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REVIEW ARTICLE

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Oil Eating Microbes: A Boon for Environmental Cleaning

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ABSTRACT

Globally with growing industrialization and increased dependence on crude oil for products such as fuel, plastics, asphalt led to unintentional release of some million gallons of oil to the environment. This poses a serious threat to our environment as these hydrocarbon components are toxic, mutagenic and carcinogenic to both aquatic and terrestrial environment. The traditional physico-chemical methods employed for clean up are laborious, expensive and also do not completely remove the oil contaminants. The popular effective, safe and economic alternative in today's world is bioremediation which uses biological organisms to detoxify environmental contaminants into harmful forms. Bioremediation by oil eating microbes are acknowledged to be the most eco-friendly and sustainable technology in the present world. Thus this paper briefly reviews of the various oil eating microbes existing today to remediate the remains of crude oil contaminants in the environment.

Keywords: crude oil, oil eating microbes, bioremediation, eco-friendly, sustainable

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INTRODUCTION

Crude oil contamination of soil and water from industrial emissions (continuous, eg. industrial emission or accidental, eg.oil spills) is a major concern as it causes hazards to both environment and human health. Unintentional spillages occurring frequently from pipelines, tankers although catastrophic are unavoidable. This spillage contains large amount of hydrocarbons which are toxic and persistent in soil and water causing a long term ecological effect [1].Natural disasters such as hurricanes and wars also initiate oil spill. Besides these, spillage also initiate oil spill which results from drilled platform at sea or disabled tanker and even from blowout or from broken pipeline on land [2].The spilled oil causes devastations to the aquatic habitat and their lives while the spillage in soils causes immediate harm to wild life, coastal habitats and may even threaten human health in long term due to prolonged environmental pollution [3-4]. The conventional physico-chemical methods for environmental cleaning are inefficient, expensive and labour intensive requiring waste disposal elsewhere thus spreading the pollution [5]. In today's world, the most popular, efficient, cost-effective and environmental friendly approaches for industrial wastes have been found by the application of biotechnological treatment known as bioremediation.

Bioremediation is a biotechnological approach which uses biological agent, i. e microorganisms (bacteria, fungi, yeast) to decontaminate the environment (soil or water) [6]. In this process, microorganisms are stimulated by addition of growth substances, nutrients, terminal electron acceptor/donor or both to degrade the hazardous pollutants to substances that are safe to the environment [7]. Biotransformation and biodegradation are the two processes which are involved during the bioremediation of contaminants to less toxic form. During biotransformation, microorganisms alter the molecule or structure of a compound while in biodegradation, multiple organisms breakdown the organic compounds into less toxic forms. Bioaugmentation is another process of bioremediation where microorganisms are imported to contaminated area to enhance the degradation process [8]. Among the several oil spill bioremediation techniques, remediation by oil eating microbes has received utmost attention being it to be inexpensive, sustainable and environment friendly when compared to other oil spill remediation techniques. Crude oil is liquid petroleum having numerous hydrocarbon types and this oil eating microbes are specific for certain hydrocarbon components which either enable them to degrade completely or not at all [9]. The

disastrous events of oil spill pose a serious threat to human as well as our environment. There are several incidents of such oil spillage from time to time. One such incident is in the year of April 2010 of the Deepwater Horizon oil spillage which devastated the Gulf of Mexico. This oil rig exploded during a routine drilling operation on the ocean floor which was located about 42 miles off the coast of Louisiana. Following this incident in around 87 days, about 200 million gallons of oil spilled into the ocean causing a total threat to the aquatic lives. Another recent incident of spillage occurred in 17th January, 2015 when a leakage in the pipeline has evaded 50000 gallons of crude oil into the Yellowstone River. As a result, benzene (a volatile organic compound) was found elevated in tap waters and was forbidden to use by the local people until further notice [10].

Oil waste contain total petroleum hydrocarbon which is a mixture of alkane, aromatics and asphaltene fractions [11].Oil contaminants have severe impacts on ecosystem as well as human. In human health, exposure to crude oil causes damage to internal organs such as kidney, liver, lungs. Polycyclic aromatic hydrocarbons inhalation leads to nausea, headache, dizziness and may also cause cancer; while benzene, toluene, xylene causes nervous disorders, congenital defects, liver diseases, cancers etc [12-13].

As all types of hydrocarbons are susceptible to microbial degradation is an established fact, therefore use of microbial bioremediation biotechnological intervention for treating crude oil contamination is a justifiable approach along with the additional impact of being efficient, cost effective and eco friendly [14-15].

Formation and composition of crude oil (or petroleum)

Crude oil or petroleum is a complex liquid mixture of hydrocarbons with some amounts of nitrogen, oxygen and sulphur [16]. The process of petroleum formation began some millions of years ago when small marine organisms existed in the sea and when they died; it settled at the sea bottom and got buried in layers of silt, clay and sand. Later on with gradual decay by pressure and heat led to formation of numerous compounds (hydrocarbons). These hydrocarbons accumulate in porous sandstone, reservoir rock or limestone. Cap rock (impervious rock covering reservoir rocks) with salt deposits acting as cap provides excellent reservoir for oil deposits found throughout the world [17].

Composition

The main components of petroleum or crude oil are- carbon (93%-97%), hydrogen (10%-14%), sulphur (0.5%-6%), oxygen (1%-1.5%), nitrogen (0.1%-2%) with few trace elements. Depending on the geographical region, the present composition of hydrocarbons may vary giving crude oil a particular uniqueness. The hydrocarbon usually present in petroleum are- naphthalenes (30%-60%), paraffins (15%-60%), aromatics (3%-30%) with the remaining percent of asphaltics.

Raw petroleum typically black or dark brown although some fields may produce greenish or yellow crude oil[18]. Crude oil consist of all normal alkenes ranging from C_1 to C_{120} . In highly paraffinic, the percentage rises to 35% while it decreases to zero in highly biodegraded oils [19-20]. Toluene and xylene are the most common aromatic compounds while thiols, sulphides and thiophenes are the sulphur compounds found in crude oils. Methane and alkanes ranging from pentane to pentadecane are the chief constituent of petrol [21-22]. Nitrogen compounds as quinolines, pyridines, indols, pyroles while oxygen compounds as organic acids occur in crude oil [23].

Various microbes eating crude oil with their process of degradation

The number of microbes present in the soil and their catabolic activity plays a vital role in degrading soil's hydrocarbon contaminants. The different microorganisms present in the soil such as bacteria, fungi, protozoa, algae, actinomycetes have varying capacity to degrade the hydrocarbons. The rate of degradation by microorganisms depends on several factors such as - number of microbes present in the soil, contaminants catabolic ability to destroy microbes, microbes activity and contaminant's molecular structure [24]. Microbes catabolic activity has to be achieved for hydrocarbon biodegradation to be successful and these include- enrichment of selective organisms to attack contaminants, genetic modification in the microbes for enhance metabolic ability and induction of specific enzymes[25]. Bioremediation can be done via- insitu or exsitu approach. In insitu, bioremediation is done with the help of indigenous microorganisms while in ex-situ, contaminated soil are removed and treated elsewhere. Bioremediation by microbes can degrade inorganic contaminants (metals, metalloids, small molecules like ammonia) and organic contaminants (chlorinated organic, organophosphates, polychlorinated biphenyls, halogenated aryl hydrocarbons) as most of these xenobiotics(organic contaminants) and metals (inorganic contaminants) are essential for microbes as macro or micro nutrients [26]. Chromium, a toxic contaminant in industrial effluents is removed by biosorption by *Pseudomonas spp.* or detoxification by Arthrobacter oxydans while marine seaweed(Eucheuma sp.) associated fungal strains of Aspergillus flavus and A. niger can absorp around one fourth of chromium present [27-29].

Zinc (Zn²⁺) bioremoval is done by bioprecipitation and biosorption by a sulphate reducing bacterium *Desulfotomaculum nigrificans* [30].Bacteria such as *Pseudomonas putida, Salmonella typhimurium,*

Streptomyces griseus, Rhizobium sp., Rhodococcus sp., E. coli, Streptomyces griseus, Bacillus megaterium, Deinococcus sp. [31] while fungi like Neurospora crassa, Penicillium citrinum, Aspergillus niger, Aspergillus flavus, Mucor racemosus help remove cobalt from soil [32].

Chlorophenol contaminated soil can be treated by *Arthrobacter chlorophenolicus* [33]. *Stenotrophomonas maltophilia* & *Pseudomonas sp.* synthesizes bioemulsifiers such as lipopolysaccharides, lipoproteins, amphipathic polysaccharides, glycolipids and lipopeptides which have several advantages in commercial applications such as enhanced oil recovery, bioremediation of oil polluted soil, cleaning up of oil contaminated pipes by replacement of chlorinated solvents [34].

Bioaugmentation is the process where microorganisms are carried to the contaminated site to enhance the process of biodegradation by decreasing the lag phase [35]. The microorganisms involved in bioremediation in varying environments are as follows- Arthrobacter, Acaligenes, Actinobacter, Acinethobacter, Bacillins, Berijerinckia, Mycobacterium, Nocardia, Nitrosomonas, Penicillium, Pseudomonas, Phanerochaete, Xanthofacter, Rhizoctomia, Serratio, Trametes [36]. They are also active members of microbial consortium. *Pseudomonas pudita* strain MHF 7109 help in biodegradation of petroleum hydrocarbons such as o-xyluene and benzene [37]. The oil degrading microbes live in groups hence referred as microbial consortia. Bacteria are the most predominant oleophilic microorganisms in the environment and are also referred to as hydrocarbonoclastic bacteria [38-39].

Some of the oleophilic microorganisms (bacteria/fungi) are mentioned below-

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Process of oil degradation by microbes

Oil eating microbes feed on the hydrocarbons present in oil. The hydrocarbons composed of numerous amounts of hydrogen and carbons. The microbes break these hydrocarbons which ultimately combine oxygen in warm temperature to give up CO_2 and H_2O . The microbes increase in size and number when they consume fertilizers. Thus these large microbes take up more oil from the environment. This is how microbes clean oil spill in the Gulf of Mexico. The left over oil not consumed by microbes are carried away by water currents and wind [51]. Some of the species of marine bacteria consuming oil are Marinobacter, Oceanospiralles, Pseudomonas and Alkanivorax. These are the known bacterial species that depend solely on oil [52]. The rate of bacterial degradation depends on the following factors such as water temperature, amount of nutrients and oxygen in water, kind of oil and the surface area of the oil. Light petroleum products (diesel, gasoline) are more easily consumed than the heavy petroleum products (heavy crude oil, fuel oil) [53]. Microbial remediation of heavy metals takes place in three different ways. Firstly by the biosorption or bioaccumulation process in which microbes integrate the metal contaminants into its cellular structure. Secondly by the process of extracellular precipitation or uptake by purified biopolymer and thirdly by other specific molecules eg. biosurfactant molecules; obtained from microbial cells [55-56].

Biosorption

Extracellular material containing cationic metals such as Fe, Zn, Cd can immobilise metal through binding to anionic functional groups present in the cell surface. Slime layers composed of carbohydrate, polysaccharide which helps in the extracellular binding. Several binding mechanisms such as Van der Waals forces, covalent bonding, redox interactions, electrostatic interactions help bind metal ions to the cell surface. Bacteria act as a good biosorbents due to active chemosorption sites (such as teichoic acid in bacteria wall) and high surface to volume ratios [57-58].

Siderophore

Bacteria, fungi, plants produces low molecular wt. chelating agents which ranges from 200-2000 Dalton and are known as siderophore. These agents help uptake of iron and also can chelate numerous other metals as well. Thus siderophores reduces metal toxicity by binding heavy metals eg. copper toxicity, reduction in cyanobacteria [59-60].

Biosurfactant molecules

Biosurfactant molecules production by microbial cells help in bioremediation by heavy metals and complex metals such as Pb, Zn, Cd through electrostatic interactions and increases metal solubility resulting in the formation of metal salts which are less soluble such as phosphate precipitates and sulphide [61-62;56].

Oilzapper Technology in India (by TERI)

Oilzapper is a cost effective technology developed by Tata Energy Research Institute (TERI), New Delhi. It is an indigenous drill cutting /oil sludge degrading bacterial consortium derived from bacterial cultures of four species of oil degrading bacteria which could degrade different fractions of Total Petroleum Hydrocarbon (TPH) present in crude oil spills to CO_2 , water and ecofriendly dead biomass .Under laboratory condition, oilzapper is multiplied and mixed with carrier materials for transportation in polybags.

In many cases carried out by TERI the initial TPH content of 5% to 52% has been successfully biodegraded to less than 1% within 2 to 6 month duration. Thus this is a challenging technology for oil industries for their hazardous oil waste management and are in use by many companies in India and abroad such as- Indian Oil Corporation Ltd. India; Oil India Ltd. India; Hindustan Petroleum Corporation Ltd, India; Bharat Petroleum Corporation Ltd India; Reliance Industries Ltd. India; Indian Petro Chemicals Corporation Ltd.; Abu Dhabi National Oil Company, Abu Dhabi; Kuwait Oil Company, Kuwait [63].

Benefits of Oilzapper technology

Oilzapper microbes have been isolated from different geographical locations of India and are adapted to degrade the toxic hydrocarbons not only between temperatures 15° C to 60° C but also from saline environment upto 1.5 depth. The organisms used in the consortium are provided with nutrients which increases its efficiency. They are non pathogenic and can degrade different components of crude oil or oily sludge like aromatic, alkane, asphaltane, nitrogen, oxygen and sulphur containing compounds. Sludge degrading bacteria multiplies in presence of oily sludge but once bioremediated to CO_2 , H_2O and fatty acids these bacteria decreases in number [64].

Conclusion

Oil eating microbes are playing a vital role in the present century in making our environment cleaner and greener in a very safe way. It is indeed a boon to the entire mankind which requires further extensive research on variety of microbes available worldwide to develop more microbial consortia so as to get an accelerated efficient degradation of the spilled petroleum. But safety and efficiency of these microbes should be the top priority before using such microbes commercially and in a much wider scale.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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