

Study of the Potential of Two Isolated Microorganisms to Degrade Various Petroleum Fractions

Sonali Sahoo, Dipa Biswas* and Sriparna Datta

Department of Chemical Technology, University of Calcutta, 92,
A.P.C. Road, Kolkata - 700 009 (India).

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Biodegradation of petroleum by the microorganisms in polluted site has been gaining attention from the environment clean-up point of view. Two isolated organisms *Acinetobacter junii* CTA₃ and *Pseudomonas aeruginosa* OCD₁ were tested for their petroleum degrading ability in liquid Bushnell-Hass broth. Both the organisms degraded higher boiling petroleum fractions more efficiently. The strain OCD₁ showed better degrading ability than the strain CTA₃. The highest degradation of 47% and 57% was obtained with diesel after 20 days of incubation by the organism CTA₃ and OCD₁ respectively. Also it was observed that, most of the oils were degraded in first 10 days of incubation for both the isolates.

Key words: *Acinetobacter junii*, Biodegradation, Petroleum fractions, *Pseudomonas aeruginosa*.

Petroleum is the great source of various essential products used in our modern life; however, despite its important uses, petroleum hydrocarbon results in environmental pollution due to industrial waste, transport and storage accidents and it is a serious global problem. This contamination are hazardous to the health of plants and are also carcinogenic, mutagenic and potent immuno-toxicants, posing a serious threat to human and animal health^{1,2,3,4,5}. The demand for the clean-up of contaminated sites increased with increasing public concern towards the preservation of the environment and hence microbial degradation of hydrocarbons become an important issue. Leahy

and Colwell demonstrated that microorganisms are main degraders of petroleum hydrocarbons in contaminated ecosystems⁶. Li et al. examined the biodegradation of diesel pollution by *Mycobacterium* and filamentous fungi⁷. The biodegradation of diesel oil in a polluted soil by *Bacillus subtilis* was also reported by few authors⁸. A diesel degrading bacteria (strain IU5) isolated from oil-contaminated soil in Korea was found to degrade many other petroleum hydrocarbons including crude oil, gasoline, benzene, toluene, xylene, naphthalene, phenanthrene and pyrene⁹. A psychotropic bacterium *Pseudoalteromonas* sp. P29 was studied for its crude oil degrading ability¹⁰. Muthuswamy et al. examined that biodegradation of crude oil by mixed bacterial consortium is higher than the individual cultures¹¹. Biodegradation of hydrocarbon contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contaminants, has been

* To whom all correspondence should be addressed.
E-mail: dipa_bis@yahoo.com

established as an efficient, economic, versatile and environmentally sound treatment¹².

The study therefore, aimed to observe the potential of two bacterial sp. to degrade various petroleum fractions.

MATERIAL AND METHODS

Microorganisms

The two microorganisms *Acinetobacter junii* CTA₃¹³ and *Pseudomonas aeruginosa* OCD₁¹⁴ were isolated in the laboratory by the present authors from oil contaminated soil samples and air respectively.

Petroleum fractions

Gasoline (unleaded), Kerosene, High Speed Diesel (HSD) – Retail Market; Atmospheric Gas Oil, Vacuum Gas Oil (VGO), Diesel Hydrodesulphurization (DHDS) feed stock, Lubricating Oil Base Stock (LOBS) – Indian Oil Corporation Ltd., Haldia Refinery, W. B., India.

Media, Chemicals and Solvents

Bushnell Haas (BH) broth, Peptone, Beef extract powder, Agar agar type I – Hi-Media; NaCl, Chloroform, Ethanol, Methanol – Merck; Acetone, Dichloromethane – SRL and other chemicals and solvents of AR Grade were procured from local supplier.

Treatment of petroleum fractions

Shake flask studies were performed for treatment of petroleum fractions viz. gasoline, kerosene, diesel, atmospheric gas oil, VGO, DHDS feed stock and LOBS with the strains *A. junii* CTA₃ and *P. aeruginosa* OCD₁. Fresh overnight culture was taken to inoculate the BH media containing 2% oil. The flasks were kept under shaking condition in a shaker incubator (ORBITEK L J E, Scigenics Biotech (Pvt.) Ltd., India) at optimum culture conditions (35°C temperature, 175 r.p.m and pH 7 for *A. junii* CTA₃; 30°C temperature, 125 r.p.m and pH 6 for *P. aeruginosa* OCD₁) for four days. The experiment was also repeated with higher percentage (upto 90 %v/v) of each petroleum fractions.

Assay of degradation of petroleum fractions by the two bacterial strains

The percentage degradation of various petroleum fractions was determined using the gravimetric analysis^{15,16}. The organisms were cultivated with 100 mL of BH media with 2 mL of C-

source at optimized culture conditions. After 10 days and 20 days of incubation, the whole culture broth was transferred to the separating funnel and aqueous phase was separated. The oil was extracted using dichloromethane¹⁷. The culture flask was also rinsed with the solvent to avoid any loss of oil. Then the percentage of oil degraded was calculated as follows:

% of oil degraded = {(weight of oil tested – weight of unconverted oil)/weight of oil tested} × 100

RESULTS AND DISCUSSION

Treatment of petroleum fractions

The growth potential of two strains on some petroleum fractions are given in Table 1. The table shows that weak growth in gasoline containing flask, medium growth in kerosene containing flask whereas, diesel, atmospheric gas oil, VGO, DHDS feed stock and LOBS shows huge growth of the organisms. Therefore, the study reveals that both the strains are suitable for degrading higher boiling petroleum fractions. The organisms CTA₃ and OCD₁ also showed significant growth with up to 90% (v/v) of petroleum fractions viz., diesel, VGO, LOBS etc. i.e. the higher percentage of oil does not inhibit the growth of organisms.

Assay of degradation of petroleum fractions by the isolated strains

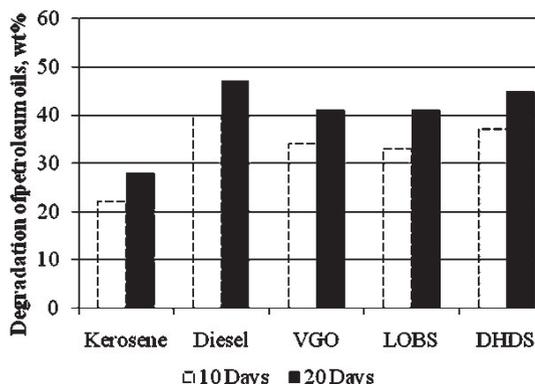
The two isolate *A. junii* CTA₃ and *P.*

Table 1. Growth potentials of CTA₃ & OCD₁ on some petroleum fractions

Petroleum fractions/ intermediates	Growth potentials of <i>A. junii</i> CTA ₃ ^a	Growth potentials of <i>P. aeruginosa</i> OCD ₁ ^b
Gasoline	+	+
Kerosene	++	++
Diesel	+++	+++
Atmospheric Gas Oil	+++	+++
VGO	+++	+++
DHDS feed stock	+++	+++
LOBS	+++	+++

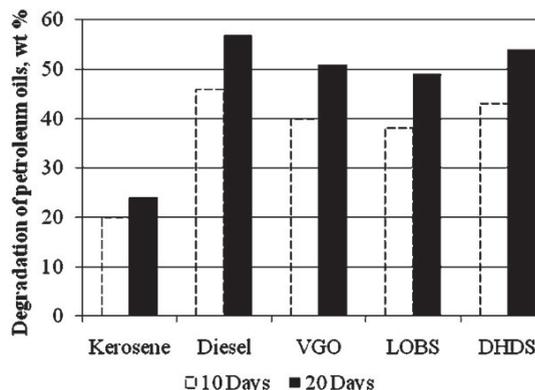
Characteristics were scored as: +, weak growth; ++, medium growth; +++, heavy growth.

Growth of strain at a: 35°C, 175 r.p.m and pH 7.0 b: 30°C, 125 r.p.m and pH 6.0



VGO, Vacuum gas oil; LOBS, Lubricating oil base stock; DHDS, Diesel hydrodesulphurization feed stock

Fig. 1. Degradation of petroleum oils by *A. junii* CTA₃



VGO, Vacuum gas oil; LOBS, Lubricating oil base stock; DHDS, Diesel hydrodesulphurization feed stock

Fig. 2. Degradation of petroleum oils by *P. aeruginosa* OCD₁

aeruginosa OCD₁ were studied for their degradation abilities of diesel and other petroleum fractions. Results of the experiment are shown in Figure 1 and Figure 2. The figures shows that the isolates have the good potential of degrading petroleum fractions like diesel, DHDS feed stock, VGO, LOBS. The highest degradation of 47% and 57% was obtained with diesel by the organism CTA₃ and OCD₁ respectively after 20 days of incubation. Biodegradation of kerosene by the isolates was observed in lower extent. The result of this figures was also supported with the result of Table 1. Also from figures it was observed that, most of the oils were degraded in first 10 days of incubation for both the isolates. However, strain *P. aeruginosa* OCD₁ showed better degradation ability of petroleum oils than the strain *A. junii* CTA₃.

Therefore, the strains *A. junii* CTA₃ and *P. aeruginosa* OCD₁ may be used for degradation of petroleum oil from the environment. Also, the isolates can be used for making bacterial consortium with other aromatic or paraffinic degraders for removal of more and more percentage of oil from the soil and aquatic system.

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