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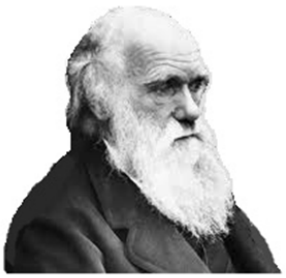

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# Diesel hydrocarbons biodegradation by *Myroide odoratimimus*

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**ABSTRACT**

Biodegradation can be a possible and effective retort to soil contamination by petroleum hydrocarbons. Nowadays, there are many cases of accidental releases of petroleum products into the environment. Use of biological agents, especially microorganisms, is effective in degradation of complex organic contaminants to other simpler organic compounds. In our present study, role of *Myroide odoratimimus* in degrading the diesel components present in soil to simpler units were checked. The study demonstrates that *Myroides odoratimimus* (SKS 05) showed the ability to degrade diesel after 40 days of incubation in the diesel adsorbed soil with a growth rate of $6.2\times10^6$ CFU/mL and from the GC study *Myroides odoratimimus* degraded the component Dotriacontane (25.471), Hexatriacontane (27.98), Tetracosane (30.296), Pentatriacontane (32.42), Tetrapentacontane (34.39) present in the diesel adsorbed soil, which was confirmed by the reduction in the peak height and the peak area in comparison to the control without *Myroides odoratimimus* (SKS 05).

***Keywords*:** Diesel, *Myroide odoratimimus*, GC-MS analysis, Soil, Diesel hydrocarbon, Biodegradation

## 1. INTRODUCTION

The genus *Myroides* was established by the reclassification of *Flavobacterium odoratum* as *Myroides odoratus*, on the basis of genomic chemotaxonomic and phenotypic studies. In recent years, many *Flavobacteria* have been reclassified into at least four different genera (Pokrywka *et al*., 1993). Members of the genus *Myroides* are Gram negative, rod, non-motile, and produce yellow pigment. This genus produces DNase, Urease, Oxidase, Catalase, and

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Phosphatase (Bernardet *et al*., 2002). Although members of the family *Flavobacterium* differ widely in their enzymatic abilities, many of them can degrade different kinds of organic macromolecules (Reichenbach, 1989). Interestingly, the recent description of several new taxa in the family, actually resulted from enzymatic screenings performed on environmental isolates aimed at discovering potentially useful exoenzymes (Humphry *et al*., 2001; Sakai *et al*., 2002). Every year, 1.3 million tonnes of oil are discharged into the environment due to leakage from underground and above ground storage tanks, as well as other accidental releases (Gallego *et al*., 2001 and Juteau *et al*., 2003).

Prolonged exposure to a high concentration of diesel may result in the development of liver and kidney disease, and potential damage to the bone marrow (Mishra *et al*., 2001; Atlas and Philp, 2005). Due to their widespread use, diesel fuel and other petroleum distillates are among the most common environmental pollutants (Watanabe, 2001). Bio-stimulation is a type of natural remediation in which conditions are manipulated and optimized in order to improve degradation of pollutants by indigenous microorganisms (Margesin *et al*., 2000) and is currently considered as the most appropriate remediation technique for diesel removal in soil. The petroleum hydrocarbon degradation is also affected by the molecular composition of the hydrocarbons, its characteristics which are directly related with the bioavailability of these compounds, and as a consequence, the biodegradation rate may be altered (Huesemann, 1995). The ecological principles that influence biodegradation with the native microorganism population will also govern the effectiveness of the inoculation, regardless of whether they are natural isolates or genetically engineered microorganisms (Liu and Suflita, 1993).

The successful use of microbial inocula in soils requires that the microorganisms contact the contaminant. Physical adsorption to soil particles or filtration through small pores may limit the transport of organisms (Margesin and Schinner, 1997). The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes (Perry, 1984). Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all. Bioremediation, especially, *in situ* bioremediation, has been identified as one technology in which an accurate scale-up can be problematic, and the prediction of how long a remediation treatment will require and how well that treatment will work can at times be uncertain (Blackburn, 1998). In this work, a bioremediation method to degrade diesel hydrocarbon present in soil using *Myroides odoratimimus* (SKS 05) was studied.

## 2. MATERIALS AND METHODS

### 2. 1. Microbial culture

*Myroides odoratimimus* (SKS 05) was obtained from Research Group, School of Biotechnology, Dr. G R Damodaran College of Science, Coimbatore, and maintained in Food Flavobacterium Medium.

Composition of Food Flavobacterium Medium (FFM):

| | | |
|---|---|---|
| Beef extract | : | 1 g |
| Peptone | : | 1 g |
| Sodium chloride | : | 0.5 g |
| Agar | : | 1 g |
| Distilled water | : | 100 mL. |

### 2. 2. Soil collection

One kg of soil was collected from the campus layout of GRD College of Science, and was dried at 80 °C in a hot air oven (this serves to eliminate indigenous bacteria from the soil). After that, 100 g of soil was given to Tamil Nadu Agricultural University (TNAU), Coimbatore, to check the sulfur, phosphorus, and nitrogen content in the soil.

### 2. 3. Soil preparation

Once dried, 10 mL of diesel collected from a petrol bunk near SITRA (Southern Institute of Textile Research Association), was added uniformly to 100 g of soil and left for 30 min for its adsorption to the soil (0.177 g diesel per gram of soil). After adsorption of diesel, the humidity was adjusted to approximately 10%.

### 2. 4. Inoculum preparation

A loopful of cells from freshly grown slant of *Myroides odoratimimus* (SKS 05) was transferred into 250 mL conical flask containing 50 mL of Food Flavobacterium Broth and incubated at 30 °C in a shaking incubator at 180 rpm for 72 h.

Composition of Food Flavobacterium Broth (FFB):

| | | |
|---|---|---|
| Beef extract | - | 1 g |
| Peptone | - | 1 g |
| Calcium chloride | - | 0.5 g |
| $NH_4SO_4$ | - | 0.5 g |
| Olive oil | - | 1% |
| Glucose | - | 0.5 g |
| Distilled water | - | 100 mL |
| pH | - | 7.5 |

### 2. 5. Biodegradation Conditions

#### 2. 5. 1. Laboratory Scale

3 mL of the inoculum prepared was added to 20 g of diesel adsorbed soil present in the treated flask ($3.5 \times 10^3$ CFU/mL), and was mixed properly. Control flask contained no inoculum. The flask was covered properly with aluminium foil and aeration was facilitated. The flasks were kept in a shaker at 180 rpm for 40 days.

#### 2. 5. 2. Bacterial count

A soil suspension was made by dissolving 1 g of soil from the treated flask at different days of interval in 100 mL of distilled water. The soil suspension was serially diluted to $10^{-10}$. 0.1 mL of each dilution was spread onto the Food Flavobacterium medium and incubated for 24 h at 37 °C.

### 2. 6. GC-MS analysis

Soil samples (20 g each) from the control and treated, were analyzed by GC/MS to determine the quantity and composition of the total hydrocarbons. Solid liquid extraction was carried out by taking 5 g of soil and extracted twice with 50 mL of dichloromethane.

The organic phase was passed through $NaSO_4$ and concentrated to 0.2 mL. Analysis by gas chromatography was carried out using a Thermo GC – Trace Ultra VER: 5.0, Thermo MS DSQ II equipped with TR 5 - MS detector and TR 5 - MS Capillary Standard Non – Polar Column.

| | | |
|---|---|---|
| Dimension | : | 30 Mts, ID: 0.25 mm, Film: 0.25 μm |
| Carrier gas | : | He |
| Flow | : | 1.0 mL/min |
| Temp Program | : | Oven temperature 80 °C rose to 250 °C, at 8 °C /min |
| Injection Volume | : | 1 μL. |

## 3. RESULTS

The *Myroides odoratimimus* (SKS 05), obtained from Research Group, School of Biotechnology, showed yellow colony on Food Flavobacterium Medium (**Fig. 1**).

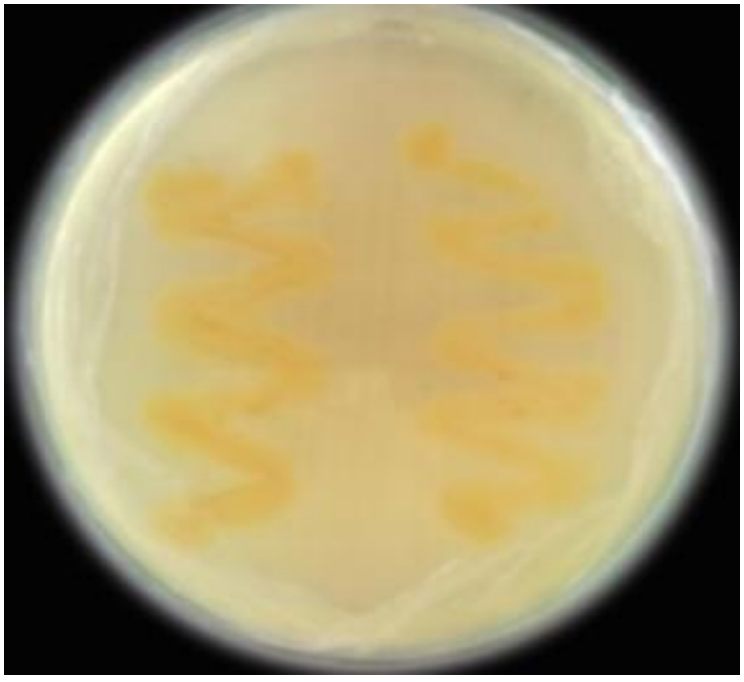


**Fig. 1.** Yellow colony formed by *Myroides odoratimimus* (SKS 05) on Food Favobacterium Medium

### 3. 1. Nature of soil

The available nitrogen, phosphorous, and potassium content of the soil, collected from the campus layout Dr. G. R. Damodaran College of Science, was tested in the soil testing and technology advisory centre of Tamil Nadu Agriculture University, Coimbatore, India. The collected soil was acidic in nature and contained a high potassium content of 422 $kg \cdot ha^{-1}$ present in 100 g of soil (**Table 1**).

**Table 1.** Report of the soil test for the soil used for biodegradation studies

| Soil physical and chemical characteristics | | |
|---|---|---|
| 1 | Sample depth | 3 cm |
| 2 | Soil texture | Sandy Loam |
| 3 | Soil type | Black |
| 4 | pH | 6.3 (Acidic soil) |
| 5 | Available N | 244 kg·ha$^{-1}$ |
| 6 | Available P | 35 kg·ha$^{-1}$ |
| 7 | Available K | 422 kg·ha$^{-1}$ |

### 3. 2. Bacterial count

The growth pattern of *Myroides odoratimimus* (SKS 05) in diesel adsorbed soil was checked, as shown in **Fig. 2**.

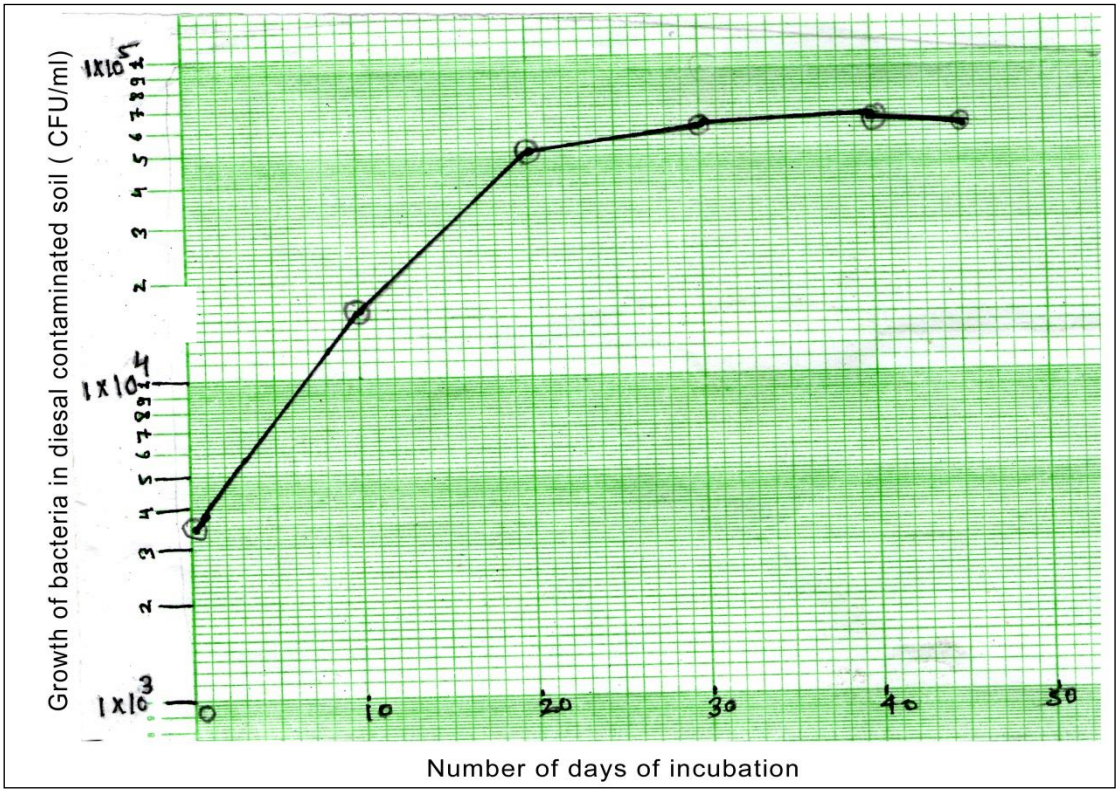



**Fig. 2.** Growth of *Myroides odoratimimus* (SKS 05) in diesel contaminated soil at different days of incubation

### 3. 3. GC analysis

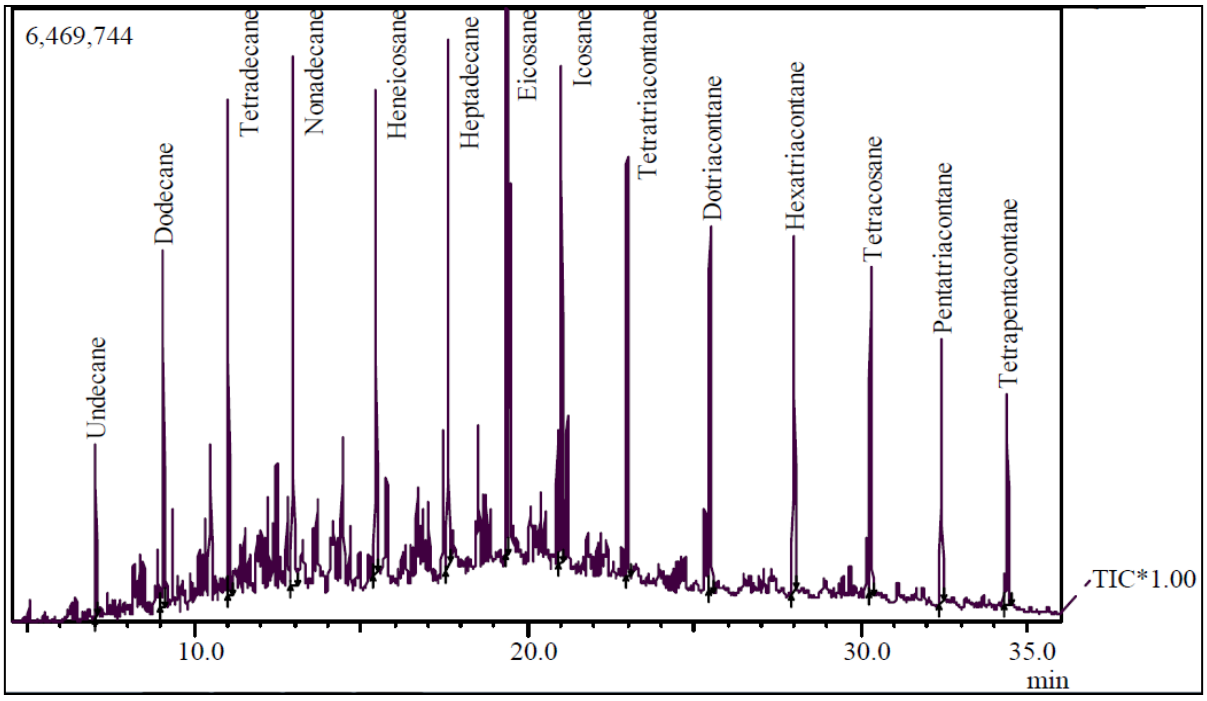



**Fig. 3.** Chromatogram showing the peak of relative absorbance of different hydrocarbons present in the control (Soil + diesel)

**Table 2.** The intensity of the different hydrocarbon present in the control with a retention time from 4.5 to 35.5 min.

Peak Report TIC

| Peak# | R.Time | I.Time | F.Time | Area | Area% | Height | Height% | A/H | Mark | Name |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7.040 | 6.992 | 7.083 | 3715846 | 2.10 | 1816167 | 3.10 | 2.05 | | Undecane |
| 2 | 9.072 | 8.967 | 9.133 | 9162589 | 5.17 | 3757288 | 6.42 | 2.44 | V | Dodecane |
| 3 | 11.014 | 10.967 | 11.125 | 14439558 | 8.15 | 5232971 | 8.94 | 2.76 | V | Tetradecane |
| 4 | 12.959 | 12.892 | 13.083 | 16649513 | 9.40 | 5576299 | 9.53 | 2.99 | V | Nonadecane |
| 5 | 15.440 | 15.383 | 15.508 | 15647805 | 8.83 | 5117049 | 8.74 | 3.06 | V | Heneicosane |
| 6 | 17.619 | 17.525 | 17.692 | 15057727 | 8.50 | 5543832 | 9.47 | 2.72 | V | Heptadecane |
| 7 | 19.379 | 19.308 | 19.425 | 14450290 | 8.15 | 5745647 | 9.82 | 2.51 | | Eicosane |
| 8 | 21.020 | 20.958 | 21.075 | 14478196 | 8.17 | 5231338 | 8.94 | 2.77 | V | Icosane |
| 9 | 22.989 | 22.917 | 23.100 | 15058850 | 8.50 | 4429481 | 7.57 | 3.40 | | Tetratriacontane |
| 10 | 25.480 | 25.400 | 25.617 | 15574049 | 8.79 | 3845695 | 6.57 | 4.05 | V | Dotriacontane |
| 11 | 27.990 | 27.908 | 28.075 | 13717611 | 7.74 | 3747485 | 6.40 | 3.66 | | Hexatriacontane |
| 12 | 30.296 | 30.225 | 30.425 | 12246317 | 6.91 | 3458833 | 5.91 | 3.54 | V | Tetracosane |
| 13 | 32.423 | 32.350 | 32.533 | 9659011 | 5.45 | 2806243 | 4.79 | 3.44 | | Pentatriacontane |
| 14 | 34.392 | 34.333 | 34.492 | 7349964 | 4.15 | 2219985 | 3.79 | 3.31 | V | Tetrapentacontane |
| | | | | 177207326 | 100.00 | 58528313 | 100.00 | | | |

The GC analysis was done for $0^{th}$ day sample (without inoculum) and after 40 days of incubation with *Myroides odoratimimus* (SKS 05). The chromatogram showing varied peaks for different hydrocarbon present in diesel adsorbed soil was obtained (**Fig. 3**). WILEY8 library was used to obtain the different hits for the components.

A significant increase in diesel degradation was observed in the treated group in comparison with the control. The result indicates that the diesel components were used by the bacteria *Myroides odoratimimus* (SKS 05), as there was an increase in bacterial population from $3.5 \times 10^3$ ($0^{th}$ day) to $6.2 \times 10^5$ CFU/ mL ($40^{th}$ day).

Fourteen components were found with different retention time (**Table 2**) and are like Undecane (7.040 RT), Dodecane (9.072 RT), Tetradecane (11.014 RT), Nonadecane (12.959), Heneicosane (15.440 RT), Heptadecane (17.619 RT),Eicosane (19.379 RT), Icosane (21.020 RT), Tetratriacontane (22.989 RT), Dotriacontane (25.480 RT), Hexatriacontane (27.990 RT), Tetracosane (30.296 RT), Pentatriacontane (32.423 RT),Tetrapentacontane (34.392 RT) along with the area, which describes the concentration of the component in the soil mixed with diesel.

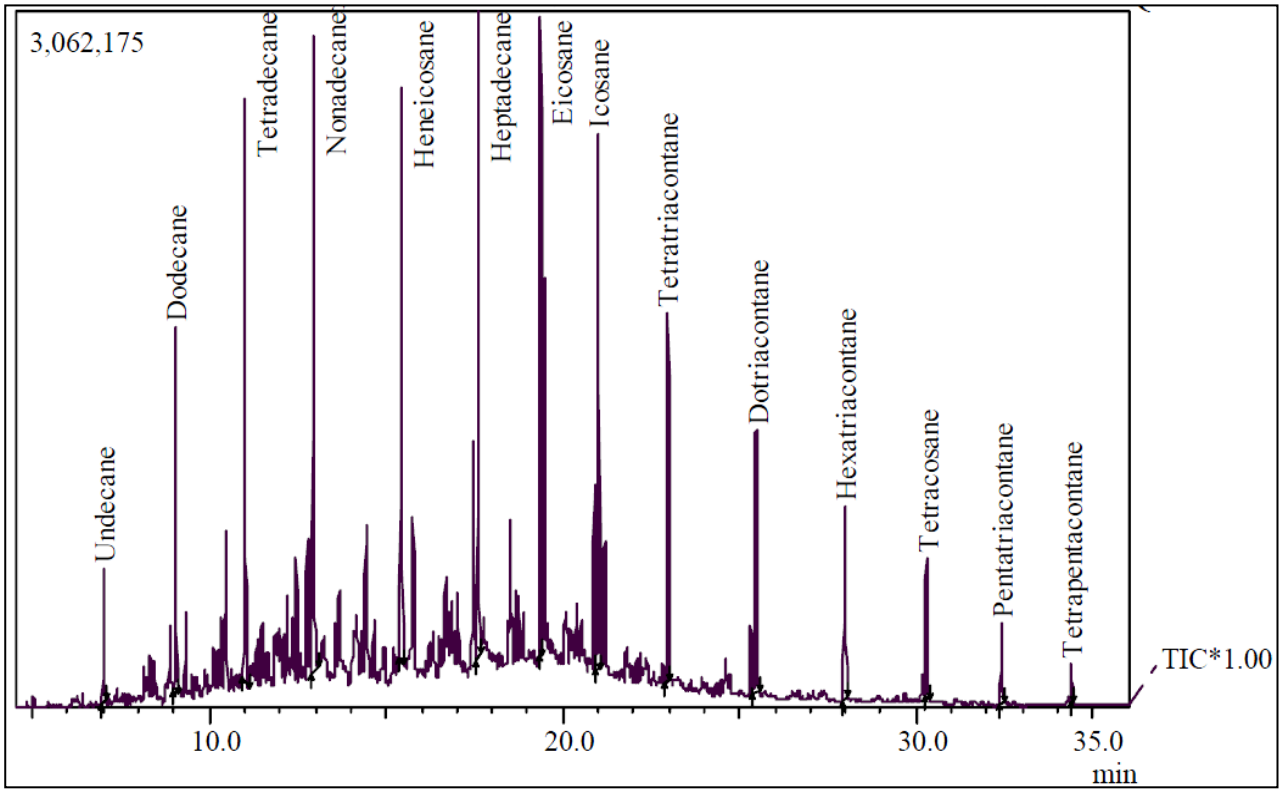



**Fig. 4.** Chromatogram showing the peak of relative absorbance of different hydrocarbons (Diesel adsorbed soil), after 40 days of incubation with *Myroides odoratimimus* (SKS 05)

Chromatogram obtained from the treated group also gives fourteen components with retention time from 4.5 to 35.5 min (**Fig. 4**). From the **Table 3** we can observe the intensity of different hydrocarbons present in the treated sample, *i.e.* the sample containing *Myroides odoratimimus* (SKS 05) through the gas chromatography.

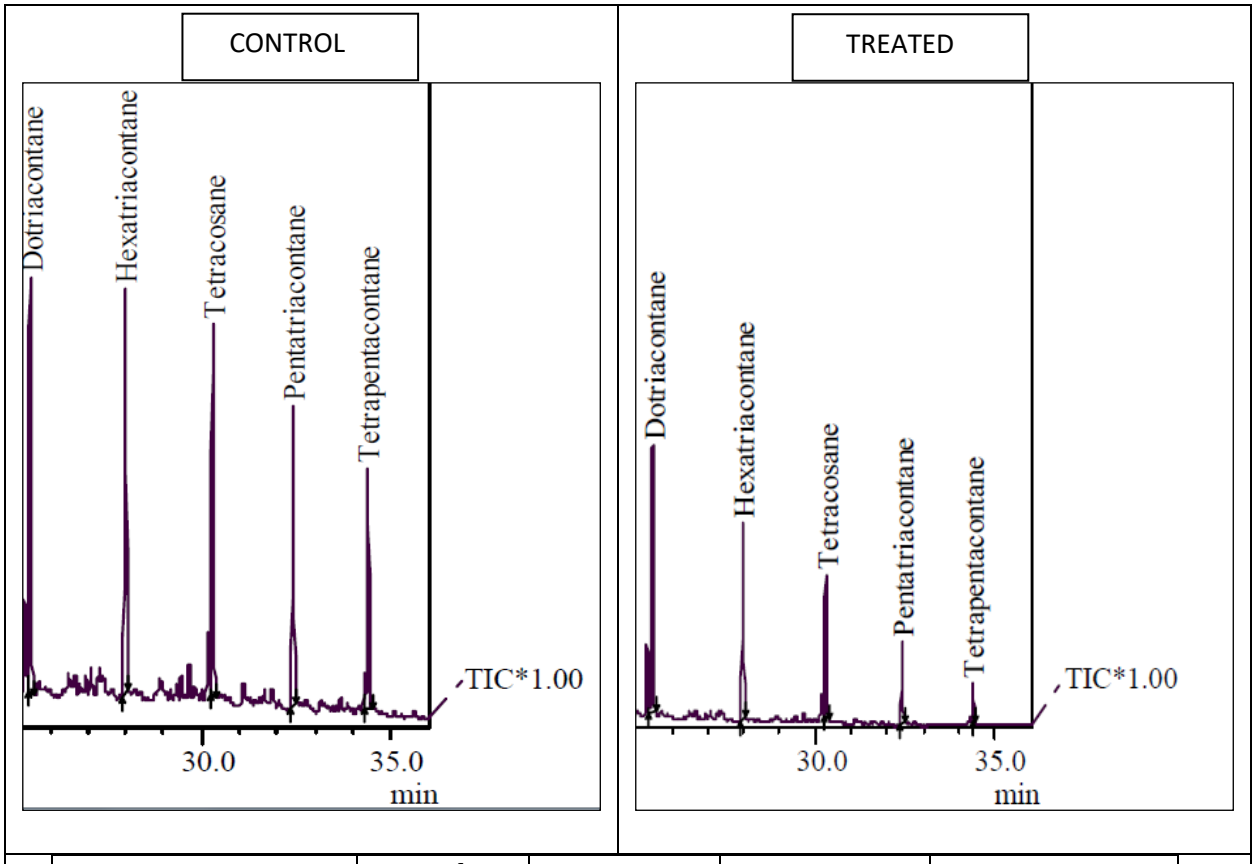
**Table 3.** The intensity of the different hydrocarbon present in the treated sample after 40 days of incubation with *Myroides odoratimimus* (SKS 05) through gas chromatography.

Peak Report TIC

| Peak# | R.Time | I.Time | F.Time | Area | Area% | Height | Height% | A/H | Mark | Name |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7.018 | 6.958 | 7.067 | 1461622 | 2.34 | 587884 | 2.57 | 2.49 | | UNDECANE |
| 2 | 9.055 | 8.967 | 9.117 | 3844477 | 6.16 | 1616573 | 7.08 | 2.38 | V | Dodecane |
| 3 | 11.003 | 10.942 | 11.117 | 6638134 | 10.63 | 2558558 | 11.20 | 2.59 | | TETRADECANE |
| 4 | 12.949 | 12.883 | 13.075 | 7602979 | 12.17 | 2795094 | 12.24 | 2.72 | | Nonadecane |
| 5 | 15.431 | 15.367 | 15.500 | 7222705 | 11.57 | 2520819 | 11.04 | 2.87 | | Heneicosane |
| 6 | 17.613 | 17.525 | 17.683 | 7469327 | 11.96 | 2832634 | 12.40 | 2.64 | V | Heptadecane |
| 7 | 19.375 | 19.308 | 19.425 | 6569829 | 10.52 | 2808599 | 12.30 | 2.34 | | Eicosane |
| 8 | 21.014 | 20.958 | 21.075 | 5991226 | 9.59 | 2350299 | 10.29 | 2.55 | V | Icosane |
| 9 | 22.978 | 22.908 | 23.058 | 5020010 | 8.04 | 1623381 | 7.11 | 3.09 | | Tetratriacontane |
| 10 | 25.471 | 25.392 | 25.558 | 4162384 | 6.66 | 1152393 | 5.05 | 3.61 | V | DOTRIACONTANE |
| 11 | 27.980 | 27.908 | 28.067 | 2951232 | 4.73 | 847834 | 3.71 | 3.48 | | Hexatriacontane |
| 12 | 30.288 | 30.217 | 30.367 | 1992698 | 3.19 | 621470 | 2.72 | 3.21 | V | Tetracosane |
| 13 | 32.417 | 32.358 | 32.483 | 1033840 | 1.66 | 349848 | 1.53 | 2.96 | | PENTATRIACONTA |
| 14 | 34.388 | 34.342 | 34.450 | 491475 | 0.79 | 171823 | 0.75 | 2.86 | | Tetrapentacontane |
| | | | | 62451938 | 100.00 | 22837209 | 100.00 | | | |

The compound Dotriacontane (25.471 RT), Hexatriacontane (27.980 RT), Tetracosane (30.296 RT), Pentatriacontane (32.423 RT) and tetrapentacontane (34.392 RT) has shown a lesser peak height and area in comparison with the control (**Table 4**).

**Table 4.** Comparison of notable changes in the peak height and area of the components present in the uninoculated control (soil+ diesel) and the 40-day treated sample [soil+ diesel + *Myroides odoratimimus* (SKS 05)], as shown in Gas chromatogram.




| Compounds (RT) | Peak height (control) | Peak area (control) | Peak height (treated) | Peak area (treated) |
|---|---|---|---|---|
| Dotriacontane (25.471) | 3845695 | 15574049 | 1152393 | 4162384 |
| Hexatriacontane (27.98) | 3747485 | 13717611 | 847834 | 2951232 |
| Tetracosane (30.296) | 3458833 | 12246317 | 621470 | 1992698 |
| Pentatriacontane (32.42) | 2806243 | 9659011 | 349848 | 1033840 |
| Tetrapentacontane (34.39) | 2219985 | 7349964 | 171823 | 491475 |

## 4. DISCUSSION

Formation of yellow coloured colony on the Food Flavobacterium medium confirms *Myroides odoratimimus* (SKS 05), as discussed by Bernardet *et al*., in 2002 that *Myroides* are Gram negative, rod, non-motile, and produce yellow pigment.

Diesel consists mostly of linear and branched alkanes with different chain lengths and aromatic compounds. It is one of the major products of crude oil. Many of these compounds, especially linear alkanes, are known to be easily biodegradable. However, due to their low water solubility, the biodegradation of these compounds is often limited by slow rates of dissolution, desorption, or transport. In general, the bioavailability of hydrophobic compounds is determined by the desorption characteristics, dissolution or partitioning rates, and the transport process to microbial cell (Sticher *et al*., 1997). Diesel biodegradation does not appear to be limited by the metabolic capabilities of the *Myroides odoratimimus* (SKS 05), since a significant fraction of high molecular weight hydrocarbons (>C12) were biodegraded at the same rate. However, bacterial inability for the total hydrocarbon utilization seems to be due to a mass transfer limitation, in which hydrocarbons are not available to microorganisms.

Chemical analyses have shown that crude oil is a complex mixture of polar and non- polar compounds (Dragun, 1988). It has also been shown, that crude oil contains more of non- polar compounds than polar compounds (Subhas and Robert, 1998). Carbon atoms of these polar and non-polar constituents of crude oil serve as growth substrates and non-growth substrates for the microbial enzymes during degradation of this biogenic (hydrocarbons) compound (Polleroni, 1994). Moderate utilization of crude oil hydrocarbons in all treatment samples, may result from the reaction of intermediates and products that are highly reactive and that inactivate the microbial enzymes by a covalent binding to it and changing its structure (Rasche *et al*., 1991). *Myroides odoratimimus* (SKS 05) was able to degrade some components present in the diesel adsorbed soil showing retention time from 25.47 to 34.38 min, which may be due to the nature of the diesel or due to the physical characteristic of the soil chosen for experiment; the soil type should be an important factor in bioremediation (Ghazali *et al*., 2004). The small pore space in loam soil might cause low oxygen diffusion rate and limits the accessibility of the target compound for degradation by microbes, as suggested by Tisdale and Nelson (1975).

Facunda (2000) gave a similar report regarding the microbial activity in hydrocarbon contaminated soil**,** where microbial consortium was used for the degradation purpose. A laboratory scale bioremediation of diesel hydrocarbon in soil by indigenous bacterial consortium was carried by Sharma and Rehman (2009). They discussed that the chromatogram obtained from the soil supplemented with diesel showed varied peak and was significantly different from the control. They showed the presence of dodecanne, tetradecane, pentadecane, tetracosane in soil adsorbed with diesel through GC-MS analysis.

The collected soil was acidic in nature and contains a high potassium content of 422 $kg{\cdot}ha^{-1}$ present in 100 g of soil. Bacterial count was carried out after every 10 days of incubation till 40 days, which confirmed the growth of the *Myroides odoratimimus* (SKS 05) culture from intial $3.5 \times 10^3$ CFU/mL to final $6.2 \times 10^5$ CFU/mL. Similar results were discussed by Padayachee and Jin in 2010; they showed the increase in the bacterial population in the diesel contaminated soil. Similar biodegradation studies have been performed with *Exigubacterium aurantiacum* and *Burkholderia cepacia* for over 15 days. The culture broth contains diesel (1%) and there has been 3-4-fold increase in the culture condition (Mohanty and Mukherjee, 2007).

Biostimulation of indigenous microorganisms by the addition of inorganic nutrients, such as nitrogen, phosphorous, and potassium to balance the high carbon content due to hydrocarbon contamination has been widely used in diesel contaminated soils (Molina-Barahona *et al*., 2004; Perfumo *et al*., 2006). The culture of *Myroides odoratimimus* (SKS 05) was able to grow in the diesel adsorbed loam soil having 244 $kg{\cdot}ha^{-1}$ available nitrogen, 35 $kg{\cdot}ha^{-1}$ available phosphorous, and 422 $kg{\cdot}ha^{-1}$ available potassium in the soil. No extra nutrients were added to the treated sample**.** In numerous field trials, the feasibility of adding inorganic nutrients has been demonstrated as a means of sustaining elevated nutrient cones within the sediments for effective bioremediation (Lee and Levy, 1991 and Venosa *et al*., 1996).

Alkane degradation ability by the microorganisms depends on the structure and length of hydrocarbon source. Longer carbon chain compounds are easier to degrade than those shorter than $C_9$ with the exception of methane (Cookson, 1995; Whyte *et al*., 1998). A wide variety of microorganisms can readily degrade longer chain aliphatic hydrocarbons. Different patterns can be proposed for liquid *n*-alkanes, $C_{12}$ -$C_{16}$, low solid *n* alkanes $C_{17}$ -$C_{28}$, and high solid alkanes above $C_{28}$ (Setti *et al*., 1993). Similar results are obtained with *Myroides odoratimimus* culture on diesel adsorbed soil. From the GC analysis, it was confirmed that the compounds with carbon chain from $C_{24}$ to $C_{36}$ were degraded by the bacteria.

The susceptibility to degradation is proportional to increasing carbon numbers (Del' Arco and de Franca, 2001; Plohl and Leskovsek, 2002). It was found that the chain compounds shorter than $C_9$ were more difficult to degrade than the longer chains. *Acetinobacter* sp. isolated in our Laboratory (Mandri and Lin, 2007; Singh and Lin, 2008) capable of degrading diesel and used engine oil effectively, failed to utilize the short chain hydrocarbons (less than 9) as the sole carbon source (Nadoo, 2007). Padayachee and Lin (2011) discussed a significant reduction in diesel content ($C_{11}$-$C_{36}$) which was observed through GC-MS analysis of diesel contents of diesel-contaminated loam soil samples with culture.

Margesin *et al*. (2007) found that hydrocarbon concentration and incubation time are important factors during bioremediation of diesel-contaminated soil. *Myroides odoratimimus* (SKS 05) degraded hydrocarbons in diesel contaminated soil after 40 days of incubation.

## 5. CONCLOSUON

Biodegradation can be a viable and effective response to the soil contamination by petroleum hydrocarbons. The study demonstrates that *Myroides odoratimimus* (SKS 05) showed the ability to degrade diesel after 40 days of incubation in the diesel adsorbed soil with a growth rate of $6.2 \times 10^6$ CFU/mL and from the GC study *Myroides odoratimimus* degraded the component Dotriacontane (25.471), Hexatriacontane (27.98), Tetracosane (30.296), Pentatriacontane (32.42), Tetrapentacontane (34.39) present in the diesel adsorbed soil, which was confirmed by the reduction in the peak height and the peak area in comparison with the control {without *Myroides odoratimimus* (SKS 05)}.

**ACKNOWLEDGEMENT**

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## References


[1] Atlas R.M. and Philp J. Bioremediation: applied microbial solutions for real-world environmental cleanup. ASM Press, Washington D.C (2005)1-292.

[2] Bernardet J.F., Nakagawa Y., and Holmes B. Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. *International Jounal of Systamatic and Evolutionary Microbiology* 52 (2002) 1049– 1070.

[3] Blackburn J.W. Bioremediation Scale-up Effectiveness: A review, *Bioremediation Journal* 1(3) (1998) pp 265–280.

[4] Cookson J.T. Bioremediation engineering: Design and application, McGraw-Hill Inc. New York (1995) 232-234

[5] Del'arco P.J. and Franca F.P. Influence of oil contamination levels on hydrocarbon biodegradation in sandy sediments. *Environmental Pollution* 110 (2001) 515-519.

[6] Dragun J. (1988). The soil chemistry of harzardous materials. Harzardous materials control research institute. *Silver Spring*. 325-445

[7] Facundo J.M.R., Rodriguez V.H., and Lamella M.T. Biodegradation of diesel oil in soil by a microbial consortium. *Biodegradation* (2000) 313-320.

[8] Gallego J.R., Loredo J., Llamas J.F., Vazquez F., and Sanchez J. Bioremediation of diesel-contaminated soils: evaluation of potential *in situ* techniques by study of bacterial degradation. *Biodegradation* 12 (2001) 325–335.

[9] Huesemann M.H. Predictive Model for Estimating the Extent of Petroleum Hydrocarbon Biodegradation in Contaminated Soils. *Environmental Science and Technology* 29 (1) (1995) 7.

[10] Humphrey S.P. and Williamson R.T. A review of saliva: Normal composition, flow, and function. *Journal of Prosthetic Dentistry* 85 (2001) 162-169.

[11] Juteau P., Bisaillon G., Lepine F., Ratheau V., Beaudet R., and Villemur R. Improving the biotreatment of hydrocarbon-contaminated soils by the addition of activated sludge taken from the wastewater treatment facilities of an oil refinery. *Biodegradation* 14 (2003) 31-40.

[12] Lee K. and Levy E.M. International Oil Spill Conference, American Petroleum Institute, Washington DC (1991) 541-547

[13] Liu S. and Suflita J.M. Ecology and evolution of microbial populations for bioremediation. *Trends Biotechnol* 11(8) (1993) 344.

[14] Mandri T. and Lin J. Isolation and Characterization of Engine Oil Degrading Indigenous Microorganisms in KwaZulu-Natal, South Africa. *Afr. J. Biotechnol* 6(1) (2007) 23-27.

[15] Margesin R. and Schinner F. Efficiency of Indigenous and Inoculated Cold-Adapted Soil Microorganisms for Biodegradation of Diesel Oil in Alpine Soils. *Appl. Environ. Microbiol*. 63(7) (1997) 2660-2664.

[16] Margesin R., Hämmerle M., and Tscherko D. Microbial activity and community composition during bioremediation of diesel-oilcontaminated soil: effects of hydrocarbon concentration, fertilizers, and incubation time. *Microb. Ecol.* 53(2) (2007) 259-69.

[17] Margesin R., Zimmerbauer A., and Schinner F. Monitoring of bioremediation by soil biological activities. *Chemosphere* 40 (2000) 339-346.

[18] Mishra S., Jyot J., Kuhad R.C., and Lal B. Evaluation of inoculum addition to stimulate *in situ* Bioremediation of oily-sludgecontaminated soil. *Applied and Environmental Microbio*logy 67(4) (2001) 1675-1681.

[19] Mohanty G. and Mukherji S. Biodegradation rate of diesel range n-alkanes by bacterial cultures *Exiguobacterium aurantiacum* & *Burkholderia cepaci*. *Int Biodet Biodegradation (*2007) 60.

[20] Molina-B.L., Rodriguez V.R., Hernandez V.M., Vega J.C., Zapata P.O., Mendoza C.A., and Albores A. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. *Applied Soil Ecol*ogy (2004) 27, 165-175.

[21] Nadoo E. A study on the substrate specificity of oil degrading *Acetobacter* isolates, Honours dissertation, University of KwaZulu-Natal, South Africa (2007).

[22] Padayachee D. and Lin J. The effect of fertilizer amendment on diesel biodegradation in contaminated soils. *African Journal of Microbiology Research* 5(14) (2011) 1729-1739.

[23] Perfumo A., Banat I.M., Marchant R., and Vezzulli L. Thermally enhanced approaches for bioremediation of hydrocarbon contaminated soils. *Chemosphere* 66(1) (2006) 1-6.

[24] Perry J.J. Microbial metabolism of cyclic alkanes in Petroleum Microbiology. Atlas RM. Ed. (1984) 61–98.

[25] Plohl K. and Leskovsek H. Biological degradation of motor oil in water. *Acta Chimica Slovenica* 49 (2002) 279-289

[26] Pokrywka M., Viazanko K., Medwick J., Knabe S., McCool S., Pasculle A.W., and Dowling J.A. *Flavobacterium meningosepticum* outbreak among intensive care patients. *American Journal of Infection Control* 21 (1993) 139–145.

[27] Propst T.L., Lochmiller R.L, Qualis C.W, and McBee J.K. In situ mesocosm assessment of immunotoxicity risks to small mammals inhabiting petrochemical waste sites. *Chemosphere* 38 (1999) 1049-1067.

[28] Rasche M.E., Hyman M.R and Arp D.J. Biodegradation of halogenated hydrocarbon fumigants by nutrifying bacteria. *Applied Environmental Microbiology* 56 (1990) 25688-25711

[29] Sakai Y., Koller Reichenbach H., and Order I. Cytophagales Leadbetter. Bergey's manual of systematic bacteriology. 3 (1989) 2011-2013

[30] A., Rangell, L.K., Keller G.A., and Subramani S. Peroxisome degradation by microautophagy in Pichia pas identification of specific steps and morphological intermediates. *Journal of Cell Biology* 141 (2002) 625–63.

[31] Setti L., Lanzarini G., Pifferi P.G., and Spagna G. Further research into the aerobic degradation of n-alkanes in heavy oil by a pure culture of a Pseudomonas sp, *Chemosphere*. 26 (1993) 1151-1157.

[32] Sharma A. and Rehman M.B. Laboratory scale bioremediation of diesel hydrocarbon in soil by indigenous bacterial consortium. *Indian Journal of Experimental Biology* 47 (2009) 766-769.

[33] Sticher P., Jaspers M.C.M., Stemmler K., Harms H., Zehnder A.J.B. and Vander M.J.R. *Applied Environmental Microbiology* 63(10) (1997) 4053.

[34] Subhas K.S. and Robert L.I. Bioremediation. Fundamentals and applications. Technomic publishing company (1998) 1, 17604.

[35] Tisdale L.S. and Nelson W.L. Soil fertility and fertilizers. 3rd Ed. Macmillan Publishing Co. Inc (1975) 23-45.

[36] Venosa A.D., Suidan M.T., Wrenn B.A., Strohmeier K.L., Heines J.R., Eberhart B.L., King D., and Holder E.L. Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. *Environmental Science Technology* 30(5) (1996) 1764-1775.

[37] Watanabe M.E. Can Bioremediation Bounce Back? *Nature Biotechnology* 19 (2001) 1111–1115.

[38] Whyte L.G., Hawari J., Zhou E., Bourbonniere L., Inniss W.E., and Greer C.W. Biodegradation of variable-chain-length alkanes at low temperatures by a psychrotrophic *Rhodococcus* sp. *Applied Environment Microbiology* (1998) 223-238.