AN ARTICULATED OVERVIEW ON DEVELOPED TRENDS IN BIOREMEDIATION OF COMPLEX HYDROCARBON CONTAMINATION

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ABSTRACT

Fuel is a major source of energy for sustenance of human life and this energy is obtained in the form of crude from the earth's crust. In order to obtain energy from earth's crust, it is recovered from the ground followed by drilling, sucking and extracting from underground. The contamination of fertile land occurs due to anthropogenic activities such as the spillage of crude after the decommissioning of the oil recovery machinery after recovery process, while recovering oil through artificial lift or naturally, transportation, etc. This leads to the exploitation of natural resources. This can be reclaimed again into clean and fertile land by giving proper remediation treatment to soil that is contaminated with Poly Aromatic Hydrocarbons. The method of reclamation involves different kinds such as, physical, chemical and biological methods. The biological method again involves several of its types and kinds. It was proved in literature that the biological method is the most reliable and environment friendly hence acquired considerable significance. Bioremediation is a technique that uses non-pathogenic environment friendly microorganisms to remediate contaminated soil. The significance of bioremediation is stated by certain observations resulted from experiments that are carried out in literature. The effect and applications are observed from studies till date are briefly articulated in current document.

Keyword: - *Bioremediation, biodegradation, complex hydrocarbons, microorganism, indigenous bacteria, crude oil, PAHs, biological methods.*

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1. INTRODUCTION

The discovery of crude oil has changed man's way of life considerably. It has improved our life economically and has also led to the availability of quick and accessible source of energy. Fuel is a major source of energy for human life and this fuel is obtained in the form of crude from the earth's crust. To obtain fuel from earth's crust, drilling and pumping and extracting are carried out from underground. Crude oil is obtained below the subsurface of the earth. It generally occurs at depths below 1500 meters and is recovered through boreholes within the earth. The liquid and gaseous phases of crude oil occur naturally under- ground, within pore spaces of sedimentary rocks. Crude oil occurs naturally in many parts of the world, particularly in the USA, Russia, Iran, Iraq, Kuwait, Saudi Arabia, Persia, Mexico, Romania, Libya, India and Nigeria. Crude oil, or petroleum, is a complex mixture of hydrocarbons with varying molecular weight and structure. It constitutes of three main chemical groups, that are; paraffinic (aliphatic), naphthenic (alicyclic) and aromatic. These hydrocarbons range from simple, highly volatile substances to complex waxes and asphaltic compounds which cannot be distilled [6]. The major problem associated with crude oil exploration is the pollution/contamination of the environment. Crude oil contamination is a global phenomenon affecting all aspects of the environment. Cases of crude oil contamination of the soil have been documented [6]. Crude oil contamination has been an long term phenomenon and possess threat to environment.

The contamination of fertile land occurs due to the spillage of crude after the decommissioning of the oil recovery machinery after recovery process. This leads to the exploitation of natural resources.

The effect of oil on soil depends on the size, quantity and grade of oil spilled. Crude oil contamination does not cause permanent damage to the soil instead of has some adverse effects on crops and other vegetation. Since years attempts have been made to find the cheapest, most efficient and environment friendly method for reclaiming crude oil contaminated soil [6]. Costly damages have been caused on coastal lines in different parts of the world by offshore oil spills [4]. Crude oil comes into contact with the soil naturally through natural oil seeps or man-made through accidental or deliberate spills and leakages such as intentional or accidental bursting of pipelines [4]. Over the years, several methods have been devised for the cleanup of crude oil contaminated soil using physical, chemical, thermal and biological treatments. This can be reclaimed again into clean and fertile land by giving proper remediation to soil after recovery process is done [8][5]. This method of reclamation involves different kinds such as, physical, chemical and biological methods. The biological method again involves several of its types and kinds. Crude oil contaminated soil can be cleaned up using physical, chemical, thermal and biological treatments [8]. The physico-chemical methods have been found to be approximately inadequate and ineffective, and may result in further contamination. Biological methods including remediation through plants and microorganisms have received considerable attention during the last few years as the most promising and environmentally benign technique for effective clean-up of crude oil contaminated soil. A wide range of bioremediation strategies is being developed to treat contaminated soil [8][51].

2. BIOREMEDIATION – AN EFFICIENT TECHNIQUE

Bioremediation is one of the processes of reclamation that uses microorganisms, non-pathogenic bacterias, fungi and green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Biodegradation generally refers to the breakdown of organic compounds by living organisms eventually resulting in the formation of carbon dioxide and water or methane. It is one of the reclamation techniques.

2.1 Nutrient availability and reaction mechanism

Each and every living organism requires carbon and with addition to this, the macronutrient like nitrogen and phosphorous is also needed by bacteria to ensure effective degradation of the oil [9]. The nutrient balance required for hydrocarbon remediation is Carbon: Nitrogen: Phosphorus which equals 100:10:4 that is in general, at least 1 ppm of ammonium nitrogen and 0.4 ppm of orthophosphate needs to be present [1]. Enzymes are produced by microorganisms in the presence of carbon sources which are responsible for attacking the hydrocarbon molecules. There are different enzymes and their metabolic pathways that are involved in the degradation of petroleum hydrocarbons [7].

2.2 Bioremediation strategies

Bioremediation involves different strategies and methods for its enhancement and applications as mentioned below:

- **Bioaugmentation:** Bioaugmentation is the addition of a group of indigenous microbial strains or genetically engineered microbes to treat the contaminated soil [5][3].
- **Biostimulation:** Biostimulation is the process of adding nutrient, electron acceptor and oxygen to stimulate existing bacteria [5][3].
- **Biosparging:** Biosparging is a method that involves injection of air at a pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in saturated zone by increasing the contact between soil and groundwater [3].
- **Bioventing:** Bioventing is a promising new technology that uses low air-flow rates to provide only enough oxygen to sustain microbial activity [3].

2.3 Factors affecting bioremediation

Temperature, soil aeration and pH are environmental factors that affect degradation rate and may be modified to enhance degradation. The presence of contaminants such as metals and cyanides which are toxic to microorganisms, may hinder degradation. The other factors are enlisted as: [2]

- Properties of contaminants.
- Toxic level of contaminants.
- Microbial degradation of pollutants.
- Environmental factors affecting biodegradation.
- Surfactant addition.

2.4 Merits and economical feasibility of bioremediation

Bioremediation has advantages of being a natural process and eco-friendly as it does not involve any use of synthetic chemicals. It reduces the toxic level of pollutants and reclaims into harmless environment by complete decomposition of contaminants. It also requires less effort and economically feasible. The limitation is time dependent and it is creepy process. Detailed description of its advantages given in Shilpi et. al. [3]. It can be easily implemented at low cost and any location by simple in application. As there is no residual treatment is required, hence eliminating additional expenditures making it more cost effective.

3. ADVANCEMENTS IN BIOREMEDIATION

There are much more advancements implemented in bioremediation followed by enhancing different methods of bioaugmentation, biostimulation, etc. various approached towards innovative application of nutrients and monitoring metabolic pathways has been discovered. Also developments by genetically monitored microbes and technological involvement in natural process have been incorporated through these years. These are manifested by followed table.

Table -1: The developments and advancements in bioremediation till date is enlisted.

Pub. Year	Thrust area of research	Experimental basis	Contaminant	Treatment option	Concluded	Ref.
1990	Approach to Bioremediation of contaminated soil.	Soil	Organic contaminants	Indigenous microbes	Enhanced physical, chemical and biological methods of bioremediation discussed.	[44]
1990	Effect of Bioremediation on PAHs residues in soil.	Soil	PAHs	Indigenous microorganisms	The contamination was removed by 12 weeks.	[61]
1990	Microbial degradation of hydrocarbons in environment.	Soil	Hydrocarbons	Microbes, fungi	The adaptation of DNA for genetic modification of microbes for scale-up has been discussed.	[30]
1993	Bioremediation of soil contaminated with PAHs – review.	Soil	PAHs	Microorganisms	The importance of full site assessments and treatability studies for successful application in field emphasized.	[51]
1996	Bioaugmentation as soil bioremediation approach.	Soil	Hydrocarbon pollutant	Bioaugmentation	Merits of Bioaugmentation discussed and	[50]

					significance is reviewed.	
1996	Bioremediation of oil contaminated soil in Kuwait (I) by landfarming.	Soil	Crude oil, PAHs	Landfarmin g	The contamination was reduced more than 80% within 15 months along with substantial PAH degradation.	
1997	Bioremediation processes for treatment of PAH contaminated sediments	Soil and aquatic sediments	PAHs	Indigenous microbes	Reviews several factors that currently complicate the implementation	[26]
1998	Microbiological methods for feasibility assessments and field evaluation.	Ex-situ applicatio n	Oil contaminated soil	Microorgan isms	It is cost effective and environmentally sound but has environmental impacts on rate limiting factors that should be optimized.	[14]
1999	Probe of enhanced bioremediation through addition of macro and micro nutrients.	Soil	PAHs	Low level macronutri ents and high level micronutrie nts with phosphorou s	Bioactivity of the foreign consortium was the greatest when a high level of micronutrients was used.	[10]
1999	Bioremediation of phenols and PAHs in creosote polluted land.	Soil	Creosote, PAHs, phenols	Indigenous microorgan isms, Ex-situ treatment by slow fertilizer addition, aeration, soil mixing	Extensive degradation was apparent with the 2- and 3-ring PAH, with decreases of 97% and 82%, and higher molecular weight 3- and 4-ring PAH were degraded at slower rates, with reductions of 45% and 51%, six-ring PAH were degraded with average reductions of 35%.	[25]
2000	Review on microbial degradation of benzo(a)pyrene.	Soil	HMW-PAHs, Benzo (a) pyrene	Microbes, fungi, algae	Occurrence of Benzo(a)pyrene and its degradation along with pathway is addressed.	[28]
2000	Metabolism of alkylbenzenes, alkanes, hydrocarbons in anaerobic bacteria.	Soil	Hydrocarbons	Anaerobic bacteria	The bacterial metabolism to paraffins, naphthenes and PAHs has been discussed and reviewed.	[11]

2002	Isolation and optimization of PAH degrading bacteria.	Liquid medium	Phenanthrene	Isolated PAH degrading bacteria	Isolated Burkholderia cocovenensus BU-3 demonstrated to be feasible strain for degradation of phenanthrene at neutral pH upto concentration of 1000 mg/l.	[52]
2002	Environmental pollution by PAHs and bioremediation	Soil	Organic contaminants	Indigenous microbes	PAH degradation and bioremediation efforts discussed.	[42]
2004	Evaluation of chemical pretreatment of contaminated soil for improved PAH remediation.	Soil	PAHs	Chemical biosurfacta nts	The efficiency of fenton oxidation may decrease when surfactants are added with fenton's reagents to contaminated soil.	[40]
2005	A review on current trends and future knowledge of PAHs bioremediation.	Review	PAHs	Bacterias, fungi	Describes the major aerobic and anaerobic breakdown pathways, and highlights some of the bioremediation technologies.	[15]
2006	Bioremediation technologies and strategies to enhance process efficiency.	Soil	PAHs	Indigenous microbes	Recent trends developed in bioremediation are reviewed and merits discussed.	[37]
2007	Clean up of crude oil contaminated soil.	Soil	Crude oil	Indigenous microbes, Chemical, Physical, Thermal, etc.	Biological methods stated to be more efficient compared to other techniques and physic-chemical methods.	[6]
2008	Overview of ex-situ decontamination techniques.	Soil	Hydrocarbon contaminants	Physical, Chemical, Thermal, Biological	Physical, chemical Thermal and biological methods are compared and discussed for ex-situ and In-situ treatments.	[8]
2009	Ex-situ bioremediation of oil contaminated soil.	Soil	Diesel oil and fuel oil	Indigenous microorgan isms	70% and 63% reduction observed in diesel and fuel oil. Residual TPH reduced to 500 mg/kg dry soil.	[31]
2009	Composition of microbial consortium in bioremediation	Soil	Petroleum hydrocarbons	Actinomyc yte	After 5-5 month treatment, TPH reduced to 89%.	[36]

	of crude oil contaminated soil.			Norcardia		
2009	Effects of soil amendment with different carbon sources for bioremediation.	Soil	PAHs	Indigenous microorganisms, sodium succinate, glucose, starch.	Optimizing the carbon source ratio, soil moisture and aeration is a feasible remediation strategy.	[45]
2009	Bioremediation of oily sludge contaminated soil by stimulating indigenous microbes.	Soil	PAHs	Indigenous microorganisms	Biostimulation by addition of manures increased degradation by 58.2% in 360 days and improved soil phisico-chemical characteristics, also reduce toxicity.	[57]
2010	Laboratory scale bioremediation of oil contaminated soil.	Soil	Petroleum contamination	Indigenous microbes and Pseudomonas aeruginosa strain	94% reduction in n-alkanes, 79% reduction in PAHs after 191 days of treatment.	[29]
2010	Comparison of different Bioaugmentation and Biostimulation treatments.	Soil	Crude oil	Indigenous bacteria using peanut hull powder	Removal of TPH ranging from 26% to 61% after 12 week treatment.	[53]
2010	Characterization of isolated indigenous petroleum degrading bacteria.	Soil and waste water	Crude oil, petroleum	Indigenous, Bacillus spp.	7 strains degraded crude oil by 87.5% and 11 strains could degrade diesel oil by 70%.	[54]
2010	Microbial degradation of petroleum hydrocarbons overview.	Soil	Crude oil, Petroleum hydrocarbons	Indigenous microbes	Effects of bioremediation and its advantages and degradation under different ecosystems along with merits of genetically modified bacteria.	[20]
2010	Successful application of novel microbial technology in SRP wells of Mehsana.	Soil	Crude oil, wax	PDS-10, mixed culture	PDS-10 is thermophilic, capable of degradation up to 90°C.	[16]
2010	Bioremediation of crude oil contaminated soil by	Soil	Crude oil	Fertilizer induced	Application of fertilizer has increased the degradation and lead to	[19]

	application of fertilizers.			microbes	greater rates of biodegradation of complex hydrocarbons.	
2010	Bioaugmentation effects by Paracoccus sp. Strain in soil microbial community and degradation.	Soil	Aged PAHs	Paracoccus sp. Strain HPD-2	The degradation percentage of 3, 4, 5-ring PAHs was 35.1%, 20.7%, and 24.3%.	[46]
2011	Bioremediation by composting and influence of bioaugmentation and biostimulation.	Soil	PAHs	White rot fungi, organic municipal solid waste microbes, rabbit food.	89% of PAHs degraded at the end of composting period. The stable compost has greater potential compared to non-stable co-substrates.	[43]
2011	Bioremediation of phenanthrene using adapted microbial consortium.	Soil	Phenanthrene	B. cereus, Spingobacterium spp., Achromobacter Insolitus	100%, 56.9% and 25.8% degradation at 100 mg/l, 250 mg/l and 500 mg/l within 14 days.	[27]
2011	Isolation of indigenous soil bacteria for bioaugmentation of PAH contaminated soil of Patagonia.	Liquid medium	PAHs	Sphingomonas paucimobilis 20006FA	The strain 22B is the most suitable strain for bioaugmentation in PAH contaminated soils of Central Patagonia.	[33]
2011	Biodegradation of bonny light crude oil by bacteria isolated from contaminated soil.	Liquid medium	Bonny light crude oil.	Psedumonas Putida C1 and P. Aeruginosa K1	90% - 92% of crude oil degraded after 21 days of incubation.	[42]
2011	Microbial communities to mitigate contamination in soil- a review on possibilities and challenges.	Soil	PAHs	Genetically Engineered Microorganisms, biosurfactants	Discussed the biotechnological possibility of genetically modified bacteria for degradation.	[22]
2011	Bioremediation of petroleum oil contaminated soil and water – review	Soil	Hydrocarbons, waxes, etc.	Indigenous bacterias	Merits and acceptance of bioremediation is discussed.	[59]
2012	PAH degradation and naphthalene metabolism in streptomyces	Soil	Petroleum and PAHs	Streptomyces sp. Isolated from oil	Streptomycin isolates are able to degrade PAHs and 98.25% removal for diesel oil 99.14%	[12]

				contamination.	naphthalene and 17.5% phenanthrene in 7 days.	
2012	Bioremediation of high molecular weight PAHs with co-contamination with metals.	Slurry, liquid medium	PAHs with metals in liquid and soil slurries	Mixed culture consortium -5 isolated from gas plant	This consortium shows great potential for degradation of HMW-PAHs at field scale.	[47]
2012	Bioremediation of PAH contaminated soil by bacterial consortium.	Soil	PAHs	Sphingobacteria and proteobacteria, Indigenous	20.2% to 35.8% of PAH removed by addition of 10% to 20% bacterial consortium.	[35]
2012	Bioremediation of PAHs by immobilized bacteria technique.	Soil	PAHs	P. putida and Indigenous bacteria	The immobilized microorganism technique, enhanced removal of PAHs.	[35]
2013	Enzymatic bioremediation of PAHs.	Soil	PAH	Fungal consortia	Lipases from fungal consortia can be used in bioremediation of motor oil, petrol, diesel, kerosene, greese.	[13]
2013	Ex-situ bioremediation of crude oil contaminated beach sand.	Beach sand	Crude oil	Nutrients and ramnolipids	97% alkanes were degraded and 95% PAHs were degraded with 45 days. The addition of nutrients enhanced activities of indigenous microorganisms.	[38]
2013	Naphthalene and crude oil degradation by biosurfactants producing streptomyces spp. isolates.	Soil	Naphthalene and crude oil	Streptomyces spp.	82% to 81% of naphthalene removal after 12 days of incubation.	[23]
2013	Comparative assessment of bioremediation approaches to recalcitrant PAH degradation.	Soil	PAHs	White rot fungi, indigenous microbes	Lignocellulose substrate, amendment promoted highest degradation in HMW-PAHs. Inhibition occur when non-ionic surfactant brij 30 ammended.	[32]
2014	Comparative study of in vitro biodegradation of spent lube	Liquid	Spent Lubricating	Aspergillus niger and	B. subtilis is better than A. niger for lube oil	[56]

	oil.	medium	oil	bacillus subtilus	degradation.	
2014	Biodegradation of petroleum compounds	Soil from automobile workshop	Petroleum compounds	B. subtilis	B. subtilis has potential to degrade petroleum compounds.	[55]
2014	Assessing the hydrocarbon degrading potential of indigenous bacteria isolated from crude oil tank bottom sludge from Azzawiya oil refinery.	Sewage sludge, liquid medium, tank bottom sludge	Crude oil	Indigenous bacteria	A rich hydrocarbonoclastic bacteria in treated soil, COTBS was identified from Hamada crude oil.	[34]
2014	Characterizing pseudomonas sp. P-1 strain as potential tool for bioremediation.	Soil	Petroleum	Pseudomonas sp. P-1 strain	Biosurfactants produced by this strain has potential to degrade.	[39]
2014	Biodegradation of complex hydrocarbons by deep sea isolated bacterial consortium.	Sea, Soil	Spent engine oil	Sea isolates, Pseudoalteromonas sp., ruegeria sp., exigubacterium, acinetobacter	These strains are capable of using complex hydrocarbon for degradation.	[24]
2014	Optimization of BH medium for an efficient biodegradation	Liquid medium	Diesel, crude oil and used engine oil	Newly isolated bacillus ceres strain DRDU 1 from hydrocarbon oil	99% of diesel, 84% of crude oil and 29% of used engine oil found degrading and was confirmed by monitoring increase in CFU w.r.t incubation time.	[58]
2015	Synergetic ex-situ biotolerant of crude oil by halotolerant bacterial consortium.	Shore sites	Crude oil	Indigenous culture strains	83.70% and 83.49% degradation of $C_8 – C_{35}$ respectively.	[60]
2015	Bioremediation by pseudomonas aeruginosa and pseudomonas flurescenes of petroleum oil.	Liquid medium	Crude oil	P. aeruginosa, P. fluorescenes	32 isolates obtained. 62.46% and 55.53% oil degradation appreciated.	[21]

2015	Bioremediation by composting.	Soil	PAHs, petroleum, pesticides, chlorophenols, heavy metals.	Indigenous microorganisms	Reviews application of composting on soil bioremediation and critical review on microbial aspects.	[18]
2015	Biodegradation of crude oil and dispersants in deep sea water from gulf of Mexico.	Sea water and Soil	Crude oil	Dispersant molecules	Dispersant molecules behaved as nutrients for microbes for better degradation proved by FC-ITR-MS.	[62]
2016	Role of rhodococcus and cordona in clean-up of contamination by batch reactor.	Liquid medium	Naphthalene and anthracene	Rhodococcus and cordona	The naphthalene and anthracene were reduced by 100% and 89% within 120 days.	[67]
2016	Multiwall carbon nanotubes application in scale up microbial community in crude oil pollutant.	Sediment and liquid medium	Crude oil	Multiwall carbon nanotubes	Combination of crude oil and multiwalled carbon nanotubes can increase the microbial population and could increase ration of several degrading microbes.	[63]
2016	Bioremediation classification based on-site applications.	Soil	All contaminants	Indigenous organisms	In-situ bioremediation merits are reviewed.	[65]
2016	Substrate inhibition: naphthalene degradation by P. putida.	Liquid medium	Naphthalene	Pseudomonas putida	Naphthalene was completely degraded by P. putida at initial stage and then inhibition is described by kinetic models.	[66]
2016	In-situ bioremediation of crude oil contaminated site – case study in Jianghan oil field.	Soil	Crude oil	Pseudomonas sp.	TPH is reduced by 95% and n-alkanes, long carbon chains, 5-6 rings PAHs were also degraded.	[68]
2016	More application of biodegradation of heavy crude oil.	Soil	Crude oil	Microorganisms, enzymes, surfactants, etc.	Better understanding the biodegradation application is reviewed.	[64]

4. CONCLUSION

Due to day to day increase in anthropogenic activities for extraction of energy resources has led to problems of land contamination by toxic chemicals. Bioremediation is a reclamation technique that enhances the use of microorganisms to treat complex hydrocarbon contaminated soil by their effective metabolism technique. It was proved in literature and also through implemented experimental values that bioremediation is an efficient remediation technique than any other techniques as it is harmless, nature friendly, cost effective, reliable and simple in operation. Due to its severe advantages and flexibility, it occupies a prominent significance in reclamation. Through these years optimization in cell engineering and methods of application has been innovated and newly indigenous cellular were also investigated. There are possibilities for further advancements by combining natural methods with co-engineered techniques and genetic monitoring of microbial digestion, such are.

5. ACKNOWLEDGEMENT

I express my gratitude to Prof. Dr. D. D. Mandaliya, Vishwakarma Government Engineering College, Chandkheda; for support of this study and their contribution to this review.

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