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PRODUCTION AND UTILIZATION OF BIOSURFACTANTS FROM RENEWABLE SOURCES: A REVIEW

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Abstract - *Surface-active compounds are commonly used in industries are chemically synthesized. However, biosurfactants have been paid increasing attention to replace the synthetic surfactants owing to their advantages such as biodegradability and low toxicity. Several microorganism are known to produce a wide variety of surface active substances, which are referred to as biosurfactants. Interesting examples for biosurfactant are rhamnolipid, galactolipids mainly known from Pseudomonas aeruginosa and Sophorolipids using Candida bombicola produce during cultivation of substrate like vegetable oils, sugars, glycerol or hydrocarbons. Used frying oils, due to their composition have great potential for microbial growth and transformation. Turkish corn oil and honey is used to produce sophorolipids. This study deals with these microorganism can use various renewal sources that leads to the greater possibility for economical biosurfactant production.*

Key Words: **Biosurfactant, Sorphorolipid, Rhamnolipid, Galactolipids, Pseudomonas aeruginosa, Candida bombicola, etc**

1. INTRODUCTION

Surfactants are an important class of amphiphilic molecules, which lower the surface tension between two phases by accumulating at the interface. With a total worldwide production of over 15 million tons per year [1], surfactants find their application in many different fields ranging from cleaning to applications in food-processing (usually as emulsifiers), enhanced oil recovery (EOR) or even the pharmaceutical sector. Due to their amphiphilic nature, these surface-active molecules facilitate the production of foam, emulsions and dispersions. Originally, surfactants were exclusively produced from renewable resources like plant oils or animal fat; however, the majority of available surfactants today are derived from petro-chemical sources [1]. Most surfactants used today are only partially or slowly biodegradable, and as such, they contribute to environmental pollution. Additionally, the production of 'ordinary' surfactants depletes the world's non-renewable petrochemical resources. To address this issue, the production of surfactant molecules should be achieved by utilizing renewable substrates.

Biosurfactant: One approach is the biotechnological production of microbial surfactants. These biologically produced molecules are generally reported to have less impact on the environment than conventional surfactants, since they are usually much better biodegradable and show less toxicity than synthetic surfactants. Some of these microbial surfactants also show excellent physico-chemical properties and hold several advantages over synthetic surfactants, like constant effectiveness over a wider range of pH and temperature.. All biosurfactants comprise at least one hydrophilic and one hydrophobic part due to their amphiphilic character. The molecular structure often also contains several hydrophobic and corresponding hydrophilic parts. The hydrophobic part usually comprises saturated or unsaturated fatty acids, hydroxyl fatty acids, or fat alcohols, with various other structures such as isoprenoids being possible as well. The chain length usually comprises between 8 and 18 carbon atoms. The hydrophilic part may be made up of either structurally relatively simple ester, hydroxyl, phosphate, or carboxyl groups, or of carbohydrates—such as mono, oligo, or polysaccharides—peptides or proteins. Many anionic and neutral biosurfactants are known. Cationic biosurfactants, in contrast, have been described extremely rarely, probably because they have a toxic effect, just like cationic surfactants in general. Within the biosurfactants, the glycolipids form the greatest share, with the non-sugar component, the aglycone, being highly versatile. These structures are particularly interesting since many biosurfactants exhibit high efficiency at concurrently good biological degradability. They can also be produced from renewable resources.

Generally, biosurfactants are assigned the following properties beneficial for industrial use:

- Great structure diversity
- Beneficial surfactant properties
- Low eco-toxicity
- Antibiotic or bioactive effects

- Complete biological degradability
- Production from renewable resources

Thus, industrial product development of biosurfactants is limited to some few biosurfactants, including spiculisporic acid, sophorolipids, rhamnolipids, and mannosylerithritollipids. Below, the best-known biosurfactants and, using some examples, the structural diversity and potential of microbial biosurfactants, in general, are illustrated based on selected structures.

TYPES OF BIOSURFACTANTS

LOW-MOLECULAR WEIGHT BIOSURFACTANTS:- Usually, low-molecular weight biosurfactants are glycolipids or lipopeptides, but may also belong to the groups of simple fatty acids and free phospholipids. The best-examined glycolipids that reduce surface tension are acylated disaccharides with long-chain fatty acid or hydroxyl fatty acid residues. Lipopeptides comprise a peptide moiety that is synthesized by non-ribosomal peptidsynthases, linked to fatty acid or hydroxyl fatty acid residues.

FATTY ACIDS AND PHOSPHOLIPIDS:- Some bacteria and fungi form free fatty acids or phospholipids when growing on *n*-alkanes .Fatty acids can be produced by microbial oxidation of alkanes. The strongest reduction of surface and interface tensions is achieved by fatty acids with chain lengths of C12–C14. In addition to un-branched fatty acids, many more complex microbial fatty acids have been described that exhibit hydroxyl groups or other alkyl residues. Some of these complex fatty acids, such as corynomycol acids are strong surfactants.

Phospholipids are the main part of microbial membranes and are usually not present in an extra- cellular form. However, *Acinetobacter* sp. HO1-N secreted extracellular phospholipid vesicles were formed when growing on hexadecane.

Rhodococcus erythropolis DSM 43215 also excreted phosphatidylethanolamines that lower sur- face tension and occur at growth on *n*-alkanes. Phosphatidylethanolamine is one of the most common phospholipids other than phosphatidylcholine and usually one of the main components of bacterial membranes.

GLYCOLIPIDS:- Glycolipids comprising mono or oligosaccharides as well as lipid moieties form the most important group of low molecular weight biosurfactants. The saccharide part can comprise glucose, mannose, galactose, galactosesulfate, glucuronic acid, or rhamnose moieties. The lipid moiety comprises either saturated or unsaturated fatty acids, hydroxyl fatty acids or fat alcohols. The four biotechnologically important groups of microbial glycolipids are rhamnolipids, sophorolipids, trehaloselipids, and mannosylerytitollipids.

TYPES OF GLYCOLIPIDS

RHAMNOLIPIDS: *Rhamnolipids* are mainly known from *Pseudomonas aeruginosa* and comprise one or two α-l-rhamnose units, linked via *O*-glycosidic linkage to one or two 3- hydroxyl fatty acid moieties. Natural rhamnolipids are present as mixtures of different congeners. The chain length of the 3-hydroxyl fatty acids varies between 8 and 16 carbon atoms, with 3-hydroxyl decanoic acid being predominant in *P. aeruginosa* and 3-hydroxyl tetradecanoic acid in *Burkholderia* species. The best-known rhamnolipid congener, being the α-l-rhamnopyr- anosyl-α-l-rhamnopyranosyl-3-hydroxydecanoyl-3-hydroxydecanoate, is displayed in Figure 1.2. A rare rhamnolipid with three hydroxyl fatty acid parts has been described only for *Burkholderia plantari.*

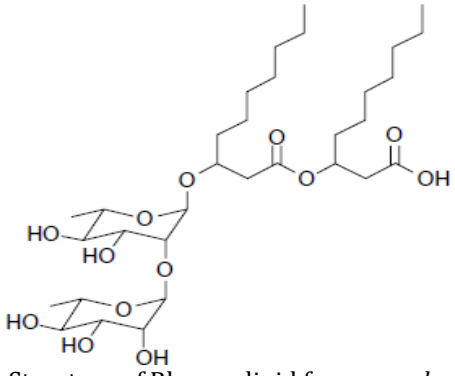

Fig -1: Structure of Rhamnolipid from *pseudomonas aeruginosa.*

SOPHOROLIPID: *Sophorolipids* contain the disaccharide sophorose and may be present in two forms, the lactonic form and the open acid form.There are many sophorolipid structures that have been mainly described for *Candida bombicola* (teleomorph *Starmerella bombicola*) and *C. apicola*. Predominantly, the hydrophobic part comprises a glycosidically bound 17-hydroxyoleic acid that is usually connected lactonically with the 4″ position of the sophorose, as well as acetyl residue in the 6′ and 6″ positions.

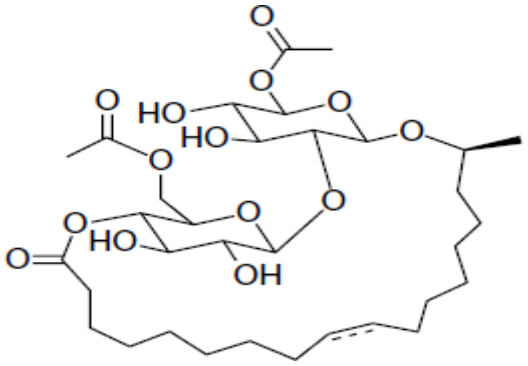

Fig -2: Sophorolipid from *candida bombicola*

MANNOSYLERYTHRITOLLIPIDS (MELs): comprise 4-*O*-β-d-mannopyranosyl-d- erythritol in their carbohydrate moiety, which may display diverse acylation patterns. The chain lengths of the acyl group vary considerably. MELs are mainly known from yeast species such as *Candida* and *Pseudozyma* (formerly *Candida*) and the closely related *Ustilago maydis.* The typical MELs with the main components of the mixtures usually being MEL-A and MEL-B. Regarding the application potential, MELs are among the most promising glycolipids. One of the reasons for this is their suitability for pharmaceutical applications.

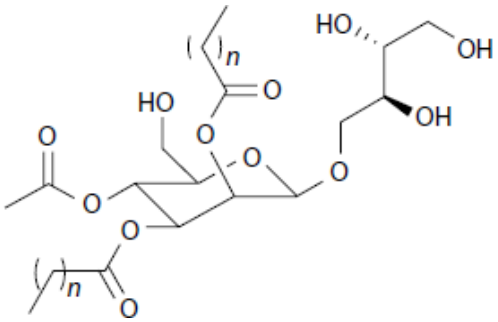

Fig -3: Mannosylerythritollipids (MELs) from *pseudozyma.*

LIPOPEPTIDE: Microbial lipopeptides are cyclic peptides that are acylated with a fatty acid. They are secreted into the growth medium by various microorganisms, including Gram-positive species, such as *Bacillus*, *Lactobacillus*, and *Streptomyces*, and Gram-negative species, such as *Pseudomonas* and *Serratia*. The natural lipopeptide that was first discovered was surfactin by *B. subtilis.*

SURACTIN*: The surfactin* from *Bacillus subtilis* is a cyclic lipopptide comprising seven amino acids and different 3-hydroxyl fatty acids. The main component is the 3-hydroxyl-13- methyl-myristin. Surfactin is a very good surfactant that also has antibacterial properties .It is synthesized by a linear, non-ribosomal peptide synthase, the surfactin synthase. When dissolved, it shows a characteristic saddle-like conformation that is essential for the wide bioactive range of surfactin . In addition to surfactin, *B. subtilis* produces two other lipopeptides as well, specifically Iturin and Fengycin.

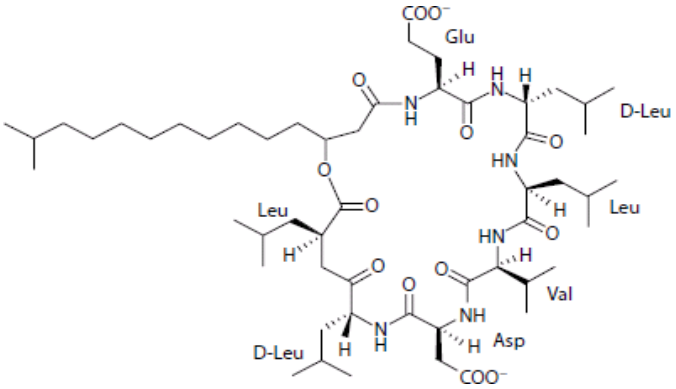

Fig -4: Surfactin from *B.subtilis.*

POLYMYXINES: It is a group of cationic, branched, cyclic dekapeptides. The polymyxines A–E have been insulated from different strains of *Bacillus polymyxa* .Polymyxine B is a decapeptide with eight amino acids forming a ring and linked to a branched fatty acid. The lipopeptid gained a certain importance as an antibiotic.

VISCOSIN: It is from *Pseudomonas fluorescens, P. libanensis*, and *P. viscosa* is a cyclic lipopeptide that reduces surface tension. The structure contains hydrophobic amino acids, linked to a fatty acid.

POLYMERIC BIOSURFACTANTS: Polymeric biosurfactants have the ability to alter the rheological properties of aqueous solutions at low concentrations. Therefore, these biopolymers are used as thickeners and to stabilize emulsions, dispersions, and suspensions in aqueous systems. Polymeric biosurfactants are high-molecular-weight biopolymers with properties like high viscosity, high tensile strength, and resistance to shear. It is because of these properties that they have found a variety of industrial uses in pharmaceuticals, cosmetics, and food industries. Emulsan and biodispersan are the best examples.

Table -1: Low cost substrate for the production of biosurfactant

Low cost or waste raw Material	Biosurfactant Type	Producer Microbial strain
Soyabean oil Refinery waste	Rhamnolipids	Pseudomonas aeruginosa AT10
Turkish Corn Oil	Sophorolipids	Candida bombicola ATCC 22214
Vegetable oil	Glycolipid	Pseudomonas fluorescens
Sunflower oil	Lipopeptidde	Serratia marcescens,Anthrobacter .
Petrol	Surfactin	Candida Tropicalis MTCC 230

PRODUCTION OF SOPHOROLIPIDS FROM CANDIDA BOMBICOLA USING TURKISH CORN OIL

Sorpholipids types and properties: Sorphorolipids have lower surface tension (72.8 mN/m to 40-30 mN/m) even in presence of salt and wide temperature range. They are suitable for dynamic appliations such as spray on coating and cleaning. Sorpholipids are good wetting agents due to their ability to decrease the contact angle of water on polyvinyl chloride from 110° C to 80° C at a minimal concentration. They are low foaming surfactant even at higher concentration.

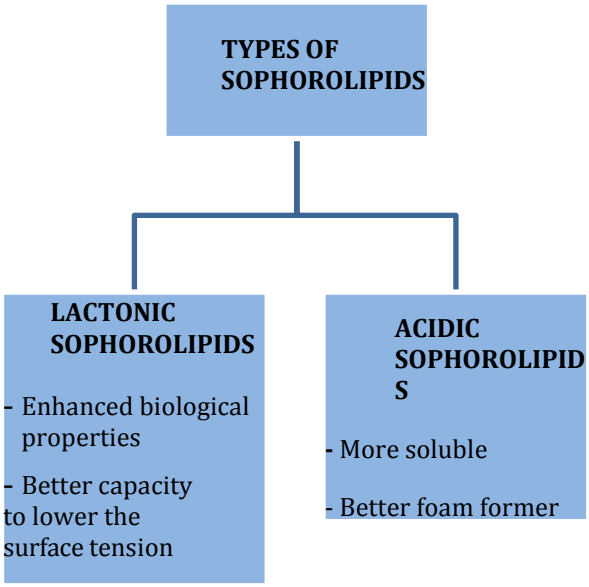

Fig -5: Types of Sophorolipids

By standard manometric respirometry and stable metabolite studies sorpholipids were demostrated to be readily biodegradable. Aquatic toxicity of sorpholipids is 10 fold less

compared with conventional surfactants. Sorpholipids is ideal component for hard surface cleaning products and dishwashing agents. Among the biosurfactants, sophrolipid are extra cellular surface active glycolipids that are produced by the cells of Candida bombicola, Candida apicola, Candida bogorenis. This article is related to the ability of Candida bombicola to produce sophorolipids using Turkish corn oil and honey. Shake flask experiment were carried out both with and without the addition of Glucose as the second carbon source. The organism produce organism under both condition but higher production was obtained when corn oil was combined with glucose.

The 3L bioreactor was operated in batch mode using corn and glucose. When all the glucose was consumed ,1/4th of the broth was pumped out and was replaced by freshly prepared medium containing 10% (w/v) of cheap market honey as the sole carbon source.

Raw material: Turkish corn oil

Organism: The yeast Candida bombicola ATCC 22214.

Method: Shake flask experiment with and without glucose as second carbon source.

Experimental Procedure

1)shake flask experiment:- The ph medium adjusted with phoshoric acid. Corn oil and glucose were autoclaved separately and added to the sterlized media.

Table -2: Cultivation conditions for shake flask experiments.

Chemicals and parameter	Two carbon sources (glucose+ corn oil)	One carbon Sources (corn oil)
Yeast extract (g/l)	10	10
D-glucose(g/l)	100	None
Corn oil (g/l)	100	100
Urea (g/l)	1	1
PH	3.5	3.5
Temperature	25	25
Stirring rate (rpm)	100	100
Total volume	200	200

Preparation of Pre culture and Inoculum for batch bioreactor: Pre culture- Preapred using 200 ml production medium .Two loops of organism taken from agar slant culture and then it incubated at 25 C and 100 rpm for 2 days. Inoculum- 2 ml of preculture added with 200 of production to prepared inoculum and incubated it at 25 c for 24 hour. In this study two stages were required as, Stage 1:- During batch bioreactor cultivation ,14 sample were taken from glucose and biomass determination .First appearance of SLPs was detected by TLC on 14 hour of Cultivation.It appaered as abrownish yellow precipitate. This was just after the first sharp decrease of dissolved oxygen concentration. The second sharp decrease in dissolved oxygen concentration around 57 hour and production of SLPs detected in bioreactor at 60 hour. A dens yellowish brown layer formed at the bottom of bioreactor.After 60 hour of fermentation samples were taken into 15 ml of weighe graduated eppendorf tubes, centrifuged and volume of SLP layer formed.These indicate SLP production has direct effect on metabolic activity and the production could be followed by change in dissolved oxygen concentration. Fig 6 shows the change of glucose consumption along with biomass production during batch reaction cultivation.

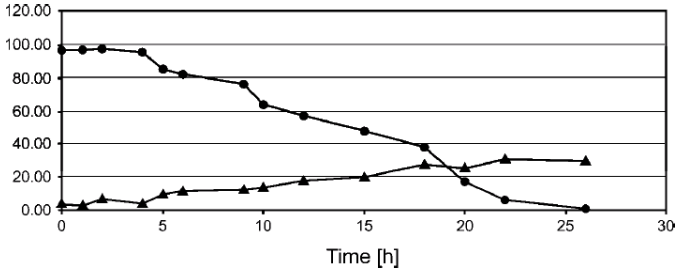

Fig -6: Change of glucose consumption along with biomass production during batch reaction cultivation. ● biomass [g/L]; ▲ glucose [%].

Stage 2:- 3L L biorector was started in batch mode, when dissolved oxygen increased to above 80% on day 4 , ¼ of broth was pumped out and replaced by honey (*bombicola*) as second carbon source. Sterilized corn oil fed on first 5 days to turn honey into co-substrate. For next feed changes in dissolved oxygen (DO) monitered. This stage lasted for 333 hours and had to ended on day 13 because of high viscosity of broth due to which stirring was difficult. Dissolved oxygen was 35% at the end, organism was still able to produce SLPs.

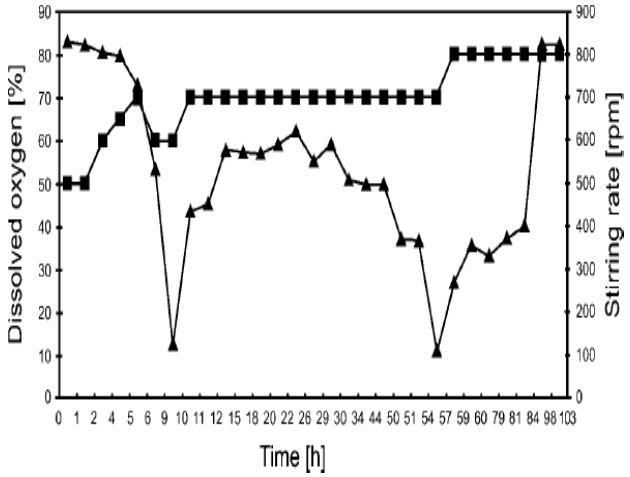

Fig -7: Change of the stirring rate along with the change of the % dissolved oxygen during the batch reactor cultivation. ▲ dissolved oxygen [%]; ■ stirring rate [rpm].

Table -3: Overview of sophorolipid production methods with respect to yield.

Characteristics	Production (g/L)	Yield (g/g)	Time (hr)	Volumetric productivity (g/l/hr)	References
Turkish corn oil with glucose	>400	>0.6	436	>0.9	Pekin et al. (2005)
Oleic acid fed batch with glucose	300	0.68	125	2.4	Rau et al. (2001)
Rapeseed oil with glucose	120	0.41	240	0.5	Felse et al. (2007)
Tallow fatty acid residue fed batch	200	-	54	3.7	Gao et al. (2012)

Applications of sophorolipids:

- Food
- Sources of Chemical Compound
- Bioremediation
- Cosmetics
- Plant Protection
- Enzyme Induction
- Nanotechnology
- Cleaning
- Medical

WASTE FRYING OIL FOR PRODUCTION OF RHAMNOLIPID USING PSEUDOMONAS AERUGINOSA

Rhamnolipid properties: Rhamnolipid potentially fulfil several function such as solubilization and uptake of hydrophobic molecules, contact to hydrophobic surfaces and intrusion into tissue due to hemolytic activity combined with a wide range of antimicrobial activity. They can be produced in relatively higher yields in comparably shorter time. They display higher surface activities and reduce the surface tension of water from 72 to 31 nM/m and critical micelle concentration between 20 and 225 mg/L in water. Rhamnolipid have high biodegradability. Also the aquatoxicity is 12 times lower than that of synthetic surfactant.

Raw material: waste frying oil

Organism: pseudomonas aeruginosa

Method: Isolation of candidate strains and random mutagenesis

Experimental Procedure: Soil samples (50g) were suspended in flasks with 200ml of sterile phosphate-buffered saline (PBS) stirred. Supernatant was inoculated into cetyltrimethyl- ammonium bromide (CTAB) methylene blue agar plates with 3% (m/v) glycerol. After the incubation of plate at 36° C for 7 day, selected strain was transferred to shaking flask with 60ml basal medium with 4% waste frying oil. For UV mutagenesis, 5 ml of culture broth subcultured for 3 days was inoculated into sterilized 60 mm culture plates and plates were set 10 cm away under 15 W UV light and exposed to radiation of wavelength 253.7 nm for 15,30,45,60,75 and 90 seconds respectively. Then culture solution was dilued with 10 ml sterilized 0.85% NaCl and 0.5 ml of dilution plated on CTAB plates. After 4 days culture in incubator (37°C), plates exposed to UV light for 60 seconds.

Growth conditions: Experiment were carried in 250 ml baffled Erlenmeyer flask containing 60 ml medium. Final pH of the medium was 6.8. The subsequent microbial cultures were conducted at 35° C on a reciprocal rotary shaker (140 r/min). Microorganisms were preserved at -20° C with 10% glycerol.

Bioreactor experiment conditions: Fermenter was performed with a 50-L aerated stirring fermenter of volume 25 L, operating with foam recycling system. The culture medium was inoculated with a 24 hour inoculum. The reactor was aerated at 0.6 vvm (volume/volume/min) for the first 48 hour and at 0.5 vvm after 48 hour culture. Temperature was 35° C and stirring speed was 300 r/min. The pH was regulated at 6.8 by adding 0.67 mol/L H_3PO_4 or 2 mol/L KOH.

Rhamnolipid characterization: Crude extracts were analyzed by thin layer chromatography (TLC) on silica gel G plates. Chromatograms were developed with chloroform/methanol/acetic acid (65/15/2) and visualized with an anthrone solution in sulfuric acid.

Outcomes: Gram reaction and cell morphology of more than 40 isolates were examined by the dark-blue halos on CTAB agar plates. Rhamnolipid productivity at 5 day incubation and gram reaction of the isolated strains are shown in table 4.

Table -4: Rhamnolipid productivity at 5 day incubation and gram reaction of the isolated strains

Isolate	Source of isolation	Ramnolipids concentration (g/L)	Gram reaction
zju.e1	Sludge at oil refinery plant	8.46	Negative
zju.e2	Sludge at oil refinery plant	7.74	Negative
zju.o1	Sewer sludge at university catering	11.61	Negative
zju.o2	Sewer sludge at university catering	10.25	Negative
zju.o3	Sewer sludge at university catering	9.97	Negative
zju.o4	Sewer sludge at university catering	9.24	Negative
zju.u1	Sewer sludge at university catering	12.47	Negative
zju.u2	Sewer sludge at university catering	9.83	Negative
zju.u3	Sewer sludge at university catering	10.47	Negative
zju.b1	Activated sludge in aeration tank	10.25	Negative

Zju.u1 has high yield of biosurfactant. Morphological observations and a battery of conventional taxonomic tests were performed to provide a generic description of zju.u1 later identified as *Pseudomonas aeruginosa.* To investigate if the initial carbon substrate of the waste frying oil (4%, v/v) is under the preferred condition, the rhamnolipid productivity of the zju.u1 was evaluated by adding waste oil at concentrations ranging from 2% (v/v) to 5% (v/v). The rhamnolipid productivities at two culture periods (2 d and 5 d) are listed in fig.

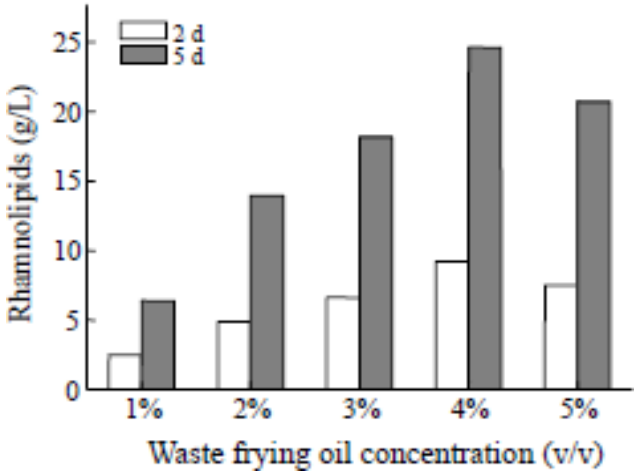

Fig -8: The various rhamnolipid productivity of naturally isolated strain zju.ul.

Fig.1 showed that the mutagenization did not alter its optimal frying oil concentration although the productivity of rhamnolipid increased by one fold. Moreover, the mutant was quite stable for at least 10 subcultures in producing rhamnolipids as shown in fig.2.

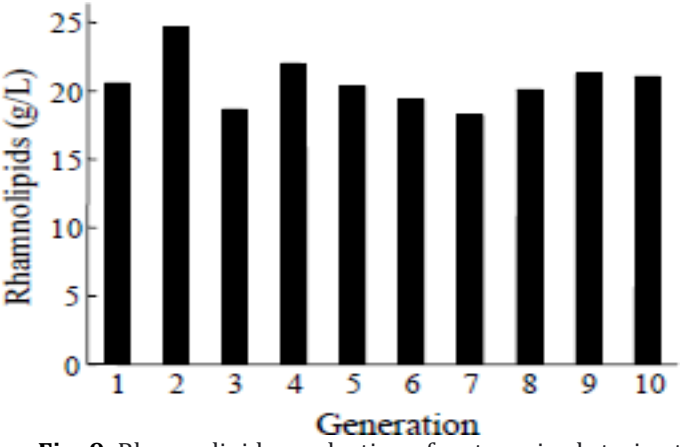

Fig -9: Rhamnolipids production of metagenized strain at different generations after 5 days culture in shaking flasks with the use of waste frying oil at 4% (v/v).

To investigate the feasibility of rhamnolipid production using waste frying oil, the mutant strain was transferred to culture in a bioreactor with a total volume of 50 L at an agitation speed of 300 (r/min). The waste frying oil was supplemented

in the medium at a concentration of 4% (v/v), or 35 g/L according to the density of the waste oil of 0.875 g/ml. According to Fig.4, bacteria increased in the initial 24 h and then reached a stationary phase while the oil substrate decreased drastically before 48 h. Most rhamnolipids were accumulated after the growth phase. At the end of the growth phase, rhmnolipids productivity reached 6.13 g/L. At the end of the stationary phase, productivity increased to 19.53 g/L.

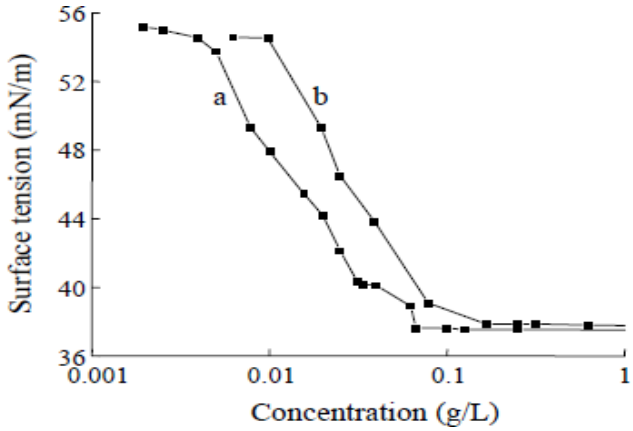

Fig -10: The surface tensions of the serially diluted solutions of the cell free culture broth (a) and the crude extract (b).

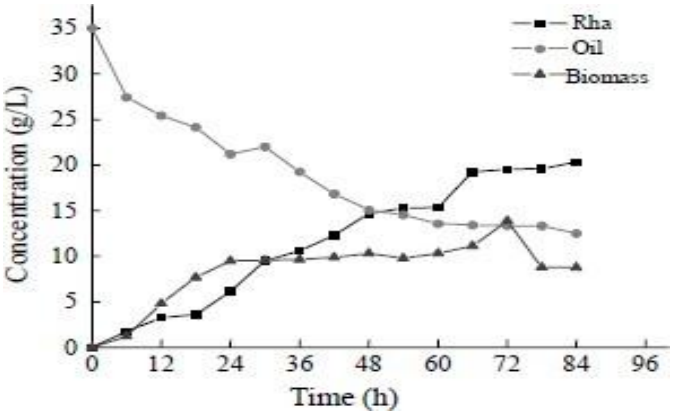

Fig -11: Time variation of growth, rhamnolipid production and substrate consumption during cultivation of Pseudomonas aeruginosa zju.u1M in a 50 L tank using an agitation speed of 300 r/min

FACTORS AFFECRING BIOSURFACTANT PRODUCTION:

The ability to produce a primary or secondary metabolite is regulated by the genetic makeup of the producer organism; however, nutritional and environmental factors have impact on the overall output of the metabolite.

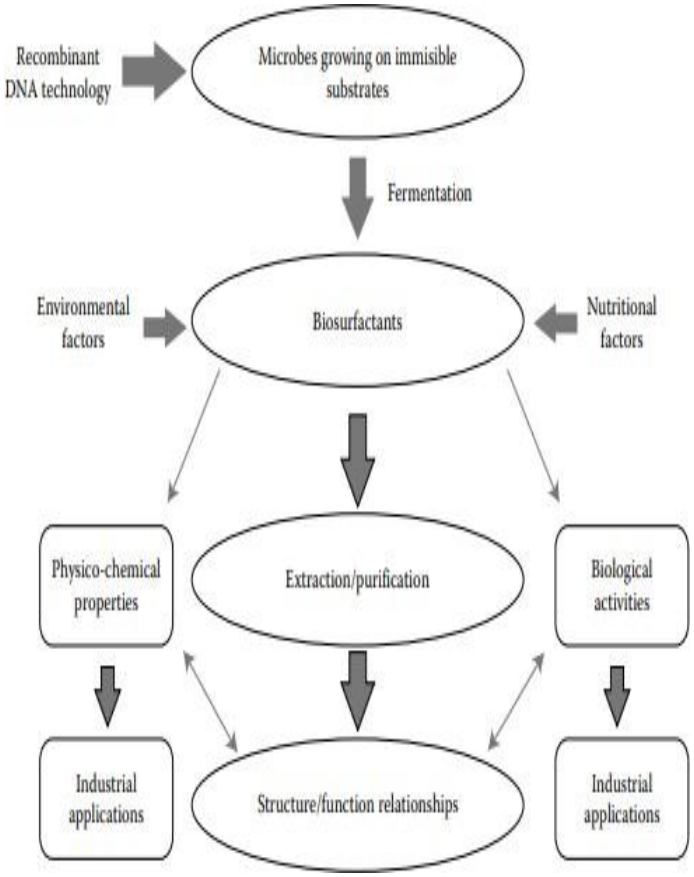

Fig -12: Diagram showing the chain of events in biosurfactant production.

Nutritional Factors

Effect of carbon source: The biosurfactant production was triggered when all the soluble carbon was consumed and when water-immiscible hydrocarbon was available in the medium. In Pseudomonas species, rhamnolipid production is regulated by the presence of n-alkanes in the medium while in Pseudomonas aeruginosa UW-1 higher production rates were achieved when grown on vegetable oil in comparison to liquid hydrocarbon (hexane). It was also shown that rhamnolipid production in P. aeruginosa under denitrification conditions with carbon substrates

palmitic acid, stearic acid, oleic acid, linoleic acid, glycerol, vegetable oil, and glucose was successful and was free from problems such as foaming and respiration limitation.

Effect of nitrogen sources: Nitrogen limitation is also important in the production of sophorose lipids. According to Kosaric and Sukan (2000), the production of biosurfactants by the genera Pseudomonas, Acinetobacter, and Torulopsis can be regulated by the C/N ratio. Among the inorganic salts tested, ammonium salts and urea were preferred nitrogen sources for biosurfactant production by Arthrobacter paraffineus (Cooper and Paddock 1983), whereas nitrate ions supported maximum biosurfactant production in P. aeruginosa (MacElwee et al. 1990), Rhodococcus sp. (Abu-Ruwaida et al. 1991), and in B. subtilis (Makkar and Cameotra 1998).

Environmental Factor: There are many scientific reports where the individual effects of oxygen availability, salinity, pH, and temperature on biosurfactant production have been examined. Study of the synergistic effects on Lactobacillus pentosus reflected the inter- dependence of these factors (Bello et al. 2012). In case of B. subtilis C9, a threefold higher yield of lipopeptides was observed under oxygen-limited conditions compared with oxygen-sufficient conditions (Kosaric and Sukan 2000) whereas, using a dissolved oxygen concentration of 30% in the medium showed a biosurfactant production of 4.92 g/L in B. subtilis SPB1.

APPLICATIONS OF BIOSURFACTANTS

Lipopeptides form the most widely reported class of biosurfactants with antimicrobial activity due to their ability to disrupt lipid membranes. Studies on lipopetide mechanisms of action have shown that pore formation in membranes occurs after lipopeptide oligomer binding, some of which are Ca2+-dependent multimers (Scott et al. 2007). Some lipopeptides have reached a commercial antibiotic status, like daptomycin (Robbel and Marahiel 2010), echinocandins caspofungin (Ngai et al. 2011), micafungin (Emiroglu 2011), and anidulafungin (George and Reboli 2012). Compositions containing rhamnolipids have been invented for inducing re- epithelization in adult skin tissue, to provide wound healing with reduction of fibrosis, and for the treatment of burn shock. Interestingly, these rhamnolipids compositions were also indicated in the treatment and prevention of atherosclerosis, rejection of transplanted organ, and in the treatment of depression and schizophrenia (Piljac and Piljac 1999).

PURIFICATION TECHNIQUES OF BIOSURFACTANT

For downstream processing of various biosurfactants, different unit operations have been described, such as washing, filtration, foam separation, solvent extraction, ultrafiltration, pre- cipitation, crystallization, adsorption, or chromatography as shown in figure. In the case of detergents, for example, it is not necessarily required to obtain only one particular form of a surfactant, in this case even a range of products might be desirable. In contrast, in medical or cosmetic use purity and conformity requirements would be higher. For both applications, it is mandatory to remove any cells, debris, and proteins due to possible allergic reactions. Any use of hydrocarbons (extraction, chromatography, and adsorption) is problematic if the product will be used in food industry, since complete removal without trace amounts in the product is difficult.In the case of oil recovery and soil remediation such increased purity requirements are not necessary and a raw product can be applied. Corresponding to the different purity requirements of the applications, the numerous unit operations have different operation windows in terms of product/contaminant concentration, yield, and purity.

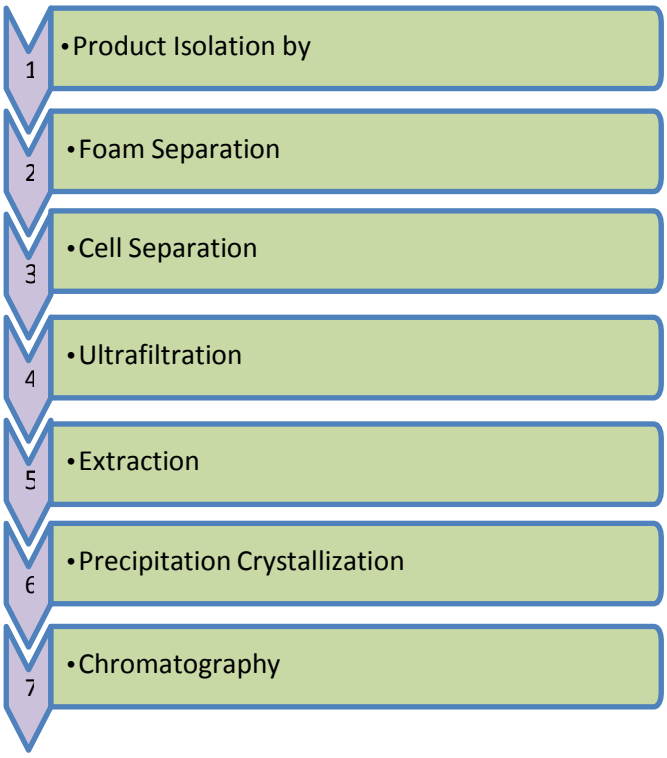

Fig -13: Downstream processing of surfactants.

ECONOMIC ASPECTS

BIOSURFACTANT YIELDS AND COST PARAMETERS

microbial surfactants possess diverse structures and better chemical properties than the synthetic equivalents, they could not overcome the chemical surfactants in cost and production capacity. Table 10.1 summarizes the product yields of different Biosurfactants. Among the various microbial surfactants, glycolipids such as sophorolipids and rhamnolipids are the promising candidates for the mass production and successful commercialization owing to their superior physicochemical properties and high product yields.

Yield and volumetric productivity of various microbial surfactants:

Surfactin are present in *Bacillus subtilis* ATCC21332 Microorganism, glucose substrate are present and Yield 6.45(g/L), Productivity 190 (mg/L/h) Yeh et al. (2006).

Surfactin+Fengycin are present in *R circulans* Microorganism, glucose substrate are present and Yield 6.98(g/L), Productivity 320 (mg/L/h) Sivapathasekaran and Sen (2013a).

Trehalsolipid are present in *Rhodococcus wratislaviensis* BN38 Microorganism, Hexadecane substrate are present and Yield 3.1(g/L), Productivity 82 (mg/L/h) Tuleva et al. (2008).

Mannosylerythritol lipid+ cellobiose lipid are present in *Ustilago maydis* Microorganism, Glucose substrate are present and Yield 23(g/L), Hewald et al. (2005).

Rhamnolipid are present in *P.aeroginosa*

DSM 7107 Microorganism, Soyabean oil substrate are present and Yield 112(g/L), Productivity 424 (mg/L/h), Lang and Wullbrandt (1999

Operating cost: Operating expenditure of biosurfactant production includes the cost of raw materials, consumables, utilities, labor, waste treatment and disposal, and facility-dependent items. Fig 16 shows the cost range of these different items with respect to the total operating expenses.

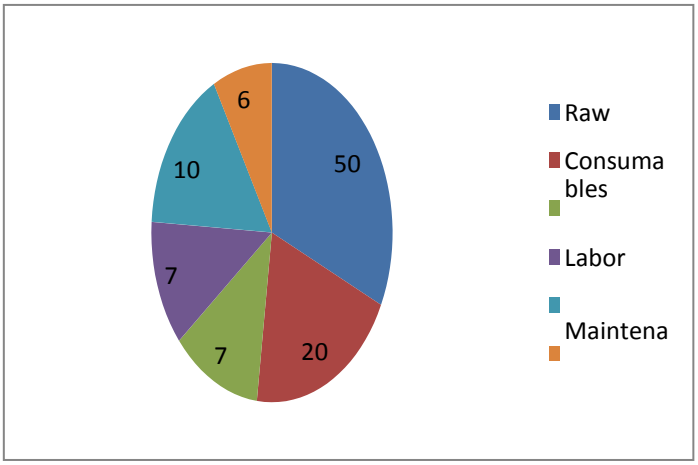

Fig -14: Operating cost distribution

Unit production cost of biosurfactants can be estimated as the ratio of the annual operating cost to the annual rate of biosurfactant production. The unit manufacturing cost is inversely proportional to the market volume of biosurfactants.

FEASIBLE COMMERCIAL BIOSURFACTANT PRODUCTION

Since the use of expensive substrates and low product yield are the main reasons for the high production cost of biosurfactants, implementation of the following strategies can facilitate the successful commercialization of these molecules: Although diverse microbes are capable of producing biosurfactants, only *Bacillus*, *Pseudomonas*, and *Candida* sp. are primarily focused. Hence, other hyper-producing genera have to be closely examined for large-scale biosurfactant production (Mukherjee et al. 2006). Microorganisms from contaminated soils, effluents, and wastewater sources can be isolated and screened for surfactant production since they are capable of utilizing industrial wastes. Apart from naturally occurring biosurfactant-producing strains, hyper-producing microorganisms can be engineered by genetic recombination and mutation. By doing so, not only can the product yield be enhanced but also the characteristics of biosurfactants can be improved (Shaligram and Singhal 2010).

Systems biology is an interesting approach and can be applied to enhance biosurfactant production by increasing the metabolic fluxes toward the product and reducing the formation of other undesired metabolites. In addition to random and targeted genetic alteration, this knowledge-based genetic and metabolic engineering approach can greatly enhance biosurfactant production (Muller et al. 2012).

The type and amount of biosurfactants produced depend on the medium components and environmental conditions. Hence, different statistical and mathematical tools can be used to optimize these variables in order to enhance the product yield and volumetric productivity (Sen 1997; Sen and Swaminathan 1997; Sivapathasekaran et al. 2010a).

Since raw materials constitute 30–80% of the overall production cost of biosurfactants, use of industrial and agro-based wastes and low-cost renewable substrates can lead to significant reduction in the operating cost involved in the process (Mukherjee et al. 2006; Nitschke and Pastore 2003; Noah et al. 2005).

Comparing the selection of raw materials, choice of purification steps is equally an important factor in establishing an economic process and, therefore, use of cost-effective downstream processes is a positive step toward successful commercialization of biosurfactants.

Another interesting approach in making profitable biosurfactants is co-production of these molecules with other metabolites like industrially important enzymes (Ramnani et al. 2005) and polyhydroxyalkanotes (Hori et al. 2002). Furthermore, *in situ* production of biosurfactants in oil reservoirs, where they are used to enhance oil recovery, can make the process economically viable (Youssef et al. 2007).

FUTURE ASPECTS

The commercial prosperity of any product, however, depends on its market demand, production cost, and ease of availability of raw material. The expensive downstream processing, low productivity, and the lack of proper understanding of the bioreactor systems used for their production are the major obstacles in case of biosurfactant production. Biosurfactants can be easily produced using cheap agro-industrial substrates and their market demand is high at the moment. Conversely, the cost of production is one such hurdle that prevents biosurfactants from capturing the chemical surfactants market. Even though the natural biosurfactant-producing strains are widely distributed, the low-yield factor remains the matter of concern. Yield is an important parameter as it determines the expense of the substrate for any biotechnological product. In order to overcome this problem, many recombinants or GMOs are being created using transgenics targeting the very gene responsible for the production of Biosurfactants. The current body of knowledge of biosurfactant production is more or less based on the genomic and proteomic studies of genera's *Bacillus* and *Pseudomonas.* The need of the hour is to explore the promising properties of other poorly researched microbes such as *Rhodococcus, Arthrobacter, Brevibacterium, Mycobacterim, Corynebacterium, Acinetobacter, Torulopsis, Candida, Pseudozyma, Yarrowia, Kluveromyces,* and *Saccharomyces* so as to achieve the required breakthrough for the biosurfactant mass production. Complete understanding of the molecular genetics of the hyper-producing strains in also necessary.

CONCLUSIONS

Nowadays surfactants are one of the most important substances for many fields of industry such as pharmacy, food industry, and design of washing agents, petroleum industry, agriculture, environmental protection and remediation. An excessive use of chemical surfactants leads to technogenic load on environment, flora and fauna, affects on food products. Biosurfactant can satisfy the needs of the modern market in natural products, particularly surface-active substances of new generation. Limitation in biosurfactants is the much higher production costs as compared with chemically produced surfactants, preventing widespread use of these interesting com- pounds. The economic efficiency of the production processes strongly depends on suitable and effective methods of isolation and cleaning of the products.

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