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SIMULTANEOUS PRODUCTION OF BIOSURFACTANTS AND LIPASES FROM *ASPERGILLUS NIGER* AND OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY AND DESIRABILITY FUNCTIONS

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Abstract - Microbial conversion for the synthesis of high added-value compounds, such as biosurfactants and lipases, is one of the most promising fields within the biotechnology industry, given its current development. In this study the simultaneous production of biosurfactants and lipases by *Aspergillus niger* in a submerged bioprocess was analyzed. Full Factorial Design (2^3) was conducted to assess the influence of the malt extract ($g \cdot L^{-1}$; 25, 50, 75) and soybean oil concentrations (% v/v; 0.0, 1.25, 2.5) and agitation rates (rpm; 0.0, 100, 200) on biosurfactant and lipase production. Higher levels of the factors favored microbial growth, while their lower levels favored the production of biosurfactants and lipases. The variables were optimized through the response surface methodology and desirability functions, obtaining simultaneous maximized values of 0.48 $g \cdot L^{-1}$ (biomass); 42.03% ($EA_{w/o}$);2.17 UE ($EA_{w/o}$) and 3.28 U (LA). This indicates the possibility of combined biosurfactant and lipase production.

Keywords: Filamentous fungus; Biosurfactants; Lipases; Optimization.

INTRODUCTION

Advances in the production of microbial compounds have opened up new possibilities for more extensive industrial scale-up applications (Vannelli et al., 2007). The potential of synthesis of the biocompounds produced by fungi is already known, and biosurfactants and lipolytic enzymes may be mentioned (Roveda et al., 2010; Araujo et al., 2013).

Biosurfactants are surface-active compounds produced by living beings on microbial cell surfaces or extracellularly excreted. These compounds contain hydrophobic and hydrophilic moieties that make them good agents for emulsification (Paramaporn et al., 2010; Morita et al., 2012; Sarafin et al., 2014). They possess significant anti-biological and anti-microbial activities, low toxicity, low irritancy, and good compatibility with human skin, making them favorable for pharmaceutical

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and cosmetic formulations. The emulsification properties allow their use in food additives (emulsifiers) and their surface active properties are very promising for oil tank cleaning, decontamination of polluted areas, microbial-enhanced oil recovery, industrial cleaning, and soil remediation (Paramaporn et al., 2010; Bhardwaj et al., 2013; Souza et al., 2014).

Lipases (EC 3.1.1.3) are enzymes that can catalyze partial hydrolysis reactions or total triglycerides decomposition into free fatty acids, mono- and diglycerides, and also acting in esterification, transesterification and interesterification in low water environments (Reinehr et al., 2014). Lipolytic enzymes have been explored and used for novel biotechnological applications such as detergent, food, pharmaceutical and cosmetics production, chemical synthesis and in waste treatment (Carvalho et al., 2003; Colla et al., 2010; Colla et al., 2012; Kumar et al., 2012).

The fungal production of these compounds depends on its physiology, as well as on the substrate formulation and process parameters. Complex substrates, such as malt extract, sugarcane molasses, corn steep liquor, vegetable oils, animal fat and oil derivatives are used for the biosynthesis of enzymes and biosurfactants (Di Luccio et al., 2004; Paramaporn et al., 2010; Bharali et al., 2011; Gudiña et al., 2015; Bonugli-Santos et al., 2015).

Due to the great influence of culture conditions on the production of enzymes and biosurfactants, the use of experimental design and statistical analysis is a successfully applied methodology (Bonugli-Santos et al., 2015). This methodology provides an efficient approach for the determination of the most important process parameters such as temperature, pH, aeration and substrate selection, which in turn can lead to process optimization. These parameters are best controlled in processes which involve submerged cultivation (Submerged Bioprocess - SmgB) due to the great homogeneity of the culture medium (Colla et al., 2010; Colla et al., 2016). Aeration of growing microbial culture can be ensured by inserting air into the culture medium and by agitation, resulting in an increased interface between gas and liquid capable of reducing the size of the air bubbles, making oxygen more easily accessible to the cells (Bakri et al., 2011). However, the intensity of agitation should remain within a narrow range to keep the damage effect in the system at a minimum level (Kozma et al., 2006).

Filamentous fungi are largely used for industrial lipase production. However, few fungi are already known as biosurfactant producers. *Aspergillus fumigatus, Aspergillus niger, Candida bombicola, Candida rugosa, Pseudozyma* sp. *Penicillium* sp. and *Yarrowia lipolytica* are fungi normally used for the production of biosurfactants and lipolytic enzymes (Fontes et al., 2008; Fukuoka et al., 2008; Castiglioni et al., 2009; Kannahi and Sherley 2012; Kumar et al., 2012; Bhardwaj et al., 2013; Santos et al., 2013; Reinehr et al., 2014). *Aspergillus niger* is a well-known lipase producer with suitable properties for industrial applications (Mahadik et al., 2002; Basheer et al., 2011). It is also reported as a biosurfactant producer (Hussain et al., 2014). Therefore, this study aims to verify the impact of different factors (malt extract, agitation and soybean oil) on the simultaneous production of biosurfactants and lipases by *Aspergillus niger* in a submerged bioprocess, optimizing this process through the use of the response surface methodology (RSM) and desirability functions.

MATERIALS AND METHODS

Microorganism

The fungal strain *Aspergillus niger* isolated from an oil sample of a vegetable oil refining company located in Gaspar, SC - Brazil, was identified and previously selected as both a biosurfactant producer and a secretor of lipases (Sperb et al., 2015). The fungus was maintained at 4 ºC in Petri dishes containing potato dextrose agar (PDA) and transferred to new dishes every two months.

Culture medium and experimental apparatus

The culture medium was prepared with malt extract (Kasvi®) and soybean oil (Soya®), according to the concentrations indicated in the experimental design (Table 1). In all assays, a saline solution containing KH_2PO_4 (2 $g \cdot L^{-1}$), $MgSO_4$ (1 $g \cdot L^{-1}$), $MnSO_4$ (0.01 $g \cdot L^{-1}$), $FeSO_4$ .7 H_2O (0.63 $mg \cdot L^{-1}$), and $ZnSO_4$ (0.62 $mg \cdot L^{-1}$) was added.

The experiments were carried out in 250 mL Erlenmeyer flasks with 100 mL of the culture medium in a rotary shaker at 25 ºC and agitation rates as specified in the experimental design (Table 1). The inoculation was accomplished using circular areas of 20 mm diameter containing mycelium grown in Petri dishes. According to preliminary laboratorial studies the best incubation period for this experiment was seven days. After fermentation, the mycelial biomass was separated by filtration through a vacuum filter and the cell free broth was assayed for biosurfactant and lipase activities.

Table 1. Factorial design matrix for *ME, AG, SO* and experimental values obtained for *biomass*, $EA_{w/o}$, $EA_{w/o}$ and *LA* assays.

Assays	***ME***(g·L^{-1})	***AG***(rpm)	***SO***(%v/v)	***Biomass*** (g·L^{-1})	$EA_{w/o}$(%)	$EA_{w/o}$(UE)	***LA***(U)
1	25(-1)	0(-1)	0(-1)	0.48±0.03	42.03±2.51	2.17±0.02	3.28±0.10
2	75(+1)	0(-1)	0(-1)	1.33±0.26	36.82±3.54	2.24±0.31	3.89±0.48
3	25(-1)	200(+1)	0(-1)	0.83±0.06	23.19±2.51	2.59±0.07	0.44±0.21
4	75(+1)	200(+1)	0(-1)	1.95±0.05	30.21±8.36	1.00±0.24	2.72±0.54
5	25(-1)	0(-1)	2.5(+1)	3.45±0.11	27.54±6.64	2.15±0.08	2.56±0.34
6	75(+1)	0(-1)	2.5(+1)	3.39±0.35	23.26±2.64	2.08±0.09	2.72±0.10
7	25(-1)	200(+1)	2.5(+1)	2.68±0.42	20.29±2.51	1.99±0.21	1.44±0.17
8	75(+1)	200(+1)	2.5(+1)	4.10±0.09	25.51±8.08	1.54±0.09	3.14±1.05
9*	50(0)	100(0)	1.25(0)	2.13±0.3	23.67±1.67	1.28±0.35	2.64±0.24

Mean ± standard deviation of the triplicates for *biomass*, $EA_{w/o}$, $EA_{o/w}$ and *LA* assays.* Center Point

Biomass quantification

Biomass quantification in the culture medium of SmgB was determined by the dry mass technique. After fermentation, the mycelial biomass was separated by vacuum filtration using membranes with 0.8 µm pores. This biomass was dried at 50 ºC until constant mass. The resulting values were converted to grams per liter of culture medium.

Determination of emulsifying activity

The water in oil (w/o) emulsifying activity ($EA_{w/o}$) was determined by the method recommended by Paraszkiewicz (2002) using 2 mL of soybean oil and 3.5 mL of cell free broth. The mixture was vortexed at 7000 rpm for 2 min. After 24 hours, the height of the emulsified phase was verified, using a caliper rule, comparing it to the total liquid height. The $EA_{w/o}$ was determined according Eq. 1.

$$EA_{w/O} = \frac{H_e}{H_t}.100 \quad (1)$$

$EA_{w/o}$ is the emulsifying activity water-in-oil (%); H_e is the height of the emulsified phase (mm); H_t is the total height of the medium (mm).

Oil-in-water (o/w) emulsifying activity ($EA_{w/o}$) was determined by the method of Johnson et al., (1992), using 2 mL of soybean oil and 3.5 mL of the cell free broth. The mixture was vortexed at 7000 rpm for 2 min. After 60 min, the absorbance of the emulsified phase was read in a spectrophotometer (Shimadzu UV-1650) at 610 nm. The emulsifying activity was obtained by Eq. 2. Water was used as the blank medium.

$$EA_{O/w} = Abs_{sample} - Abs_{blank} \quad (2)$$

$EA_{w/o}$ is the emulsifying activity oil-in-water (units of emulsification, UE); Abs_{sample} is the absorbance of the sample (ABS); Abs_{blank} is the absorbance of the blank medium (ABS).

One unit of emulsification (UE) was defined as the amount of absorbance read in this assay.

Lipase activity

Lipase activity was analyzed in crude extracts, using a modified titrimetric method as described by Pastore et al., (2003). The method is based on the titration with NaOH of the fatty acids released from the hydrolysis of olive oil, previously emulsified with xanthan gum, by the action of the lipase present in the cell free broth. Reaction mixture containing 5 mL of the emulsion (1% of xanthan gum (w/v) and 25% of olive oil (v/v)), 2 mL of phosphate buffer (pH 7) and 1 mL of culture medium was incubated at 37 ºC during 15 min. Reaction was stopped by adding 15 mL of (1:1) acetone:ethanol solution and the amount of the fatty acids was quantified through the titration assay with a solution of 0.05M NaOH until achieving pH 7. The lipase activity was determined according to Eq. 3:

$$LA = \frac{V_{NaOH} \cdot Cm_{NaOH}}{t.V_{sample}} \quad (3)$$

where *LA* is the lipase activity (µmol· mL^{-1}·min^{-1}); V_{NaOH} is the volume of NaOH used for titration (mL); Cm_{NaOH} is the molar concentration of NaOH (µmol·mL^{-1}); *t* is the time (min); Vsample is the volume of sample used (mL).

One unit (U) of lipase activity was defined as the amount of enzyme which releases 1 µmol·mL^{-1}·min^{-1} of fatty acids under the assay conditions.

Experimental design

A full factorial design (2^3) with 1 center point and 2 replicates was used to identify the factors that have significant impacts on biomass, biosurfactant and lipase production in SmgB. A set of 9 experiments was used to determine the relative effect of different malt extract (*ME*) and soybean oil (*SO*) concentrations

and agitation rates (*AG*) in 2 levels, high (+1) and low (-1). In Table 1 the real values used and their levels are shown, as well as the experimental values obtained for Biomass, $EA_{w/o}$, $EA_{w/o}$ and *LA* assays.

The experimental values were fitted to the multivariate model, given by Eq. 4, through the least squares method. Surface response graphs were built by using these regression equations.

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{i<j} \sum \beta_{ij} x_i x_j + \beta_{ijk} x_i x_j x_k + \epsilon \quad (4)$$

where Y is the predicted response; β_0, β_j, ... β_n are the regression coefficients; x_i, x_j, x_k are the real values of the independent variables; ϵ is the associated error of the model.

Model curvature analysis for each response surface was generated, according to Eq. 5. The curvature analysis is based on the sum of the curvature squares.

$$SS_{curv} = \frac{n_f n_c (\bar{y}_f - \bar{y}_c)^2}{n_f + n_c} \quad (5)$$

where SS_{curv} is the summation of the curvature squares; n_f is the number of experiments carried on the main factorial matrix; n_c is the number of experiments carried out on center points; $\bar{y}_f$ is the mean value obtained in the main factorial matrix; $\bar{y}_c$ is the mean value obtained on the center points.

The regression coefficients of the multivariate model, curvature analysis, response surface graphs and all statistical analysis were performed with a 95 % significance level, using the software Statistica® 7.0 (*Statsoft*).

Optimization of the responses

The optimization method of non-linear programming desirability functions, described by Derringer and Suich (1980), was used to obtain the best responses under the factorial design of this study. The one-sided transformations were used, as stated in equations 6 (for maximization) and 7 (for minimization) below:

$$d_{\max} = \begin{cases} 0 & if \quad Y < L \\ \left(\frac{Y-L}{T-L}\right)^S & if \quad L \le Y \le T \\ 1 & if \quad Y > T \end{cases} \quad (6)$$

$$d_{\min} = \begin{cases} 0 & if \quad Y > H \\ \left(\frac{H-Y}{H-T}\right)^t & if \quad T \le Y \le H \\ 1 & if \quad T < Y \end{cases} \quad (7)$$

where d_{max} is the one-sided desirability value for maximization; d_{min} is the one-sided desirability value for minimization; Y is the predicted response; L is the lowest response value obtained in this study; H is the highest response value obtained in this study; T is the target for the response; s and t are exponential values defined by user's criteria.

The process optimization criteria were the maximization of $EA_{w/o}$, $EA_{w/o}$ and *LA* values (Desirability for max values = 1 and min. values = 0) while minimizing biomass quantities (Desirability for max values = 0 and min. values = 1). Unitary values were used in the exponents s and t, as linear desirability functions were preferred.

The overall desirability is calculated by the geometric mean of all individual desirability values, according to Eq. 8. The optimization routine consists of changing the factor values (according to the factorial matrix) until the maximum overall desirability value is achieved.

$$D = \left(d_{Biomass} \cdot d_{EA_{w/O}} \cdot d_{EA_{O/w}} \cdot d_{LA}\right)^{1/4} \quad (8)$$

where D is the overall desirability; $d_{Biomass}$ is the desirability for *Biomass*; $d_{EA_{w/o}}$ is the desirability for the $EA_{w/o}$; $d_{EA_{o/w}}$ is the desirability for the $EA_{o/w}$; d_{LA} is the desirability for the *LA*.

The criteria for choosing the minimization of biomass and the maximization of the remaining responses were due to the consideration that the products are of extracellular nature. Therefore, the least biomass produced in the process represents the least purification costs and less residue formation.

The optimization routine as well as the drawing of desirability contour plots were performed in Statistica® 7.0 software (*Statsoft*), by the use of the Response Desirability Profile tool. The optimization study considered the whole response surfaces, described by the regression model in Eq. 4.

RESULTS AND DISCUSSION

The effects and relationships among the factors *ME*, *AG* and *SO* in the production of biomass, biosurfactants and lipases for *Aspergillus niger* were determined according to the design of experiments. Biomass concentration, biosurfactant and lipase production for each assay with the experimental responses are presented in Table 1.

The full factorial design led to the following regression equations (9 - 12), where the numbers with an asterisk are not significant at the 95% level. Null values of the regression coefficients were omitted in this article.

The fit of the regression equations, as well as the adjusted determination coefficients (adj. R^2) can be visualized in Fig. 1.

$$Biomass = 0.050 + 0.017\ ME + 0.001\ AG + 1.374\ SO - 0.007\ ME.SO - 0.003\ AG.SO \quad (9)$$

$$EA_{w/O} = 44.631 - 0.104\ ME - 0.125\ AG - 5.984\ SO + 0.001\ ME.AG + 0.007\ ME.SO + 0.026\ AG.SO \quad (10)$$

$$EA_{O/w} = 2.140 + 0.001\ ME + 0.006\ AG - 0.019\ SO - 0.001\ ME.SO - 0.002\ AG.SO \quad (11)$$

$$LA = 2.972 + 0.012\ ME - 0.0183\ AG - 0.200\ SO - 0.003\ ME.SO + 0.004\ AG.SO \quad (12)$$

Regression models showed a good degree of adjustment with ane overall adjusted R^2 of 0.80. The lowest adjusted R^2 was 0.65 for the $EA_{w/o}$ response. However, the model is considered suitable for representation of the results.

Analysis of the regression coefficients and their significance (95% level) showed that all main factors impact positively on biomass production, with *SO* being the major contributor to its growth. This indicates that the fungus *Aspergillus niger* is capable of using the lipids from soybean oil for its development.

The biosurfactant production was monitored using as parameters the emulsifying activities $EA_{w/o}$ and $EA_{w/o}$. It is also known that emulsification activities from *Aspergillus* strains strongly correlate with the production of biosurfactants (Castiglioni et al., 2009; Ishaq et al., 2015). The response of $EA_{w/o}$ was impacted negatively by the factors *AG* and *SO*, while *ME* was not significant (at the level of 5%). The interaction values *ME.AG* and *AG.SO* were positive and significant but their absolute values compared to the ones of the main factors. The interaction factor of *ME.SO* was not significant. The response $EA_{w/o}$ was

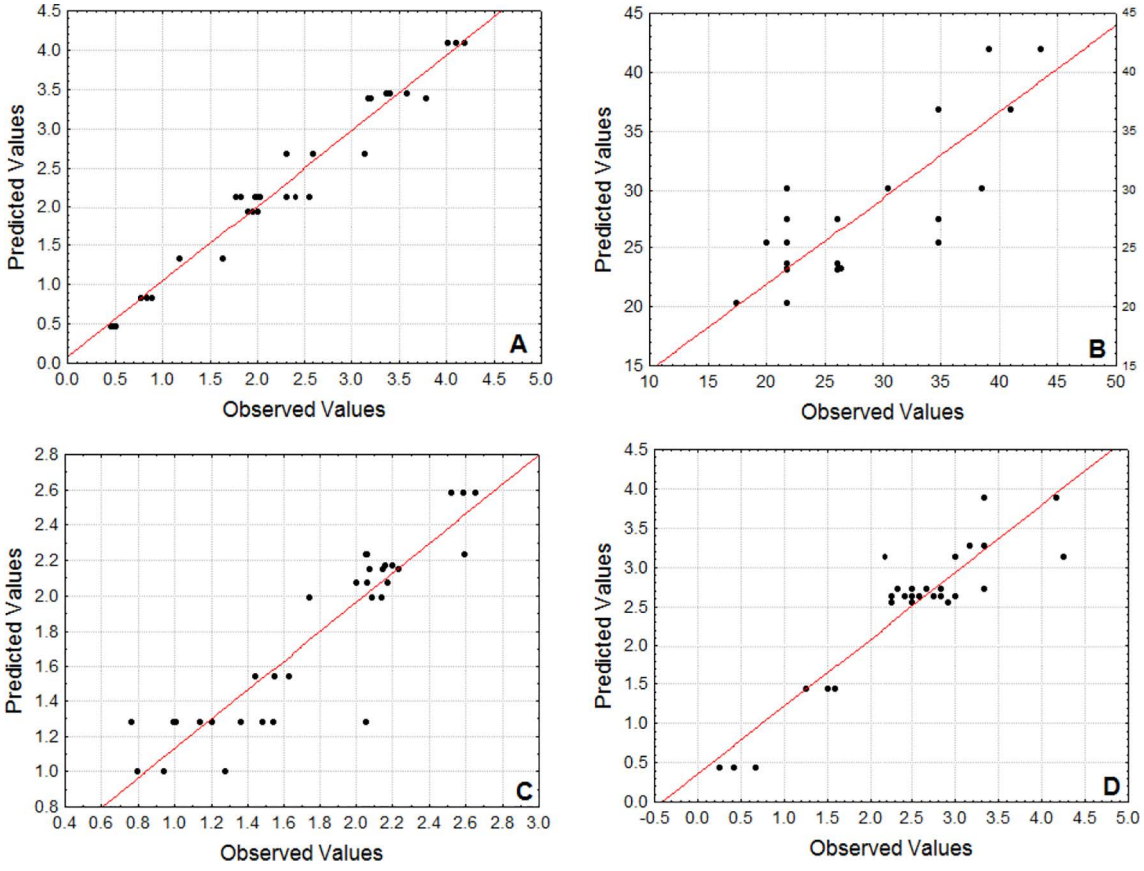

Figure 1. Representation of the Predicted values x Observed values: Biomass (A) adj. $R^2 = 0.96$; $EA_{w/o}$ (B) adj. $R^2 = 0.65$; $EA_{w/o}$ (C) adj. $R^2 = 0.78$; *LA* (D) adj. $R^2 = 0.81$.

impacted positively by *ME* and *AG*; however, their absolute values were low. SO was not significant for this emulsification activity. The effect of interaction of ME and SO for $EA_{w/o}$ was significant and impacted negatively on this response. The remaining interaction factors were either to low or not significant.

According to Table 1, it was verified that low SO is favorable for obtaining $EA_{w/o}$ *Aspergillus niger*. Furthermore, lower *AG* increases both $EA_{w/o}$ and $EA_{w/o}$ values. Fatty acids and maltose are both substrates which can be used by *Aspergillus niger* as carbon and energy sources (Lu et al., 2010; Papanikolaou et al., 2011). It can be clearly seen (Table 1) that the addition of soybean oil into the culture medium stimulated the biomass production (assays 5, 6, 7, 8 and 9). Similar values for biomass were observed by Colla et al., (2010) with *Aspergillus* spp. in the presence of soybean oil, achieving maximum values of 4.49 $g \cdot L^{-1}$. A wide range of oils have been reported as substrates used in culture media. Sarkar and Laha (2013) observed biomass concentrations (derived from 20 mL of the medium) of 13.8 $mg \cdot mL^{-1}$, 15.2 $mg \cdot mL^{-1}$ and 13.1 $mg \cdot mL^{-1}$ for sunflower oil, olive oil and coconut oil respectively. Higher values (13.3 $g \cdot L^{-1}$) of biomass were obtained by Papanikolaou et al., (2011) for *Aspergillus niger* LFMB 1 with waste cooking olive oil in 168 hours. The maximum biomass concentration (4.10 $g \cdot L^{-1}$) in this study was obtained in the assay that had the higher values (+1) of *ME*, *AG* and *SO*.

Lipidic carbon sources (as vegetable oils) are cited as essential inducers for obtaining significant biosurfactant and lipase amounts (Dutra et al., 2008; Saharan et al., 2011). However, the mechanism that regulates biosynthesis widely varies for different microorganisms (Dalmau et al., 2000). In this study the lipase production was independent of the addition of lipids to the culture medium, achieving its maximum value of 3.89 U (Table 1) in the assay with the high level (+1) of *ME* and low levels (-1) of *AG* and *SO*. Colla et al. (2010) in their study obtained a maximum lipolytic activity of 4.52 U using soybean oil as a supplementary carbon source for *Aspergillus* spp. Sarkar and Laha (2013) obtained higher lipase production values when using glucose and olive oil as substrates and reduced lipolytic activity values were observed when using glucose as the sole carbon source. Higher soybean oil concentrations impacted negatively lipase production by *Aspergillus niger* and *Aspergillus flavus* according to Colla et al. (2016).

Low ME levels and the absence of soybean oil favored emulsification activities, with $EA_{w/o}$ value of 42.03% and $EA_{w/o}$ value of 2.59 UE. Biosurfactant molecules are mainly formed as microbial secondary metabolites and play critical roles in the survival of their producing microorganisms by facilitating nutrient transport, interfering in microbe-host interactions and quorum sensing mechanisms or by acting as biocide agents (Gudiña et al., 2013). Carbon source restriction may have been favorable for the activation of microbial secondary metabolism, which resulted in little fungal biomass formation: 0.48 and 0.83 $g \cdot L^{-1}$ in assays 1 and 3, respectively. An $EA_{w/o}$ value of 42.67% and an $EA_{w/o}$ value of 2.95 UE were obtained by Colla et al., (2010) by using *Aspergillus* spp. Santos et al., (2013) studied the production of biosurfactants by *Yarrowia lipolytica,* achieving the best $EA_{w/o}$ results of 47% in a cell-free broth, having corn steep liquor (2.5%) and animal fat (5%) as substrates and soybean oil as the nonpolar phase of the emulsification test. In this study, it was verified that higher (+1) *SO* values resulted in lower emulsifying activities.

It is possible to verify that, agitation played a significant role in all of the acquired responses. Agitation ensures the supply of nutrients, especially oxygen, facilitating mass transfer phenomena. However, high agitation rates lead to high energy dissipation rates connected with high shear stress, which may result in fragmentation and damage of cells and mycelial networks (Kelly et al., 2004; Bakri et al., 2011). The impact of colony morphology on the production of metabolites has been discussed by the academia, but there are many contrasting results (Ibrahim et al., 2015; Quintanilla et al., 2015). Visual impacts were observed in the fungal colony in this study. In the static process (*AG,* level -1) the colony grew as a biofilm on top of the liquid substrate solution and under agitation there was the formation of pelletized colonies. No clear relationship between the morphology of fungal colonies and the studied responses was noted.

The significance analysis of the curvature of the models was carried out, and the assays of $EA_{w/o}$ and $EA_{w/o}$ showed significant values while biomass and lipase activity had no significant values for this analysis. The plotting of response surface curves for $EA_{w/o}$ and $EA_{w/o}$, Fig. 2 and 3 respectively, show the interaction between the factors malt extract (*ME*), agitation (*AG*) and soybean oil (*SO*). The variables not represented on each plot are fixed at their lower levels (-). Through this analysis it is possible to verify the nearness to critical values of $EA_{w/o}$ and $EA_{w/o}$, under the conditions of this study.

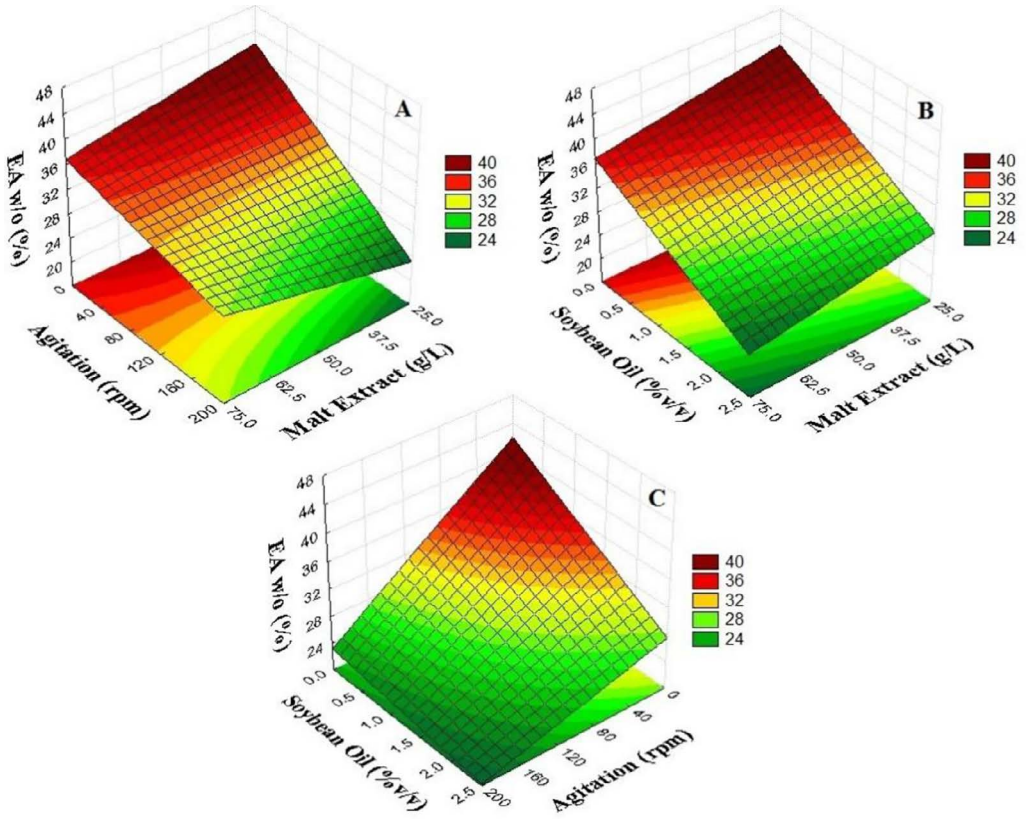

Figure 2. Response Surface curves of $EA_{w/o}$ production from *Aspergillus niger* showing interaction between: *AG* and *ME* (A); *SO* and *ME* (B); *SO* and *AG* (C).

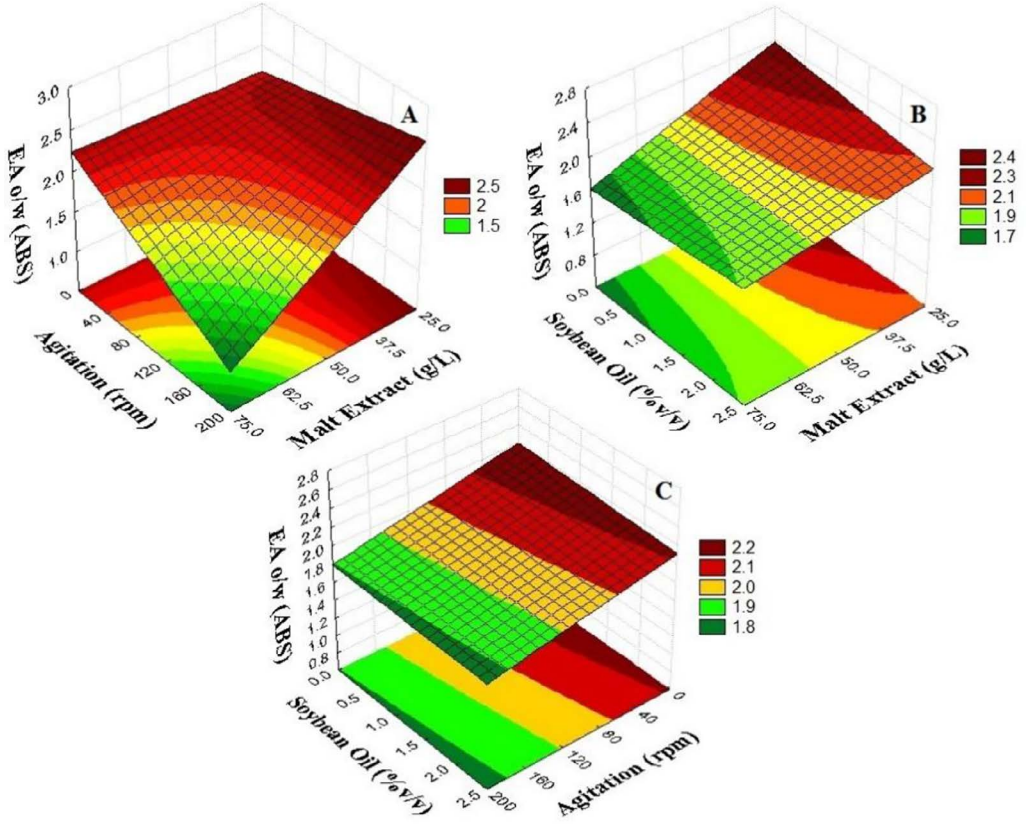

Figure 3. Response Surface curves of $EA_{w/o}$ production from *Aspergillus niger* showing interaction between: *AG* and *ME* (A); *SO* and *ME* (B); *SO* and *AG* (C).

In Fig. 2 and 3 it is verified that the critical values are found in the region with the lower response values, indicating the presence of a point of minimum productivity. Considering that, among the objectives of this work is the simultaneous maximization of the $EA_{w/o}$ and $EA_{w/o}$ responses, and the maximal response regions lie far from the critical points, the first order model is suitable for the optimization routine.

The optimization step using the desirability functions was conducted and the results obtained by this procedure are demonstrated on the contour plots of Fig. 4.

The surfaces generated by the desirability functions allow one to verify the points where the answers are closest to those values defined as objectives. Fig. 4 shows the strong interactions between the factors *ME* and *SO* and *ME* and *AG*, which generated two regions (Fig. 4A and 4B) of maximum desirability, one of them in the higher (+1) and the other in lower (-1) values of *ME*, but with the values of *SO* and *AG* always at their lower levels (-1). This trend is confirmed in Fig. 4C where there is only one region of maximum desirability, found in the region of the lowest values of *SO* and *AG*. This corroborates earlier analysis that accounted for a region of minimum $EA_{w/o}$ and $EA_{w/o}$ with all factors at their highest levels.

The optimized desirability function value was 0.85, which is a very good desirability value, at the lower levels of all factors. The values of the responses, under optimized conditions, are 0.48 $g \cdot L^{-1}$ of *Biomass*; $EA_{w/o}$ of 42.03%; $EA_{w/o}$ of 2.17 UE and *LA* of 3.28 U.

CONCLUSIONS

Submerged cultures of filamentous fungi are widely used to produce commercial biocompounds. Simultaneously obtaining compounds in a singular bioprocess would result in higher process efficiencies. Hence, the possibility of simultaneous production of biosurfactant and lipase was assayed in this study by using *Aspergillus niger*, cultivated in SmgB, varying malt extract (*ME*) and soybean oil (*SO*) concentrations and agitation rates (*AG*). It was found that higher *SO* led to poor emulsification and lipase activities and greater biomass growth, the same occurs for *AG*. Limited access to substrate was a very important factor that affected the production of biosurfactants and lipases positively, in contrast with biomass production, which is affected positively by substrate availability in the medium. Further studies are needed in order to verify the mechanism used by the fungus in this process of converting soybean oil into fungal biomass.

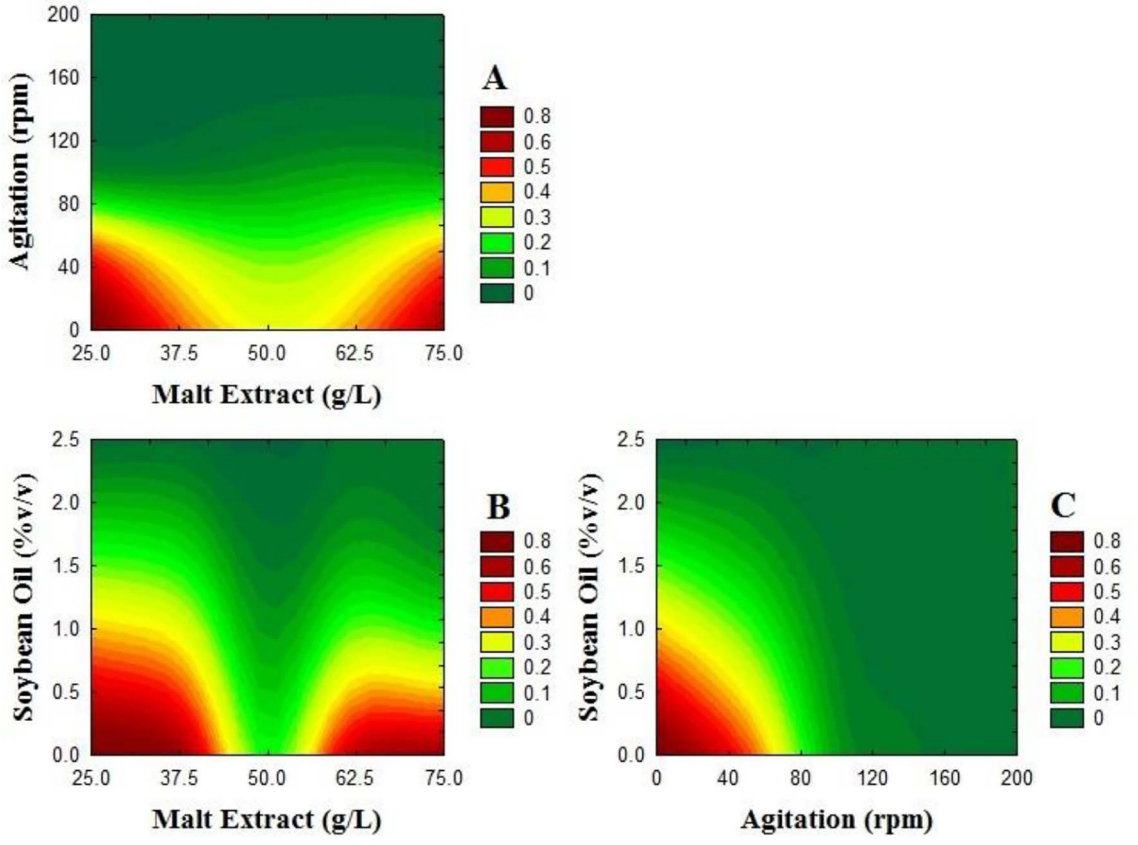

Figure 4. Desirability contour plot from *Aspergillus niger* showing interactions between: *AG* and *ME* (A); *SO* and *ME* (B); *SO* and AG (C).

The application of the design of experiments, response surface and desirability function methodologies proved to be valuable and important tools in the comprehension, development and optimization of bioprocesses. Due to their application it was possible to verify the impacts caused by the factors (*ME, AG* and *SO*), their interaction values and the best response values to a given condition (desirability), in an objective and practical way. Therefore, according to this study factorial matrix, the lowest values of the factors should be used for optimum biosurfactant and lipase production. Due to the diversity of biomolecules synthesized by microorganisms, characterization studies and identification of the molecules produced will be necessary.

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