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Isolation, Screening and Identification of Biosurfactant producing Bacterial strains

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Abstract

Polyethylene plays an important role in packaging of food materials, goods, medicines, garbage bags and many more. But in today's scenario degradation of polyethylene became a great threat and important cause for environmental pollution. This study aimed to explore methods of biodegradation rate by physical, chemical and biological ways. Bacterial species RG2 was tested for its potential in using polyethylene as its sole carbon source. This microbe produced surface active compounds that enhance the degradation process surface treatment. Pretreatment of polyethylene strips with chemicals helps in faster degradation. Inoculation of surface treatment of polyethylene strips with bacterial species and addition of its biosurfactant proved to most efficient with greater weight loss of polyethylene.

Key words: Polyethylene; degradation; biosurfacatnt; pollution; weight loss.

Introduction

Biosurfactants are surface-active amalgams blended by a wide variety of microorganisms (Vimala *et al.*, 2016) Surfactants like wetting agents or petroleum derivatives lower the surface tension between two liquids or between a liquid and a solid. But, they are non- degradable leading to toxic effects. To solve this difficulty, eco-friendly microbes were discovered for biosurfactant property with diverse applications.

Though plastic and polyethylene are resilient and strong with wide applications, they seem to be non-degradable prominent to severe pollution issues by disturbing the whole ecosystem. Even their reprocessing is not followed widely due to economic viability.

Similar to plastic issues even the hydrocarbons like plastic, petrol, diesel spills seem to be the global environmental pollutant causing lethal pollution. Successful application of bioremediation technology solely

depends on in-situ application of microbial consortium with higher biodegradation capacity. (Mrinalini & Padmavathy 2015)

Biosurfactants are surface- active compounds synthesized by a wide variety of microorganisms. They are molecules that have both hydrophobic and hydrophilic domains comprising an acid, peptide cations or anions, mono/ di or polysaccharides and a hydrophobic moiety of saturated or unsaturated hydrocarbon chains or fatty acids. Due to their amphilic structure, biosurfactants increase the surface area of hydrophobic water-insoluble substances, increase the water bioavailability of such substances and change the properties of the bacterial cell surface. Because of their potential advantages biosurfactants are widely used in many industries such as agriculture, food production, chemistry, cosmetics and pharmaceutics.

There have been focuses on the recent hypotheses and experimental findings regarding the biodegradation of polyethylene. Ambika *et al.,* 2009 made a review on different approaches to enhance the biodegradation of polyolefin (Vimala and Lea 2016, Ambika *et al.,* 2009. Pre-treatment of the polymer using physical means prior to biodegradation have been found to enhance the process considerably. UV radiation was used as a pre-treatment by (Mahalakshmi *et al.,* 2012, Sowmya *et al.,* 2014, Mathew 2016)

Research works on biosurfactant its production, analysis and applications were also measured. There is ample research literature in the fields of polymer degradation and on the various aspects of biosurfactants such as its isolation, production, extraction from different microbes, its applications in heavy metal removal and biodegradation of hydrocarbons. However, the use of biosurfactant in polymer degradation is an inadequate area of study. The screening of biosurfactant producing microorganisms is generally carried out by monitoring parameters that estimate surface activity, such as surface tension, interfacial tension and ability to emulsify oils or hydrocarbons (Cooper & Zajic 1980, Revathi *et al.,* 2018)

Materials and Methods:

Collection of polymer sample:

Polyethylene films (LDPE) were purchased. PE films were cut in required size of approximately 3cmX 3cm and they were subjected to UV treatment for 96 h.

Collection of soil sample:

Soil samples were collected from the municipal dumpsite in Raichur. Total of about five soil samples were collected from five different dumpsites of same place (Raichur)

Isolation of microorganisms:

Enrichment procedure was used for the isolation of bacteria; polyethylene was used as sole source of carbon. Isolated bacteria were identified based on microscopic observation followed by biochemical tests. Isolated bacterial strains were grown separately on minimal salt broth incubated at 30°C on a rotary shaker for 5 days.

Screening technique for biosurfactant assay:

Preliminary test:

Phenol: H_2SO_4 method:

One ml of supernatant was added with 5% one ml phenol and 2-5 ml of conc H_2SO_4 was added carefully drop by drop until orange colour persisted which indicated the presence of lipids containing biosurfactant.

Biuret test:

This test was performed to detect the lipopeptide containing biosurfactant. Two ml of supernatant was heated at 700°C and then was mixed with one ml of 1 M NaOH solution. Few drops of 1% $CuSO_4$ was slowly added until violet/pink ring developed. Formation of violet or pink ring indicated the presence of lipopeptides containing biosurfactant.

Phosphate test:

Ten drops of 6M HNO_3 was added to 2ml of culture supernatant, and was heated at 70°C, 5% (W/V) ammonium molybdate was added to this mixture drop by drop slowly until the formation of yellow colour and then the yellow precipitate. This indicates the presence of phospholipids containing biosurfactant. (Kalyani *et al.,* 2014)

Secondary/ Confirmatory test:

Biosurfactant assay was determined for the pure culture of bacterial strain by different methods namely, Haemolysis, drop collapse, Oil spreading technique, Emulsification index, Penetration assay.

Haemolysis activity:

Biosurfactant assays of the isolate was evaluated by the haemolysis test on blood agar and the plates were incubated at 28°C for 7 days. Plates were observed for zone of clearance around the colonies.

Drop collapse method:

A clean glass slide was taken at the end of the slide indicator mixed oil drop was added, then 10 microliter cell free culture was added to the oil drop. After 2min the drop was collapsed indicating the presence of biosurfacatnt in the cell free suspension. (Singh & Sedhuraman 2015, Deepika & Kannabiran 2010 and Yousaf *et al.,* 2010)

Oil- spreading technique:

5ml of distil water was taken in the Petri plate, 2ml of crude oil (petrol), 1ml of olive oil was added onto the Petri plate and 1ml of culture filtrate was placed on the Petri plate at the centre of the oil layer. The presence of biosurfactant would displace (spread) the oil and a clear zone on the oil surface would be visualized under visible light and UV after 30 sec. The zone increased after 10min from 3mm to 5mm diameter which is also known as displacement activity (Vimal & Mathew 2016, Singh & Sedhuraman 2015).

Emulsification index/assay:

Emulsification assay was carried out using petroleum as described by Cooper *et al,* Singh & Sedhuram and Revathi *et al.,* 2018). 2ml of cell-free supernatant was taken in the test tube, 4ml of water and 6ml of petrol (hydrocarbon)was added and vortexed for 2min to ensure the homogenous mixing of the liquids to obtain maximum emulsification. After, 24-48h emulsification index (Ellaiah *et al.,* 2002, Revathi *et al.,* 2018, Singh & Sedhuraman 2015). The emulsification activity was observed after 24h and it was calculated by using the formula given below:

$$\frac{\text{Total height of the emulsion}}{\text{Height of the aqueous layer}} \times 100$$

Penetration assay:

This assay relies on the contact of two insoluble phases which leads to a colour change as described by Maczek *et al.,* 2007, Singh & Sedhuraman 2015). In this assay, the cavities of microplate were filled with 150 µl of hydrophobic paste made up of oil and silica gel. This separated paste was covered with 20 µl of oil. Then, 10 µl of red- staining solution i.e. safranin was added to 90 µl of the supernatant. The coloured supernatant was then placed on the surface of oil covered paste.

Results and Discussion:

Isolation and screening:

From five soil samples used, 08 bacterial colonies were identified and isolated and out of them 05 isolates showed growth and these isolates were purified and selected for the further studies.

Preliminary tests for biosurfactant production:

Phenol: H_2SO_4 method:

All the isolates showed the appearance of orange colour indicating production of glycolipids containing surfactants which is a positive result.

Fig: 1 Phenol: Sulphuric acid

Biuret test:

After the addition of NaOH and $CuSO_4$ to the bacterial supernatant, violet ring was observed in all bacterial strain which indicated that it has the lipopeptide containing surfactants producing capability except in RG1 which indicated negative for lipopeptide.

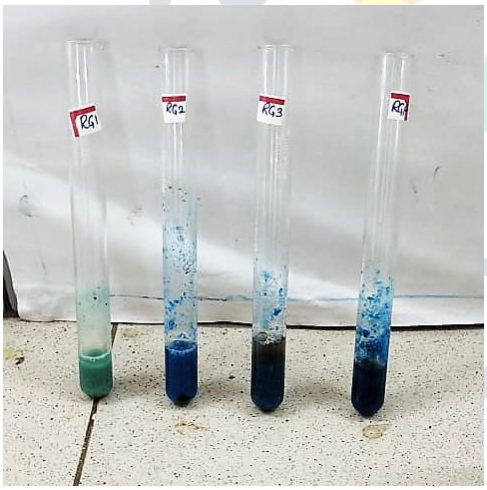

Fig: 2. Biuret test

Phosphate test:

After the addition of 5 % w/v ammonium molybdate solution drop by drop to the culture supernatant containing 6M HNO_3 white precipitate was observed. This indicates the presence of phospholipid containing surfactant producing capability.

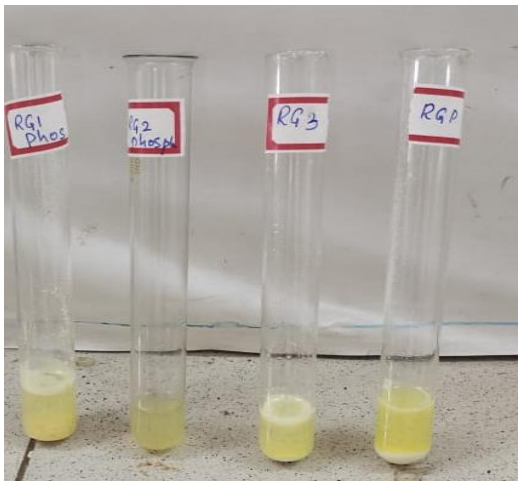

Fig: 3 Phosphate Test

Secondary/ Confirmatory test:

Haemolytic assay:

On blood agar, it showed a clear zone around the colonies using lysis of blood.

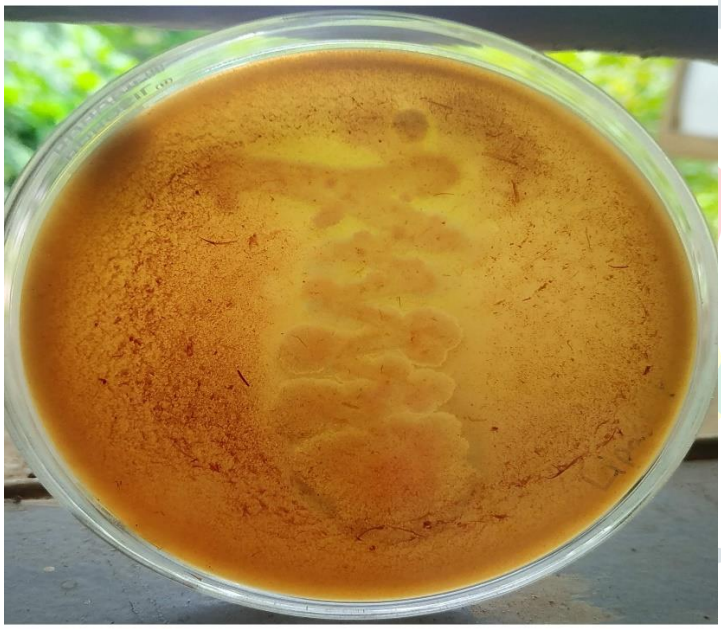

Fig: 4 Haemolytic assay

Drop collapse method:

In presence of surfactant, the culture supernatant drop spreads over hydrophobic surface as the interfacial tension between the droplet and hydrophobic surface is reduced. In contrast the droplet remains beaded or round in the absence of surfactant.

Fig: 5 Oil drop collapse

Oil spread method:

The presence of biosurfactant activity of the supernatant of the isolates was observed by the displacement / spread of oil formation of clear zone.

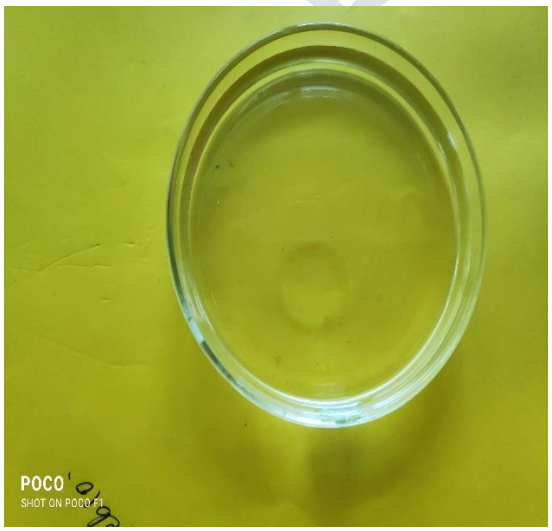

Fig: 6 Oil spread method

Emulsion index:

High emulsion index was observed in RG isolate and other bacterial isolates were RG1 to RGP*. The culture showed good emulsification activity was reported to be positive in RG1, the lowest emulsion activity was observed in RG1*.

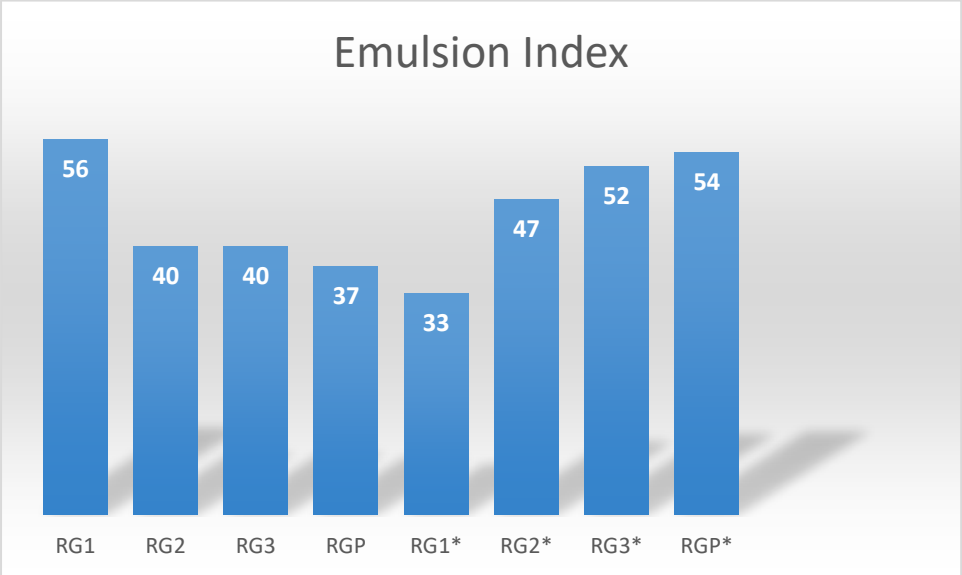

Fig: 7 Emulsion index

Penetration assay:

The presence of biosurfactant was confirmed since the hydrophilic liquid break through the oil film barrier into the paste. Silica is entering the hydrophilic phase and the upper phase changed from clear red to cloudy white within 10 min. The described effect relies on the phenomenon that silica gel is entering the hydrophobic phase from the hydrophobic paste much more quickly if biosurfactants are present. Biosurfactant free supernatant will turn cloudy but stay red.

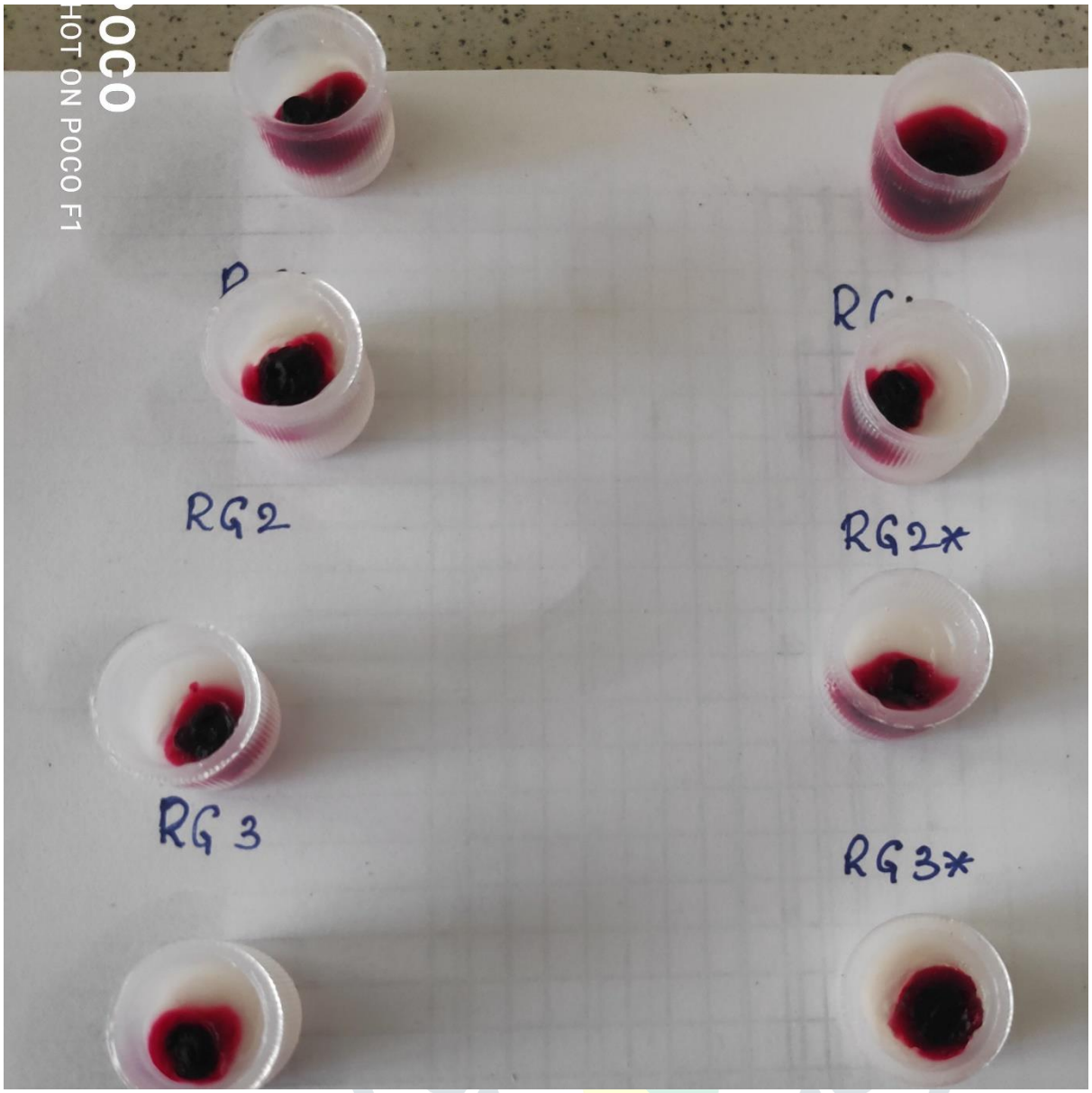

Fig: 8 Penetration assay

Conclusion:

It was determined that all the isolates have been isolated were screened and found to possess biosurfactants producing capabilities from good supporting medium. Biosurfactants are natural surface active agents produced by bacteria these products have shown to be efficient in process of microbial enhanced oil recovery and bioremediation in hydrocarbon contaminated environments. They possess potential applications in agriculture, pharmaceutical and cosmetic industries. They are more advantageous than the synthetic derived ones when considering their biodegradability, low density, low toxicity and better environmental compatibility.

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