

Original Research Article

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## Factorial Design based Medium Optimization for the Improved Production of Biosurfactant by *Mucor polymorphosphorus*

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### ABSTRACT

#### Keywords

Optimization;  
Factorial Design;  
*Mucor polymorphosphorus*;  
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The biosurfactants are compounds of microbial origin that exhibit surfactant property, low toxicity and high biodegradability. In this study was to evaluate the production of surfactant by *Mucor polymorphosphorus*, isolated from Caatinga soil from Brazil, using agro-industrial residues. The experiments were monitored by Central Composite Rotational Design (CCRD) using as variable response the reduction of the surface tension. The factors selected for study were soybean waste oil and corn steep liquor as substrates. The spore suspension containing  $10^7$  sporangiospores/mL were inoculated in Erlenmeyer flasks containing the alternative medium according factorial design; The flasks were grown during 96 hours, at 28°C and 150 rpm. All factors studied were important within the ranges investigated. The empirical forecast model developed regarding effective nutritional factors was adequate for explain 84.79% of the variation observed on biosurfactant production. Maximal reduction in surface tension of  $33.2 \text{ mN.m}^{-1}$  with final pH 6.5 was obtained under the optimal conditions of corn steep liquor 4.61% and soybean oil after frying 7.5%. These results demonstrated that the factorial design is adequate for identifying the optimal conditions for biosurfactant production by fungi.

### Introduction

Surfactants are amphipathic molecules, which reduce the interfacial tensions between liquids, solids and gases and confer

excellent detergency, emulsifying, foaming and other versatile chemical process. Biosurfactants constitute one of the main

classes of natural surfactants produced by the microorganisms, being classified in accordance with their chemical composition or microbial origin. These polymers have attracted, in the last few years, considerable interest due to biodegradable nature, low toxicity and diversity of applications (Al-Sulaimani *et al.*, 2011; Rodrigues *et al.*, 2015).

The high cost in the production of biosurfactants, mainly determined by the high cost raw material and low product concentration, inhibited their competition with chemical surfactants. A positive strategy to reduce biosurfactant production costs is the use of inexpensive substrates or high-efficiency strain to increase its productivity. The used frying oils in large quantities are hence considered as a problematic waste product contributing to the pollution of the environment. Overall, the waste frying oil disposal is a growing problem needing effective solution. Biotransformation of frying oils by microorganisms into biosurfactants may be the effective way to solve the problem above (Luo and Zhi *et al.*, 2013; Coronel-León *et al.*, 2016).

Reducing the overall costs of biosurfactant production usually depends on improving the strain, the use of low cost raw materials such as agricultural and industrial wastes as substrates, the use of process scale-up and the use of advanced computer-based techniques for process control and optimization (Shekhar *et al.*, 2015).

Optimizing the composition of the medium is an important issue in developing economically feasible biosurfactant production processes. Microbial produced surfactants or biosurfactants have attracted attention because of their low toxicity, biodegradability, and ecological

acceptability. However, they must compete with surfactants of petrochemical origin in three respects-cost, functionality, and production capacity to meet the needs of the intended application (Ahmad *et al.*, 2016; Rossi *et al.*, 2016).

The aim of the present study was to optimize the production of biosurfactants by *Mucor polymorphosporus* using low cost substrates, on a flask scale.

## Experimental Section

### Microorganism

*Mucor polymorphosporus* (URM 1044) was kindly supplied from the Culture Collection Mycology Department UFPE, and maintained in the Culture Collection UCP (Universidade Católica de Pernambuco) and registered in the World Federation for Culture Collection – WFCC, at 5°C on Sabouraud dextrose agar medium (SAB). The culture was transferred each four months to fresh agar slants SAB medium to maintain viability.

### Biosurfactant Production

All optimization experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of basal medium. The basal medium consisted of corn steep liquor and soybean oil after frying dissolved in distilled water, in various concentrations, according to the experimental designs. The media were sterilized by autoclaving at 121°C for 20 min. The pH of the media was adjusted to 5.5. The experiments were monitored by Central Composite Rotational Design (CCRD) 2<sup>2</sup>, (Table 1), using as variable response the reduction of the surface tension. Aliquots (1 mL) of the spore suspension containing 10<sup>7</sup> spores/mL were inoculated in Erlenmeyer flasks. The

cultures were grown during 96 hours at 28°C, and kept under agitation (150 rpm).

### Surface Activity Assay

Surface tension was determined on cell-free broth obtained by centrifuging the cultures at  $10,000 \times g$  for 15 min with a Tensiometer model Sigma 70 (KSV Instruments Ltd., Finland) using the Du Nouy ring method at room temperature ( $\pm 28^\circ\text{C}$ ). Measurements of surface tension from distilled water were used as controls (Kuyukina *et al.*, 2005).

### Results and Discussion

This work describes the use of post-frying soybean oil and corn steep liquor as low cost medium components for biosurfactant production by the filamentous fungus *Mucor polymorphosporus* (URM 1044). This production was detected by surface tension becoming lower when the microorganism was cultivated on water to which of post-frying soybean oil and corn steep liquor were added, in various concentrations. The optimization process was conducted for Factorial Design DCCR  $2^2$ . Decesaro *et al* (2013), considered as good producer of biosurfactant those microorganisms which reduce the surface tension of water of 72 mN/m to 40 mN/m. Table 2 presents the surface tension of metabolic liquid, free of cells, produced by *M. polymorphosporus*. The results showed activities surfactants in assay 5 (4.61% of corn steep liquor and 7.5% after frying oil) 33.2 mN m final pH of 6.5. Qazi *et al* (2014) using sucrose and yeast extract, as carbon and nitrogen sources, respectively for the production of biotensioactives with *Fusarium sp.* BS-8 obtained a reduction of the surface tension (air / water interface) of 72 mN/m to 32 mN/m. In another study, the biosurfactant from *Candida bombicola* URM 3718 cultivated in a low cost medium formulated

with 5% corn steep liquor, 5% molasses and 5% soybean waste frying oil as substrates reduced the surface tension to values around 30 mN/m after 144 h (Luna *et al.*, 2016), while the biosurfactant from *Candida guilliermondii* UCP0992 cultivated in 2.5 % waste frying oil, 2.5 % corn steep liquor and 4.0 % molasses a reduced the surface tension to 28 mN/m (Sarubbo *et al.*, 2016).

The analysis of the surface tension values is listed in Table 2. The effects of corn steep liquor and post-frying oil on surface tension, as well as the interaction between them, in the Central Composite Rotational Design (CCRD)  $2^2$  are shown in Figure 1. The Pareto chart clearly shows that corn steep liquor concentration is by far the most important factor affecting the reduction of surface tension of cell-free culture broth, followed by post-frying oil concentration and corn steep liquor - post-frying oil interaction. As can be seen in the Pareto chart, the increase in post-frying oil concentration influenced negatively, in a statistically significant way, the increase in surface tension, leading to lower surface tension. The increase in corn steep liquor concentration also influenced negatively, in a statistically significant way, the increase in surface tension, leading to lower surface tension. On the other hand, the corn steep liquor - post-frying oil interaction contributes in a statistically significant way to the increase of surface tension in the culture medium. Figure 1 also shows that curvature effect crosses the 95% confidence level, indicating the proximity of the optimum point.

Table 3 displays a summary of ANOVA representing the results of the fit of linear response model. According to Box (1973), Fisher's variance ratio must be large enough to justify a very high degree of adequacy of the model and indicate that the treatment

combinations are highly significant. In this table, the value of the explained variance ( $R^2 = 84.79$ ) ensures adequate fit ( $R = 0.92$ ); thereby validating the model.

According to Nathans *et al.*, (2012), a model is more than another template set, their values when the correlation coefficient approaches 1.0, the value of the variance approaches 100%. These authors state that

the larger the calculated value of the test F1 is more significant tabulated model and the smaller the calculated F2 test than the more predictive F2 test is tabulated model. Thus, it can be stated that the model represented by Equation 1 is statistically significant, is fitted to the experimental data and thus can predict the experimental results with better accuracy than other models.

**Table.1** Factorial design - Delineation central composite rotational DCCR 22 for *Mucor polymorphosporus*

Factor	Level				
	-1.41	-1	0	+1	+1,41
Corn Steep Liquor (%v/v)	0.38	1	2.5	4	4.61
Soybean Oil After Frying (%v/v)	3.98	5	7.5	10	11.02

**Table.2** Surface Tension according to first full factorial design - DCCR 22 using *Mucor polymorphosporus* after 96 h incubation with shaking

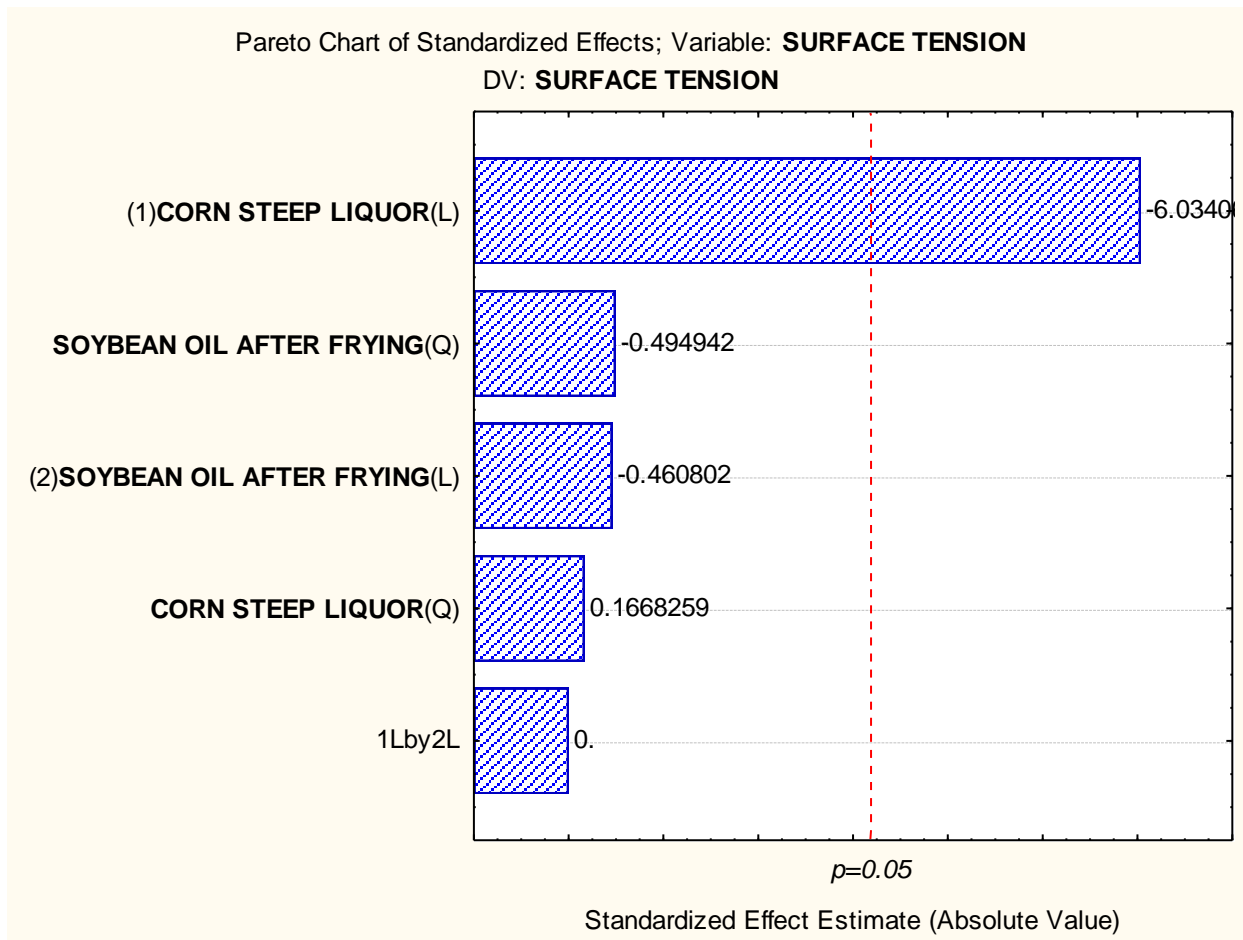
Assay	Components Culture medium		Surface tension (mN/m)	pH
	Corn steep liquor (%)	Soybean oil After frying (%)		
1	+1	+1	34.3	6.45
2	-1	+1	35.7	5.71
3	+1	-1	34.4	5.93
4	-1	-1	35.8	5.54
5	<b>+1.41</b>	<b>0</b>	<b>33.2</b>	<b>6.50</b>
6	0	-1.41	35.0	6.00
7	-1.41	0	37.0	5.66
8	0	+1.41	34.7	6.35
9	0	0	35.6	5.48
10	0	0	34.9	6.32
11	0	0	35.3	6.46
12	0	0	34.5	6.30

**Table.3** The analysis of variance (ANOVA) model that best fit the experimental data at 95% confidence level

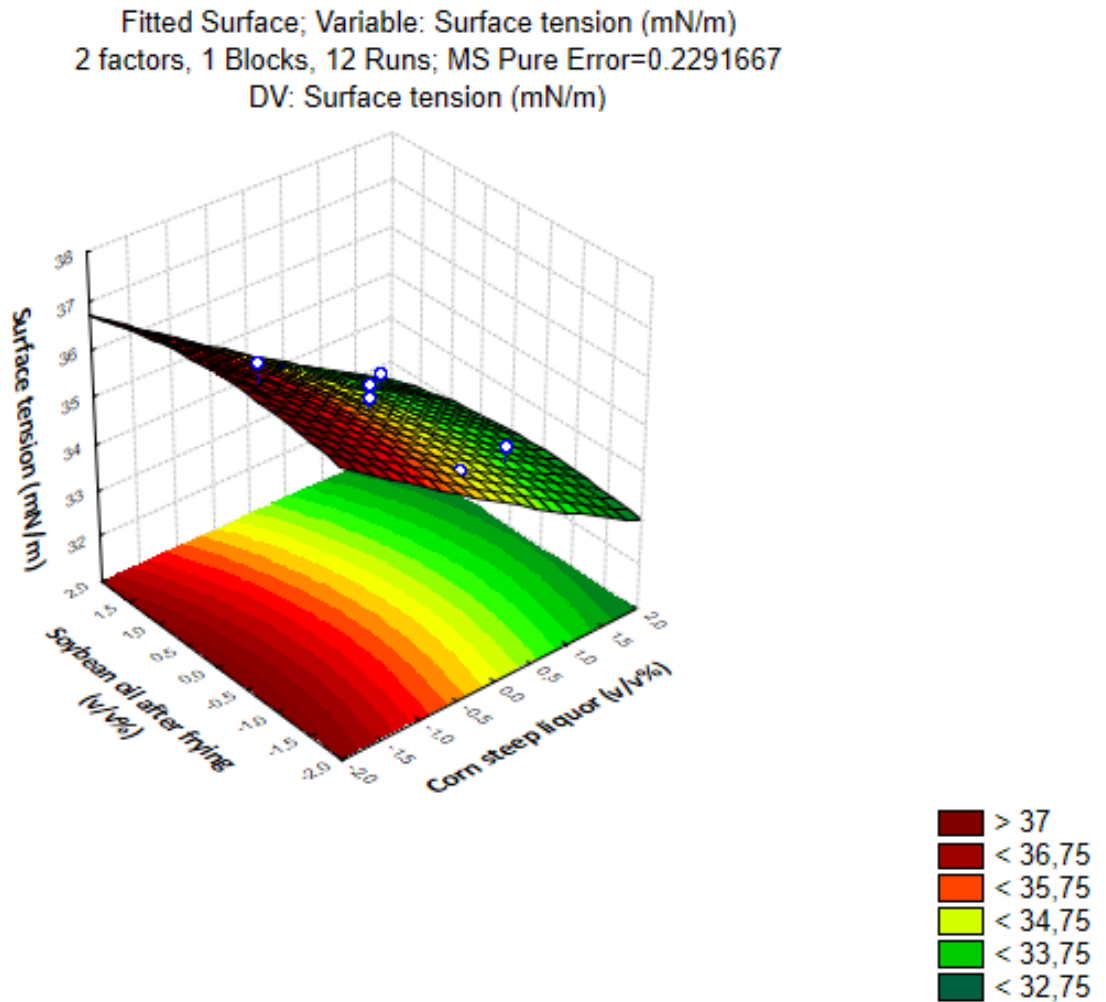
Source of variation	Sum of squares	Degrees of freedom	Mean square	F <sub>calc</sub>
Regression	8.485	4	2.121	
Residual	1.542	7	0.220	9.631
Lack of fit	0.854	4	0.214	
Pure error	0.687	3	0.229	0.932
Total	10.007	11		
% explained variance				84.79
% maximum explainable variance				93.13
Correlation Coefficient (R)				0.9208

$F_{\text{tab } 1(4, 7)} = 4.12; F_{\text{tab } 2(4, 3)} = 9.117$

**Fig.1** Pareto's Chart of standardized effects for (1) corn steep liquor and (2) soybean oil post-frying using surface tension as response variable. The point at which the effect estimates were statistically significant ( $p = 0.050$ ) is indicated by dashed line



**Fig.2** Surface tension response surface showing interaction between soybean post-frying oil and corn steep liquor concentrations



Equation 1 represents the model that best fit the experimental data and experimental. This model has a greater influence on surface tension corn steep liquor than soybean oil. However, one can not overlook the influence of soybean oil, because in a second it can influence response (Figure 2).

$$[>> Y = 36,2592-0,7531*x1+0,1935*x2 +0,0143*x1.^2-0,0150*x2.^2]$$

(Eq.1)

Observing the analysis according to Nathans *et al.*, (2012), quoted above, we note that the

model, although it was the best that stood out among the rest, although it can be considered highly predictive ( $F_{2calc} \ll F_{2tab}$ ). This way, you can use it in process optimization study therefore within the range of the study; it can predict the experimental results with better accuracy than other models.

Equation 2 represents the model used to optimize the process under study. This model has a greater influence on the pH of corn steep liquor, but the influence of soybean oil was not negligible as in the first.



$$[>> Y_2 = 5,0646 + 0,2083 * x_1 + 0,0938 * x_2 - 0,0380 * x_1^2 - 0,0062 * x_2^2 + 0,0233 * x_1 * x_2]$$

Eq.2)

In conclusion, the *Mucor polymorphosphorus* was able to produce a surfactant using as culture medium, corn steep liquor (4.61%) and post-frying soybean oil (7.5%), thereby reducing the surface tension of water from 72 mN m to 33.2mN/m. The results suggest the effectiveness and feasibility of using Central Composite Rotational Design (CCRD) to identify the best medium composition for the enhanced production of biosurfactant. formulation of an alternative and inexpensive medium, based on the corn steep liquor, as well as reuse of soybean oil post-frying to produce biosurfactants could be reduce the production cost, enhancing the feasibility of the commercial application of this promising biomolecule.

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### Conflicts of Interest

The authors declare no conflict of interest.

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