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AN ASSESSMENT OF PETROLEUM HYDROCARBON DEGRADATION BY BACTERIAL ISOLATE UNDER PHYSIOLOGICAL CONDITIONS

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ABSTRACT

Petroleum is a complex mixture of different hydrocarbons and is very fluid, viscous and volatile. They escape into the environment through the various sources and contribute modern pollution situation of environment. The levels of oil contaminant into the soil may be as high as 10% w/w and thus creating enormous problem due to their mutagenic and carcinogenic effect. They also highly toxic to plants, animals & humans and showed serious toxic effect on bone marrow and expose to benzene may cause leukemia.

Therefore, present study was conducted to determine the degradation ability under different physiological conditions of isolated bacteria from oil contaminated site. Total 13 bacteria have been isolated from collected soil sample from Karari station depot, Jhansi U.P. India. Among isolated bacteria, only KDJ-9 have been screened out and identified as Cornybacterium species. The maximum rate of oil degradation was found by bacterial isolate at 3.0 v/v concentration of petrol as carbon source and ammonium nitrate was the best nitrogen source. In different environmental conditions, the isolate showed their maximum degradation at pH 7.0 and temperature 35°C. Further understanding of the metabolic process of this organism on the crude oil will increase possibilities of develop strategies for removal of crude oil pollutants from oil-impacted environments.

Keywords: *Petroleum hydrocarbon, Cornybacterium species Physiological conditions, Bioremediation,*

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INTRODUCTION

Petroleum is the chief source of hydrocarbon, it is found in huge underground deposits in many parts of the world. Petroleum is a complex mixture of hydrocarbons and other organic compound, they escape into the environment and caused modern pollution situation of an environment. Large quantities of hazardous substances are carefully of in the environment and are thus creating enormous problems in soil, water around the world.

The physical and chemical treatments of waste are generally expansive and are not able to reduce quantities of pollutants. Bioremediation is a cheaper alternative and could remove contaminants. Till date numerous bioremediation of industrial waste have been attempted and several have been successful (Bishnoi et al, 2006, Potentini et al, 2006, Demir et al, 2007, Singh et al, 2008). The extent of hydrocarbon biodegradation in contaminated soil is critically dependent upon several parameters. Environmental and bioavailability of the contaminants to microorganism (Fantrouse and Agathos 2005, Romantsechuk et al, 2000). In this work, we reported that isolated diesel oil degrading bacteria was able to grow on relatively high diesel oil concentration at pH-7.0 and temperature 35 °C.

MATERIAL AND METHOD

1. **Sample collection –**Petroleum contaminated soil was collected from Karari Station Depot Jhansi, U.P. India. Samples were taken from the superficial to the depth 05-15 cm and transferred to the laboratories.
2. **Isolation of bacteria from Petroleum oil contaminated soil –** The bacterial cultures were isolated from the collected soil sample. 10 gram soil sample was suspended in 100 ml sterile distilled water and shaken for 5 minute & spread the suspended on the Bushnell Hass (BH) agar medium plate and incubated at 37°C for 24hrs.
3. **Screening of isolated bacterial culture –** The screening of petroleum hydrocarbon degrading isolates were carried out on the basis of diameter of clearing zones formed around the bacterial colonies on BH agar medium plate(Adeline *et al.*,2009)
4. **Identification and characterization of bacterial isolate-** Identification and characterization of bacterial isolate was done by cell morphology, cell physiology and biochemical characteristics include IMViC test, starch hydrolysis, Casine hydrolysis, Gelatin hydrolysis, TSIA test, Catalase test, H_2S test, carbohydrate fermentation and nitrate reductase test (Cappuccino and Sherman (2007).
5. **Optimization of physiological conditions for the potent isolates-** The influence of Petroleum oil concentration, nitrogen source, pH and temperature, on the growth of selected isolate was assessed using BH medium.

5.1 Optimization of Petroleum oil concentration – The flasks of (BH) medium contain different Petroleum oil concentration (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 % v/v) were inoculated with 1ml of broth culture of potent bacterial isolate. The inoculated media & control were incubated

on the orbital shaker (120 rpm) at 37°C until growth was decreased. The growth was observed in term of optical density at 600nm.

5.2 Optimization of Nitrogen source- The flasks of BH medium, replace by different nitrogen source like- Ammonium nitrate, ammonium sulphate, ammonium chloride and Ammonium nitrite were inoculate with 1ml of broth culture of potent bacterial isolate. The inoculated media & control were incubated on the orbital shaker (120 rpm) at 37°C until growth was decreased. The growth was observed in term of optical density at 600nm.

5.3 Optimization of pH- The flasks of BH medium containing different pH range (4, 5, 6, 7, 8, 9) were inoculated with 1 ml of potent bacterial isolate. The inoculated media & control were incubated on the orbital shaker (120 rpm) at 37°C until growth was decreased. The growth was observed in term of optical density at 600nm.

6. Optimization of Temperature - The flasks of BH medium were inoculated with 1 ml of potent bacterial isolate. The inoculated media & control were incubated on the orbital shaker (120 rpm) at different temperature range 25, 30, 35, 40, 45°C until growth was decreased. The growth was observed in term of optical density at 600nm

7. RESULTS AND DISCUSSION

5.1 Isolation and Screening of potent oil degrading bacteria- Total 13 petroleum oil degrading bacteria (KDJ-1 to KDJ-13) were isolated from different petroleum oil contaminated soil samples. Out of 13, only one bacterial isolate KDJ-9 showed maximum hydrocarbons degrading ability. (Fig-1) Adeline *et al.*, (2009) reported that the rate of increase in the diameter of zone of inhibition was formed corresponds to rate of biodegradation of isolates on a specific medium. Zhang *et al.*,(2004) reported that formation of zone of inhibition on Bushnell Hass agar medium supplement with naphthalene was not clearly visible in naphthalene solid cultures because this substrate is highly volatile.

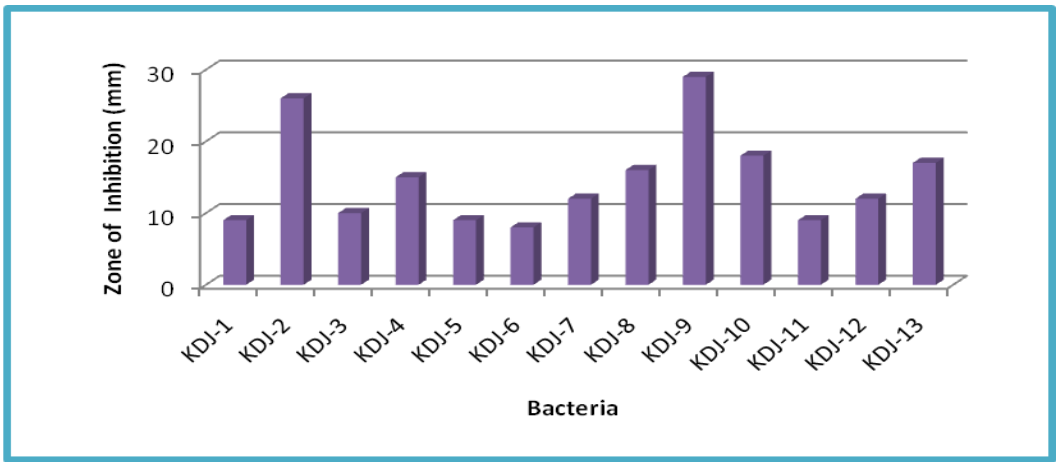

Fig.1 Zones of inhibition detected on BH agar plates by isolated bacterial Cultures

Identification and characterization of bacterial isolate- Selected bacterial isolate was identified as *Cornybacteriaum* species by cell morphology, cell physiology and biochemical characteristics (Table-1)

Table-1 Characteristics of bacterial isolate KDJ-9

S.No.	**Characteristics**	**Results**
1	Shape	Rod
2	Size (µm)	0.6× 0.8
3	Gram staining	-ve
4	Indole production	+ ve
5	Methyl red	+ve
6	Voges proskauer	-ve
7	Citrate utilization	+ve
8	Phenyl alanine diaminase test	- ve
9	Carbohydrate fermentation Glucose Lactose Sucrose	 +ve -ve -ve
10	Nitrate reductase	+ ve
11	Casein hydrolysis	- ve
12	Starch hydrolysis	+ ve
13	Gelatin hydrolysis	+ ve
14	Catalase	+ ve
15	Urease	+ ve
16	H_2S	+ ve
17	Lipid hydrolysis	+ ve
18	Oxidase	+ ve
19	TSI test	- ve

5.3 Optimization of physiological conditions for the potent isolates

5.3.1 Petroleum oil concentration – The result of carbon source optimization showed that the maximum rate of oil degradation was found by bacterial isolate at 3.0 v/v concentration of Petroleum oil as carbon source (Fig.2). Although bacterial growth started to decreased above this. Petroleum is needed as a carbon source but at certain concentrations, petroleum oil can be toxic to microorganisms due to the solvent effect of petroleum oil which destroy bacterial cell membrane. Thus many biodegradation studies on petroleum are carried out using lesser diesel concentrations ranging from 0.5 to 1.5% (Mukherji *et al.,* 2004; Lee *et al.,* 2005,2006; Hong *et*

al., 2005; Ueno *et al.*, 2007; Rajasekar *et al.*, 2007). Degradation at a much higher concentration (6% v/v diesel) has been reported but degradation requires glucose (0.2% w/v) and yeast extract (0.1% w/v) (Kwapisz *et al.*, 2008). The results suggest that bacterial culture was able to grow at relative high concentration of diesel oil.

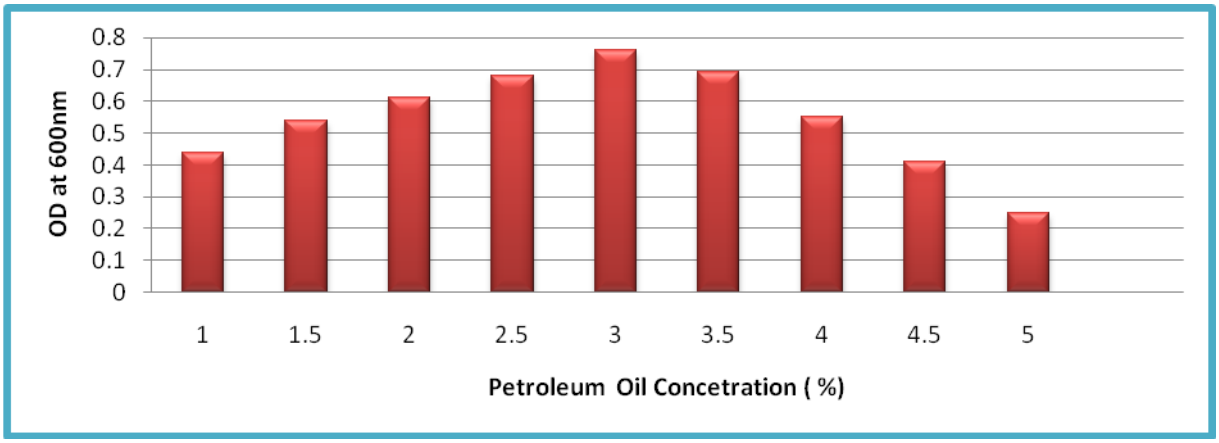

Fig.2 Optimization of different oil concentrations for bacterial growth of KDJ-9

5.3.2 Nitrogen source- The results of optimization of nitrogen source showed that ammonium nitrate was the best nitrogen source for bacterial isolate (Fig.3). The optimization of nitrogen source is important because low level of fixed form of nitrogen source in the bacterial growth environment limit the rate of petroleum hydrocarbon degradation (Atlas and Cerniglia, 1995) the ability to use nitrite as a nitrogen source is an advantage. It is usually added as a nitrogen source for cellular growth, but it can also serve as an electron acceptor (Leeson and Hinchee, 1997). Ammonium nitrate was chosen as the principal nitrogen source due to it widespread usage as a cheap source of nitrogen source of nitrogen for petroleum hydrocarbon degradation.

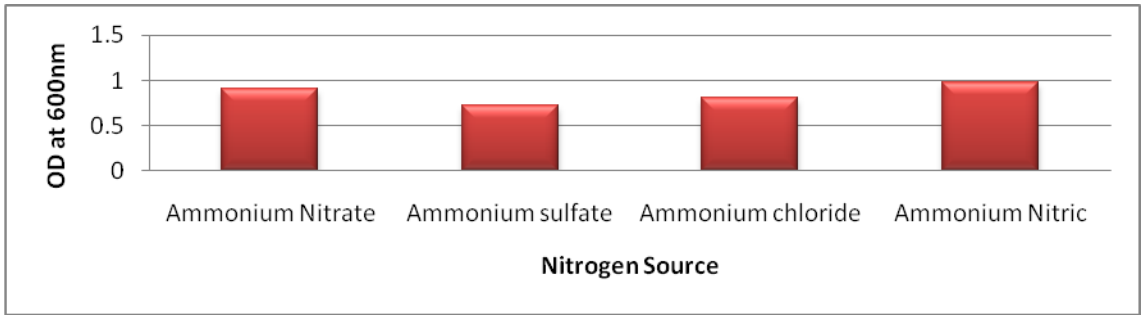

Fig.3 optimization of nitrogen source for bacterial growth of KDJ-9

5.3.3 Optimization of pH- The result of optimization pH showed that the maximum rate of oil degradation was found by potent bacterial isolate at pH-7.0 (fig.4). The result showed that the optimum pH value is required for the maximum growth of potent bacterial isolate because above

& below pH value caused low growth of potent bacterial isolate. The requirement of neutral or near neutrality for optimal growth of bacteria on diesel is also exhibited by many other bacterial strains (Espeche *et al.,* 1994; Chapman and Shelton, 1995; Bicca *et al.,* 1999; Margesin, 2000; Cavalca *et al.,* 2000; Mukherji *et al.,* 2004; Hong *et al.,* 2005; Marquez-Rocha *et al.,* 2005; Lee *et al.,* 2005, 2006; Rajasekar *et al.,* 2007; Ueno *et al.,* 2007; Kwapisz *et al.,* 2008).

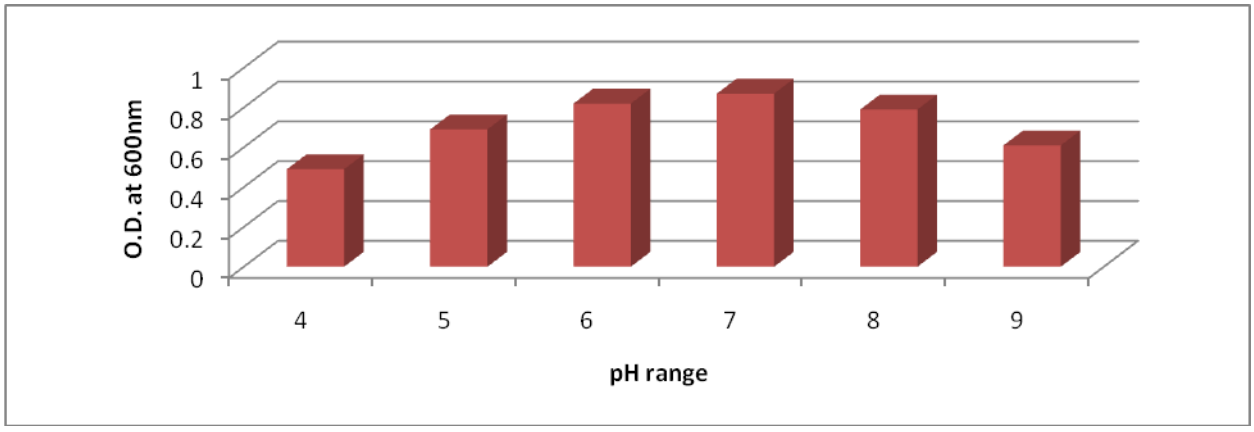

Fig.4 Optimization of different pH range for bacterial growth of KDJ-9

5.3.4 Optimization of Temperature - The result of optimization temperature for growth of potent isolate showed that the maximum rate of oil degradation was found by potent bacterial isolate at 35°C (fig.5). Potent isolate was able to grow at 30-40°C significantly but optimum temperature was noted 35°C for maximum growth. One of the most reported temperature optima supporting diesel degradation is at 30°C (Cavalca et al., 2000; Mukherji et al., 2004; Hong et al., 2005; Lee et al., 2006; Kwapisz et al., 2008). Lower temperature optima have been reported such as between 10 and 25oC (Margesin, 2000), at 20°C (Chapman and Shelton, 1995; Lee et al., 2005 and Ueno et al., 2007) and at 27°C (Rajasekar et al., 2007). Higher growth optima were reported by Marquez-Rocha et al. (2005) at 37°C for a tropical diesel-degrading bacterium from Mexico. Pepi *et. al.*,(2005) reported that psychrotropic bacteria, *Halomonas* species has an optimum growth at 15°C using 2% hexadecane as a substrate.

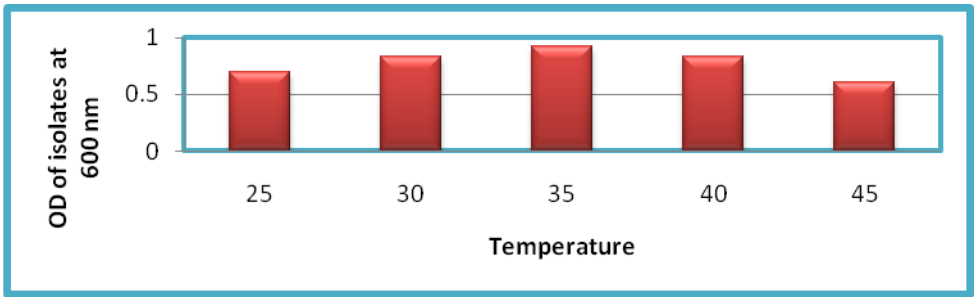

Fig.2 Optimization of different temperature for bacterial growth of KDJ-9

8. **CONCLUSIONS-** This study showed that isolated bacterial culture was identified as *cornybacterium* species that have the ability to utilized petroleum hydrocarbon as carbon source and removing from hydrocarbon contaminated environment. Growth optimizations of physiological conditions study of potent isolated bacterial culture were performing to promoting higher degradation rate.

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