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Kinetics of Growth and Enhanced Sophorolipids Production by *Candida bombicola* Using a Low-Cost Fermentative Medium

Achlesh Daverey · Kannan Pakshirajan

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Abstract In this study, effect of various parameters on sophorolipid (SL) production by the yeast *Candida bombicola* was investigated for the enhancing of its production by employing L18 orthogonal array design of experiments. At optimum conditions of sugarcane molasses 50 gl^{-1}, soybean oil 50 gl^{-1}, inoculum size 5% (*v*/*v*), temperature 30 °C, inoculum age 2 days, and agitation 200 rpm, the yeast produced almost equal amounts of the product in batch shake flasks and in a 3-l fermentor without any pH control (45 and 47 gl^{-1}, respectively). However, the yield increased to 60 gl^{-1} in the fermentor under controlled pH environment. Time course of SL production, yeast biomass growth, and utilization of sugarcane molasses and soybean oil at these optimized conditions were fitted to existing kinetic models reported in the literature. Estimated kinetic parameters from these models suggested that conventional medium containing glucose can very well be replaced with the present low-cost fermentative medium.

Keywords Sophorolipids · *Candida bombicola* · Design of experiments · Sugarcane molasses · Kinetics

Introduction

Sophorolipids (SLs) are a group of glycolipid type of biosurfactants produced by several *Candida* species mainly *Candida bombicola*, *Candida apicola*, *Candida bogoriensis*, and *Candida batistae* [1–4]. SLs and derivatives have shown promise as clinical agents in improving the rate of sepsis survival [5], in reducing asthma severity in an *in vivo* asthma model [6], in decreasing IgE production, and in killing human pancreatic cancer cells [7]. SLs have also been reported as an inducer of cellulose production [8], as a capping agent for Co nanoparticles [9], and for preparing glucolipids and specialty fatty acids [10]. They have also found applications as cosmetics [11], deodorants [12], and in the manufacture of

A. Daverey · K. Pakshirajan (✉)
Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781039 Assam, India
e-mail: pakshi@iitg.ernet.in

detergents [13]. In the environmental sector, they have been tested for heavy metal removal from soil sediments [14] and degradation of insoluble aromatic compounds [15].

However, for successful commercialization of SLs over existing synthetic surfactants, the process should be carried out with minimal cost, but with higher productivity. Since raw materials in any process contribute up to 75% of the total production cost, the cost can be kept low by utilizing agro-industry wastes such as deproteinized whey, soy molasses, and industrial fatty acid residues. Although *C. bombicola* has been shown to utilize these agro-industry wastes [16–19], the yield of SL production is not sufficient enough to replace conventional costly medium and therefore necessitates optimization of medium and culture conditions.

In our earlier work, sugarcane molasses was evaluated for the first time as a low-cost fermentative hydrophilic carbon source compared to costly glucose for the production of SLs using *C. bombicola* [20]. However, no attempt has been made so far to systematically investigate the effect of such low-cost medium and other process parameters on the SL production to further enhance its yield. Therefore, in the present work, effect of various physical and chemical variables influencing the SL production by the yeast using sugarcane molasses as the cheap raw material was studied and optimized by employing the statistically valid orthogonal array (OA) and experimental design technique. The yield of SL production under optimized process conditions was also determined, and the time courses of SL production, yeast biomass growth, and sugarcane molasses and soybean oil consumption were modeled with an aim to estimate the biokinetic constants in the process.

Materials and Methods

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^{-1}): glucose, 10; yeast extract, 3; peptone, 5; and agar, 20 (GYP-agar). The microorganism was sub-cultured every 2 weeks and maintained at 4 °C in a refrigerator.

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. Low-cost media constituents of soybean oil and sugarcane molasses used in the fermentation of the yeast were purchased from a local market in Guwahati, India.

Effects of Various Process Parameters on SL Production

A well-known experimental design technique, namely L18 OA design [21–23], was employed to study the effect of six parameters, viz inoculum size, sugarcane molasses concentration, soybean oil concentration, temperature, inoculum age, and agitation on the SL production. Essentially, the design consisted of a total of 18 experiments with the six parameters—one parameter having two levels and the others having three levels, i.e., L18 ($2^1 \times 3^5$). Table 1 presents the experimental combinations adopted as per the design along

Table 1 Experimental design matrix showing parameters and their levels along with the observed response.

Experimental run no.	Parameters						SL[a] production (g l^{-1})	*S/N* ratio (dB)
	Inoculums size (%)	Sugarcane molasses (g l^{-1})	Soybean oil (g l^{-1})	Temperature (ºC)	Inoculums age (d)	Agitation (rpm)		
1	5 (1)	50 (1)	50 (1)	20 (1)	2 (1)	150 (1)	28.62±1.17	29.14
2	5 (1)	50 (1)	100 (2)	25 (2)	3 (2)	200 (2)	32.4±1.98	30.21
3	5 (1)	50 (1)	150 (3)	30 (3)	4 (3)	250 (3)	15.8±1.84	23.97
4	5 (1)	100 (2)	50 (1)	20 (1)	3 (2)	200 (2)	31.2±1.13	29.88
5	5 (1)	100 (2)	100 (2)	25 (2)	4 (3)	250 (3)	14.2±1.27	23.05
6	5 (1)	100 (2)	150 (3)	30 (3)	2 (1)	150 (1)	30.5±0.71	29.69
7	5 (1)	150 (3)	50 (1)	25 (2)	2 (1)	250 (3)	17.78±1.10	25.00
8	5 (1)	150 (3)	100 (2)	30 (3)	3 (2)	150 (1)	16.5±0.85	24.35
9	5 (1)	150 (3)	150 (3)	20 (1)	4 (3)	200 (2)	14.25±1.34	23.08
10	10 (2)	50 (1)	50 (1)	30 (3)	4 (3)	200 (2)	38.67±2.36	31.75
11	10 (2)	50 (1)	100 (2)	20 (1)	2 (1)	250 (3)	12.9±1.13	22.21
12	10 (2)	50 (1)	150 (3)	25 (2)	3 (2)	150 (1)	26.1±0.99	28.33
13	10 (2)	100 (2)	50 (1)	25 (2)	4 (3)	150 (1)	24.3±2.26	27.71
14	10 (2)	100 (2)	100 (2)	30 (3)	2 (1)	200 (2)	36.8±1.70	31.32
15	10 (2)	100 (2)	150 (3)	20 (1)	3 (2)	250 (3)	10.5±2.12	20.42
16	10 (2)	150 (3)	50 (1)	30 (3)	3 (2)	250 (3)	16.3±1.41	24.24
17	10 (2)	150 (3)	100 (2)	20 (1)	4 (3)	150 (1)	13.25±1.06	22.44
18	10 (2)	150 (3)	150 (3)	30 (3)	2 (1)	200 (2)	15.65±0.78	23.89

dB decibels

[a] Results are the average of three experiments

with SL yield as the observed response in each run. All experiments in this study were performed in 250-ml Erlenmeyer flasks by varying the medium composition and culture conditions according to the experimental plan (Table 1). To study the SL production in the batch shake flasks, 50 ml of the production medium was inoculated with seed culture and agitated in an incubator shaker for 8 days. Medium used for the developing the yeast seed culture consisted of (g l^{-1}): glucose, 100; yeast extract, 10; urea, 1, pH6.0 [24]. To vary the age of seed culture/inoculum, yeast grown at different times—as per the experimental design in Table 1—in three flasks each containing 50 ml of the seed culture medium was incubated at 30°C and 180 rpm in a rotating orbital incubator shaker. Samples were taken periodically for the analyses of the concentrations of biomass, residual sugarcane molasses, oil, and SLs in the media. All batch shake flasks experiments in this study were conducted in triplicate, and average of results from these triplicate experiments is presented with a standard deviation of ±3%.

Sugarcane molasses used was diluted twice with distilled water to overcome its high viscosity and then centrifuged at 10,000 rpm for 15 min to remove any nonvolatile suspended solids. The final concentration of molasses, required as per the experimental design, was adjusted by suitably diluting with distilled water. Sugarcane molasses contained approximately 20% water, 62% sugar, 10% nonsugar, and 8% inorganic salts as ash

contents [25]. Of the total sugar content, 35% *w*/*v* of sugarcane molasses contains sucrose as the major sugar with the remaining composed of glucose and fructose. In each experimental run, the response was recorded as the SL production and corresponding signal-to-noise (*S*/*N*) ratio was calculated using Eq. 1 with an overall objective of estimating the effects of various parameters on SL production, where a large *S*/*N* ratio is preferred.

$$\frac{S}{N} = -10 \times \log\left(\frac{1/Y^2}{n}\right) \tag{1}$$

where Y is the response, and n is the number of experimental runs.

Statistical analysis of the results in the form analysis of variance (ANOVA) was performed by using the statistical software package MINITAB® Release 15.1, PA, USA.

Analytical Methods for the Estimation of Yeast Biomass, Sugarcane Molasses, Soybean Oil, and SLs

Samples were extracted twice with equal volume of ethyl acetate to separate unutilized oil and SLs from the fermentation broth. Following separation of the two layers, the aqueous layer was centrifuged at 12,000×*g* for 15 min at 25 °C, and the cell pellets were washed twice with distilled water and dried to constant weight at 80 °C to determine the yeast biomass concentration. The resulting supernatant was utilized to analyze residual sugarcane molasses concentration in the sample, in terms of total carbohydrate content, by anthrone method [26].

The solvent layer, i.e., ethyl acetate extract was vacuum-dried at 40 °C to remove the solvent. The residue was washed twice with hexane to remove any remaining oil and any hydrophobic substances, viz. fatty acids and alcohols, formed during fermentation. SLs were thus obtained after vaporizing the residual hexane at 40 °C under vacuum and its yield was calculated gravimetrically [19]. Concentration of unutilized soybean oil in the samples was determined gravimetrically from the hexane extract following extraction of SLs by ethyl acetate and vacuum evaporation [27], as mentioned before.

Results and Discussion

Major limitations for commercial exploitation of any bioproduct is its production cost, which can, however, be kept low by using low-cost fermentative medium based on cheap raw materials. A number of low-cost fermentative substrates, compared to costly glucose, have been tested for SL production, but all these substrates have often resulted in a decreased yield of the product [16, 17, 19]. Hence, there is a need to search for a better yet cheap substrate for the production of SLs. In our earlier study, conducted in batch shake flasks, it was found that *C. bombicola* produced more SLs when grown on media containing only sugarcane molasses and soybean oil compared to those produced on media containing sugarcane molasses, yeast extract, urea, and soybean oil [28]. The study thus indicated the possibility of eliminating the use of unnecessary nitrogen sources (yeast extract and urea) in the medium for keeping the process cost low and also for better yield of the product. Therefore, in this study, both sugarcane molasses and soybean oil concentrations along with the physical parameters temperature, agitation, inoculum size, and age were chosen to study their effect on SL production.

Table 2 Values of average *S*/*N* ratio of the process variables at various levels and their ranking based on delta *S*/*N* ratio.

Level	Inoculums size	Sugarcane molasses	Soybean oil	Temperature	Inoculums age	Agitation
1	26.48	27.60	27.95	24.53	26.87	26.94
2	25.81	27.01	25.60	26.37	23.24	28.35
3		23.83	24.90	27.55	25.33	23.15
Delta[a]	0.67	3.77	3.06	3.02	1.54	5.20
Rank	6	2	3	4	5	1

[a] Difference between maximum and minimum *S*/*N* ratio values

Influence of Individual Parameters on the SL Production

As noted earlier, Table 1 presents the experimental design and SL production obtained in each run of the study; the table also depicts the *S*/*N* ratio obtained in the experiments. It is clear from the table that depending upon the combinations of the process variables and their levels, SL production in each run varied largely thus indicating strong influence of the variables and their levels on the response. Further, to understand which of these variables affected SL production in a significant manner, their ranking was performed based on the calculated delta *S*/*N* ratio. In general, delta value for a factor, calculated by measuring the difference between the highest and lowest characteristic average *S*/*N* ratio of the factor, indicates its relative significance over others on a given response: higher value of delta for a factor denoting a larger significant effect than others. And, while *S*/*N* ratio indicates effect of factors on a response, delta *S*/*N* ratio can be used as a criterion for ranking factors of their effects on the response [22]. In this study, based on the delta *S*/*N* ratio obtained for each factor, the six parameters were ranked accordingly, and the results are presented in Table 2. This ranking suggests that agitation had the maximum effect while inoculum age had the least effect on SL production by *C. bombicola*. To validate these findings on the significance of the individual parameters and their contribution on SL production, ANOVA of the results was employed. Table 3 presents the ANOVA of SL production at 192 h of culture that shows a term for error, the value of mean sum of squares (MS) for which

Table 3 Analysis of variance (ANOVA) of SL production.

Source	DF[a]	SS[b]	Adj SS	MS[c]	*F* ratio (*F*)	*P* value (*P*)	Confidence level (%)	% Contribution
Inoculums size	1	2.56	2.56	2.56	0.17	0.695	30.5	0.196
Sugarcane molasses	2	368.47	368.47	184.24	12.18	0.008	99.2	28.148
Soybean oil	2	170.46	170.46	85.23	5.63	0.042	95.8	13.021
Temperature	2	160.75	160.75	80.37	5.31	0.047	95.3	12.28
Inoculums age	2	39.85	39.85	19.92	1.32	0.336	66.4	3.044
Agitation	2	566.95	566.95	283.48	18.74	0.003	99.7	43.310
Error	6	90.76	91.76	15.13				
Total	17	1399.80						100

[a] Degree of freedom

[b] Sum of squares

[c] Mean sum of squares

indicates that the amount of variation in the response data left unexplained is low. Table 3 also shows the main effects of the factors on the SL production. In general, low *P* value of a term in ANOVA indicates high significance of the term; hence, in this study, agitation was found to have a maximum significant effect on SL production followed by sugarcane molasses concentration. Guilmanov et al. [29] also reported that agitation largely impacts SL production compared to any other factors. Soybean oil concentration and temperature were next found to have a significant effect on the SL production with *P* values 0.042 and 0.047, respectively. The other two variables, i.e., inoculum age and size, did not, however, show any significant effect on the SL production. These findings of the effects of parameters on SL production by ANOVA are in good agreement with those observed earlier from the factors ranking based on their delta *S*/*N* ratio. The percent contribution of variance for each term in the ANOVA table (Table 3) shows that agitation and concentration of sugarcane molasses contributed more than 70% of the total SL production.

Selection of Optimum Levels of Parameters for Enhancing the SL Production

Figure 1 illustrates the effects of the six process variables investigated in the study on SL production. It can be seen from this figure that for each of the six variables tested at different levels, particular levels of the variables caused a significant increase in the mean response compared to other levels of the variables. For e.g., level 1 of each of the variables on inoculum size and age, concentrations of sugarcane molasses and soybean oil, level 3 of temperature and level 2 of agitation were found to exhibit a significant positive effect on the response. These levels of the variables were therefore chosen optimum for the maximum production of SLs in the medium [22]. Based on this observation, optimum levels of the process parameters were chosen to enhance the SL production, and Table 4 presents the optimum levels of the variables.

At the optimum values of the parameters (Table 4), SL production was investigated in a 250-ml Erlenmeyer flask with 50 ml working volume and later verified in a batch-operated 3-l fermentor (with or without pH control; Applikon, Holland) of 1 l working volume. The results showed that in the simple batch shake flask, the obtained SL production value of 45 gl^{-1} was very high compared to any of those values shown in Table 1, i.e., values

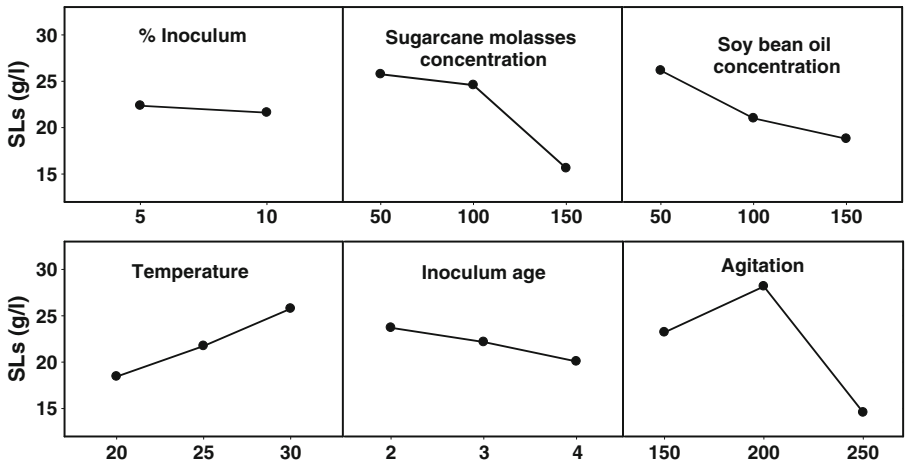

Fig. 1 Main effects plot of the process variables

Table 4 Optimum levels of the process parameters chosen for enhancing the SL production.

Process parameter	Optimum level
Inoculums size	5%
Molasses concentration	50 gl^{-1}
Soybean oil concentration	50 gl^{-1}
Temperature	30°C
Inoculums age	2 days
Agitation	200 rpm

obtained at un-optimized process conditions. However, in the fermentor operated without pH control, the yeast was able to produce 47 gl^{-1} of SL, which was even slightly higher than the value observed from the shake flask experiment, probably due to better control of aeration, temperature, and agitation conditions in the fermentor. These values are 90.02% more (or 1.92-fold higher) than earlier reported SL yield using the un-optimized low-cost medium and process conditions [28]. Further, batch fermentation was carried out using the same fermentor, but under controlled pH condition, as reported by Hu and Ju [27]. The initial pH of the production medium was set to 6.0 before autoclaving, as in the previous experiments, and during the fermentation when the pH dropped to 3.5, it was then controlled at this value throughout for this case. And after 192 h of fermentation, the yeast was able to produce 60 gl^{-1} of SLs, which was 27.6% higher than the production obtained in the fermentor operated with control of pH. Kim et al. [30] reported a maximum SL production of 80 gl^{-1} when the yeast *C. bombicola* was grown on glucose–soybean oil containing medium with yeast extract and other nutrients in a 5-l fermentor under continuous mode of fermentation.

On the other hand, when soy molasses and oleic acid were used in addition to yeast extract and urea in the fermentation medium with repeated feeding of soy molasses and oleic acid in a 12-l fermentor, Solaiman et al. [17, 18] observed that the yeast produced only 21 gl^{-1} of SLs; however, after refinement of the product isolation procedure, they were able to improve the yield up to 75 gl^{-1} of SLs. But the yield decreased to 53 gl^{-1} SLs when the yeast was grown on medium containing only soy molasses and oleic acid under fed-batch mode of fermentation [18]. On the contrary, in the present study, 60 gl^{-1} of SLs was produced by the yeast under simple batch mode of fermentation even without any yeast extract or urea in the medium with further scope of enhancing its production, such as by applying fed-batch mode of fermentation. In view of this, it could be well said that the SL production using only sugarcane molasses and soybean oil in the present study was comparable to that reported by Solaiman et al. [17, 18] and also seems more practicable and more attractive, as well, for large-scale application.

Kinetics of SL Production, Yeast Biomass Growth, and Utilization of Carbon Source

The profiles of SL production, yeast biomass growth, and utilization of sugarcane molasses and soybean oil in the above three different systems at the optimized process conditions are illustrated in Fig. 2. It can be seen from Fig. 2b, c that there was no lag in the yeast growth with an exponential phase of up to 48 h fermentation time, and during this period the amount of SLs produced was less. This observation is also found consistent with our earlier report on the yeast biomass growth and SL production [20]. However, following this

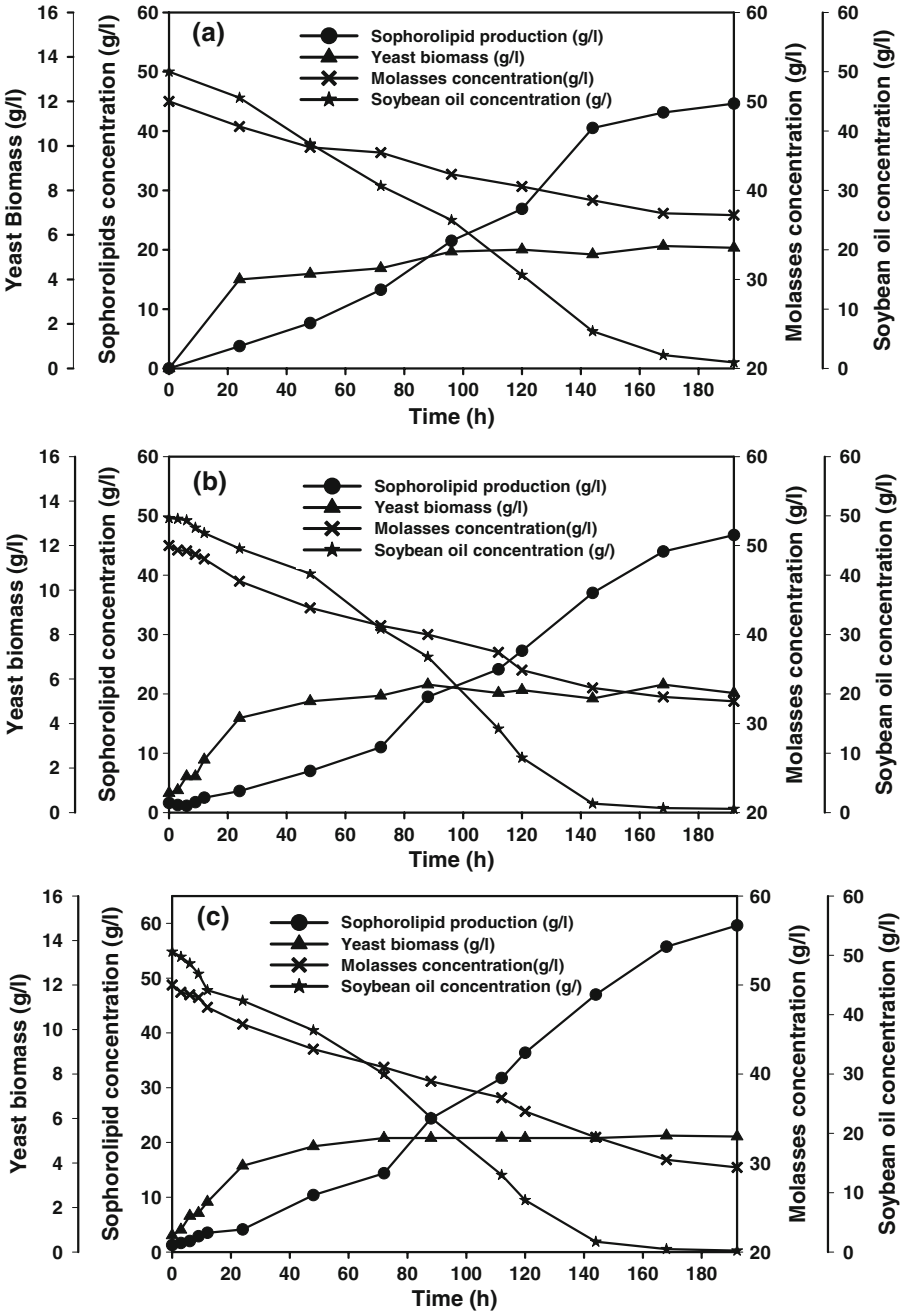

Fig. 2 Profiles of SL production, yeast biomass growth, and utilization of sugarcane molasses and soybean oil in **a** batch shake flask, **b** 3-l fermentor operated without pH control, and **c** 3-l fermentor operated with pH control

exponential growth phase, when the yeast entered its stationary growth phase, the SL yield was observed to increase with time (Fig. 2). These results are in good agreement with the literature-reported results that the SL production by the yeast is a nongrowth associate phenomenon [27, 28, 31]. On the other hand, compared to SL production in batch shake flask, where it remained constant after 144 h of fermentation (Fig. 2a), the results from the fermentor showed that the biosurfactant production could be maintained high even after 192 h (Fig. 2b, c). From the utilization profiles of the carbon sources, it was clear that soybean oil compared to sugarcane molasses in all the three systems was completely utilized by the yeast during its fermentation. Further, to estimate the biokinetic constants involved in the process, certain models reported in literature were fitted to these experimental data on SL production, yeast biomass growth, and utilization of sugarcane molasses and soybean oil.

Table 5 presents the kinetic models applied in this study along with the estimable kinetic parameters from these models. These models are essentially unstructured logistic models originally proposed by Mercier et al. [32] for describing the kinetics of biomass growth, substrate consumption, and product accumulation. Recently, these models were applied by Rodrigues et al. [33] to explain the biosurfactant production kinetics in their study. For the fitting of the models to the experimental data in the present study, nonlinear regression using the least-squares method was used employing Matlab® 7 and Microsoft Excel Solver 2003. The estimated kinetic parameters values obtained from these models are mentioned in Table 6, which also shows that the determination coefficient (R^2) values obtained by fitting the various models to the experimental data were found to be very high (≥0.95) thus revealing good accuracy of the models. From the kinetic parameter values presented in the table, it could be seen that the values of P_r and μ were found to be higher in the batch-operated fermentor compared to their values obtained in batch shake flask, which is attributable due to better control of physical parameters, mainly temperature, agitation, and aeration, in the fermentor than in the simple shake flask. Also, the values of product yields ($Y_{P/M}$ and $Y_{P/S}$) were found to be higher than those reported by Garcia-Ochoa and Casas [34] suggesting that the low-cost fermentative medium can replace the costly conventional SL production medium with a better yield. However, these authors found high values for the biomass growth in their study (μ=0.14 h^{-1}, X_{max}=5.98 gl^{-1}), which is mainly due to the fact that they utilized extra nitrogen source, i.e., yeast extract, for fermenting the yeast.

It is reported that while hydrophilic carbon source in fermentation of the yeast is utilized for both its growth and SL production, lipophilic carbon source (oil) is spent mainly for

Table 5 Models applied for the estimation of biokinetic constants involved in the process.

Kinetic model equation		Estimable kinetic parameters
SL production	$P = \frac{P_0 P_{max} e^{P_r t}}{P_{max} - P_0 + P_0 e^{P_r t}}$	P_r, P_0, P_{max}
Yeast biomass growth	$X = \frac{X_0 X_{max} e^{\mu t}}{X_{max} - X_0 + X_0 e^{\mu t}}$	μ, X_0, X_{max}
Sugarcane molasses utilization	$(S_{M0} - S_M) = \frac{1}{Y_{P/M}}(P - P_0) + \frac{1}{Y_{X/M}}(X - X_0)$	S_{M0}, $Y_{P/M}$, $Y_{X/M}$
Soybean oil utilization	$(S_{S0} - S_S) = \frac{1}{Y_{P/S}}(P - P_0) + \frac{1}{Y_{X/S}}(X - X_0)$	S_{S0}, $Y_{P/S}$, $Y_{X/S}$

t time (h), P SL concentration (g l^{-1}), P_{max} maximum concentration of SLs (g l^{-1}), P_r ratio between the initial volumetric rate of product formation and the initial product concentration P_0 (g l^{-1}), X yeast biomass concentration (g l^{-1}), X_{max} maximum concentration of yeast biomass (g l^{-1}), μ *(1/h)* ratio between the initial volumetric rate of biomass formation and the initial biomass concentration X_0 (g l^{-1}), $Y_{P/M}$ *(g* g^{-1}*)* SL yield due to molasses, $Y_{X/M}$ *(g* g^{-1}*)* biomass yield due to molasses, S_M molasses concentration (g l^{-1}), S_{M0} initial molasses concentration (g/l), $Y_{P/S}$ *(g* g^{-1}*)* SL yield due to soybean oil, $Y_{X/S}$ *(g* g^{-1}*)* biomass yield due to soybean oil, S_S soybean oil concentration (g l^{-1}), S_{S0} initial soybean oil concentration (g l^{-1})

Table 6 Kinetic parameters estimated by fitting the various models to experimental data obtained for different systems in the study.

Fermentation type	Sophorolipids production				Yeast biomass production				Sugarcane molasses utilization				Soybean oil utilization			
	P_0 (g l^{-1})	P_{max} (g l^{-1})	P_r (1/h)	R^2	X_0 (g l^{-1})	X_{max} (g l^{-1})	μ (h^{-1})	R^2	S_{M0} (g l^{-1})	$Y_{P/M}$ (g g^{-1})	$Y_{X/M}$ (g g^{-1})	R^2	S_{S0} (g l^{-1})	$Y_{P/S}$ (g g^{-1})	$Y_{X/S}$ (g g^{-1})	R^2
Batch shake flasks	1.692	48.48	0.028	0.990	0.638	5.054	0.109	0.937	49.99	0.719	2.438	0.999	49.99	0.783	0.431	0.999
Fermentor (no pH control)	1.784	51.88	0.029	0.996	0.804	5.410	0.122	0.988	49.99	0.721	2.625	0.963	49.62	0.785	0.417	0.963
Fermentor (under pH control)	2.373	65.42	0.030	0.997	0.840	5.117	0.114	0.996	49.99	0.730	2.425	0.977	50.60	1.025	0.470	0.977

product formation and for maintenance of the biomass [34]. This aspect was found true in the present study as it was observed that (a) the biomass yield due to sugarcane molasses ($Y_{X/M}$) is higher than that due to soybean oil ($Y_{X/S}$), and (b) the SL yield due to sugarcane molasses ($Y_{P/M}$) is less compared to the yield from soybean oil ($Y_{P/S}$). The findings on the kinetics parameters from the different models were also in agreement with those of Rodrigues et al. [33]. These kinetic results further suggest the low-cost medium composed of only sugarcane molasses and soybean oil to be a better alternative than conventional glucose containing medium for production of the biosurfactant.

Conclusions

A low-cost fermentative medium composed of sugarcane molasses and soybean oil together with the physical parameters, inoculum volume and size, temperature, and agitation, were investigated of their effects for enhanced production of SLs by the yeast *C. bombicola*. Based on the L18 OA and experimental design methodology for screening the various process parameters, agitation, concentrations of sugarcane molasses and soybean oil, and temperature were found to be the most influential factors affecting SL production at 192 h of fermentation. At an optimum combination of inoculum size 5%, inoculum age 2 days, sugarcane molasses concentration 50 gl^{-1}, soybean oil concentration 50 gl^{-1}, temperature 30 °C, and agitation 200 rpm, 90.02% enhancement in SL production was achieved in batch shake flask and in a 3-l fermentor operated without any pH control. Compared to these values, experiments under pH-controlled condition in the fermentor resulted in 27.6% further enhancement in the SL production. Kinetics of SL production, yeast biomass growth, and utilization of sugarcane molasses and soybean oil revealed complete utilization of the lipophilic carbon source together with a high yield of the product.

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References

1. Spencer, J. F. T., Gorin, P. A. J., & Tulloch, A. P. (1970). *Antonie van Leeuwenhoek, 36*, 129–133.
2. Gorin, P. A. J., Spencer, J. F. T., & Tulloch, A. P. (1961). *Canadian Journal of Chemistry, 39*, 846–855.
3. Tulloch, A. P., Spencer, J. F. T., & Deinema, M. H. (1968). *Canadian Journal of Chemistry, 46*, 345–349.
4. Bluth, M. H., Fu, S. L., Stanek, A., Smith-Norowitz, T. A., Wallner, S. R., Gross, R. A., et al. (2008). *Journal of Allergy and Clinical Immunology, 121*, S2.
5. Konishi, M., Fukuoka, T., Morita, T., Imura, T., & Kitamoto, D. (2008). *Journal of Oleo Science, 57*, 359–369.
6. Hardin, R., Pierre, J., Schulze, R., Mueller, C. M., Fu, S. L., Wallner, S. R., et al. (2007). *Journal of Surgical Research, 142*, 314–319.
7. Fu, S. L., Wallner, S. R., Bowne, W. B., Hagler, M. D., Zenilman, M. E., Gross, R., et al. (2008). *Journal of Surgical Research, 148*, 77–82.
8. Lu, C. M., & Ju, L. K. (2009). *Enzyme and Microbial Technology, 44*, 107–111.
9. Kasture, M., Patel, P., Prabhune, A., Ramana, C., Kulkarni, A., & Prasad, B. (2008). *Journal of Chemical Sciences, 120*, 515–520.
10. Saerens, K., Bogaert, I. V., Soetaert, W., & Vandamme, E. (2009). *Biotechnology Journal, 4*, 517–524.
11. Maingault, M. (1997) Canada Patent. No. 126,242874.
12. Mager, H., Rothlisberger, R. and Wagner, F. (1987) European Patent. No. EP0209783.
13. Hall, P. J., Haverkamp, J., van Kralingen, C. G. and Schmidt, M. (1996) US Patent, No. 5,520, 839.

14. Mulligan, C. N., Yong, R. N., & Gibbs, B. F. (2001). *Journal of Hazardous Materials, 85*, 111–125.
15. Schippers, C., Gessner, K., Muller, T., & Scheper, T. (2000). *Journal of Biotechnology, 83*, 189–198.
16. Daniel, H. J., Otto, R. T., Reuss, M., & Syldatk, C. (1998). *Biotechnological Letters, 20*, 805–807.
17. Solaiman, D. K. Y., Ashby, R. D., Nunez, A., & Foglia, T. A. (2004). *Biotechnological Letters, 26*, 1241–1245.
18. Solaiman, D. K. Y., Ashby, R. D., Zerkowski, J. A., & Foglia, T. A. (2007). *Biotechnological Letters, 29*, 1341–1347.
19. Felse, P. A., Shah, V., Chan, J., Rao, K. J., & Gross, R. A. (2007). *Enzyme and Microbial Technology, 40*, 316–323.
20. Daverey, A., & Pakshirajan, K. (2008). *Applied Biochemistry and Biotechnology.* doi:10.1007/s12010-008-8449-z.
21. Dasu, V. V., Panda, T., & Chidambaram, M. (2003). *Process Biochemistry, 38*, 877–880.
22. Saudagar, P. S., & Singhal, R. S. (2007). *Bioresource Technology, 98*, 2010–2017.
23. Hao, L. M., Xing, X. H., Zheng, L., Zhang, J. C., Sun, J. X., Jia, S. R., et al. (2009). *Applied Biochemistry and Biotechnology.* doi:10.1007/s12010-008-8507-6.
24. Asmer, H. J., Lang, S., Wagner, F., & Wray, V. (1988). *Journal of the American Oil Chemists' Society, 65*, 1460–1466.
25. Prescott & Dunn's. (1987). *In industrial microbiology* (4th ed., pp. 710–715). New Dehli: CBS.
26. Scott, T. A., Jr., & Melvin, E. H. (1953). *Analytical Chemistry, 25*, 1656–1661.
27. Hu, Y. H., & Ju, L. K. (2001). *Enzyme and Microbial Technology, 29*, 593–601.
28. Daverey, A., & Pakshirajan, K. (2009). *Food Research International, 42*, 499–504.
29. Guilmanov, V., Ballistreri, A., Impallomeni, G., & Gross, R. A. (2002). *Biotechnology and Bioengineering, 77*, 489–494.
30. Kim, K. D., Choi, D. W., Choa, Y. H., & Kim, H. T. (2007). *Colloids and Surfaces A Physicochemical and Engineering Aspects, 311*, 170–173.
31. Deshpande, M., & Daniels, L. (1995). *Bioresource Technology, 54*, 143–150.
32. Mercier, P., Yerushalmi, L., Rouleau, D., & Dochain, D. (1992). *Journal of Chemical Technology and Biotechnology, 55*, 111–121.
33. Rodrigues, L., Moldes, A., Teixeira, J., & Oliveira, R. (2006). *Biochemical Engineering Journal, 28*, 109–116.
34. Garcia-Ochoa, F., & Casas, J. A. (1999). *Enzyme and Microbial Technology, 25*, 613–621.