BIOSURFACTANTS - A REVIEW

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SURFACTANTS

Surface-active substances or surfactants are molecules having amphiphilic characteristics, i.e. both hydrophilic and hydrophobic properties (Hutchinson et al., *1967; Van Dyke* et al., *1991). Because of the presence of hydrophilic and hydrophobic groups within the same molecule, surfactants partition preferentially at the interface between fluid phases of different degrees of polarity and hydrogen bonding. The formation of an ordered molecular film at the interface lowers the interfacial tension and is responsible for the unique properties of surfactant molecules (Georgiou* et al., *1992).*

The hydrophilic part of a surfactant may be anionic, cationic or neutral, while the hydrophobic portion normally consists of hydrocarbon chains. Depending on the type of charge present, there are four possible kinds of surfactants, i.e. anionic (negatively charged), cationic (positively charged), nonionic (no charge) and amphoteric (positive and negative charges within the same molecule) (Georgiou et al., *1992).*

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BIOSURFACTANTS - WHAT ARE THEY ?

Many microorganisms produce surface-active compounds when grown in media containing a particular carbon source (Parra *et al.*, 1989). These compounds, called biosurfactants (bioS), consist of simple and complex lipids or their derivatives (Gerson *et al.*, 1979; Kosaric et al., 1987). Like chemically synthesized surfactants, they are amphiphilic in nature. The hydrophobic moieties are usually long-chain carboxylic acids covalently linked by ester or amide bonds to the hydrophilic moieties which are drawn from a wide range of organic functional groups (nonionic, positively charged, negatively charged or amphoteric).

BioS are synthesized extracellularly or are associated with the cell wall (Zajic *et al.*, 1984). When they are extracellular, they cause emulsification of the carbon source; when associated with the cell wall, they facilitate the penetration of the carbon source into the periplasmic space by changing the structure of the cell wall (Lang et al., 1987).

Based on the structure of the hydrophilic moieties, the bioS which have been reported to date are classified into five types, namely; lipopeptides, glycolopids, lipopolysaccharides, neutral lipids and fatty acids or phospholipids (Jenny *et al.*, 1991; Mulligan *et al.*, 1989; Sasidharan *et al.*, 1993b; Wagner, 1988). *Table 1* shows different bioS produced by microorganisms and their properties.

The most frequently used indices for the performance of bioS are surface tension, interfacial tension and critical micelle concentration (Georgiou *et al.*, 1992). Representative values for the most promising and extensively studied microbial surfactants are listed in *Table 2.*

The increasing interest in bioS derives from their biodegradability and the broad range of their functional properties (Fiechter, 1992). They exhibit excellent surface-active properties, such as the lowering of surface and interfacial tension, wetting or penetrating action, dispersing and emulsifying actions, detergency, gelling and vesicle formation, foaming, demulsifying and flocculating actions, metal sequestration, microbial growth enhancement in some cases, and antimicrobial activity in others (Ishigami, 1993).

The release of bioS promotes an emulsification of the water and hydrocarbon phases. These interfaces are very suitable for microbial growth (Zajic *et al.*, 1984). Addition of these compounds to oil and water culture media, therefore, stimulates the growth of certain microorganisms (Cooper *et al.*, 1980; Van Dyke *et al.*, 1991).

BioS which are more of a hydrophobic nature form micelles in the hydrocarbon phase and stabilize water-in-oil emulsions, whereas predominantly hydrophilic bioS form micelles in water and stabilize oil-in-water emulsions (Kosaric *et al.*, 1987).

Some bioS also exhibit good thermal and chemical stability and possess antibiotic activity against various microbes (Georgiou *et al.*, 1992).

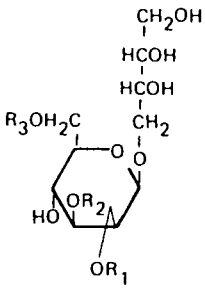
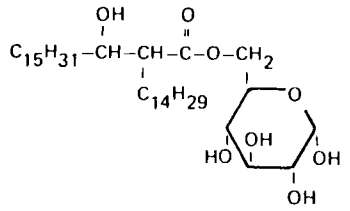
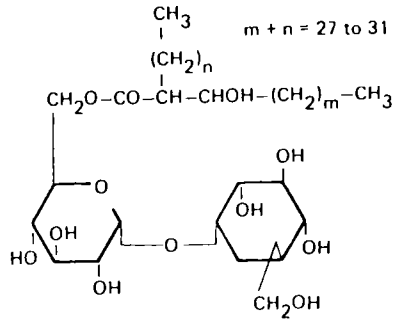
PRODUCTION OF BIOSURFACTANTS

BioS are synthesized by bacteria, yeasts and fungi (Falbe, 1987; Fiechter, 1992; Lang *et al.*, 1987; Wagner, 1988). Yeasts and fungi utilize preferably linear and saturated n-alkanes while some bacteria in addition degrade isoalkanes and cycloalkanes as well as unsaturated aromatic compounds. The syntheses are frequently regio-, stereo- and group-selective (Slydatk *et al.*, 1987).

During the biosynthesis of bioS, there are various parameters that contol the type and amount produced. Among these are :

- nature of the carbon source
- nature of growth and nutritional limitations, e.g. nitrogen source and concentration and the C:N ratio
- physical and chemical parameters, such as aeration, temperature and pH

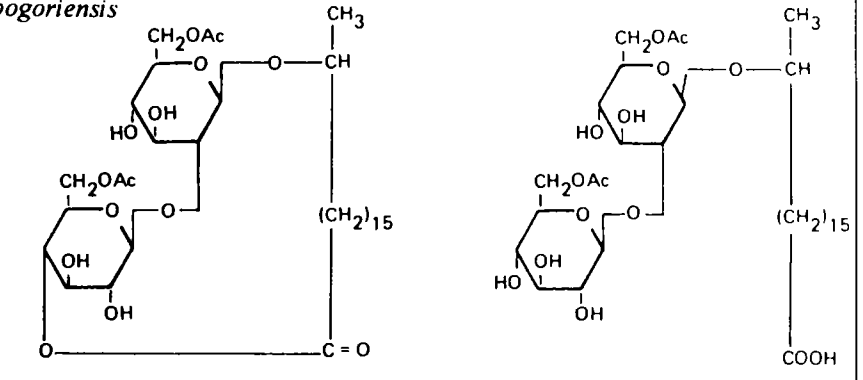
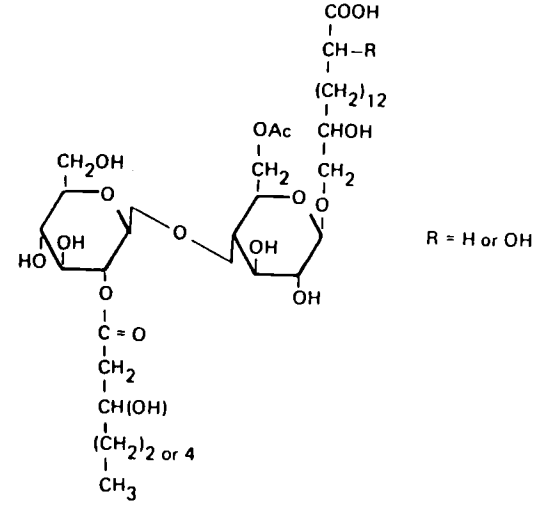
TABLE 1. BIOSURFACTANTS PRODUCED BY MICROORGANISMS

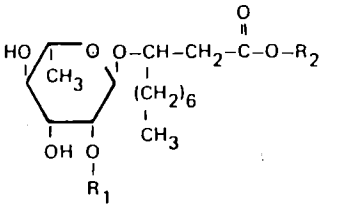
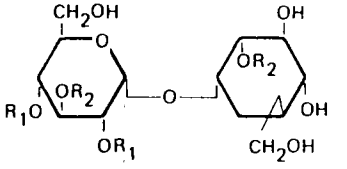
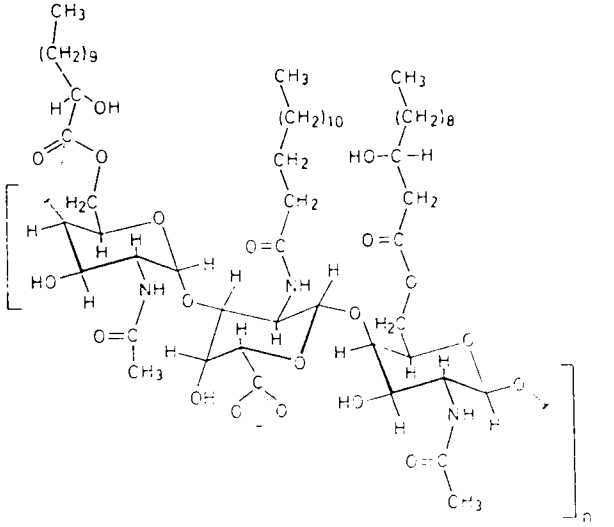
Group	Compounds	Properties	Microorganisms	Structure
Glycolipids	Mannosylerythritol lipids	Nonionic, extracellular	*Candida* species	
	Mycolates of mono-, di- and trisaccharides	Nonionic, cell wall associated	*Arthrobacter* sp., *Nocardia* sp., *Corynebacteria, Brevibacteria*	
	Trehalose corynomono- and di-mycolates	Nonionic, cell wall associated	*Arthrobacter paraffineus, Rhodococcus erythropolis, Mycobacteria*	

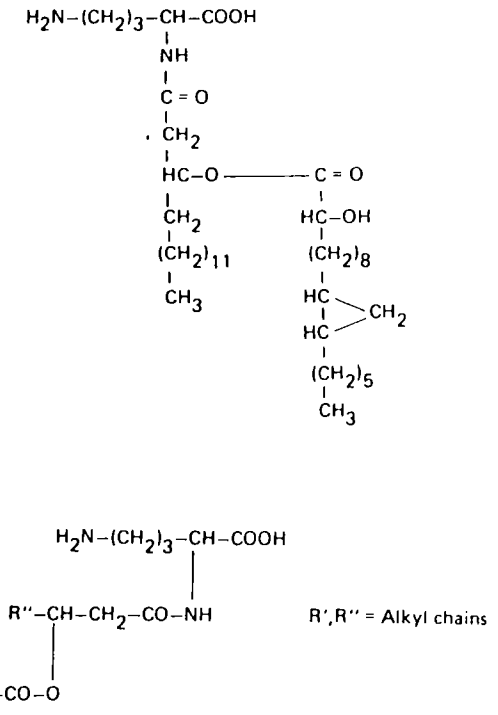
	R_1	R_2	R_3
A:	Ac	n-$C_{15}H_{31}CO$•	H
B:	Ac	n-$C_{15}H_{31}CO$•	Ac

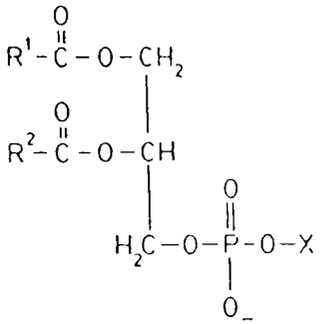
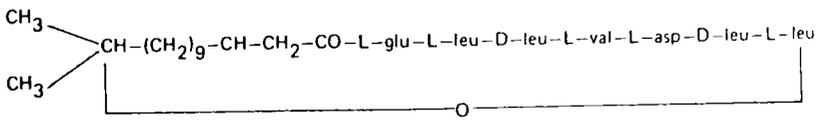
• In admixture with other long chain residues

m + n = 27 to 31

Group	Compounds	Properties	Microorganisms	Structure
	Sophorolipids	Nonionic or anionic, extracellular	*Torulopsis* sp. *Candida bogoriensis*	
	Cellobioselipids	Nonionic, extracellular	*Ustilago maydis*, *Ustilago zeae*	

Group	Compounds	Properties	Microorganisms	Structure
	Rhamnolipids	Anionics, extracellular	*Pseudomonas* sp.	 RL 1: R_1=L–α–Rhamnopyranosyl– R_2=β–Hydroxydecanoic acid RL 2: R_1=H R_2=β–Hydroxydecanoic acid RL 3: R_1=L–α–Rhamnopyranosyl– R_2=H RL 4: R_1=H R_2=H
	Trehalose tetraesters	Anionic, extracellular and cell wall associated	*Rhodococcus erythropolis*, *Arthrobacter paraffineus*, *Corynebacterium hydrocarboclastus*	 R_1 = OC $(CH_2)_mCH_3$ and OC $(CH_2)_2COOH$ m=6 (main component) R_2 = OC $(CH_2)_nCH_3$ n=8 (main component)
Lipopoly-saccharides	Emulsan	Polyanionic, extracellular	*Acinetobacter tumefaciens*	

Group	Compounds	Properties	Microorganisms	Structure
	Lipoteichoic acid	Extracellular	*Streptococcus sanguis*	n.a.
	Lipopolysaccharides of different structures	Cell wall associated	*Candida, Pseudomonas*	n.a.
Ornithine-lipids/ lysinelipids	Ornithine or lysine as polar compound	Cell wall associated	*Gluconobacter cerinus, Pseudomonas rubescens, Thiobacillus ferrooxidans*	

Group	Compounds	Properties	Microorganisms	Structure
Other products	Phospholipids, fatty acids, neutral lipids, glycerides, alcohols	Cell wall associated or extracellular	All microorganims, enrichment during growth on hydrocarbons	
Lipoproteins, lipopeptides, peptidoglyco-lipids, protein emulsifiers	Surfactin, substilisin	Extracellular	*Bacillus substilis*	
	Siolipin	Cell wall associated	*Streptomyces sioyaensis*	n.a.
	Peptidoglycolipid	Extracellular	*Pseudomonas aeruginosa*	n.a.

Group	Compounds	Properties	Microorganisms	Structure
	Lipoproteins of different structures	Cell wall associated and extracellular	*Corynebacteria*, *Endomycopsis lipolytica*, *Agrobacterium tumefaciens*	n.a.
	Protein emulsifiers	Extracellular	*Pseudomonas* sp., *Candida petrophilum*, *Torulopsis petrophilum*	n.a.

n.a. : not available
Source : Falbe, 1987; Kosaric *et al.*, 1987; Wagner, 1988.

TABLE 2. SURFACE ACTIVE PROPERTIES OF TYPICAL BIOSURFACTANTS

C-source	Microorganisms	Biosurfactant	Surface tension (mN/m)	Interfacial tension (mN/m)	Critical micelle concentration
n-Alkanes	*Rhodococcus aurantiacus*	glycolipids	26	0.35	n.a.
	Nocardia corynebacteroides	pentasaccharide lipid	26	< 1	25 mg/l
		trehalose-mono, dicorynomycolates	32-36	14-17	4 mg/l
	Rhodococcus erythropolis	trehalose-mono, dicorynomycolates	32-36	14-17	4 mg/l
		phosphatidylethanolamines	30	< 1	30 mg/l
Hexadecane	*Rhodococcus* sp. strain H13A	glycolipids	n.a.	0.02	1.5 g/l
	Nocardia erythropolis	fatty acids and neutral lipids	32	< 3	n.a.
	Corynebacterium insidiosum	n.a.	28.5	0.55	n.a.
n-Paraffin	*Psedomonas aeruginosa* KY 4025	rhamnolipid	25-30	< 1	5-200 mg/l
Kerosene	*Corynebacterium lepus*	fatty acids	< 30	2	150 mg/l
Glucose	*Pseudomonas aeruginosa*	rhamnolipid	29	0.25	n.a.
	Bacillus licheniformis JF2	lipopeptides	27	0.016	0.02 mg/l
	Bacillus licheniformis 86	lipopeptides	27	0.36	10 mg/l
	Bacillus substilis	surfactin	27-32	1	23-160 mg/l
	Ustilago maydis	ustilagic acid	30	< 1	20 mg/l

C-source	Microorganisms	Biosurfactant	Surface tension (mN/m)	Interfacial tension (mN/m)	Critical micelle concentration
Sucrose	*Pseudomonas fluorescens*	protein-carbohydrate complex	27	n.a	< 10 mg/l
Mannose, glucose, cellobiose, maltose, maltotriose	*Arthrobacter* sp. DSM 2567	8 different glycolipids containing the corresponding carbohydrate moiety and 1,2 or 3 α-branched, β-hydroxy fatty acids	33-46	1-19	4-50 mg/l
Fructose	*Arthrobacter paraffineus* KY 4303	fructose lipids	n.a.	25	n.a.
Glycerol	*Serratia rubidaea*	rubiwettins	25.5-25.8	n.a.	10 mg/l
	Pseudomonas fluorescens	viscosin	26.5	n.a.	150 mg/l
	Serratia marcescens	serrawettin	28.8-33.9	n.a.	n.a.
Olive oil	*Pseudomonas* 42A2	dihydoxyoctadecanoic acid	30	n.a.	n.a.
Alkane/ carbohydrate	*Torulopsis apicola*	glycolipids	30	< 0.9	n.a.
Glucose/oleic acid	*Torulopsis bombicola* ATCC 22214	sophorolipids	33	1.8	n.a.
Glucose/NBD palm	*Candida bombicola* ATCC 22214	sophorolipids (major component is a sophorose ester of a monounsaturated 17-hydroxyoleic acid)	35	n.a.	48 mg/l

n.a. : not available
Source : Georgiou *et al.*, 1992.

CARBON SOURCE

BioS can be produced from a variety of renewable substrates (Fiechter, 1992). The structure of bioS, particularly the hydrophobic tail, may reflect the structure of the substrate provided in the culture, and changing the substrate often alters the structure, and hence the properties of the product (Kosaric *et al.*, 1987). Certain microorganisms produce bioS only when grown on hydrocarbons while others require water-soluble substrates such as carbohydrates and amino acids (Georgiou *et al.*, 1992). Oils, fats and fatty acids have also been used.

The carbon source also determines whether the bioS are extracellular or intracellular (Fiechter, 1992). *Table 3* summarizes, by chemical type, the substrates for the production of bioS and the advantages, disadvantages and possibilities of using both refined and waste substrates.

The potential substrates from waste water treatment should be rich in sugars which could be partly converted to lipids and partly used for the production of bioS. Usually the carbohydrates are provided from an external source or obtained by hydrolysis of complex components (*e.g.* cellulose) within the waste (Kosaric *et al.*, 1987).

Apart from the influence of different substrates, microorganisms also produce different products depending on the physical and chemical conditions under which thay are grown. *Table 4* shows some of the bioS that are produced by certain microorganisms using different substrates and methods of production.

NATURE OF GROWTH AND NUTRITIONAL LIMITATIONS

There are a number of possibilities for the production of bioS. They can be made during the various stages of microbial growth or under various growth-limiting conditions. Different possibilities for bioS production are shown in detail in *Table 5* (Slydatk *et al.*, 1987); the main categories are :

a) production during cell growth
b) production during cell growth but under growth-limiting conditions
c) production by resting cells, and
d) production with addition of precursors.

The production of bioS is often related to growth. They may be formed during the stationary phase of growth under certain conditions or released into the culture medium throughout the exponential phase; some are also produced by resting cells or by immobilized biocatalysts (Georgiou *et al.*, 1992). The effect of nutrients will vary depending on the way particular bioS are produced.

a) Production of bioS during cell growth

This method is often connected with foam formation, a lowering of the surface and interfacial tension and an emulsion of the lipophilic substrate in the culture broth. The carbon source, nitrogen source, the composition of the medium, temperature and pH are important factors which can influence surfactant production.

b) BioS production by growing cells under growth-limiting conditions

An overproduction of bioS has been observed in some cases, when some of the growth factors such as nitrogen or multivalent cations (Fe^{2+}, Mg^{2+}, Ca^{2+}) were limiting. Unfortunately, the effects of limiting N or multivalent cations are nonspecific and are thought to be due to some changes in the physiological state of the microorganisms used.

c) BioS production by resting cells

Some bioS are secondary metabolites produced during the stationary phase of growth, *i.e.* by resting cells. After cultivation under optimal conditions, the microorganisms are separated from the culture broth by centrifugation. The wet biomass is washed and used for the production of secondary metabolites under specific conditions.

TABLE 3. FEEDSTOCK FOR BIOSURFACTANT PRODUCTION

Feedstock	Advantages	Disadvantages
Carbohydrates	Abundant in most geographic regions from biomass resources (silvaculture, agriculture). Present (sometimes with lipids) in wastes which might have a cost credit.	Lowest conversion efficiency of all substrates to biosurfactant unless lipids or hydrocarbons are also added.
Hydrocarbons	Abundant in some geographic regions from petroleum resources. High yields of glycolipid biosurfactants when provided with carbohydrates.	Cost of biosurfactant production from non-waste hydrocarbons is tied to the cost of petroleum. Seldom present together with carbohydrates in wastes. Use of hydrocarbon wastes for biotechnology has been studied little.
Triglycerides, fatty acids, seed oil and animal fat	Abundant in some geographic regions from agriculture resources. High yield of glycolipid biosurfactants when provided with carbohydrates. Present (sometimes with carbohydrates) in wastes which might have a cost credit.	Cost of biosurfactant production from non-waste seed oil and animal fats is tied to the real positive costs of these substrates.
Microbial oil (single cell oil, CSO)	Potential of being produced from lipid-poor carbohydrate-containing wastes which may have a cost credit. High yields of glycolipid biosurfactants when provided with carbohydrates.	–

Source : Kosaric *et al.*, 1984.

TABLE 4. PRODUCTION OF BIOSURFACTANTS : PROCESS, MEDIA AND PRODUCTS

Process	Media	Products	Source
Growing cells	Basic medium (glucose, yeast extract and urea) + oleic acid or Basic medium (oleic acid, yeast extract and urea) 1) Best basic medium : 10% glucose, 1% yeast extract and 0.1% urea. 2) pH = 6.0 before sterilization. 3) Incubation temperature = 23°-30°C. 4) Stepwise or continuous addition of the second C-source to the basic medium after 24 hours.	sophorose lipids	Asmer *et al.* (1988)
Growing cells	Mineral salts medium supplemented with metal salts (Fe and Mn). Also with nutrient broth, yeast extract and hexane.	surfactin (lipopeptide)	Cooper *et al.* (1981)
Growing cells	Mineral salts medium, glucose	lipopeptides	Jenny *et al.* (1991)
Growing cells	Mineral salts medium + Imperial oil no. 9 kerosene and nutrient broth	lipopeptide (corynomycolic acids, small amount of phospholipids and neutral lipids)	Cooper *et al.* (1979)
Growing cells	Mineral salts medium, olive oil	dihydroxyoctadecanoic acid	Mercade *et al.* (1988)

Process	Media	Products	Source
Resting cells	1) Growth medium - glucose, mineral salts, yeast extract and tap water 2) Reaction medium - soyabean oil and distilled water 3) Fermentation medium - soyabean oil, mineral salts, yeast extract and distilled water	mannosylerythritol lipids	Kitamoto *et al.* (1992)
Resting cells	1) Medium A - mineral salt solution, glucose 2) Medium B - mineral salts and glucose, phosphate deficient medium - limitation of nitrogen	glycolipids	Ramana *et al.* (1989)

TABLE 5. METHODS OF BIOSURFACTANT PRODUCTION BY MICROORGANISMS

1. Cell growth-associated production of biosurfactants

   1.1 Induction of production by lipophilic substrates
   1.2 Increase of production by optimization of medium composition
   1.3 Increase of production by optimization of environmental influence such as pH, temperature, aeration, agitation speed, *etc.*
   1.4 Increase of production by adding reagents which cause change of cell wall permeability, such as penicillin, ethambutol, EDTA, *etc.*
   1.5 Increase of production by addition of reagents which cause a detachment of cell wall-bound biosurfactants into the medium as alkanes, kerosene, EDTA, *etc.*

2. Biosurfactant production by growing cells under growth-limiting conditions

   2.1 Production under N-limitation
   2.2 Production under limitation of multivalent cations
   2.3 Increase of production under growth-limiting conditions by a change of environmental conditions such as pH or temperature.

3. Biosurfactant production by resting cells

   3.1 Production by resting free cells
   3.2 Production by resting immobilized cells
   3.3 Production by resting immobilized cells with simultaneous product removal.

4. Biosurfactant production by growing, resting free, and resting immobilized cells with addition of precursors.

Source : Slydatk *et al.*, 1987.

There are several advantages in using resting cells to produce bioS (Kitamoto *et al.*, 1992) :

- The effect of possibly disturbing by-products (e.g. foam) can be eliminated.
- In most cases, the resting cells show a higher conversion rate than growing cells.
- The resting cell method requires only a carbon soource (no nutrients of buffer systems).
- Resting cells can be grown on various substrates and they exhibit a stable productivity.
- The cells can be stabilized by immobilization, thus allowing a continuous production process.

d) BioS production by microbial cells with addition of precursors

It is possible to increase the yield of some bioS by addition of lipophilic compounds to the culture broth, to act as precursors. Further, the nature of glycolipids produced can be directed by using a specific carbohydrate as carbon source. This method will probably be of great interest in future since it allows the production of new bioS whose chemical and physical properties can be changed by changing the C-source used.

PHYSICAL AND CHEMICAL PARAMETERS

The N-source and/or N-limitation play an important role in the production of some bioS, both in resting and growing cells. If inorganic salts are used as N-source, organisms generally prefer ammonium ion to nitrate. The effect of N-limitation on the synthesis of bioS is frequently nonspecific (Slydatk *et al.*, 1987). It can often be replaced by other limitations which can bring about the same effect.

Other parameters such as pH, temperature, agitation speed and aeration can also influence the formation of bioS.

APPLICATION OF BIOSURFACTANTS

Bios have several advantages over synthetic surfactants, such as :

- they show surface-active properties differing in some cases from those of synthetic surfactants, providing new possibilities for industrial applications.
- they have been shown to be more effective and specific than many conventional synthetic surfactants in particular applications.
- they are usually non-toxic, biodegradable and ecologically harmless.
- they are produced from renewable resources.
- they have the capacity to be modified and thus tailored to meet specific requirements.

There are many areas of industry where synthetic surfactants could be replaced by bioS, such as agriculture, building and construction, food and beverage, industrial cleaning, leather, *etc.* (Fiechter, 1992). BioS are specifically utilized in the removal of oil pollution in the oceans or on beaches, cleaning oil-contaminated tankers, storage tanks or pipelines; they can be used as emulsion stabilizers and as protective substances in cosmetics (sophorolipid), in enhanced oil recovery, for facilitating the degradation of organic pollutants in soil, and as food additives (Banat *et al.*, 1993; Falbe, 1987). The future of bioS is in the area of fine chemicals (Ishigami, 1993). This is based on the discovery of the multiple hydrophilic and hydrophobic moieties of some bioS which are advantages for the desired functions such as dispersant.

Naturally-occuring bioS can also lead to problems. Fuel tanks can be contaminated by microorganisms producing bioemulsifiers, and these can cause water dispersion into the oil in the tank and lead to corrosion (Cooper *et al.*, 1980).

ECONOMIC ASPECTS

So far, the use of bioS has been limited to a few specialized areas as they are unable as yet to compete economically with the chemically synthesized compounds in the wider market, because of high production costs, lack of public acceptance and stringent purification processes which are required before they can be used in the cosmetics, food and pharmaceutical industries. These difficulties are due mainly to inefficient bioprocessing methodology, the use of strains of organisms with poor productivity, and the need to use expensive substrates (Fiechter, 1992).

BioS must compete with surfactants of petrochemical origin in three aspects - cost, functionality and production capacity to meet the needs of the intended application. High production costs can be tolerated for bioS used in small volume in high value-added products such as cosmetics and pharmaceuticals. In applications where cheap products are required in large volumes, e.g. in the case of surfactants for enhanced oil recovery, bioS may not be able to compete with the surfactants that are presently used.

Production of large quantities of bioS which are cost-competitive with surfactants of petrochemical origin has been studied by Kosaric *et al.* (1984). They concluded that only if cost-free or cost-credit wastes were used as feed stock for microbial growth could the synthesis of bioS be economically competitive. Emphasis was therefore placed on sludges from municipal waste water treatment to produce a cost-competitive bioS and to offset the cost of high quality waste water treatment.

In the 'multi-organism strategy' (*Figure 1*) proposed by Kosaric et al. (1984), the appropriate lipogenic bacteria or yeast and the appropriate lipogenic alga are co-cultured to produce triglycerides in the form of oil. The microbial triglycerides and the same sugar used to feed the lipogenic microbes are used by *Torulopsis bombicola* ATCC 22214 to produce a glycolipid.

The biomass from *Torulopsis*, produced during bioS synthesis, can be recycled for treatment or separated and sold as a yeast-rich feed supplement. Gaseous by-products (CH_4) serve the energy requirements of the process and four final products are obtained (treated water, high-grade and low-grade biomass, and bioS) with the latter product having sale value that could help to offset the water treatment costs.

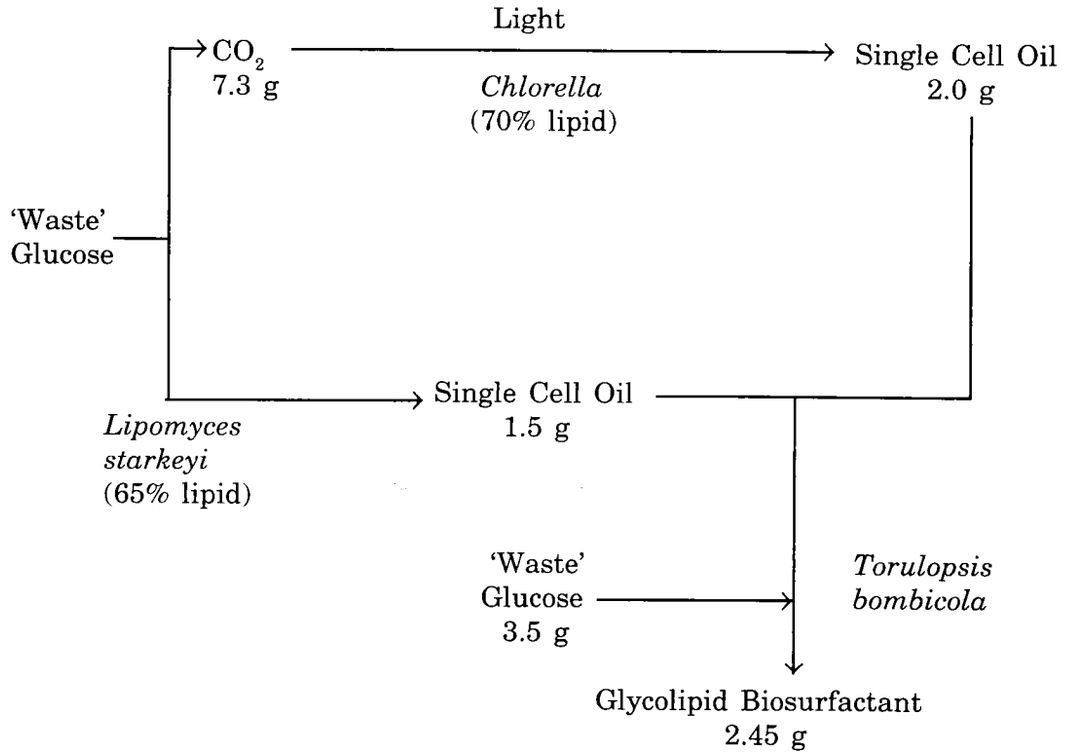
FUTURE POTENTIAL OF BIOSURFACTANTS

BioS are of increasing interest for commercial use because of the continually growing spectrum of available substances (Slydatk *et al.*, 1987). They are biodegradable, often nontoxic and they can be produced by microbial processes, in which simple substrates such as n-alkanes, vegetable oils and carbohydrates or even industrial waste products can serve as substrates (Sasidharan *et al.*, 1993a). The only commercial industrial bioS in the market at present is Emulsan. It is a protein-associated lipopolysaccharaides which is used in cleaning oil-contaminated vessels, in oil spill management and in microbially-enhanced oil recovery (MEOR) (Fiechter, 1992).

Industry uses at least one million tonnes/ year of synthetic surfactants. Many different types are being used, but it is important to develop even more to broaden the spectrum of properties available. No one surfactant is suitable for all the potential applications (Cooper *et al.*, 1980).

BioS could replace the relatively expensive petroleum sulphonates or lignosulphonates that are currently used for enhanced oil recovery. A potential area for the application of bioS is the cosmetic industry, especially in shampoos and skin-care products (Fiechter, 1992).

Many of the potential applications for bioS depend on whether they can be produced economically. Much effort is still needed on optimizing processes at the engineering and biological levels. Legal factors, such as stricter regulations concerning environmental pollution by industrial activities, as well as health regulations, could also strongly influence the chances of biodegradable bioS replacing their chemical counterparts.



Source : Kosaric *et al.* (1984).

Figure 1. A multiorganism strategy for glycolipid production from simple waste using co-cultured lipogenic alga (Chlorella) and lipogenic yeast (Lipomyces) to produce the lipid substrate for a biosurfactant production by Torulopsis.

POSSIBILITIES OF USING PALM OIL AS SUBSTRATE FOR THE PRODUCTION OF BIOSURFACTANTS

Several researchers have succeeded in producing bioS using vegetable oil and glucose as carbon sources. These natural surfactants are being evaluated in relation to environmental protection, safety and mildness. Palm oil is a renewable resource and its production is expanding.

Asmer *et al.*, (1988) succeeded in producing a mixture of sophorose lipids by the cultivation of *Torulopsis bombicola* ATCC 22214 on a mixture of glucose and oleic acid or oleic acid alone. Glucose as substrate gave a lower yield of crude product, while the best result was found when oleic acid was used as the sole carbon source. In general, a 17-hydroxyoctadecanoic acid at C-6'- and C-6"-positions of sophorose were found as substituents in the lactone and acidic forms of these lipids.

Koh *et al.*, (1983) conducted physiological studies of yeasts for cell production from palm oil. Most of the strains tested could assimilate crude palm oil (CPO) better than the refined oil. *Torulopsis candida* Y-128 assimilated CPO effectively and showed a higher yield of protein on both crude and refined palm oil than any other strains. When specific fatty acids were used as the sole carbon source, unsaturated

fatty acids such as oleic and linoleic acids were found to be more easily assimilated than saturated acids.

Seino *et al.*, (1984) successfully synthesized carbohydrate esters of fatty acids enzymatically using microbial lipases. Fatty acids such as stearic, oleic and linoleic acids, and carbohydrates such as sucrose, glucose, lactose and sorbitol were of interest. It was reported that lipase from *Candida cylindracea* was the most active enzyme for the synthesis of carbohydrate esters.

Sasidharan *et al.*, (1993a,b) used the same strain as Asmer *et al.* (1988), namely *Torulopsis bombicola* ATCC 22214, for the production of sophorolipids using glucose and/or palm oil as substrate. Enhanced production was observed when NBD palm olein was used together with glucose in the medium. The crude mixture of sophorolipids consisted of one major compound that was identified as sophorose ester of a mono-unsaturated 17-hydroxyoctadecanoic acid. The crude product was comparable to the commercial surfactant, lauryl alkyl-sulphonate (LAS) in terms of soil removal efficiency.

CONCLUSION

Biosurfactants are very varied and a lot of work has still to be done in perfecting economic methods for their production. However, the various characteristics exhibited by bioS, allow a product chemist to expect a variety of potential specialized applications based on their structure.

Various raw materials can be used as substrates and these in turn result in the production of various types of bioS. Among the various raw materials, oils, fats and oleochemicals are of special interest to PORIM since these raw materials can be found in abundance in Malaysia. It is heartening to note that research and development carried out using palm oil, albeit limited, has indicated promising findings.

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