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Full Length Research Paper

Biodegradation of mineral oil by bacterial strains isolated from contaminated soils

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Potential mineral oil degrading bacterial isolates (*Pseudomonas putida*, *Rhodococcus erythroplolis* and *Bacillus thermoleovorans*) were studied from the contaminated soils. The bacterial populations of these polluted soils were 5.25 × 10^5, 1.76 × 10^6 and 5.11 × 10^5 CFU/ml with three different colony types of bacterial strains, respectively. Microbial population diversity studies were carried out by microbial enumeration, identification, and determination of growth responses of bacterial isolates in different concentrations of mineral oil sample. Phenotypic examination of the heterotrophic bacteria belonged mainly to the genus Pseudomonas, Rhodococcus and Bacillus. The mixed populations are capable of degrading mineral oil up to 120 ppm. Mineral oil biodegradation was fast by the mixed culture comparing to the biodegradation of each strain separately. Since the biological treatment can efficiently destroy the hydrocarbons and does not allow the contaminant to accumulate, it is considered to be a superior technology. This study revealed that the mixed bacterial consortium achieved maximum crude oil degradation at pH 7. Hence, it is suggested that the use of the aforementioned mixed bacterial consortium under optimized conditions will be an effective and eco-friendly technology for the degradation of hydrocarbons from BH crude oil.

Key words: Mineral oil, biodegradation, *Pseudomonas putida, Rhodococcus erythroplolis*, *Bacillus thermoleovorans*.

INTRODUCTION

Soil contaminated with mineral oil has posed a great hazard for terrestrial and marine ecosystems. Mineral oil, water-in-oil emulsion, and hydraulic fluids all have components in common with a very large number of other products that are based on mineral oil and synthetic mineral oils (polyalphaolefins) (Regina et al., 2006). These products include motor oils, fuel oils, and petroleum distillates. Mineral oil is highly refined oil that consists of saturated hydrocarbons. According to the U.S. Coast Guard Emergency Response Notification System (ERNS), "mineral oil" is one of the most commonly spilled petroleum products in the U.S. Possibly, many different mineral oil substances, including the many industrial use mineral oils are lumped into the general heading of "mineral oil" when spilled (Abdel-Megeed et al., 2010). Mineral oil use can cause a variety of untoward effects (such as decreased absorption of vitamin A), so habitual use on mineral oil should be avoided. Mineral oils used as lubricants for metal workers have been associated with increased occupational risk of skin cancer. In humans, several potential problems or symptoms have been observed (Deppe 2003). If it gains access to the lungs, mineral oil produces lipid pneumonitis. So far, biodegradation suggests an effective method (Morgan et

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al., 1989). Biodegradation of mineral oil, agricultural agrochemicals, and other environmental pollutants in natural ecosystems is quite complex, as it occurs relatively slow. Mineral oil products with its multitude of potential primary substrates provide an excellent chemical environment in which cometabolism can occur. During biodegradation, mineral oil is used as an organic carbon source by a microbial process, resulting in the breakdown of mineral oil components to low molecular weight compounds. The feasibility of bioremediation starts with an assessment of the degradation potential of the contaminants degradation potential of the contaminants (Noordman and Janssen, 2002). This assessment includes evaluations of the easy or difficulty of degradation, the ability to achieve total mineralization, as well as the environmental conditions necessary for mineralization. Regulatory officials and citizens demand information on the potential degradation products and their potential hazard (Whyte et al., 1999). Microbial transformations of organic compounds are frequently described using the terms detoxification, degradation, and mineralization. Microorganisms which actually grow on other hydrocarbons within the mixture, is a phenomenon called cometabolism. Detoxification is the transformation of the compound to some intermediate form that is less toxic. Degradation means that the parent compound no longer exists. Mineralization refers to the complete conversion of the organic structure to inorganic forms e.g. H_2O and/or CO_2. The cleanup of soils and ground water contaminated with hydrocarbons is of particular importance in minimizing the environmental impact of petroleum and petroleum products and in preventing contamination of potable water supplies (Abdel-Megeed et al., 2010). The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry (Das and Chandran, 2011). Consequently, there is a growing industry involved in the treatment of contaminated topsoil, subsoil and groundwater (Morgan and Watkinson, 1989). Although the autochthonous microorganisms in soils show a broad capability to degrade a variety of contaminants, they are, in some cases, extracted from the contaminated soil, cultivated in a reactor, and re-introduced into the soil as a start-up culture. In other cases, site-independent cultures with known degradation capabilities are introduced in the soil as superbugs, but their usefulness is questionable because they are not adapted to the local environmental soil conditions (Abdel-Megeed et al., 2010).

One important requirement is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9 (Atlas and Bragg, 2009).

This research is aimed at determining identifying microorganisms capable to degrade mineral oil with views to a future employment in the bioremediation of polluted soils. Understanding the microbial degradation process of mineral oil will increase possibilities of developing models and strategies for removing hydrocarbon from contaminated sites (Das and Chandran, 2011).

MATERIALS AND METHODS

Sample collection

The soil used in this study was collected from the bus areas, Hanover, Germany, from the surface layer to plow depth (5 to 40 cm) according to method described by American Society for Testing and Materials (1998). During operation, sterile plastic bottles were used in collecting the samples after which they were transported and stored at 40°C. The soil was air-dried, sieved through a 2 mm sieve to homogenize. The soil texture was Sand 4%, Silt 85%, and Clay 11% and the soil texture was Silt. Extraction of the micro-organisms from soil was carried out according to the method described by Lindahl and Bakken (1995). The soil properties were determined according to the methods described by Ngole (2011) with some modifications. The chemical properties of the soil were as follows: pH (1:1 w/v water) 4.72, EC (1:1) 1.20 dS/m, organic carbon 0.41%, $CaCO_3$ 2.14%, available nitrogen 23.20 mg/kg soil, available P 9.3 mg/kg soil (Olsen).

Extraction and culturing conditions

Microorganism isolation was carried out using selective media as described by Abdel-Megeed (2004). Solid and liquid media were used for the isolation of mineral oil degrading microorganisms. Nutrient Broth and Mineral Salt Medium (MSM) (Abdel-Megeed and Al-Harbi, 2010) were the liquid media while Mineral Salt Oil Agar and Sabouraud Dextrose Agar (Abdel-Megeed 2004) were the solid media. The extraction of the mineral oil was carried out according to Deppe (2003). Heterotrophic bacterial sample were enumerated by preparing serial dilutions of the soil extraction liquid sample (Lindahl and Bakken, 1995) using nutrient broth as the diluent. Mineral Salt Oil Agar was employed in determining the density of mineral oil degrading microorganisms. The isolated strains were characterized and identified by determination of the CFU wall composition. Initial identification was based on the criteria of Bergey's Manual of Determinative Bacteriology (Bergey's Manual, 1985). Further classification and identification were performed by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DSMZ) by fatty acids analysis and 16S rDNA sequencing.

Degradation capacity of mineral oil by various isolates

Three dominant isolates which grew profusely on the mineral salt hydrocarbon agar medium were used for this study. The isolates were streak inoculated onto nutrient agar plates from the stock culture and incubated at 20°C for 24 h to check for viability and purity. The isolates from the pure plates were inoculated aseptically into 20 ml of sterile nutrient broth in screw-capped flasks. These were incubated at 20°C for 24 h. Serial dilutions of the broth cultures were prepared and 0.1 ml volumes were inoculated onto duplicate plates using the pour plate technique. These were

incubated aerobically at 20°C for 24 h. The mean counts on the duplicate plates were used to calculate the colony formation units (CFU) of CFU/ml of the original broth culture (Abdel-Megeed, 2010; Das and Chandran, 2011).

Flasks with different concentrations (30, 60, and 120 ppm) of mineral oil (Merk) were prepared. The mineral oil was mixed properly with the mineral salt medium. One ml of the isolates was added to each flask. The flasks were incubated at 20°C for a period of 12 days. To measure microbial growth and pH, sample aliquots were withdrawn at 2 day interval. Serial dilutions of samples were carried out using sterilized mineral salt solution as the diluents. Aliquot of 0.1 ml of samples were inoculated onto duplicate plates employing the pour plate technique. All inoculated plates were incubated aerobically at 20°C for 24 h, after which CFU were counted and mean counts recorded.

Gas chromatography analysis

Mineral oil was analyzed according to the method reported by Tzing et al. (2003) with a Varian Gas Chromatograph-Mass Spectrometer (GC-MS; model CP-3800 gas chromatograph and Saturn 2200 mass spectrometer, Varian Technologies Japan, Inc.). Resulting chromatograms were analyzed by Saturn Software GC/MS Workstation Version 5.52 to identify mineral oil components. All analyses were carried out with the split ratio of 20:1. Helium was used as the carrier gas with a flow rate of 0.8 ml min^{-1}. Injector temperature was set at 250°C.

RESULTS AND DISCUSSION

Identification and characterization of the isolates

Three bacterial isolates were identified on the basis of their cultural and biochemical characteristics and with reference to Bergey's Manual of Determinative Bacteriology (9^{th} edition). The bacterial isolates were *Pseudomonas putida*, *Rhodococcus erythroplolis* and *Bacillus thermoleovorans*. Further identifications and characterization by 16S rDNA sequence and fatty acids analyses were performed by DSMZ (Table 1).

Effect of mineral oil on mixed culture

The standard cultures of the isolates *P. putida, R. erythropolis* and *B. thermoleovorans* were found to be 5.25×10^5, 1.76×10^6 and 5.11×10^5 CFU/ml, respectively, in the extraction sample (experimental soil extract). According to the preliminary experiment with maximum concentration utilized of mineral oil (120 ppm), the mixed culture exhibited high efficiency of assimilating the mineral oil. The bacterial growth reached its maximum value of 6.66×10^7 CFU/ml. Fluctuations in bacterial counts expressed as number of bacteria (CFUs/ml) over the incubation period of 12 days in mineral salt medium containing mineral oil as sole carbon source was monitored (Figure 1). There was no growth or degradation occurred after 8 days. The maximum concentration of mineral oil utilized by the mixed culture was 120 ppm.

Regarding to the existence of the heterotrophic bacteria ranged from 4.20×10^4 to 9.86×10^5 CFU/ml, the indigenous microbial communities are likely to contain microbial populations of different taxonomic characteristics, which are capable of degrading the contaminating chemicals (Barth and Atlas, 1977).

This observation is in line with reports of Atlas and Bragg (2009), that there was an increase in heterotrophic bacterial population in the presence of dispersant agents. Full agreement with Das and (2011), that the medium employed for isolation of mineral oil degrading bacteria may have a significant selective effect on the bacterial population, that are sampled. The mixed population was capable of degrading maximum concentration utilized of mineral oil (120 ppm). Similar patterns of mineral oil degradation in which preferred degradation occurs for mineral oil have been observed with CFUs of *Flavobacterium* sp. ATCC 39723 (Steiert et al., 1987; Abdel-Megeed, 2004), and with CFU extracts from *Arthrobacter* sp. This strongly suggests the complete degradation of mineral oil without the accumulation of inhibitory or toxic metabolites for these microorganisms. This is considered to be a very important issue to be taken into account in degradation processes and bioremediation strategy.

Bacillus thermoleovorans

There was a steady increase in the CFU from 8.1×10^5 to 1.5×10^8 CFU/ml within 9 days. From microbiological point of view, the delay in the *Lag* phase of *B. thermoleovorans was* expected since a number of different chemical reactions are involved in mineral oil utilization. It was observed that the growth fluctuated according to the various concentrations (Figure 2). By the 3^{rd} day, there was a steady rise from 4.1×10^7 CFU/ml till the 7^{th} day, where there was decrease and final rise on the 12^{th} day. There was a fall in bacterial growth estimated by 3.9×10^7 CFU/ml and the substrate was totally assimilated. However, the pH was steady within this period and ranged from 6.56 to 8.38.

Pseudomonas putida

The CFU had a steady increase of 8.25×10^5 to 1.02×10^8 CFU/ml by the 9^{th} day. This was followed by a gradual decrease to reach 2.10×10^6 CFU/ml by the 12^{th} day (Figure 3). The pH however rose steadily within this period. One can notice that after the 3^{rd} day, they began to utilize mineral oil. 100% of the mineral oil concentration was assimilated after the 12^{th} day. A similar trend in pH to the previous strain was observed during the growth pattern.

Rhodococcus erythropolis

The CFU had a steady increase from 8.1×10^5 to

Table 1. Morphological and physiological properties of *P. putidas, B. thermoleovorans* and *R. erythropolis.*

Properties	*P. putida*	*R. erythropolis*	*Bacillus thermoleovorans*
Motility	-	+	+
Gram reaction	-	+	-
Adipate	-	+	+
Caprate	+	+	+
Arabinose	+	+	+
m-inositol	+	+	+
Catalase	+	+	-
n-acetylglucosamin	+	+	+
Pigment fluorescent	+	-	-
D-xylose	+	+	-
Malat	+	-	-
Maltose	-	-	-
Mannitol	+	+	+
Shape of the CFU	rods	short rods	coccus
NO_3- and NO_2-	-	-	-
Oxidase	+	+	+
Phenylacetate	+	+	+
Pigment fluorescent	+	-	+
Sorbitol	+	+	+

+: growth -: no growth

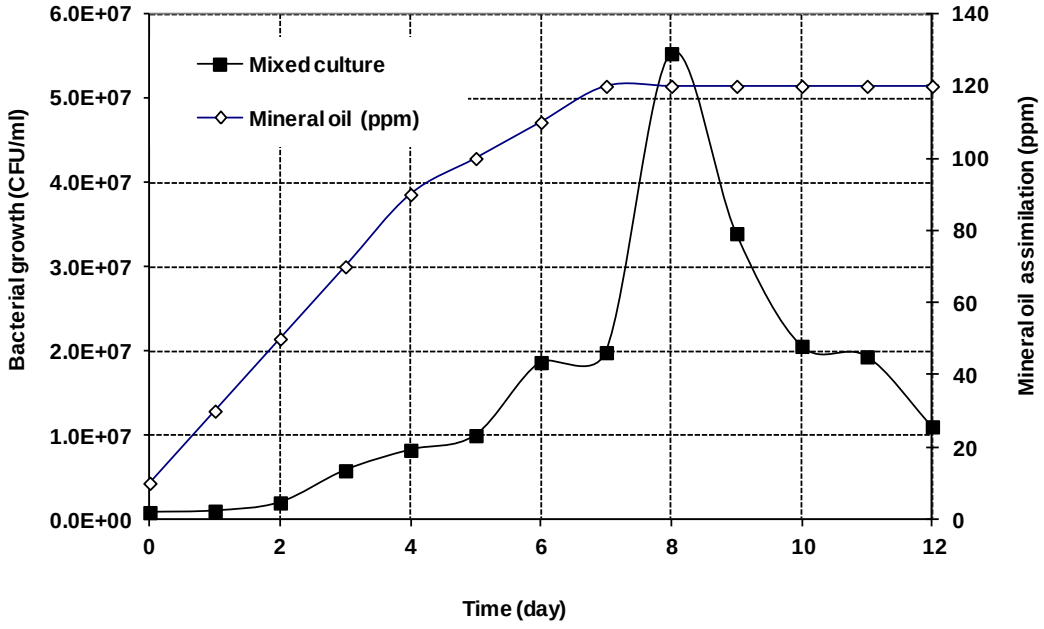

Figure 1. Biodegradative capability of the mixed culture grown on mineral oil.

7.9×10^7 CFU/ml by the 10^{th} day (Figure 4). The pH rose steadily to 7.64 by the 12^{th} day, while the CFU rose till the 9^{th} day. After the 9^{th} day, there was a gradual fall in CFU. By the 12^{th} day, there was a fall in bacterial growth estimated by 4.02×10^7 CFU/ml. It was clear that the mixed culture exhibited high efficiency compared to the isolates in assimilating of mineral oil (Figure 5).

Moreover, if a comparative study was held between the

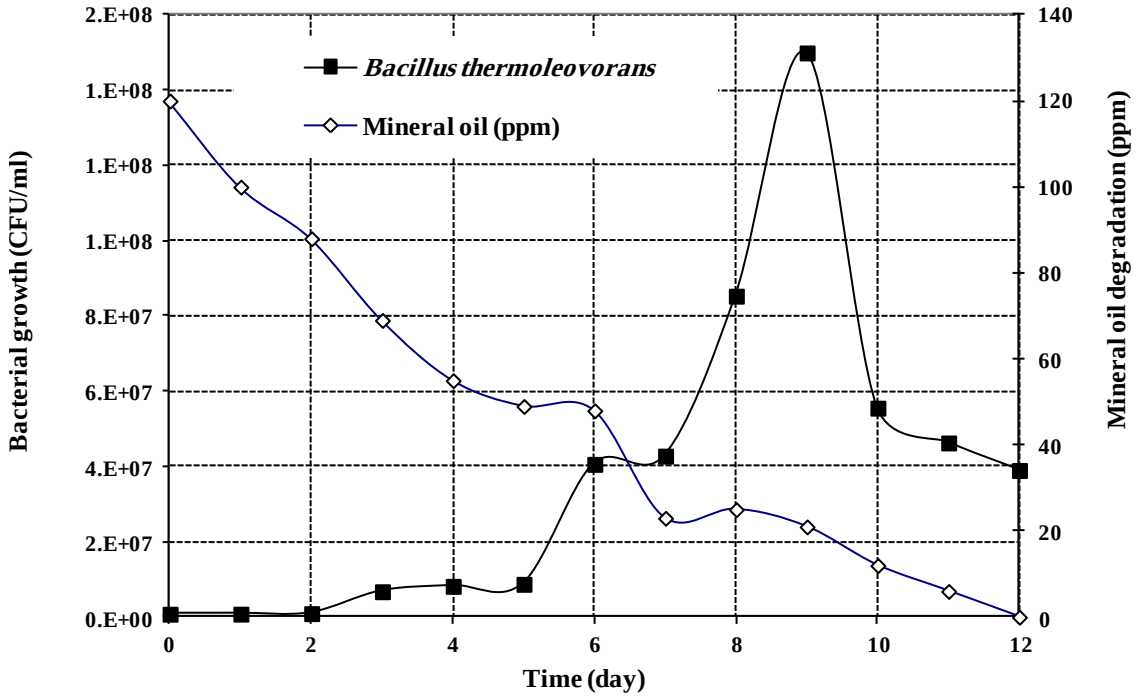

Figure 2. Growth and biodegradation of mineral oil by *Bacillus thermoleovorans*.

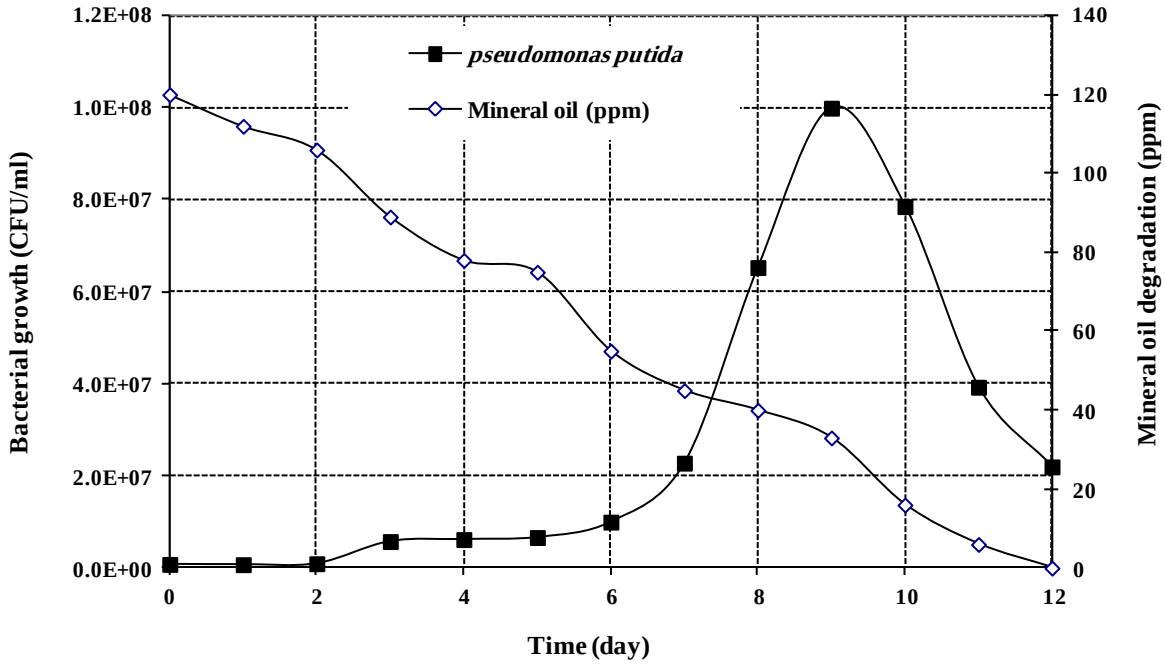

Figure 3. Growth and biodegradation of mineral oil by *Pseudomonas putida.*

isolates and the mixed culture. It was concluded from Figure 6 that up to 75% of mineral oils was actually degraded after the 2nd day and 100% was utilized within 8 days.

The biodegradation of mineral oil was fast compared to the biodegradation of each strain separately. In this respect, these isolates would have great application in bioremediation of mineral oil contaminated sites.

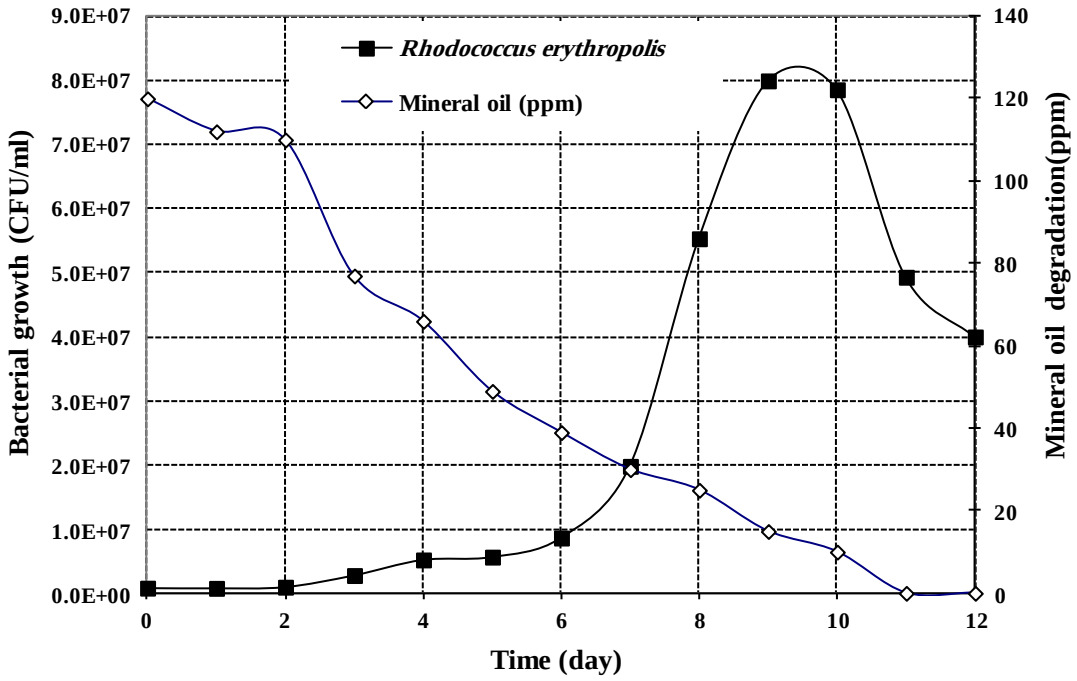

Figure 4. Growth and biodegradation of mineral oil by *Rhodococcus erythropolis*.

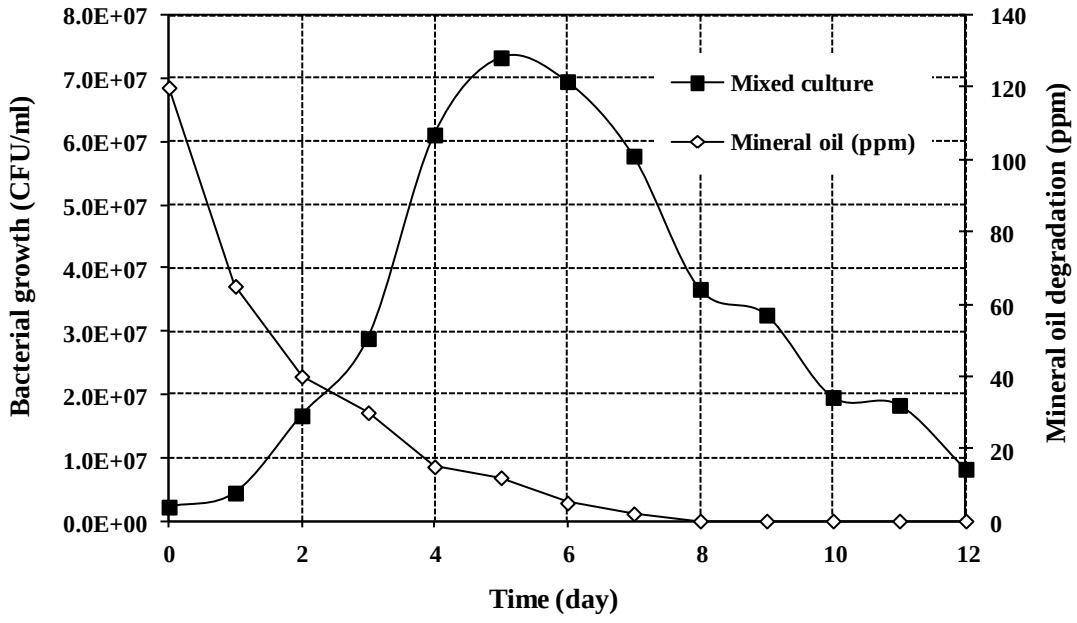

Figure 5. Cometabolite and biodegradatve capability of *Geobacillus thermoleovorans*, *Pseudomonas putida* and *Rhodococcus erythropolis*.

Bioaugmentation treatment with such a wide spectrum of degraders would be the preferable choice of treatment over many others. The biodegradation increased fast and the metabolism was high enough to maintain the bacterial activity stable. It was also observed that the first day of incubation was the most important and critical stage for the biodegradation of the mixture. Results obtained in this study are similar with results obtained from soil samples at Hokaiddo and Japanese Costal water (Tanaka et al., 1993; Obayori et al., 2009).

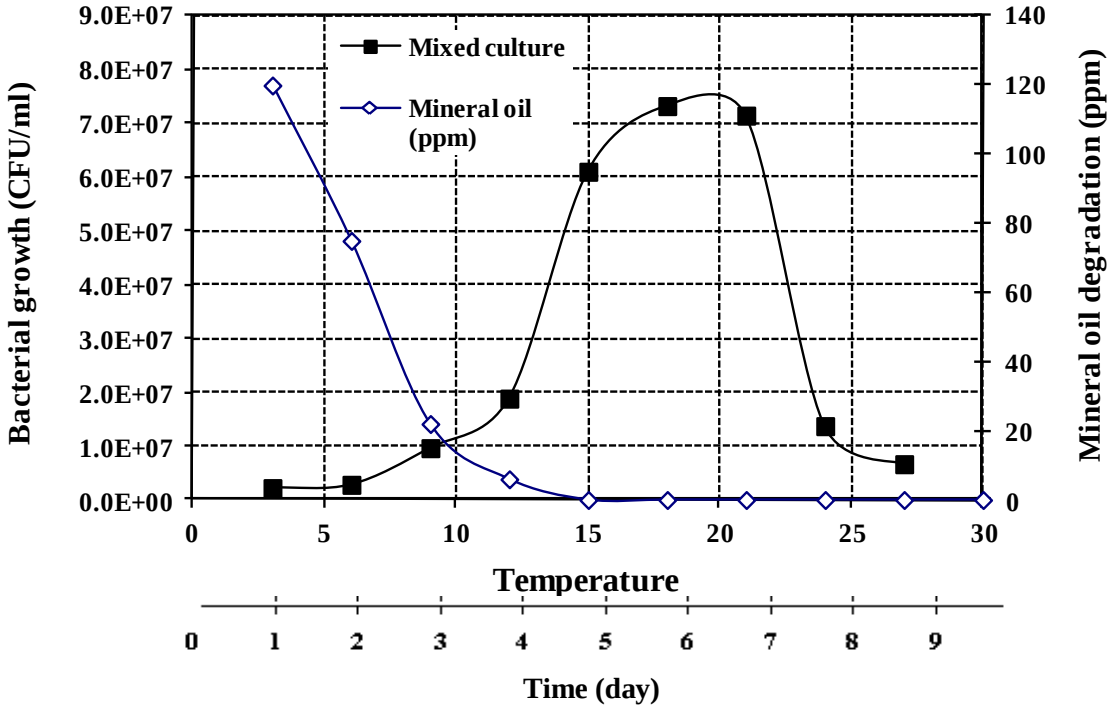

Figure 6. Temperature dependence of mineral oil degradation by the mixed culture.

The behavioral patterns of the mineral oil-utilizing bacteria in media containing different concentrations of mineral oil present an interesting observation.

To carry out the bioaugmentation successfully, it would be necessary to select bacteria having a high capacity and the versatility to degrade the many components of petroleum products. Consequently, bacteria able to grow on this carbon source would easily acquire the ability to degrade a wide variety of hydrocarbon components of different petroleum products. Therefore, bacterial screening was conducted using mineral oil as a sole carbon source. Among the three isolated candidates, strains *P. putida* and *B. thermoleovorans* appeared to be two best degraders. Indeed, the mixed culture was capable of degrading an excess amount of mineral oil (120 ppm) present in the media in an efficient manner. It was observed that the mixed culture utilized mineral oil effectively compared with each isolate separately. As such, *R. erythropolis* would be a unique strain possessing the ability to degrade a wide spectrum of hydrocarbons. Currently, evaluation of *B. thermoleovorans* regarding its ability to decontaminate soils from mineral oil at laboratory scale is in progress.

Temperature dependence of growth by mixed culture grown on mineral oil

Another interesting observation during the growth of mixed culture on mineral oil was that the increase in the temperature value enhanced the biodegradation of the mineral oil (Figure 6). It was also observed that the degradation efficiencies were dependent on the temperature fluctuations.

Additionally, the increase in temperature makes biological membranes more fluid due to increased vibrational activity of the fatty acid chains in the phospholipid bilayer. This increase in the rate of fluidity helps in increasing the rate of substance uptake from a CFU's surrounding medium. The ability of the mixed culture to function well between 17 and 21°C depends to a great degree on the availability of the substrates. Compared to the biodegradation of three strains separately, the mixed culture could assimilate the whole amount of mineral oil within the first five days. The results conform to Colwell and Walker (1977) and Jain et al. (2005) report on two major microbial responses to mineral oil, which was an increase in microbial biomass.

The GC profile of the remnant compounds of mineral oil in mixed culture was compared to that of the control. Minor peaks between two *n*-alkanes include those of iso-alkanes and cycloalkanes. Figure 7 shows the GC profile of the control culture (12 days) with mineral oil, which included a mixture of hydrocarbons from nC_9 to nC_{20} consisting of *n*-alkanes.

All components in mineral oil were highly reduced by mixed culture. However, the degradation of hydrocarbons shorter than C_{21} was not so detectable. In general, there was a common reduction of all components of aliphatic hydrocarbons. These findings suggest that mixed culture

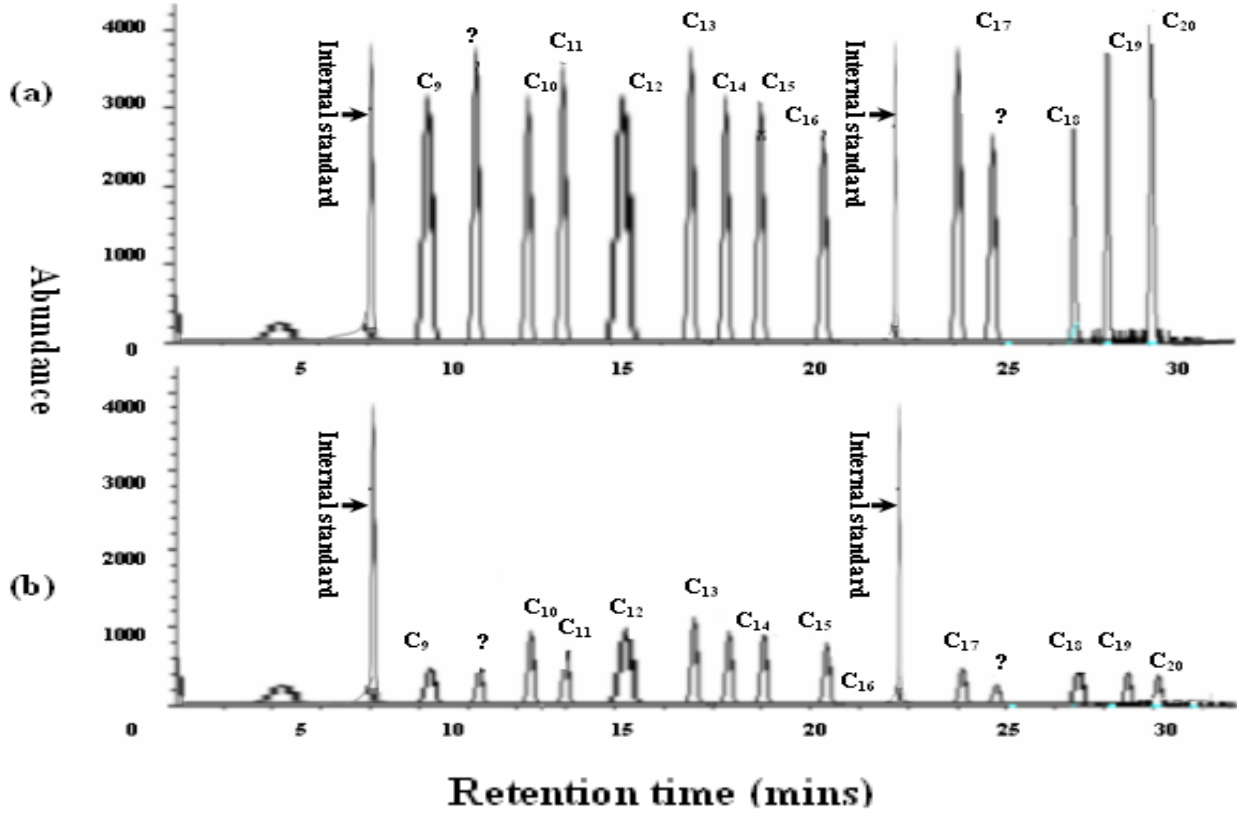

Figure 7. GC-MS profile of mineral oil quantified by Gas Chromatography –M. a) Before the incubation with the mixed culture; b) After the incubation with the mixed culture.

had the substrate preference from C_9 to C_{20}. Also, studies have shown that lower doses of mineral oil were more highly utilized than higher doses. This could account for the high growth response in the low dose of mineral oil (10 ppm). The result is consistent with that of Lizarraga-Partida et al. (1982), who observed that mineral oil has little or no effect on the total heterotrophic bacteria of an environment. The presence of certain microorganisms in both locations shows the interrelationship of the microorganisms in the complex environment (Adebusoye et al., 2007; Okoh et al., 2006).

In fact when bioremediating mineral oil occurred, the following should be considered. Firstly, the oxygen is required because biodegradative pathways are aerobic processes. Secondly, many microorganisms are capable of aliphatic hydrocarbons degradation. Thirdly, soil normally contains an adequate inoculum of natural organisms for bioremediation. Friendly soil microbes may be too efficient in breaking down chemicals, acting before pesticides have had a chance to protect crops. The tiny soil creatures adapt so successfully to the new wave of non-residual chemicals that the chemicals may disappear before they can kill the pests. The beneficial microbes literally eat the poison, thus the more pesticide applied, the quicker they devour it (Massoud et al., 2010).

An enabling environment facilitates the degradation of mineral oil carried out by microorganisms; pH, temperature and other growth factors required by the organisms should be optimal. Microbial degradation of mineral oil and its derivatives is an important field of biotechnology research because of the impact of oil spills in the environment.

The isolation of pure strains from such a consortium has also been achieved, its mineral oil degradation ability confirmed, and the different effects of mineral oil on their degrading capacity have been shown. Preliminary identification of these strains has been carried out and further work continues on their characterization. More research is necessary to understand the fundamental mechanisms of enhancement and inhibition in the microbial degradation of super high concentration of toxic compounds. However, further research could be carried out on these isolates, on genetic manipulation for improvement and exploitation as bioremediation vehicles (Obayori et al., 2008).

In conclusion, cleaning up and detoxification of mineral oil from the environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform/mineralize the

organic contaminants (Al-Qurainy and Abdel-Megeed,2009) . Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize mineral oil as a source of carbon and energy. Therefore, knowledge obtained from this study could help in understanding the biodegradation of mineral oil in contaminated sites, as well as to design efficient biocatalyst allowing transformation of oil fractions into valuable compounds. Hence, based on the present study, it may be concluded that microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation.

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REFERENCES

Abdel-Megeed A (2004). Psychrophilic degradation of long chain alkanes. Dissertation, Technische Universität Hamburg-Harburg. p. 156.

Abdel-Megeed A, Al-Harbi N, Al Dyab SS (2010). Hexadecane degradation by bacterial strains isolated from contaminated soils. Afr. J. Biotechnol. 44:7487-7494.

Adebusoye SA, Ilori MO, Amund OO, Teniola OD, Olatope SO (2007). Microbial degradation of petroleum in a polluted tropical stream. World J. Microbiol. Biotechnol. 23:1149-1159.

Al-Qurainy F, Abdel-Megeed A (2009). Phytoremediation and detoxification of some pesticides residues in Riyadh area. World Appl. Sci. J. 6:987-998.

American Society for Testing and Materials. (1998). Designation. D4-700-791.

Atlas R, Bragg J (2009). Bioremediation of marine oil spills. when and when not—the Exxon Valdez experience. Microb. Biotechnol. 2:213-221.

Barth R, Atlas RM (1977). The microbiology of aquatic oil spills. Adv. Appl. Microbiol. 22:225-226.

Bergey's Manual of Systematic Bacteriology Book Review Int. (1985). 9th edition. J. Syst. Bacteriol. p. 408.

Colwell RR, Walker JD (1977). Ecological aspects of microbial degradation of petroleum in the marine environment. Crit. Rev. Microbiol. 5:423-445.

Das N, Chandran P (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants. Overview Biotechnol. Res. Int. 44:13-19.

Deppe U (2003). Degradation of crude oil at low temperature by a newly isolated psychrotolerant bacterial consortium. Dissertation Technische Universität Hamburg-Harburg, p. 89.

Jain RK, Kapur M, Labana SB, Lal Sarma PM, Bhattacharya D, Thakur IS (2005). Microbial diversity. Application of microorganisms for the biodegradation of xenobiotics. Curr. Sci. 89:101-112.

Jain RK, Kapur S, Labana B, Lal PM, Sarma D, Thakur IS (2005). Microbial diversity. Application of microorganisms for the biodegradation of xenobiotics. Curr. Sci. 89:101-112.

Lindahl V, Bakken LR (1995). Evaluation of methods for extraction of bacteria from soil. FEMS Microbiol. Ecol. 16:135-142.

Massoud MA, Abdel-Megeed AA, Saad AS, Khalil DH (2010). Bacterial Strains from the Rhizosphere for Remediation of Certain 348 Pesticides. J. Adv. Agric. Res. 15:1029-1049.

Morgan P, Watkinson R. (1989). Hydrocarbon degrading in soil and methods for soil biotreatment. Critical Rev. Biotechnol. 8:305-333.

Ngole VM (2011). Using soil heavy metal enrichment and mobility factors to determine potential uptake by vegetables. Plant Soil Environ. 57:75-80.

Noordman H, Janssen B (2002). Rhamnolipid stimulates uptake of hydrophobic compounds by *P. aeruginose*. Appl. Environ. Microbiol. 68:4502-4508.

Obayori OS, Adebusoye SA, Adewale AO, Oyetibo GO, Oluyemi OO, Amokun RA, Ilori MO (2009). Differential degradation of crude oil (Bonny light) by four Pseudomonas strains. J. Environ. Sci. 21:243-248.

Obayori OS, Adebusoye SA, Adewale AO, Oyetibo GO, Oluyemi OO, Amokun RA, Ilori MO (2009). Differential degradation of crude oil (Bonny light) by four Pseudomonas strains. J. Environ. Sci. 21:243-248.

Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Amund OO (2008). Pyrene-degradation potentials of *Pseudomonas* species isolated from polluted tropical soils. J. Microbiol. Biotechnol. 24:2639-2646.

Okoh AI, Trejo-Hernandez MR (2006). Remediation of petroleum hydrocarbon polluted systems. exploiting the bioremediation strategies. Afr. J. Biotechnol. 5:2520-2525.

Regina OE, Emuobonuvie IF, Roseline UE (2006). Growth responses of bacterial isolates on various concentrations of crude oil. J. Am. Sci. 2:13-16.

Steiert JG, Pignatello JJ, Crawford RL (1987). Degradation of chlorinated phenols by a pentachlorophenol degrading bacterium. Appl. Environ. Microbiol. 53:907-910.

Tanaka OH, Venkatswaran K, Komukai S, Toki H, Iwabuchi T, Miyachi S (1993). Ecodynamics of oil degrading bacteria and significance of marine mixed populations in the degradation of petroleum compounds. International Oil Spill Conference. pp. 780-785.

Tzing SH, Chang JY, Ghule A, Chang JJ, Ling YC (2003). A simple and rapid method for identifying the source of spilled oil using an electronic nose. Confirmation by gas chromatography with mass spectrometry. Rapid Commun. Mass Spectrometry 17:1873-1880.

Whyte LG, Bourbonnie L, Greer CW (1999). Biodegradation of petroleum hydrocarbons by psychrotrophic Pseudomonas strains possessing both alkane (alk) and naphthalene (nah) catabolic pathway. Appl. Environ. Microbiol. 63:3719-3723.