products. This is usually done especially if the microorganisms that are native to the affected area cannot easily degrade hydrocarbons (Adams *et al.*, 2015). Ueno *et al.* (2007) further describes autochthonous bioaugmentation as one which uses indigenous microorganisms to the site (be it soil or water). Some researchers suggested that bioaugmentation becomes a better option when biostimulation and bioattenuation have not yielded better results (El Fantroussi and Agathos, 2005). Frosyth *et al.* (1995) stated that bioaugmentation is useful for soils having low amount of contaminant degrading microorganisms, for small-scale land on which bioaugmentation is cheaper when compared to other non-biological methods and for soils polluted with toxic compounds which are persistent and have stayed a long time in the soil. However, bioaugmentation have been employed for amending soils contaminated with toxic radionuclides and heavy metals (Je'ze'quel and Lebeau 2008; Gavrilescu *et al.*, 2009).

Bioaugmentation can be achieved in any of these three ways: (i) the enhancing and/or isolating the indigenous microbes from the polluted area, and later culturing and reinoculation; (ii) isolates or enrichments are not inoculated to the source of the original culture; and (iii) with the use of modified or carefully-mutated microorganisms. Isolated single strains or enriched cultures, which can be obtained "before" or "after" the contamination of the target sites, are inoculated into the sites to be treated.

Bioaugmentation can yield positive results if the following are considered: using of microbial strains that have been well adapted to the site to be treated, fast rate of growth of the microbial consortia, resistance to high contaminant concentrations, easy to culture and its ability to thrive in different environmental conditions (Mrozik and Piotrowska-Seget, 2009). Also the increase of microorganisms used for bioaugmentation depends a lot on the chemical structure and concentration of the contaminants, the availability of the pollutant to the microbes, the amount and nature of the microbial population and the physical environment and these should all be incorporated when screening microorganisms to be used in bioaugmentation process.

Most researchers have used microbes belonging to genus *Aspergillus* (Ezeigbo *et al.*, 2014; dos Santos *et al.*, 2008), *Bacillus* (Silva *et al.*, 2009b; Nwokoro and Uju Dibua, 2014), *Pseudomonas* (Heinaru *et al.*, 2005; Sinha *et al.*, 2011; Nwokoro and Uju Dibua, 2014), *Mycobacterium* (Jacques

et al., 2008), Penicillium (Mancera-Lopez *et al.*, 2008), Flavobacterium (Jogdand, 1995) and *Mucor* (Szewczyk and Długonski, 2009). Sometimes the biodegradation of soil contaminated with hydrocarbon can be carried out using single strains of microorganisms (Niu *et al.*, 2009; Dams *et al.*, 2007) or the use of mixed culture (microbial consortia) of many hydrocarbon-degrading microorganisms (Ghazali *et al.*, 2004; Yu *et al.*, 2005). A consortium of microorganisms has proved more effective than using the single strains due to the fact that one strain can have multiple catabolic pathways which can undergo further degradation by other strains having suitable catabolic pathway (Heinaru *et al.*, 2005).

Another way bioaugmentation process can be employed is by the use of genetically engineered microbes (GEMs) to enhance the ability of the modified strains to degrade and withstand toxic compounds like xenobiotics than when using indigenous strains (Mrozik and Piotrowska-Seget, 2009). This method focuses on improving the biodegradating ability of the microbes by transferring packaged catabolic genes from one or more strains (donor) to indigenous microflora existing in polluted sites. These catabolic pathways are situated on plasmids like transpozons, TOL/pWW0, TOL/pWW53, TOL/pDK1, BPH/ pWW110, NAH/NAH7 and PHE/pVI150, and other mobile or integrative elements (Jussila et al., 2007; Top et al., 2002). These catabolic pathways (plasmid - based) are beneficial because they are flexible systems and are easily removed from one bacteria species to another or its genera (Sayler and Ripp, 2000). Another advantage towards this method is that it has other mechanism (like transformation and transduction) which plays a vital role in assisting microorganisms to adapt to stressed or unfavourable conditions. Experimental findings have proved the use of GEMs to be effective. For example, Filonov et al., (2005) used a plasmid bacteria strain of Pseudomonas putida KT2442 to degrade naphthalene in a soil contaminated with naphthalene. On introducing the strain KT2442 20 days later, the bacterial count increased from 10^5 to 6 x 10^6 CFU/g of soil which is about 90% of the total population of naphthalene degraders. Others also investigated the efficiency of using genetically cloned or engineered strains and their results showed that it was more effective to use these engineered strains in removing contaminants from the soil than the wild strains (Massa et al., 2009; Mishra et al., 2004; Rodrigues et al., 2006)

(vi) **Biostimulation**

Biostimulation is the adding of nutrients, oxygen, or other electron donors and acceptors to the contaminated soil in order to increase the microbial activity or population (Perfumo *et al.*, 2007). It is also defined as a remediation process that occurs naturally such that the degradation of pollutants is improved by maximizing conditions such as aeration, nutrient addition, changes in pH and temperature (Margesin and Schinner, 2001). Thus, biostimulation entails the environment of the soil being altered so that indigenous bacteria capable of bioremediation can be stimulated (Adams *et al.*, 2015). To achieve this, different types of limiting nutrients and electron acceptors like phosphorus, carbon, nitrogen or oxygen (in the form of molasses) are added. The essence of this is to provide bacterial communities with a favourable environment, in which they can effectively degrade nutrients (Mohan *et al.*, 2006; Ueno *et al.*, 2007). Biostimulation under aerobic conditions requires the supply of oxygen to the soil to stimulate microbial activity and increase biodegradation rates (Zawierucha and Malina, 2011). Oxygen is commonly introduced to the soil

either by tilling, forced aeration, or chemical methods (Atlas, 1991; Brown and Crosbie, 1994; Riser-Roberts, 1998). Tilling is usually employed during land farming but can only be effective when it involves top soils; whereas forced aeration techniques include injection of aerated water, pure oxygen (air) which is usually used for improving the biodegradation in soils and groundwater contaminated with petroleum hydrocarbons (Brown and Crosbie, 1994). The use of chemicals such as hydrogen peroxide (H₂O₂), potassium permanganate (KMnO₄) and ozone can serve as an alternative source of oxygen (Riser-Roberts, 1998). Several researchers have employed each of the aforementioned methods: aerated water (Malina, 2007); H₂O₂ (Olexsey and Parker, 2006) and there results showed that the biodegradation rate of hydrocarbons in soil was enhanced. However, reports also had it that H₂O₂ for instance was toxic to microbes and dosage of it (100-200 mg/l) can inhibit bacteria metabolism (Riser-Roberts, 1998). Increasing the aerated water into the soil had resulted in the soil pores to be filled with it thus, limiting the transfer of oxygen and reducing the rate of contaminants biodegradation (Ramirez et al., 2009). Biostimulation can also be employed by adding nutrients be it in organic form (e.g cow dung, poultry droppings, agrowastes, etc) (Agarry et al., 2013; Aghalibe et al., 2017; Agamuthu et al., 2013) or inorganic form like NPK fertilizers, nutrient ammendments [(NH₄)₂SO₄ and K₂HPO₄] (Agarry et al., 2013; Agarry & Oghenejoboh, 2014; Adeyemo, 2013). Increasing the C: N: P ratio has enhanced the biodegradation of petroleum hydrocarbons (Zawierucha et al., 2008). However, the effectiveness of the biostimulation process depends on the C: N: P ratio and the type of soil as was observed in the works of (Aspray et al., 2008). Surfactants can also be used to enhance biostimulation. It may be natural or synthetic in form (Lai et al., 2009). Hydrocarbon-degrading microorganisms produce a variety of surface-active natural agents called biosurfactants, which enhances the bioavailability of the contaminants. However, synthetic surfactants are used especially when the contaminants are hydrophobic and/or firmly sorbed in clay particles or soil organic matter (Menendez-Vega et al., 2007). Surfactants have both hydrophobic and hydrophilic features which help in reducing the tension between the interfaces of hydrocarbons and soil water and hence increasing the water solubility of the hydrophobic contaminants. In other words, surfactants increase the bioavailability of hydrocarbons to microbes and hence increasing their biodegradation. Whang et al. (2009) investigated the effect of biosurfactants such as surfactin (SF) and rhamnolipid (RL) to enhance the biodegradation of diesel and yielded positive results. Lai et al (2009) compared the effectiveness of biosurfactants and that of synthetic surfactants in heavily-polluted soil. Further research works have testified to the effectiveness of surfactants in enhancing the microbial activity and biodegradation of hydrocarbons (Bento et al., 2005; Xie, 2003). Their result showed that the removal efficiency of TPH was higher in biosurfactants than in synthetic ones. Biosurfactants are better than the synthetic ones because they are less toxic and have shorter persistence in the environment (Nievas et al., 2008). Another possible reason for their effectiveness over the synthetic ones is because of their unusual structural diversity that may give them their special properties and biodegradability (Mulligan, 2005).

The main advantage of biostimulation is that bioremediation will take place since the soil contains indigenous bacteria that are best suitable or adaptable to the environment. However, its challenges include not always working well due to the insufficient indigenous oil-degraders or very high concentration of contaminants (Ueno *et al.*, 2007), uneven distribution of additives due to the

subsurface of the soil being impervious (may due to tight clay or other fine-grained material) (Adams *et al.*, 2015).

3.0 Mechanisms of Bioremediation

a) Heavy metals uptake by microorganisms

Bioremediation by microorganisms usually undergo several processes like binding, oxidation, volatilization and immobilization or by chemical transformation of the pollutants (Malla *et al.*, 2018). Thus, this detoxification mechanism could involve mobilization, immobilization, biotransformation and bioaccumulation. Mobilization mechanism mainly uses bioleaching while immobilization mechanism is primarily by biosorption process, which encompasses other processes such as adsorption, precipitation, ion exchange and complexation (Bahafid *et al.*, 2017).

Microorganisms can bind with heavy metals by either adsorption or absorption. Heavy metal complexes are adsorbed on the cell surface of microbes after which they are absorbed into the cell. This is made possible due to the structure of the cell surface – i.e cell wall or membrane being permeable and having mucus layer. Ions in the cell surface functional groups such as nitrogen, phosphorus, oxygen, sulphur and phosphorus form complexes with metal ions. Additionally, anions such as phosphoric acid and carboxyl groups which are on the surface of the cell wall of microbes interact with cations of heavy metals (Jin *et al.*, 2018). Thus, metal ions can bind or pass through the cell membrane as depicted in **Figure 1**.



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Figure 1: Biosorption mechanisms of microorganisms. Source: Jin *et al.*, 2018.

Bioleaching also referred to as biomining involves heavy metals being transformed to soluble metals using microorganisms (Marcinčáková *et al.*, 2015). The metabolic reactions of microorganism yields secretions having organic acids of low molecular weight that can easily dissolve heavy metals and soil particles having heavy metals metal minerals (Jin *et al.*, 2018). Prokaryotic microbes participate in redox reactions and change the valency of heavy metals thereby changing their activity, which affects their mobility as well as toxicity (Marchenko *et al.*, 2015) (**Figure 2**).



Figure 2. Biomining mechanism of microbes. Source: Jin *et al.*, 2018.

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b) Heavy metals uptake by plants

Mechanisms used by plants to efficiently remove heavy metals involve the uptake, translocation, and storage of toxic elements in their tissues. Specialized proteins found in the cell plasma membrane of plants do serve as transport systems that help in metal ion uptake and translocation. They are: (i) proton pumps (___ ATPases that consume energy and generate electrochemical gradients); (ii) co- and antitransporters (proteins that use the electrochemical gradients generated by ___-ATPases to drive the active uptake of ions); and channels (proteins that facilitate the transport of ions into the cell). During this process, a range of ions are taken up (Tangahu *et al.*, 2011). A major problem is the interaction of ionic species during uptake of various heavy metal pollutants. After uptake of pollutants, translocation into shoots becomes easy since harvest of the root biomass is usually not possible. The transportation of metal ions from the roots to the shoots is not fully known. Generally, plants can only accumulate 10 to 15 ppm of most trace elements that they really need for metabolic reactions with the exceptions of "hyperaccumulator" plants which can take up toxic metal ions at levels in the thousands of ppm. Storage of toxic metal ions is in the vacuoles of plants via several mechanisms.

Water, evaporating from the leaves of plant, helps to pump nutrients and other soil substances into the roots of the plant. This process is referred to as evapotranspiration, and it accounts for the movement of contaminants into the plant shoots as well. The plants are harvested with contaminants already in their shoots, thus amending the soil.

Heavy metals uptake by plants follows any of these mechanisms - phytoextraction, phytostabilisation, rhizofiltration, and phytovolatilization as depicted in **Figure 3**. (Tangahu et al., 2011; Baah, 2011).

Phytoextraction- Phytoextraction is the absorption (uptake) and translocation of contaminants by plant roots into the shoots and leaves of the plant, after which the plant can be harvested, burnt and the metals are recovered from the ash (Tangahu *et al.*, 2011) Phytoextraction mechanism is best used for metals and other inorganic contaminants (Baah, 2011).

Phytostabilisation- This involves the use of special plants to reduce the mobility of contaminants in the soil and ground water via processes such as absorption and accumulation in the tissues of the plant, adsorption onto roots, or precipitation within the root zone thereby preventing the contaminants from migrating into the soil, or their moving into the soil by erosion and deflation (Erakhrumen and Agbontalor, 2007). It can be used to remove toxic metals or inorganic contaminants from the soil or sediments (Baah, 2011).

Rhizofiltration- Rhizofiltration involves the adsorption or precipitation of heavy metals/ contaminants onto the roots of plants or absorption into and sequesterization of contaminants in the roots of the plant. This process is suitable for removing metals and inorganic contaminants that may found in groundwater, surface water and wastewater (Baah, 2011).

Phytovolatilization- Phytovolatilization is the uptake and transpiration of a volatile contaminant by a plant, or the escape of the degraded contaminant to the atmosphere via the plant. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at relatively low concentrations (Tangahu *et al.*, 2011). This mechanism ensures a solid or liquid contaminant is transformed to a vapour form, which can be pure or metabolized by the plant before vapourizing to the atmosphere. Phytovolatilization can occur in contaminants such as volatile organic compounds (e.g trichloroethene) and volatile inorganic chemicals (e.g selenium, mercury, and arsenic) that are found in soil, sediments, or water (Baah, 2011).



Figure 3: The mechanisms of heavy metals uptake by plant through phytoremediation technology (Source: Tangahu *et al.*, 2011).

c) Heavy metals uptake by yeast

Yeasts use different detoxifying mechanisms to mobilize, immobilize or transform metals. The mobilization mechanism uses mainly bioleaching by producing acids which combines with metal ions to form insoluble complex. The immobilization mechanisms require (a) biosorption which involves metals interacting with cell membranes using processes like ion exchange, crystallization, adsorption, complexation, and precipitation; (b) biotransformation- which deals with toxic metals being reduced to less toxic forms; and (c) bioaccumulation which is intracellular uptake of metal ions by microorganisms (Siddique *et al.*, 2015).

Biosorption is a physicochemical process that occurs between compounds of cellular organisms and metal. The biological species (e.g yeast and fungi) should be able to concentrate and bind heavy metals by means of metabolic pathways (Abbas *et al.*, 2014). Biosorption mechanism is a complex one that involves several processes like ion exchange, complexation, adsorption and precipitation (Siddique *et al.*, 2015; Ruchita *et al.*, 2015). Ion exchange involves an absorbed ion or an ion that is easily exchanged (usually in a solid phase) by another ion which it is in contact with. Ion exchange also means the migration of cations and anions in the soil. Ion exchange could also be referred to as cation exchange as the process entails interchanging of cation in water solution around the particles of the soil with another cation that is held to the surface of clay. Many researchers have proven ion exchange as a mechanism in biosorption process (Ahluwalia, and

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Goval, 2007; Davis *et al.*, 2003) even though it is not a major mechanism for heavy metals biosorption. In soil precipitation, a chemical substance or nutrient present in a solution of soil particles and water is changed into a solid and this takes place during a chemical reaction. Metal precipitation produces precipitates of inorganic metal that is usually insoluble. This happens when metals are held to the surface or outer cells of polymeric compounds that is secreted by microbes (yeast and fungi). The precipitates formed are stuck to the internal cells of the microbes (Macaskie *et al.*, 1994).

Bioaccumulation is defined as the absorption and transportation of pollutants by living cells into the cell (Malik, 2004). It is a growth-dependent process that depends only on living organisms (Karna et al., 1996). The mechanism of intracellular uptake is more complex than biosorption itself and is not fully grasped yet. However, the process proceeds in two stages. The first stage referred to as passive biosorption occurs quickly (about few minutes). It proceeds via any or all of the following mechanisms that binds metal (which include complexation, coordination, ion exchange or physical adsorption). Metal ions are adsorbed to the cell surface through reactions between the metal functional groups held on the cell surface. The second stage is slow and takes place when ions from metal permeate the membrane of the cell and goes into the cells (Chojnacka, 2009). A study by Raspor et al. (2000) showed that there is an active transportation mechanism in the cells after initial rapid biosorption on cell walls that allows metal ions to permeate the membrane of the cells and enter the cells. Metal ions around the cell membrane are converted into other species or precipitated by active cells, including transporting them within the cell. The process of metal accumulation can be useful for metal ions that are essential and non-essential in form. However, cytoplasmic detoxification is the principal method used in non-essential metal (Assis, 2011). This cytoplasmic detoxification could be accomplished by the transporting of metal to the outside of the cell or to less sensitive cellular compartments, thus making the metal ions unavailable to cytotoxic effects (Tamas and Wysocki, 2010). Some of the mechanisms used by microbes are cell membrane metal efflux (Kamizono et al., 1989), intracellular chelation by metallothionein proteins and peptides obtained from glutathione (also called phytochelatins) (Kneer et al., 1992; Presta and Stillman, 1997), in addition to compartmentalization of metal in vacuoles (Volesky, 1994), but that of intracellular accumulation is not clearly understood. Metals generally go into the cells of yeast by means of special transport systems that are often subject to negative regulation when the unique metal is present in excess intracellularly. Researchers have reported the transport of metal ion by baker's yeast (Portnoy et al., 2001; Eide, 1998). The baker's yeast was known to have at least two substrate-specific transport systems which may be used to accumulate only a metal ion. Upon entering the cell, the metal ions react with thiol compounds found in the residues of cysteine. The thiolated peptides are the glutathione (GSH), phytochelatins (PC), and the metallothioneins (MT) (Tamas and Wysocki, 2010). The metal-thiolated peptide complexes formed may act as a substrate for metal/metalloid extrusion to the outside of the cell, or for accumulation in cellular compartments (the vacuole). However, thiolated peptides can be converted to chelate metals, thereby reducing their reactivity and availability to the cells. The ions of metal could be detoxified by vacuoles as explained by many researchers. Its role is observed in molecular degradation, metabolite storage and regulation of metal ion cytosolic concentrations and detoxifies easily toxic ions. The results obtained by (Ramsay and Gadd, 1997; Thorsen et al., 2012) indicated that vacuole-deficient strain was highly sensitive while the uptake of As, Zn, Mn, Co and Ni was significantly reduced. Avery and Tobin (1992) also confirmed the accumulation of Sr^{2+} in the vacuole of *S. cerevisiae* living yeast cell.

Bioaccumulation process is preferred to biosorption in the accumulation of heavy metals using yeast. However, biosorption process is more feasible when applied in a large scale than when bioaccumulation process is used. This is because microorganisms need extra nutrients for effective uptake of heavy metals (Ksheminska *et al.*, 2008). Hence, extensive studies had been done on the biosorptive ability of yeast than for bioaccumulation process.

Bioreduction: metal ions could be detoxified either by oxidation or reduction. When a metal's redox state is reduced, this also reduces its mobility and toxicity thus paving way for bioremediation process (Siddique et al., 2015). Available information on the detoxification of metals using reduction mechanisms by yeasts considered only neutrophilic yeasts and little information on prokaryotes or eukaryotic cells. The detoxification mechanism of As(V) was proven by Tamás and Wysocki (2010) to be the reduction of As(V) to As(III) as catalysed by arsenate reductase enzymes. Various types of biomass were used to remove toxic Cr(VI) from aqueous solution through biosorption process (Tahri et al., 2015). The reduction of Cr(III) by Cr(VI) using cellular-reducing systems follows pathways that requires enzymes and non-enzymes. Mechanism used for detoxifying chromium is mainly on Cr(VI) reducing intracellularly to Cr(III). The microbial reduction of Cr(VI) under aerobic condition, is catalyzed by soluble enzymes called chromate reductase (Tahri et al., 2015). Yeasts such as Cyberlindnera fabianii, P. anomala, Rhodotorula pilimanae D-76, and Pichia guilliermondii ATCC 201911 were known to enzymatically reduced Cr(VI) to Cr(III) (Ksheminska et al., 2008; Bahafid et al., 2013; Bahafid et al., 2011). Cr(VI) may be removed from aqueous solutions by reducing it to Cr(III) on contacting with the biomass, S. cerevisiae, C. tropicalis, P. anomala and Penicillium chrysogenum (Bahafid et al., 2011; Park et al., 2005). For microbial cells, cysteine and glutathione are known to be the strongest chromate reducing agents without enzymes while ascorbate are for higher organisms (Siddique et al., 2015; Elangovan et al., 2006). Two mechanisms (direct and indirect reduction) is used by dead cells to remove Cr(VI) from aqueous solution. For the former, Cr(VI) is directly reduced to Cr(III) by contact with the biomass acting as donor of electron while the later proceeds in three stages- adsorption of Cr(VI) anionic species to the surface of positively charged biomass groups; Cr (VI) being reduced to Cr(III) by adjacent electron-donor groups and lastly the removal of Cr (III) ions in the aqueous phase due to the electronic repulsion between the Cr (III) ions and positively charged groups, or adsorption of Cr(III) with adjacent groups.

Bioleaching is a process in which naturally occurring microbes dissolve metals from their mineral source (Bahafid *et al.*, 2017). It could also be described as the transformation of solid metal values into forms that are soluble in water using microorganisms (Hamidian, 2011). In order words, leaching transforms metals to more soluble forms so that they are removed easily from soils and sediments. Metals are usually held on soils using sorption and ion exchange processes, metals forming complex ions with organic materials in the soil or as precipitates of pure or mixed solids (Knox *et al.*, 2000). But, in acidic soils, metals can be chemically converted to more soluble or mobile forms. Research showed that microorganisms especially fungi had interacted with metals

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and minerals in both natural and artificial soils, thereby changing their physical and chemical state and also resulted in metals existing in soluble forms (Gadd, 1999).

Thus, fungi and other microbes can biochemically affect metal speciation and mobility in the soil, thereby altering their biogeochemical cycles. Acidolysis and complexolysis are the most essential mechanisms used by fungi to make metal and minerals soluble. It was discovered that some metabolites excreted with characteristics of metal-complex (e.g. phenolic compounds) plus organic acids enhance metals dissolution (Gadd, 2004). Organic acids with low molecular weight (e.g. citric acid and oxalic acid) are the most essential products gotten from yeast and chemical fungal and were used to solubilize heavy metal (Gadd, 1999). Other studies also revealed high mobility on the part of metal citrates. Oxalic acid has been demonstrated to be a leaching agent for metals (e.g. Al and Fe), resulting in soluble oxalate complexes being formed (Strasser et al., 1994). Organic acids are known to possess protons used for changing metals to soluble state as well as in the form of anion metal-chelates which forms complexes with metal cation (Devevre et al., 1996). These are achieved as the substrate is acidified by organic acids, thus increasing the solubility of ions and complexes are formed with dissolved ions, which make them to be mobile. Bioleaching process is usually done in the mining industry especially in processing ores of low-grade and ores containing arsenic which could not have been processed effectively by high temperature smelting. There are two types of bioleaching processes - bioleaching in the stirred tank reactors (STR) used for high-grade ores and heap leaching used for low grade ores. Bioleaching process has also been employed in the extraction as well as recovery of metals from their sulphides. For instance, the extraction of copper from its sulphide ores [secondary copper sulfides such as covellite (CuS), chalcocite (Cu₂S) and bornite (Cu₅FeS₄) and the primary copper sulfide, chalcopyrite (CuFeS₂)] have been used successfully in places like Toromocho and United States (Dreisinger, 2006; Watling, 2006). Apart from using bioleaching process to produce copper, it has been used in a large scale (industrially) to obtain precious metals like gold especially in countries like Brazil, Australia, South Africa, Ghana, Peru, China and Kazakhstan. For example, the process is used to leach minerals containing sulphides like pyrite, FeS₂ and arsenopyrite, FeAsS, with gold particles being encapsulated microscopically by them. On dissolving these sulphide compounds, the gold particles are uncovered and can be recovered using further treatments (Neale, 2006).

However, the use of yeasts in the field of bioleaching has been limited. It was reported that 0.17% of lithium was recovered from lepidolite by bioleaching using the heterotrophic microorganism of *R. rubra*, and effectively mobilizing or leaching copper from sedimentary Rock using strains of *R. mucilaginosa sp. lm*9 isolated from Kupferschiefer black shale that contains oxalic and malic acids (Marcinčáková *et al.*, 2015; Rajpert *et al.*, 2013). So, the use of yeasts for bioleaching process should be carried out on a larger scale as well as developing the technology that will achieve this objective.

d) Biodegradation of Hydrocarbons

Hydrocarbons can serve as carbon and energy sources for bacteria isolates as they are composed mainly of carbon and hydrogen only. The degradation process can be aerobic (oxygen is present) or anaerobic (uses ferric iron, nitrate, sulphate and electron acceptors) (Widdel and Musat, 2010).

Thus, the mechanism by which hydrocarbons are degraded will be described in different perspectives such as:

- (i) Aliphatic versus aromatic hydrocarbons
- (ii) Aerobic versus anaerobic degradation pathways
 - I. Aerobic Degradation
- (i) Aliphatic hydrocarbons: These include n-alkanes (straight chain), branched chain alkane and cyclic alkane.
- N-alkane: The degradation of n-alkanes is usually by monooxygenases/hydroxylases enzymes as shown in Figure 4. The n-alkanes are oxidized to their corresponding alcohol, which is then further oxidized to the corresponding aldehyde, and finally converted to a fatty acid (Sierra-Gracia and de Oliveira, 2013). The β-oxidation of the fatty acid produces a β-keto acid, which is lastly decarboxylated to a degraded alkane (Ivey, 2006).



Figure 4: Biodegradation pathway for n-alkanes Source: Ivey (2006)

• **Cyclic alkane:** The biodegradation of cyclic compounds occurs in the following stepsstepwise oxidation of the cyclic alkane to form cycloketone which is further oxidized by an oxygenase enzyme to produce a lactone (Ivey, 2006). Lactone undergoes hydrolysis, which results in cyclic ring being splitted to form an oxidized n-alkyl fatty acid that can further be degraded to n-alkane as explained in the degradation pathway of n-alkane (**Figure 5**).

Figure 5: Biodegradation pathway for cyclic alkanes Source: Ivey (2006)

(ii) Aromatic hydrocarbons: The biodegradation of aromatic compounds occurs in two metabolic pathways (catechol and gentisate). In both pathways, the following takes place - activation and cleavage of the ring, and later the conversion of the fission products (Ivey, 2006). The oxidative degradation of these compounds can be through monooxygenases or mainly by dioxygenases via these three superfamilies- the Rieske non-heme iron oxygenases (RNHO), the flavoprotein monooxygenases (FPM) and the soluble diiron multicomponent monooxygenases (SDM). For example, toluene, benzene, naphthalene and other polycyclic aromatics usually undergo aerobic degradation by Rieske non-heme iron oxygenases (RNHO). Further intermediates can be catalyzed by two enzymes (intradiol and extradiol dioxygenases) as depicted in Figure 6 (Sierra-Gracia and de Oliveira, 2013; Karigar and Rao, 2011).

Figure 6: Aromatic compounds degraded by dioxygenase Source: Karigar and Rao (2011)

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• **Polycyclic Aromatic Hydrocarbons (PAHs):** The aerobic degradation of PAH usually occurs by first, the oxidation of the PAH to a dihydrodiol using a multi-component enzyme system. Then, the dihydrodiol intermediates formed may then pass through either an ortho cleavage type pathway or a meta cleavage pathway, resulting in the formation of central intermediates (like protocatechuates and catechols), which are further converted to tricarboxylic acid cyclic intermediates (Ivey, 2006).

II. Anaerobic Degradation

Aliphatic Hydrocarbons: Anaerobic degradation of n-alkanes proceeds via two pathways. First, the sub-terminal carbon is activated by the addition of fumarate then secondly, carboxylation reaction takes place by sulfate-reducing strain Hxd3 (Figure 7). However, carboxylation of alkanes is not feasible when viewed from energy requirement except when the concentration of fatty acid derived during the process is in micromolar or less (Sierra-Gracia and de Oliveira, 2013; So *et al.*, 2003).

Figure 7: Mechanism, stereochemistry and co-metabolism of anaerobic degradation of *n*alkanes. Source: Rabus *et al.*, 2016

ii) Aromatic Hydrocarbons: Since oxygen is absent, aromatic compounds are degraded anerobically by microbes using reduction reactions which alters the aromatic ring (Fuchs, 2008). Anaerobic degradation of aromatic compounds undergo the following mechanisms – addition of fumarate by glycyl radical, methylation of aromatics that are

not substituted, hydroxylation with water using molybdenum cofactor as enzymes and carboxylation reaction (**Figure 8**) (Sierra-Gracia and de Oliveira, 2013; Rabus *et al.*, 2016).

Figure 8: Biodegradation pathways of phenanthrene in thermophilic bacteria. Source: Nzila, 2018

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4.0 Factors Affecting Bioremediation process-

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients) (Sharma, 2012). These factors are further explained as follows:

(i) Biotic factors

Biotic factors also termed biological factors are factors which involves the interactions of microorganisms with organic pollutants in the soil as the little amount of carbon present is shared amongst one another. The rate of biodegradation of contaminant depends on the concentration of the contaminant as well as the numbers of microbes present. Some of the biotic factors include enzyme activity, horizontal gene transfer, mutation, competitive and predators' interactions and biomass growth with respect to the size of the population and structure (Madhavi and Mohini, 2012).

(ii) Environmental factors

The successful interaction between the enzymatic or metabolic performance of microorganisms and that of the physicochemical characteristics of the main contaminants depends a lot on the environmental conditions of the contaminated soil. The growth of microorganisms and their degrading ability are affected by temperature, moisture, soil structure, solubility of it in water, redox potential and oxygen content, nutrients and soil pH, the bioavailability of contaminants due to its nature, concentration, toxicity level, solubility, type and molecular structure (Abatenh *et al.*, 2017).

(iii) Temperature

Of all the abiotic factors affecting rate of biodegradation, temperature is the most important, since it ascertains the extent to which microorganisms can thrive while in soil. It can also affect the composition of the contaminant in the soil (Das and Chandran, 2011). For cold climate, such as the Artic, the degradation of hydrocarbons is slow and forces the microorganisms to do more in cleaning the spilled oil. This is so because, at low temperature, the transport systems in the cells of the microbes are locked together with their cytoplasm being frozen, thus making them to be metabolically inactive (Macaulay, 2015). Temperature can either increase or decrease the rate of biodegradation. Each contaminant possesses a particular temperature for degradation to take place. In a nutshell, the rate of microbial action increases with temperature, and reaches its maximum (optimum) temperature and any further increase or decrease in temperature will only reduce the metabolic rate of the microbes (Abatenh *et al.*, 2017).

(iv) Moisture

Water (moisture) affects the rate of pollutant degradation because microbes need water to survive. It also affects the type and quantity of soluble substances that the soil contains, likewise the osmotic pressure plus the pH of aquatic and terrestrial systems. The amount of water in the pore

spaces of the soil also affects the exchange of oxygen. Under this condition, the quantity of oxygen absorbed by the microbes is more than when it is low. Conversely, the moisture content of the soil should be between 25–85% of the water holding capacity and 50–80% for an enhanced biodegradation. The water holding capacity of soil also known as the "field capacity" is the percentage of water left in a saturated soil and gravitational drainage has stopped (Eskander and Saleh, 2017).

(v) pH

Soil pH is the degree of the acidity or alkalinity of water. The soil's pH can affect the metabolic rate of microbes. The pH value of the soil is an index to the metabolic rate of the microorganisms (Enim, 2013). The rate of removal of soil contaminants can either increase or decrease due to metabolic activity of microbes. Optimum biodegradation can take place at a pH value ranging from 6.5-8.5 for mostly aquatic and terrestrial systems (Abatenh *et al.*, 2017). Generally, a pH value that is within 5-9 is acceptable. The pH of the soil may affect the availability of nutrients. For instance, phosphorus is very soluble in a soil of pH 6.5, but above or below this value, it becomes insoluble (Eskander and Saleh, 2017).

(vi) Availability of nutrients

For microorganisms to thrive and indeed continue in their degradation activities, they require some amount of nutrients like nitrogen, phosphorus and carbon. When the C: N: P ratio is small, the degradation of hydrocarbon is reduced. Thus, the absence or low level of essential nutrients can affect microbial growth or metabolism. The addition of the right amount of nutrients can enhance the rate of microbial metabolism likewise biodegradation efficiency especially in cold environment (Couto *et al.*, 2014). Optimal amount of nutrients is required for increase oil degrading bacteria, but too much of these nutrients can negatively influence the biodegradation of hydrocarbons due to low metabolic rate of the microorganisms (Koshlaf and Ball, 2017). The microbial count for surface soils is higher than for subsurface soils and lower in aquifer sediments or aquatic regions due to limited supply of nutrients (Eskander and Saleh, 2017).

(vii) Amount of oxygen

Bioremediation process takes place both in aerobic and anaerobic conditions, which is made possible because oxygen is available to increase the metabolism rate of microorganisms on petroleum pollutants (Macaulay, 2015). The quantity of oxygen present in the soil depends on the soil type, moisture content and the biodegradation rate. Oxygen is required in respiration process as well as oxygenases participating in the degradation pathway of the crude oil pollutants.

(viii) Soil characterization

The structure and conditions of the soil will determine the movement of the contaminants, which in turn will affect its biodegradation rate. Higher amount of organic matter in the soil yields higher biomass as well as improved bacteria growth resulting in increased biodegradation rate of hydrocarbons (Scherr *et al.*, 2007; Chaerun and Tazaki, 2005).

(ix) Bioavailability

Bioavailability is the fraction of contaminant available for microorganisms to degrade. The rate of degradation is determined by the bioavailability of hydrocarbons and the extent to which the contaminants are degraded is a direct function of the concentration of bioavailable contaminant (Eskander and Saleh, 2017). The higher the molecular weight of hydrocarbon pollutants, the lower will be its solubility thereby reducing its availability by microorganisms (Koshlaf and Ball, 2017). The hydrophobicity nature of poly aromatic hydrocarbons (PAHs) makes them less bioavailable for microbial degradation activity together with its fast sorption to organic matter and soil matrix makes them more toxic and recalcitrant (Sihag *et al.*, 2014).

(x) Concentration and characteristics of toxic contaminants

When the concentrations of the contaminants are high, this can result to slow rate of decontamination of the toxic compounds by microbes. This is as a result of the toxic effect of the contaminants on the growth and microbial metabolism in the soil. The extent of contamination and its degradative mechanisms differ with some pollutants, their concentration, with also the microbes that are affected. Some organisms are easily attacked by certain organic and inorganic compounds (Madhavi and Mohini, 2012).

Additionally, the metabolic breakdown of hydrocarbons depends also on the characteristics as well as chemical structure of the hydrocarbon pollutants (Sihag *et al.*, 2014). For instance, hydrocarbons with carbon length of (C_{10} - C_{25}) can easily degrade whereas those with long chains of (C_{25} - C_{40}) cannot easily be degraded due to their hydrophobic nature, poor water solubility and low bioavailability (Koshlaf and Ball, 2017; Sihag *et al.*, 2014). Complex and less soluble compounds results in low biodegradation of it. Branched chain alkanes and cycloalkanes degrade more slowly than the corresponding straight chain alkanes (Koshlaf and Ball, 2017).

5. Advantages of Bioremediation

- 1. It is a natural process and generally acceptable by the public.
- 2. It is eco- friendly.
- 3. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants.
- 4. Bioremediation is less expensive than other technologies that are used for clean-up of hazardous waste.
- 5. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
- 6. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.

6. Disadvantages of Bioremediation

- 1. Bioremediation is limited to biodegradable compounds. Thus, not all compounds are susceptible to rapid and complete degradation.
- 2. It is time-consuming compared to other treatment options, like excavation and removal of soil or incineration.
- 3. There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.
- 4. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- 5. It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- 6. Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Some of the contaminants may be present as solids, liquids, and gases.

7. Conclusion

Over the years bioremediation process has gained world-wide recognition and acceptance, due to its environmental friendly nature. It could be applied in-situ (on site) or ex-situ, with the former being less expensive. There are several processes incorporated into it, which include bioaugmentation, biostimulation, bioventing, bioreactors, landfarming, phytoremediation, and others. Bioremediation mechanism generally involves enzymatic degradation of pollutants by microbes to less toxic products. The degradation of hydrocarbons is mainly done by enzymes belonging to oxygenases (monooxygenase and dioxygenase), while the uptake of heavy metals via microbes (yeast) and plants is as follows: yeast- biosorption, bioaccumulation, complexation, adsorption, ion exchange and so on and for plants- phytoextraction, phytostabilization and so on. From the review, it was clear that some factors must be put into consideration for bioremediation to be effective. They are biological factors (microbes like bacteria, fungi and algae) and environmental factors (pH, amount of oxygen, temperature and nutrient) as well as the extent of the pollutant in the soil. Indeed, bioremediation processes as outlined in this paper have their advantages as well as limitations and can be applied on the soil depending on the nature and extent of toxicity of the pollutant, the amount of microorganisms present in the soil, and cost requirements. Generally, the advantages of bioremediation far outweigh its limitations. Thus, bioremediation process continues to be an effective method to use when it comes to reducing and even eliminating contaminants in the soil.

Future perspectives of Bioremediation

Based on the review, the following points should be considered for future learnings:

- 1. Bioremediation techniques should be applied to more contaminants other than hydrocarbons.
- 2. Further understanding of the mechanisms involved in bioremediation processes should be opted for.
- 3. Newer bioremediation techniques such as nanobioremediation, OMICS, etc should be employed.

4. More research should be aimed at simultaneously applying more than one or two of these bioremediation techniques in a larger scale (field scale).

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