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COMPARATIVE STUDY BETWEEN SURFACTANT AND BIOSURFACTANT (SOPHOROLIPID) WITH CHARACTERIZATION

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ABSTRACT

Biosurfactants are the type of amphiphilic compounds that are produced on living surfaces or excreted extra-cellular polar and non-polar portions that have the ability to accumulate between two liquid phases, thereby lowering the surface and interfacial tension. Biosurfactants are biodegradable, having low toxicity, foaming ability, stable activity over wide pH range and temperatures. Biosurfactants have various applications in the field of cosmetics, pharmacy, agriculture, food and medicine industries. They possess anti-microbial, anti-cancer and anti-viral activities. They are classified according to their chemical structure and microbial origin as- Glycolipids, lipopeptides and lipoproteins, fatty acids, phospholipids and polymeric biosurfactants. The main aim of this study is to isolate Sophorolipid which is a type of glycolipid biosurfactant on laboratory scale, by using yeast [*Starmerella bombicola*] and to evaluate Sophorolipid on the basis of surface tension, critical micelle concentration, density, pH and boiling point and to compare its activity with synthetic surfactant sodium lauryl sulfate.

KEYWORDS: Anti-microbial, Biodegradable, Biosurfactant, Sophorolipid, Yeast (*Starmerella bombicola*).

1. INTRODUCTION

Sophorolipids are the important class of glycolipids which are generally produced by yeast, which consists of dimeric carbohydrate called sophorose, linked with long-chain hydroxyl fatty acid by a glycoside linkage. These sophorolipids are mixture of almost six to nine different hydrophobic sophorolipids and lactone form of sophorolipids and has many applications.[1] Generally, biosurfactants can be classified according to their chemical structure and microbial origin. They are classified into two categories, high molecular weight and low molecular weight, following table no. 1 represents classification of biosurfactants. Low-molecular weight includes: glycolipids, lipopeptides and phospholipids, whereas high-molecular weight includes: polymeric and particulate surfactants.[2]

Table No. 1: Classification of Biosurfactant.

Biosurfactant Type	Properties
Glycolipid Biosurfactant: Glycolipds are carbohydrates that combines with long-chain aliphatic acids or hydroxyl fatty acids that links with either ester or an ether group. The best known glycolipids are rhamnolipids, trehalolipids, and sophorolipids.[2]	Types of Glycolipids are: Rhamnolipids: The well-known glycolipids are rhamnolipids. It contains one or two molecules of rhamnose linked to one or two molecules of β-hydroxydecanoic acid. In *Pseudomonas aeruginosa* the production of rhamnose-containing glycolipids was first described.[2] Trehalolipids: Several structural sorts of microbial trehalolipid biosurfactants are reported which consists of trehalose (a disaccharide of two glucose molecules) connected to long chain fatty acids. Disaccharide trehalose linked at C6 and C69 to mycolic acids is related to most of the species. Mycolic acids are long chain, α-branched-β-hydroxy fatty acids. Sophorolipids: Sophorolipids are the important class of glycolipids which are generally produced by yeast, which consists of dimeric carbohydrate called sophorose, linked with long-chain hydroxyl fatty acid by a glycoside linkage. Mannosylerythritol- lipids: The other high yielding glycolipids from yeasts are mannosylerythritol-lipids (MEL), which are receiving much attention owing to their biomedical applications, Mannosylerythritol lipid (MEL), 2,3-di-O-alka(e) noyl-β-D-mannopyranosyl-(1→4)-Omeso-erythritol partially acetylated at C4 and/or C6, is a glycolipid that contains mannose and the sugar alcohol erythritol as hydrophilic moiety and acetyl groups as well as fatty acids as the hydrophobic moiety.[3]
Lipopeptides and lipoproteins:	Lipopeptides and lipoproteins are a class of biomolecules known for their bio-surfactant activities. They are cyclic lipopeptide containing a lipid linked to a polypeptide or amino acid chain. Cyclic lipopeptides like gramicidins (decapeptide antibiotic) and polymyxins (lipopeptide

	antibiotic) show remarkable surface active properties.[4] Example: Surfactin produced by Bacillus subtilis is another well studied cyclic lipopeptide of this type and a powerful bio-surfactant, made of seven amino acid ring attached with fatty acid, hydroxy-methyl tetradecanoic acid. Lichenysinis another of this type which act synergistically and exhibit excellent temperature, pH and salt stability.[4][5]
Fatty Acids:	Fatty acids produced from alkanes are also considered as surfactants. They have - OH group and alkyl branch. Example is Corynomucolic acid. The hydrophylic and lipophylic balance of fatty acids are clearly related to length of hydrocarbon chain. For lowering surface tension most active saturated fatty acids are in the range of C12-C14.[6]
Phospholipids:	Structures are similar to many organisms. For e.g. Biosurfactant from *Corynebacterium lepus*; Neutral lipids, Fatty acids and hydrophobic proteins.[5]
Polymeric Biosurfactants:	Examples are Liposan, Emulsan, biodispersan, alasan, mannoprotein and polysaccharide-protein complexes. Liposan is composed of 83% carbohydrate and 17% protein and is produced by *Candida lipolytica*. Mannoproteins are produced by Saccharomyces cerevisiae and contain 44% mannose and 17% protein.[6]

2. Properties of biosurfactants

a. Surface and interfacial activity: Biosurfactants at a lower concentration decreases surface tension; giving greater effectiveness and efficiency in comparison to standard surfactants.

b. Tolerance to temperature, pH and ionic strength: Biosurfactants can be used under extreme conditions, for e.g. the lipopeptide from *Bacillus licheniformis* which is stable at temperatures around 75°C for up to 140 hours and within a pH range of 5 to 12. They can also tolerate salt concentrations up to 10%, whereas 2% NaCl is sufficient to inactivate conventional surfactants.

c. Biodegradability: They get easily degraded by bacteria and other microorganisms in water or soil, helpful in microbial bioremediation and in waste treatment.

d. Low toxicity: The low degree of toxicity of compounds allows their use in food, cosmetic and pharmaceutical products and thus received greater attention due to the increasing concern on the part of the population regarding the allergic effects of artificial product.[5]

e. Availability: Biosurfactants can be produced from widely available raw materials and can also be produced from industrial waste.

f. Specificity: As complex organic molecules with some particular functional groups, being definite in their actions, which are of considerable interest regarding the detoxification of specific pollutants also as in particular applications in many industries.

g. Biocompatibility and digestibility: Due the biocompatibility and digestibility of biosurfactants, they have various applications in the food, cosmetic and pharmaceutical industries.[5]

3. Cosmeceutical Applications Of Biosurfactant

a. Anti-aging skin care products: Mannosylerythritol lipid (MEL): To reduce or slower the effect of skin aging certain antioxidants containing products like anti-aging facial gel, anti-aging creams are used.[7]

b. Sunscreen products: Surfactin biosurfactant: Biosurfactant from agro-industrial stream as sunscreen agent, biosurfactant which could possibly be used to increase the protective effect of mica minerals against ultraviolet light (UV light). The sunscreen protection factor (SPF) of various biocomposites which is predicated on different mica minerals alone or mixed with a biosurfactant extract are often obtained from the corn industry.[8]

c. Face wash Rhamnolipid: Rhamnolipids are a naturally occurring class of compounds that have surface active properties. It is mild when we use it to wash our skin and hair.[9]

d. Shampoo Rhamnolipid biosurfactant: Rhamnolipid bio-surfactant obtained from *Pseudomonas aeruginosa* to formulate a shampoo comprising 2% of rhamnolipid dissolved in water. The antimicrobial effect of the said bio-surfactant kept the scalp free from odour for three days and maintain luster.[5]

e. Hair Conditioners Mannosylerythritol lipids (MELS) biosurfactant: MELS have a hair care properties like Repair the Damaged Hair. MELS are proposed to be the new hair care ingredient, which is highly useful agent not only for the recovery of damaged hair but also for providing the smooth and flexible hair.[10]

4. Table no. 2: Comparison between Biosurfactant and Surfactant.[11]

Sr.No	Biosurfactant	Surfactant
1.	More effective and efficient	Less effective and efficient
2.	CMC is lower than synthetic surfactant	CMC is higher
3.	Biodegradable	Non-biodegradable
4.	Nontoxic	Some surfactant may produce toxicity
5.	Compatible	Non-compatible
6.	Cost Effective	High Cost

5. **Need of work:** Biosurfactant in recent years have been reported to possess several properties of therapeutic and biomedical importance. The need of the work is to search biosurfactant as an alternative to synthetic surfactant because they are biodegradable in nature, shows high activity at extreme temperature and pH and long storage time, high washing activity and good compatibility.

6. **Plan of work:** To synthesize biosurfactant (sophorolipid) by using yeast (*Starmerella bombicola*) and to compare its activity with synthetic surfactant as an eco-friendly alternative.

7. **Preparation of Sophorolipid**[12]

a. **Microorganism procurement and its maintenance:** The yeast used in this study *starmerella bombicola* MTCC* 1910 was procured from Microbial Type Culture Collection And Gene Bank [MTCC] INSTITUTE of microbial technology, Chandigarh India. The yeast available in freeze dried cultures form.

b. **Preparation of slant of yeast:** Subculture the organism immediately to yeast extract peptone dextrose agar (YPD) growth medium by Streak a few drops of the freeze dried culture suspension to YPD agar medium into a Petri plate and incubate at room temperature. Following table no.3 represents composition of growth medium (YPD).

Table no. 3: Composition of growth medium (YPD).

Chemical	Quantity given for liter	Quantity taken for 100ml
Yeast extract	3g	0.3g
Peptone	10g	1g
Dextrose	20g	2g
Agar Agar	15g	1.5g
Distilled water	1000 ml	100 ml

The slants were prepared and pH of the growth medium was 6.2. The microorganism was enriched in every two weeks and maintained in a refrigerator at 4^0c.

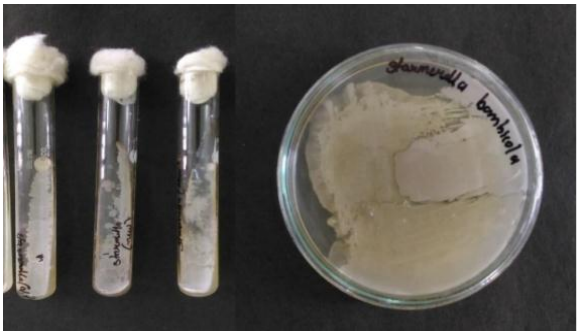


Fig no. 1: Slant prepared in test tube and petri dish.

c. Seed culture preparation for sophorolipid production: The medium used for developing the seed culture contained (grams per liter), glucose 10g, yeast 1g, urea 0.1g and oleic acid 5ml in 100ml distilled water, 2 conical flask (500ml) containing 200 ml of the seed culture medium each were autoclaved at 121^0**c** for 20 minutes and inoculated with a loop full of the microorganism freshly grown slant.[12] Table no.4 represents seed culture medium composition.

Table no. 4: Seed culture medium composition.

Chemical	Quantity given for liter	Quantity taken for 200 ml
Glucose	100g	20g
Yeast	10g	2g
Urea	1g	0.2
Oleic acid	50ml	10ml
Distilled water	1000 ml	200ml

d. Innoculum development and Biosynthesis of Sophorolipid

Fig. no. 2: Rotary Shaker.

The culture was then incubated for 48 hours at room temperature and 180 rpm on a rotary shaker. Production medium containing 100 g/l glucose, 10g/l yeast extract, 1g/l urea and 50

g/l oleic acid (sterilized) was used for sophorolipid biosynthesis in batch cultures. Batch fermentations were carried out in 500ml conical flasks containing 200ml production medium for 192h at 30^0c in a rotary shaker at 180 rpm. All other media composition and culture condition remained the same.

e. Isolation (estimation) of sophorlipid

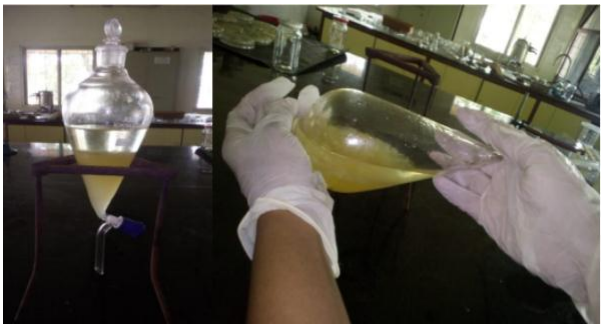


Fig. no. 3: Separation of sophorolipid.

The yeast biomass was extracted twice with equal volume of ethyl acetate to remove unutilized oleic acid and sophorolipid in the fermentation broth. For isolation of sophorolipid, the previously obtained ethyl acetate extract was dried at 40^0c to remove the solvent. Two times the residue was washed with hexane to remove the remaining oil if any, and any hydrophobic substances such as fatty acids and alcohols, if any formed during the fermentation. Partially purified sophorolipid was obtained after vaporizing the residual hexane at 40^0 c under vacuum and therefore the yield was assessed by gravimetric measurements.

f. Yield of sophorolipid

- Weight of empty beaker(w1)=147.33 gm
- Weight of the beaker + sophorolipid after process (w2)= 257.63 v/w
- Weight of sophorolipid (w2-w1)= 110.3 ml
- Weight of sophorolipid before process =400 ml
- Percentage yield = 110.3/400 x 100 =27.57%

The percent yield of sophorolipid is found to be **27.57%**

g. Identification test for Sophorolipid: The crude biosurfactant thus collected from the bacterial isolate were subjected to a series of test like carbohydrate test, lipid test, solubility test and glycerol test. Table no.5 represents identification test for Sophorolipid.

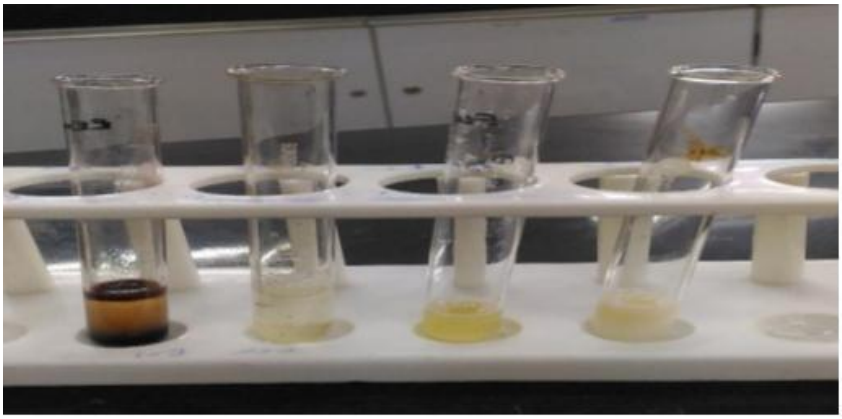


Table No. 5: Identification test for Sophorolipid.

Sr. No.	Identification test	Observation	Result
1	Carbohydrates (Molisch reagent)	Reddish violet ring was formed at junction	Passes the test
2	Saponification test	Formation of soap bubbles	Passes the test
3	Glycerol test	No color change	Passes the test
4	Solubility test	Soluble in water but insoluble in organic solvent	Passes the test

From above table 5, identification test of sophorolipid passes as per standard.

8. Biosurfactant Characterization

8.1 Determination of Boiling Point: The boiling point of sophorolipid was determined by capillary tube method using Thiele's tube. The boiling point of sophorolipid was found to be 104.5^0c, which was within the range i.e. 120^0c as per standard value.[13]

8.2 Determination of Density of Sophorolipid: Density of the sophorolipid was found to be 1.031g/cm^3 within the range of 0.9 to 1.20 g/cm^3 as per standard value. Surface tension is directly proportional to density. If the liquid with highest density has highest surface tension, while the liquid with lowest density, has the lowest surface tension. Hence, density of the sophorolipid is low then surface tension of sophorolipid will also be low. Sophorolipid produce a lower surface tension at a lower concentration, it gives greater effectiveness and efficiency in comparison to conventional surfactants.[14]

8.3 Determination of surface tension of sophorolipid: The drop count method was employed for determination of surface tension. The surface tension of the sophorolipid was found to be 40.89dynes/cm. The standard surface tension value of sophorolipid was 43.2

dyne/cm and the resultant value was found to be 40.89dynes/cm due to experimental error or personal error.[14]

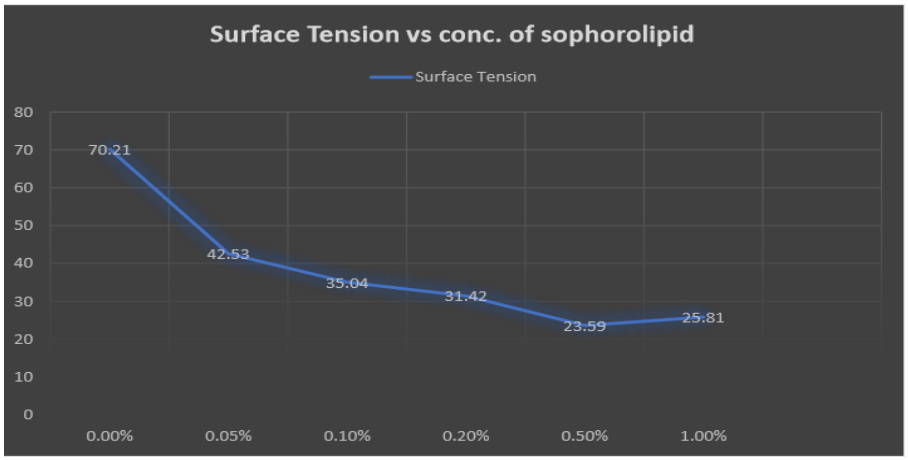
8.4 Determination of pH: pH, which is the logarithm of the reciprocal of the hydrogen ion concentration of sophorolipid, was measured using a glass electrode pH meter. pH of sophorolipid was found to be pH 6.3. The pH of sophorolipid was found to be 6.3 within the range of 6 to 8 pH as per standard value.[13]

8.5 Critical micelle concentration (CMC): The CMC of sophorolipid was determined by using stalagmometer.

Table no. 6: Observations.

Sr. no.	Concentration surfactant % (w/w)	No.of drops of sophorolipid 1	2	3	Average number	Density g/cm3	Surface tension dyne/cm
1	0.0%	53	51	50	52	1.000	70.21
2	0.05%	87	85	87	86	1.002	42.53
3	0.1%	98	108	109	105	1.008	35.04
4	0.2%	118	115	118	117	1.007	31.42
5	0.5%	159	156	152	156	1.008	23.59
6	0.1%	142	145	142	143	1.011	25.81

A graph was plotted by taking concentration of surfactant solution on x axis and the corresponding surface tension on y axis. A sudden change in the trends of surface tension corresponds to the CMC of the surfactant.



Graph No. 1: Determination of critical micelle concentration.

From the graph the critical micelle concentration of sophorolipid was found to be 0.5% to 1.0%. The resultant value was found within the range of 40 to 100(0.4% to 1%) as per standard. The critical micelle concentration (CMC) value indicates the amount of surfactant required to reach maximum surface tension reduction. The lower the CMC, the less surfactant required to effectively emulsify, solubilize and disperse soils at the surface hence, sophorolipid is more effective for emulsification, solubilization and to disperse soils at the surface.[13]

9. RESULT

The main aim of the study is to synthesize Sophorolipid from yeast in lab scale and to evaluate its characterization and also to study the comparison between sophorolipid and synthetic surfactant.

Table no. 7: Result and comparison between biosurfactant and synthetic surfactant.

SR.No.	Characterization	Standard sophorolipid biosurfactant[14]	Observed sophorolipid biosurfactant	Sodium lauryl sulfate[15]
1	Boiling point	120°c	104.5°c	100°c
2	Density	0.95-1.20g/cm^3	1.031 g/cm^3	Greater than 1.1 g/cm^3
3	Surface tension	43.2 dynes/cm	40.89 dynes/cm	39.5 dynes/cm
4	Critical micelle concentration	0.4%-1%	0.5% - 1.0%	Around 0.5%
5	pH	6-8	6.3	6- 8

10. CONCLUSION

The Biosurfactant sophorolipid was synthesized on laboratory scale successfully using yeast (*Starmerella bombicola*). The sophorolipid was identified by the tests which were performed. The yield value of sophorolipid is found to be 27.57%. It is comparatively cost effective than synthetic surfactant, synthesis of biosurfactant used renewable raw substrates and fermentation technology which reduces their production cost. From the observed result it is concluded, the values which were obtained are almost same. So, biosurfactant can be used as an alternative to synthetic surfactant.

11. Future scope: Biosurfactant have many advantages as compared to synthetic surfactant such as biodegradable, ecofriendly, cost effective, nontoxic and tolerance to high temperature

and pH. Therefore, biosurfactant can be used in sulfate free formulations and other formulation for their antimicrobial and anti-fungal properties.

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