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Bioremediation of Petroleum Oil spills and Hydrocarbon Waste Using Microbes

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Abstract

Crude oil spills, oily sludge and petroleum industry effluents are considered to be a major problem due to their high toxicity and polluting nature. Terrestrial and aquatic life is being affected by it. Safe disposal of them is a serious problem, due to their polluting nature. Bioremediation is a natural and effective phenomenon to minimize their toxicity. Bioremediation simply uses the transforming nature of bacteria and other microorganisms, through which they use few toxic compounds of crude oil and uses or degrade them into simpler forms. There are several methods which can alter the rate of bioremediation such as temperature, pH, salinity, etc. carcinogenic nature of toxic components of crude can certainly affect living organisms in several ways. They have their impact on most of the systems and relate organs of the human body, such as reproductive system, digestive system, nervous system, circulatory system etc. harmful affects of crude oil are also noticed and studied on plants and animals

Keywords: Bioremediation, Transformation, Toxic, Effluent, Indigenous, Consortium, Crude oil, Volatile, Emulsification

Introduction

The petroleum industry effluents, oily sludge and oily spills are a major threat to the environment, an as they cause pollution wherever they are dumped .India, USEPA (United States Environmental Protection Agency) and OECD (Organization for Economic Co-operation and Development) countries designated oily wastes as hazardous wastes (Zhu et al., 2001; Ministry of Environment and Forest, Government of India, 2000) . The release of crude oil into the environment through several ways leads to hazardous pollution problems (thouand et al., 1999).The oily waste consist of total petroleum hydrocarbons (TPH), sediments, water. The TPH constitute a mixture of alkane; aromatic; nitrogen, sulfur, and oxygen containing compounds; and asphaltene fractions (Bhattacharya et al., 2003). Oil contamination has adverse effects on animal and plant ecosystem including human health. Crude oil may cause damage to kidneys, liver, lungs, and other organs as well. Polycyclic aromatic hydrocarbons (PAH), a cancer causing agent is present in oily waste. Its inhalation causes headache, dizziness, respiratory irritation and nausea. BTEX(Benzene, toluene ,ethyl benzene and xylene) causes nervous disorder, depression, irregular heartbeat and mutation, etc. (Gomer et al., 1980; Knafla et al.,2006; Zhang et al., 1992; Carpenter et al., 1977;Lee et al., 2006; Chen et al., 2008; Lewis et al.,2008 and Rice et al., 2007).

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These pollution problems mostly causes big disturbances in ecosystem components e.g. biotic and abiotic (Mueller et al., 1992), more in way that few hydrocarbons have been known to relate to the family of neurotoxic and carcinogenic organic pollutants (Hallier- Soulier et al., 1999).Oil causes soil pollution resulting into loose soil and has affect on seed germination (Yoshida et al.,2006 and Gong et al., 2001). Since long years various conventional methods such as incineration, land filling and spurging, etc. are applied to mineralize crude oil (Vidali, 2011 and Mandal et al., 2007). And tests show all methods affects environment (Sood et al., 2009). Being a temporary solution to the environment, these methods are not pocket friendly. (Mandal et. al., 2007, and Ouyang et. al.,2005).

Studies shows that microbes can degrade virtually all types of hydrocarbons and hence the process of bioremediation can be used with biotechnological approach in order to properly treat the hazardous waste (Atlas, 1991; Head, 1998). Bioremediation, which uses the biodegrading capacity of microorganisms, is a technology that shows its impact on both toxicity and high volume thus lessening the toxicity and reducing volume, further recovering the sites affected from hazardous oil and wastes (Caplan 1993). Bioremediation showing a great impact on contaminated oil, and is considered as one of the best treatment for oil waste. (Bragg et al., 1994 and Prince et al.,1994). Bioremediation is an efficient, environmental friendly and also a cost effective treatment technology for the treatment of hydrocarbon polluted soils (Chikere et. al., 2009). Bioremediation is a natural process during which a microorganism acts on harmful substance and convert it into a non toxic compounds (Lal et al.,1996 and Bartha et al., 1984). Several practical and tests have verified that oil bioremediation on contaminated shorelines can augment oil bio degradation. (Prince,1993 and Swannell et al., 1996). Hazardous oil pollutant's biodegradation concept is largely studied in open field experiments (Chaineau et al., 2003; Gogoi et al., 2003) and in controlled conditions (Sugiura et al., 1997; Chaillan et al., 2004). The proper working of Bioremediation is dependent on the suitable number of microorganisms with favourable conditions and amount and constituents of the contaminant. Although large laboratory based research have been carried out on bioremediation of soil, field tests with little quantity of oily effluent have been carried out in limited number in India and abroad. (Raghavan et. al., 1999; Mishra et. al., 2001; B K Gogoi et. al., 2003, Ouyang et. al., 2005, Chikere et. al., 2009 and Liu et. al.,2009).

Degradation of petroleum hydrocarbons by microbes

To make the bioremediation technology effective on the contaminated site and to obtain good results, it requires proper knowledge of the contaminated site and the properties of the microbial transformation of pollutants (Sebate et al., 2004). petroleum hydrocarbon biodegradation is known to be affected by fair number of limiting factors. Oil pollutant composition is major factor that effects Biodegradability. In same manner biodegradability of crude oil is quantitative, even in the favourable conditions only 11% biodegradation of heavy asphaltic-naphthenic crude oils is possible in a reasonable period of time (Bartha 1986 a). It has been reported that biodegradation efficiency, starter oil concentration and growth rate specificity does not share any relation (Thouand et al., 1999), such cases points towards the fact that biomass is needed to make up certain amount of enzyme only so that the degradation process runs without any obstacle even when biomass production is stopped (Okoh 2002). Reports related to sunlight irradiation impact, point towards the physiochemical transformation of crude oil rather than its biodegradation

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(Jacquot et al., 1996; Nicodem et al., 1998), photo oxidation elevates petroleum hydrocarbon biodegradability by enhancing its bioavailability and ultimately resulting in boosting the microbial activity, it is been reported by the recent studies(Maki et al., 2005). Temperature is also an essential factor in the transformation of petroleum hydrocarbons, as it changes the pollutant chemistry and besides this the physiology of microbial population is reported to get affected by temperature. Environmental temperature causes changes in the microbial activity as well as crude oil (Venosa and Zhu, 2003). Rise in the viscosity of oil is reported at low temperatures, whereas toxic hydrocarbons with low molecular weight becomes less volatile due to which the onset of biodegradability consumes more time (Atlas 1981). Microbial degradation of crude oil seems to be a natural process in which a large amount of polluting oil is taken as an organic carbon source, that further result into the degradation of petroleum constituents into smaller or another organic compounds e.g. Biosurfactants (Chhatre et al., 1996). Previous studies shows that addition of nonionic surfactant, e.g. primary constituent of spilled oil dispersants, is likely to effect the transformation rate of Alkane (Bruheim and Eimhjelle, 1998; Rahman et al., 2003).Studies shows that the surfactant use in case of oil spill contamination may show neutral effect or accelerate or slow down the oil components biodegradation (Liu et al., 1995).The reason to predict the function of Biological and chemical surfactants is that their role related to biological systems may be discovered (Rocha and Infante 1997; Lindstrom and Braddock 2002). Bacteria, fungi, yeasts, and algae, few species of these microorganisms are able to mineralize petroleum hydrocarbons completely and besides that they metabolize the carbon components in order to generate more cell mass. for example bacteria for new cell production converts PAHs into dihydrodiols under aerobic conditions (Atlas, 1984).

70 genera of oil-degrading microorganisms are there, such as bacillus, acinetobacter, spirillum, actinomyces, vibrio, pseudomonas, streptomyces, are among the bacterial genera and few such as mucor, debayomyces, penicilium, candida, asprgillus, and saccharomyces are among the genera of fungi or yeast. In most of areas these microorganisms are less abundant in comparison to the total no of identified microorganisms under natural conditions, however when it comes to the petroleum hydrocarbon polluted sites these microorganisms grows well and are found in abundance as they use petroleum hydrocarbon as a carbon source and hence the no increases. (Ahn et al., 1999; Aldrett et al., 1997; Altas, 1981; Bento et al., 2005; Chaerun et al., 2004; Das and Mukherjee, 2007; Gallego et al., 2001; Hua et al., 2003; Mohanty and Mukherji, 2008; Palittapongarnpim et al., 1998; Supaphol et al., 2006).

Biotransformation of naphthalene to catechol is a fine example showing bacterial oxidation pathway. Naphthalene, a potentially toxic compound of petroleum and related products is a water soluble compound. Indigenous microorganisms residing in groundwater and soil are capable of degrading big amount of petroleum hydrocarbons, while degrading hydrocarbons they need sufficient amount of oxygen, as well as water and nutrients, usually nitrogen and phosphorus (Bartha and Bossert, 1984).Crude oil biodegradation and cell growth were observed when rhamno lipid or glycerol were added in small quantity. As only 0.02% crude oil shows solubility in water, crude oil emulsification becomes important to improve bioavailability of insoluble hydrocarbons to microorganisms by enlarging the area of interface between water insoluble hydrocarbons (Gerson, 1993). Prokaryotic action on aromatic hydrocarbons transforms them into trans-dihydrodiols by an initial dioxygenase attack and afterwards these trans-dihydrodiols are oxidized to dihydroxy compounds, e.g. like in case of benzene catechol is oxidized (Atlas and Bartha, 1998). Heavy crude oil maya was reportedly biodegraded between 8.8 to 29% by mixed bacterial consortium in soil microcosm, and 6.5 to 70% was the reduction noticed in the major

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peak components of the oil (okoh et al., 2003). Degradation level of petroleum hydrocarbon was noticed to rise when group of bacteria was taken into account rather than using mono species (Ghazali et al., 2004). Nutrients being one of the most important factors for the efficiency of biodegradation hazardous hydrocarbon, especially phosphorus, nitrogen and also iron in some cases (Cooney 1984). Occurrence of oil spills in cases of freshwater and marine water, in such cases the availability of phosphorus and nitrogen acts as a limiting factor in degradation of oil, while on the other hand carbon supply is enhanced (Atlas 1984).

Factors altering rate of bioremediation

Temperature

Temperature has its impact on both the microbial consortium and physiochemical characteristics of petroleum. Organic substrates biodegradation with low level or null level are recorded in case of low temperature (Alexander, 1999). Including arctic petroleum hydrocarbon biodegradation has been noticed in aquatic and terrestrial cold regions ecosystems (braddock et al, 1997). Microbial degradation of diesel at 10C has been reported (margesin & schinner, 1998). Psychrotropic *Rhodococcus* species strain is reported to transform short-chain alkanes at 0*C (Whyte et al, 1998, 1999). A rise in incubation temperature (5-20C) of psychrotrophic and/or psychrophilic consortium would result in the enhancement of biodegradation rates (Siron et al, 1995).

Fall in the biodegradation rates of petroleum could be the result of fall in metabolic activities of microbial consortium and their growth, due to poisonous contents of crude oil and low temperature. Oil viscosity is inversely proportional to temperature which lessens the spreading of oil in soil and water. Volatility of short chain alkanes is decreased in low temperature that enhances their solubility in soil and water. As a result toxicity towards microorganisms is increased (Leahy & Colwell, 1990). With rise in enzymatic activity of thermophilic and mesophilic microbes associated with rising temperatures it is possible that biodegradation rates will increase to certain level, commonly in range of 30 to 40C. Hydrocarbons toxicity of membranes is enhanced thus ceasing biodegradation (Bossert & Bartha, 1984).

pH

pH closer to neutral is recorded to have highest biodegradation rates (Alexander, 1999; Leahy & Colwell, 1990; Zaidi & Imam, 1999). In environments with low pH, petroleum bioremediation is normally taken over by fungal consortium, which is usually more adapted to acidic pH environments (Jones et al, 1970). An indigenous microbial consortium consist of several bacteria, yeast and a fungus was reported to mineralize mono and PAHs in the coal pile’s run off at pH 2. It was concluded that the starting fungal action on the hydrocarbons might have transformed hydrocarbons into intermediates for remaining degradation by bacteria (Stapleton et al 1998). At other extreme an alkaliphilic bacteria have been isolated from industrial effluents and highly alkaline lake that were successfully transforming phenol from wastewater at pH 7.5-10.6 (Kanekar et al, 1999, Sarnaik & Kanekar, 1995).

Salinity

Generally bacteria isolated from soil, freshwater and estuaries are able to survive in salinity compared to seawater salinity. However some species whether freshwater or terrestrial (ZoBell, 1973) are capable of reproducing in seawater with salinity 3.5%. Marine microbial growth

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retards in salinities less than 1.5 to 2% due to need of salt. In bacterial consortium of seawater, highest biodegradation of petroleum was noticed at 0.4 M NaCl (natural seawater) while noticeable crude oil degradation was noticed between 0.1 to 2.0 M NaCl. The mineralization of hydrocarbons is mainly caused by *pseudomonas* sp., Enterobacteria and a very less number of negative aerobes (Bertrand et al, 1993). The retarded biodegradation capacity in hyper saline niches would contribute more to a general decrease in activity of microorganism related with extreme environments instead of the impacts on the hydrocarbon forms (Ward & Brock, 1978).

Oxygen

In hydrocarbon degradation molecular oxygen is important. In ecosystems of water bodies, microbial degradation is limited due to the improper amount of oxygen mainly when spilled oil came up on water surface. However, in water sediments, water mobility is highly restricted by the little size of pores and heterotrophic activity usually renders all but the thin layer anaerobic. Any oil moving below 1-2 cm top soil layer is generally believed to remain for long time period. Sinking oil are generally tar balls that usually are not biodegraded. Respiration rate of microorganisms, kind of soil, and the available substrates presence that can result in oxygen exhaust, all these factors determines oxygen availability in soil (Bossert & Bartha, 1984).

Studies show that generally 3.1 mg/l oxygen needed in biodegradation of 1 mg/l hydrocarbons. As groundwater is contains dissolved oxygen at 6-12 mg/l (depending on temperature), fully filled groundwater can be expected in degrading 2-4 mg/ml of hydrocarbons. However these calculations do not take microbial cell growth into account. In this case, oxygen mass needed for the degradation of 1 mg benzene decreases to 1.03 mg (Curtis & Lammey, 1998).

Anaerobic biodegradation persist in limited oxygen and can result in a significant reduction in contamination by actions of anaerobic microbial consortium like sulphate-reducing bacteria, metal-reducing bacteria, methane producing bacteria and denitrifiers. A bacteria that is capable of degrading 80% of toluene carbon to CO_2 under totally anaerobic conditions takes sulphate as electron acceptor was isolated from oil contaminated subsurface oil (Beller et el., 1996). So and young (1999) isolated bacterium from petroleum contaminated estuarine sediment that was capable of degrading alkane and reducing sulphate. A strain AK-01 was characterized to catabolise alkane (C_{13}-C_{18}). Anaerobic degradation of benzene coupled to nitrate reduction to nitrogen gas was observed in enriched cultures produced from soil and groundwater microorganism (Burland & Edwards, 1999). Anaerobic consortiums play an important role in the biodegradation of crude oil hydrocarbons in environment with anaerobic conditions (Curtis and Lamney, 1998; Rooney-Varga et al, 1999; Vargas et al, 2000).

Nutrients

It is necessary to maintain ratio of Carbon: Nitrogen : Phosphorus at about 120:10:1 in any environment to continue any microbial activity (Thomas et al, 1992). Generally, the nutrient supply except C increases the need of the resident microbial consortium given the less readily available C. However, condition alters considerably if a compound that is potentially easily usable is introduced into the environment in noticeable amount. When oil occurs, the sudden rise in the carbon amount disturbs this ratio by making high C: N or C: P ratios, thus harming microbial growth. Water in saline sources, lakes, soils etc, containing crude oil normally have very low concentrations of these inorganic components at the interface between aqueous phase and water insoluble polluting substances to make the microbial activity going on that might

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increase transformation (Alexander, 1999). Because of small levels of N and P in marine water, the rate of petroleum and sea oil spills biodegradation as well is decreased unless P or N or both are to stimulate the indigenous microbial consortium (Rosenberg et al, 1992; Zaidi and Imam, 1999). Both sources of N and P whether organic or inorganic have been searched for their in activating biodegradation in terrestrial and aquatic environments. Marine biodegradation carried out in enclosures showed that inorganic salts works well, it is tough to resist the dilution effect related with mixing water-soluble salts to an open aqueous body. The concentration of N and P salts added into surface of water or very close to oil-water interface area soon gets lowered due to the introduction of these salts to activate biodegradation less economical and effective in bioremediation efforts. N and P addition through organic sources was greatly studied through the application of fertilizers following the Exxon Valdez spill. Out of three types of fertilizers formulations, the oleophilic fertilizer generated the most far-reaching effects in activating biodegradation in time period of 2 to 3 weeks on the cobble shorelines at Prince William Sound (Atlas, 1995;Pritchard and Costa, 1991). Oleophilic fertilizers remain bound with the oil and activate hydrocarbon-transforming microbes (Swannell et al, 1996).

Chemical composition of petroleum

Due to different molecular weights and chemical structures, crude oil hydrocarbons different in their affect to microbial action as follows: n-alkanes > branched alkanes> low-molecular weight aromatics> cyclic alkanes (Perry, 1984; Leahy and Colwell, 1990). Efficacy for biodegradation is maximum for saturates, followed by light aromatics with high molecular weight aromatics and polar compounds being lowest susceptible to biodegradation (Leahy and Colwell, 1990; Obuekwe et al, 2001). Cooney et al studied that more naphthalene than hexadecane was transformed in sediment and water mixtures.

Physical state of the environment

The oil crosses different types of processes such as evaporation, dissolution, emulsification and photo oxidation with water to make emulsions and perhaps finally results in the formation of tar balls. Oil emulsion formation extends the surface area of soil and thus making it more susceptible for microbial action (Al-hadhrami et al, 1995; Leahy and Colwell, 1990). Biodegradation rate in tar balls is minimum or null because of their solid state and volume to low surface area ratios.

PHAs are highly abundant in the environment because of their high hydrophobicity and hence less availability to biodegrading microbes. Microbial degradation of low molecular PAHs occurs quickly when they are in liquid phase (Volkering et al, 1992). Hence, the less solubility and dissolution rates of bigger complex PAHs may inhibit their occurrence for microbial degradation. Water solubilities of anthracene and phenanthrene are 0.07 and 1.29 mg/l respectively (Poeton et al, 1999). Phenanthrene in crystalline form is reported to be less biodegradable than in solution form (Bouchez et al, 1995).

Concentration of the petroleum hydrocarbons

Microorganism uptake rate and rate of microbial degradation of water-soluble compounds are generally directly proportional to the concentration of the compound, the same relation cannot be taken in case of compounds with low water solubility and those apply toxicity to membrane at high concentrations. The microbial degradation rate of high molecular weight PAHs like naphthalene and phenanthrene are connected to their water solubility instead of their

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concentrations in a given solution. On the other side, greater concentrations of highly volatile or soluble compounds may be harmful for microorganisms because of their toxicity. Dibble and Bartha (1972) noticed that microbial degradation process in oil sludge occurred between oil concentrations of 1.25 and 5%. Oil loadings (>5%) causes decrease in microbe population because of rise in toxicity level. Del' Arco and Franca (2001) agreed to this using sandy sediment slowly contaminated with crude oil. Tarabily and Khalid (2002) studied that ceasing of activities when the concentrations of aqueous soluble parts exceed from 50%. With toxicity, greater concentrations of crude oil may also limit growth of microorganisms by disturbing the C: N: P ratios. Oxygen limitation makes it difficult for microbe to grow when a broad layer of oil forms on the surface of a water body, inhibiting oxygen transfer into the water phase.

Impacts of hydrocarbon waste

Living beings, either directly or indirectly comes in the exposure of petroleum. Refining of petroleum results into some highly poisonous byproducts, which are further used in the production of some other products. The continuous release of these poisonous and hazardous compounds into the environment and finally affects living matter, in same way spilled crude oil in marine and freshwater harms life either directly or indirectly, and both of these petroleum forms together results into the a major form of environmental pollution. Although petroleum products constitute a huge group of hydrocarbons, only some of them are of toxic nature. A large variation of boiling point and molecular weight are found in the molecules of petroleum hydrocarbons, are a reason for the various level of toxicity towards environment. Physical and chemical properties of hydrocarbon molecules are responsible for their toxicity and the effect of microorganism metabolic effects on it. Petroleum is poisonous and can be fatal in many cases, the constituents of petroleum, one comes in contact with it and for how long time period one remains in contact to it are main factors. Different compounds present in crude oil can result into a broad range of health related problems in humans and other living organisms depending on the period of time of exposure and their exposure. Crude oil constitute some highly poisonous compounds that most probably shows impact on the functioning of the organ and can damage organ sometimes, further resulting in the damage of whole system of the body. Body systems that might be affected by the toxins are e.g. circulatory system, nervous system, reproductive system, immune system, respiratory system. Crude oil compound certainly causes various diseases and disorders and syndromes (Costello, 1979). Maximum risk of problems caused by crude oil contaminants are to pregnant women and her foetus, infants, kids, and also to people living in environment that forces them to generate stress in them and people already suffering from any major disease or disorder. The symptoms and the effect of toxic chemicals on body parts can be sudden or prolonged (Singh et al., 2004). The adsorption amount of petroleum hydrocarbons through oral, respiratory or dermal routes changes due to the large extent of the physiochemical properties of these compounds. Human health risks due to oxygenated and aromatic constituents of gasoline, and also impact of lead and manganese. Petroleum compounds introduction in the body can certainly harm human reproductive system (Menkes and Fawcett 1997). Volatiles obtained from the petroleum are reportedly one of the reasons for the cancer in very young age across the globe. And the non-volatiles product of the petroleum is absorbed by soil and tends to remain at release site that can certainly affect when comes in contact with flora, fauna and humans through water and source of food. Poly-aromatic and napthene-aromatic are major constituents of petroleum, are found to cause major harm to the nervous system and can

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also develop cancer and tumors in the body. Crude oil constitute aromatic compounds, these are found in various forms environment and acts as pollutants. Presence of these aromatic compounds are found in refinery generated waste, emissions from petrochemical industry, oil spills, waste from oil storage, etc. mobility of toxic petroleum compounds can harm the land and aquatic life including humans. Nitrogen, oxygen and sulphur are the part of polar organic compounds which are present in the petroleum. According to the reports, the oil spilled sites constituted the nitrogen, oxygen and sulphur which move into groundwater making the big petroleum hydrocarbons fraction in the potable water source (Mahatnirunkul et al., 2002, Delin et al., 1998, Oudot, 1990). Therefore such water sources are considered unsafe for terrestrial, aquatic plants and animals and humans (Griffin and Calder, 1976). Benzene was once produced through coal carbonization but now is obtained mainly from petroleum. (Verschueren, 1983).

Conclusion

Being toxic in nature, crude oil can be transformed into less toxic, non harmful compounds if bioremediation enabled species are taken for its treatment, such as pseudomonas, streptococcus, etc. while various other factors are need to keep in mind while carrying out the bioremediation process. Different factors e.g. temperature, media, pH, bacterial consortium, etc can greatly alter the entire process. As crude oil is a bunch of several toxic compounds, mainly organic compounds it should be handled with highly specified instruments and experts. Related new searches still having space, can be known by carrying different species and different methods. long time period taken for transformation of crude oil turns out to be a small weak point for this solution. However, treatment of crude oil and its transformation is the strongest key point of this treatment. Time period can certainly be reduced by mixing different microbial consortium to get results in short duration of time, various tests regarding multi-species treatment has been carried out to get better results in both senses i.e. time period and transformation efficiency.

Bioremediation can be seen as a successful future solution for various industrial effluents, pollutants and waste.

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