Microbial production of biosurfactants and their importance

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A large variety of microorganisms produce potent surface-active agents, biosurfactants, which vary in their chemical properties and molecular size. While the low molecular weight surfactants are often glycolipids, the high molecular weight surfactants are generally either polyanionic heteropolysaccharides containing covalently-linked hydrophobic side chains or complexes containing both polysaccharides and proteins. The yield of the biosurfactant greatly depends on the nutritional environment of the growing organism. The enormous diversity of biosurfactants makes them an interesting group of materials for application in many areas such as agriculture, public health, food, health care, waste utilization, and environmental pollution control such as in degradation of hydrocarbons present in soil .

BIOSURFACTANTS (BS) are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces, or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension (ST) and interfacial tensions between individual molecules at the surface and interface, respectively. Since BS and bioemulsifiers both exhibit emulsification properties, bioemulsifiers are often categorized with BS, although emulsifiers may not lower surface tension. A biosurfactant may have one of the following structures: mycolic acid, glycolipids, polysaccharide–lipid complex, lipoprotein or lipopeptide, phospholipid, or the microbial cell surface itself.

Considerable attention has been given in the past to the production of surface-active molecules of biological origin because of their potential utilization in food-processing[1–3], pharmacology, and oil industry. Although the type and amount of the microbial surfactants produced depend primarily on the producer organism, factors like carbon and nitrogen, trace elements, temperature, and aeration also affect their production by the organism.

Hydrophobic pollutants present in petroleum hydrocarbons, and soil and water environment require solubilization before being degraded by microbial cells. Mineralization is governed by desorption of hydrocarbons from soil. Surfactants can increase the surface area of hydrophobic materials, such as pesticides in soil and water environment, thereby increasing their water solubility. Hence, the presence of surfactants may increase microbial degradation of pollutants. Use of biosurfactants for degradation of pesticides in soil and water environment has gained importance only recently. The identification and characterization of biosurfactant produced by various microorganisms have been extensively reviewed[4–6]. Therefore, rather than describing the numerous types of biosurfactants and their properties, this article emphasizes the production of biosurfactants and their role in biodegradation of pesticides.

Microbiology

Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a hydrocarbon (C_xH_y), microorganisms facilitate their diffusion into the cell by producing a variety of substances, the biosurfactants. Some bacteria and yeasts excrete ionic surfactants which emulsify the C_xH_y substrate in the growth medium. Some examples of this group of BS are rhamnolipids which are produced by different *Pseudomonas* sp.[7–11], or the sophorolipids which are produced by several *Torulopsis* sp.[12–14]. Some other microorganisms are capable of changing the structure of their cell wall, which they achieve by synthesizing lipopolysaccharides or nonionic surfactants in their cell wall. Examples of this group are: *Candida lipolytica* and *C. tropicalis* which produce cell wall-bound lipopolysaccharides when growing on *n*-alkanes[15,16]; and *Rhodococcus erythropolis*, and many *Mycobacterium* sp. and *Arthrobacter* sp. which synthesize nonionic trehalose corynomycolates[14,17–23]. There are lipopolysaccharides, such as Emulsan, synthesized by *Acinetobacter* sp.[22,23], and lipoproteins or lipopeptides, such as Surfactin and Subtilisin, produced by *Bacillus subtilis*[24–26]. Other effective BS are: (i) Mycolates and Corynomycolates which are produced by *Rhodococcus* sp., *Corynebacteria* sp., *Mycobacteria* sp., and *Nocardia* sp.[24,27,28]; and (ii) ornithinlipids, which are produced by *Pseudomonas rubescens*, *Gluconobacter cerinus*, and *Thiobacillus ferroxidans*[29–31]. BS produced by various microorganisms together with their properties are listed in Table 1.

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Classification and chemical nature of biosurfactants

The microbial surfactants (MS) are complex molecules covering a wide range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc. Microorganisms also produce surfactants that are in some cases combination of many chemical types: referred to as the polymeric microbial surfactants (PMS). Many MS have been purified and their structures elucidated. While the high molecular weight MS are generally polyanionic heteropolysaccharides containing both polysaccharides and proteins, the low molecular weight MS are often glycolipids. The yield of MS varies with the nutritional environment of the growing microorganism. Intact microbial cells that have high cell surface hydrophobicity are themselves surfactants. In some cases, surfactants themselves play a natural role in growth of microbial cells on water-insoluble substrates like C_xH_y, sulphur, etc. Exocellular surfactants are involved in cell adhesion, emulsification, dispersion, flocculation, cell aggregation, and desorption phenomena. A broad classification of BS is given in Table 2. A very brief description of each group is given below.

Glycolipids

Glycolipids are the most common types of BS (ref. 32). The constituent mono-, di-, tri- and tetrasaccharides include glucose, mannose, galactose, glucuronic acid, rhamnose, and galactose sulphate. The fatty acid component usually has a composition similar to that of the phospholipids of the same microorganism. The glycolipids can be categorized as:

Trehalose lipids: The serpentine growth seen in many members of the genus *Mycobacterium* is due to the presence of trehalose esters on the cell surface[33,34]. Cord factors from different species of *Mycobacteria*[33,35–37], *Corynebacteria*[38], *Nocardia,* and *Brevibacteria* differ in size and structure of the mycolic acid esters.

Sophorolipids: These are produced by different strains of the yeast, *Torulopsis*. The sugar unit is the disaccharide sophorose which consists of two β-1,2-linked glucose units. The 6 and 6′ hydroxy groups are generally acetylated. The sophorolipids reduce surface tensions between individual molecules at the surface, although they are effective emulsifying agents[13,39,40]. The sophorolipids of *Torulopsis* have been reported to stimulate[41,42], inhibit[41,43], and have no effect[8] on growth of yeast on water-insoluble substrates.

Rhamnolipids: Some *Pseudomonas* sp. produce large quantities of a glycolipid consisting of two molecules of rhamnose and two molecules of β-hydroxydecanoic acid[44,45]. While the OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the OH group of the second acids is involved in ester formation. Since one of the

Table 1. Structural types of microbial surfactants

Surfactant	Source
Trehalose dimycolates	*Mycobacterium* sp.
Trehalose dicorynemycolates	*Nocardia* sp., *Rhodococcus* sp. *Arthrobacter* sp. *Corynebacterium* sp.
Rhamnolipids	*Pseudomonas* sp.
Sophorolipids	*Torulopsis* sp.
Aminoacid–lipids	
Lipopeptides	*Bacillus* sp., *Streptomyces* sp. *Corynebacterium* sp. *Mycobacterium* sp.
Ornithine–lipid	*Pseudomonas* sp., *Thiobacillus* sp. *Agrobacterium* sp. *Gluconobacter* sp.
Phospholipids	*Candida* sp., *Corynebacterium* sp. *Micrococcus* sp. *Thiobacillus* sp.
Fatty acids/ natural lipids	*Acinetobacter* sp., *Pseudomonas* sp. *Micrococcus* sp., *Mycococcus* sp. *Candida* sp., *Penicillium* sp. *Aspergillus* sp.

Table 2. Classification of biosurfactants

1. Glycolipids
- Trehalose lipids
- Sophorolipids
- Rhamnolipids

2. Fatty acids

3. Phospholipids

4. Surface active antibiotics
- Gramicidin S
- Polymixins
- Surfactin
- Antibiotic TA

5. Polymeric microbial surfactants
- Emulsan from *Acinetobacter calcoaceticus* RAG-1 (ATCC 31012)
- The polysaccharide protein complex of *Acinetobacter calcoaceticus* BD4
- Other *Acinetobacter* sp. emulsifiers
- Polysaccharide liquid complexes from yeasts
- Emulsifying protein PA from *Pseudomonas aeruginosa*
- Emulsifying and solubilizing factors from *Pseudomonas* sp. PG-1
- Bioflocculant and emulcyan from the filamentous *Cyanobacterium phormidium* J-1

6. Particulate surfactants
- Extracellular vesicles from *Acinetobacter* sp. 1101-N
- Microbial cells with high cell surface hydrophobicities

carboxylic acid is free, the rhamnolipids are anions above pH 4.0. Rhamnolipids are reported[46] to lower surface tension, emulsify C_xH_y, and stimulate growth of *Pseudomonas* on *n*-hexadecane. Formation of rhamnolipids by *Pseudomonas* sp. MVB was greatly increased by nitrogen limitations[47]. The pure rhamnolipid lowered the interfacial tension against *n*-hexadecane in water to about 1 mN/m and had a critical micellar concentration (cmc) of 10 to 30 mg/l depending on the pH and salt conditions[48].

Fatty acids

The fatty acids produced from alkanes by microbial oxidations have received maximum attention as surfactants[49]. Besides the straight-chain acids, microorganisms produce complex fatty acids containing OH groups and alkyl branches. Some of these complex acids, for example corynomucolic acids, are surfactants[24,28,50].

Phospholipids

These are major components of microbial membranes. When certain C_xH_y-degrading bacteria[51–53] or yeast[54–56] are grown on alkane substrates, the level of phospholipids increases greatly. Phospholipids from hexadecane-grown *Acinetobacter* sp. have potent surfactant properties. Phospholipids produced by *Thiobacillus thiooxidans* have been reported to be responsible for wetting elemental sulphur, which is necessary for growth[57,58].

Surface active antibiotics

Gramicidin S: Many bacteria produce a cyclosymmetric decapeptide antibiotic, gramicidin S. Spore preparations of *Brevibacterium brevis* contain large amounts of gramicidin S bound strongly to the outer surface of the spores[59,60]. Mutants lacking gramicidin S germinate rapidly and do not have a lipophilic surface[61]. The antibacterial activity of gramicidin S is due to its high surface activity[62–65].

Polymixins: These are a group of antibiotics produced by *Brevibacterium polymyxa* and related bacilli[66]. Polymixin B is a decapeptide in which amino acids 3 through 10 form a cyclic octapeptide. A branched chain fatty acid is connected to the terminal 2,4-diaminobutyric acid (DAB). Polymixins are able to solubilize certain membrane enzymes[67].

Surfactin (subtilysin): One of the most active biosurfactants produced by *B. subtilis* is a cyclic lipopeptide surfactin[26,68]. The yield of surfactin produced by *B. subtilis* can be improved to around 0.8 g/l by continuously removing the surfactant by foam fractionation and addition of either iron or manganese salts to the growth medium[24].

Antibiotic TA: *Myxococcus xanthus* produces antibiotic TA which inhibits peptidoglycan synthesis by interfering with polymerization of the lipid disaccharide pentapeptide[69]. Antibiotic TA has interesting chemotherapeutic applications[70].

Polymeric microbial surfactants

Most of these are polymeric heterosaccharide containing proteins.

Acinetobacter calcoaceticus RAG-1 (ATCC 31012) emulsan: A bacterium, RAG-1, was isolated during an investigation of a factor that limited the degradation of crude oil in sea water. This bacterium efficiently emulsified C_xH_y in water[71]. This bacterium, *Acinetobacter calcoaceticus*, was later successfully used to clear a cargo compartment of an oil tanker during its ballast voyage[22,72]. The cleaning phenomenon was due to the production of an extracellular, high molecular weight emulsifying factor[22], emulsan.

The polysaccharide protein complex of Acinetobacter calcoaceticus BD413: A mutant of *A. calcoaceticus* BD4, excreted large amounts of polysaccharide together with proteins. The emulsifying activity required the presence of both polysaccharide and proteins[73,74].

Other Acinetobacter emulsifiers: Extracellular emulsifier production is widespread in the genus Acinetobacter. In one survey[75], 8 to 16 strains of *A. calcaoceticus* produced high amounts of emulsifier following growth on ethanol medium[76,77]. This extracellular fraction was extremely active in breaking (de-emulsifying) kerosene/water emulsion stabilized by a mixture of Tween 60 and Span 60.

Polysaccharide-lipid complexes from yeast: The partially purified emulsifier, liposan, was reported to contain about 95% carbohydrate and 5% protein[78]. A C_xH_y-degrading yeast, *Endomycopsis lipolytica* YM, produced an unstable alkane-solubilizing factor[79]. *Torulopsis petrophilum* produced different types of surfactants depending on the growth medium[39]. On water-insoluble substrates, the yeast produced glycolipids which were incapable of stabilizing emulsions. When glucose was the substrate, the yeast produced a potent emulsifier.

Emulsifying protein (PA) from Pseudomonas aeruginosa: The bacterium *P. aeruginosa* has been observed to excrete a protein emulsifier. This protein PA is produced

from long-chain *n*-alkanes, 1-hexadecane, and acetyl alcohol substrates; but not from glucose, glycerol or palmitic acid. The protein has a MW of 14,000 Da and is rich in serine and threonine[80].

Surfactants from Pseudomonas PG-1: *Pseudomonas* PG-1 is an extremely efficient hydrocarbon-solubilizing bacterium. It utilizes a wide range of C_xH_y including gaseous volatile and liquid alkanes, alkenes, pristane, and alkyl benzenes[79,81,82].

Bioflocculant and emulcyan from the filamentous Cyanobacterium phormidium J-1: The change in cell surface hydrophobicity of *Cyanobacterium phormidium* was correlated with the production of an emulsifying agent, emulcyan[85]. The partially purified emulcyan has a MW greater than 10,000 Da and contains carbohydrate, protein and fatty acid esters. Addition of emulcyan to adherent hydrophobic cells resulted in their becomeing hydrophilic and detach from hexadecane droplets or phenyl sepharose beads.

Particulate surfactants

Extracellular vesicles from Acinetobacter sp. H01-N: *Acinetobacter* sp. when grown on hexadecane, accumulated extracellular vesicles of 20 to 50 mm diameter with a buoyant density of 1.158 g/cm^3. These vesicles appear to play a role in the uptake of alkanes by *Acinetobacter* sp. HO1-N. (refs 57, 84).

Microbial cells with high cell surface hydrophobicities: Most hydrocarbon-degrading microorganisms, many nonhydrocarbon degraders, some species of *Cyanobacteria*[85], and some pathogens have a strong affinity for hydrocarbon-water[70] and air-water[86,87] interfaces. In such cases, the microbial cell itself is a surfactant.

Factors affecting biosurfactant production

Biosurfactants (BS) are amphiphilic compounds. They contain a hydrophobic and hydrophilic moiety. The polar moiety can be a carbohydrate, an amino acid, a phosphate group, or some other compound. The nonpolar moiety is mostly a long-carbon-chain fatty acid. Although the various BS possess different structures, there are some general phenomena concerning their biosynthesis. For example, BS production can be induced by hydrocarbons or other water-insoluble substrates[88]. This effect, described by different authors, refers to many of the interfacially active compounds. Another striking phenomena is the catabolic repression of BS synthesis by glucose and other primary metabolites. For example, in the case of *Arthrobacter paraffineus*, no surface-active agent could be isolated from the medium when glucose was used as the carbon source instead of hexadecane[89]. Similarly, a protein-like activator for *n*-alkane oxidation was formed by *P. aeruginosa* S7B1 from hydrocarbon, but not from glucose, glycerol, or palmitic acid[80,81]. *Torulopsis petrophilum* did not produce any glycolipids when grown on a single-phase medium that contained water-soluble carbon source[13]. When glycerol was used as substrate, rhamnolipid production by *P. aeruginosa* was sharply reduced by adding glucose, acetate, succinate or citrate to the medium[8,10].

Olive oil mill effluent, a major pollutant of the agricultural industry in mediterranian countries, has been used as raw material for rhamnolipid biosurfactant production by *Pseudomonas* sp. JAMM. Many microorganisms are known to synthesize different types of biosurfactants when grown on several carbon sources[6,90]. However, there have been examples of the use of a water-soluble substrate for biosurfactant production by microorganisms[91,92]. The type, quality and quantity of biosurfactant produced are influenced by the nature of the carbon substrate[93], the concentration of N, P, Mg, Fe, and Mn ions in the medium[9,24,94,95], and the culture conditions, such as pH, temperature, agitation and dilution rate in continous culture[9,95–97].

Biosurfactant production from *Pseudomonas* strains MEOR 171 and MEOR 172 are not affected by temperature, pH, and Ca, Mg, concentration in the ranges found in many oil reserviors. Their production, on the other hand, in many cases improves with increased salinity. Thus, they are the biosurfactants of choice for the Venezuelan oil industry and in the cosmetics, food, and pharmaceutical markets.

The nitrogen source can be an important key to the regulation of BS synthesis. *Arthrobacter paraffineus* ATCC 19558 preferred ammonium to nitrate as inorganic nitrogen source for BS production. Urea also result in increased BS production[89]. A change in growth rate of the concerned microorganisms is often sufficient to result in over production of BS (ref. 27). In some cases[24], addition of multivalent cations to the culture medium can have a positive effect on BS production. Besides the regulation of BS by chemicals indicated above, some compounds like ethambutol[20,98], penicillin[99], chloramphenicol[23], and EDTA[79,100] influenced the formation of interfacially active compounds. The regulation of BS production by these compounds is either through their effect on solubilization of nonpolar hydrocarbon substrates or by increased production of water-soluble (polar) substrates. In some cases, BS synthesis is regulated by pH and temperature. For example in rhamnolipid production by *Pseudomonas* sp.[101,102], in cellobioselipid formation by *Ustilago maydis*[103], and in sophorolipid formation by *Torulopsis bombicola*[42], pH played an important role, and in the case

of *Arthrobacter paraffineus* ATCC 19558 (ref. 104), *Rhodococcus erythropolis*[101,102], and *Pseudomonas* sp. DSM 2874 (refs 47, 102) temperature was important. In all these cases however the yield of BS production was temperature dependent.

Applications of biosurfactants in pollution control

The identification and characterization of microbial surfactants produced by various microorganisms have been extensively reviewed[6,88,105–107]. Therefore rather than describing numeric types of MS, it is proposed to examine potential applications of MS.

Microbial enhanced oil recovery

An area of considerable potential for BS application is microbial enhanced oil recovery (MEOR). In MEOR, microorganisms in reservoir are stimulated to produce polymers and surfactants which aid MEOR by lowering interfacial tension at the oil–rock interface. To produce MS *in situ*, microorganisms in the reservoir are usually provided with low-cost substrates, such as molasses and inorganic nutrients, to promote growth and surfactant production. To be useful for MEOR *in situ*, bacteria must be able to grow under extreme conditions encountered in oil reservoirs such as high temperature, pressure, salinity, and low oxygen level. Several aerobic and anaerobic thermophiles tolerant of pressure and moderate salinity have been isolated which are able to mobilize crude oil in the laboratory[108,109]. Clark *et al.*[110], based on a computer search estimated that about 27% of oil reservoirs in USA are amenable to microbial growth and MEOR. The effectiveness of MEOR has been reported in field studies carried out in US, Czechoslovakia, Romania, USSR, Hungary, Poland, and The Netherlands. Significant increase in oil recovery was noted in some cases[111].

Hydrocarbon degradation

Hydrocarbon-utilizing microorganisms excrete a variety of biosurfactants. BS being natural products, are biodegradable and consequently environmentally safe. An important group of BS is mycolic acids which are the α-alkyl, β-hydroxy very long-chain fatty acids contributing to some characteristic properties of a cell such as acid fastness, hydrophobicity, adherability, and pathogenicity. Enriching waters and soils with long- and short-chain mycolic acids may be potentially hazardous. Daffe *et al.*[112] reported trehalose polyphthienoylates as a specific glycolipid in virulent strains of *Mycobacterium tuberculosis*. Kaneda *et al.*[113] reported that granuloma formation and hemopoiesis could be induced by C36–C48 mycolic acid-containing glycolipids from *Nocardia rubra*. Biolid extract (BE), obtained as a byproduct during the production of fodder yeast, is a dark brown heavy fluid with a characteristic odour and high interfacial activity. This product has many applications in agrochemistry, mineral flotation, and bitumen production and processing. Potentially, the product may be used as an emulsifying and dispersing agent while formulating herbicides, pesticides, and growth regulator preparations. Including phospholipids in formulations, facilitate penetration of active substances into the plant tissues[114], making it possible to apply only very low concentrations of the substances[115]. The constituent fatty acids of biolipid extract have antiphytoviral and antifungal activities and therefore, can be applied in controlling plant diseases[116]. These fatty acids also increase stress tolerance of plants, leading thereby to higher yields despite physiological drought[117].

Hydrocarbon degradation in the soil environment

C_xH_y degradation in soil has been extensively studied[31,95,118–122]. Degradation is dependent on presence in soil of hydrocarbon-degrading species of microorganisms, hydrocarbon composition, oxygen availability, water, temperature, pH, and inorganic nutrients. The physical state of C_xH_y can also affect biodegradation. Addition of synthetic surfactants or MS resulted in increased mobility and solubility of C_xH_y, which is essential for effective microbial degradation[122].

Use of MS in C_xH_y degradation has produced variable results. In the work of Lindley and Heydeman[123], the fungus *Cladosporium resiuae*, grown on alkane mixtures, produced extracellular fatty acids and phospholipids, mainly dodecanoic acid and phosphatidylcholine. Supplement of the growth medium with phosphatidylcholine enhanced the alkane degradation rate by 30%. Foght *et al.*[124] reported that the emulsifier, Emulsan, stimulated aromatic mineralization by pure bacterial cultures, but inhibited the degradation process when mixed cultures were used. Oberbremer and Muller-Harting[125] used mixed soil population to assess C_xH_y degradation in model oil. Naphthalene was utilized in the first phase of C_xH_y degradation; other oil components were degraded during the second phase after the surfactants produced by concerned microorganisms lowered the interfacial tension. Addition of biosurfactants, such as some sophorolipids, increased both the extent of degradation and final biomass yield[126].

Biodetox (Germany) described a process to decontaminate soils, industrial sludges, and waste waters[127]. They also described *in situ* bioreclamation of contaminated surface, deep ground and ground water. Microorganisms were added by means of a biodetox foam that contained bacteria, nutrients and surfactants; and was biodegradable.

Another method to remove oil contaminants is to add BS into contaminated soil to increase C_xH_y mobility. The emulsified C_xH_y could then be recovered by using a production well, and subsequently degrading above ground in a bioreactor. *In situ* washing of soil was studied using two synthetic surfactants, Adsee 799 and Hyonic NP-90 (ref. 128). Removal of PCBs and petroleum C_xH_y from soil by adding surfactants to the wash water, has met with some success[129].

Several strains of anaerobic bacteria produce biosurfactants[130,131]. However, the observed reduction in surface tension (45 to 50 mN/m) was not as large as the observed reduction in surface tension by anaerobic organisms (27 to 50 mN/m) (ref. 106). MS can also be used to enhance solubilization of toxic organic chemicals including xenobiotics. Berg *et al.*[132], using the surfactant from *Pseudomonas aeruginosa* UG2, reported an increase in the solubility of hexachlorobiphenyl added to soil slurries, which resulted in a 31% recovery of the compound in the aqueous phase. This was about 3-times higher than that solubilized by the chemical surfactant sodium ligninsulfonate (9.3%). When the *P. aeruginosa* bioemulsifier and sodium ligninsulphonate were used together, additive effect on solubilization (41.5%) was observed. *Pseudomonas ceparia* AC 1100 produced an emulsifier that formed a stable suspension with 2,4,5-T, and also exhibited some emulsifying activity against chlorophenols[133]. Thus, this emulsifier can be used to enhance bacterial degradation of organochlorine compounds.

Hydrocarbon degradation in aquatic environment

When oil is spilled in aquatic environment, the lighter hydrocarbon components volatilize while the polar hydrocarbon components dissolve in water. However, because of low solubility (< 1 ppm) of oil, most of the oil components will remain on the water surface. The primary means of hydrocarbon removal are photooxidation, evaporation, and microbial degradation. Since C_xH_y-degrading organisms are present in seawater, biodegradation may be one of the most efficient methods of removing pollutants[95, 134]. Surfactants enhance degradation by dispersing and emulsifying hydrocarbons. Microorganisms that are able to degrade C_xH_y have been isolated from aquatic environment. These microorganisms which exhibit emulsifying activity as well as the soil microorganisms which produced surfactants may be useful in aquatic environment. Chakrabarty[136] reported that an emulsifier produced by *P. aeruginosa* SB30 was able to quickly disperse oil into fine droplets; therefore it may be useful in removing oil from contaminated beaches[135]. BS produced by oil-degrading bacteria may be useful in cleaning oil tanks. When an oil tanker compartment containing oily ballast water was supplemented with urea and K_2HPO_4 and aerated for 4 days, the tanker was completely free of the thick layer of sludge that remained in the control tanker[137]. Presumably this was owing to the surfactant produced, when growth of the natural bacterial population was enhanced.

Surfactants have been studied for their use in reducing viscosity of heavy oils, thereby facilitating recovery, transportation, and pipelining[138,139]. Emulsan, a high MW lipopolysacharide produced by *A. calcaoceticus* RAG-1, has been proposed for a number of applications in the petroleum industry such as to clean oil and sludge from barges and tanks, reduce viscosity of heavy oils, enhance oil recovery, and stabilize water-in-oil emulsions in fuels[140,141]. Specific solubilization of various C_xH_y types during growth of prokaryotic organism was demonstrated by Reddy *et al.*[79,81]. The specific solubilization of C_xH_y was strongly inhibited by EDTA which was overcome by excess Ca^{++}. It was concluded that specific solubilization of C_xH_y is an important mechanism in the microbial uptake of C_xH_y.

Pesticide-specific biosurfactants

Due to biodegradative property of biosurfactants, they are ideally suited for environmental applications, specially for removal of the pesticides—an important step in bioremediation. Survey of the literature reveals that application of biosurfactants in the field of pesticides is still in its infancy compared to the field of hydrocarbons. In India, a number of laboratories have initiated studies on BS. Some of the earlier works are by: (i) Banarjee *et al.*[133] on 2,4,5-tricholoacetic acid, (ii) Patel and Gopinath on Fenthion[142], and (iii) Anu Appaiah and Karanth[143] on alpha HCH. Very recently reports on production of microbial BS, based on preliminary studies by several groups, have appeared in posters/proceedings of symposia[144–148]. The noteworthy feature being the increasing interest shown by the various researchers on: (i) degradation of pesticides[149–152], (ii) production and exploitation of BS for the removal of pesticides from the environment, and (iii) postulates on the possible replacement of synthetic surfactants with the biosurfactants in the pesticide formulation and clean-up[153–156].

Biosurfactant and HCH degradation

Hexa-chlorocyclohexane (HCH) is still the highest ranking pesticide used in India and many other countries. Of the eight known isomers of HCH, the alpha-form constitutes more than 70% of the technical product, which is not only noninsecticidal but also a suspected carcinogen. The use of technical HCH, which is a mixture of isomers, will continue in the Indian market because of their all-time availability with good insecticidal efficiency

and at a price which is 10–12 times less than that of the pure gamma HCH (Lindane). It is pertinent to note that the environment burden of already-dumped HCH continues to pose threat to all forms of 'life'. The poor solubility is one of the limiting factors in the microbial degradation of alpha-HCH. Presence of six chlorines in the molecule is another factor that renders HCH lipophilic and persistent in the biosphere.

Even though several reports are available on biodegradation of specific isomers of HCH in animals, plants, soil and microbial systems, literature on metabolism of alpha-HCH by microorganisms is limited. Furthermore, the exact mechnism of translocation of HCH to the site of destruction and degradation of alpha-HCH in bacteria is not well understood.

During the course of our work at CFTRI on the bacterial degradation of alpha-HCH, we isolated several bacterial strains capable of degrading HCH. One of the strains efficient in HCH degradation was characterized as *Pseudomonas Ptm*$^+$ strain. The CFTRI isolate produced extracellular biosurfactant in a mineral medium containing HCH. While this BS emulsified the solid organochlorine-HCH to a higher extent, it emulsified other organochlorines such as DDT and cyclodienes to a lesser extent[156], implying thereby the specificity of the BS in dispersing HCH. It was also demonstrated that the peak in production of the emulsifier appeared before the onset of HCH degradation by the *Pseudomonas* growing in liquid culture. The role of biosurfactant in the HCH degradation was ascertained using partially purified BS. The extracellular BS was a macro-molecule containing lipid, carbohydrate, and protein moieties. The carbohydrate part was identified as rhamnose by different analytical methods. The rhamnose part of the BS was stable and was necessary for the BS activity. Careful investigations revealed that the protein fraction represented the proximal enzymes of HCH metabolism. In the presence of BS, HCH was converted through the involvement of isomerase and dechlorinase to tertachlorohexenes and then to chlorophenols[157].

Figure 1. Biosurfactant as a cleaning agent: *left*, HCH sticking to the walls of the bottle which does not get cleaned by rinsing in water; *right*, biosurfactant becoming turbid as it cleans and removes HCH from the glass surface.

The BS acted by increasing the surface area of HCH, which accelerated this transformation. Hence, it is evident that extracellular BS has a definite role in HCH degradation by CFTRI strain of *Pseudomonas Ptm*$^+$. Production of BS for Fenthion, a liqiud OP insecticide, has also received attention. *Bacillus subtilis* excreted the BS both in liqiud as well as in solid state fermentation system[146,147]. The microbial surfactant produced by these two organisms also shows properties of a good cleansing agent for dislodging the pesticides from used containers, mixing tanks, cargo docks, etc. Attempts have also been made to standardize parameters for BS production both in liquid and solid state fermentations. A limited number of scale-up studies indicate good scope for expolitation of BS in industries.

Table 3. Varied industrial uses of biosurfactants for their desired functions and properties

	Industrial uses										
Function	Agriculture	Building/ construction	Elastomers/ plastics	Foods and cleaning	Insdustrial cleaning	Leathers	Metals	Paper	Paint/ protective coating	Petroleum/ petrochemicals production	Textiles
Emulsification	x		x	x		x	x		x	x	x
Deemulsification										x	
Wetting, spreading, penetration	x	x	x	x	x	x	x	x	x	x	x
Solubilization and soild dispersal	x		x	x						x	x
Air entrapment foaming		x	x	x			x			x	
Detergency				x	x	x		x		x	x
Defoaming				x				x			
Antistatic			x						x		x
Corrosion inhibition					x					x	

In a separate study, it has been shown that addition of BS from *Pseudomonas Ptm*$^+$ strain facilitied 250-fold increase in dispersion of HCH in water. Addition of either this organism or BS dislodged surface-borne HCH residues from many types of fruits, seeds and vegetables[158] as well. Laboratory-scale studies have revealed that BS is very efficient in cleaning the containers where HCH residues were sticking to the wall (Figure 1). Studies using fermentor for large-scale production of this BS from *Pseudomonas Ptm*$^+$ have been carried out[159]. A bioformulation is planned from this BS for effective removal of HCH from contaminated soils.

Table 4. Consumption rate and estimated demand for synthetic surfactants[162]

Surfactant	1994	2000	%Annual growth (1994–2000)
	($kg \times 10^6$)		
Anionic	2162	2518	26.0
Nonionic	850	1008	2.9
Cationic	315	387	3.5
Amphoteric	24	34	6.0
Total	**3351**	**3947**	**2.8**

Other applications

By virtue of properties of biodegradability, substrate specificity, chemical and functional diversity, and rapid/controlled inactivation, biosurfactants are gaining importance in various industires like agriculture, food, textiles, petrochemicals, etc. The potential applications of biosurfactants having desired functions and properties are listed in Table 3[94,160,161]. The current consumption rate and estimated demand pattern for synthetic surfactants are shown in Table 4. Number of patents available on the subject are given in Table 5.

BS from some other bacterial taxa may be of public health concern. Methylrhamnolipids from *Pseudomonas aeroginosa* have cytotoxic effects[163]. Lipopolyglycans from mycoplasmas show endotoxic properties, potentially inducing procoagulant activity in human leukocytes[164]. The toxicity and antigenic properties of mycobacterial

Table 5. Patents on biosurfactants

Microorganisms/process	Name	Patent detail
Torulopsis bombicola	Spencer *et al.*, 1965	US Pat. 3,205,150
	Spencer *et al.*, 1967	US Pat. 3,312, 684
	Spencer *et al.*, 1969	S Pat. 3,445,337
Corynebacterium hydrocarboclastus	Zajic and Knettig; 1976	Can Pat. 990,668
	Zajic and Knettig, 1976	US Pat. 3,997,398
	Zajic *et al.*, 1977	Can Pat. 1,125,683
Candida sp. of synthetic fibers	Hieke et *al.*, 1979	Ger. Pat. 139,069
	Research Institute, 1985	Jp. Pat. 60,35,003
Nocardia rhodochrous	Wagner *et al.*, 1979	DE Pat. 2,805,823
Yeast sp.	Wagner *et al.*, 1984	DE Pat. 3,248,167
Acinetobacter calcoaceticus	Gutnick and Rosenberg, 1980	Jp. Pat. 80,112,201
	Gutnick *et al.*, 1980	US Pat. 4,234,689
Nocardia BPM 1613	Agency of Industrial Science and Technology, 1982	Jp. Pat. 57,150,391
		Jp Pat. 57,150,392
Corynebacterium lepus OSGB-1 and *Acinetobacter* ATCC 31 012	Canadian Patent Development Ltd., Japan Kokai Tokyo, Koho, 1980	Can. Pat. 1,114,759
		Jp. Pat. 80,112,201
Corynebacterium salvinicum SFC	Zajic *et al.*, 1982	Can. Pat. 1,125,683
	Zajic and Gerson, 1982	Can. Pat. 1,114,759
Biosurfactant recovery in petroleum industry	Wagner *et al.*, 1982	Can. Pat. 1,119,794
	Sculz *et al.*, 1983	UK Pat.GB2,953, 182
Acinetobacter sp.	Gutnick *et al.*, 1983	UK Pat. GB 4,395,354
Corynebacterium sp., *Nocardia* sp., *Bacillus* sp., *Arthrobacter* sp., and *Alcaligenes* sp.	KAO Chemicals Co., 1984	Aust. Pat. 8317-555
Quaternary oil recovery process using MEOR	Hitzman, 1984	US Pat. 4,450,908
Pseudomonas mendocina	Jarman and Hacking, 1984	Eur Pat. 78,643
Bacillus polymyxa	Cox and Steer, 1984	US Pat. 4,483,782
Alcaligenes ATCC 31853	Peik *et al.*, 1985	Eur. Pat. EP 64,354
Arthrobacter terregens	Zajic and Gerson, 1987	US Pat. 4,640,767
Trehalose lipid-producing organisms	Lang, 1988	US Pat. 4,720,456
Acinetobacter calcoaceticus	Gutnick *et al.*, 1989	US Pat. 4,883,757
	Hayes, 1989	US Pat. 4,870,010
Streptomyces setonii and *S. Viridiosporus*	Strandberg and Lewis, 1990	US Pat. 4,914,024
Pseudomonas putida	Vandenbergh, 1990	US Pat. 4,910,143
P. aeruginosa SB-30	Banerjee *et al.*, 1991	US Pat 5,013,654

glycolipids, produced by pathogenic mycobacteria such as *M. avium-intracellure*, *M. scrofulaceum*, and *M. fortulitum*, which are habitats of water polluted with industrial and domestic residues, are well known[165,166]. The varied uses of BS also imply scope for MS, and the need to strengthen the research in this emerging area.

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MEETINGS/SYMPOSIA/SEMINARS

36th Annual Convention and Meeting on Earth System Sciences in the next Millennium

Date: 21–23 December 1999
Place: Pondicherry

Earth System Sciences in the next Millennium (Special reference to Groundwater & Echo System Management, Oil and Mineral Sector, Space Applications and Ocean Resources).

Besides the above theme, original research contributions are invited for presentation in the Annual Convention on the following fields: Imaging techniques in exploration; Integrated geophysical techniques: Solid earth geophysics; Studies on deep continental structure; Geophysical exploration; Isotope studies; Marine geosciences; Atmospheric sciences; Geodynamics; Space sciences and planetology.

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