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MINI-REVIEW

I. M. Banat · R. S. Makkar · S. S. Cameotra

Potential commercial applications of microbial surfactants

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Abstract Surfactants are surface-active compounds capable of reducing surface and interfacial tension at the interfaces between liquids, solids and gases, thereby allowing them to mix or disperse readily as emulsions in water or other liquids. The enormous market demand for surfactants is currently met by numerous synthetic, mainly petroleum-based, chemical surfactants. These compounds are usually toxic to the environment and non-biodegradable. They may bio-accumulate and their production, processes and by-products can be environmentally hazardous. Tightening environmental regulations and increasing awareness for the need to protect the ecosystem have effectively resulted in an increasing interest in biosurfactants as possible alternatives to chemical surfactants. Biosurfactants are amphiphilic compounds of microbial origin with considerable potential in commercial applications within various industries. They have advantages over their chemical counterparts in biodegradability and effectiveness at extreme temperature or pH and in having lower toxicity. Biosurfactants are beginning to acquire a status as potential performance-effective molecules in various fields. At present biosurfactants are mainly used in studies on enhanced oil recovery and hydrocarbon bioremediation. The solubilization and emulsification of toxic chemicals by biosurfactants have also been reported. Biosurfactants also have potential applications in agriculture, cosmetics, pharmaceuticals, detergents, personal care products, food processing, textile manufacturing, laundry supplies, metal treatment and processing, pulp and paper processing and paint industries. Their uses and potential commercial applications in these fields are reviewed.

I. M. Banat (✉)
Biotechnology Group, Faculty of Science,
University of Ulster, Coleraine, BT52 1SA, Northern Ireland
e-mail: IM.Banat@ulst.ac.uk
Tel.: +44-1265-323062
Fax: +44-1265-324906

S. S. Cameotra · R. S. Makkar
Institute of Microbial Technology (IMTECH), Sector 39-A,
Chandigarh-160036, India

Present addresses:
R. S. Makkar
Biomaterials Conversion Laboratory,
National Food Research Institute,
Tsukuba, Ibaraki 305-8642, Japan

S. S. Cameotra
Soil Biochemistry Laboratory,
129 Land and Water Building,
Pennsylvania State University,
University Park PA 16802

Introduction

In the new era of global industrialization where many classical industries are being de-emphasized and redirected towards emerging technologies, biotechnology has a challenging edge that is opening several research opportunities. The biotechnology world market was US $25 million in 1980. It increased to around US $1.7 billion in 1992 and is expected to go beyond US $500 billion by the end of century (Muller et al. 1997).

Surfactants constitute an important class of industrial chemicals widely used in almost every sector of modern industry. During the last decade demand for surfactants increased about 300% within the US chemical industry (Greek 1990). Current worldwide production exceeds three million tonnes per annum (at an estimated value of US $4 billion) and is expected to rise to over four million tonnes by the end of the century (Greek 1991; Sarney and Vulfson 1995). About 54% of the total surfactant output is utilized in household/laundry detergents with only 32% destined for industrial use. Most of the commercially available surfactants are chemical surfactants, mainly petroleum-derived. However, rapid advances in biotechnology and increased environmental awareness among consumers, combined with expected new legislation, has provided further impetus for serious consideration of biological surfactants as possible alternatives to existing products.

Biosurfactants have therefore gained considerable interest in recent years due to their low toxicity, biodegradable nature and diversity. Their range of potential industrial applications includes enhanced oil recovery, crude oil drilling, lubricants, surfactant-aided bioremediation of water-insoluble pollutants, health care and food processing (Fiechter 1992; Muller-Hurtig et al. 1993; Velikonja and Kosaric 1993; Finnerty 1994; Lin 1996; Desai and Banat 1997; Sullivan 1998). Other developing areas for biosurfactants are in cosmetic and soap formulations, foods and both dermal and transdermal drug delivery systems, as reflected in Japanese patent literature.

Synthetic surfactants and biosurfactants

Surfactants are amphiphilic molecules consisting of a hydrophilic and a hydrophobic domain. The non-polar, hydrophobic part is frequently a hydrocarbon chain. The polar component appears in many variations (Georgiou et al. 1992). The most common non-ionic surfactants are ethoxylates, ethylene and propylene oxide co-polymers and sorbitan esters. Examples of commercially available ionic surfactants include fatty acids, ester sulphonates or sulphates (anionic) and quartenary ammonium salts (cationic). Microbial compounds which exhibit particularly high surface activity and emulsifying activity are classified as biosurfactants. Biosurfactants are structurally diverse compounds, mainly produced by hydrocarbon-utilizing microorganisms which exhibit surface activity. Biosurfactants can be produced using relatively simple and inexpensive procedures and substrates (Kosaric 1992; Lang and Wullbrandt 1999; Makkar and Cameotra 1999).

Some structural types of surfactant are produced using biological systems and cannot easily be synthesized by chemical processes (Gerson and Zajic 1979). These molecules can be tailor-made to suit different applications by changing the growth substrate or growth conditions (Fiechter 1992). Biosurfactants are both biodegradable, which is a positive ecological aspect (Zajic et al. 1977a; Shoham et al. 1983; Oberbremer et al. 1990; Kesting et al. 1996) and non-toxic or less toxic than chemical surfactants (Poremba et al. 1991a, b; Van Dyke et al. 1991; Flasz et al. 1998). They occur naturally in soil, which makes them acceptable from a social and ecological point of view. There are many potentially useful biosurfactants, including both ionic and non-ionic surfactants which range from short fatty acids to large polymers (Table 1). This wide range results in a broad spectrum of potential industrial applications.

In this article we review the latest developments in biosurfactant applications and discuss their increased potential role in newly emerging fields for their application.

Biosurfactant applications

The largest possible market for biosurfactant is the oil industry, both for petroleum production and for incorporation into oil formulations (Van Dyke et al. 1991). Other applications related to the oil industries includes oil spill bioremediation/dispersion, both inland and at sea, removal/mobilization of oil sludge from storage

Table 1 Major types of biosurfactants produced by microorganisms

Microorganism	Biosurfactant type	Reference
Norcardia SFC-D	Trehalose lipid	Kosaric et al. 1990
Rhodococcus sp. H13 A	Trehalose lipid	Singer et al. 1990
Rhodococcus sp. ST-5	Trehalose lipid	Abu Ruwaida et al. 1991a
Pseudomonas aeruginosa GL-1	Rhamnolipiod	Arino et al. 1996
Pseudomonas aeruginosa UW-1	Rhamnolipid	Sim et al. 1997
Pseudomonas aeruginosa GL-1	Rhamnolipid	Patel and Desai 1997
Alcanivorax borkumensis	Glycolipid	Abraham et al. 1998
Tsukamurella sp.	Glycolipid	Vollbrecht et al. 1998
Serratia rubidea	Glycolipid	Matsuyama et al. 1990
Serratia marcescens	Glycolipid	Pruthi and Cameotra 1997b
Candida antarctica	Mannosylerythritol lipids	Kitamoto et al. 1993
Candida bombicola	Sophorose lipid	Brakemeier et al. 1995
Candida apicola IMET 43747	Sophorose lipid	Hommel et al. 1994
Bacillus pumilus A1	Surfactin	Morikawa et al. 1992
Bacillus subtilis	Surfactin	Makkar and Cameotra 1997
Bacillus subtilis C 9	Surfactin	Kim et al. 1997
Bacillus licheniformis	Lichenysin A	Yakimov et al. 1995
Bacillus licheniformis JF-2	Lichenysin B	Lin et al. 1994
Arthrobacter sp. EK1	Trehalose tetraester	Schulz et al. 1991
Arthrobacter sp. MIS 38	Arthrofactin	Morikawa et al. 1993
Lactobacillus sp.	Surfactin	Velraeds-Martine et al. 1996b
Pseudomonas fluorescens	Viscosin	Laycock et al. 1991
Streptomyces tendae TU901/8c	Streptofactin	Richter et al. 1998
Acinetobacter radioresistens	Alasan	Navon-Venezia et al. 1995
Pseudomonas marginalis PD 14 B	Particulate-surfactant (PM factor)	Burd and Ward 1996
Pseudomonas maltophilla CSV 89	Biosur Pm	Phalle et al. 1995

tanks and enhanced oil recovery (Georgiou et al. 1992; Khire and Khan 1994a, b). The second largest market for biosurfactants is emulsion polymerization for paints, paper coatings and industrial coatings. Layman (1985) described other uses of surfactants including asphalt, cement, textile and fiber manufacturing, in addition to metal treatment, mining, water treatment, coal slurry defoamers and as wood preservatives.

Surfactants are also used in food and cosmetic industries, industrial cleaning of products and in agricultural chemicals to dilute and disperse fertilizers and pesticides and to enhance penetration of active compounds into plants (Kosaric et al. 1987). Comprehensive details of various potential applications for biosurfactants as fine specialty chemicals is shown in Table 2. Ishigami (1997) has speculated various on potential applications for biosurfactants in bioengineering including their use as cryopreservatives, protein solubilizers, enzyme stabilizers, DNA isolating agents, preservatives for cut flowers, growth enhancers for plants, recovery enhancers for wounds and swelling and for the control of biomembranous functions.

Biosurfactants and bioremediation

Bioremediation in general aims at providing cost effective, contaminant-specific treatments to reduce the concentration of individual or mixed environmental contaminants (Head 1998). Bartha (1986) estimated that approximately 0.08–0.4% of the total worldwide production of petroleum eventually reaches the oceans. Several oil spill accidents in recent years have resulted in significant contamination of oceans and shoreline environments. Well known examples include the Amoco Cadiz oil spill in Brittany coastal waters in 1978, the Exxon Valdez spill in the Prince William Sound in 1989 and the Haven spill off the coast of Italy in 1991. More recent examples include the Nakhodka tanker oil spill off the Oki Islands in the Sea of Japan, 1997, the San Jorge tanker spill on the shores of Punta Del Este in Uruguay in 1997 and the Nissos Amorgos spill in the Maracaibo Channel in the Gulf of Venezuela in 1997. Apart from these accidental spills, deliberate releases of oil have also caused considerable contamination. During the Gulf War in 1991 over 10^5 tonnes of oil were released in the Gulf waters, threatening desalination plants and the coastal ecosystem of the Gulf (Pearce 1993).

Such incidents have intensified attempts to develop various chemicals, procedures and techniques for combating oil pollution both at sea and along the shoreline. Biosurfactants are just such chemicals, and were applied to parts of the Exxon Valdez oil spill (Harvey et al. 1990). The ability of biosurfactants to emulsify hydrocarbon–water mixtures enhances the degradation of hydrocarbons in the environment. The presence of hydrocarbon-degrading microorganisms in seawater renders biodegradation one of the most efficient methods for removing pollutants (Gutnick and Rosenberg 1977; Leahy and Colwell 1990; Atlas 1991). Most biosurfactants, in comparison to chemical surfactants, have lower possible toxicity and shorter persistence in the environment (Zajic et al. 1977b; Georgiou et al. 1992). The ability of a surfactant to enhance the biodegradation of slightly soluble organic compounds depends on the extent to which it increases the bioavailability of the compound (Begley et al. 1996).

Biosurfactant and marine bioremediation/dispersion

Microorganisms capable of hydrocarbon degradation have often been isolated from aquatic environments (Brown and Braddock 1990). Chakrabarty (1985) reported that an emulsifier produced by *Pseudomonas aeruginosa* SB30 was able to quickly disperse oil into fine droplets, and inferred that it may be useful in removing oil from contaminated beaches. Mattei et al. (1986) studied crude oil degradation in a 20-l continuous-flow fermentor using a mixed bacterial community isolated from seawater and reported an enhanced degradation rate of crude oil. In a similar study on biodegradation of a mixture of hydrocarbons with *P. aeruginosa* S8, Shafeeq et al. (1989) demonstrated the presence of biosurfactants in the culture medium.

Harvey et al. (1990) tested a biosurfactant from *P. aeruginosa* for its ability to remove oil from contaminated Alaskan gravel samples under various

Table 2 Potential applications of biosurfactants as fine and specialty chemicals (adapted from Ishigami 1997)

No.	Function	Application field
1	Emulsifiers and dispersants	Cosmetics, paints, additives for rolling oil
2	Solubilizers and microemulsions	Toiletries, pharmaceuticals
3	Wetting and penetrating agents	Pharmaceuticals, textile industry, paints
4	Detergents	Household, agriculture products, high tech products
5	Foaming agents	Toiletries, cosmetics, ore floatation
6	Thickening agents	Paints
7	Metal sequestering agents	Mining
8	Vesicle forming materials	Cosmetics, drug delivery systems
9	Microbial growth enhancers	Sewage sludge treatments for oily wastes, fermentation
10	Demulsifiers	Waste treatment
11	Viscosity reducing agents	Pipeline transportation
12	Dispersants	Coal-oil mixture, coal-water slurry
13	Resource recovery agents	Tertiary recovery of oil

conditions, such as different concentrations of surfactant, time of contact, temperature of the wash and presence or absence of gum. They reported increased oil displacement (about 2–3 fold) in comparison to water alone. The necessary contact time for maximum effect was also reduced from 1.5–2 min for water to 1 min. These results demonstrated the capacity of biosurfactants to remove oil from a naturally occurring substrate. The Environmental Technology Laboratory at the University of Alaska, Fairbanks, conducted a field trial in July 1993 in Sleepy Bay on LaTouche Island in Prince William Sound to test the effectiveness of a biosurfactant in removing weathered crude oil from subsurface beach material. They reported complete removal of diesel-range petroleum hydrocarbons (to the limit of 0.5 mg kg^{-1}) while semi-volatile petroleum hydrocarbons were reduced to the 70% level, a removal of 30% (Tumeo et al. 1994). All of these studies are laboratory-based and successful bioremediation of exposed marine open sites using biosurfactants remains a challenge.

Biosurfactant and soil bioremediation

Growing interest in biosurfactant applications for treating hydrocarbon-contaminated soils has developed recently (Bartha 1986; Van Dyke et al. 1993b; Banat 1995b). Hydrocarbon degradation by microbes present in the contaminated soil is the primary method for removing hydrocarbon pollutants from the soil. Partially purified biosurfactants can either be used in bioreactors or in situ to emulsify and increase the solubility of hydrophobic contaminants. Alternatively, either surfactant-producing microorganisms or growth-limiting factors may be added to the soil to enhance the growth of added or indigenous microorganisms capable of producing biosurfactants (Lang and Wagner 1993).

Variable results exist as to the utility of using biosurfactants in hydrocarbon biodegradation. Oberbremer et al. (1990) used a mixed soil population to assess hydrocarbon degradation in a model oil and reported a statistically significant enhancement of hydrocarbon degradation when sophorose lipids were added to a model system containing 10% soil and 1.35% hydrocarbon mixture of tetradecane, pentadecane, hexadecane, pristane, phenyldecane and naphthalene in mineral salt medium. In the absence of surfactant, 81% of the hydrocarbon mixture was degraded in 114 h while, in the presence of biosurfactant, up to 90% of the hydrocarbon mixture was degraded in 79 h. In another study, Fought et al. (1989) found the emulsifier Emulsan inhibited alkane mineralization by pure and mixed bacterial cultures. This emulsifier stimulated aromatic mineralization by pure cultures but inhibited aromatic degradation by mixed cultures.

Biodetox (Germany) described a process to decontaminate soils, industrial sludge and waste waters (Van Dyke et al. 1991). The procedure involves transport of contaminated materials to a biopit process for microbial degradation. Biodetox also performs in situ bioreclamation for surface, deep ground and ground water contamination. Microorganisms are added by means of "Biodetox foam", which is not harmful to the environment, contains bacteria, nutrients and biosurfactants and can be biodegraded. Jain et al. (1992) found that the addition of *Pseudomonas* biosurfactant enhanced the biodegradation of tetradecane, pristane, and hexadecane in a silt loam with 2.1% organic matter. Similarly, Zhang and Miller (1995) reported the enhanced octadecane dispersion and lightened biodegradation by a *Pseudomonas* rhamnolipid surfactant. Falatko and Novak (1992) studied biosurfactant-facilitated removal of gasoline overlaid on the top of a coarse-grain sand-packed column. Up to a 15-fold increase was observed in the effluent concentration of four gasoline constituents; toluene, *m*-xylene, 1,2,4-trimethylbenzene and naphthalene, upon the addition of biosurfactant solution (600 mg l^{-1}).

Herman et al. (1997) investigated the effects of rhamnolipid biosurfactants on in situ biodegradation of hydrocarbon entrapped in a porous matrix and reported a mobilization of hydrocarbon entrapped within the soil matrix at biosurfactant concentrations higher than critical micelle concentration (CMC). At concentrations lower than CMC, they detected enhanced in situ mineralization of entrapped hydrocarbon. One of the methods for removing oil contaminants is to add biosurfactants to soil, increasing hydrocarbon mobility. The emulsified hydrocarbon can then be recovered by a production well and degraded above ground in a bioreactor. Ermolenko et al. (1997) used the strain *Mycobacterium flavescens* Ex 91 for the development of Ekoil, a bacterial preparation for the decontamination of areas polluted with oil. According to trials by the Nizhnevolzhskneft enterprise, this product could decontaminate oil-polluted water and was efficient in the treatment of oil-contaminated wastewater at Zaporzhskaya nuclear power station (Ermolenko et al. 1997). Bai et al. (1997) used an anionic mono-rhamnolipid biosurfactant from *P. aeruginosa* to remove residual hydrocarbons from sand columns. They recovered approximately 84% of residual hydrocarbon (hexadecane) from sand columns packed with 20/30 mesh sand and 22% of hydrocarbons from 40/50-mesh sand, primarily because of increased mobilization. They reported the optimal concentration of rhamnolipid as 500 mg l^{-1} with a potentially useful range of 40–1500 mg l^{-1}.

Biosurfactants and polyaromatic hydrocarbon bioremediation

Only limited numbers of microorganisms are capable of degrading polyaromatic hydrocarbons (PAHs) with four or more fused aromatic rings (Harayama 1997). Their biodegradation is limited by their poor availability to the microorganisms, which is due to their hydrophobicity, low aqueous solubility and strong adsorptive capacity in

soil (Mihelcic et al. 1993; Volkering et al. 1995). Ganeshlingam et al. (1994) suggested that applying surfactants as immobilizing agents might be one way to enhance the solubility of PAHs. Surfactants help degradation by solubilization or emulsification, to release hydrocarbons sorbed to soil organic matter and increase the aqueous concentrations of hydrophobic compounds, resulting in higher mass transfer rates (Aronstein et al. 1991). In a critical survey of literature on the effects of the use of synthetic and biologically produced surfactants on PAH biodegradation, Rouse et al. (1994) revealed many contradictory reports about these compounds' efficiency on PAH removal.

Other investigations indicate a potential use for synthetic surfactants to enhance PAH degradation by increasing microbial accessibility to insoluble substrates (Tiehm 1994). Providenti et al. (1995) studied the effects of *P. aeruginosa* UG2 biosurfactants on phenanthrene mineralization in soil slurries and detected an increase in phenanthrene mineralization combined with reduced lag period prior to the onset of mineralization. The efficiency of biosurfactants in the remediation of soil contaminated by metals, phenanthrene and polychlorinated biphenyls (PCBs) has also been reported (Miller 1995a). Berg et al. (1990) described an emulsifying agent produced by *P. aeruginosa* UG2 that increased the solubility of hexachlorobiphenyl added to soil slurries, resulting in a 31% recovery of the compound in the aqueous phase.

Churchill et al. (1995) demonstrated that rhamnolipids from bacteria, in combination with the oleophilic fertilizer Inipol EAp-22, increased the degradation rate of hexadecane, benzene, toluene, *o*- and *p*-cresol and naphthalene both in aqueous-phase bioreactors and in those containing soil. They also reported increased rates of biodegradation of aliphatic and aromatic hydrocarbons by pure bacterial cultures. In a similar study Van Dyke et al. (1993a) surveyed a variety of biosurfactants for the removal of hexachlorobiphenyl from soil. Out of 13 biosurfactants tested, seven removed hexachlorobiphenyl more efficiently compared to the control. Two strains of *P. aeruginosa* and one strain of *Acinetobacter calcoaceticus* RAG-1 produced the most efficient biosurfactants. Robinson et al. (1996) carried out a batch investigation to evaluate the impact of biosurfactant (rhamnolipid RI) on microbial utilization of PCBs and concluded that the addition of biosurfactants followed by augmentation with the pure culture is a promising approach for the treatment of non-aqueous phase and soil-bound PCBs.

In an investigation of the capacity of PAH-utilizing bacteria to produce biosurfactants using naphthalene and phenanthrene, Daziel et al. (1996) detected biosurfactant production that was responsible for an increase in the aqueous concentration of naphthalene (31 mg l^{-1}). This indicates a potential role for biosurfactants in increasing the solubility of such compounds. Similarly Zhang et al. (1997) tested the effects of two rhamnolipid biosurfactants on the dissolution and bioavailability of phenanthrene and reported increases in both solubility and degradation rate of phenanthrene. Kanga et al. (1997) applied glycolipid biosurfactants produced by *Rhodococcus* sp. H13A and a synthetic surfactant (Tween 80) for enhanced substrate solubility. Using naphthalene and methyl-substituted derivatives in crude oil as representative of the PAH content, they observed that both surfactants lowered surface tension in solutions from 72 dynes cm^{-1} to 30 dynes cm^{-1}. The biosurfactants were more efficient in increasing the solubility of hydrocarbons, particularly the substituted derivative. In a laboratory column study, Noordman et al. (1998) applied rhamnolipid biosurfactants for the enhanced removal of phenanthrene from phenanthrene-contaminated soil eluting with an electrolyte solution containing rhamnolipid (500 mg l^{-1}). Rhamnolipids enhanced the removal of phenanthrene (2- to -5-fold shorter time for 50% recovery and 3.5-fold for 90% recovery) compared to controls. The enhanced removal of phenanthrene occurred mainly by micellar solubilization. We observed that the effects of adding biosurfactants on PAH bioremediation were generally unpredictable. Further studies are needed to select the best microorganisms and surfactants for application to PAH bioremediation and to provide a clearer understanding of the interaction between sorbed PAHs and surfactants.

Biosurfactants and metal-contaminated soils remediation

It is well known that microbial cells may chelate metals from solution. Little information is available however, concerning the use of biosurfactants to chelate metals. There are several reports of exopolysaccharide use for metal chelation (Kaplan et al. 1987; Scott and Palmer 1988; Marques et al. 1990). Miller (1995b) reported that the addition of biosurfactant may promote desorption of heavy metals from soils in two ways. The first is through complexation of the free form of the metal residing in solution which decreases the solution-phase activity of the metal and therefore promotes desorption. The second occurs under conditions of reduced interfacial tension; the biosurfactants accumulate at the solid–solution interface, which may allow direct contact between the biosurfactant and the sorbed metal.

Exopolysaccharides however, differ from biosurfactants in their size (high molecular weights) and minimal surface activity, although they have strong affinities for oil–water interfaces (Gutnick and Shabtai 1987). The advantage of biosurfactant use in bioremediation over exopolymers is their smaller size and over chemical chelating agents is their biodegradability. Tan et al. (1994) investigated the potential of rhamnolipid biosurfactants produced by *P. aeruginosa* ATCC 9027 in the removal of metals from soils contaminated with cadmium and reported 92% complexation of Cd^{2+} in a 0.5 mM solution of $Cd(NO_3)_2$ using a 5 mM solution of rhamnolipid (22 μg mg^{-1} rhamnolipid). In a similar study, Herman et al. (1995) added rhamnolipid solu-

tions (12.5, 25, 50 and 80 mM) to soil containing either sorbed Cd^{2+} (1.46 mmol kg^{-1}), Pb^{2+} (1.96 mmol kg^{-1}) or a mixture of Pb^{2+} – Cd^{2+} – Zn^{2+} (3.4 mmol kg^{-1}). At 12.5 and 25 mM rhamnolipid concentration they reported 78% sorption to soil; and less than 11% of soil-bound Cd^{2+} and Zn^{2+} was desorbed. However, ion exchange of bound metals with K^{+} present in the rhamnolipid matrix may account for the removal of 16–48% of the sorbed Cd^{2+} and Zn^{2+}. At 50 mM and 80 mM, rhamnolipid sorption to soil decreased to 20–77% and the removal of Cd^{2+} and Zn^{2+} therefore exceeded the removal by ion exchange by up to three-fold. The behavior of Pb^{2+} was quite different. Less than 2% of soil-bound Pb^{2+} was desorbed due to ion exchange, although up to 43% was desorbed by 80 mM rhamnolipid, which may have been due to the insensitivity of Pb^{2+} to ion exchange effects. Hong et al. (1998) used Aescin as a biosurfactant for remediation of soil contaminated with Cd^{2+} and Pb^{2+} by a soil-washing process. Maximum desorption occurred at 45 mM Aescin concentration (32% removal) whereas 30 mM Aescin removed only 8% Pb^{2+} from soil. In control experiments with water only 5% Cd^{2+} and 0.2% Pb^{2+} could be removed from soil.

Although the use of rhamnolipid biosurfactants in the bioremediation of metal contaminated soils has promise, to achieve better metals-removal and develop remediation technologies it is important to understand the factors affecting rhamnolipids sorption to soil. These factors include ionic strength, mineral composition and pore water chemistry within metal-contaminated soils. Future success of biosurfactant technology in bioremediation initiatives will require targeting their use to the physical conditions and chemical nature of the pollution-affected site to maximize their efficiency and economical viability.

Biosurfactants and oil storage tank cleaning

Another application of biosurfactants is oil storage tank cleaning. Surfactants have been studied for use in reducing the viscosity of heavy oils, thereby facilitating recovery, transportation and pipelining (Bertrand et al. 1994). A glycolipid surfactant produced by Gram-negative, rod-shaped bacterial isolate H13A has been reported to reduce the viscosity of heavy crude oil by 50% (Finnerty and Singer 1985). Earlier Zajic et al. (1974) isolated a *Pseudomonas* strain which produced an emulsifying agent capable of emulsifying heavy grade VI fuel oil. Hayes et al. (1986) have demonstrated the ability of Emulsan to reduce the viscosity of Boscan (Venezuelan heavy oil) from 200,000 to 100 cP, making it feasible to pump heavy oil in 26,000 miles of commercial pipe line.

In a pilot field investigation, Banat et al. (1991) tested the ability of biosurfactant produced by a bacterial strain (Pet 1006) to clean oil storage tanks and to recover hydrocarbons from the emulsified sludge. Two tonnes of biosurfactant-containing whole-cell culture were used to mobilize and clean 850 m^3 oil sludge. Approximately 91% (774 m^3) of this sludge was recovered as re-sellable crude oil and 76 m^3 non-hydrocarbon materials remained as impurities to be manually cleaned. The value of the recovered crude covered the cost of the cleaning operation (US $100,000–150,000 per tank). Such a clean-up process is therefore economically rewarding and less hazardous to persons involved in the process compared to conventional processes (Lillenberg et al. 1992). It is also an environmentally sound technology leading to less disposal of oily sludge in the natural environment. To our knowledge however, further commercial applications of this technology have not been carried out.

Biosurfactant and microbial enhanced oil recovery

An area of considerable potential for biosurfactant application is in the field of microbial enhanced oil recovery (MEOR). Enhanced oil recovery methods were devised to recover oil remaining in reservoirs after primary and secondary recovery procedures. It is an important tertiary recovery technology, which utilizes microorganisms and/or their metabolites for residual oil recovery (Banat 1995a). In MEOR, microorganisms in reservoirs are stimulated to produce polymers and surfactants, which aid MEOR by lowering interfacial tension at the oil–rock interface. This reduces the capillary forces preventing oil from moving through rock pores. There are several reports that describe various methods used in laboratory studies of MEOR (Table 3).

Biosurfactants can also aid oil emulsification and assist in the detachment of oil films from rocks (Banat 1995a, b). In situ removal of oil is due to multiple effects of the microorganisms on both environment and oil. These effects include gas and acid production, reduction in oil viscosity, plugging by biomass accumulation, reduction in interfacial tension by biosurfactants and degradation of large organic molecules. These are all factors responsible for decreasing the oil viscosity and making its recovery easier (Jack 1988).

The strategies involved in the MEOR depend on the prevalent oil reservoir conditions, including temperature, pressure, pH, porosity salinity, geologic make-up of the reservoir, available nutrients and the presence of indigenous microorganisms. These factors should be considered before devising a strategy for use in an oil well. There are three main strategies for the use of biosurfactants in enhanced oil recovery (EOR) or mobilization of heavy oils (Shennan and Levi 1987; Banat 1995a):

1. Production in batch or continuous culture under industrial conditions followed by addition to the reservoir in the conventional way along with the water flood (ex situ MEOR).
2. Production of surface-active compounds by microorganisms at the cell–oil interface within the reservoir

Table 3 Emulsification index and sand pack recovery of some of the microorganisms. The emulsification index (E24) was determined by adding 6 ml motor oil to 4 ml culture broth in a graduated tube, followed by vortexing at high speed for 2 min. The emulsion stability was determined after 24 h. The E24 was calculated by measuring the emulsion layer thus formed. The sand pack technique described by Abu-Ruwaida et al. (1991b) was used for oil recovery. A glass column (40.0 × 2.5 cm) was packed with 100 g acid-washed sand. The column was then saturated with 100 ml kerosene oil. The ability of the isolated surfactant to recover oil was estimated by pouring 100 ml aqueous biosurfactant (1.0 mg ml^{-1}) into the column. The amount of oil released was measured. *NA* Data not available

Microorganism	Emulsification index (E24)	Sand pack oil recovery (%)	Reference
Bacillus subtilis MTCC 2423	90	62	Makkar and Cameotra 1997a, b
Bacillus subtilis MTCC 1427	33.3	56	Makkar and Cameotra 1998
Arthrobacter protophormiae	60	90	Pruthi and Cameotra 1997a
Serratia marcescens	94	82	Pruthi and Cameotra 1997b
Rhodococcus sp.	NA	80	Abu Ruwaida et al. 1991b
Bacillus sp. AB-2	80–90[a]	90–100	Banat 1993
Pet 1006	NA	95	Banat et al. 1991

[a] The oil used was Burgan Kuwait Oil

formation, implying penetration of metabolically active cells into the reservoir.

3. Injection of selected nutrients into a reservoir, thus stimulating the growth of indigenous biosurfactant-producing microorganisms.

The first strategy is expensive due to the capital required for bioreactor operation, product purification and introduction into oil-containing rocks (Moses 1987). The second and third strategies require that the reservoir contains bacteria capable of producing sufficient amounts of surfactant. For the production of biosurfactants, microorganisms are usually provided with low-cost substrates such as molasses and inorganic nutrients, which promote growth and surfactant production (Makkar and Cameotra 1997b). Alternatively surfactant-producing strains may be introduced into the well (Springham 1984). The introduced organism faces competition from the indigenous population of microbes for binding sites on rocks and for the added nutrients. Another problem with inducing microbial growth in a reservoir is that the quality of oil may be affected by undesirable microorganisms, e.g. sulphate-reducing bacteria are known to cause souring of the crude oil and subsequent corrosion of equipment (Brown et al. 1985).

Apart from the problems discussed above, for the microorganisms to be suitable and useful in MEOR in situ, they must be able to grow under the severe environmental conditions encountered in oil reservoirs, such as high temperature, pressure, salinity and low oxygen levels (Cameotra and Makkar 1998). At these extreme conditions, the temperatures and pressures have been reported to be 96 °C and 2×10^4 kPa in the North Sea Forties Field, and 125 °C and 5×10^4 kPa in the Ninian Field (Shennan and Levi 1987). Bacteria capable of growth and producing surface-active compounds under these conditions have been isolated. Post and Al-Harjan (1988) reported the isolation of a halobacterium capable of producing surface-active agents, while several anaerobic thermophilic bacteria tolerant of pressure and moderate salinity have also been reported to mobilize crude oil in the laboratory (Levi et al. 1985). We have also observed good sand-pack oil recovery using strains of *Bacillus subtilis* at 45 °C (Makkar and Cameotra 1997a; 1998). Biosurfactant produced by two strains of *B. subtilis* (MTCC 1427 and MTCC 2423) accounted for 56% and 62% oil recovery from oil-saturated sand columns. The added advantage of being thermotolerant and stable over a wide range of pH values (4.5–10.5) makes them suitable candidates for in situ MEOR. The two strains grew at 45 °C and utilized molasses, a cheap source of nutrient additive. Yakimov et al. (1997) investigated the applicability of *B. licheniformis* isolates for MEOR under conditions occurring in the oil reservoirs of Northern Germany. They used three different methods: flask cultivation, static batch culture and core flooding experiments to assess the MEOR capability of the strains. Strain BNP29 was chosen as candidate for the static batch culture and core flood experiments and exhibited potential application for the development of enhanced oil recovery processes. Oil recovery efficiencies varied over 9.3–22.1% of the water flood residual oil saturation.

Most of the laboratory studies on MEOR utilize core samples and columns containing the desired substrate. Banat (1995a, b) reviewed the state of the art for this strategy and its effectiveness in field studies carried out in Czechoslovakia, Hungary, the Netherlands, Poland, Romania, the United States and the USSR, with a significant increase in oil recovery noted in some but not all cases. Behlugil et al. (1992) applied MEOR to Turkish heavy oil. They injected an anaerobic bacterium *Clostridium acetobutylicum* into a model reservoir (a stainless steel tube 100 cm long, 6.2 cm diameter) containing a Turkish heavy oil (Raman oil) at 38 °C. They found an overall increase of 12% in MEOR effectiveness, compared to controls. This increase was attributed to changes in viscosity and pH of crude oil caused by the biosurfactant produced by the added bacterium. The lipopeptide biosurfactant produced by *B. licheniformis* JF2 has been used in coreflood experiments for enhanced oil recovery (Thomas et al. 1993). The efficiencies of different biosurfactants used in MEOR laboratory experiments are shown in Table 4.

Table 4 Effectiveness of biosurfactants used in microbial enhanced oil recovery (MEOR). Adapted from Muller-Hurtig et al. (1993)

Type of biosurfactant	Time (h)	Oil removal (%)
Control	114	81
Sophorolipid	75	97
Rhamnolipid	77	94
Trehalose-6,6′ dimycolate	71	93
Cellobiose lipid	79	99

Biosurfactants as therapeutic agents

Biosurfactants have some therapeutic applications. Rhamnolipids produced by *P. aeruginosa* (Itoh et al. 1971), lipopeptides produced by *B. subtilis* (Sandrin et al. 1990; Leenhouts et al. 1995; Vollenbroich et al. 1997a) and *B. licheniformis* (Jenny et al. 1991; Fiechter 1992; Yakimov et al. 1995) and mannosylerythritol lipids from *Candida antarctica* (Kitamoto et al. 1993) have all been shown to have antimicrobial activities. Surfactin, one of the earliest known biosurfactants, has various pharmacological applications such as inhibiting fibrin clot formation and hemolysis (Bernheimer and Avigard 1970) and formation of ion channels in lipid membranes (Sheppard et al. 1991). It has also been reported as having an antitumor activity against Ehrlich's ascite carcinoma cells (Kameda et al. 1974), inhibiting cyclic adenosine 3′,5′-monophosphate phosphodiesterase (Hosono and Suzuki 1983) and having anti-fungal properties (Vater 1986b). Thimon et al. (1995) described another anti-fungal biosurfactant, Iturin, a lipopeptide produced by *B. subtilis*, which affects the morphology and membrane structure of yeast cells.

Naruse et al. (1990) demonstrated a significant inhibitory effect of pumilacidin (surfactin analog) on herpes simplex virus 1 (HSV-1). They also reported an inhibitory activity against H^+,K^+-ATPase and protection against gastric ulcers in vivo. Itokawa et al. (1994) have reported the potential of surfactin against human immunodeficiency virus 1 (HIV-1). Vollenbroich et al. (1997b) have reported a potential use for surfactin in the virus safety enhancement of biotechnological and pharmaceutical products. They also suggested that the anti-viral action of surfactin is due to a physiochemical interaction between the membrane-active surfactant and the virus lipid membrane.

There is increasing interest in the effect of biosurfactants on human and animal cells and cell lines. Lipopeptides produced by *Streptosporangium amethystogenes* subsp. *fukuiense* Al-23456, which have a similar nature to some biosurfactants, were shown to have the ability to induce granulocyte colony stimulating factor and granulocyte–macrophage colony stimulating factor (Hida et al. 1995). Takizawa et al. (1995) reported significant stimulation of the proliferation of bone marrow cells from BALB/c female mice by S. *amethystogenes* lipopeptides. Isoda et al. (1997) investigated the biological activities of microbial glycolipids of *C. antarctica* T-34 and reported an induction of cell differentiation in the human promyelocytic leukemia cell line HL60. These glycolipids also induced the human myelogenous leukemia cell line K562 and the human basophilic leukemia cell line Ku812 to differentiate into monocytes, granulocytes and megakaryocytes. The reports on antibiotic effects (Neu et al. 1990) and inhibition of HIV virus growth in white blood corpuscles have opened up new fields for their applications. Kosaric (1996) describes possible applications as emulsifying aids for drug transport to the infection site, for supplementing pulmonary surfactant and as adjuvants for vaccines. Respiration failure in premature infants is caused by a deficiency in pulmonary surfactant (Tayler et al. 1985). With the bacterial cloning of the gene for the protein molecule of the surfactant, the fermentative production of this product for medical application is now possible (Lang and Wullbrandt 1999). The succinoyl-trehalose lipid of *Rhodococcus erythropolis* has been reported to inhibit HSV and influenza virus with a lethal dose of 10–30 μg ml^{-1} (Uchida et al. 1989a, b). To our knowledge, commercial production of biosurfactants for use as antimicrobial agents has not taken place yet.

The involvement of biosurfactants in microbial adhesion and desorption has also been reported. A dairy *Streptococcus thermophilus* strain produced a biosurfactant which caused its own desorption from glass, leaving a completely non-adhesive coating (Busscher et al. 1990). Pratt-Terpstra et al. (1989) reported a release of biosurfactant by an oral *S. mitis* strain, which was responsible for a reduction in the adhesion of *S. mutans*. Similarly Velraeds-Martine et al. (1996a) reported on the inhibition of adhesion of pathogenic enteric bacteria by biosurfactant produced by a *Lactobacillus* strain and later showed that the biosurfactant caused an important, dose-related inhibition of the initial deposition rate of *Escherichia faecalis* and other bacteria adherent on both hydrophobic and hydrophilic substrata (Velraeds-Martine et al. 1997). They also speculated on other possible therapeutic agents through the development of anti-adhesive biological coatings for catheter materials to delay the onset of biofilm growth.

Biosurfactants for agricultural use

Concerns about pesticide pollution have prompted global efforts to find alternative biological control technologies

While Stanghellini et al. (1996) were investigating the effects of synthetic surfactants on controlling the root rot fungal infections of cucumbers and peppers caused by *Pythium aphanidermatum* and *Phytophora capsici*, they observed lysis of fungal zoospores due to some bacterial metabolites in the nutrient solution. The metabolites were thought to be biosurfactants, as their mode of action was similar to the synthetic surfactants. Subsequently the bacterium was identified as *Pseudo-*

monas aeruginosa and the biosurfactant as a rhamnolipid (Stanghellini and Miller 1997). The biosurfactant has zoosporicidal activity against species of *Pythium*, *Phytophora*, *and Plasmopara* at concentrations ranging over 5–30 μg ml^{-1}. The proposed mechanism for the biosurfactant action is that the biosurfactant intercalates with and disrupts the plasma membrane, although this was not established. Staghellini and Miller (1997) also evaluated the biological control potential of rhamnolipid-producing strains and concluded that biosurfactants have potential for the biological control of zoosporic plant pathogens.

Surface-active agents are needed for the hydrophilization of heavy soils to obtain good wettability and also to achieve equal distribution of fertilizers and pesticides in the soils. Biosurfactants have also been used in formulating poorly soluble organophosphorus pesticides. Two *Bacillus* strains producing an emulsifier, possibly a glycolipopeptide, were able to form a stable emulsion in the presence of the pesticide fenthion (Patel and Gopinathan 1986). The compound had some activity against other liquid-immiscible organophosphorus pesticides, but not solid organophosphorus or organochlorine pesticides, or hydrocarbons. A biosurfactant produced by *P. aeruginosa* has been reported to solubilize toxic organic chemicals and increase the solubility and recovery of hexachlorobiphenyl from soil slurries by 31% (Berg et al. 1990). In recent collaborative research, we found that the addition of a biosurfactant (400 μg ml^{-1}) produced by *B. subtilis* MTCC 2423 enhanced the rate of biodegradation of the chlorinated pesticide α- and β-endosulfan by 30–40%, in both flask-coated and soil-bound conditions. It also mobilized the residual endosulfan isomers towards biodegradation. These would otherwise have remained undegraded (Awasthi et al. 1999).

Biosurfactants use in mining

Biosurfactants may be used for the dispersion of inorganic minerals in mining and manufacturing processes. Rosenberg et al. (1988) described the production of *Acinetobacter calcoaceticus* A2, an anionic polysaccharide called biodispersan, which prevented flocculation and dispersed a 10% limestone in water mixture. Biodispersan served two functions: dispersant and surfactant; and catalyzed the fracturing of limestone into smaller particles. To elucidate the mechanism of this action, Rosenberg and Ron (1998) suggested that the pH should be alkaline (9–9.5) during the grinding process, and that biodispersan is an anionic polymer at that pH. The polymer enters microdefects in the limestone and lowers the energy required for cleaving the microfractures. Kao Chemical Corporation (Japan) used *Pseudomonas*, *Corynebacterium*, *Nocardia*, *Arthrobacter*, Bacillus and *Alcaligenes* to produce biosurfactants for the stabilization of coal slurries to aid the transportation of coal (Kao 1984. Australian Patent 8317–8555). Similarly Polman et al. (1994) tested biosurfactants for solubilization of coal and achieved partial solubilization of North Dakota Beulah Zap lignite coal using a crude preparation of biosurfactants from *Candida bombicola* (Breckenridge and Polman 1994).

Biosurfactants and personal care

Biosurfactants have found a niche in the personal care market because of their lower moisturizing properties and skin compatibility (Brown 1991). Sophorolipids are produced both by *C. bombicola* KSM-36 in quantities of 100–150 g l^{-1} using palm oil and glucose as carbon source (Itoh 1987) and by *C. apicola* to about 90 g l^{-1} using glucose and sunflower oil as substrates (Stuwer et al. 1987). A product containing one mole sophorolipid and 12 moles propylene glycol has specific compatibility to the skin and has found commercial utility as a skin moisturizer (Yamane 1987). Kao Chemical Corporation at present uses sophorolipids commercially as humectants for cosmetic makeup brands such as Sofina. Kosaric (1992) has speculated on the expanding role of biosurfactants in various products used in the cosmetic industry. Recently much higher concentrations of sophorolipids, up to 300 g l^{-1} (Davila et al. 1997) and 422 g l^{-1} (Daniel et al. 1998), have been reported using *C. bombicola* in two different two-stage fermentation techniques utilizing rapeseed oil as the main carbon source.

Biosurfactants use in the food industry

In the food industry, biosurfactants are used as emulsifiers for the processing of raw materials. Emulsification plays an important role in forming the right consistency and texture as well as in phase dispersion. Other applications of surface-active compounds are in bakery and meat products, where they influence the rheological characteristics of flour and the emulsification of partially broken fat tissue (Vater 1986a). Lecithin and its derivatives are currently in use as emulsifiers in food industries worldwide (Bloomberg 1991). *C. utilis* bioemulsifier has been used in salad dressing (Shepherd et al. 1995). Busscher et al. (1996) found that a biosurfactant produced by thermophilic dairy *Streptococcus* spp could be used for fouling control of heat exchanger plates in pasteurizers, as they retard the colonization of *S. thermophilus* responsible for fouling.

Other applications of biosurfactants

Some other potential commercial applications of biosurfactants are in the pulp and paper industry (Rosenberg et al. 1989), textiles, ceramics (Horowitz and Currie 1990) and uranium ore processing (McInerney et al. 1990). Pellerin et al. (1991) have successfully applied

heteropolysaccharides from *Macrocystis pyrifera* and *Azotobacter vinelandii* as dispersants in the ceramic processing industry. Biodispersan, a polymeric biosurfactant from *Acinetobacter calcoaceticus* A2 has potential use in paint industries (Rosenberg and Ron 1998). The suspension made in the presence of the biodispersant is easy to handle, as particles settle very slowly. This is an important aspect for paints, because it gives better spreadability and improved mixing properties.

The Research Institute of Synthetic Fibers in the USSR described a surfactant produced by *Candida* yeasts for which there are uses in textile, pharmaceutical and cosmetics industries (Research Institute of Synthetic Fibers 1984. Russian Patent 1006–1481). Bengmark (1998) has suggested a possible use for biosurfactants in immunonutrition. Mulligan and Cooper (1985) used biosurfactants as dewatering agents in pressing peat. The addition of surfactants to peat before pressing resulted in enhanced water release. However, the problem of releasing organic matter along with the water was observed. To minimize loss, peat pressate was used as substrate for biosurfactant production.

Conclusion

During the last 2–3 decades a wide variety of microorganisms have been reported to produce numerous types of biosurfactants. Their biodegradability and lower toxicity gives them an advantage over their chemical counterparts and therefore may make them suitable for replacing chemicals. While many types of biosurfactants are in use, no single biosurfactant is suitable for all potential applications. To date, biosurfactants are unable to compete economically with chemically synthesized compounds in the market, mainly due to their high production costs and the lack of comprehensive toxicity testing. Measures to simplify product types for selected applications, such as using sterilized or pasteurized fermentation broth without any need for extraction, concentration or purification of the biosurfactant may significantly reduce the cost of production. Such crude product may be directly utilized in most applications related to both the oil industries and environmental bioremediation. Other strategies involving medium and downstream process-optimization may also have a positive impact on cost reduction. It is puzzling however that large chemical companies seem not to be interested in research in these areas.

The usefulness of biosurfactants in bioremediation is however expected to gain more importance in coming years. Their success in bioremediation will require precise targeting to the physical conditions and chemical nature of the pollutant-affected areas. Encouraging results have been obtained for the use of biosurfactants in hydrocarbon pollution control in marine biotopes in closed systems (oil storage tanks) and, although many laboratory studies indicate potential for use in open environments, a lot remains to be demonstrated in pollution treatment in marine environments or coastal areas. The possible use of biosurfactants in MEOR has many advantages, yet more information is required about structures and factors such as interaction with soil, structure function analysis of surfactant solubilization, scale-up and cost analysis for ex situ production. Another critical factor for the application of biosurfactants in industry is a detailed knowledge of their genetics, as this may hold the key to their future economical production using enhanced recombinant strains.

The usefulness of biosurfactants in other fields is emerging, especially in personal and health care and as therapeutic agents. Enzymatic synthesis of tailor-made surfactants by lipases has given a new dimension to biosurfactant production, especially in the application of biosurfactants in health care and cosmetics. With increased efforts on developing improved application technologies, strain improvement and production processes, biosurfactants are expected to be among the most versatile process chemicals for use in the near future.

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