Diesel Biodegradation Capabilities of Indigenous Bacteria Isolated From Ganga River, West Bengal

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Abstract: *The present work was conducted to isolate and biochemically identify some bacteria from oil-contaminated soil to evaluate their role in biodegradation of commercial diesel under laboratory conditions. Diesel fuels are used by different vehicles, generators and especially heavy transport vehicles. Its manufacturing, transportation, utilization and disposal have the threat to pollute the surrounding environment as they retain in the environment for the long period of time. Biodegradation is one of the biological processes to remediate the pollutants. This is the cheaper and easy method as compared to other methods like direct burning, land foaming and bioventing. Water sample was isolated from Ganga River and five colonies were isolated & show diesel degradation capabilities. Several different biochemical tests were performed to preliminary identify them & growth curve was measured. All of the isolates show growth in Bushnell Hass media containing 2% crude diesel as a sole carbon source. Therefore they can be used to bio remediate diesel contaminated water or soil although their interaction with other soil bacteria needs to be understood.*

Keywords: *Biodegradation; bioventing; Bushnell Haas; crude diesel; bioremediate.*

1. INTRODUCTION:-

Crude oil are composed of hydrocarbons & other organic compounds (Paraffin, Alicyclic & aromatic hydrocarbons) including some organometallic constituents that is used to lubricate the parts of an engine, in order to keep everything running smoothly (Hagwell et al., 1992). But as the usage of petroleum hydrocarbon products increases, soil contamination with diesel & engine oil is becoming one of the major environmental problems.

Mechanical method to reduce hydrocarbon pollution is very expensive & time consuming. Bioremediation provide an effective & efficient strategy to speed up the clean up process. Petrophiles (oil consuming microorganism) are very unique organisms that can naturally degrade large hydrocarbons & utilize then as a food source (Harder, 2004). Petroleum hydrocarbons can be degraded by microorganisms such as bacteria, fungi, yeast & microalgae (Riser-Roberts, 1992; Bundi *et al.* , 2004). However bacteria play the central role in hydrocarbon degradation. The driving force for petroleum biodegradation is the ability of the microorganism to utilize hydrocarbons to satisfy their cell growth & energy needs. Mixed cultures carry out more extensive biodegradation of petroleum that pure cultures (Ghazali et al., 2004; Oteyza et al., 2005; Sun et al., 2004; Gardis et al., 2004; Trindade et al., 2004). In this study, the petroleum degrading potential of one or more bacterial cultures are examined with the hope of isolating & stocking useful organisms with high crude oil degrading potential from the nearby areas of Ganga River. Diesel was used as a sole carbon in Bushnell Haas broth & all the isolates were incubated up to 33 days to check their ability of degradation of crude diesel.

2. Materials & Method:-

2.1 Isolation of Bacteria:- Blackish water from AhiritolaGhat, Ganga, West Bengal was isolated and inoculated in Bushnell Haas broth containing 1% Crude diesel oil as a sole carbon source. It was incubated for 7 days & then serial dilution & standard plate count method were performed on normal Nutrient agar plate for 24 hours at 37˚C. 5 different colonies (I_1- I_5) were isolated & tested further.

2.2 Colony Character & morphology determination by Staining:- Each of the five colonies were characterised according to colonial morphology & both Simple stain & Gram Stain were performed to check their microscopic morphology & Gram Character.

2.3 Biochemical Tests:- Several different biochemical tests were conducted such as – catalase, Urease, Starch Hydrolysis, hydrogen sulphide production, Lactose utilization, IMViC test according to normal conventional protocol.

2.4 Growth Curve:- Bacterial Growth curve were performed in Nutrient broth media by inoculating 1% overnight culture. Each interval of 1 hour, 3ml sample were taken & absorbance were measured at 590nm with respect to fresh nutrient broth as control. Approximately 24 hours growth measurement were performed.

2.5 Diesel degradation potential of the bacterial isolates:- To check the ability of utilizing diesel as a sole carbon source, all the isolates were inoculated in Bushnell Haas broth containing 0.2% crude diesel for 20days & then OD were taken against blank. Similar experiment were conducted but with 10time higher concentration of diesel (2%) as a carbon source & incubated for 33 days at 37˚C. OD were taken at 590nm after incubation.

3. Result:-

3.1 Colony character of bacteria

Table:1

Pure Culture	Colony Character
Green pigmented colony(I_1)	Colonies are round, regular surface, glossy, Pigment got diffused throughout the agar
Brown pigmented colony(I_2)	Colonies are very small round shaped, matty, dry surface. Pigment diffused throughout the agar
Yellow pigmented colony(I_3)	Colonies are round glossy have sticky surface. Pigment did not diffuse throughout the agar
Orange pigmented colony(I_4)	Colonies are irregular in shape, glossy have sticky surface. Pigment did not diffuse throughout the agar
White colony(I_5)	Nucleated colonies, Non pigmented, small irregular in size.

Fig-1. Plate showing the colonial growth of I_1- I_4 respectively

3.2 The morphology of bacteria determined by simple stain

Table:2

Bacterial isolates	Morphology
I_1	Short rods, arranged singly
I_2	Elongated rods, scattered in single ‘v’ or ‘L’ shaped
I_3	Rods singly arranged
I_4	Slender rods
I_5	Rod shaped, arranged both in isolated & in chain form.

3.3 Identification based on Gram character

Table: 3 The gram character of bacterial isolates

Bacterial isolates	Gram character
I_1	Gram negative
I_2	Gram positive
I_3	Gram positive
I_4	Gram positive
I_5	Gram positive

3.4 Identification based on Biochemical character

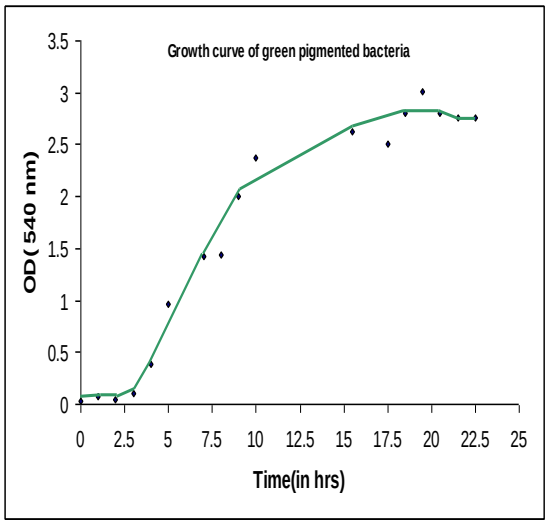
Table: 4 Biochemical reactions given by bacterial isolates

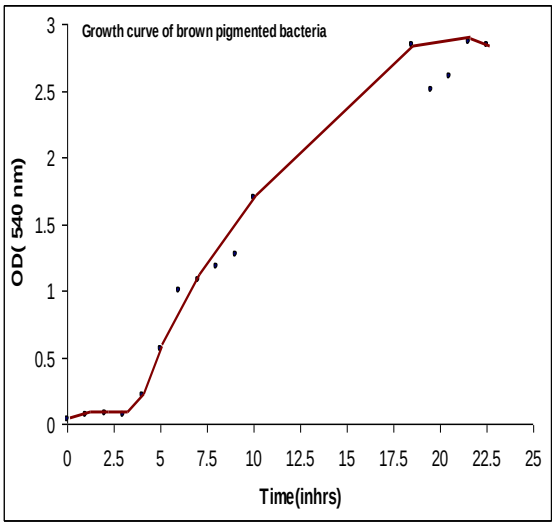
Biochemical test	I_1	I_2	I_3	I_4	I_5
Catalase test	+ve	+ve	+ve	+ve	+ve
Urease test	+ve	−ve	−ve	−ve	−ve
Starch hydrolysis	−ve	+ve	+ve	−ve	−ve
H_2S production	−ve	−ve	−ve	+ve	−ve
Lactose utilizing	−ve	−ve	−ve	−ve	−ve
Indole production	−ve	−ve	−ve	−ve	+ve
Methyl Red Test	−ve	−ve	−ve	+ve	−ve
V P test	−ve	−ve	+ve	+ve	+ve
Citrate utilization	−ve	+ve	−ve	+ve	+ve

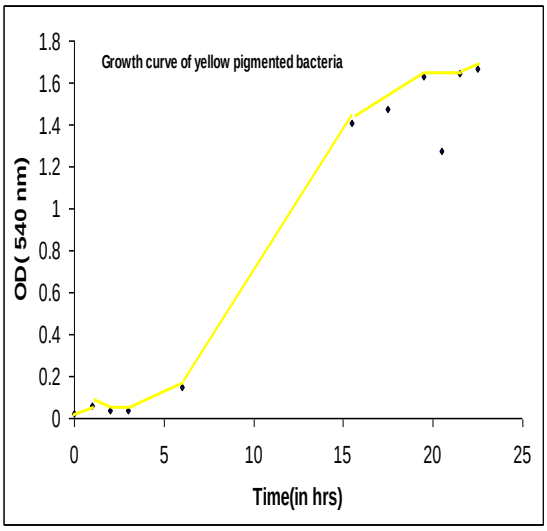
(+)ve= Shows the reaction.
(−)ve= Does not show the reaction.

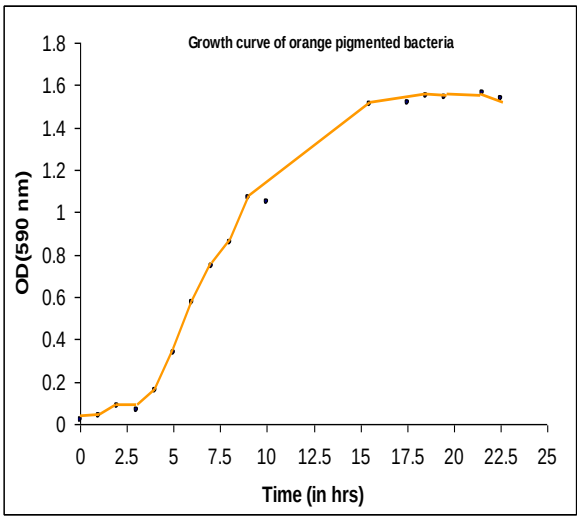
Fig-2-Citrate utilization test & indole production test of the bacterial isolates.

3.5 Growth curve determination in Nutrient Broth:-









3.6 Petroleum degrading potential of bacterial isolates:- After 2nd enrichment in BH media for 20days, 2ml from each grown bacterial cultures (I_1-I_5) were taken in a cuvette & O.D was taken at 590 nm using 0.2% crude diesel containing BH media as control.

Table:5

Bacterial cultures	O.D at 590 nm
I_1	0.473 (++++)
I_2	0.131 (+)
I_3	0.126 (+)
I_4	0.186 (++)
I_5	0.381 (+++)

+++ : strong growth,+++ :good growth,++ : medium growth, + : poor growth

Results of bacterial isolates (good degraders) after growing in BH media with 2% diesel as a carbon source. O.D. was taken on each consecutive days (20 days).

Table:6 Absorbance values of the bacterial suspension grown in 2.0% crude diesel containing BH medium for 33 days at 590 nm against blank containing BH medium +2.0% crude diesel

Bacterial isolates	Absorbance at 590 nm
I_1	0.749(+++)
I_2	0.155(+)
I_3	0.160(+)
I_4	0.206(+)
I_5	0.440(++)

+++: strong growth, +: poor growth, ++: moderate growth

4. DISCUSSION:

The green pigmented bacteria(I_1) was found to be gram (-)eve, short rods, gave positive reaction for catalase and urease but negative for indole, citrate and lactose non utilizing (non fermentative). The I_1 bacteria resembled the *Pseudomonas sp*. in their gram character and biochemical reactions. The brown pigmented bacteria (I_2) was found to be gram (+)ve elongated rods. The young cells of brown pigmented bacteria were elongated rods but old culture cells were coccus in appearance. They have a rod-coccus cycle like that of *Arthrobacter* species. They gave positive reaction for catalase but negative for urease, indole, lactose non utilizing, citrate utilizing, hyrolysed starch. The yellow pigmented bacteria(I_3) was found to be gram (+)ve, gave positive reaction for catalase, negative for indole hydrolysed starch but lactose and citrate non utilizing. The orange pigmented bacteria (I_4) ,rods and cocci elements was found to be gram (+)eve, gave positive reaction for catalase,H_2S producing, positive for VP test with lactose and citrate non utilizing.The non pigmented I5 bacteria was gram positive ,Catalase positive(aerobic), urease negative, non utilizing for starch and lactose, positive for indole production , VP positive with citrate utilizing.

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For **I_1 bacteria,** pigment release was started after 7 hrs of growth(during the late log phase)**.** For **I_2 bacteria,** pigment was released after 9 hrs (log phase) of growth, it had an extended log phase compared to other three bacteria isolates. For **I_3 bacteria**(yellow pigmented), it had an extended lag phase in comparison to other bacterial isolates, the pigment release time could not be assessed due to the yellow colour of the medium. For **I_4 bacteria**, pigment was released after at 8hrs of growth (late log phase) in Luria broth . The bacterial cells were also stained to observe their morphology during the growth. After 3 hrs, I_1 cells appeared to be very small rods, after 4 hrs they were rods. In case of I_2, after 3 hrs the cells appeared to be very small rods but after 9 hrs, they became elongated rods. The orange pigmented bacteria (I_4) after 5 hrs of growth, the cells appeared as short rods with swollen ends. For **I_5 bacteria** , the lag phase is very short. After 5 hrs of growth, the cells appeared as slender rods.

All the isolates show capabilities to degrade crude diesel as a sole carbon source both in 0.2% concentration & 2% concentration. The rate of degradation increases with increasing time of incubation. The degradation rate shows maximum in case of I_1 bacterial isolates in both cases although I_5 shows good degradation activity compared to other isolates. Further identification is required to characterise them. The application of the isolates in natural environment may show good bioremidial activity.

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