GOLDEN RESEARCH THOUGHTS

DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS(PAH'S)EMPLOYING BIOSURFACTANT PRODUCING BACTERIA FROM DIESEL CONTAMINATED SOIL .

Prof. Pharande N.S.
M. S. Kakade college, Someshawarnagar,Baramati, Pune,India.

ABSTRACT:

Soil sample where collected from oil contaminated sites at Saswad region. Experiments were conducted in laboratory to determine the efficiency of strain to degrade polycyclic aromatic hydrocarbons. Mineral salt supplemented with oil was use and most abundant species isolated from an oil slugged soil Micrococcus spp.Pseudomonas spp. [2]. Biosurfactant production was measured and result of the study show that this native biosurfactant producing bacterial strain has great potentiality in the degradation of hydrocarbon[1].

KEY WORDS: *Biosurfactant, PAH's degradation, Hydrocarbons, Pseudomonas.*

INTRODUCTION:

The principle chemical element (90% wt. Of the crude oil) are carbon and hydrogen which are combined in a series of compounds called hydrocarbons. Polycyclic aromatic hydrocarbons constituent a large and diverse class of organic compounds and are generally described as molecules which consist of three or fused rings in various structural configurations. [1]. Petroleum hydrocarbon are not easily degradable.[6]. Microorganisms have the capacity to degrade majority of hydrocarbon component the saturated and unsaturated alkanes,monoaromatic and low molecular weights polycyclic aromatic hydrocarbons. The majority of hydrocarbons are found in diesel, petrol and kerosene, such hydrocarbon utilizing microorganism shows emulsifying activity. The degradation rate is affected by several physicochemical and biological parameter such as p^H ,nutrients, quantity of hydrocarbon.[5].

Materials:

1.For Isolation of PAH degrading and screening of biosurfactant producing organism[1]:

a. Soil sample were collected from oil contaminated sites of Saswad.
b. Mineral salt media
c. 2% crude oil.
d. 2% glucose solution

2. For drop collapse assay.

a. Fermented broth.
b. 2% crude oil.

3. For Identification of microbes:

A. Gram staining reagent.
B. Biochemical reagent:
a. Catalase test-2% H_2O_2

b. Oxidase test-NNNN-Tetramethyl P-Phenylenediamine Dihydrochloride.
c. Sugar Utilization test-Phenol red indicator
d. Nitrate reduction test-0.2% KNO_3
Indicators-Sulphonic acid, alpha naphthalamine, acetic acid.

4. Extraction and Characterization of biosurfactants:

a. 6N HCL
b. Ethyl acetate
c. Ninhydrin reagent
d. Anthrone reagent
e.3M NaOH

5. For media optimization[5]:

a. for Carbon source: Glucose, Fructose, Mannitol, Peptone
b. for p^H: Sodium hydroxide(NaOH), Hydrochloric acid(HCL)

6. For fractional recovery rate (FRR):

a. 2ml oil
b. 10gm soil
c. 10ml Dichloromethane

Methods :

1] Isolation of PAH's degrading microorganisms:

1gm of soil sample was added to sterile 100ml NB+MS medium to which 2% crude oil was added. Incubated at 35^0c in a rotary shaker at 150 rpm for 3day's,serial dilution was prepared.

2] Identification of microbes by using Bergey's manual:

By reffering Bergey's manual of determinative bacteriology biochemical tests was selected. Different biochemical tests was performed.

3] Screening for biosurfactant producing bacteria:

5ml culture+100ml MS medium each with 2% glucose. Incubated at 35^o c at 150 rpm for 48 hrs in rotary shaker. Growth observed and proceed for biosurfactant producing organism by drop collapse assay.

a. Drop collapse assay(for strain 1^{st} ,2^{nd} and 3^{rd}):

1 drop oil on slide +1drop of 48hrs grown culture from mother culture 1. Drop collapse activity was observed

4] Screening of most efficient hydrocarbon degrading bacterial isolates:

Both cultures were prepared from all biosurfactant producing bacterial isolates in NB. 100ml MS medium+ 2% crude oil + 5ml mother culture (for strain 1^{st} and 2^{nd}). Incubated at 35^0c at 150 rpm for 7 days. O.D. measured at 600nm by UV visible spectrophotometer.

5] Extraction of Biosurfactant:

48 hrs old culture was centrifuged at 10,000 rpm for 20 min at 4^0c.Supernatant+ 6N HCL adjusted p^H 2, kept at 4^0c for overnight. Biosurfactant was extracted with Ethyl acetate A mixture of supernatant and ethyl acetate shaken, left for phase separation. The organic phase was then transferred to rotary evaporator. A viscous solid product was recovered after solvent evaporation at 40^0c under reduced pressure.Biosurfactant determined gravimetrically.

6] Characterization of biosurfactants:

a) Ninhydrin test**:** 1ml of broth solution was taken in a test tube and add a few drops of Ninhydrin reagent and vortex the contents. Place the test tube in boiling water bath for 4-5 min and cool to room temperature. b) Anthrone test: 1ml of broth was taken in test tube and add Anthrone reagent. Kept in water bath for 10 min. Cool the test tube at room temperature. Observed the colour and take O.D. at 600nm.c) Saponification test: Approximately 0.5ml of liquid is added to 0.5ml 3M NaOH. After the sample is heated for 15min then 10ml water is added and solution shaken vigorously.

7] Fractional recovery rate:

2ml of the oil+10gm of soil. 10ml of Dichloromethane was poured into tube containing oil polluted soil with seeded organism. It was shaken vigorously and decanted into sterile test tube. The total extract which

contain the residue oil was then heated over a hot water bath for evaporation.The residual crude oil weighed and volume calculated based on fractional recovery rate.

8] Media optimization:

a) Effect of p^H on hydrocarbon degradation:

100ml Mineral salt medium broth was prepared of varying p^Hlike 5,6,7 and sterilized 1ml crude oil was added to that broth. 5ml of bacterial inoculum were added. Incubated at 28^0c for 5 days at 120 rpm on shaker. Observed growth and O.D. was taken at 600nm.

b) Effect of carbon source on hydrocarbon degradation:

100ml MSM broth with three different carbon sources Glucose, Mannitol, Fructose were prepared and sterilized. 1ml crude oil was added to the broth. 5ml of bacterial inoculum was added. Incubated at 28^0c for 5 days at 120 rpm on shaker. Observed growth and O.D. was taken at 600nm.

Observation and Result:

1] Isolation of biosurfactant producing microorganism:

A total three morphologically different bacterial colonies were isolated from the collected soil sample. The two isolates were screened out for biosurfactant production.

2] Identification of microbes by using Bergey's manual:

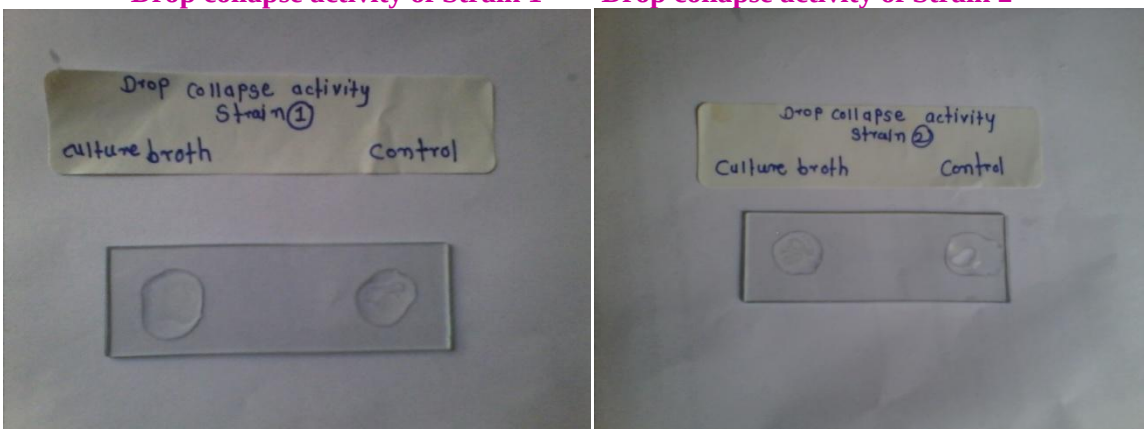
Morphological and Biochemical Characterization was done by referring Bergey's manual, The isolated organism may be *Micrococcus* spp. *Pseudomonas* spp. *Marinococcus spp*.

3] Screening for biosurfactant producing bacteria:

a. Drop Collapse Assay:

Among the three bacterial isolates ,culture broth of two isolates could collapse the drop of crude oil indicating the presence of biosurfactant in the culture medium .The drop of Crude Oil was collapsed immediately within one minutes of addition of culture broth .The remaining bacterial culture broths could not collapse the drop of crude oil even after one minutes.

Drop collapse activity of Strain 1 **Drop collapse activity of Strain 2**



4]Screening of Most Efficient Hydrocarbon Degrading Bacterial Strain:

The *Pseudomonas spp* .and *Micrococcus spp*. showed the maximum growth(OD 600nm) = 0.376 ,0.238) in crude oil containing medium after 7 day of incubation.

5] Extraction Of Crude Biosurfactant:

The yield of biosurfactant from *Pseudomonas spp.* was 1.12g/L . The colour of the crude biosurfactant was dark Brown.

6] Characterisation of Biosurfactant:

Ruhemann′s purple Complex Formation was absent in Ninhydrin test ,indicating absence of amino acid or protein in the biosurfactant.In the Anthrone test for Carbohydrates , a blue –green colour formation was observed , which indicates the presence of Carbohydrates in the Sample .In Saponification test , NaOH

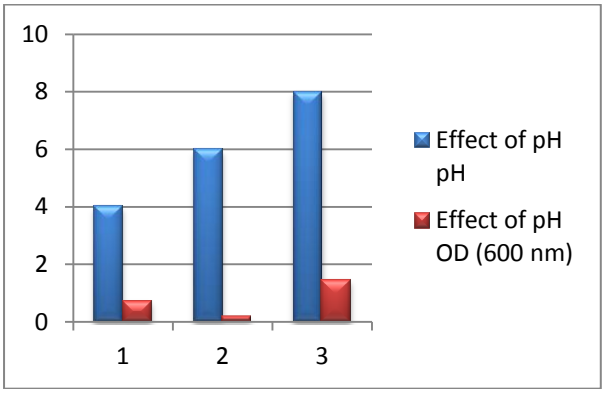
saponifies the lipids present in the biosurfactants , indicating the presence of lipids . These results indicates that the Crude biosurfactant produced by a bacterial strains Contains sugar and lipid molecules but not proteins molecules.

7] Fractional recovery rate:

Amount of Oil introduced	2ml
Amount of Oil Recovered	1.8ml
Recovery per ml of the Oil	1.8/2=0.9 / ml
Percentage Recovery	0.9 * 100= 90.0 %
The fractional Recovery Rate (FRR)	1.8
The Fractional lost	0.2

8] Media Optimization:

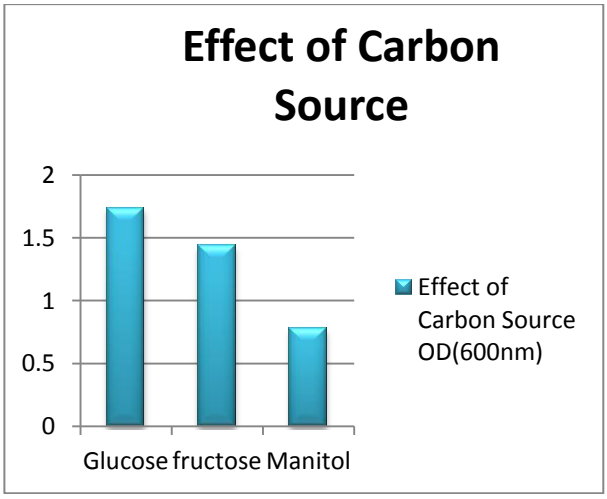
a) Effect of pH on Hydrocarbon Degradation:



pH	OD (600 nm)
4	0.72
6	0.18
8	1.46

The maximum degradation rate was obtained at pH 8 which showed the optimum pH for degradation of hydrocarbons.

b) Effect of carbon source on hydrocarbon degradation:



Carbon Source	OD(600nm)
Glucose	1.74
fructose	1.44
Mannitol	0.78

Mannitol showed less degradation .Glucose showed higher degradation of hydrocarbon.

DISCUSSION:

The rate of biodegradation of crude oil by hydrocarbociastic organism isolated from crude oil sludge environment was assessed. The biodegraders which were *Pseudomonas spp*., *Micrococcus spp*., showed different abilities in breakdown of crude oil. The highest growth exhibited by *Pseudomonas spp*., was not surprising not only because it was isolated from oil sludge environment but also because it is known to possessed a more competent and active hydrocarbon degrading enzymes than other biodegraders(Robert et al 2000).

REFERNCES:

1)Kaustuvmani patowary ;Mohan Kalita 2 and Suresh Deka′* April 2015, Degradation of Polycyclic Aromatic Hydrocarbons (PAHS) employing biosurfactant producing *Pseudomonas aeruginosa KS3*,**.** Vol.14, Indian Journal Of Biotechnology.

2)Deepika Paramanik a and G. Rajalakshmi b, July –Sept-2013, Biodegradation of Petroleum Hydrocarbons Pollutants in soil using microbial Consortium, Volume -3 Issue-3. International Journal Of Plannt , Animal and Ennvironmental Sciences

3) Robert –A Kanaly *and Shigeaki Haryana, April ,2000, Biodegradation of High molecular weight Polycyclic Aromatic Hydrocarbons by Bacteria. Marine Bacteriology Institute ,Kamaishi Laboratories ,Kamaishi city , Iwade 026-0001,Japan.

4) P.Anna Joice 1* and R. Parthasarathi 2 , 2014, Optimization Of Biosurfactant production from Pseudomonas aeruginosa PBSC1, International Journal Of Current Microbiology and Applied Sciences. ISSN -2319 – 7706 Volume 3 , NO -9, Division Of Micobiology , Faculty Of Science .

5) Talat Yasmeen Mujahia , Abdul Wahab, 2015,) Isolation and Characterization of Hydrocarbons Degrading Bacteria from petrol Contaminated soil. Journal of Basic and Applied Sciences, Department of Microbiology ,University of Karachi -75270,Pakistan.