Rhizoremediation of Petroleum hydrocarbon, Prospects and future

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Abstract

Oil refineries generate several tones of oily waste which is dumped in an open pit within the vicinity of oil field. The disposal or removal of such waste through excavation is costly and laborious. Therefore oil refineries are looking for rapid and economic methods to remediate such waste. Rhizoremediation is successfully adapted to remediation hydrocarbon contaminated soils by the oil refineries. This is a technique in which plants and microbes are used to remediate oily waste contaminated sites**.** The main aim of the study was to introduce an economic and rapid rhizoremediation technique to oil refineries in which plant and microbes are used to enhance the natural degradation process of oily sludge contaminated soils. The rhizosphere of oily sludge is composed of several genera of hydrocarbon degrading microbes naturally. When such microbes are inoculated to oily sludge rhizosphere are capable of degrading hydrocarbons more likely because of presence of root exudates that can provide enough carbon, nutrients and oxygen for microbes to increase in number thus accelerate the rate of degradation of hydrocarbons via β-oxidation. The use of microbes+plants to remediate oily sludge remained an area of interest by researchers over the last several years. Several laboratory scale and field trials have been conducted to evaluate the combine effect of hydrocarbon degrading microbes with plants to accelerate the natural rehabilitation process of oily sludge contaminated soils. However the mode of degradation of hydrocarbons in combination with plants and microbes remained an area of interest for scientist to remediate oily sludge contaminated soils. Oil refineries generate several tonnes of oily waste which is dumped in an open pit within the vicinity of oil field. The disposal or removal of such waste through excavation is costly and laborious. Therefore oil refineries are looking for rapid and economic methods to remediate such waste. Rhizoremediation is successfully adapted to remediation hydrocarbon contaminated soils by the oil refineries. This is a technique in which plants and microbes are used to remediate oily waste contaminated sites**.**

The suitability of plant growth promoting rhizobacter and plant species to remediate oily sludge was discussed in detail in this review. The factors affecting the degradation of hydrocarbons and under oily sludge contaminated rhizosphere are also examined.

Key Words: Rhizoremediation, Phytoremediation, Bioremediation, Plant, Microbial degradation, Hydrocarbons, Oily Sludge, Toxic Hydrocarbon, Soil Contamination

1. Introduction

The increased interest in understanding the role of hydrocarbon degrading microorganisms isolated from oily sludge has undoubtedly been stimulated in bioremediation because of their significance in a variety of industrial considerations, such as the removal of deleterious activities of fungi growing in aviation turbine fuel, the possibility of bioremediation of hydrocarbon contaminated soils from hydrocarbon degrading microorganisms and exploiting hydrocarbon fractions as substrate for microbial growth (Wanga *et al*., 2016: Okoh 2006). Over the last several decades, huge amount of oily sludge is removed during drilling and stored in an open pit within the vicinity of oil fields. In addition to that oil refineries are also generating oily waste which is released to the environment without treatment hence poses constant threat to the agro-environmental ecosystems. Oily sludge contains a range of carcinogenic and toxic organic or inorganic compounds. When oily sludge enters into the terrestrial or aquatic environment may cause toxicity to microorganisms and plants. In addition to that toxic hydrocarbon may accumulate in the food chain of aquatic and marine life hence disrupts their response to chemoreception (Siddiqui *et al.,* 2001). Natural rehabilitation of oily sludge contaminated sites is slow and may take several years to rehabilitate. Bioremediation involves the use of microorganisms to remove pollutants (Joo *et al.,* 2008).

Some recent review on remediation of oily sludge with compost (Parkash *et al*., 2015), inoculation with consortium known as bioremediation (Dizenok *et al*., 2016; Adams *et al*., 2015; Joo *et al.,* 2008), role of plants to remediate oily sludge contaminated soils (phytoremediation) (Daryabeigi and Hoveidi, 2016) provide a comprehensive information on the degradation of organic contaminants via plants. The combine effect of plants and microbes (Rhizoremediation) to remediate oily sludge contaminated soils is reviewed recently by Shahzad *et al*., (2016) and Das and Chandran, (2011). The Bioremediation involve the use of microbes to remediate hydrocarbon contaminated soils whereas rhizoremediation also involve microbes but in combination with plant root exudates to enhance remediation at depth. Thus increase in microbial number and activity in rhizosphere soil enhance the degradation of hydrocarbons than non rhizosphere soil. Rhizoremediation can be suitable for subsurface degradation of hydrocarbon via interaction between microbes and plants whereas bioremediation can degrade hydrocarbon at surface and subsurface degradation may not be achieved with rhzioremediation.

1.1. What is Oily Sludge and its source?

Oily sludge is a mixture of complex emulsion of a variety of petroleum hydrocarbons (PHCs), water, heavy metals, and solid particles, generated by petroleum industries. Oily sludge is composed of saturates (straight chain alkanes or paraffins), naphthenes (saturated rings), and aromatics (unsaturated rings) whereas asphaltenes and resins are present in traces (Loubna *et al*., 2016: Bojes and Pope, 2007)**.** Oily sludge contains a range of carcinogenic and toxic organic or inorganic compounds. In addition to that toxic hydrocarbon may accumulate in the food chain of aquatic and marine life hence disrupts their response to chemoreception. Natural rehabilitation of oily sludge contaminated sites is slow and may take several years to rehabilitate. Therefore it is necessary to remediate oily sludge contaminated sites through adapting cost effective technique in order to accelerate the natural rehabilitation process. Both the upstream and downstream operations in petrochemical and oil refineries can generate a large amount of oily wastes. The upstream operation includes the processes of extracting, transporting, and storing crude oil, while the downstream operation refers to crude oil refining processes. The oily waste generated in petroleum industry can be categorized as either simple oil or sludge depending on the ratio of water and solids within the oily matrix (Patowary *et al*., 2016: Futaisi *et al*., 2007)

1.2. Class composition, range and sources of carbon numbers in oily sludge

The composition of oily sludge primarily depends on the geochemical characteristics of the source rocks from which crude oil is produced and secondly on the status of environment under anaerobic conditions. Therefore the chemical composition of oily sludge varied from region to region more likely because of the difference in the geochemical characteristics of the region from which crude oil is produced.

Table 1. Class composition of oily sludge generated from various oil companies around the world.

Location of oily sludge	Saturates (mg/g)	Aromatic (mg/g)	NSO (mg/g)	Asphaltenes (mg/g)	Références
India	52	31	7	10	Mishra *et al*., (2001)
Turkey	15	23	43	16	Karayildirim *et al*., (2006)
Jordanian	66.7	14.7	7.8	12.0	Tahhan and Abu-Ateib. (2009)
Theran-Iran	69.2	12.2	22.2	-	Salehi *et al*., (2009)
Western Canada	21.2	47.8	9.6	21.4	Warid *et al*. (2003)
Western(USA)	45.4	37.8	3.9	12.9	Do
South East Asia	44.7	40.8	6.5	8.0	Do

1.3. Terrestrial contamination of environment

Terrestrial environment nearby oil field remained under constant threat more likely because of discharge of organic contaminants from oily waste pit. It has been estimated that more than 10% of the terrestrial oil contamination is because of dumping of oily sludge which have resulted into serious environmental pollution and environmental hazards Ubani *et al*., 2016:Onwurah *et al.,* 2007. Presence of oily sludge into the terrestrial environment may cause anaerobiosis, nutrients deficiency and retardation of microbial population (Pichtel *et al*., 2016; Tang *et al.,* 2010). Other disrupting possibilities could be that toxic and carcinogenic compounds in oily sludge could lead to the elimination of many plants and aquatic species and are resistant to degradation and may move up the aquatic or marine food chains.

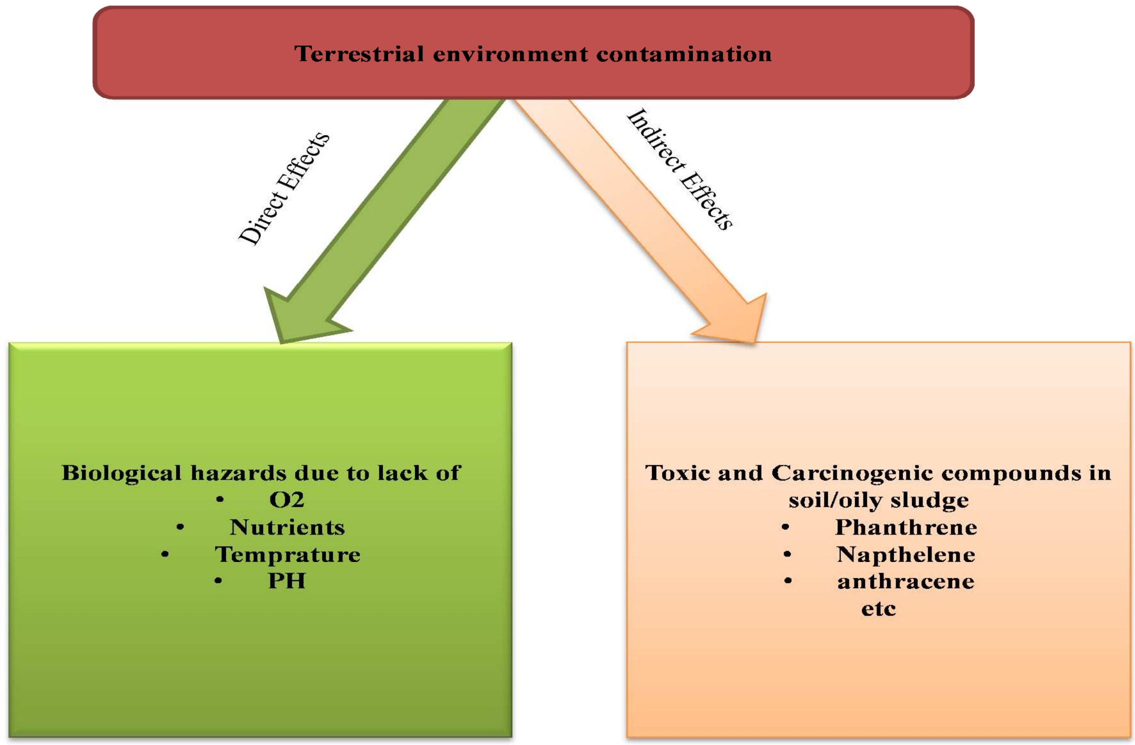

Fig 1. Direct and indirect effects of contamination on terrestrial environment

1.4. Factors involved in bacterial degradation of hydrocarbons

1.4.1. Oxygen

Under aerobic environment porosity of soil enhance the activity of indigenous microorganisms their activity, required to degrade hydrocarbons (Marimuthu and Sundaram., 2016; Agnieszka and Zofia 2010) Once hydrocarbon enters the rhizosphere it occupies the pore spaces hence less oxygen is available for the microorganisms to degrade hydrocarbons. The rate of degradation of hydrocarbons is slow in oxygen deficient soil more likely because biodegradation is well known as aerobic processes in which the degradation of hydrocarbons into biomass, CO_2 and intermediate product is proceed further by bacteria through adapting aerobic metabolic pathways in the presence of oxygen (Rohrbacher and Marc St-Arnaud, 2016: Reshma and Anu 2014; Rentz *et al.,* 2008;). However, under anaerobic conditions biodegradation of hydrocarbons also occur but the mode of degradation of hydrocarbons was different than aerobic degradation (Rabus *et al*., 2016;). **Oxygen availability for biodegradation of hydrocarbons remained a challenge for hydrocarbon degrading bacteria under waterlogged conditions** (Ladino-Orjuela *et al*., 2016;). Reduction in pore spaces, replacement of gases by water and anaerobic conditions up to 2 m poses indirect effect on oxygen movement within soil pores hence causes delay in the degradation of hydrocarbons. In rhizosphere environment, plant roots and microorganisms provide enough oxygen for specific genera of hydrocarbon degrading microorganisms to degrade hydrocarbons than non rhizospheric soils ((Saleem, 2016; Martina and David 2009; Lee *et al.,* 2006). Martía *et al.,* (2009) reported that the degradation of oily sludge was rapid in rhizosphere with sufficient oxygen and organic carbon than rhisosphere deficient in oxygen. In contrast to that Moreno *et al.,* (2005) reported that the rate of degradation of oily sludge was more in oxygen deficient soils with increased carbon source. Guolin *et al.,* (2011) reported that increased aeration to oily sludge contaminated soils enhance the rate of degradation of hydrocarbons then under anaerobic conditions.

1.4.2. Nutrients

Microorganisms require nitrogen and phosphorus and micronutrients to degrade petroleum hydrocarbons. However, nutrient availability to microorganisms to degrade hydrocarbon under the rhizosphere impregnated with hydrocarbons usually depends on the amount of hydrocarbons and secondly on the plant species growing under such environment (François and Aurélie Cébron, 2016; Liu *et al.,* 2009: Martina and David, 2009; Kuiper *et al.,* 2004). There is conflicting evidence on the need to add N and P supplements to contaminated soils and on the levels of N or P required (John *et al*., 2016; Das and Chandran 2011).

Once rhizosphere become contaminated with oily sludge enough carbon substrate become available for microorganisms to increase in number. Thus nitrogen availability increases in the rhizosphere necessary to degrade hydrocarbons under stress environment. Microorganisms degrade hydrocarbons into biomass, CO_2 and water under aerobic condition (Prakash *et al.,* 2015). Azari and Abu Bakar. (2016) reported that C:N ratio of petroleum contaminated soils was reduced from 600:1 to 97:1 and 167:1 when 10.7 mg of N as KNO_3, NH_4Cl to 5 and 10 mg of petroleum per g of sandy soil. They concluded that increased microbial activity under nitrogen enriched petroleum contaminated soils enhanced the rate of degradation of petroleum. Jiang *et al*. (2016) reported that C:N:P ratio of 100:1:0.1 was enough to degrade hydrocarbon contaminated soils.

Wang *et al.* (2012) found that C:N ratio of 15:1 was enough to degrade 4.9g of oily sludge per kg of rhizosphere over 3 months of incubation. Reza *et al.,* (2008) reported that C:N ratio from 15-33:1 was enough to degrade 5-6 g of hydrocarbon per kg of petroleum contaminated rhizosphere soil. Walworth *et al.,* (2007) reported that microbial activity was increased 4 fold in petroleum impregnated soils when 256 µg N as NH_4NO_3/g soil was added than in same soil without nitrogen. They concluded that C:N ratio of 170:1 is enough to degrade 44 mg of hydrocarbons per g of oily sludge impregnated soil. Furthermore when C:N ratio was reduced to 11:1 (4 µg N as NH_4NO_3/g of soil was added to oily sludge contaminated soils) the microbial activity was retarded and degradation of hydrocarbons was minimized., microbial activity was reduced. Amellal *et al.,* (2001) reported that C:N ratio of 35:1 and 15:1 was enough to degrade hydrocarbons in silty soil than clayey soil. Dahlhem (1998) reported that C:N ratio of 30:1 is enough to degrade 100 mg of hydrocarbon in petroleum impregnated rhizospheric soil.

In some cases the addition of N and P to rhizosphere contaminated with oily waste did not accelerate the rate of degradation of hydrocarbons. Langley *et al*. (2015) reported that nitrogen addition did not accelerate the rate of degradation of hydrocarbons. They concluded that this is because of plant species grown over such soil. Mohammadi *et al*. (2011) reported that addition of nitrogen and phosphorus fertilizer to oily sludge contaminated rhizsosphere did not increase the soil respiration and degradation of hydrocarbons over 2 months of incubation. They concluded that the 2 months of incubation was insufficient to exhaust the mineral nutrient reserves of the soil. Zhou *et al.,* (2009) reported that C:N:P ratio of 137:14:1 was not enough to degrade 500 mg of oily sludge per kg of soil over 42 days of incubation. Reza *et al.,* (2008) reported that the addition of phosphorus reduced the C:N ratio more likely because of enhanced microbes utilization of available carbon and nitrogen substrate. Similar results were reported by Siciliano *et al.,* (2003) while studying the effect of phosphorus addition to oily sludge contaminated rhizosphere, possibly due to interaction among grass roots and nutrient in the soil. Ayotamuno *et al.,* (2006) and Tyagi *et al.,* (2011) reported that addition of 20:10:10-NPK-fertilizer had effect on the hydrocarbon degradation over a period of 6 to 12 weeks of petroleum contamination. They concluded that this could be due to the mineral nutrient reserves of the soils. In some soils N or P or N and P reduced the rate of degradation of hydrocarbons and soil respiration. (Margesin *et al*., 2007)

1.4.3. Temperature

Temperature is another environmental factor which influences the hydrocarbons degradation exactly affecting on the physical nature and chemical composition of the oil, rate of hydrocarbon metabolism by microorganisms and composition of the microbial community (Jiang *et al*., 2016; Pelletier *et al*., 2004). The rate of degradation of petroleum hydrocarbon was negligible at low temperature (Rajendiran, *et al*., 2016; Delille *et al*., 2004). The degradation of petroleum hydrocarbons has been previously reported under antarctic, sub-antarctic, alpine, artic, sub-arctic and sea-ice water conditions (Margesin, 2000; Delille and Delille, 2000). Eriksson *et al.,* (2003) compared the biodegradability of naphthalene, 2-methylnaphthalene, fluorene, and phenanthrene under mesophilic (22^{o}C) and psychrophilic (7^{o}C) conditions and showed that the most extensive polyaromatic hydrocarbon (PAH) degradation under nitrate-reducing conditions occurred at 7^{o}C, 39% removal, occurred in a culture from fuel-contaminated Arctic soil. Walworth *et al.,* (2013) found that when the rate of degradation of hydrocarbons was increased under sub-antarctic condition when O2 was added to the soil. Whereas negligible hydrocarbons were degraded under anaerobic conditions even at low temperature. Literatures indicate that under low temperature the degradation of hydrocarbons was carried out by cold adapted microorganisms-psychrophilic or physchotolerant (Margesin *et al.,* 2007; Giudice *et al.,* 2010).

Bacterial isolates obtained from bioremediation experiments with Arctic and Antarctic sea-ice as well as water from Antarctic sea-ice was tested for their hydrocarbonoclastic capabilities at low temperatures. Gerdes (2005) studied the fate of *Oleispira* and *Pseudomonas* (*Gammaproteobacteria*) as well as *Dietzia* and *Rhodococcus* (*Actinobacteria*) isolated from sea ice once inoculated to hydrocarbon contaminated soils at temperature as low as 0 ^{o}C and -3 ^{o}C. They found that *Pseudomonas* isolate as well as *Marinobacter* strains were also able to grow on various aromatic compounds. Several *Shewanella* strains, one *Psychrobacter* sp. isolate and one *Marinomonas* sp. isolate (all *Gammaproteobacteria*) as well as a *Loktanella* sp. and a bacterium, related to the genus *Jannaschia* (both of the *Alphaproteobacteria*) were able to grow on short chain alkanes as well as on the 2-ring aromatic compounds naphthalene and acenaphthene even at very low temperature.

1.4.4. pH

The rate of degradation of hydrocarbons depends on the pH of the soil, sediments or water. Most heterotrophic bacteria favour a pH near 7, whereas fungi are more tolerant to acidic conditions (Sukumar and Nimala., 2016; Das and Chandran, 2011). Hydrocarbon mineralization is favored by near neutral pH values. It is common practice to add lime to bioremediate acid soils containing harmful organic compounds (Semple *et al*., 2003). Biodegradation was reported to proceed well in aquifers at natural pH values of 4.5–5 (Roling, 2014), and petroleum hydrocarbon utilizers were found in a tropical, acidic forest soil (pH varied from 4 to 6) 17 years after an extensive oil spillage (Amadi *et al.,* 1996). Where the pH has been shifted away from neutral by manmade changes, biodegradability was likely to be impaired (Norris, 1994). Raymond *et al.,* (1998) reported that degradation of poly aromatic hydrocarbons was greater under acidic conditions. They concluded that this is more likely because of increased activity of acidophilic bacteria.

1.5. Bacterial diversity in oily sludge

Bacteria and fungi are the most dominant microorganisms found in oily sludge (Adams *et al.,* 2015). The classical technique to assess microbial activity has been to count the number of microorganisms present (Gómez-Ullate et al., 2008). Crivelaro *et al.,* (2010) performed microbial counts in oily sludge and found considerable variation between samples of oily sludge and non-oily sludge soil. Siddiqui *et al.,* (2001) studied the effect of adding diesel to soil with previous history of hydrocarbon contamination on microbial population and reported that bacterial population increased to 100 fold than soils without previous history of hydrocarbon contamination. Fulekar (2010) investigated two different soils and found that an increase in total microbial count was experienced with oily sludge contaminated soils, but clay soil showed no change after oil contamination. Bacterial population and diversity within contaminated soils primarily depends on the soil depth. Ismail *et al.,* (2008) found that bacterial population decreased to 65% with increase in depth in soil contaminated with oily waste. They concluded that this difference in bacterial population in relation to depth is more likely because of the difference in C:N ratio between horizons within contaminated soil profile. Colleen *et al.,* (2008) reported that bacterial population and communities differ between horizons within contaminated soil profile. They concluded that this difference in bacterial count and genera is more likely because of the difference in the geochemical characteristics of horizons within a soil profile.

Soil characteristics are very important for successful hydrocarbon biodegradation. The main limiting factors involved are soil texture, permeability, pH, water holding capacity, soil temperature, nutrient content and oxygen content. Soil with low permeability like in clay soils delays the transportation and the distribution of water, nutrients and oxygen. To enable the bioremediation of such soil, it should be mixed with amendments or bulking materials (straw, sawdust etc.), as the bioremediation processes rely on microbial activity, and microorganisms require oxygen inorganic nutrients, water and optimal temperature and pH to support cell growth and sustain biodegradation (Jain *et al.,* 2011). Table 1.2. demonstrated that bacteria play an important role in oil decomposition.

Table 2. Bacterial diversity in oily sludge

Sources	Bacterial species	References
Oily Sludge	*Aeromonas, Alcaligenes, Acinetobacter, Arthobacter, Bacillus, Brevibacterium, Geobacillus, Mycobacterium, Pseudomonas, Rhodococuss, Sphingomonas, Thermus and Zainthomonas*	Zobell, 1950; Atlas, 1977; Atlas and Bartha, 1993; Nitu *et al*., 2010).
Petrochemical oily sludge	*Stenotrophomonas acidaminiphila, Bacillus megaterium and Bacillus cibi,*	Cerqueira *et al*., (2011)
Petrochemical waste	*Pseudomonas aeruginosa* and *Bacillus cereus*	Cerqueira *et al*., (2011)
Oil field of China	*Bacillus amyloliquefaciens*	Liu *et al*., (2012)
Hydrocarbon contaminated sites	*Pseudomonas, Sphingomonas, Moraxella, Acinetobacter, Alcaligenes, and Proteus*	Abed *et al*., 2001
Oily Sludge	*Bacillus , Burkholderia ,Paenibacillus , Pseudomonas ,Bacillus , Stenotrophomonas Enterobacteria Pseudomonas , Bacillus , Pandoraeaand Kocuria*	Roy *et al*., (2014)
Oily Sludge	β-*Proteobacteria , Firmicutes , δ-Proteobacteria ,Bacteroidetes , Acidobacteria , Proteobacteria, Lentisphaerae ,Spirochaetes ,*	Das and Kazy (2014)
Oil-contaminated soil	*Bacillus sp., Corynebacterium sp., Pseudomonas sp., and Pseudomonas sp.*	Muthuswamy *et al*., (2008)
Petroleum oil contaminated site of India	*Bacillus thuringiensis*	Arunkumar *et al*., (2012)

1.6. Techniques to remediate oily sludge contaminated soils

Natural rehabilitation process of oily sludge contaminated soils is extremely slow and may take several decades for hydrocarbons to disappear completely. This is more likely because once oily sludge enters in the soil may sorbed within the clay particles or organic matter hence become less available to microbes for degradation. Therefore petrochemical industries are looking for rapid and economic ways to remediate oily sludge contaminated soils.

1.6.1. Landfarming

The remediation of oily sludge through land farming is the most common practice adapted by oil companies around the world (Blanca *et al*., 2008) Landfarming is considered as an inexpensive and cost effective technique. In landfarming oily sludge is surface spread and cultivated as farmers plough and fertilized agricultural land. Landfarming is not recommended for toxic hydrocarbons and best suitable for rapidly degradable hydrocarbons. The bioremediation and phytoremediation is recommended to be an inexpensive and cost effective technique best suited for remediation of oily sludge contaminated sites.

1.6.2. Bioremediation

Bioremediation means remediation of oily sludge contaminated soils through microorganism (Sharma 2012). Such microbes are usually isolated from oily sludge contaminated environment and are capable of degrading organic contaminants to non-toxic compounds (Pankaj and Vivek 2012). These microbes degrade hydrocarbon through beta-oxidation in which organic contaminants are degraded into biomass, CO_2 and intermediate products (Calvo *et al.,* 2009; Segura *et al.,* 2009; Thapa, 2012; Luke *et al*., 2013).

A bioremediation field study was carried out by Mishra *et al.,* (2001) at Mathura oil refinery in India to remediate oily sludge contaminated soils sporadically becomes contaminated over the several years. A 576m^2 area within the vicinity of an oil refinery was contaminated with an average of 0.92 (w/w) of oily sludge. The site was treated with 1kg of inoculum (*Acinetobacter baumannii, Burkholderia cepacia,* and *Pseudomonas* isolated from oily sludge) and 50kg of nutrient mixture per sq.ft. was ploughed monthly.

Total petroleum hydrocarbons were reduced to 4700 mg per kg of soil in 120 days after adding inoculum and nutrients. The rehabilitation of the field occurred in less than a year and was aided by the low relative molecular mass of the petroleum hydrocarbons and the light sandy soil.

Nkeng *et al.,* (2012) conducted a bioremediation study at oil refinery site of West Africa. An area of 37 m x 37 m (1,369 m^2) was contaminated with oily sludge over the last 15 years. Three plots of 10 m x 10 m (100 m^2) were prepared. Consortium (*Bacillus subtilis, Aspergillus* sp, and *Penicillium* sp) was added and it was observed that around 94% of hydrocarbons were removed after six months.

Mandel *et al.,* (2012) carried out a bioremediation study at 22 oil refinery sites in India. They reported that total content of hydrocarbons ranged from 83.50 to 531.30 g/kg that inoculation of oily sludge with consortium (bacteria isolated from oily sludge) of total petroleum hydrocarbons were reduced to 10 mg/kg over a period of year.

Peter (2011) demonstrated that corn material efficiently remediate hydrocarbon-contaminated soils. The hydrocarbon contaminant is contacted with the corn material in the presence of nutrients and microbial consortia comprising bacterial strains *Acinetobacter bauminii*, *Alcaligene odorans*, *Bukhardica cepacer*, *Pseudomonas aeruginosa*, effective in bioremediation and degradation (97%) occurred at 38 d. Gregory *et al*., (2008) described bioremediation process that enables direct and on-site treatment of hydrocarbon sludge**.** The process comprised steps of contacting the sludge with a microbial biofilm. Boulos *et al.,* (2010) demonstrated in-situ method for treating contaminants in soil and groundwater using an oxidizing agent that generated free radicals e.g., iron (II) and iron (III), copper (II) salts etc. hydroxyl radicals (peroxide, ozone) and neutral agent comprising an effective amount of metal catalyst . Arthur and Renfro (1991) demonstrated a method for onsite bioremediation of hydrocarbon contaminated soils. This method includes mixing of soil with cationic ion exchange resin to promote growth of organism capable of degrading the alkanes and other petroleum derived hydrocarbons. Eric(2004) described a process for the absorption and removal of hydrocarbon contaminated soil using powered cellulose containing essentially 3- 8% of ammonium sulphate biologically active media , which preferentially absorbed hydrocarbons in the presence of water and supports the growth of naturally occurring hydrocarbon reducing bacteria.

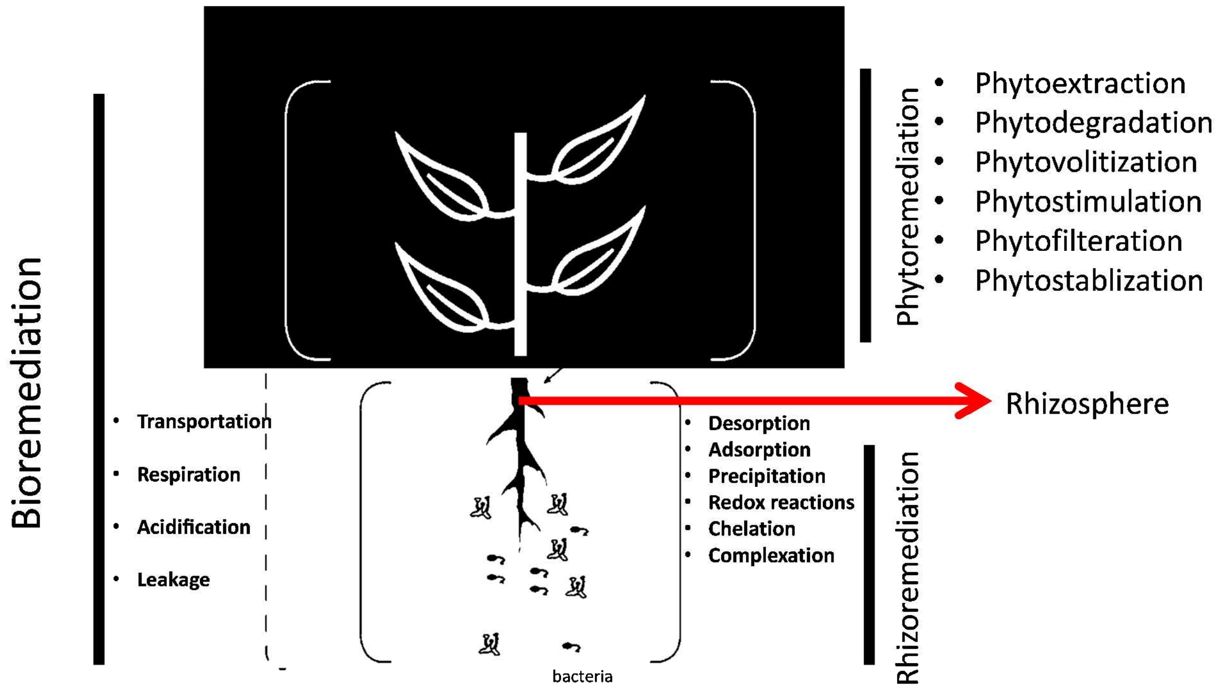

Fig 2. Sketch of plant involved in Remediation process

1.6.3. **Phytoremediation**

Plants are environments friendly and low cost remediation technique than some other physical and chemical remediation techniques (Glick, 2003; Huang *et al.,* 2004, 2005; Greenberg, 2006; Gerhardt *et al.,* 2009). Plant once grown over hydrocarbon stress environment may adapt a mechanism of tolerance to such contamination (Greenberg *et al.,* 2006; Abhilash, 2009). Some important mechanisms are biophysical and biochemical processes e.g. adsorption, transport and translocation, as well as transformation, degradation and mineralization of contaminants (Meagher, 2000).

Apart from plants tolerance to hydrocarbon contamination the deleterious effect of hydrocarbons to edible and non-edible parts of plants grown over hydrocarbon stress environment cannot be ignored. It has been previously reported that when diesel oil wasspilled over agricultural crops such as wheat or maize, it penetrates inside plant tissues rupture the cell membranes. It covers the stomata and reduces the transpiration rate hence anoxic conditions are created which effect the rate of photosynthesis and ultimately plant dies (Muratova *et al.,* 2008). Volatile oil is considered to be more toxic to sensitive plants than viscous oil (Panchenko *et al.,* 2002). This is more likely because volatile oil is composed of short chain hydrocarbons (nC_5 to $_{nC9}$) which are considered to be more toxic to germination than medium and long carbon chain hydrocarbons (nC_{13} to nC_{20} and nC_{20} to nC_{44})(Babu, 2014). Apart from aliphatic hydrocarbons plants can also degrade aromatic hydrocarbons present in the oil. The mechanism of degradation of polychlorinated biphenyls, polycyclic aromatic compounds, nitroaromatics, or linear halogenated hydrocarbons is not yet clear. Literature documented that degradation of tri-chloroethylene (TCE) (Gordon *et al.,* 1998); the explosives 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), glycerol trinitrate (GTN), and nitroglycerin and PCBs (Kuiper *et al*., 2004) was slightly different than *n*-alkanes. Uptill now the mode of degradation of aromatic hydrocarbon inside plant tissue has not yet been reported. However there are evidences in the previous literature that the pathways followed by microbes at soil-root interface are through oxidative cleavage of benzene ring in the presence of enzymes and oxygen (Patzelt 2007).

Plant root exudates releases enzymes and gel which increases microbial population and their activity in the rhizosphere thus accelerate the rate of degradation of hydrocarbons. However the release of root exudates primarily depends on the plant species. In contrast to alfalfa, the rate of degradation was enhanced in soils with ryegrass more likely because of increased release of succinate necessary for catabolic activity of microbes involved in the degradation of hydrocarbons (Phillips, 2008). Muratova *et al.,* (2009) concluded that the enhanced activity of oxidase, peroxidase and tyrosinase in the soil become contaminated with phenantherene and *Sorghum bicolor* (L.) Moench was grown over such soils may accelerate the degradation of phenantherene. Etienne *et al.,* (2014) reported that when plants were grown over hydrocarbon contaminated soils, root exudates accelerate the microbial activity and increase the degradation of hydrocarbons in oily sludge contaminated soils. Plant release enzymes in the soil when exposed to hydrocarbon contamination. Cytochrome P-450 is a heme protein detoxicating enzyme in plant which develops tolerance to xenobiotics, industrial chemicals and herbicides in plants once grown over hydrocarbon stress environment (Anzenbacher and Anzenbacherová, 2001; Ioannides and Lewis, 2004; Chuang *et al.,* 2012).

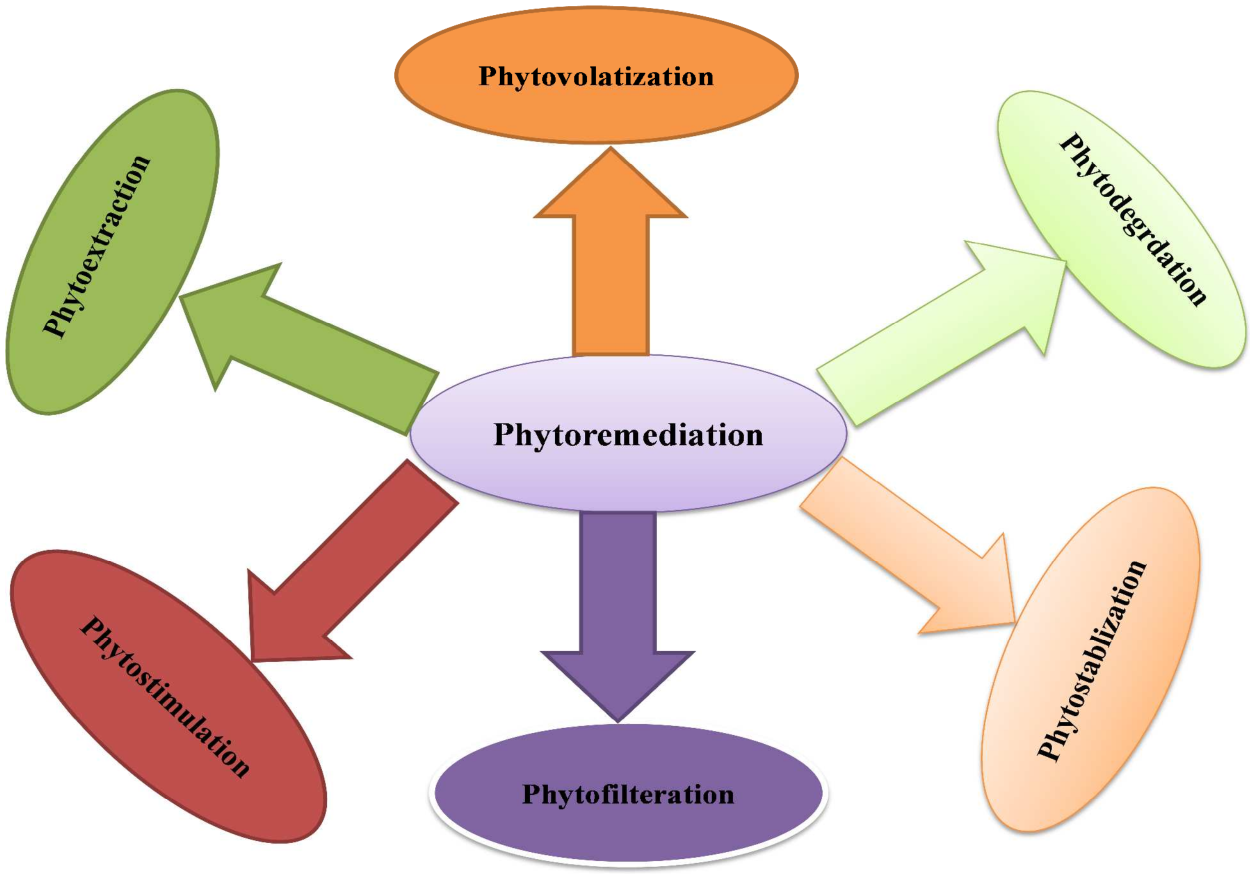

Fig 3. Schematic diagram of Phytoremediation

1.6.4. Rhizoremediaton :

Rhizoremediation includes both bioremediation and phytoremediation. Bioremediation means inoculation of oily sludge contaminated sites with hydrocarbon degrading bacterial consortium. In addition to that phytoremediation means to use hydrocarbon tolerant plants such as alfalfa, soyabean, perennial ryegrass, fescue or Kaller grasses or others to degrade hydrocarbons of oily sludge contaminated sites. Plants or rhizosphere will provide a unique environment for hydrocarbon degrading microorganisms to grow and increase in number and because of combined effect of release of root exudates (sugars, amino acids and organic acid etc) and microorganisms the rate of degradation of hydrocarbons is more rapid than in non rhizosphere environment of oily sludge contaminated sites. Through rhizoremediation natural rehabilitation process of degradation of oily sludge is more rapid than either through bioremediation or phytoremediation.

The rhizosphere of petroleum hydrocarbons contaminated soils provides a complex and dynamic environment for diverse genera of plant associated-hydrocarbon degrading microbial population to increase and degrade certain toxic hydrocarbons into biomass, CO_2 and H_2O through β-oxidation (Siddiqui and Adams, 2002; Ely *et al.,* 2008). The mode of degradation of various groups of hydrocarbons inside microbial body varies such as for isoalkanes the formation of a cyclic alcohol subsequently dehydrogenated to a ketone, whereas for aromatics the formation of a diol that spontaneously decays to catechol (Jones *et al.,* 2008). Numerous genera of aerobic microorganisms that occur in rhizosphere environment are reported in the previous literature among which the most common are *Corneybacterium spp, Pesudomonas methancia spp, Pesudomonas aeruginosa spp, Pesudomonas putida spp, Arthrobacter, Rhodochcous, Bacillus* (Nair *et al.,* 2008). Mishra *et al.,* (2001) isolated *Acinetobacter baumannii* capable of degrading straight chain hydrocarbons, *Burkholderia cepacia* can accelerate the degradation of aromatic and NSO compounds in oily sludge whereas *Pseudomonas has the capability to remove* asphaltene and alkane fractions *from oily sludge*. Bhattacharya*et al.,* (2003) isolated four standard bacterial strains,*Pseudomonas putida*MTCC 978, *Pseudomonas citronellolis*MTCC 1191,*Pseudomonas aeruginosa*MTCC 1034, and*Pseudomonas aeruginosa*MTCC 2642 from various soils become contaminated with oily sludge over the last 15 to 100 years. They found that strains accelerated the rate of degradation of hydrocarbons and total hydrocarbons were reduced to 25 to 4% in oily sludge over a period of a year. The concluded that the rate of degradation of aliphatic hydrocarbons in oily sludge once inoculated with hydrocarbon degrading strain was more rapid than aromatics.

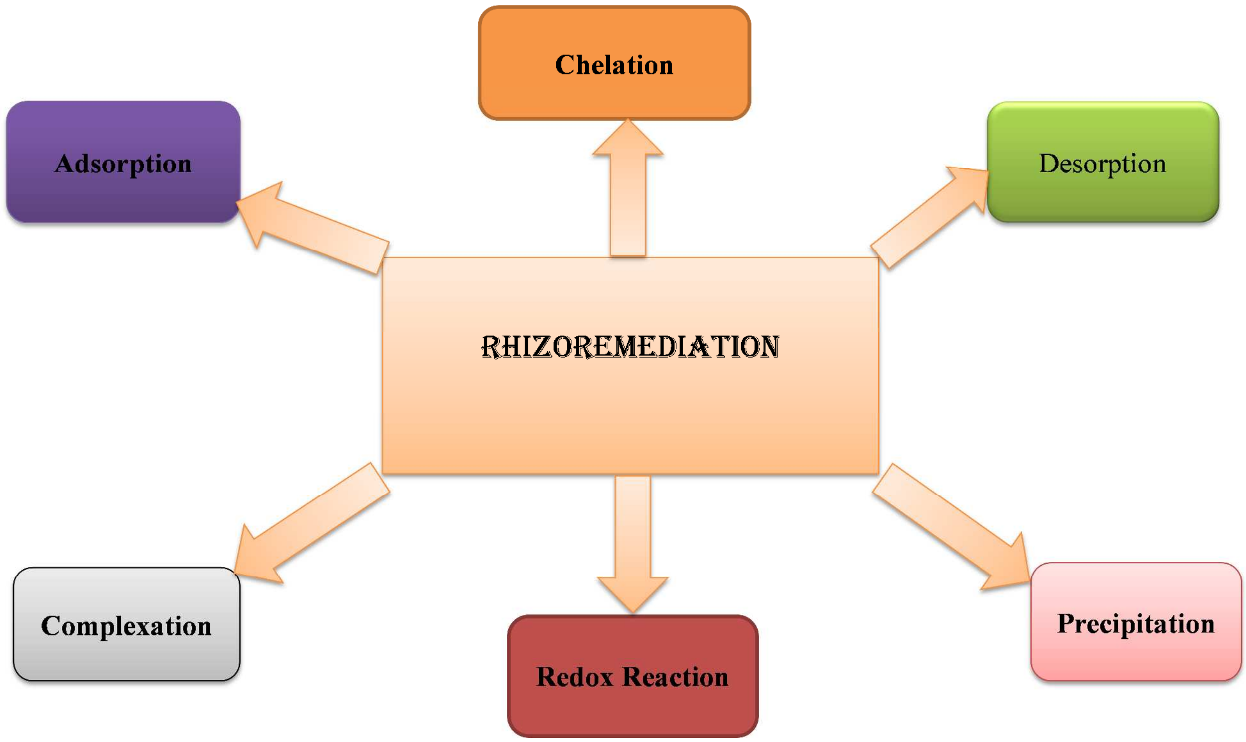

Fig 4. Schematic Diagram of Rhizoremediaton

1.6.4.1. Advantages of Rhizoremediation

Rhizoremediation is considered to be rapid, low cost, economic and environmentally acceptable technique than bioremediation or phytoremediation (Susarla *et al*., 2002, Pilon-Smits, 2005, Chaudhry *et al.,* 2005)Several other techniques such as conventional ex situ methods, soil excavation or incineration, disposal of contaminated soils away from the source, removing of organic contaminants in the laboratory through soil washing, and in situ capping or sealing of organic contaminants through stabilization are very expensive. The estimated cost of all ex-situ remediation techniques for large area of contaminated soil is between \$200–\$1500, whereas the cost for remediation of oily sludge contaminated soils is around \$10–\$50/ton through rhizoremediation (Pilon-Smiths*et al*., 2005 and Schnoor *et al*., 2008). Oil refineries are usually looking for low cost technique and rhizoremeidation is an economic and rapid technique therefore is considered to be best suitable to remediate oily sludge contaminated soils for oil refineries. Apart from low cost, rhizoremediation can enhance the oxygen and nutrient availability under oily sludge contaminated soils and improves the soil texture and structure. Use of plants can reduce the soil degradation and erosion. Rhizoremediation can be applied at any geographical area that can support plant growth. An additional advantage, albeit an unscientific one, is that there is high public acceptance for rhizoremediation which makes it an attractive option for industry and regulators.

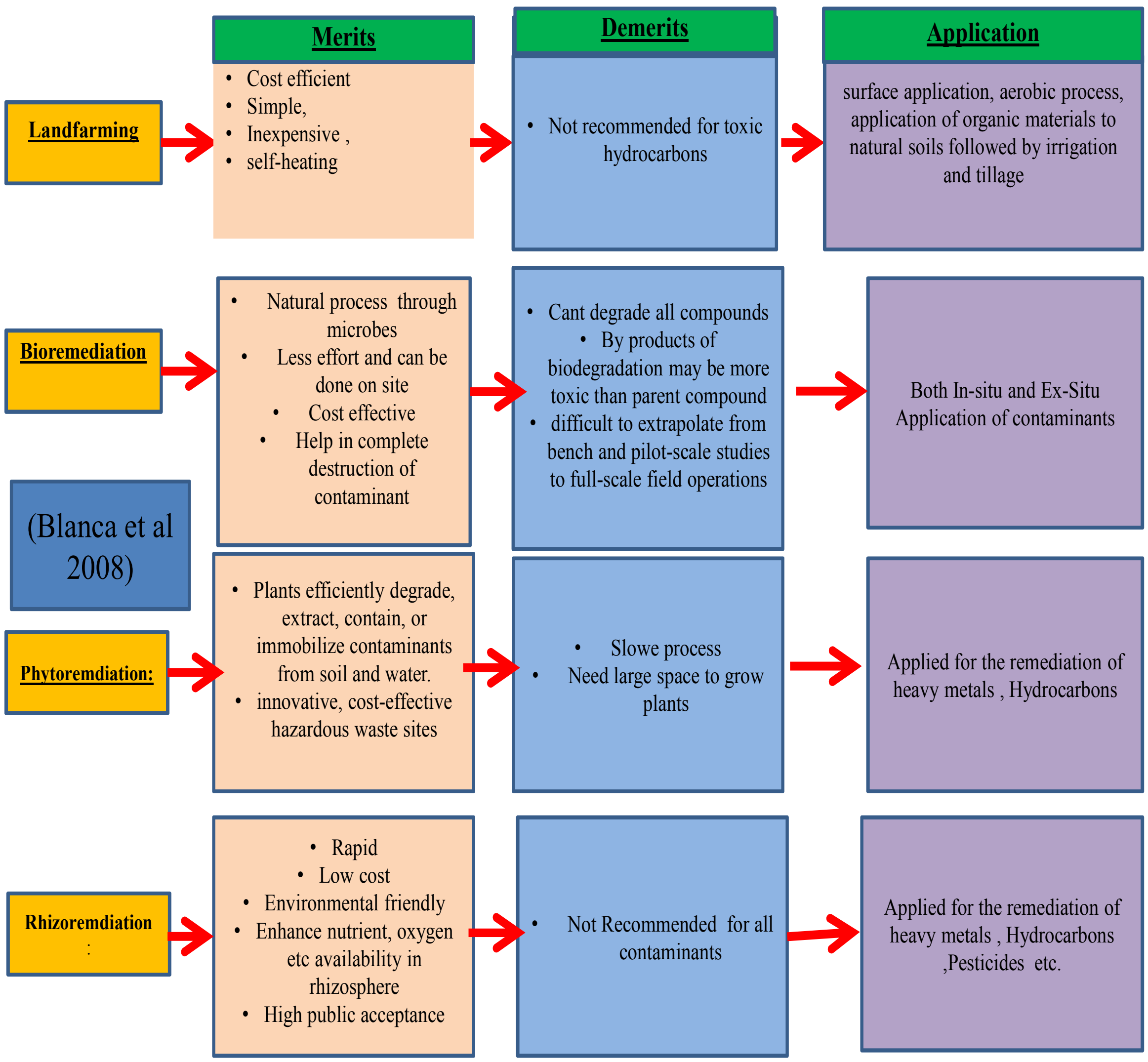

Fig 5. Flow diagram of degradation strategies

1.7. Oily sludge effect on plant metabolism

Indigenous microbial activity was reduced in oily sludge contaminated soils more likely because once microorganisms are exposed to hydrocarbon stress conditions they may develop a cyst around them and become protected from this new source of carbon

Nicholson *et al*., 2002; Takamatsu and Watabe, 2002). Therefore, specific genera of microorganisms survived under such conditions (Odu, 1989). It has been observed that under oily sludge contaminated rhizosphere hydrocarbons degrading microbes were increased in number which may accelerate the rate of degradation of such hydrocarbons in such environment (Siciliano *et al.,* 2003). Kirk *et al.,* (2005) found that increase in hydrocarbon degrading microorganism was observed in oily sludge contaminated soils in presence of alfalfa. This is more likely because of increase in root biomass which may result in tolerance to toxic hydrocarbons by alfalfa (Huang *et al.,* 2004). Merkl *et al.,* (2004) found that cereals such as wheat and beans are non-tolerant to oily sludge. Similarly, non-leguminous plants show lack of tolerance to oily sludge (Adam and Duncan, 2002). The antioxidant system and its importance for the acclimation of plants to contaminants have been reviewed precisely (Rennenber *et al.,* 2006),

Plants show stunted growth more likely because of anaerobic conditions and change in the membrane permeability. Inhibition to germination of grasses was commonly observed when grown under hydrocarbon stress environment Ekpo and Nwaankpa (2005).

Apart from organic compounds contamination oily sludge is a source of inorganic compounds contamination once enters in the soil. Nutrient imbalance in the oily sludge contaminated soils was also observed by Odjegbaand Atebe (2007). They reported that reduced growth of ***Amaranthus hybridus*** L is more likely because of low nutrient content in oily sludge contaminated soils. Availability of some major elements such as N, K, Mg, Na and P was increased in plants of oily sludge contaminated soils. Agbogidi *et al.,* (2001) and Sandalio *et al.,* (2001) found that high Cd content may leads to deficiency of N and P in plants more likely due to alterations in the antioxidant defense system.

Plant varies for their tolerance to hydrocarbon contamination such as grasses and leguminous plants are more tolerant to hydrocarbon contamination than non-leguminous plants (Kuiper *et al.,* 2001). This is more likely because of fibrous root system and more surface area is available for bacteria to grow and increase in number.

Huang *et al.,* (2004reported that when beans and wheat were grown over oily sludge contaminated soils the germination was inhibited. Adam and Duncan (2002) reported that volatile fraction of diesel causes Inhibition to germination of seed plants. Nonetheless diesel fuel contaminated treatments with minimal volatile diesel fuel components were never as high as the control germination results. This suggests that the influence of the volatile fraction of diesel fuel is not the only factor inhibiting seed germination. The remaining diesel fuel in the soil still had some level of toxicity to the germinating seeds

Bossert and Bartha (1985) also found that inhibition to germination was mainly because of short chain hydrocarbons. This is more likely because of rupture of cell wall of plants.

Poly aromatic hydrocarbons are considered to be phytotoxic. Nonetheless several plant species such as woody, herbaceous and grasses are considered to be more tolerant to PAHs contamination. Some tree such as poplar and jack pine, grasses such as rye, oat, wheat, and maize, as well as agricultural crops including sunflower, soybean, pea and carrot are tolerant to poly aromatic hydrocarbon contamination (Ke *et al.,* 2003; Liste and Alexander, 2000; Kulakow and Schwab, 2000).

Gao and Ling (2006) reported that the rate of degradation of phenantheren and pyrene was increased in soils with Amaranth, a selenium accumulator plant was grown over such soils.

Plants once exposed to hydrocarbon contamination may undergo some biochemical and physiological changes of plants than morphological changes (Mok and Mok, 2001). However changes in enzyme regulation mechanisms involved in the growth and development of plants are also observed under stress environment. Mok and Mok (2001) reported changes in phytohormone levels controlled by the respective hormone system are essential steps in assimilation of the plant under stress environment.

With the release of root exudates the microbial activity was enhanced in the rhizosphere hence the rate of degradation of poly aromatic hydrocarbons was increased. Muratova *et al.,* (2003) performed a study to understand the difference in the response of indigenous microbial population between the alfalfa (*Medicago sativa*) and reed (*Phragmites australis*) in rhizosphere and bulk soil impregnated with hydrocarbons over the last several years. Furthermore the tolerance of alfalfa (*Medicago sativa*) and reed (*Phragmites australis*) to hydrocarbon contamination was also studied. They concluded that total microbial population reduced in the bulk soil than alfalfa (*Medicago sativa*) and reed (*Phragmites australis*) rhizosphere impregnated with hydrocarbons. Furthermore the rate of degradation of hydrocarbon by indigenous microorganisms was more in the alfalfa (*Medicago sativa*) rhizosphere than reed (*Phragmites australis*) rhizosphere impregnated with hydrocarbons. They also reported some structural changes in the microbes in the alfalfa (*Medicago sativa*) rhizosphere than reed (*Phragmites australis*) rhizosphere impregnated with hydrocarbons.

Plant root exudates contain enzymes and gel which increases microbial population and their activity in the rhizosphere thus accelerate the rate of degradation of hydrocarbons. However the release of root exudates primarily depends on the plant species. Such as alfalfa releases malonate, hence because of absence of microbes necessary for catabolic plasmid transfer for degradation of hydrocarbons. In contrast to alfalfa, the rate of degradation was enhanced in soils with ryegrass more likely because of increased release of succinate necessary for catabolic activity of microbes for degradation of hydrocarbons (Phillips, 2008).

1.8. Antioxidant system of plants aWhen plants are grown over oily sludge contaminated soils the effect of oily sludge on their physiology is not well understood. There are evidences in the previous literature that oily sludge contaminated soils usually deficient in oxygen and nutrients but when plants are grown over such soils they may suffers from various stresses the most commonly observed stress is changes in their defense mechanisms towards hydrocarbon contamination. Meyer (2008) and Romero-Puertas *et al.,* (2007) reported that when plants are exposed to stress environment their natural mode of metabolism of reactive oxygen species such as superoxide radical O2 and H2O2 and hydroxyl radical (-OH) within plant tissues is effected because of this increase in the ROS concentration is observed which directly causes damage to the cell membrane because of lack of oxygen and the message carried by ROS towards plants development and defensive mechanism is retarded thus plant antioxidant system is damaged. Foyer *et al.,* (2005) reported that the activity of superoxide dismutase (SOD) is reduces under stress conditions which may leads to the reduction in the release of Glutathione (GSH) and ascorbate (ASC) thus plant oxidative defense system is disturbed.

Very little information is available about the response of anti-oxidative system of plants under hydrocarbon stress environment. The most recent study was performed by Martía *et al.,* (2009) who reported that inhibition to germination of alfalfa was observed under oily sludge stress environment which might be because of lack of oxygen which has increased the protein oxidation and disturbed the balance of superoxide dismutase isoenzymes, peroxidase, and those enzymes involved in the ascorbate–glutathione cycle showed significant activity increases, parallel to an enhancement of total homoglutathione, allowing plants being tolerant to this situation. Nwaogu and Ukowundu (2010) found that the concentration of ascorbic acid and β-carotene was less in *Psidium guajava* grown over petroleum contaminated soils. However no information about the effect of inoculation of plant growth promoting rhizobacter to hydrocarbon contaminated soils over which plants are grown on antioxidant system of plants has been reported previously.

1.9. Plant microbe interaction involved in bioremediation

The mutual exchanges between plant and microbes results in increased nutrient availability in the rhizosphere (Imran *et al*., 2014), enough carbon sources are available for indigenous microbial population to grow and increase in their numbers (Heinonsalo *et al.,* 2000), increased release of root exudates necessary for the degradation of hydrocarbons in the rhizosphere impregnated with hydrocarbons (Hou *et al.,*2001) enhanced microbial activity (Bank *et al.,* 2003) may accelerate the fate of hydrocarbons degradation in the rhizosphere. Moreover, the mutual exchanges between plants and microbes is effected by the amount and the type of hydrocarbons (Mezzari *et al*., 2011) and the period of exposure to the contamination by plant species either grasses or leguminous or herbaceous (Siciliano *et al.,* 2003; Parrish *et al.,* 2004; Liste and Prutz, 2006;; Phillips, 2008; Rezek *et al.,* 2008).

In rhizospheric environment of hydrocarbon contaminated sites, plants because of detoxification usually develop tolerance under hydrocarbon stress condition in rhizospheric soils. This suggests that plants adapt a mechanism to degrade toxic hydrocarbons into non-toxic intermediate products by the action of enzyme or in the presence of root exudates inside or outside their tissue. This confirms that plants undergone some physiological changes when utilizing hydrocarbons as a food source than plants growing under non rhizophere environment of uncontaminated sites.

Some information's are available in the previous literature in which the fate of degradation of hydrocarbons by plants through phytodegradation process has been reported (Mougin 2002; Newman and Reynolds, 2004). They found that only 2% of radiolabelled anthracene was degraded by soybean. They concluded that the degradation of radiolabelled anthracene is more likely occurred because of catabolized by soybean plants. Moreover the rate of degradation and the by-products of degradation of radiolabelled anthracene were not studied. Like Edwards and others studies are performed at laboratory scale, soil used was not sterilized and the increase in the population of microbes was not reported. The other factors which facilitate the process of hydrocarbon degradation had not been discussed in detail and similarly the interaction of microbes in the degradation process has been ignored. The amount of isotope-labeled anthracene extracted from the soil after inoculation was subtracting from the anthracene extracted from the root and leaves. The difference between the concentrations of anthracene obtained from the total concentration of anthracene in the soil from the concentration of anthracene in the root was used to evaluate the uptake and translocation of anthracene. Balasubramaniyam (2012) reported that once *fescue arundinacea* was grown over clay soils with low organic matter impregnated with naphthalene develops tolerance because of combined synergistic interactions and integrated redox system resulted in a detoxification mechanism. It is well accepted in the previous literature that once hydrocarbons enters in a soil a portion of such hydrocarbons is sorbed on the roots hence become unavailable for uptake by roots. Gao and Ling (2012) reported that clover (*Trifolium pretense* L.) and hyssop (*Hyssopus officinalis* L.) grown well in the bulk than rhizosphere soil impregnated with poly aromatic hydrocarbons over the last several years. They concluded that this is more likely because of the sorption of such hydrocarbons by clay rather than of detoxification mechanisms for uptake of such PAHs by grasses.

1.10. Mechanism involved in Rhizoremediation

The importance of plant microbe interaction in the remediation of oily sludge contamination was confirmed in studies at the level of rhizosphere (Gerhardt *et al*., 2009 and Ho *et al*., 2007), the phyllosphere and inside the plant (Kidd *et al*., 2008 and Sandhu *et al*., 2007). Rhizoremediation is considered as the most potential approach for hydrocarbons remediation in oily sludge (Mohan *et al*., 2006). Soil microorganisms play key role in rhizoremediation of xenobiotics (Barac *et al*., 2009) The interaction among microbial degrader, plant and hydrocarbons in oily sludge might be regulated through rhizosphere processes (Ma *et al*., 2009) Rhizoremediation systems for hydrocarbons rely on a beneficial interaction between suitable plants and their root associated bacterial populations(Barac *et al*., 2009). Degradation was assisted through a rhizosphere effect where plants root exudates containing organic compounds increase the density and activity of potential hydrocarbon degrading microorganisms in the rhizospheric zone, surrounding the roots (de Carcer *et al*., 2007) The biodegradation abilities of bacteria and the expression and maintenance of bacteria in the rhizosphere are extremely important for the effective removal of contaminants in rhizoremediation (Phillips *et al*., 2012). The bioremediation, phytoremediation and rhizoremediation contribute significantly to the fate of toxic contaminants and can be used to remove these unwanted compounds from the rhizosphere (Ma *et al*., 2011). Plant beneficial bacteria, such as rhizospheric bacteria have been shown to contribute in the bioremediation of toxic hydrocarbons in oily sludge and might have ability to improve remediation potential of plants (David *et al*., 2009). Other important mechanisms include direct phytohormonal action, increase of plant nutrient availability and the enhancement of other plant beneficial microorganisms during rhizoremedaiton process(Dodd *et al*., 2010). Under suitable rhizospheric conditions isolated strain can be introduced together with a suitable plant, which inhabits on the root along with indigenous population, thereby enhancing the bioremediation process (Bisht *et al*., 2010). Moreover, these capabilities for root colonizing, pollutant degrading bacteria utilize the growing root system and hence this acts as an injection system to spread the bacteria through soil (Harms *et al*., 2006). Plant root accomplished certain specialized roles such as ability to synthesize, to accumulate and to secrete a diverse array of nutrient compound consequently no requirement of exogenous carbon source, roots may regulate the soil microbial community in their immediate vicinity, cope with herbivores, encourage beneficial symbioses, change the chemical and physical properties of the soil and inhibit the growth of competing plant species Walker *et al*., 2003) A effective rhizoremediation mehtod could depend on the highly branched root system of the plant species where a large number of bacteria harbor, establishment of primary and secondary metabolism, survival and ecological interactions with other organisms .Plant roots can performed as an alternative for the tilling of soil to incorporate additives (nutrients) and to improve aeration in soil.

Summarizing the rhizoremediation, the process is assisted through a rhizosphere effect where plants root exudates containing organic compounds increase the density and activity of potential hydrocarbon degrading microorganisms in the rhizospheric zone, surrounding the roots. Other important mechanisms include direct phytohormonal action, increase of plant nutrient availability and the enhancement of other plant beneficial microorganisms during rhizoremedaiton process. Moreover, these capabilities for root colonizing, pollutant degrading bacteria utilize the growing root system and hence this acts as an injection system to spread the bacteria through soil. Plant root accomplished certain specialized roles such as ability to synthesize, to accumulate and to secrete a diverse array of nutrient compound consequently no requirement of exogenous carbon source, roots may regulate the soil microbial community in their immediate vicinity, cope with herbivores, encourage beneficial symbioses, change the chemical and physical properties of the soil and inhibit the growth of competing plant species

1.11. Plants suitable for rhizoremediation

Research on phytoremediation, through trial and error, has focused on densely rooted, fast growing grasses and plants, such as Brassica sp., with fine root systems. Mulberry (Morus alba L.) and poplar (Populus deltoides) trees have been used successfully in the rhizoremediation of chlorophenols and chlorinated solvents such as trichloroethylene (TCE) Various grass varieties and leguminous plants have shown to be suitable for rhizoremediation (Kuiper *et al*., 2001, 2004). Shahzad *et al*., (2016) reported maize with bacterial consortium significantly reduced hydrocarbons in oily sludge at very short period of time. Similarly Bano *et al*., (2015) also reported beneficial interaction of alfalfa plant with bacterial strains in hydrocarbon degradation. Kala 2014 also reported several plants species(Sugercane, rice, alfalfa, rye grass, maize, wheat and grasses) that found suitable for rhizoremdeation of different contaminants

1.12. Genes involve in degradation of Hydrocarbons:

The genes encoding enzymes which are located on chromosomal or plasmid DNA are well documented in the literature as expression of gene capable of degrading hydrocarbons under aerobic conditions (**Peixoto** *et al*., 2011)).

Several novel techniques are used at laboratory scale to isolate the enzyme encoding genes has been developed such as screening for a specific gene or activity of interest, gene quantification, and DNA and mRNA sequencing. Each of the above mentioned techniques has been reported with success when applied to hydrocarbon contaminated soils (Jeon *et al.,* 2003; Witzig *et al.,* 2006; Lorenzo 2008). The *n*-Alkanes are saturated hydrocarbons and are the dominant hydrocarbons in oily sludge (Ulrik**e** *et al.,* 2015). The *n*-Alkanes act as a source of carbon and energy to alkane-utilizing bacteria (Wentzel *et al* 2007, Aislabie *et al*., 2012). The *n*-Alkane utilizing bacteria usually have *Alk* enzyme systems which possess the metabolic pathways for the degradation of alkanes (Rojo, 2009). The functional Alk enzyme system contains the trans membrane alkane monooxygenase *AlkB* which is encoded by the *alkB* gene and involved in the initial activation step of aerobic aliphatic hydrocarbon metabolism. Bacteria that can own the Alk enzyme system are valued in environmental bioremediation and biocatalysis for the synthesis of industrial compounds, including drugs, pravastatin, and other compounds (Koch *et al.,* 2009)

The aerobic bacterial catabolism of aromatic compounds involves a broad diversity of peripheral pathways that activate structurally diverse substrates into a limited number of common intermediates that are further cleaved and processed by a few central pathways to the central metabolism of the cell (Carmona *et al.,* 2009). The intermediates of these metabolic pathways can be catalyzed by two different kinds of enzyme, intradiol and extradiol dioxygenases, which symbolize two classes of phylogenetically different proteins (Jouanneau, 2010)

The degradation pathways in Aerobic microorganisms generally start with the activation of aromatic nucleus through oxygenation reactions. Some central intermediates such as catechols, protocatechuates, gentisates and (hydroxy)benzoquinols, are produced by the introduction of hydroxyl groups, usually in ortho- or para-position to one another (peripheral reactions). All these intermediates are subject to oxygenolytic ring cleavage followed by channeling of the ring- cleavage products into the central metabolism. Otherwise aromatic hydrocarbons, even under aerobic conditions, can be metabolized through the corresponding CoA thioesters and subject of non-oxygenolytic ring cleavage.

Conclusions:

The success of rhizoremediation in the studies reported in this review suggests that selection of microbial genera and plant species is a prerequisite prior to develop their combination to degrade toxic hydrocarbons into intermediate products under such rhizosphere. However there is a debate on whether a single or multiple genera of microbes is responsible for degradation of hydrocarbons in oily sludge contaminated soils alone or in close interaction with plant species. Role of plant species in the degradation of hydrocarbons cannot be ignored. Nonetheless the mode of degradation of hydrocarbons by plant species needs to be examined at laboratory scale in future. Furthermore the closer study of the genetic makeup responsible to degrade hydrocarbons both in microbes and plants is required. The selection of plant species, microbes and release of root exudates under oily sludge contaminated soils needs to be address in detail in future.

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