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Biodegradation of Used Engine Oil by Bacterial Cultures

Pravinkumar A. Domde[1], Hemant J. Purohit[2], Rajpal Singh Kashyap[3], Shardul S. Wagh[4]
[1]Assistant Professor, Biochemistry Department, Kamla Nehru Mahavidyalaya,Nagpur, Maharashtra, India
[2]Ex-Cheif Scientist G, National Environmental Engineering and Research Institute (NEERI), Nagpur
[3]Director Research, Central India Institute of Medical Sciences (CIIMS), Nagpur
[4]Assistant Professor, Biochemistry Department, Kamla Nehru Mahavidyalaya, Nagpur

Abstract: *In the present study biodegradation of used engine oil by four bacterial cultures was targeted. Versatile catabolic capability of four bacterial cultures Bacillus subtilis PD6, Bacillus sp. PD9, Enterobacter sp. PD11 and Bacillus sp. PD14 was assessed by subjecting them for the utilization of used engine oil in 250ml 0.1XM9 media containing flasks. Biodegradation of used engine oil was analysed by COD analysis and observation were statistically processed by ANOVA. Out of four bacterial cultures, Bacillus sp. PD9 and Bacillus sp. PD14 were found to be efficient cultures which were able to degrade 67.8% and 65.5% used engine oil within six days. Other two cultures Bacillus subtilis PD6 and Enterobacter sp. PD11 also exhibited successful biodegradation of used engine oil but less efficiently compared to Bacillus sp. PD9 and Bacillus sp. PD14. This study accentuate the role of isolated four bacterial cultures in removal of used engine oil and its possible application in bioremediation studies.*

Keywords: Biodegradation, Bioremediation, Bacterial cultures, COD, Used engine oil, ANOVA

I. INTRODUCTION

Man developed new technologies to facilitate the work as well as to obtain the comfort at every step of life. In doing so various aspects related to environmental health knowingly or unknowingly were ignored. The outcome of most of these developmental processes is environmental deterioration. To facilitate the comfortable transportation automobile vehicles were developed across the world. These vehicles have certainly expedited the transport but at the same time played and still playing major role in environmental deterioration.

The demand for petroleum as a source of energy and as a primary raw material for chemical industries in recent years has resulted in sudden increase in its consumption worldwide. This dramatic increase in production, refining and distribution of crude oil has brought with it, an ever increasing problem of environmental pollution (Atlas and Bartha, 1992).

Petroleum products used in the automobile vehicles include gasoline, diesel, engine oils of variable densities, grease etc. All these products are derived from the crude oil, extracted from the oil wells dig dip into the earth. Environmental deterioration by automobile vehicles, running on petroleum products such as gasoline, diesel occurs at every level. Various gases released during combustion of petroleum products have contaminated air significantly. Engine oil which is used to provide lubrication to engine parts, has also contributed heavily in the pollution of water and soil.

The fate of petroleum hydrocarbons in the environment is largely controlled by abiotic factors which influence rates of microbial growth and enzymatic activities that determine the rates of petroleum hydrocarbon utilization (Leahy and Colwell, 1990). The persistence of petroleum pollution depends on the quantity and quality of hydrocarbon mixture and on the properties of the affected ecosystem. In one environment, petroleum hydrocarbon persists indefinitely whereas under another set of conditions the same hydrocarbons may be completely biodegraded within a few hours or days (Atlas and Bartha, 1972). Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats (Atlas and Bartha, 1973). The ability to isolate high numbers of certain oil-degrading microorganisms from oil-polluted environment is commonly taken as evidence that these microorganisms are the active degraders of that environment (Okerentugba and Ezeronye, 2003). Although, hydrocarbon degraders may be expected to be readily isolated from an oil-associated environment, the same degree of expectation may be anticipated for microorganisms isolated from a total unrelated environment such as domestic wastewater.

Petroleum compounds are considered to be recalcitrant to microbial degradation and persist in ecosystems because of their hydrophobic nature (low water solubility) and low volatility, and thus they pose a significant threat to the environment (Abed *et al*., 2002)

Crude oil, the source material of nearly all petroleum products, contains a wide variety of elements combined in various forms. The principal constituents are carbon and hydrogen which in their combined form are hydrocarbons. In the refining process petroleum products are strongly enriched with hydrocarbons, leaving most crude-based inorganic materials and other types of organic compounds containing sulfur, nitrogen and oxygen in the residual material.

Petroleum hydrocarbons are organic compounds comprised of carbon and hydrogen atoms arranged in varying structural configurations. In the broadest sense, they are divided into two families, *aliphatics* (fatty) and *aromatics* (fragrant). Aliphatics are further divided into three main classes, *alkanes* , *alkenes* and *cycloalkanes* .

Engine oils or **motor oils** used in the automobile vehicles consist of variety of different types of hydrocarbons. Its primary function is lubrication of the engine parts. The motor oil used freshly is viscous and contains base lubricating oil (a complex mixture of hydrocarbons, 80 to 90% by volume) and performance enhancing additives (10 to 20% by volume). Engine oils are altered during use because of the breakdown of additives, contamination with the products of combustion, and the addition of metals from the wear and tear of the engine. Therefore, the composition of waste oil is difficult to generalize in exact chemical terms. It is recognized that the major components consist of aliphatic and aromatic hydrocarbons (such as phenol, naphthalene, benz(a)anthracene, benzo(a)pyrene, and fluoranthene) Chinenyeze and Ekene (2015).

It is observed that, in India, in spite of presence of strict guideline for safe disposal of used engine oil, garages and automobile service stations throw it without any treatment to the storm drains. As engine oil used in all types of vehicles are mostly derived from crude oil which is composed of variety of different types of hydrocarbons, its direct release into the environment poses potential threat to the components of ecosystem.

Fate of Used Engine oils

New motor oil contains fresh and lighter hydrocarbons that would be more of a concern for short-term (acute) toxicity to aquatic organisms, whereas used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic (long-term) hazards including carcinogenicity. Metals of concern include lead; and often to a lesser degree, zinc, chromium, barium, and arsenic Chinenyeze and Ekene (2015).

Aromatic compounds are considered to be the most acutely toxic component of petroleum products, and are also associated with chronic and carcinogenic effects. Aromatics are often distinguished by the number of rings they possess, which may range from one to five. Lighter, mono-aromatic (one ring) compounds include benzene, toluene, ethylbenzene, and xylenes. Aromatics with two or more rings are referred to as polyaromatic hydrocarbons (PAHs). Used motor oil contains several toxic components including up to 30% aromatic hydrocarbons, with as much as 22 ppm benzo(a)pyrene (PAH). Used motor oil typically has much higher concentrations of PAHs than new motor oil for example naphthalene present as one of the component of motor oil exhibits its toxic effect on liver, kidneys, heart, lungs and nervous system. Benzo(a)pyrene which is also present as one of the component of used motor engine oil is potential mutagen and carcinogen.

The hydrocarbons present in the engine oil are hydrophobic in nature with little tendency to solubilise in the water and persists in the soil and water for a longer period. Apart from this, these hydrocarbons are recalcitrant to biodegradation and posses potential for various chronic effects (like carcinogenicity). Some of the compounds present in the engine oil have also been found to exhibit immunologic, reproductive, hepatotoxic, fetotoxic, and genotoxic effect.

Waste motor oil not only affects human life but it also affects other lives (like amphibians reproduction was affected in the environment of high concentration of motor oil contamination). Hydrocarbons from oil can move to atmosphere or settle through water to bottom sediments, where they may persist for years. Metals from oil may build up in various media. The concentration of various PAHs is much higher in used oil than in fresh lubricating oil. For example, Grimmer *et al*. (1981) reported concentrations of ibenz(a,c)-anthracene, 4-methylpyrene, fluoranthene, benz(a) anthracene, benzo(e)pyrene, benzo(g,h,i)perylene, and benzo(a)pyrene, respectively, 36, 49, 253, 720, 1,112, 4,770, and 7,226 times higher in "used" compared to "fresh" oil. As oil is used in a crankcase, motor oil breaks down to give a wide variety of oxygenated and aromatic hydrocarbons. Other organic compounds found in waste oil include toluene, benzene, xylenes, and ethylbenzene. Also present are organic and inorganic compounds of chlorine, sulphur, phosphorus, bromine, nitrogen, and metals such as zinc, magnesium, barium, and lead resulting from oil additives and contamination during use or disposal. Used engine oil is a contaminant of concern, with large volumes entering aquatic ecosystems through water runoff. The major source of petroleum contamination in urbanized estuaries comes from waste crankcase oil (WCOs).

Another source of WCOs entering the aquatic environment is through improper disposal of used motor oil. Many people change their own car motor oil instead of having it done professionally. Shops that perform oil changes have special receptacles for used motor oil. Many private citizens do not dispose of their used oil properly. For example, some people pour waste oil over their gravel/dirt driveways to keep the dust down, let it sit in their garage or backyard for extended periods of time, or illegally pour it down storm drains.

Contamination of one component of ecosystem by used motor oil leads to the imbalance of other components of the ecosystem. Used engine oils or petroleum products from the automobile garages or service stations may contaminate different components of the environment. These service stations release oil contaminated wastewater directly into the common sewer or storm drains without treatment. This oil and other additives used for cleaning the vehicles contaminate not only water in the sewer but also this water contaminate soil and nearest fresh water sources like wells, ponds, rivers etc. Used motor oils are used by people for various other purposes like application to road surfaces, application to tin pans and rafters used in the building construction activities for making roof. Here oil is applied to the tin pans for rendering repulsive nature to the tin pans and rafters towards the cement so that cement should not stick to the tin pans and rafters and facilitate easy removal of it after proper solidification and consolidation of cement in the roofs, beams or pillars.

From contaminated soil surfaces, roads or other anthropogenic applications, oil constituents enter the surrounding environment by volatilization to air; transport of windborne dust to air and subsequent transfer to land and water; and runoff to adjacent soil or drainage ditches and eventually to local water systems. Burning waste engine oil leads to air emissions of metals and polycyclic aromatic hydrocarbons (PAHs) that are generally adsorbed to particulate matter and deposited to soil and water (Katiyar et al 2010).

Microbial degradation of oil

Varieties of microorganisms are involved in the biodegradation of petroleum oil and its components. Researchers all over the world have studied microorganisms able to degrade crude oil aliphatic components such as Dodecane, hexadecane, octadecane, Eicosane, Tetracosane, and aromatic and PAH compounds such as phthalic acid, xylene, Toluene and Naphthalene etc. (Cerniglia 1980; Holden, 2002; Hadibarata *et al.,* 2009; Kropp 2000; Kim, 2001; Noordman *et al.,* 2002; Patrauchan, 2005; Suenaga *et al.,* 2001). Adoki and Orugbani (2007) worked on heterotrophic bacteria for the removal of crude petroleum hydrocarbons from soil. This soil was amended with nitrogenous fertilizer plant effluents. They found *Pseudomonas* and *Bacillus* species as the predominant bacterial species present in the soil contaminated with oil while a total of ten genera of petroleum degraders were isolated, namely, *Micrococcus*, *Pseudomonas*, *Acinetobacter*, *Proteus*, *Bacillus*, *Actinomyces*, *Corynebacterium*, *Enterobacter*, *Brevibacteria* and *Citrobacter*. Okparanma *et al.,* (2009), studied the effectiveness of 2 bacterial isolates (*Bacillus subtilis* and *Pseudomonas aeruginosa*) in the restoration of oil-field drill-cuttings contaminated with polycyclic aromatic hydrocarbons (PAHs).

Active involvement of *Bacillus* species in petroleum hydrocarbons or oil degradation has been corroborated by the study performed by Wang *et al.,* (2019).

II. EXPERIMENTATION AND RESULTS

Versatile catabolic capability of four bacterial cultures *Bacillus subtilis* PD6, *Bacillus sp.* PD9, *Enterobacter sp.* PD11 and *Bacillus sp.* PD14 was assessed by subjecting them for the utilization of used engine oil. Used engine oil was collected from automobile garage located in Hingna, Nagpur (Maharashtra, India) in a sterile plastic container and kept at -20^{o}C. This oil was subjected to biodegradation by the four bacterial cultures, which was carried out in 250ml 0.1XM9 media containing flasks. All flasks containing 0.1XM9 media were sterilized by autoclaving at 120 lbs. 1250 µl used engine oil was added as a sole carbon source to sterilized medium. Four selected bacterial cultures *Bacillus*

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subtilis PD6, *Bacillus sp.* PD9, *Enterobacter sp.* PD11 and *Bacillus sp.* PD14 from freezer stocks were inoculated into sterile 5 ml Luria Bertani (L.B.) broth tubes. All the inoculated tubes were incubated at 28^{o}C and after overnight growth 100 µl of each of these four bacterial cultures was inoculated into respective minimal medium flasks containing used engine oil as substrate.

All flasks were incubated at 28^{o}C at 100 rpm in a shaker incubator and samples were withdrawn from each control and experimental flask at regular interval of 24h for COD (Chemical Oxygen Demand) analysis. Experiments were performed in triplicate and observations taken were statistically analysed by ANOVA (Analysis of Variance). COD removal by all the selected bacterial cultures was found to be significant ($P<0.01$). Figure 1 shows time course for engine oil degradation by *Bacillus subtilis* PD6, *Bacillus sp.* PD9, *Enterobacter sp.* PD11 and *Bacillus sp.* PD14 and Figure 2 show percent degradation of used engine oil by all the four selected bacterial cultures.

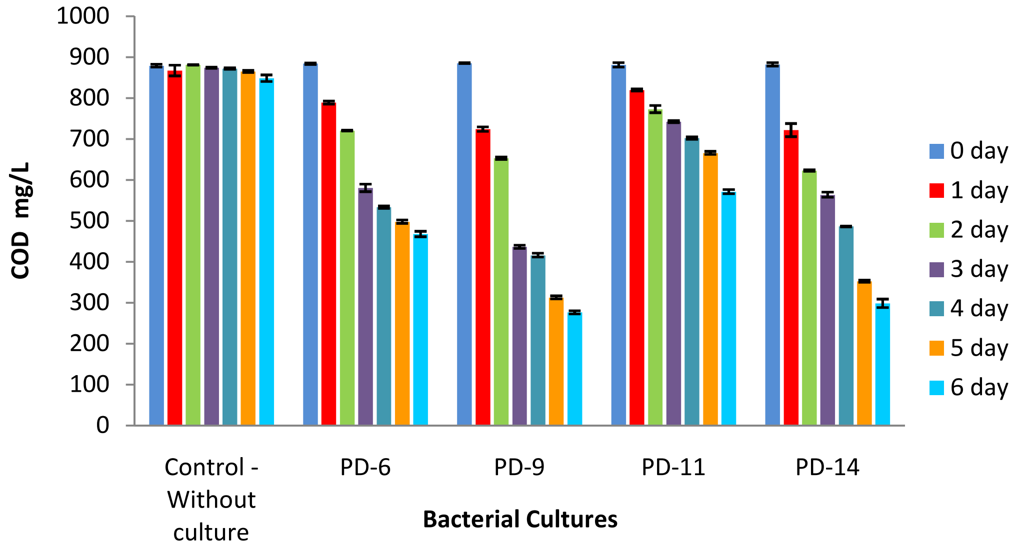

Figure 1: Biodegradation of used engine oil by bacterial cultures *Bacillus subtilis* PD6, *Bacillus sp.* PD9, *Enterobacter sp.* PD11 and *Bacillus sp.* PD14.

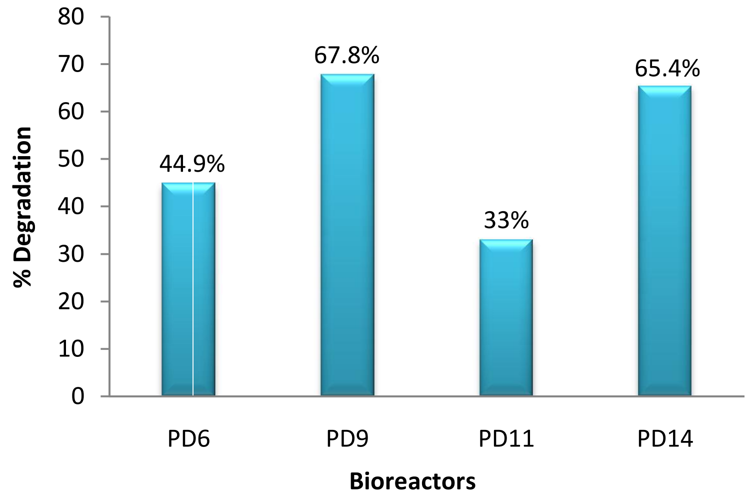

Figure 2: Percent biodegradation of used engine oil by bacterial cultures *Bacillus subtilis* PD6, *Bacillus sp.* PD9, *Enterobacter sp.* PD11 and *Bacillus sp.* PD14.

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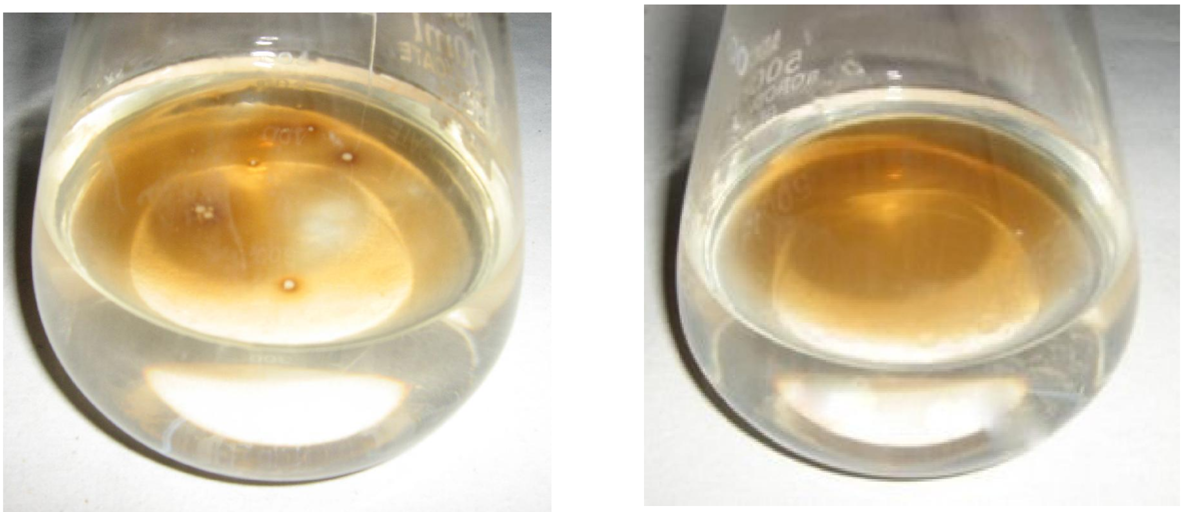
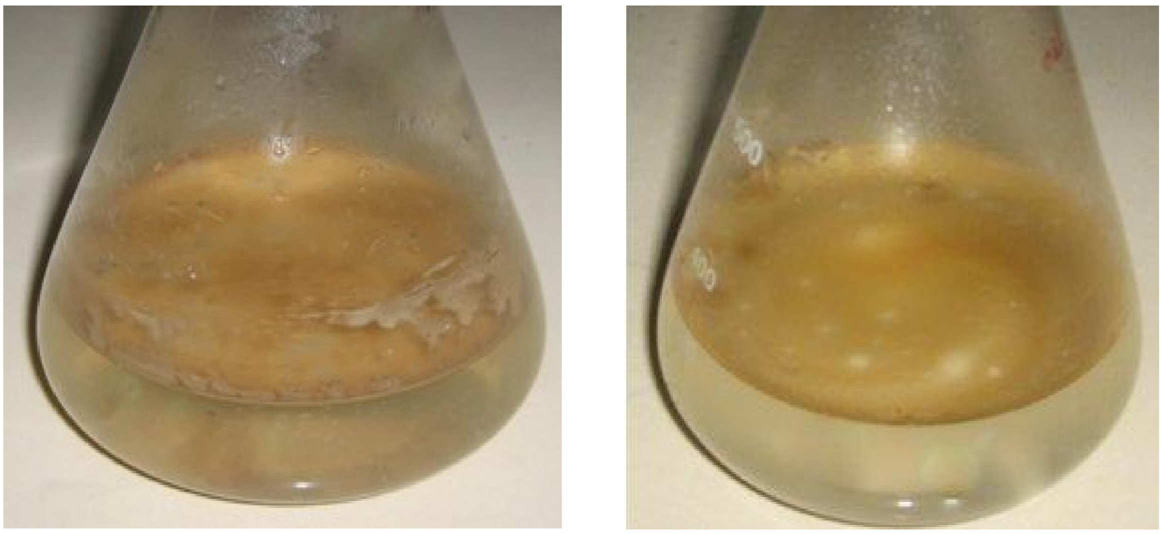
Comparative assessment of COD values after 6 days incubation revealed that in presence of *Bacillus subtilis* PD6 COD decreased from 884 ± 2 mg/l to 468 ± 7 mg/ml which corresponds to 44.9%, while in presence of *Bacillus sp.* PD9 COD decreased from 885±1 mg/l to 276±4 (67.8% COD removal) *Enterobacter sp.* PD11 displayed comparatively lower COD removal of 33% (decrease from 881±5 mg/l to 571±5 mg/l) and PD14 showed 882±4 mg/l to 298±10 (65.4% COD removal). Biodegradation and mineralization of the used engine oil at different time points is shown in Figure 3 a, b and c. These photographs show the development of the intense growth in the flask indicating the versatile catabolic and oil mineralizing capability of the inoculated bacterial cultures.



Bacillus subtilis PD6 *Bacillus sp.* PD14

Figure 3a: Flasks before inoculation of bacterial cultures containing 0.1X M9 media and used engine oil as a sole source of carbon.



Bacillus subtilis PD6 *Bacillus sp.* PD14

Figure 3b: Flasks after inoculation and 24 h incubation with bacterial cultures *Bacillus subtilis* PD6 and *Bacillus sp.* PD14.

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Bacillus subtilis PD6 *Bacillus sp.* PD14

Figure 3c: Flasks after two days incubation with bacterial cultures *Bacillus subtilis* PD6 and *Bacillus sp.* PD14 show development of heavy growth and complete mineralization of used engine oil.

III. DISCUSSION

Microorganisms are equipped with metabolic machinery to use petroleum as a carbon and energy source (Van Hamme *et al*., 2003). In the present study catabolic potential of four selected bacterial cultures was assessed for used engine oil (So *et al.,* 2001). The catabolic potential of bacterial cultures towards different components or compounds of crude oil is dependent on presence of catabolic pathways in the bacterial cell (Whyte *et al.,* 1997). The catabolic capability of soil isolates has been assessed by various researchers towards different aromatics (Khardenavis *et al.,* 2008, 2009; Seo, 2009; Thangaraj *et al.,* 2008; Wrenn 1998), polycyclic aromatic (Vincent, 2000) and aliphatic hydrocarbon compounds (Kas *et al.,* 1997; Bogan *et al.,* 2003). All cultures were found to be able to degrade Dodecane, hexadecane, octadecane, Eicosane, Tetracosane, Phthalic acid, Xylene, Toluene and Naphthalene. All these compounds are constituents of crude oil and engine oil (Paxéus, 1996; Hamme *et al.,* 2003). Bacterial cultures capable of degrading all the above compounds may also possess the potential to degrade engine oil. With this objective biodegradation of automobile engine oil was attempted. During this study all the bacterial cultures were found to biodegrade used engine oil significantly ($P<0.01$). Out of four bacterial cultures, *Bacillus sp.* PD9 and *Bacillus sp.* PD14 showed comparable degradation of used engine oil up to 67.8% and 65.5% removal of COD within six days respectively. Remaining cultures, *Bacillus subtilis* PD6 and *Enterobacter sp.* PD11 displayed only 44.9% and 33% COD removal within six days (Figure 2). Thus of the four cultures, *Bacillus sp.* PD9 and *Bacillus sp.* PD14 showed complete solubilisation and mineralization of oil (Figure 3a, 3b and 3c) indicating presence of bio-surfactant producing potential in these bacterial cultures which has been known to be essential for solubilisation of oil by various researchers (Naim Kosaric, 2001; Tabatabaee *et al*., 2005).

Natural surfactants increase the solubility of hydrocarbon, which in turn increases its bioavailability to micro-organisms (Kaczorek *et al.,* 2005). Results of previous research work demonstrated that biosurfactants affect the rate of hydrocarbon biodegradation in two ways: by increasing solubilization and dispersion of the hydrocarbon and by changing the affinity between microbial cells and hydrocarbons by inducing increases in cell surface hydrophobicity (Zhang *et al*., 1995). In the present study, *Bacillus subtilis* PD6 and *Enterobacter sp.* PD11 also exhibited the mineralizing activity but it was comparatively less and presence of used engine oil droplets was witnessed in the flasks even after three days whereas complete removal of oil was observed in the *Bacillus sp.* PD9 and *Bacillus sp.* PD14 flasks. Bacterial cultures were found to degrade crude oil as a whole or in fractions or components collected from it

(Chikere *et al.,* 2009). In this study total four bacterial cultures were tested for used engine oil biodegradation on individual basis. These cultures can be mixed and used as bacterial consortia for the cleanup of used oil contaminated water (Chhatre *et al.,* 1996; Kapley *et al.,* 1999). Findings of the present study are in agreement with work done by Joshi and Pandey (2011) in which *Bacillus sp*. isolated from the cow dung was found to degrade petroleum products such as xylene, benzene, toluene, kerosene and diesel oil. In this study *Bacillus sp.* was appeared to be the efficient degrader of used engine oil. This observation is in congruence with the observation obtained by Nwaogu *et al.,* (2008) who found *Bacillus sp*. as the better degrader of diesel oil compared to other isolates. Kebria *et al.,* (2008) also isolated *Bacillus sp.* and attributed the bioremediation of pollutants by the *Bacillus sp* in the extreme environment to the presence of resistant endospore in the *Bacillus sp*.

Prevalence of *Bacillus sp.* in the contaminated soil and its role in the used engine oil utilization was demonstrated by the Dominguez-Rosado *et al.,* (2004) which supported the utilization of used engine oil by *Bacillus* sp. in the present study. Frequent occurrence of *Bacillus* sp. as an effective agent for the degradation of hydrocarbons (Benkacaker and Ekundayo, 1997; Diaz *et al.,* 2000) was already proved. Ghazali *et al.* (2004) isolated *Bacillus* sp. from hydrocarbon polluted soils and studied this genus relative to their potential to biodegrade crude oil, benzene, ethylbenzene, 0-xylene, n-tetradecanol, octanol and decanol (Moneke *et al.,* 2011). A study performed by Sebiomo *et al*. (2011) has found *Bacillus sp* among the best utilizer of crude oil and gasoline. In this study, presence of used engine oil utililzing capability was also observed in the *Enterobacter sp.* PD11. This finding agrees with the finding of the Jain *et al.,* (2010) who isolated *Enterobacter* strain from petroleum contaminated soil from high altitude Mussoorie, India and found it capable to degrade PAH and n- alkane etc. of 2T engine oil.

IV. CONCLUSION

In the present study biodegradation of used engine oil by four bacterial cultures was carried out successfully. Out of four bacterial cultures, *Bacillus sp.* PD9 and *Bacillus sp.* PD14 were found to be efficient cultures which were able to degrade 67.8% and 65.5% used engine oil within six days. Other two cultures *Bacillus subtilis* PD6 and *Enterobacter sp.* PD11 also exhibited successful biodegradation of used engine oil but less efficiently compared to *Bacillus sp.* PD9 and *Bacillus sp.* PD14. From the above study it was substantiated that the selected four bacterial cultures possess the capability to eliminate used engine oil rapidly from the waste water and these cultures can be used to bioremediate the wastewater laden with used engine oil or hydrocarbon compounds.

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