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Analytical modeling and numerical optimization of the biosurfactants production in solid-state fermentation by *Aspergillus fumigatus*

Gabriel Luis Castiglioni[1*], George Stanescu[2], Luiz Alberto Oliveira Rocha[3] and Jorge Alberto Vieira Costa[1]

*[1]Laboratório de Engenharia Bioquímica, Universidade Federal do Rio Grande, Cx Postal 474, Rio Grande, Rio Grande do Sul, Brazil. [2]Centro Politécnico, Universidade Federal do Paraná, Curitiba, Paraná, Brazil. [3]Escola de Engenharia, Universidade Federal do Rio Grande, Rio Grande, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: g_castigli@yahoo.com.br*

ABSTRACT. This is an experimental, analytical and numerical study to optimize the biosurfactants production in solid-state fermentation of a medium containing rice straw and minced rice bran inoculated with *Aspergillus fumigatus*. The goal of this work was to analytically model the biosurfactants production in solid-state fermentation into a column fixed bed bioreactor. The Least-Squares Method was used to adjust the emulsification activity experimental values to a quadratic function semi-empirical model. Control variables were nutritional conditions, the fermentation time and the aeration. The mathematical model is validated against experimental results and then used to predict the maximum emulsification activity for different nutritional conditions and aerations. Based on the semi-empirical model the maximum emulsification activity with no additional hydrocarbon sources was 8.16 UE·g^{-1} for 112 h. When diesel oil was used the predicted maximum emulsification activity was 8.10 UE·g^{-1} for 108 h.

Keywords: *Aspergillus fumigatus*, biosurfactant, solid-state fermentation.

Modelagem analítica e otimização numérica da produção de biossurfactantes por *Aspergillus fumigatus* em fermentação sólida

RESUMO. Este trabalho trata de um estudo experimental, analítico e numérico para otimizar a produção biossurfactante por fermentação em estado sólido, utilizando *Aspergillus fumigatus* em substrato contendo casca e farelo de arroz. O objetivo do trabalho foi modelar analiticamente a produção biossurfactantes em biorreator de coluna com leito fixo. O Método dos Mínimos Quadrados foi utilizado para ajustar os valores experimentais de atividade de emulsificação no modelo semiempírico. As variáveis de controle foram as condições nutricionais, tempo de fermentação e aeração. O modelo matemático foi validado experimentalmente e usado para prever a atividade emulsificante nas diferentes condições nutricionais e de aeração. Com base no modelo semiempírico a atividade emulsificante máxima encontrada para os experimentos sem fonte adicional de carbono foi de 8,16 EU g^{-1} em 112h. Quando o óleo diesel foi utilizado, a atividade emulsificante máxima foi de 8,10 EU g^{-1} em 108h.

Palavras-chave: *Aspergillus fumigatus*, biossurfactante, fermentação em estado sólido

Introduction

Biosurfactants are active compounds produced at the microbial cell surface or excreted and which reduce surface and interfacial tension producing emulsification (ILORI et al., 2005). Based on their characteristics, the biosurfactants are useful in remediation of insoluble organic pollutants in soil and marine environments (MARTINS et al., 2006).

Either adherent to the cell surface or extracellularly excreted in the growth medium (CHANG et al., 2005), they are surface-active macromolecules synthesized by microorganisms adapted to water-insoluble substrates (INOH et al., 2004).

Several researches have been carried out to check the best ratio between carbon, nitrogen and other nutrients needed to obtain high production yields. The optimizing of these factors that affect growth in biosurfactant producing organisms with potential for commercial exploitation is of major importance (SOUMEN et al., 2006).

Due to their environment-friendly composition, low toxicity, effectiveness at extreme temperature, pH, salinity and ease of synthesis, the biosurfactants have special advantage over their commercially manufactured counterparts (THANOMSUB et al., 2006). These properties allow them to be extensively used in cosmetic, pharmaceutical, and food processes as emulsifiers, humectants, preservatives, and detergents (BANAT et al., 2000). Moreover, are ecologically safe and can be applied in

bioremediation and waste treatments (CAMEOTRA; MAKKAR, 2004). The most attractive applications of biosurfactants are related to the oil industry to aid clean up, may be added to reduce the interfacial tension at the oil–water interface and increase the solubility of petroleum (GRISHCHENKOV et al., 2000; O'CONNOR, 2002). The physical nature of the medium has been shown to affect biosurfactant production in different way and strains. The biosurfactants synthesized by microbial cells can vary in their composition and hence in their chemical and physical properties (RODRIGUES et al., 2006).

Mathematical modeling evolved simultaneously to the advance of scientific knowledge. One of the approaches applied for obtaining increased yields in fermentative production is the medium optimization (SCHUGERL, 2001).

Combining different physical concepts and computational techniques into a mathematical model must be done very carefully. The accuracy of numerical simulations results should allow the calibration of trustful mathematical models (LENZ et al., 2004). The main difficulties related with the SSF mathematical modeling are the evaluation of microorganisms growth parameters and the analysis of cellular growth. Determination of the substrate consumption is particularly difficult given its structural and nutritional complex nature (PANDEY, 2003).

This paper presents an experimental, numerical and analytical study of the optimal nutritional conditions for maximum biosurfactants production in solid-state fermentation by *Aspergillus fumigatus*. The goal of this work is to analytically model the biosurfactants production in solid-state fermentation by the fungus *Aspergillus fumigatus* into a column fixed bed bioreactor. The analytical model was validated against experimental results and then used to predict the optimal nutritional conditions for maximum biosurfactants production. Control variables were the nutritional conditions, the fermentation time and the aeration. The objective function to be maximized was the emulsification activity.

Material and methods

Microorganism and culture medium

The experimental work has been carried out using the filamentous fungus *Aspergillus fumigatus.* Microorganisms were maintained at 4ºC suspended in test tubes containing Potato-Dextrose-Agar (PDA) medium with 1% glycerin.

The solid substrate was a medium containing rice straw and 0.42 to 0.50 mm pieces of non-fat thermally stabilized rice bran (moisture 13.44%; protein 17.62%; fiber 8.55%; ash 10.81%; fat 3.36%; carbohydrate 46.22%). The rice bran was first milled into 1 mm pieces and then sieved between the 35 and 32 Tyler sieves (0.425 and 0.500mm, respectively**)**. Substrate also contained a nutritious solution of (g L^{-1} of distilled water): $MgSO4.7H_2O$ (0.5), $NaNO_3$ (3.0), KH_2PO_4 (1.0), yeast extract (1.0) and peptone (0.3). The moisture was expressed on a wet basis and maintained at 50%.

The defatted rice bran was donated by the Company IRGOVEL - Riograndense Vegetable Oil Industry Ltd. (pellets, Brazil), the reagents brand used in the experiments is Synth and diesel oil was donated by Ipiranga Oil Refinery (Rio Grande do Sul State, Brazil).

The fermentation medium was sterilized in polypropylene bags, to which, after cooling, the inoculum was added and mixed.

Biosurfactants production with no additional hydrocarbon sources was compared with experimental results obtained when 1% (w w^{-1}) of diesel oil (in order to verify the ability of the microorganism to produce biosurfactant under this condition) has been added. Experimental runs were in triplicate, conducted without aeration and for air flow rates of: 40, 60, 100, 120 and 200 ($cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$).

Experimental apparatus

Figure 1 shows the sketch of the experimental apparatus used to perform the experiments (HASAN et al., 1998).

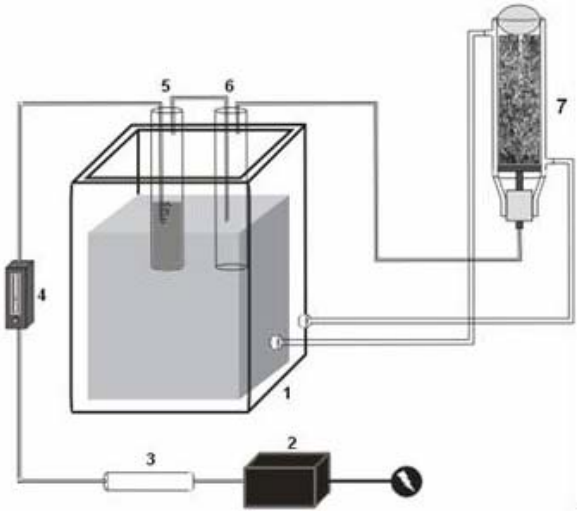

Figure 1. Experimental device. Thermostatic bath (1), Diaphragm pump (2), air filter (3), Flow-meter (4), Humidifier (5), Droplets separator (6), Column fixed bed bioreactor (7).

Fermentation tests were conducted during 144h, into a column fixed bed bioreactor of 50 mm internal diameter and 250 mm height. The water inside the external heating-cooling jacket has been maintained at 30°C during all the experimental runs. To maintain proper humidification of the substrate, the air delivered by the diaphragm pump was firstly filtered through the glass silk filter, then into the humidifier and thirdly into the droplets separator. The air flow rate entering the column bioreactor has been also controlled by an inlet valve at the bioreactor bottom.

Biosurfactants Extraction

The biosurfactants produced was extracted with distilled water (solute-solvent, 1:5 w v^{-1}). After the addition of distilled water, samples were stirred for 30 minutes at 160 rpm and 50°C. Then, samples were vacuum filtered (qualitative filter paper used had 80 g m^{-2}, porosity of 3 microns, 7 cm diameter) to obtain the extract for the emulsification activity analysis.

Emulsification activity determination

The emulsification activity determination was done using the methodology described by Johnson et al. (1992). Vacuum filtered samples and soybean oil were then vortexed for 1 min and then, after 60 min left at rest, analyzed into a spectrophotometer at 610nm. It was part of the medium for analysis, 3.5 mL of extract and 2 mL of soybean oil.

To convert the results for the dry basis, the moisture of each sample has been determine employing the Association of Official Agricultural Chemists (AOAC, 1995) methodology.

Analysis of results and mathematical modeling

Results were evaluated using the software Statistica for Windows 6 and mathematical models were developed using the software Matlab.

The validation of the mathematical models was made experimentally using full factorial design 2^2 with triplicate center point. The center point used was the maximum result from the developed model. The inferior and superior level of the experimental design varied symmetrically in 20 $cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$.

Results and discussion

Experimental results

Tables 1 and 2 summarize the experimental results for biosurfactants production by *Aspergillus fumigatus* SSF with no additional hydrocarbon sources and those obtained when diesel oil has been added as an additional hydrocarbon source, respectively.

Tables 1 and 2 report that, with the exception of the results for aeration equal to 40 $cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$, higher values of the emulsification activity correspond to the A*spergillus fumigatus* SSF process with no additional hydrocarbon sources. These results could be explained, as suggested by Sullivan (1998), based on the supposition that the production of biosurfactants is correlated with the microorganism cell density and reflect an indirect correlation with the availability of energy, carbon and nitrogen. It is possible that the production of biosurfactants at high biomass density has a selective advantage.

Table 1. Emulsification activity (UE g^{-1}) and standard deviation for biosurfactants production with no additional hydrocarbon sources under different aeration conditions.

t (h)	Aeration ($cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$)					
	0	40	60	100	120	200
0	2.48 (± 0.12)	3.98 (± 0.13)	4.21 (± 0.18)	3.63 (± 0.12)	3.37 (± 0.13)	2.68 (± 0.16)
24	2.50 (± 0.11)	4.10 (± 0.15)	4.78 (± 0.11)	5.18 (± 0.17)	4.49 (± 0.17)	2.39 (± 0.10)
48	2.86 (± 0.13)	4.30 (± 0.14)	7.09 (± 0.17)	6.80 (± 0.17)	5.64 (± 0.10)	2.62 (± 0.19)
72	2.90 (± 0.16)	4.69 (± 0.17)	7.75 (± 0.19)	6.21 (± 0.11)	7.59 (± 0.13)	3.14 (± 0.12)
96	2.76 (± 0.11)	4.40 (± 0.10)	6.52 (± 0.10)	6.49 (± 0.21)	9.13 (± 0.18)	3.88 (± 0.09)
120	2.06 (± 0.14)	4.10 (± 0.13)	7.13 (± 0.20)	6.29 (± 0.16)	10.83 (± 0.10)	4.44 (± 0.06)
144	1.83 (± 0.16)	3.86 (± 0.09)	6.73 (± 0.09)	5.79 (± 0.10)	8.90 (± 0.13)	3.88 (± 0.10)

Table 2. Emulsification activity (UE g^{-1}) and standard deviation for biosurfactants production with diesel oil added as an additional hydrocarbon source under different aeration conditions.

t (h)	Aeration ($cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$)					
	0	40	60	100	120	200
0	1.35 (± 0.19)	3.14 (± 0.19)	4.59 (± 0.21)	4.82 (± 0.10)	3.03 (± 0.17)	1.66 (± 0.16)
24	1.94 (± 0.18)	3.19 (± 0.18)	4.66 (± 0.22)	5.76 (± 0.28)	3.33 (± 0.20)	1.72 (± 0.17)
48	3.12 (± 0.11)	4.12 (± 0.21)	5.14 (± 0.08)	7.73 (± 0.22)	5.10 (± 0.20)	1.97 (± 0.19)
72	3.88 (± 0.12)	4.13 (± 0.12)	5.82 (± 0.13)	7.58 (± 0.29)	5.92 (± 0.19)	3.97 (± 0.20)
96	2.57 (± 0.17)	5.01 (± 0.14)	6.32 (± 0.24)	7.04 (± 0.34)	9.21 (± 0.21)	3.97 (± 0.19)
120	2.90 (± 0.12)	4.47 (± 0.22)	6.74 (± 0.12)	6.40 (± 0.23)	9.81 (± 0.22)	3.03 (± 0.28)
144	3.44 (± 0.12)	4.80 (± 0.29)	5.79 (± 0.20)	7.22 (± 0.20)	6.58 (± 0.29)	4.65 (± 0.22)

Biosurfactant are produced when the cell density is high enough to cause a localized attack on the host. Since the microorganisms growing on hydrocarbons are growing at the oil–water interface, the production of biosurfactants when the density is high will increase the surface area of the drops, allowing more microorganisms to feed. Alternatively, when the utilizable fraction of the hydrocarbon is consumed, as in the case of oil that consists of many types of hydrocarbons, the production of the surfactants allows the microorganisms to detach from the 'used' droplet and find a new one.

Mathematical modeling

Experimental results in Table 1 and 2 reveal a strong dependence of the emulsification activity on the aeration all through the process of biosurfactants production by *Aspergillus fumigatus* SSF. Through the analysis of variance and Tukey test it was observed significant differences in the results ($p < 0.00001$), for both experiments, with no additional hydrocarbon sources as for diesel oil used as an additional hydrocarbon source. The experiments without aeration and 200 $cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$ showed statistically the same behavior.

Therefore, the time and the aeration are the two independent variables to be considered for the mathematical modeling. The experimental data obtained for each run were adjusted to fit a polynomial function of time with variable coefficients C_1, C_2 and C_3 depending on the nutritional conditions and aeration. To develop the model, preliminary tests were made using polynomials up to grade 4, but the models are unstable and inaccurate, leading us to choose polynomial of degree 2.

$$EA = C_1 + C_2.t + C_3.t^2 \quad (1)$$

where EA is the emulsification activity ($UE{\cdot}g^{-1}$).

For biosurfactants production with no additional hydrocarbon sources the numerical values of coefficients C_1, C_2 and C_3 are listed in Table 3. Table 4 shows the numerical values of C_1, C_2 and C_3 when biosurfactants production occurs with diesel oil added as an additional hydrocarbon source.

Based on numerical values in Tables 3 and 4, the coefficients C_1, C_2 and C_3 in Equation (1) were each one fitted to a different linear function. For each experimental condition, the results were polynomially fitted. The combination of each set of the polynomial was fitted to a linear equation, where 'Z' represents another function (sine). This function Z takes into account the variables in the process (Equations 2, 3 and 4).

Table 3. Numerical values of C_1, C_2 and C_3 for biosurfactants production with no additional hydrocarbon sources.

Aeration, V ($cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$)	Polynomial coefficients			R^2
	C_1	C_2	C_3	
0	2.387	1.620 E-2	-1.428 E-4	0.876
40	3.895	1.646 E-2	-1.170 E-4	0.810
60	4.044	6.838 E-2	-3.540 E-4	0.811
100	3.823	6.490 E-2	-3.640 E-4	0.890
120	2.833	8.839 E-2	-2.753 E-4	0.912
200	2.374	1.113 E-2	0.153 E-4	0.782

Table 4. Numerical values of C_1, C_2 and C_3 for biosurfactants production with diesel oil added as an additional hydrocarbon source.

Aeration, V ($cm^3_{air}\ g^{-1}_{substrate}\ h^{-1}$)	Polynomial coefficients			R^2
	C_1	C_2	C_3	
0	1.410	3.708 E-2	-1.785 E-4	0.652
40	2.969	2.507 E-2	-0.870 E-4	0.858
60	4.279	3.045 E-2	-1.190 E-4	0.809
100	4.956	5.454 E-2	-2.982 E-4	0.692
120	2.065	9.649 E-2	-3.836 E-4	0.755
200	1.412	2.662 E-2	-0.444 E-4	0.731

$$C_1 = B_1 + B_2.Z \quad (2)$$

$$C_2 = B_3 + B_4.Z \quad (3)$$

$$C_3 = B_5 + B_6.Z \quad (4)$$

where B_1 to B_6 are numerical coefficients to be determined and $Z = \sin[a_1.\log(a_2.V)]$ is a new function, accounting for the effects of aeration (V) on the biosurfactants production.

Table 5 shows the numerical values of coefficients B_1 - B_6 and a_1 - a_2 for different nutritional conditions, when the parameters a_1 and a_2 have been determined such that the correlation coefficient R^2 belongs to the interval ($0.9 \leq R^2 \leq 1.0$), over to those of Tables 3 and 4 when polynomially fitted.

Table 5. Numerical values of coefficients B_1 to B_6 and parameters a_1 and a_2 in Equations 2 to 4.

Nutritional conditions	Coefficients	$10^3{\cdot}B^*_i$	$10^3{\cdot}B^*_j$	a_1	a_2	R^2
no additional hydrocarbon sources	C_1	3250.00	900.00	2.61	4.09	0.995
	C_2	-6.80	90.00	1.65	1.29	0.901
	C_3	-0.12	0.26	2.28	1.57	0.992
additional hydrocarbon source (diesel oil)	C_1	2992.00	-1861.00	3.36	2.56	0.955
	C_2	52.00	-39.60	3.75	4.73	0.954
	C_3	-0.22	-0.19	4.17	1.30	0.992

*Bi stands for B1, B3 and B5; Bj stands for B2, B4 and B6.

The algebraic system represented by equations (1) – (4) and the coefficients in Table 3 represent the semi-empirical model for the biosurfactants production in solid-state fermentation by the fungus

Aspergillus fumigatus into the column fixed bed bioreactor.

Numerical optimization

Figure 2 presents the experimental data against the numerical approach for biosurfactants production with no additional hydrocarbon sources and with diesel oil added as an additional hydrocarbon source for aerations of 40, 60, 100 and 120 $cm^3_{air}\,g^{-1}_{substrate}\,h^{-1}$.

Time variation of the emulsification activity in Figure 3 shows the existence of an optimal fermentation time, t_0, for optimal (maximum) emulsification activity, EA_0, when the aeration is maintained constant. The subscript 'o' in this figure means that the emulsification activity was optimized in terms of fermentation time. The optimized parameters were obtained analytically.

The optimized emulsification activities EA_0 and the corresponding optimal fermentation time t_0 are plotted in Figure 3 for different aerations and nutritional conditions. It is worth noting in Figure 3 the existence of a maximum value of the optimized emulsification activity, EA_{00}. Here, the subscript '$_{00}$' means that the emulsification activity was optimized twice, firstly in terms of fermentation time and secondly in terms of aeration.

Numerical values of the maximum emulsification activity and optimal fermentation time in Figure 3 are EA_{00} = 8.16 UE·g^{-1} and t_0 = 112h (V = 90 $cm^3_{air}\,g^{-1}_{substrate}\,h^{-1}$) and EA_{00} = 8.10 EU g^{-1} and t_0 = 108h (V = 100 $cm^3_{air}\,g^{-1}_{substrate}\,h^{-1}$) for SSF with no additional hydrocarbon sources and SSF with diesel oil added as an additional hydrocarbon source, respectively.

Results in Figure 3 agree with those previously reported by Veenanadig et al. (2000). Factors controlling the production of biosurfactants include the physical and chemical conditions affecting the metabolic capability of microorganisms such as pH, water, aeration and presence of oxygen, nutrients and temperature. Experimental results published by Veenanadig et al. (2000) indicated that biosurfactants production by *Bacillus subtilis* SSF in fixed bed column bioreactors has increased with the aeration increase. This is due to an increase of the fermentation process efficiency based on the presence of more oxygen. On the other hand, as mentioned by Hongzhang et al. (2002), despite improvements in the microorganisms metabolic capability, higher values of aeration may reduce the substrate moisture and become prejudicial. Therefore, as shown in Figure 3, there is some optimal aeration for the maximum production of surfactants. The repeatability of the experiment can be demonstrated by the results of the triplicate at the central point when the model validation was made. The results for the center point were the largest determined, reaching values 8.65 UE g^{-1} (± 0.24) for the experiments with no additional of hydrocarbon sources 8.03 UE g^{-1} (± 0.69) that were diesel oil was used as an additional hydrocarbon source.

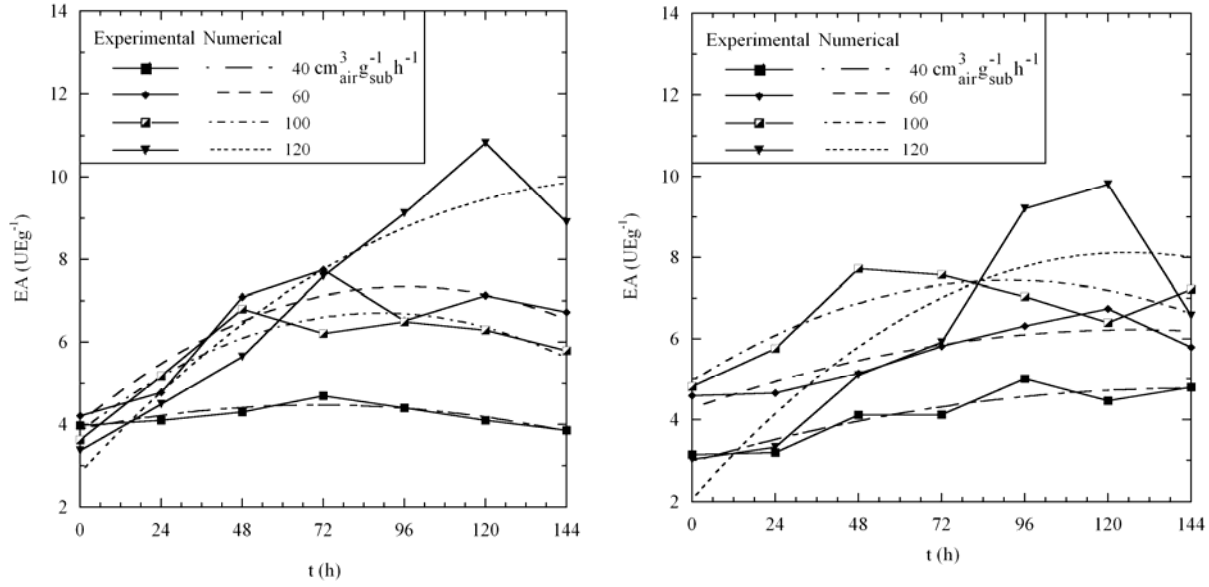

Figure 2. Time course of the emulsification activity for different nutritional conditions (experimental data versus numerical approach): (a) SSF with no additional hydrocarbon sources; (b) SSF with diesel oil added as an additional hydrocarbon source.

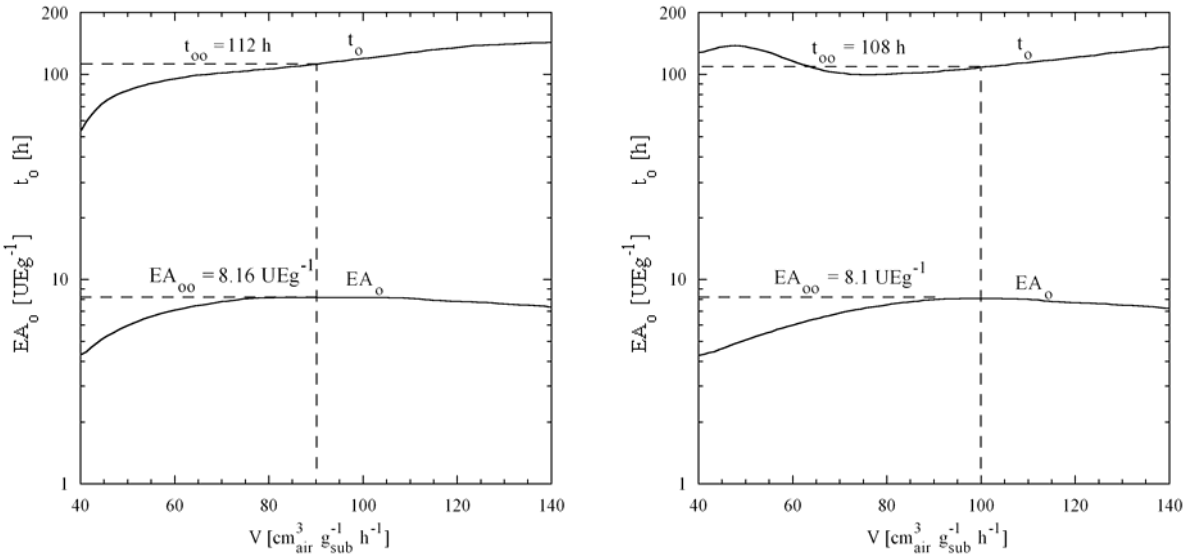

Figure 3. Influence of the aeration on the maximum emulsification activity and the corresponding fermentation time for different nutritional conditions: (a) SSF with no additional hydrocarbon sources; (b) SSF with diesel oil added as an additional hydrocarbon source.

Conclusion

Experimental results for the biosurfactants production by solid-state fermentation of a medium containing rice straw and minced rice bran inoculated with A*spergillus fumigatus* showed a good emulsifying activity. With the exception of the results for aeration equal to 40 cm^3_{air} $g^{-1}_{substrate}$ h^{-1}, higher values of the emulsification activity corresponded to the A*spergillus fumigatus* SSF process with no additional hydrocarbon sources. Based on the semi-empirical model and experimental validation was possible to optimize the values of emulsification activity with no additional hydrocarbon sources (EA_{00} = 8.16 UE g^{-1} for t_0 = 112h and V = 90 cm^3_{air} $g^{-1}_{substrate}$ h^{-1}) and diesel oil was used as an additional hydrocarbon source (EA_{00} = 8.10 UE g^{-1} for t_0 = 108h and V = 100 cm^3_{air} $g^{-1}_{substrate}$ h^{-1}). These results provide important information for a new alternative for process optimization and behavior of *Aspergillus fumigatus* in the experimental conditions examined in this study.

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