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OPTIMIZATION OF THE OPERATING CONDITIONS FOR RHAMNOLIPID PRODUCTION USING SLAUGHTERHOUSE-GENERATED INDUSTRIAL FLOAT AS SUBSTRATE

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Abstract - Biosurfactants have a wide range of applications in emulsions, separations, and solubilization because these chemicals reduce the surface tension and viscosity of solutions. This work studied rhamnolipid production using a batch bioreactor with a working volume of 1.5 liters, *Pseudomonas aeruginosa* ATCC (American Type Culture Collection) 10145 strain, and the greasy effluent from the slaughter of poultry and pigs as the substrate. The main goal of this research was to evaluate the level of aeration, agitation speed and inoculum concentration using a Central Composite Design (CCD). Experimental conditions were selected using the surface response technique obtained from the CCD, and the results were validated to test the reproducibility. The following operating conditions were selected: 1.2 vvm level of aeration, 600 rpm agitation speed, and 1.0 g/L biomass inoculum concentration. Under these conditions, the following results were obtained: the rhamnose production, surface tension and emulsifying index were 5.37 g/L, 25.6 dyne/cm and 100%, respectively.
Keywords: Biosurfactant; Agitation speed; Aeration level; Rhamnolipid.

INTRODUCTION

Surfactants are surface-active molecules that, beyond serving other functions, allow the growth of microorganisms in water-immiscible substrates by reducing the surface tension, which makes the substrate available for assimilation and metabolism by the microorganisms (Kronemberger, 2007).

Biosurfactants can be classified according to their biochemical composition and the microorganism used for production (Banat, 2000). According to Tuleva *et al*. (2002), rhamnolipids are biosurfactants that belong to the glycolipids class and are produced mainly from hydrocarbons by the bacterium *Pseudomonas aeruginosa* (Gram-negative). Rhamnolipids are efficient in the bioremediation of soils polluted by oily compounds (Santa Anna *et al*., 2007). According to Pirollo (2006) and Moussa *et al* (2014), rhamnolipids consist of one or two rhamnose molecules and one or two fatty acid chains of 8 to 12 carbon atoms, which may be saturated or unsaturated.

Biosurfactants can be modified using genetic and/or biochemical techniques, which have major advantages because of the need to meet specific bio-

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degradability standards. Unlike biosurfactants, chemical surfactants are not biodegradable and create environmental problems due to toxicity and accumulation in natural ecosystems (Kronemberger, 2007).

Nitschke and Pastore (2002) report that biosurfactants have a wide range of applications in emulsions, separations, and solubilization because these chemicals reduce the surface tension and viscosity of solutions. These features allow biosurfactants to be used widely in the food, agricultural, textile and pharmaceutical industries. According to Barbosa and Peace (2007) and Gallert and Winter (2002), biosurfactants have the properties of a detergent, which makes these biomolecules versatile products for reducing environmental pollution.

Several authors have also studied the operating conditions for biosurfactant production. Benincasa *et al*. (2002) studied rhamnolipid production by *Pseudomonas aeruginosa* using agitation speeds from 500 to 800 rpm in a 2 L Biostat B reactor (B. Braun Biotech International) with a working volume of 1.2 L. The process also had a foam recycling system and was aerated using a sparger at 3 L air/min. For each agitation speed, the O_2 solution transfer coefficients, K_La, were calculated. Lee *et al*. (2004) observed rhamnolipid production in batch and fed-batch fermentation using *Pseudomonas aeruginosa*, and the agitation speed and aeration rate in the batch culture ranged from 100 to 250 rpm and 1.0 to 3.0 L/min. The optimum conditions for the batch culture, using a 7-L jar fermentor, were an agitation speed of 200 rpm and an aeration rate of 2.0 L/min.

According to Mulligan (2005) and Rahman and Gakpe (2008), biosurfactants are used in bioremediation processes to degrade substrates that are insoluble in water, fats and oils. These factors suggest the possibility of biosurfactant production from industrial float from the slaughterhouse. The slaughterhouse effluent is usually subjected to physical and chemical treatments, such as flotation and centrifugation. The organic compost produced from this treatment is called industrial float.

These floats have high percentages of crude protein (44.03%), ether extract (32.74%), and dry matter (35.12%), and approximately 1 million tons of industrial float is produced per year (Zimmer and Klein, 2011).

Therefore, this study aimed to optimize rhamnolipid production with the fermentation of industrial float obtained from an effluent treatment plant for pig and poultry slaughterhouses. A Central Composite Design (CCD) was used to evaluate the experimental parameters, which were the level of aeration, agitation speed, and initial inoculum concentration. The optimization of the operating conditions was performed using the response surface technique obtained from the results of the CCD.

MATERIAL AND METHODS

Microorganisms

The strain *Pseudomonas aeruginosa* ATCC 10145, obtained from Culture Collection's Tropical André Tosello Foundation, Campinas-SP, Brazil, was used in this study.

Culture Medium for Maintenance of Strains and Growth Conditions

The bacterial cultures were grown in inclined test tubes with a nutrient agar medium containing 20.0 g/L sucrose, 3.0 g/L meat extract, 5.0 g/L bacteriological meat peptone, and 20.0 g/L bacteriological agar. This medium was adjusted to pH 7 and sterilized at 121 °C for 15 min.

The growth medium (inoculum) was prepared according to Lima *et al*. (2007) and Santos *et al*. (2002) and contained 10.0 g/L glucose, 11.0 g/L brewery residual yeast, 5.7 g/L NH_4NO_3, 0.2 g/L $MgSO_4.7H_2O$, 7.0 g/L Na_2HPO_4, and 3.0 g/L KH_2PO_4. The medium containing the inoculum was adjusted to pH 7 and sterilized at 121 °C for 15 min.

The fermentation medium used for biosurfactant production was prepared according to Lima *et al*. (2007) and contained 0.2 g/L $MgSO_4.7H_2O$, 7.0 g/L Na_2HPO_4, and 3.0 g/L KH_2PO_4. The concentrations (in g/L) selected for the fat, ammonium nitrate and residual brewery yeast were 12, 0 and 15, respectively, according to Borges *et al*. (2012).

Characterization of Industrial Float from the Slaughterhouse

In the preliminary tests, the fat percentage and nitrogen concentration of the industrial float from the slaughterhouse was determined. The Bligh-Dyer method, presented by Cecchi (1999), was used to determine the fat percentage and the total nitrogen concentration was determined by the Kjeldahl method, according to Apha *et al*. (1998).

Bioreactor and Statistical Analyses

All the experiments were conducted in a batch bioreactor (model B. Braun Biotech International model Biostat - Sartorius AG; Goettingen, Germany) with a

total volume of 2.0 L and a working volume of 1.5 L. The bioreactor was composed of a mechanical shaker to control agitation, an aeration flow meter to control dissolved oxygen and a cooling jacket with a water inlet to maintain the temperature at 30 ± 0.5 °C during the 48-hour fermentation period, as reported by Lima *et al*. (2009).

A Central Composite Design (CCD) was used to maximize the production of the biosurfactant by selecting operating conditions for the aeration levels (X_1), agitation (X_2) and microorganism inoculum concentration (X_3). This CCD was performed for a total of 15 experiments – three replicates at the central point, six experiments at the central point, and six experiments at the orthogonal (α) of 1.3533. In this CCD, the aeration level ranged from 0.03 to 1.37 vvm, agitation from 94 to 906 rpm and inoculum concentration from 0.32 to 1.68 g/L. All the experiments were performed in duplicate, for a total of 34 experiments.

The Central Composite Design is needed to optimize the process, to develop formulations within the established parameters and to evaluate the effects and impacts that factors have in relation to the desired response. Proper planning and use of the scientific method enabled the reliable completion of the experiments. These steps should be followed during experimental analyses: the completion of the planning and statistical analysis of the data (Rodrigues and Iemma, 2005). This methodology of planning experiments coupled to an analysis of the response surfaces allows us to statistically verify the individual effects and interactions between variables and to evaluate the experimental errors and the empirical equation results (Box *et al*., 1978).

The response surface technique is a set of statistical techniques used to model processes where these responses are influenced by input factors, which are the independent variables. This method is based on a full factorial design over a schedule with replicas at the midpoint. In planning, the input variables are coded (Lopera *et al*., 2012).

All the studied variables were made dimensionless (coded) using Equation (1),

$$X_n = \frac{(X - X_0)}{\left[\frac{X_{+1} - X_{-1}}{2}\right]} \quad (1)$$

where X_n is the coded value of the variable (n = 1, 2...), X is the value of the variable to be calculated, X_0 is the value of the variable at the central point, X_{+1} is the value of the variable at the top level, and X_{-1} is the value of the variable at the lower level.

Statistical calculations were performed using *Statistica 7.1* software *(*StatSoft, Inc.; Tulsa, Oklahoma, USA*)*, and the responses were maximized using the surface response technique together with an algorithm implemented in *Maple 9.5 (Cybernet Systems Co, Japan)*.

After maximizing the results using the CCD, the best conditions for biosurfactant production, with respect to rhamnose concentration, surface tension and the emulsifying index, were determined in this experimental work.

Kinetics of Rhamnose Production in Optimized Conditions

Kinetics were investigated when the conditions (aeration level, agitation speed, and inoculum concentration) reached the optimal point, as determined by the CCD. The fermentation medium used for biosurfactant production is described above. The reaction kinetics were monitored every 6 hours for a total period of 78 hours. The medium was contained in a reactor with a 1.5 L working volume, and 5 to 30 mL samples were taken from the reactor at 0, 6, 12, 18 and 24 hours. Two additional brews were tested in the same reactor and under the same conditions. Aliquots were fermented for 24, 30, 36, 42 and 48 hours and then again for 48, 54, 60, 72 and 78 hours, respectively.

Analytical Procedures

The rhamnose concentration was determined according to the method described by Rahman *et al*. (2002).

The surface tension was determined using a previously calibrated tensiometer (*Fisher* Scientific Co., *model 21 Tensiomat*, Hanover Park, Illinois, USA). Centrifugation was performed on 30 mL samples of fermented medium in a Beckman Coulter centrifuge (Model Avanti J-25, Beckman Coulter do Brasil**,** Santana de Parnaíba- SP – Brasil.) at 12500 rpm (corresponding to a relative centrifugal field of 18900 x g) at 25 °C for 20 minutes to sediment the cells. The supernatant was removed, and aliquots of approximately 10 mL were placed in petri dishes and analyzed in the tensiometer. The tensiometer was used with a platinum-iridium ring 2 cm in diameter and 6.0 cm in height. The ring was immersed in each of the three Petri dishes that contained the samples, and the results are expressed as the mean value of these readings.

The emulsifying index was determined using the method described by Cooper and Goldenberg (1987).

The dry mass was determined as described by Faria *et al*. (2010).

RESULTS AND DISCUSSION

Characterization of Greasy Wastewater

The concentration of fat in the greasy effluent was 19.48 ± 1.5%, and the residue had 0.2 ± 0.02 g/L of nitrogen.

Central Composite Design (CCD) for the Operating Conditions: Aeration Level, Agitation Speed and Inoculum Concentration

The results obtained from the CCD for aeration level (X_1), agitation speed (X_2), inoculum concentration (X_3), rhamnose concentration (RM), surface tension (ST), and emulsifying index (EI) are shown in Table 1.

Table 1: Results of rhamnose production, surface tension and emulsifying index with respect to the operating conditions – level of aeration, agitation speed and inoculum concentration.

Exp.	Aeration (vvm)	Agit. (rpm)	Inoc. (g/L)	RM (g/L)	ST (dyne/cm)	EI (%)
1	0.2 (-1)	200 (-1)	0.5 (-1)	0.91	31.8	67.5
2	0.2 (-1)	200 (-1)	1.5 (+1)	0.97	32.1	72.2
3	0.2 (-1)	800 (+1)	0.5 (-1)	1.85	30.3	84.2
4	0.2 (-1)	800 (+1)	1.5 (+1)	2.17	29.3	89.4
5	1.2 (+1)	200 (-1)	0.5 (-1)	2.15	29.9	86.0
6	1.2 (+1)	200 (-1)	1.5 (+1)	2.72	29.1	90.0
7	1.2 (+1)	800 (+1)	0.5 (-1)	4.29	26.3	100.0
8	1.2 (+1)	800 (+1)	1.5 (+1)	5.57	25.3	100.0
9	0.03 (-α)	500 (0)	1 (0)	1.66	30.4	75.3
10	1.37 (+α)	500 (0)	1 (0)	4.12	26.5	100.0
11	0.7 (0)	94 (-α)	1 (0)	1.37	30.7	75.3
12	0.7 (0)	906 (+α)	1 (0)	3.49	27.7	99.0
13	0.7 (0)	500 (0)	0.32 (-α)	2.46	29.2	93.0
14	0.7 (0)	500 (0)	1.68 (+α)	3.35	27.2	100.0
15	0.7 (0)	500 (0)	1 (0)	3.95	27.8	100.0
16	0.7 (0)	500 (0)	1 (0)	3.91	27.3	99.0
17	0.7 (0)	500 (0)	1 (0)	3.93	27.2	100.0

Agit. – agitation speed; Inoc. – inoculum concentration; RM – rhamnose concentration; ST – surface tension; EI – emulsifying index.

Analysis of the CCD

To analyze the data presented in Table 1, a 5% rejection probability for the null hypothesis in *Student's t-test* was used for the following independent variables: rhamnose concentration, surface tension and emulsifying index.

Equations (2), (3) and (4) describe the empirical models adjusted for rhamnose concentration, surface tension and emulsifying index, respectively, with respect to the tested variables. For these equations, the determination coefficients (R^2) were 0.96, 0.97 and 0.98 for rhamnose concentration, surface tension and emulsifying index, respectively. These results indicate that 96%, 97% and 98% of the experimental data fit the models presented in Equations (2), (3) and (4), respectively.

$$\text{Rhamnose} = 3.748 + 1.041X_1 + 0.857X_2 + 0.295X_3 - 0.345X_1X_1 - 0.596X_2X_2 - 0.335X_3X_3 + 0.355X_1X_2 \quad (2)$$

$$\text{Surface tension} = 27.466 - 1.559X_1 - 1.351X_2 - 0.446X_3 + 0.514X_1X_1 + 0.924X_2X_2 + 0.378X_3X_3 - 0.388X_1X_2 \quad (3)$$

$$\text{Emulsifying index} = 98.411 + 8.236X_1 + 7.711X_2 + 2.013X_3 - 5.955X_1X_1 - 6.228X_2X_2 \quad (4)$$

The positive values for the coefficients of X_1, X_2 and X_3 in Equations (2) and (4) and the negative values for these coefficients in Equation (3) indicate that, when the fermentation is performed at higher values of aeration, agitation speed and inoculum concentration, the rhamnose production and the emulsifying index increase, whereas the surface tension decreases. This result is illustrated by comparing experiments 1 and 8 listed in Table 1.

To validate the model for rhamnose concentration (Equation (2)), optimal values were calculated to determine the conditions that maximize rhamnolipid production by applying the algorithm generated using Maple 9.5 software. Rhamnose concentration was also determined experimentally for three responses at this condition. The conditions that maximized the levels of aeration, agitation speed and inoculum concentration were 2.1 vvm, 1011 rpm and 1.8 g/L of *Pseudomonas aeruginosa* ATCC 10145, respectively. For these conditions, the experimentally obtained rhamnose concentration, surface tension and emulsifying index were 5.26 g/L, 25.4 dyne/cm and 80%, respectively. When the coded values for the optimal conditions were substituted into Equations 2, 3 and 4, the rhamnose concentration, surface tension, and emulsifying index were 5.00 g/L, 25.9 dyne/cm and 73%, respectively. Benincasa and Accorsini (2008) and Abalos *et al.* (2004) reported that the rhamnolipid concentration is approximately three times that of rhamnose. These results indicate that the use of industrial float as the substrate provided high rhamnolipid concentrations. Lofabad *et al*. (2010)

used soybean oil as the substrate and *Pseudomonas aeruginosa* MR01 and produced 5.5 g/L rhamnolipid in a 48-hour period.

The optimized rhamnose concentration of 5.26 g/L can be compared to the results reported by Wu *et al*. (2007), who produced 7.5 g/L rhamnolipid using *Pseudomonas aeruginosa* EM1, glucose and glycerol as substrates and an agitation speed of 200 rpm. Santos *et al*. (2002) reported producing a large amount of rhamnolipids when working with high values of a mixture of glycerol and vegetable oils, reaching up to 7.4 g/L rhamnolipids using *Pseudomonas aeruginosa* PA1.

To optimize the results generated by the Maple 9.5 algorithm, the best conditions for the minimization of the surface tension occurred at the following operating conditions: level of aeration = 1.8 vvm, agitation speed = 897 rpm and inoculum concentration = 7 g/L. The experiment resulted in a surface tension of 25.8 dyne/cm, a rhamnose concentration of 5.35 g/L and an emulsifying index of 95%. The surface tension value of 25.8 dyne/cm characterizes a more efficient rhamnolipid than a surfactant with a surface tension of 26.5 dyne/cm, which was the surface tension of a biosurfactant produced by Wu *et al*. (2007) from glucose and glycerol using *Pseudomonas aeruginosa* EM1 at 200 rpm.

The optimal conditions for rhamnose concentration, surface tension and emulsifying index were 5.25 g/L, 25.3 dyne/cm and 90%, respectively, and these conditions are consistent with the results obtained from the models presented in Equations (2), (3) and (4). According to Reiser *et al*. (1993), the minimum value for surface tension is in the range of 25 to 28 dyne/cm.

Yin *et al*. (2009) reported that a reduction in surface tension from 72 dyne/cm to 33.9 dyne/cm occurred for biosurfactant production by *Pseudomonas aeruginosa* S6 in wastewater containing oil. A decrease in surface tension was also reported by Nawawi *et al*. (2010), who studied biosurfactant production from palm oil with 21% to 25% fatty acids by fermentation at 180 rpm and 30 °C in a mixed culture of micro-organisms. The surface tension of the initial production medium was 54.5 mN/m before inoculation. A decrease in surface tension, which had a minimum value of 30.1 mN/m, was actually coupled with microbial growth.

With a subroutine added to the program in the Maple 9.5 software, the emulsifying index was maximized at 100% for a level of aeration of 1.0 vvm, an agitation speed of 661 rpm and an inoculum concentration of 1.3 g/L. Experiments conducted under these conditions resulted in an emulsifying index of 100%, a rhamnose concentration of 4.91 g/L and a surface tension of 26.5 dyne/cm. When the coded values (X_1, X_2 and X_3) for this condition were substituted into the model equations for rhamnose concentration (Equation (2)), surface tension (Equation (3)) and the emulsifying index (Equation (4)), the results were 4.71 g/L, 26.0 dyne/cm and 100%, respectively. These model results show good agreement with the experimental results. High emulsifying index values were also reported by Barros *et al*. (2007), who obtained an 80% emulsifying index for a biosurfactant produced by a bacterial strain of *Bacillus* that was grown in manipueira.

Oliveira *et al*. (2009) studied the emulsification potential of a biosurfactant produced by *Rhodococcus sp*. with various hydrocarbons as carbon sources (substrate). The biosurfactant showed an emulsifying index of up to 90% when palm oil was used as a substrate. The authors reported that the emulsifying index of a biosurfactant depends on the direct interaction of the hydrophobic portion of the biosurfactant with the substrates (hydrocarbons).

Figures 1, 2 and 3 show the contour curves for rhamnose concentration, surface tension and emulsifying index for the studied operating variables. These curves allow us to simultaneously evaluate the best working range for the studied responses.

From Figure 1, the maximum rhamnose concentration occurs when the aeration level is from 1.2 vvm to 2.4 vvm and the agitation speed is from 600 to 1200 rpm. This range corresponds to a rhamnose concentration of 4.91 g/L on the contour curve. Within this range the surface tension is 25.7 dyne/cm when the aeration level is from 1.2 to 2 vvm and the agitation speed is between 600 and 1000 rpm. The emulsifying index is 100% when the aeration is from 0.8 to 1.4 vvm and the agitation is from 550 to 850 rpm. According to Cooper and Zajic (1980), surfactants are compounds with a surface tension below 40 dyne/cm; therefore, the effectiveness of a biosurfactant with a surface tension below 35 dyne/cm must be proven.

Figure 2 shows that the maximum rhamnose concentration and the minimum surface tension values occur when the level of aeration is from 1.2 to 2 vvm and the inoculum concentration is from 1 to 1.5 g/L. This track has a rhamnose concentration of 5.33 g/L and surface tension of 25.3 dyne/cm. The charts for this dataset show that a 99% emulsifying index occurs when the inoculum concentration is greater than or equal to 1 g/L and the aeration is from 0.7 to 1.4 vvm.

Figure 3 shows that the rhamnose concentration is 4.83 g/L and surface tension is 25.8 dyne/cm for agitation speeds ranging from 600 to 1000 rpm and

inoculum concentrations ranging from 0.6 to 1.6 g/L. By adopting a narrower range of agitation speed and inoculum concentration, a higher rhamnose concentration and lower emulsifying index can be obtained. As expected, the rhamnose concentration increased to 4.94 g/L and the surface tension decreased to 25.6 dyne/cm. A higher emulsifying index is favored when working with a higher inoculum concentration and agitating the broth from 500 to 900 rpm. The surface tension values for all the experiments in the CCD were less than or equal to 32.1 dyne/cm. These values are close to the minimum surface tension value of 28 dyne/cm found by Oliveira *et al*. (2009) for rhamnolipid production by a strain of *Pseudomonas alcaligenes* using palm oil as the carbon source.

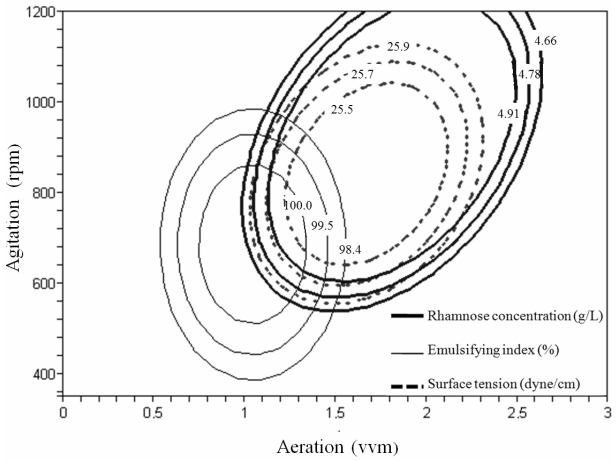

Figure 1: Surface response and contour curves for the surface tension, emulsifying index and rhamnose concentration relative to the level of aeration and agitation speed.

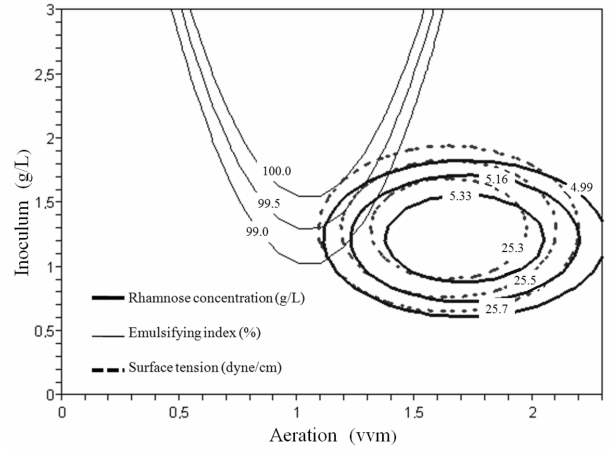

Figure 2: Surface response and contour curves for the surface tension, emulsifying index and rhamnose concentration relative to the level of aeration and inoculum concentration.

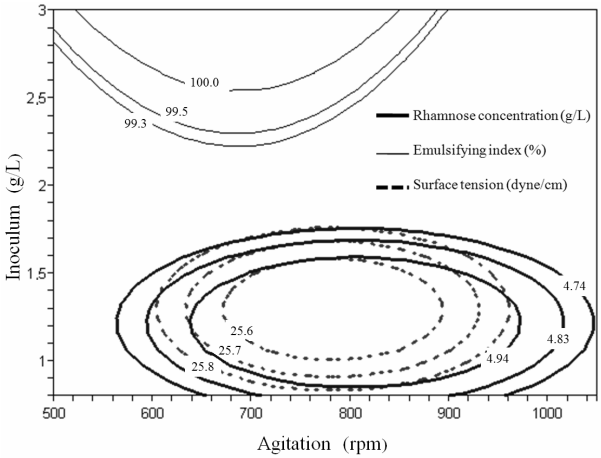

Figure 3: Surface response and contour curves for the surface tension, emulsifying index and rhamnose concentration relative to the agitation speed and inoculum concentration.

Table 2 summarizes the ranges of the variables (level of aeration, agitation speed and inoculums concentration) that provided the best results according to the response surfaces (Figures 1, 2 and 3). This summary helps facilitate a comparison of the data and to determine the most satisfactory condition according to a joint analysis of all the variables and responses.

Table 2: Best tracks for the operating conditions – level of aeration (X_1), agitation speed (X_2) and inoculum concentration (X_3) – obtained from Figures 1, 2 and 3.

Responses studied		X_1 Aeration (vvm)	X_2 Agitation (rpm)	X_3 Inoculum (g/L)
Rhamnose	**Fig. 1**	1.2 – 2.4	600 - 1200	-
	Fig. 2	1.2 – 2.0	-	1.0 – 1.5
	Fig. 3	-	600 - 1000	0.6 – 1.6
Surface Tension	**Fig. 1**	1.2 – 2.0	600 - 1000	-
	Fig. 2	1.2 – 2.0	-	1.0 – 1.5
	Fig. 3	-	600 - 1000	0.6 – 1.6
Emulsifying Index	**Fig. 1**	0.8 – 1.4	500 - 850	-
	Fig. 2	0.7 – 1.4	-	≥1.0
	Fig. 3	-	500 - 900	≥2.2

From the data shown in Table 2, the intersection of the tracks with the best results for rhamnose concentration, surface tension and emulsifying index occurs between 1.2 and 1.4 vvm for the level of aeration, 600 and 850 rpm for the agitation speed and 1 and 1.5 g/L for the inoculum concentration. Note that the range for the inoculum concentration relative to the emulsifying index response was higher than assumed. However, the results presented in Table 1 (experiments 7 and 8) showed that, when using the highest level of aeration and agitation speed, the inoculum concentration is not a significant variable.

The following conditions were selected within the range of process variables studied to optimize the minimum cost of biosurfactant production: a level of aeration of 1.2 vvm, an agitation speed of 600 rpm and an inoculum concentration of 1.0 g/L *Pseudomonas aeruginosa.* These conditions were selected to perform a kinetic study.

An agitation speed of 600 rpm was selected because the effect of aeration was determined to be superior to the effect of agitation speed. Therefore, an agitation speed of 600 rpm could likely be selected (lower than the value of 800 rpm (+1)) at a level of aeration of 1.2 vvm and still produce good results for the responses studied.

Kinetic Study to Evaluate the Optimized Conditions of Aeration, Agitation and Inoculum Concentration

The kinetic study was conducted for a 1.2 vvm level of aeration, 600 rpm agitation speed and 1.0 g/L inoculum concentration. This condition was selected from the analysis of the response surfaces. For this condition, the experimentally determined rhamnose concentration, surface tension and emulsifying index were 5.37 g/L, 25.6 dyne/cm and 100%, respectively. The rhamnose concentration, surface tension and emulsifying index, as calculated from the models in Equations (2), (3) and (4) for the selected conditions, were 4.8 g/L, 25.6 dyne/cm and 100%, respectively.

The results show that the selected conditions met expectations because the values were near the highest values determined during planning, as shown by the responses of experiment number 8 presented in Table 1 (1.2 vvm level of aeration, 800 rpm agitation speed and 1.5 g/L inoculum concentration). The data represent 48 hours of fermentation. This kinetic study shows the importance of analyzing the variables since obtaining similar results was possible at a more economical agitation speed and a lower inoculum concentration.

Figure 4 shows that cell growth occurred in the first 24 hours of fermentation and that the rhamnose concentration increased for up to 48 hours; therefore, cell growth is partly associated with biosurfactant production. The experiments were performed with the industrial float without the addition of nitrogen to the fermented medium. According to Santos *et al*. (2002), consumption of nitrogen influences the synthesis of the biosurfactant and can indirectly interfere with microbial growth. Several researchers have studied rhamnolipid synthesis in the early stationary phase of growth and by exhaustion of the nitrogen source (Ramana and Karanth, 1989; Guerra-Santos *et al*., 1984; Mulligan and Gibbs, 1989). Significant increases in rhamnolipid production were observed in nitrogen-limited conditions (Ochsner and Reiser, 1995; Lang and Wullbrandt, 1999). Syldatk *et al*. (1985) showed that nitrogen limitation is responsible not only for the increase in biosurfactant production, but also for changing the composition of the biosurfactant produced. At 24 hours, the rhamnose concentration was only 1.61 g/L, whereas at 48 hours the concentration had increased to 5.37 g/L. This result shows that most of the rhamnolipid production occurred after the growth phase. These results for rhamnose concentration were higher than those previously reported in the literature (Lima *et al*. 2007; Lang and Wullbrandt, 1999; Lofabad *et al*., 2010; Oliveira *et al*., 2009; Nawawi *et al*., 2010).

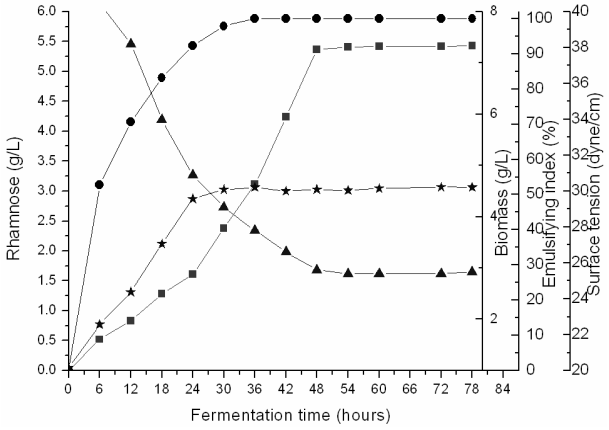

Figure 4: Fermentation kinetics for the selected condition in the region of optimization; the level of aeration, agitation speed and inoculum concentration for the responses rhamnose concentration (■), surface tension (▲), emulsifying index (●) and biomass concentration in dry mass (★); Fermentation at 30 ± 0.5 °C.

CONCLUSIONS

As a global conclusion, the main contribution of this work is that the use of industrial float from the slaughter of poultry and pigs has shown promise for rhamnolipid production. Furthermore, performing joint analysis of the variables and the response surface technique were essential to select the best conditions to increase rhamnolipid production in this study. The operating conditions within the contour curves selected for rhamnolipid production were as follows: 1.2 vvm level of aeration, 600 rpm agitation speed, and 1.0 g/L inoculum concentration. Under these conditions, the rhamnose concentration, surface tension and emulsifying index were 5.37 g/L, 25.6 dyne/cm and 100%, respectively.

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