The Use of Agroindustrial By-Products for Biosurfactant Production

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Traditionally, hydrocarbons have been used for biosurfactant production. However, urban waste, peat pressate and agroindustrial by-products, such as olive oil mill effluent and acid whey, are possible substitutes for microbial growth and biosurfactant production. The state of the art has been reviewed, augmented by some new information on *Pseudomonas* fermentation of olive oil mill effluent. More research is needed to improve yields and production economics.

KEY WORDS: Biosurfactant, olive oil mill effluent, *Pseudomonas* waste biotransformation.

Recently, a considerable number of studies have been published on surfactants produced by a wide variety of microorganisms such as bacteria, yeast and filamentous fungi. Microorganisms can produce a broad spectrum of products with excellent surface-active properties. Most microbial surfactants are complex molecules, comprising different structures that include peptides, glycolipids, glycopeptides, fatty acids and phospholipids, as reviewed recently (1–4). The most commonly isolated and widely studied group of surfactants produced by microorganisms are glycolipids (5–8).

From the economic standpoint, biological surfactants are not yet competitive with their synthetic counterparts. As stated by Cooper (9), to justify the replacement of synthetic surfactants by biological molecules, it is necessary to find a more economical production process. Different routes should be examined to reduce production costs, such as high yields and product accumulation, economical engineering processes, and use of cost-free or cost-credit feedstocks for microorganism growth and surfactant production.

Several strategies for biosurfactant production from waste materials proposed so far are reviewed, and a new strategy for rhamnolipid production from an agroindustrial waste, olive oil mill effluent (OOME), as feedstock is also discussed.

Growth substrates. It is assumed that surfactant production is induced to render hydrophobic substrate accessible to the cell (1,10). The main factor for the proposed use of hydrocarbons as a substrate for biosurfactant production was their price. Although high yields of rhamnolipids have been reported (Table 1) (3), hydrocarbons should not be used as substrate at this time because of high process cost (6).

Carbohydrates and vegetable oils are among the most widely used substrates for research on biosurfactant production (Table 1), although rhamnolipid accumulation from glucose is not high, about 2.3 $g \cdot L^{-1}$ (3,11). Vegetable oils are good substrates for surfactant production (Table 1). Substrate conversion yields ($Y_{p/s}$) reported are fairly high: 0.50 at a preparative scale from olive oil in the culture medium (12), and 0.61 with production of 46 g/L after 10 d of fermentation in a 50-L bioreactor with corn oil added to the medium (13). The advantages of these arable feedstocks are the huge surpluses and the possibility of production in regions with temperate to tropical climates.

Selection of waste substrates involves the difficulty of finding a waste with the right balance of carbohydrates and lipids to support optimal growth and production. Agroindustrial wastes with high contents of carbohydrates or lipids and urban wastes meet the requirements for use as substrates for biosurfactant production. Peat pressate, urban waste, OOME, lactic whey and soapstock oil are possible substrates for surfactant accumulation.

Few attempts at using wastes for biosurfactant production and only a few types of surfactants produced from wastes have been published to date (Fig. 1). Kosaric *et al.* (14) reported processes for sophorose-containing lipid production from urban wastes, which may involve multiorganism strategies: Carbohydrate-rich waste is converted into triglycerides by oleaginous organisms, and a second microorganism, *Torulopsis bombicola*, converts these lipids to sophorolipids. Yields reported are between 0.36 to 0.74, depending on the strategy followed.

Mulligan and Cooper (15) considered the possibility of using water that is expressed during the drying of fuel-grade peat. This waste contains a significant amount of carbohydrates (glucose, galactose and xylose) and some amino acids,

TABLE 1

Comparison of Rhamnolipid Production by Different Substrates and Different Strains of *Pseudomonas aeruginosa*

Microbial strain	Carbon source	Product concentration (g/L)	Conversion yield ($Y_{p/s}$)	Product yield ($Y_{p/x}$)	Reference
Unknown	Glucose	—	0.4	0.8	3
DSM 2659	Glucose (c. culture)	2.25	0.075	0.9	11
44T1	Olive oil	10.0	0.5	2.38	12
UI 29791	Corn oil	46.0	0.61	3.0	13
DSM 2874	*n*-Alkanes (resting cells)	13.2	0.24	3.5	22
DSM 2874	*n*-Alkanes (growing cells)	12.8	0.32	0.63	22

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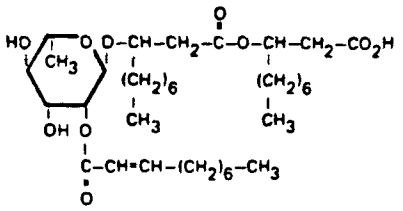

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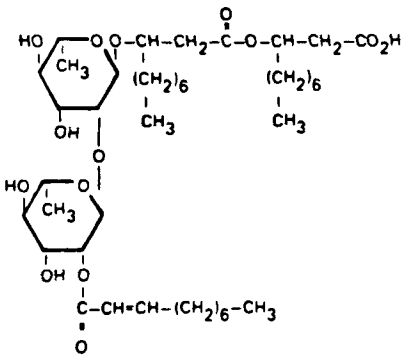

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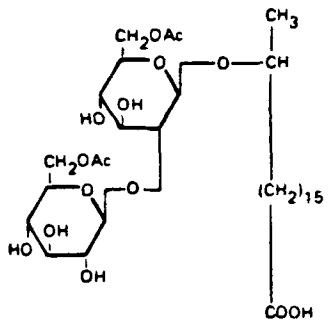

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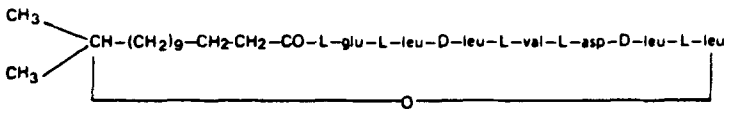

1. Biosurfactants produced from wastes. (a)(b) Rhamnolipids of *Pseudomonas aeruginosa* (22). (c) Sophorolipid (acid form) of *Torulopsis bombicola* (12). (d) Surfactant of *Bacillus subtilis* (4).

as substrate for microbial growth and surfactant production by *Bacillus subtilis*. The critical micelle concentration $^{-1}$ values of eight were observed, although no conversion yields were reported.

Another strategy, reported by Koch *et al.* (16), is the use of lactic whey from the dairy industry as a substrate for rhamnolipid production. The composition of whey generally includes high amounts of lactose (75% of dry matter), 12 to 14% of protein and smaller amounts of organic acids, minerals and vitamins. Its disposal is a major pollution problem. The strategy, involving the cloning of the lactose gene from *Escherichia coli*, LacZY, to the *P. aeruginosa* chromosome, enables this organism to utilize lactic whey as a substrate for growth and rhamnolipid production, although no production yields have so far been published.

For maximal concentration of rhamnolipids, lipoidal substrates should be used. We have reported that *P. aeruginosa* 44T1 produces 10 $g \cdot L^{-1}$ of rhamnolipids with olive oil as the sole source of carbon (12). Soapstock oil was used for rhamnolipid production with *P. aeruginosa* D10 (S. Elysseev, personal communication). Current studies carried out in our laboratory with other residual lipidic wastes from the oils and fats processing industry indicate that they are able to support microbial growth and produce rhamnolipids when supplied as the sole carbon source in a mineral medium.

As part of an effort to contribute to the recycling of waste material from local industries, residue from the olive oil industry was used for rhamnolipid production. This feedstock, OOME, is produced during olive oil extraction and contains the water of the olives themselves and also the water used during the extraction process (Table 2). Olive oil processing is carried out with the traditional discontinuous pressing process or the more recent continuous solid-liquid centrifuge system. Spain produces about 2,000,000 m^3 of this effluent annually, which has a high solids content of 40-120 $kg \cdot m^{-3}$, depending on the extraction process (17).

TABLE 2

Composition of the Olive Oil Mill Effluent

Components (%)	Discontinuous process	Continuous process
Dry material	12	4.0
Organic substances	10.5	3.5
Minerals	2.5	0.5
Organic components (%)		
Sugars	5.0	1.0
Protein	1.2	0.28
Organic acids	0.7	0.30
Polyalcohols	1.8	1.1
Pectins, tannins	1.0	1.37
Polyphenols	1.0	0.5
Lipids	0.1	1.0
Mineral components (ppm)		
P	500	96
K	3000	1200
Ca	350	120
Mg	200	48
Na	450	245
Fe	35	16

TABLE 3

Contaminating Load of Oil Mill Effluents

Parameters ($kg \cdot m^{-3}$)	Discontinuous process	Continuous process
pH	4.5–5	4.7–5.2
BOD[a]	120–130	43–60
COD[a]	90–100	35–48
Suspensed solids	1	9
Total solids	120	60
Total volatile matter	105	55
Minerals	15	5
Lipids	0.5–1.0	3–10

[a]BOD, biological oxygen demand; COD, chemical oxygen demand.

Although different technologies are being evaluated to deal with this waste, most of the waste water is stored in open basins for evaporation, which produces noxious odors, proliferation of insects and other environmental risks. Essentially, the composition is water (83.2%), organic substances and minerals, and it varies depending on the oil extraction process. The fundamental organic compounds and mineral elements are shown in Table 2. This waste water has a low pH (4.5–5) and is lightly colored. Generally, waste waters produced with the continuous process are more dilute, but the polluting organic load, in terms of the weight of olives processed, amounts to 45–55 kg biological oxygen demand (BOD_5) per ton of olives, no matter which processing method is used. Its chemical oxygen demand (COD) ranges from 35 to 100 $g \cdot L^{-1}$, depending on the oil extraction procedure (Table 3) (17,18).

Microorganisms. Biosurfactants are produced by a variety of prokaryotes and eukaryotes (Table 4) (16,19,20), although few of them so far have been grown on residual substrates (14,15,21). Microbial surfactants are lipoidal molecules; their occurrence, chemical structure and properties have been reviewed extensively (1,4,6,16).

In some cases, biosurfactants are produced exclusively during the growth of the microorganism on hydrophobic substrates. *Rhodoccocus* and *Corynebacterium* are representative microorganisms of this group (16,22). Others produced surfactants on both water-soluble and hydrophobic substrates. *Pseudomonas aeruginosa* and *T. bombicola* (15,23) are examples of microorganisms of this group. Potentially, microorganisms of this group are the most adapted for growing and producing biosurfactants in complex substrates, such as wastes. Thus, the nature and composition of the waste substrate should be evaluated for a good selection of the proper microorganism. In our laboratory, *P. aeruginosa* JAMM (NCIB 40044) was selected for its capacity to decrease surface tension when grown on OOME (21,24).

Production process. The use of waste material for surfactant production involves the difficulty of finding a suitable substate, fulfilling a number of general nutritional conditions, to achieve microbial growth, and production. It is well known that biosurfactant accumulation is strongly influenced by C:N and C:P ratios and the presence of divalent cations, such as iron or calcium (20,25).

Because of its origin, OOME has a high concentration of valuable organic substances, such as sugars (glucose, sacharose), nitrogen compounds, pectins, polyphenols and residual oil. It has enough mineral content to make this effluent an interesting substrate for microbial growth; only the addition of nitrogen should be considered (24). For rhamnolipid production with OOME, it was only necessary to add 2.5 $g \cdot L^{-1}$ sodium nitrate, as nitrogen source, to support better growth and a greater subsequent drop in surface tension (from 42 mN m^{-1} to 30 mN^{-1}), which indicates the presence of surfactants (21). In comparison, when peat pressate water was used as substrate for surfactant production by *Bacillus subtilis*, the addition of glucose or peptone was necessary (15). As stated by Kosaric *et al.* (14), sophorolipids are produced minimally with urban wastes if lipogenic substrates are not added. Few biosurfactant production processes with waste substrates have been published. We have developed the process for rhamnolipid production in a stirred tank fermentor with *P. aeruginosa* (Figs. 2 and 3). The surfactant concentration increased during incubation, achieving a final value of 1.4 $g \cdot L^{-1}$. The conversion yield ($Y_{p/s}$) was 0.058, calculated on the basis of the COD (24 $g \cdot L^{-1}$) of the culture medium.

The use of wastes as feedstock for bioprocesses generates new analytical and methodological difficulties concerning the critical measurement of the product accumulated (24). To overcome these difficulties, new methods should be developed based on the waste used.

TABLE 4

Microbial Biosurfactants

Microorganisms	Type of biosurfactant
Rhodococcus sp.	Trehalose lipids
Arthrobacter	Trehalose dicorynemicolates
Pseudomonas sp.	Rhamnolipids
	Ornitine lipids
	Fatty acids
A. calcoaceticus	Lipopolysaccharide
Corynebacterium	Corynemicolic acids
Bacillus subtilis	Lipopeptides
Candida tropicalis	Glycolipids
Torulopsis sp.	Sophorolipids

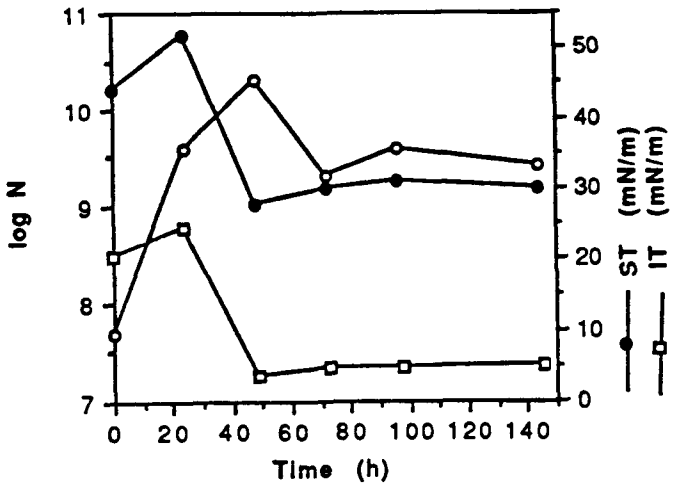

FIG. 2. Growth of *Pseudomonas* sp. JAMM (NCIB 4044) in olive oil mill effluent medium and modification of surface-active parameters. ST—surface tension, IT—interfacial tension.

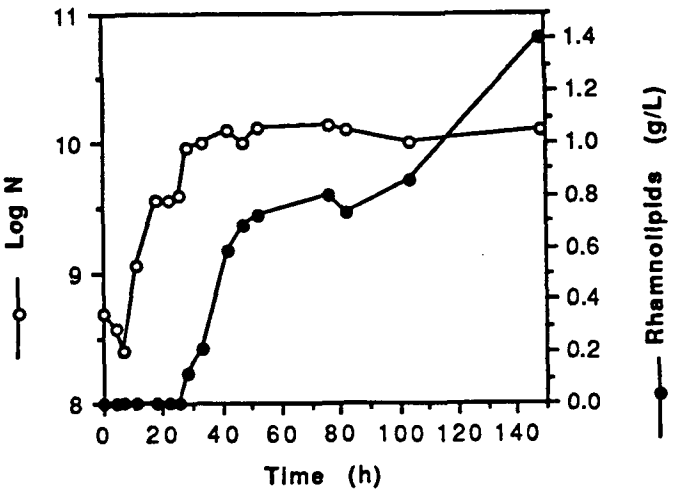

FIG. 3. Growth and rhamnolipid production of *Pseudomonas* JAMM (NCIB 4044) in olive oil mill effluent medium.

The yields for biosurfactant production from complex wastes are low. However, more research is needed to develop biotechnological processes to increase biosurfactant production yields from complex wastes, and efforts to find further alternative waste materials with nutritional capabilities to be used as fermentation feedstocks should be continued.

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