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Isolation of Bioemulsifier Producing Organism for Degradation and Aromatic Compound

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ABSTRACT:

Biosurfactants are surfactants that are surface active compounds that reduce the interfacial tension between two liquids, or that between a liquid and a solid. Surfactants are organic compounds that contain both hydrophobic (head part of the surfactant) and hydrophilic (tail part of the surfactant) moieties. Thus surfactant contains both water insoluble i.e. water repellent group as well as water soluble i.e. water loving group.The bio emulsifier producing organism was isolated from the garage soil sample and it was found to be Gram positive, rod shaped.It possesses both catalase and oxidase enzymes as well as amylase enzyme as it hydrolysed starch. The isolated organism shows zone of hemolysis around the colonies on blood agar plate. The isolated organism shows positive drops collapse test. This confirms that the isolated organism is a bio emulsifier producer.The effect of substrate concentration on the growth of the microorganism was studied at various initial concentrations of phenol ranging from 50 ppm to 200 ppm at pH 7.

Keywords: Biosurfactants, *Corynebacterium*, Emulsification index ,drop collapse ,Folin-Ciocalteu.

Introduction:

Biosurfactants are surfactants that face active emulsion that reduce the interfacial pressure between two liquids, or that between a liquid and a solid. Surfactants are organic emulsions that contain both hydrophobic(head part of the surfactant) and hydrophilic(tail part of the surfactant) halves. therefore surfactant contains both water undoable i.e. water repellent group as well as water answerable i.e. water loving group. Biosurfactants also face active emulsion like chemical surfactants but unlike the chemical surfactant, biosurfactants are synthesised by microbes like bacteria, fungi and incentive.Biosurfactants are organic compounds belonging to various classes including glycolipids, lipopeptides, fatty acids, phospholipids, neutral lipids and lipopolysaccharides. Biosurfactants comprise the parcels of dropping face pressure, stabilising mixes, promoting raging and are generally non-toxic and biodegradable.Bio emulsifiers are amphipathic polymers while biosurfactants face active chemicals produced by large numbers of bacteria, incentive and fungi.

In the literature, the terms biosurfactant and bioemulsifier are considered interchangeable, but although all bioemulsifiers are considered biosurfactants, not all the biosurfactants produce stable emulsions. Biosurfactants can reduce the surface tension between two liquids, and bioemulsifiers induce a dispersion of undissolved material throughout the liquid, by formation and stabilisation of droplets of the dispersed phase.Microbial biosurfactants are classified by their chemical composition and their microbial origin. Emulsification is the process of dispersing one immiscible liquid into another immiscible liquid is called emulsification. An emulsifier is a substance that stabilises an conflation. It's also called emulgent. Surfactants similar to cleansers are one type of emulsifier.

• Some examples of Emulsions-

▪ Egg thralldom - contains the emulsifying agent lecithin.

▪ Adulation oil painting and water admixture.

▪ Mayonnaise- a conflation of oil painting in water.

Biosurfactants are low molecular weight face-active composites extensively produced by bacteria, incentive and fungi. These amphiphilic molecules reduce the face pressure at the air/ water interfaces and the interfacial pressure at oil painting/ water interfaces numerous microorganisms similar as *E.coli, Acinetobacter junii, Aeromonas caviae, Pseudomonas fluorescens, Klebsiella pneumonia, Bacillus spp* synthesise an extracellular polymeric substances called as bioemulsifier. In addition to having many unique properties and applications, the ability to exhibit biosensor actives (surface- active) properties, which lower the surface tension and the interfacial tension of their growth media, allow biosurfactants to play diverse key beneficial roles.

Currently, the major request for biosurfactants is the petroleum assiduity, in which these composites can be used in the remittal of canvases tumbles, the junking of oil painting residue from storehouse tanks, microbial- enhanced oil painting recovery, and the bioremediation of soil and water.

Application of bio emulsifier :

The properties⁄ applications of biosurfactants includes excellent detergency, emulsification, foaming, dispersing traits, wetting, penetrating, thickening, microbial growth enhancement, metal sequestering and resource recovering (oil) which make surfactants replace some of the most versatile process chemicals. Biosurfactants are promising natural surfactants that offer several advantages over chemically synthesised surfactants, such as lower toxicity, biodegradability and ecological acceptability.

Material and method

Soil collection

The diesel, petrol, kerosene containing soil used in this study were collected from local petrol bunk and oil shops and stored separately in bottles before being added aseptically to the growth medium.

Enrichment of organism

1gm of soil sample inoculated into minimal medium broth containing 2% tributyrin as a carbon source,incubated at 37^0C at 150 rpm for 2 to 3 days.

Isolation of bacteria

Microorganisms isolated by striking a loopful of enriched culture on minimal agar plate containing 2% tributyrin. Incubated the plate at 37^0C for 24 hr.

Identification and Characterization of isolates:

The two isolated bacterial strains were further characterised based on colony characteristics and different biochemical tests.

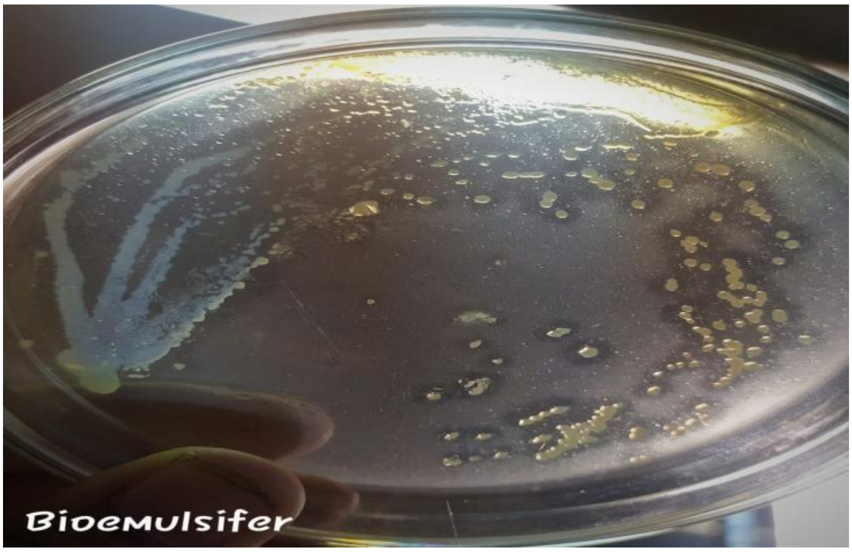


Fig: Bioemusifier producing colonies

A) Strain1-

Fig: Gram staining (Strain 1)

B) Strain2-

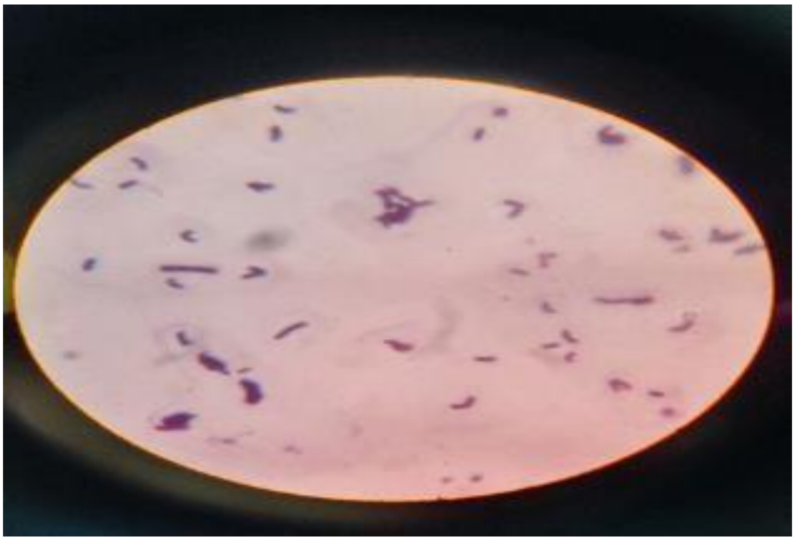


Fig: Gram staining (Strain 2-Gram positive rods)

Table: Biochemical tests of strain 1 and 2

Sr.no	Test	Strain 1	Strain 2
1	Gram character	Gram negative rods	Gram positive rods
2	Oxidase	+	+
3	Catalase	+	+
4	Glucose fermentation	+	+
5	Nitrate	+	+
6	Hydrogen sulphide	+	+
7	Mannitol	+	-
8	Na+ utilization test	+	+
9	Organism	*Aeromonas spp.*	*Corynebacterium spp.*

(+Positive , -Negative)

Test for screening Bio- emulsifier activity:

1. Hemolytic activity:

Several studies reported the impossibility of biosurfactant production without haemolytic activity, as haemolysis has been referred to as a determination of biosurfactant.An ideal assay for determining surfactant production, as it is commonly claimed that biosurfactants cause lysis of erythrocytes, and this is usually the principle adopted in the haemolysis assay for biosurfactant determination.Based on the biosurfactant-producing capacity in liquid medium,it was found to be associated with haemolytic activity, the use of blood agar lysis (haemolysis assay) was considered and recommended as appearing to be a good primary (and in few cases, secondary) screening criterion/method for biosurfactant production, by surfactant-producing microbial strains, and regarded as indicative of biosurfactant production.

Materials:

1) Blood agar containing petri plate-

2) Culture suspension-

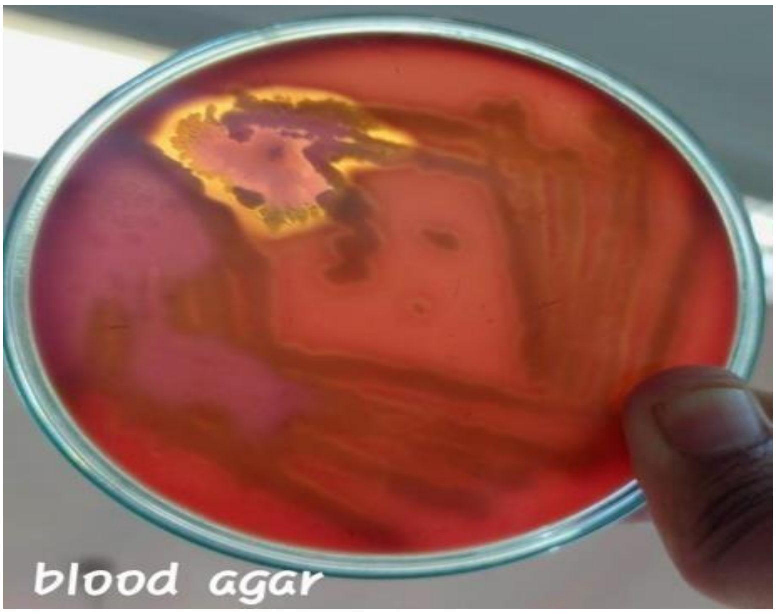


Fig: Hemolysis on Blood agar plate

Observation:

After incubation at 37^0C for 24 hours the colonies showed hemolytic activity.

Result:

The isolated organism shows a zone of hemolysis around the colonies on the blood agar plate.

2. Drops collapse assay:

This assay relies on the destabilisation of liquid droplets by surfactants. If the liquid contains surfactants, the drops spread or even collapse because the force or interfacial tension between the liquid drop and the hydrophobic surface is reduced.

Materials:

1) Paraffin oil,

2) Culture supernatant

3) methyl blue

4) Petri dish.

Observation:

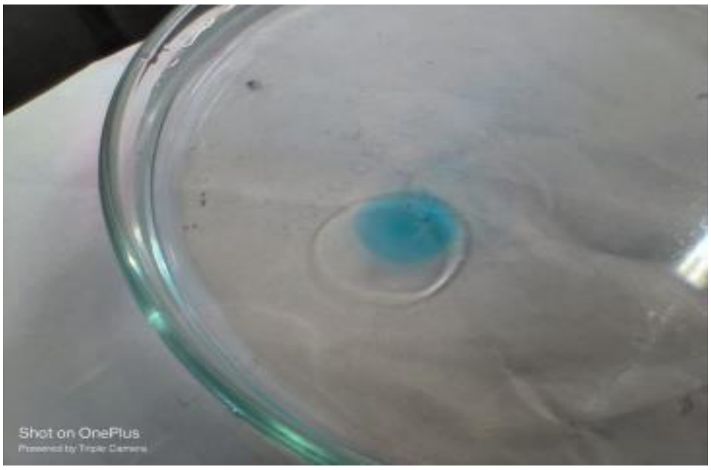


Fig: The oil drop get collapse after 10 minutes

Result:-The oil drop collapsed after adding the supernatant. This confirms that the isolated organism is a bio emulsifier producer.

Quantitative method:

1.Emulsification index-

The emulsification index test determines whether the biosurfactant has emulsifier property or not by calculating the ratio of the height of the stable emulsion layer and the total height of liquid formed after vertexing and leaving it for 24 h. Evaluating the emulsification capacity is a simple screening method suitable for a first screening of biosurfactant producing microbes .

Formula:

$$E24=\frac{Height\ of\ emulsified\ layer}{Total\ height\ of\ liquid\ colume}\times 100$$

Observations:

-Height of emulsifier layer (paraffin oil) = 2cm

-Height of emulsifier layer (petrol)=0.9 cm

Paraffin Oil **Petrol**

Fig: Emulsification index

Result: The emulsification index of paraffin oil is 38.4% greater than petrol.

Degradation of aromatic compound by using bioemulsifier producing organisms:

Bio degradation of phenol by isolated bio emulsifier organisms:

Phenol: Phenol is the common name of hydroxybenzene, an aromatic compound having one hydroxyl group attached to the benzene ring . Phenol has also been called carbolic acid, phenic acid, phenylic acid, phenyl hydroxide or oxybenzone. Phenol is the basic structural unit for a variety of synthetic organic compounds. It is a white crystalline solid which is soluble in most organic solvents. The industries like leather, paint, pharmaceutical, coking plant petrochemical, oil refinery, plastic, explosives, steel, pesticides etc and disinfectants use phenolic and its derivative compounds as their products and raw materials.

Nowadays, environmental pollution has become one of the major concerns of societies with the increase of industrial growth. Aromatic compounds are environmental pollutants that exist in different regions such as freshwater, sea and land. Phenol and phenolic derivatives are among the most well-known aromatic compounds that are hazardous due to teratogenic, toxic, carcinogenic and mutagenic properties, and their removal from the environment is important.

Materials:

Chemicals-

1) Phenol

2) Folin-Ciocalteu reagent

3) Diethyl ether

4) 20 % Sodium carbonate (Na_2CO_3)

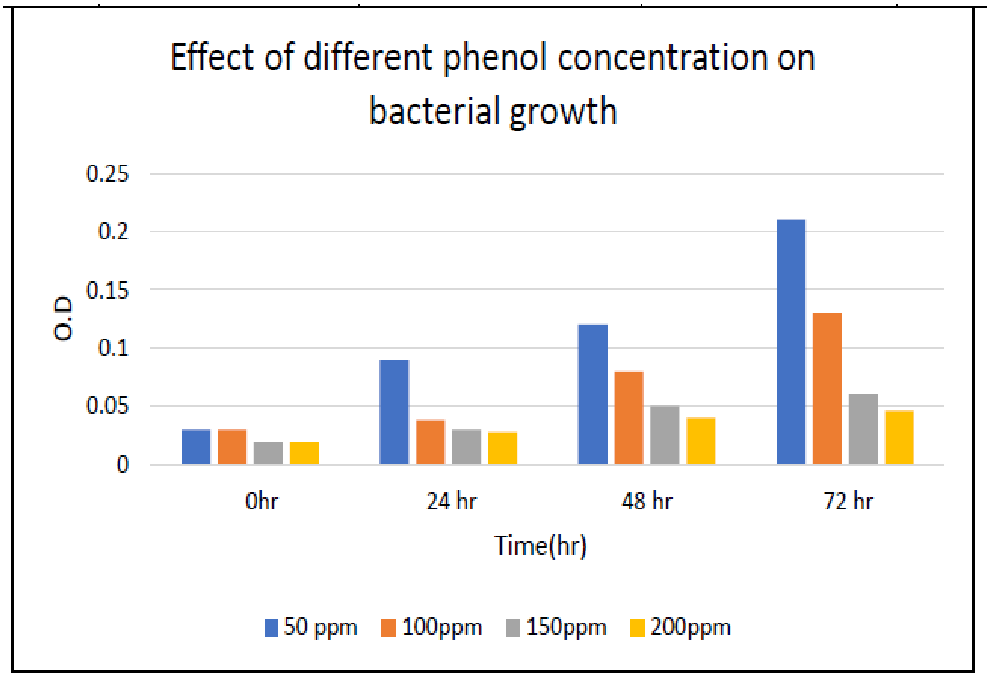
Other requirement-

1) UV spectrophotometer

2) pipette

Table for bacterial growth after 24 hr time interval-

Conc.of phenol	Absorbance at 600 nm (incubation time)			
	0hr	24 hr	48 hr	72 hr
50 ppm	0.03	0.09	0.120	0.210
100ppm	0.03	0.038	0.08	0.13
150ppm	0.02	0.030	0.05	0.06
200ppm	0.02	0.028	0.04	0.046



Result: From the graph it is found that the organism tolerates and utilises maximum phenol at concentration of 50 ppm.

Biodegradation of Phenol:

Phenol present in solids is oxidised by Folin-Ciocalteu reagent. This reagent is formed from a mixture of phosphotungstic acid, $H_3PW_{12}O_{40}$, and phosphomolybdic acid, $H_3PMo_{12}O_{40}$. After oxidation of the phenols, the reagent is reduced to a mixture of blue oxides of tungsten, W_8O_{23}, and molybdenum, Mo8O23. The blue coloration produced has a maximum absorption in the region of 600 nm to 700 nm and is proportional to the total quantity of phenol present.

Table :Bio degradation of phenol by Folin-Ciocalteu reagent assay

Sample	Distilled water (ml)	Folin-Ciocaulteu reagent (ml)	20% Sodium carbonate (ml)	Incubation time
0.1	O.4	1.5	0.5	30 min

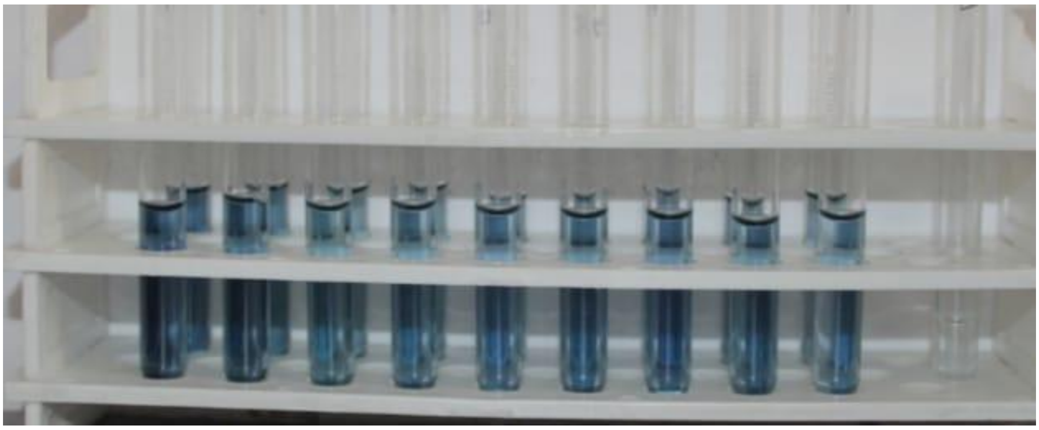


Fig: Folin-Ciocalteu reagent assay

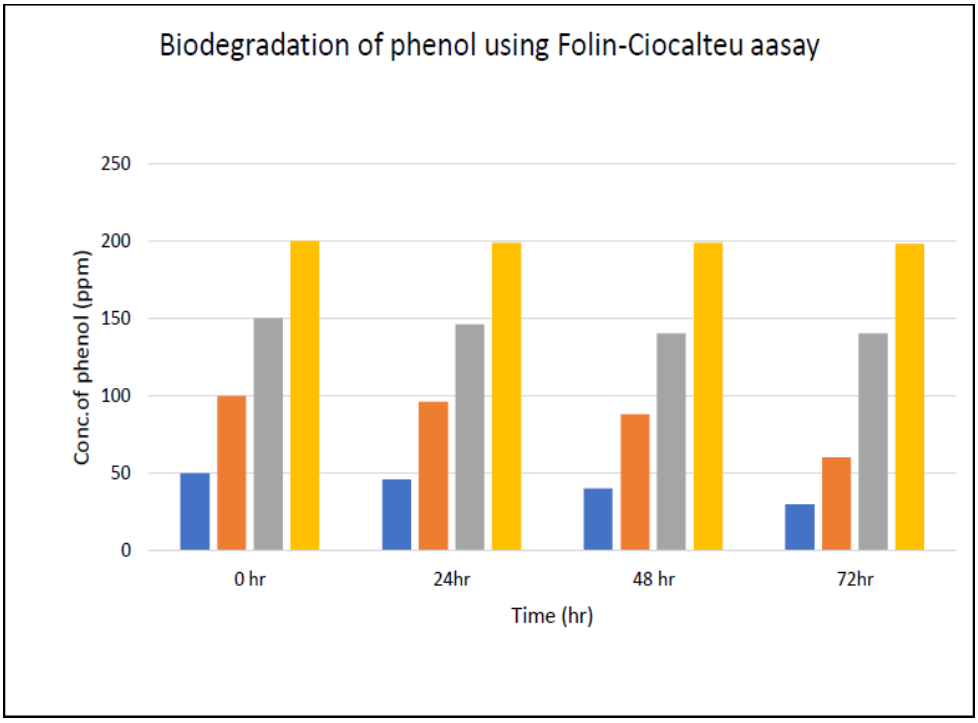


Fig: Bio degradation of phenol by *Corynebacterium spp.* using Folin-Ciocalteu assay.

Result:

It was observed that with increase in the concentration of the phenol, the substrate confers its toxicity on the growth of microorganism which can be deduced with increase in concentration, the growth of the microorganism slows down. Corynebacterium spp. shows maximum biodegradation rate of phenol at 50 ppm concentration in 72 hours is 30 ppm.

Conclusion:

Isolation of a Bioemulsifier producing organism for degradation of aromatic compound phenol was successfully done.

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