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Isolation and characterization of bacteria from oil filling stations at kakinada, India

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Abstract

Bioremediation, basically functions as biodegradation, is the promising technology for the treatment of oil contaminated sites. This may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. Many microorganisms in water and soil are capable of degrading hydrocarbon contaminants. Pseudomonas species (S6) was isolated from soil in nearby petrol bunks, its adaptation was been studied under different parameters. Degradation in diesel was identified by performing tetrazolium test. This paper presents an updated overview of diesel hydrocarbon degradation and reduction reaction by microorganisms under definite physico-chemical parameters. In the present study microorganism isolated, Pseudomonas species (S6), reduced tetrazolium (2,3,5-triphenyl tetrazolium chloride solution) utilizing hydrocarbons as nutrient source from diesel. The result was identified by change in the color from colorless to pink.

Key words: *characterization, diesel, Pseudomonas species, turbidity, tetrazolium.*

1. Introduction

Hydrocarbons enter into nature by spills, disposals, transport and storage. Hydrocarbon degrading bacteria were widely distributed in nature like water and soil. Microorganisms have the ability to degrade hydrocarbons in nature as energy sources and for metabolic activities [Adeline et al., 2009]. These micro organisms utilize these hydrocarbons released in the nature accidentally by either water or soil pollution. Industrialization and accidental spillage of hydrocarbons increased in nature. Due to increase in contamination in the environment there requires the study of isolating and characterizing oil degrading bacteria. The increase of hydrocarbons in nature can be removed by many processes by chemical oxidation, volatilization, photo oxidation and absorption. However one of the major decomposition process is by microbial degradation. Biodegradation, which is the destruction of organic compounds by microorganisms, is carried out largely by diverse bacterial populations, mostly by Pseudomonas species [Boboye et al., 2010; Dubey, 2009; Balows et al., 1992]

It is essential to isolate such species in the natural environment. Such potential degraders are highly acclimatized and adopted to natural contaminated environment. Such degraders are mostly isolated from their natural habitat, i.e., from diesel filling stations. The isolated species were been cultured in selective media and performed biochemical characterization. The aim of this study is to investigate the possibility of biodegradation of diesel. The cultures were isolated from adopted conditions and efficiency of biodegradation was tested by adding diesel during characterization tests. In the present study we characterized the isolated microorganism for its ability to degrade hydrocarbon of diesel. The complete decomposition of oil occurs over an extended period of time, requires abundant nutrients, and involves several species of microbes [Hong et al,. 2005]. Change in oil composition due to microbial breakdown can be observed in the study.

2. Materials and Methods

Soil sample were collected at nearby oil filling stations in Kakinada: of country India lies on the geographical coordinates of 16° 56' 0" N, 82° 13' 0" E. Approximately 50gms of soil was collected by observing the color and odor of oil mixed soil in a sterile screw capped tube. About one gram of soil sample is taken as test, serially diluted, and plated on nutrient agar media by spread platting methods [Aneja, 2006] for the growth of bacteria Isolates were identified and performed streaking on nutrient agar plates [Cappuccino and Sherman, 2002] Pure cultures obtained and performed gram staining, are streaked on to enrichment media [Bushnell and Haas, 1941; Atlas, 1981]. The initial ph was adjusted to 7.0 and the medium was sterilized at 1210 c for 15 min. After sterilization, the media was supplemented with 1ml of diesel separately on to agar plates .Isolates were streaked and the Colonies were identified and subculture to perform bio chemical tests according to berg's manual of determinative bacteriology [Holt et al., 1994] including gram staining, cell motility, Indole production, methyl red-Voges Proskaeur test, citrate utilization test. Isolates were cultured in LB broth and turbidity was also measured at 600nm to observe the change in growth of bacteria by taking OD values every day i.e., up to 10 days with 1%,2%,3%,4%,5% of hydrocarbons. Control was also been set up without the addition hydrocarbons in broth culture.

Isolates were also incubated in different media conditions like

1. Hydrocarbon+1ml of culture

2. Hydrocarbon+Nutient broth+1ml culture

3. Nutrient broth+culture

The change in growth of the culture in presence of hydrocarbon has been recorded up to 10 days taking OD values at 600 nm

2.1 Screening for diesel degradation

Isolated stains were inoculated in nutrient broth for 18 hours. Fresh broth culture of 1ml was added into two test tubes containing each with 2 ml of 0.2% 2,3,5-triphenyl tetrazolium chloride solution and 5drops of diesel. Test tubes were cotton plugged and incubated for 24 hrs in an incubator. Control has been set up in another tube without diesel. Test tubes were screened for reduction

reaction by micro organisms after 24 hrs of incubation [Theresa et al., 1999].

3. Results and Discussion

It is found that the isolated stains observed as gram –^{ve} rod shaped bacteria, exhibits green color fluorescence (figure 1) in UV light and was identified as *Pseudomonas* species by performing biochemical test (table1). Growth of bacteria was found to increase in diesel which was noticed by observing OD values (table 2; figure 2). Under different media conditions *pseudomonas* species turbidity increased in when cultured in nutrient broth. Whereas turbidity has been decreased in the tube where there is no nutrient broth. Turbidity is observed more at 7th day of incubation where the broth is supplemented with diesel and also in the media containing only diesel and not supplemented with nutrient broth (figure3)

Table 1: Tabulation of biochemical test for strain S6

Biochemical tests	Result
Gram staining	Gram negative rods
Indole	-ve
Methyl red	-ve
VP	+ve
Citrate	+ve
UV Fluorescence	+ve
Growth at 37^{0}C	+ve

Figure 1: *Pseudomonas* (S6) isolated strain from oil spilling station at Kakinada showing characteristic fluorescence green color under UV light.

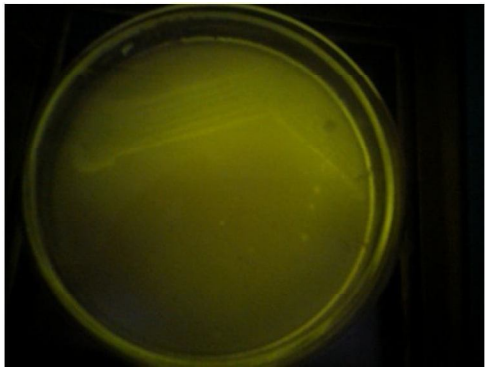


Table 2: Showing OD values for turbidity when culture was incubated in different % of hydrocarbon

% of hydrocarbon	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
1%	0.01	0.02	0.05	0.08	0.11	0.05	0.04	0.04	0.01	0.00
2%	0.02	0.05	0.06	0.11	0.10	0.10	0.07	0.04	0.00	0.00
3%	0.09	0.10	0.11	0.13	0.14	0.16	0.07	0.04	0.02	0.01
4%	0.09	0.10	0.12	0.13	0.17	0.19	0.016	0.13	0.10	0.02
5%	0.07	0.09	0.14	0.12	0.13	0.09	0.05	0.03	0.00	0.00

Figure 2: Showing graphical representation of bacterial growth in presence of hydrocarbon

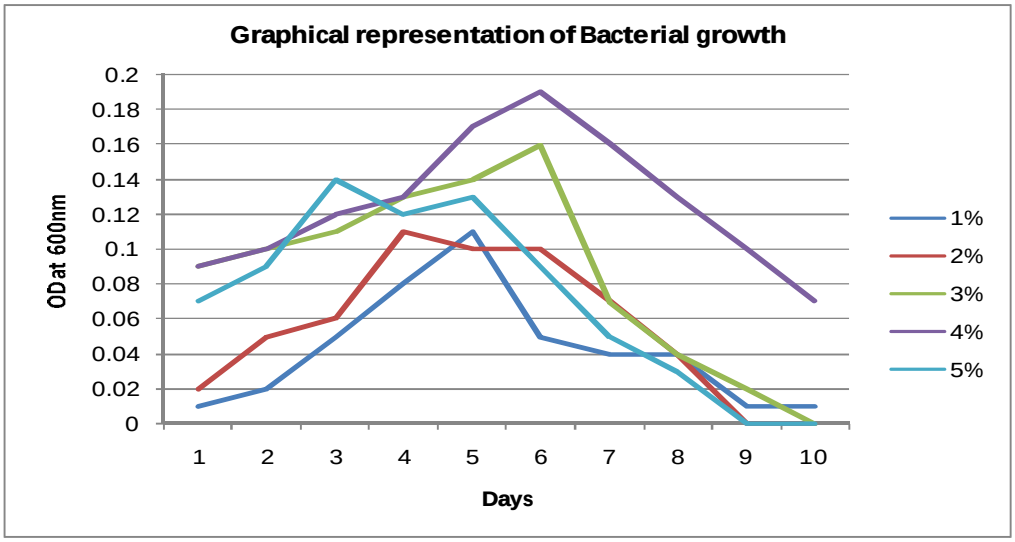
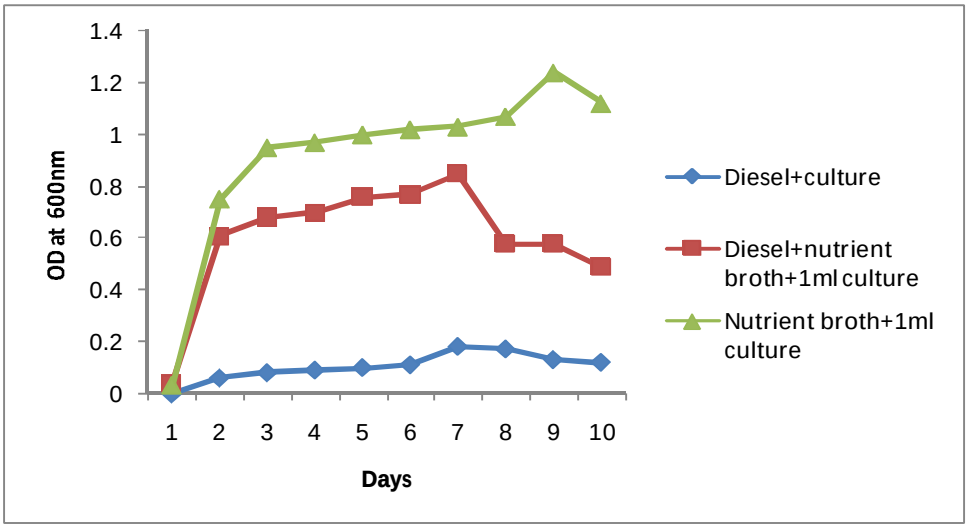


Table 3: Showing OD values when grown in different media conditions for 10 days

Days	Diesel+culture	Diesel+nutrient broth+1ml culture	Nutrient broth+1ml culture
1	0.00	0.04	0.03
2	0.06	0.61	0.75
3	0.08	0.68	0.95
4	0.09	0.70	0.97
5	0.10	0.76	1.00
6	0.11	0.77	1.02
7	0.18	0.85	1.03
8	0.17	0.58	1.07
9	0.13	0.58	1.24
10	0.12	0.49	1.12

Figure 3: Graphical representation of Bacterial growth in media containing different compositions a) Media with Diesel without nutrient broth b) Media containing nutrient broth supplemented with Diesel and c) Media with nutrient broth without Diesel.



3.1 Reduction test

By observing reduction test from the above tubes after 24 hours it was found that color of the solution has been changed to pink color in the tubes in which the microbes were added. Pink color was observed in the test tubes supplemented with diesel when incubated with microbes in presence of tetrazolium chloride. Negative result (discoloration) was observed in control test tubes where microbes were not inoculated. Tetrazolium is an indicator which is colorless, but changes to pink when it is reduced [Theresa et al., 1999].The change in color to pink indicates that the isolated bacteria incubated with tetrazolium can degrade diesel. The use of this reaction, the reduction of tetrazolium from its oxidized form (colorless) is reduced (pink) that acts as an indication of microbial metabolism.

Table 4 Showing bacterial degradation of hydrocarbon by tetrazolium reduction test

S.No	Bacterial culture (pseudomonas species)	Tetrazolium (0.2%)	(hydrocarbon) diesel	Result(pink color)
Tube.1(control)	-	2 ml	5 drops	- ve
Tube 2(test)	1ml	2 ml	5 drops	+ ve
Tube 3(test)	1ml	2 ml	5 drops	+ ve

4. Conclusion

The present study is to isolate bacteria from oil contaminants at petrol stations at Kakinada, India where the bacterium is adopted to survive. The bacteria which is isolated, identified as *Pseudomonas* species by biochemical test and Its growth is estimated by turbidity in presence of diesel and at different media conditions. These bacteria are able to degrade the oil present in soil which is one of the catalytic activities of microorganisms in the environment which was concluded and identified by observing reduction test by using 2,3,5-triphenyl tetrazolium chloride. This test confirms the ability to degrade diesel supplemented in the nutrient broth.

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