Biosurfactant Production by Microorganisms on Unconventional Carbon Sources

Randhir S. Makkar[1] and Swaranjit S. Cameotra*

Institute of Microbial Technology, Chandigarh, India

ABSTRACT: In recent years natural biosurfactants have attracted attention because of their low toxicity, biodegradability, and ecological acceptability. However, for reasons of functionality and production cost, they are not competitive with chemical surfactants. Use of inexpensive substrates can drastically decrease the production cost of biosurfactants. This review describes the use of unconventional carbon sources for biosurfactant production. These sources include urban as well as agroindustrial wastes. With suitable engineering and microbiological modifications, these wastes can be used as substrates for large-scale production of biosurfactants.

Paper no. S1084 in *JSD 2*, 237–241 (April 1999).

KEY WORDS: Agroindustrial products, *Bacillus subtilis,* biosurfactant, molasses, waste biotransformation.

In recent years biosurfactant synthesis has been studied extensively (1–3). Biosurfactants are attracting attention as promising natural surfactants because they offer several advantages over chemical surfactants, such as low toxicity, inherently good biodegradability, and ecological acceptability. In the personal-care sector, penetration by biosurfactants is expected to be rapid. By the year 2000 most cosmetic products are predicted to be biocosmetics, including color bases. In Japan, sophorose lipids from *Torulopsis bombicola* KSM 35 are in use as a high-value skin moisturizer. Biosurfactants have several applications in the petroleum industry, including microbially enhanced oil recovery (MEOR), cleaning of oil tankers, extraction of bitumen from tar sands, pumping of crude oils by use of bioemulsifiers, de-emulsification of crude oils, and viscosity reduction of heavy crude oils. In the food industry, lecithin, a biosurface-active material, is a widely used food emulsifier. Antibiotic activity of some biosurfactants and their inhibitory effect on AIDS virus growth have been reported recently (4).

Although interest in biosurfactants is increasing, these surfactants are not economically competitive with their synthetic counterparts. Microbially produced surfactants or biosurfactants must compete with surfactants of petrochemical origin in three respects—cost, functionality, and production capacity to meet the needs of the intended application. While high production costs can be tolerated for biosurfactants used in low-volume, high-priced products (e.g. in cosmetics and medicines), high production costs are incompatible with applications, such as enhanced oil recovery (EOR), that require large volumes of low-priced surfactants. Different strategies must be devised and explored to reduce production costs. Examples include high yields and product accumulation, economical engineering processes, and use of cost-free or cost-credit feed stocks for microorganism growth and surfactant production.

Traditionally, hydrocarbons have been the substrates of choice for the production biosurfactants and bioemulsifiers (5,6). It is assumed that the induction of surfactant production renders hydrophobic substrates accessible to the cell (7). Water-soluble substrates also have been used (8–10). These are cheaper and are preferred over hydrocarbons as substrates since single-phase fermentations are simpler than biphasic fermentation. Moreover, hydrocarbon substrates are unacceptable for many applications such as in food, cosmetics, and pharmaceutical industries. Nonhydrocarbon substrates include fats, oils, glycerol, or carbohydrates. Carbohydrates and vegetable oils are among the most widely used substrates for research on biosurfactant production.

The choice of inexpensive raw materials is important to the overall economics of the process because they account for 50% of the final product cost. Few attempts at using waste for biosurfactant production, and only a few types of biosurfactants produced from waste, have been reported

[1]Present address: National Food Research Institute, Tsukuba, Ibaraki 305, Japan.

*To whom correspondence should be addressed at Department of Soil Biochemistry, 129 Land and Water Bldg., Pennsylvania State University, University Park, PA 16802.
E-mail: swaranjit@excite.com

(11,12). Selection of waste substrates involves the problem of finding a waste with the right balance of carbohydrates and lipids to support optimal growth and production. Agroindustrial wastes, with high levels of carbohydrates or lipids, and urban wastes meet the requirements for use as substrates for biosurfactant production. Peat pressate, urban waste, olive oil mill effluent (OOME), lactic whey, and soapstick oil are possible substrates for surfactant accumulation. Apart from traditional carbon and nitrogenous substrates, the spectrum of available raw materials includes various agricultural and industrial by-products and waste materials. These agricultural feed stocks are attractive in that they are available in surplus and can be produced in regions with temperate to tropical climates. The foci for reduction of biosurfactant production costs are the microbes (selected, adapted, or engineered for high yields of product), the process (selected, designed, and engineered for low capital and operating costs), the microbial growth substrate, and/or the process by-products (minimized or managed as salable products rather than treated and discarded as wastes).

Kosaric *et al.* (12) examined different strategies, involving the use of mixed cultures of microorganisms, for the economical production of glycolipids from urban wastes. For example, carbohydrate-rich waste can be converted into triglycerides by oleaginous organisms, and *T. bombicola* can convert these compounds into extracellular sophorose-containing glycolipids that have been prepared for applications ranging from cosmetics formulation to recovery of petroleum crudes. These biosurfactants are unlikely to be cost-effective if extensive refining is required. Thus, process developments need to focus on biosurfactants that lend themselves to simple, inexpensive process technologies in which, for example, the surfactants can be easily recovered from the fermentation broth by gravity separation of a surfactant-rich phase, and in which they are obtained in their separated form as relatively concentrated products that are free of major contamination.

TABLE 1
Various Substrates Available for Biosurfactant Production

Feedstock	Advantages	Disadvantages
Carbohydrates	Abundant in most geographic regions from biomass resources (silviculture, agriculture) Present (sometimes with lipids) in wastes which might have a cost credit	Lowest conversion efficiency of all substrates to biosurfactants unless lipids or hydrocarbons are provided along with the carbohydrates
Hydrocarbons	Abundant in some geographic regions from petroleum resources High yields of glycolipid biosurfactants when provided with carbohydrates	Cost of biosurfactant production from nonwaste hydrocarbon tied to the cost of petroleum Seldom present together with carbohydrates in wastes Use of hydrocarbon wastes for biotechnology not yet extensively studied
Triglycerides, fatty acids		
Seed oil and animal fat	Abundant in some geographic regions from agricultural resources High yields of glycolipid biosurfactants when provided with carbohydrates Present (sometimes with carbohydrates) in wastes which may have a cost credit	Cost of biosurfactant production from nonwaste seed oils and animal fats tied to the real positive costs of these substrates
Microbial oil (single cell oil, SCO)	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	

Substrates for microbial growth and biosurfactant production must be available at low cost. In an economic atmosphere dominated by petroleum and petrochemical prices, the best way to reduce substrate cost for biotechnology at present is to use wastes which are either free or carry a cost credit for environmental benefit. Table 1 summarizes by chemical type the substrates for biosurfactant production and the advantages, disadvantages, and possibilities of using both refined and waste substrates. Depending on desirability and the considerations listed in Table 1, three strategies for utilization of wastes are possible:

- Select a waste substrate which has both carbohydrates and lipids.
- Select a waste which is either lipid- or carbohydrate-rich and transport it to and blend it with waste of the opposite nature.
- Select a waste which is carbohydrate-rich and microbially convert part of the carbohydrate to lipid as needed for biosurfactant production.

The application of these strategies is not without problems for (i) it would be difficult to find a waste with the right balance of lipid and carbohydrate for optimal substrate composition, (ii) it involves the likelihood of having to transport at least one of the wastes to be blended and hence of increasing the energy input and cost of the final biosurfactant, and (iii) it involves use of two or more microbes, hence increasing the complexity of the process.

Utilization of waste feedstocks for biosurfactant (or other bioproduct) production can potentially reduce biosurfactant production costs to a level competitive with similarly functioning petrochemicals and at the same time improve the economics of waste treatment. At a time when the necessity of countering a dependence upon petroleum is pointedly before us, it is appropriate that further research be devoted to define processes, assess the regulatory factors operating in these processes, and examine the economics for production of bioproducts from recycled wastes.

Mulligan and Cooper (14) used water collected during drying of fuel-grade peat. This waste contains a significant amount of carbohydrates (glucose, galactose, and xylose) and some amino acids suitable as substrates for microbial growth and surfactant production by *Bacillus subtilis*. In this case , a critical micelle concentration (CMC) of 8 mg/L was observed but no report of conversion yields was given. Sheppard and Mulligan (15) used peat hydrolysate for biosurfactant production.

In another strategy, Koch *et al.* (16) cloned the lactose gene from *Escherichia coli*, *Lac*ZY, to the *Pseudomonas aeruginosa* chromosome, which enabled this organism to utilize lactic whey as a substrate for growth and rhamnolipid production. Lactic whey is composed of high levels of lactose (75% of dry matter) and 12–14% protein. In addition, organic acids, minerals, and vitamins are present. Whey disposal represents a major pollution problem especially for countries depending on dairy economies.

Mercede *et al.* (11) reported use of OOME for rhamnolipid production by *Pseudomonas* sp. For enhanced rhamnolipid production, lipoidal substrates are best. For this reason, Manersa *et al.* (17) found that *P. aeruginosa* 44T1 could produce 10 $g \cdot L^{-1}$ of rhamnolipids with olive oil as the sole source of carbon. Soap stick oil has been used for rhamnolipid production with *P. aeruginosa* D10 (12). In their study Mercede and Manersa (12) used various residual lipidic wastes from the oils and fats processing industry, indicating that these wastes are able to support microbial growth and rhamnolipid production when they are supplied as the sole source of carbon in the medium. In an effort to contribute to the recycling of waste material from industry, Mercede *et al.* (11) used a residue from the olive oil industry for rhamnolipid production. OOME is produced during olive oil extraction and contains water from the olives themselves and water used during the extraction process. Although different technologies have been evaluated to deal with this waste, most of the wastewater is stored in open basins for evaporation, which produces noxious odors, proliferation of insects, and other environmental risks. In addition, one contains dry material (12%), organic substances (10.5%), and minerals (2.5%). The wastewater has a low pH (4.5-5) and is lightly colored. Because of its origin, OOME contains high levels of valuable organic substances such as sugars (glucose, saccharose), nitrogen compounds, pectins, polyphenols, and residual oils which makes it suitable for microbial growth and production of biosurfactants. Mercede and Manersa (12) developed a process for rhamnolipid production in a stirred tank reactor with *P. aeruginosa*. The reported final biosurfactant production was 1.4 $g \cdot L^{-1}$, and conversion yield was 0.058.

Ohno *et al.* (18–20) reported production of iturin and surfactin by a strain of *B. subtilis* NB 22 using wheat bran and okara (soybean curd residue). Okara is a by-product of tofu manufacturing processes. It is composed of water (81.1%), protein (4.8%), fat (3.6%), starch and sugar (6.4%), fiber (3.3%), and ash (0.8%). Ghurye *et al.* (21) put forward a practical approach to biosurfactant production using nonaseptic fermentation of mixed cultures on molasses. Sudhakar *et al.* (22) studied the kinetics of biosurfactant production by *P. aeruginosa* strain BS2 from distillery and whey wastes. The results indicated that specific growth rates (μ_{max}) and specific product formation rates (V_{max}) from both wastes were comparatively greater than those from a synthetic medium, thus showing potential use of both industrial wastes for biosurfactant production.

Efforts have recently been initiated to use some of the agroindustrial wastes available in India for biosurfactant production (23). Molasses is one such by-product of sugar cane industry. It is the major raw material for the production of baker's yeast, citric acid, feed yeasts, acetone/butanol, organic acids, and amino acids. The principal reasons for widespread use of molasses as substrate are its low price compared to other sources of sugar and the pres-

ence of several other compounds besides sucrose. For example, average values for constituents of cane molasses (75% dry matter) are total sugars, 48–56%; nonsugar organic matter, 9–12%; protein (N × 6.25), 2–4%; potassium, 1.5–5.0%; calcium, 0.4–0.8%; magnesium, 0.06%; phosphorus, 0.06–2.0%; biotin, 1.0–3.0 mg/kg; pantothenic acid, 15–55 mg/kg; inositol, 2500–6000 mg/kg; and thiamine, 1.8 mg/kg. Two strains of *B. subtilis* in minimal medium supplemented with molasses as sole source of carbon were used for biosurfactant production (24). Both strains grew and produced biosurfactant in the late stationary phase (Figs. 1,2). *Bacillus subtilis* MTCC 2423 produced larger amounts of biosurfactant than *B. subtilis* MTCC 1427.

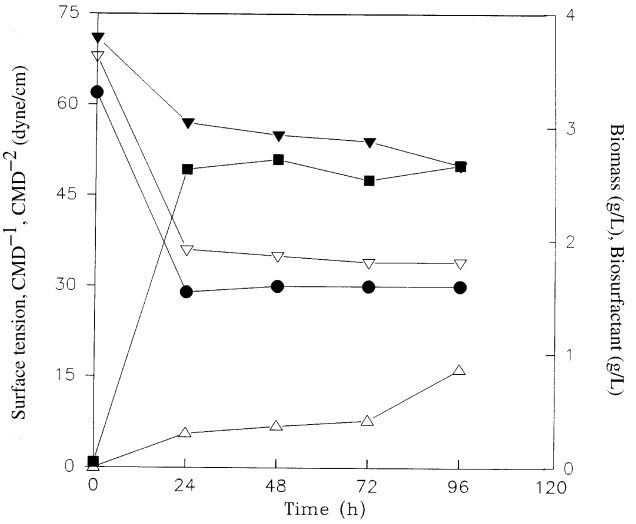

FIG. 1. Growth (■), biosurfactant production (△), and surface activity profiles of *Bacillus subtilis* MTCC 2423 grown in mineral salt medium supplemented with molasses at 45°C. Other symbols: (●) Surface tension, (▽) CMD^{-2}, (▼) CMD^{-2}, CMD, critical micelle dilution.

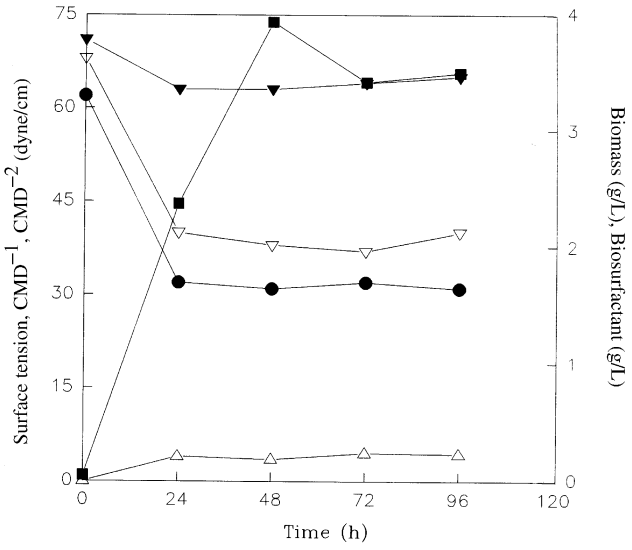

FIG. 2. Growth, biosurfactant production, and surface activity profiles of *Bacillus subtilis* MTCC 1427 grown in mineral salt medium supplemented with molasses. For symbols and abbreviation see Figure 1.

The use of wastes for bioprocesses generates new analytical and methodological problems for the critical measurement of the accumulated product. To solve these problems, methods need to be developed based on the specific wastes employed. Although the yield of biosurfactant from complex waste is presently low, one may assume that yields from complex wastes will increase with advances in biotechnology and process development.

In conclusion, the studies listed above suggest that, with suitable engineering and microbiological modifications, agroindustrial wastes may be used as substrates for the large-scale production of biosurfactants.

ACKNOWLEDGMENTS

The authors are grateful to the Director, IMTECH, Chandigarh, for permission to write this review. This is communication No. 034/98 from IMTECH, Chandigarh.

REFERENCES

1. Fiechter, A., Biosurfactants: Moving Towards Industrial Application, *Trends Biotechnol. 10*:208 (1992).
2. Georgious, G., S. Chyr, and M. Sharma, Surface Active Compounds from Microorganisms, *Bio/Technology 10*:60 (1992).
3. Kosaric, N., N.C.C. Gray, and W.L. Cairns, Biotechnology and Surfactant Industry, in *Biosurfactants and Biotechnology*, edited by N. Kosaric, W.L. Cairns, and N.C.C. Gray, Marcel Dekker, New York, 1987, p. 1.
4. Desai, J.D., and A.J. Desai, Production of Biosurfactants, in *Biosurfactants Production, Properties and Applications*, edited by N. Kosaric, Marcel Dekker, New York, 1993, p. 65.
5. Singer, M.E., Microbial Biosurfactants, *Microbes Oil Recovery. 1*:19 (1985).
6. Zajic, J.E., and W. Steffens, Biosurfactants, *Crit. Rev. Biotechnol.1*:87 (1984).
7. Hommel, R.K., Formation and Physiological Role of Biosurfactants Produced by Hydrocarbon-Utilizing Microorganisms, *Biodegradation 1*:107 (1990).
8. Cooper, D.G., and J.E. Zajic, Surface Active Compounds from Microorganisms, *Adv. Appl. Microbiol. 26*:229–253 (1980).
9. Cooper, D.G., and B.G. Goldenberg, Surface-Active Compounds from Two *Bacillus* Species, *Appl. Environ. Microbiol. 53*: 224 (1987).
10. Cooper, D.G., Biosurfactants, *Microbiol. Sci. 3*:145 (1986).
11. Mercede, M.E., M.A. Manersa, M. Robert, M.J. Epsuny, C. de Andres, and J. Guinea, Olive Oil Mill Effluent (OOME): New Substrate for Biosurfactant Production, *Bioresour. Technol. 43*: 1 (1993).
12. Mercede, M.E., and M.A. Manersa, The Use of Agroindustrial By-products for Biosurfactant Production, *J. Am. Oil Chem. Soc. 71*:61 (1994).
13. Kosaric, N., W.L. Cairns, N.C.C. Gray, D. Stechey, and J. Wood, The Role of Nitrogen in Multiorganism Strategies for Biosurfactant Production, *Ibid. 61*:1735 (1984).
14. Mulligan, C.N., and D.G. Cooper, Pressate from Peat Dewatering as a Substate for Bacterial Growth, *Appl. Environ. Microbiol. 50*:160 (1985).
15. Sheppard, J.D., and C.N. Mulligan, The Production of Surfactin by *Bacillus subtilis* Grown on Peat Hydrolysate, *Appl.*

Microbiol. Biotechnol. 27:110 (1987).
16. Koch, A.K., J. Reiser, and O. Kappeli, Genetic Construction of Lactose Utilizing Strains of *Pseudomonas aeruginosa* and Their Application in Biosurfactant Production, *Bio/Technology 6*:1335 (1988).
17. Manersa, A., J. Bastida, M.E. Mercede, C. de Andres, M.J. Espuny, and J. Guinea, Kinetic Studies on Surfactant Production by *Pseudomonas aeruginosa, J. Ind. Microbiol. 8*:133 (1991).
18. Ohno, A., A. Takashi, and M. Shoda, Production of Lipopeptide Antibiotic Surfactin by Recombinant *Bacillus subtilis* in Solid State Fermentation, *Biotechnol. Bioeng. 47*:209 (1995).
19. Ohno, A., A. Takashi, and M. Shoda, Production of Antifungal Peptide Antibiotic Iturin by *Bacillus subtilis* NB22 in Solid State Fermentation, *J. Ferment. Bioeng. 75*:23 (1993).
20. Ohno, A., A. Takashi, and M. Shoda, Production of a Lipopeptide Antibiotic Surfactin by Recombinant *Bacillus subtilis* NB22 Using Wheat Bran as Substrate, *Biotechnol. Lett. 14*: 817 (1992).
21. Ghurye, G.L., C. Vipulanandan, and R.C. Willson, A Practical Approach to Biosurfactant Production Using Non-aseptic Fermentation of Mixed Culture, *Biotechnol. Bioeng. 44*:661 (1994).
22. Sudhakar, B.P., A.N. Vaidya, A.S. Bal., R. Kapur., A. Juwarkar, and P. Khanna, Kinetics of Biosurfactant Production by *P. aeruginosa* Strain BS2 from Industrial Wastes, *Biotechnol. Lett. 18*:263 (1996).
23. Makkar, R.S., Production, Characterization and Applications of Biosurfactants with Special Emphasis to Their Synthesis by Extremophiles, Ph.D. Thesis, Panjab University, Chandigarh, India, 1997.
24. Makkar, R.S., and S.S. Cameotra, Utilization of Molasses for Biosurfactant Production by Two *Bacillus* Strains at Thermophilic Conditions, *J. Am. Oil Chem. Soc. 74*:887 (1997).

[Received April 28, 1998; accepted October 22, 1998]

Randhir S. Makkar was awarded a Ph.D. in 1999 for his thesis entitled "Production, Characterization and Applications of Biosurfactants with Special Emphasis to Their Synthesis by Extremophiles" (23). He is presently an STA Fellow in NFRI, Ibaraki, Japan.

Swaranjit S. Cameotra received his Ph.D. in 1986 for his thesis "Mechanism of Hydrocarbon Utilization by Microorganisms." He is leading a project on "Microbial Degradation of Petroleum Sludge and is employed as a Scientist "E" in the Institute of Microbial Technology, Chandigarh, India. He is also responsible for the Bacterial Wing of the Microbial Type Culture Collection (MTCC) located in IMTECH, Chandigarh, India.