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ISOLATION PRODUCTION AND POTENTIAL APPLICATION OF BIOSURFACTANT- A Review

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Abstract :
Naturally occurring surface-active compounds derived from micro-organisms are called bio-surfactants. Biosurfactant are amphiphilic compounds produced in living surfaces, mostly on microbial cell surfaces or excreted extracellular hydrophobic and hydrophilic moieties that confer on the organism the ability to accumulate between fluid phases thus reducing surface and interfacial tension. The ability to reduce surface tension is a major characteristic of surfactant. Surface-active compounds commonly used in industries are chemically synthesized. However, bio -surfactants have been paid increasing attention to replace the synthetic surfactants owing to their advantages such as biodegradability and low toxicity. Bio -surfactants can be produced with high yield by some microorganisms, especially Pseudomonas sp. These microorganisms can use the various renewal resources, especially agro industrial wastes, as the potential carbon sources. The use of bio surfactant for this purpose was found to be eco-friendly approach and also an alternative to conventional complex remediation. The current review summarizes research carried out on isolation and production of Biosurfactant and its potential applications and future scope of research in various fields.

KEYWORDS:-Biosurfactants, Emulsification, Application, Vegetable oil refining, Toxicity, Biodegradability

INTRODUCTION:-

Biosurfactant can be defined as surface active biomolecules produced by micro-organism. Biosurfactant are biologically produced surfactants which are naturally produced by bacteria, fungi and yeast. Due to their unique properties like specificity low toxicity and surface active biomolecules have attracted wide interest. Biosurfactants are amphiphilic biological compounds produced extra cellular or as part of cell membranes by a variety of yeast, bacteria and fungi from various substance including sugars, oils and wastes. Bio-surfactant have several advantages over synthetic surfactant such as higher biodegradability, lower toxicity, good biocapability with eukaryotic organism, effectiveness at wide range of tempter, pH, salinities synthesis under user friendly conditions.

The bio-surfactant are complex molecules covering a wide range of chemical types including peptides fatty acids, phospholipids, glycolipids, antibiotics, lip peptides etc. Biosurfactant lead to an increasing interest on these microbial products as alternatives to chemical surfactants [1] It has been focused that improving the method of biosurfactant production and characterizing the major properties of the biosurfactant are highly important in the commercial application of biosurfactant. This review includes the factors influencing biosurfactant production, potential industrial application and future research needs.

PRODUCTION AND POTENTIAL APPLICATION OF BIOSURFACTANT

B. Anandaraj et.al [2] carried out research on ISOLATION AND PRODUCTION OF BIOSURFACTANT PRODUCING ORGANISM FROM OIL SPILLED SOIL.

The isolation of biosurfactant producing bacteria, the sample was collected from automobile workshop, where the oil spilled in the soil.

The isolated colonies were tested for their bio-surfactant production by two methods.

Oil Spreading Technique

2) Blood Haemolysis test

The screened bio-surfactant producing organism was then characterized by using different tests. They are Gram staining, Motility Test, Indole Test , Methyl Red Test, Voges-Proskauer Test, Citrate Test, Spore staining, Starch hydrolysis, Casein hydrolysis, Gelatin hydrolysis, Lipid hydrolysis, Gelatin liquefaction Test, OxidaseTest, Catalase Test.

Results from the biochemical analysis were used to find the closest match with known bacterial genus and to assign the bacterial signature according to Bergey`s manual. Extraction of biosurfactants was carried out. The culture was incubated at 25^0C for 7 days with shaking condition. After incubation the bacterial cells were removed by centrifugation at 5000 rpm, 4^0C for 20 minutes. The supernatant was taken and the pH of the supernatant was adjusted to 2, using 1 MH_2SO_4. Now add equal volume of chloroform: methanol (2:1). This mixture was shaken well for mixing and left Overnight for evaporation. White colored sediment was obtained as a result i.e., the "Biosurfactant''.

The biosurfactant produced were characterized by using TLC plates and growth of the biosufactant producing organism using different carbon sources was measured.

They discussed that biosufactant producing organisms were isolated from the soil spilled soils of automobile workshop [3] Lai also workshop soil for isolation of the biosufactant producing organisms. In their study among the five isolate obtained from soil. The culture (C3) was found to produce the biosurfactant. This was screened by the oil displacement method. This method was also followed by KingsieyUrum et.al.[4]. The culture C3 also showed beta haemolytic activity on blood agar plate. The culture producing beta haemolysis was able to produce biosufactant. It correlates with the studies of Rashedi, et al.[5]. The biosufactant producing organism was found to be Pseudomanas spieces [6].

The biosurfactant produced were characterized by using TLC. The components were obtained as rhamnolipid i.e. a glycolipid also reported by earlier workers [5]. The molecular analysis was done by GC-Ms [7]. The components were separated into three and their molecular weight was 80.6710, 118.4550 and 126.4950.

A Tabatabaee et.al carried out research on Isolation of Biosurfactant producing Bacteria from Oil Reservoirs[8]. Isolation of biosurfactant producing bacteria was performed according to Francy et .al[9] but with there modifies: microorganisms from oil reservoirs[P (1,2,3,4,5,6) in west of Iran and E (E 0,1,2,3,4,E1P2,E3P4) and B (B1,B3)] were isolated. The isolates were tested by analytical methods –a) Hemolysis test and b)Emulsification test

Investigation of different factors was also carried out such as-a) Measurement of surface tension b) Investigation of salt different concentrations effect on surface tension c) Investigation of pH on surface tension d) Investigation of temperature effect on surface tension g) Kinetics of surfactant production

Biomass was measured by dry weight method. Surface tension and CMD were measured by Ring method. Emulsification was also estimated by Cooper and Goldenberg method [10].The biosurfactant produced by strain4 were extracted and characterized by TLC.

They discussed that haemolytic test is regarded as indicative of biosurfactant production. Identification of biosurfactant producing bacteria can be futher confirmed by measurement of surface tension. Reduction of surface tension measurement by isolated bacteria from Iranian crude oil reservoirs indicates the production of surface- active compounds. Similar results obtained by Banat et. al [11]

Salt concentration also affected biosurfactant production depending on its effect of cellular activity. Environmental factors and growth conditions a such as pH, temperature, agitation and oxygen availability also effect biosufactant production.

In this study, strains reduced surface tension in tasted temperatures but best temperature for selected strains was between 30-40^0C. According to investigation of kinetics of biosurfactant production results indicate that the biosurfactant biosynthesis from oil occurred predominantly during the exponential growth phase, suggesting that the biosurfactant is produced as a primary metabolite accompanying cellular biomass formation. Similar result obtained by Abu Ruwaida et.al.[12]

I.E. Klosowskaet.al studied on BIOSURFACTANTS – BIODEGRADABILITY, TOXICITY, EFFICIENCY IN COMPARISON WITH SYNTHETIC SURFACTANTS.[13]
In their work biodegradability, toxicity and efficiency is presented in order to compare their properties with synthetic surfactants. They collected various data on biosurfactants toxicity. As it can be seen from presented data, biosurfactants in comparison with synthetic surfactants pose haemolytic activity to human erythrocyte lower than cationic surfactants (CTAB, TTAB, BC) and anionic SDS. They do not pose detrimental effect to heart, lung, liver and kidney and interfere in blood coagulation in normal clotting time. Their inhibit luminescence of 50% of Vibriusfisheri in comparable or higher concentration than synthetic Surfactants.
Testing of Chemicals (301C Modified MITI Test), showed that biodegradation of biosurfactants starts immediately after cultivation. Moreover, biodegradability, expressed in a form of BOD/TOD (Biochemical Oxygen Demand to Total Oxygen Demand ratio), for sophorolipids after 8 days of cultivation has reached the level of 61%. Two others biosurfactants (surfactin and arthrofactin) examined were also as easy biodegradable as sophorolipids, while synthetic surfactants showed no biodegradability after 8 days [14]. Another research indicated that rhamnolipid biosurfactants are biodegradable under aerobic and anaerobic conditions.
Due to good physicochemical properties, low toxicity and good biodegradability biosurfactants are widely applied in environmental protection techniques. The efficiency of biosurfactant in removing crude oil was comparable to those of synthetic surfactant and much higher than for natural plant surfactant – saponin and synthetic Tween 60. In the case of removing hexadecane from sand biosurfactant was much more efficient than SDS and Tween 80Biosurfactants are also very effective in enhancing of oils biodegradation. Summarizing, one can state that biosurfactants display a lot of advantages over chemically synthesized surfactants.
They concluded that biosurfactants are less toxic and more easily biodegradable than synthetic surfactants. Moreover they are very effective in different applications including oil refining process, thus they are considered as very promising and prospective biotechnological product. Application of biosurfactants instead of synthetic surfactants in many branches allows to fulfil more and more restrictive environmental expectations and simultaneously ensures very good efficiency. Biosurfactants effectiveness in crude oil degumming was very high and it reaches upto 99%. The pH of washing solution did not affect efficiency of degumming significantly. Only at pH 13.6 the biosurfactant solution in concentration 5.0g/l reveal lower phosphorus removal than in other pH conditions.

Fakruddin Md studied on Biosurfactant: Production and Application [15] Biosurfactants have many advantages when compared to their chemically synthesized counterparts. Many biosurfactants are not affected by environmental factors such as temperature, pH and ionic strength tolerances.
The composition and emulsifying activity of the biosurfactant depends on the producer strain and the culture conditions, thus, the nature of the carbon source, the nitrogen source as well as the C:N ratio, nutritional limitations, chemical and physical parameters such as temperature, aeration, divalent cations and pH influence not only the amount of biosurfactant produced but also the type of polymer produced [16]

Biosurfactants have been used for various food processing application but they usually play a role as food formulation ingredient and anti-adhesive agents. In the cosmetic industry, due to its emulsification, foaming, water binding capacity, spreading and wetting properties effect on viscosity and on product consistency, biosurfactant have been proposed to replace chemically synthesized surfactants.
Biosurfactants play a major role in petroleum extraction, transportation, upgrading and refining and petrochemical manufacturing. Microbial surfactants are widely used in oil recovery in recent times.
They concluded that challenging issue as the composition of final product is affected by the nutrient, micronutrient and environmental factors. Guideline and regulation should be formulated for use of biosurfactants in different sectors. It is expected that in future, super-active microbial strains will be developed using genetic engineering for production of biosurfactants at industrial level using renewable substrates as raw material.

Need for future Research- The chemically synthesized surfactants are mainly petroleum based and are usually non biodegradable thus remain toxic to the Environment. Also these compounds may bio-accumulate and their production processes and by-products can be environmentally hazardous, due to this increasing awareness on the need to protect the ecosystem, environmental scientist have been tightening environment regulations thus necessitating an increased interest in surfactants of microbial origin as possible alternatives to chemically synthesized ones.
Biosurfactants have several potential applications in agriculture, medicine, petroleum industries.
The future of biosurfactants will be depend on their cost and applications. To reduce cost, the substrate in particular should be inexpensive like Molasses food wastes, such as whey, can be used as substrates. Other potential agricultural waste substrates could be straw, coconut shells, banana skins, starch wastewater from potato processing stillage from ethanol production and other fruit and vegetable processing wastes. Reactor design and process control developments are also required full scale production.

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