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Sophorolipids Production by *Candida Bombicola* using Synthetic Dairy Wastewater

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***Abstract*—** Sophorolipids (SLs) production by the yeast *Candida bombicola* was studied in batch shake flasks using synthetic dairy wastewaters (SDWW) with or without any added external carbon and nitrogen sources. A maximum SLs production of 38.76 g/l was observed with the SDWW supplemented with low cost substrate of sugarcane molasses at 50 g/l and soybean oil at 50 g/l. When the SDWW was supplemented with more costly glucose, yeast extract, urea and soybean oil, the production, however, got lowered to only 29.49 g/l, but with a maximum biomass production of 17.38 g/l together with a complete utilization of the carbon sources.

***Keywords*—**Candida bombicola, dairy wastewater, fat and oil, sophorolipids.

I. Introduction

SOPHOROLIPIDS (SLs), a kind of extracellular biosurfactants, are reported to be secreted by yeasts of *Candida* sp.[1]-[3]. Typically, SLs consists of a dimeric glucose (also called sophorose) linked by a glycosidic bond through a hydroxyl group located at the penultimate position of an 18-carbon fatty acid. This type of biosurfactant occurs as a mixture of macrolactone and open-chain (free acid) forms and may be acetylated at the primary hydroxyl positions of the sophorose sugars [2], [3]. SLs and their derivatives have also shown promise as surfactants, emulsifiers, antimicrobials and a source of speciality chemicals such as sophorose and hydroxylated fatty acids [4], [5]. In the environmental cleanup arena, surfactant properties of SLs have been identified to be ideal for several applications, mainly for heavy metal removal from soil sediments [6] and in biodegradation of insoluble aromatic compounds [7].

It is well known that the cost of raw materials generally contributes up to 75% of the selling price of bioproducts. Hence, in order to compete with more cheaper chemical surfactants, it is important to use suitable low-cost fermentation media for the production of SLs [8]. In other words, biosurfactants can replace synthetic surfactants by way of maintaining the cost of the raw material in its production process at a minimum. In this aspect, renewable substrates from various sources, particularly from well-known industrial wastes can be utilized for the production of biosurfactants. Soy molasses, sugarcane molasses, animal fat are some of the industrials wastes which have been used for the production of SLs in large amounts [4], [9], [10].

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Dairy industry is one of the major food industries in India, and India ranks first among the maximum major milk producing nations [11]. The wastewaters generated from dairy industries contain large amount of fats and oils that makes such wastewaters not easily biodegradable [12], [13]. Further, high levels of fats and oils in these wastewaters cause gross pollution of land and water due to their high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) [13]. Also, the high content of fats and oils often interfere with normal wastewater treatment procedure resulting in significant increase in the process cost and time. Hence, removal or pretreatment of these fats and oils is necessary before subjecting the wastewater to biological treatment operations. A large number of pretreatment methods are employed to remove fats and oils from these wastewaters prior to biological treatment, and these methods include grease-trap, tilted plate separators, dissolved air flotation systems and other physico-chemical treatment methods. However, the cost of these methods is considered high and removal efficiency of fat and oil is low [12] – [14]. There are few reports available for degradation of fats and oils by alkaline/acid/enzymatic hydrolysis [15], [16], but these methods are still not very cost effective. Therefore, utilization of dairy wastewater by microorganisms for the production of valuable bioproducts can solve both purposes: pretreatment of the wastewater and cost reduction in the bioproduct production process.

Towards this goal, synthetic dairy wastewaters (SDWW) were prepared in our laboratory and tested for SLs production by the yeast *Candida bombicola*.

II. Materials And Methods

A. Microbial Culture and its Maintenance

The yeast used in this study *Starmerella bombicola* NRRL Y-17069 (an equivalent strain of *C. bombicola* ATCC 22214) was procured from Agricultural Research Service (ARS-Culture collection), USDA, Peoria, USA. The strain was grown, according to the supplier's instructions, for 48 h at 30 °C incubation on agar slants containing (g/l): glucose, 10; yeast extract, 3; peptone, 5; and agar, 20 (GYP-agar). The microorganism was sub-cultured in every four weeks and maintained at 4 °C in a refrigerator.

B. Chemicals and Reagents

All chemicals and solvents used in the study were of analytical grade and supplied by Hi-Media Pvt. Ltd., India and Merck India Ltd., respectively. Dried milk powder, fat (ghee), sugarcane molasses and soybean oil used in the fermentation of the yeast were purchased from local market in Guwahati, India.

C. Seed Culture Preparation

The medium used for developing the seed culture contained (g/l): glucose, 100; yeast extract, 10; urea, 1, pH 6.0 [2]. 250 ml Erlenmeyer flasks containing 50 ml of the seed culture media were autoclaved at 121 °C for 20 min, and inoculated with a loop full of the microorganism freshly grown on GYP agar slant. The culture was then incubated for 48 h at 30 °C and 180 rpm in a rotating orbital incubator shaker.

D. Sophorolipids Production

The production of SLs was carried out in batch shake flasks each containing 50 ml of synthetic dairy wastewater with or without added nutrients. The SDWW was prepared in the laboratory by mixing dried milk powder and fat (ghee) with or without any added nutrients and labeled as medium A, B, C, D and E [17]. Table I shows the composition of SDWW tested for the SLs production in this study. All the experimental flasks were inoculated with 5 % seed culture and incubated at 30°C and 180 rpm.

TABLE I
COMPOSITION OF THE VARIOUS MEDIA BASED ON SDWW WITH OR WITHOUT ANY ADDED NUTRIENTS USED FOR SLS PRODUCTION BY THE YEAST C. BOMBICOLA.

Media A	
Constituents	Concentration (g/l)
Dried milk powder	2.0
Fat (ghee)	0.2
Media B	
Constituents	Concentration (g/l)
Dried milk powder	2.0
Fat (ghee)	0.2
Sugarcane molasses	50
Media C	
Constituents	Concentration (g/l)
Dried milk powder	2.0
Fat (ghee)	0.2
Sugarcane molasses	50
Soybean oil	50
Media D	
Constituents	Concentration (g/l)
Dried milk powder	2.0
Fat (ghee)	0.2
Glucose	100
Yeast extract	10
Urea	1.0
Soybean oil	100
Media E	
Constituents	Concentration (g/l)
Dried milk powder	50
Fat (ghee)	1.0

E. Analytical Methods

1) Biomass Estimation and Carbohydrate Concentration

For the yeast biomass measurement samples taken periodically along the 8-day fermentation were extracted twice with equal volume of ethyl acetate to remove unutilized oil and SL in the fermentation broth. After separating the two layers, the aqueous layer was centrifuged at 10,000 × g for 10 min at 25°C, and the cell pellets were washed twice with distilled water and dried to constant weight at 65°C under vacuum for determining the yeast biomass concentration; the supernatant was utilized for analyzing residual carbohydrate concentration in the sample. The carbohydrate analysis in media type A and E was carried out by DNS reagent with the DNS method using lactose as the standard [18]; for other media types (B, C and D) total carbohydrate content was analyzed by anthrone method with glucose as the standard [19].

2) Sophorolipids Estimation

For SLs analysis, the previously obtained ethyl acetate extract was vacuum-dried at 40°C to remove the solvent. The residue was twice washed with hexane to remove the remaining oil, and any hydrophobic substances such as fatty acids and alcohols, if any formed during the fermentation. Partially purified SLs was thus obtained after vaporizing the residual hexane at 40°C under vacuum and its yield was measured by gravimetric measurement [9], [20].

III. RESULTS AND DISCUSSION

Dairy wastewaters are considered highly rich in biodegradable carbon and nitrogen source, and hence its utilization as a low cost fermentative media for SLs production was tested with aims to reduce its production cost as well as to reduce the biological load in the wastewater.

The time course of SLs production during the yeast fermentation using different production media based on SDWW investigated in this study is presented in Fig. 1; it can be observed that a maximum SLs production of 38.76±1.3 g/l was obtained when it was supplemented with sugarcane molasses and soybean oil, which was followed by 29.49±2.9 g/l of SLs production when supplemented with glucose, yeast extract, urea and soybean oil. The SLs production was found to be very less when SDWW was not supplemented with any external carbon or nitrogen source. These results are very well matched with the results obtained from our earlier work, which also shows higher production of SLs by *C. bombicola* when the production medium contained only sugarcane molasses and soybean oil [21].

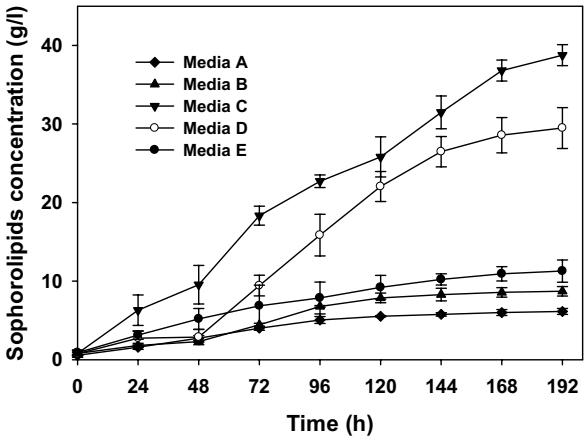

Fig. 1 Time course of SLs production by the yeast employing the different media types based on SDWW with and without any added nutrients.

On the other hand, the yeast biomass produced was more (17.38 g/l) in wastewater supplemented with glucose, yeast extract, urea and soybean oil than others. This increase in yeast biomass in media type D was attributed to the high nitrogen concentration compared to the other media types and also because the yeast was able to utilize the glucose as a

carbon source more easily compared to the sugarcane molasses. Fig. 3 shows the time course of carbohydrate utilization, which shows that the yeast was able to consume all the carbohydrate in medium type D, which contained glucose, compared to other media types.

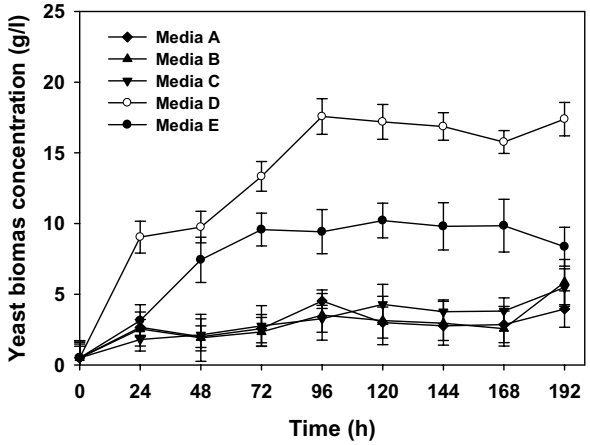

Fig. 2 Time course of yeast growth in the different media types based on SDWW with and without any added nutrients.

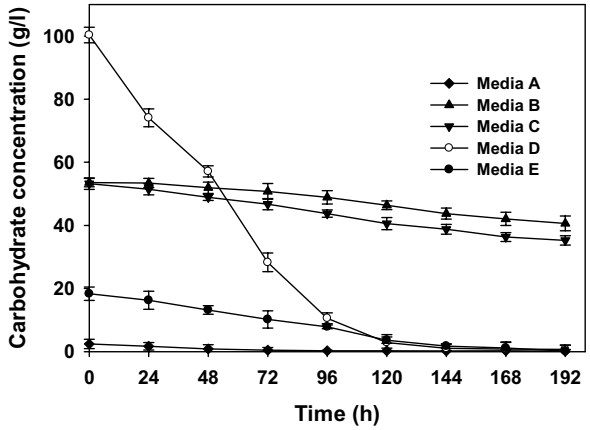

Fig. 3 Time course of carbohydrate utilization by the yeast during its growth in different media types.

From Figs. 1 – 3 it is clear that the yeast is able to grow, produce SLs and utilize carbohydrate content of the media even when extra carbon and nitrogen source is not supplied (media type A and E)suggesting its possible application in utilization as well as pretreating real dairy waste water.

IV. Conclusions

The present study showed that the yeast *C. bombicola* can utilize SDWW, with or without other nutrients, for SLs production. The production was the maximum when SDWW was supplemented with cheap carbon sources of sugarcane molasses and soybean oil. Further, the study suggested the feasibility of utilizing real dairy industry wastewater for both production of SLs and pretreatment purpose.

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