



Evaluation of a new strategy in the elaboration of culture media to produce surfactin from hemicellulosic corncob liquor

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ARTICLE INFO

Article history:

Received 30 March 2019

Received in revised form 27 June 2019

Accepted 26 July 2019

Keywords:

Biosurfactant

Design of experiments

Lignocellulosic residue

Hemicellulosic corncob liquor

Bioremediation

ABSTRACT

The biosurfactant production is characterized by high costs with substrates, which does not make them sufficiently competitive against synthetic surfactants. The insertion of alternative sources of low cost, especially agro-industrial residue, is an excellent alternative to make this competitiveness viable. An alkaline pretreatment was used to extract the hemicellulose from corncob in order to enhance its C5 fraction, common to vegetable biomasses. The hemicellulosic corncob liquor was used with glucose and mineral salt solution as carbon and nutrients sources in a fermentation process for the growth of *Bacillus subtilis*. It was performed a 2³ full factorial design to determine the best conditions for the surfactin production in relation to the following response variables: surface tension reduction rate (STRR) and emulsification index (EI₂₄), from which were obtained two optimized bioproducts under specific conditions. The optimized biosurfactants found to be effected presenting a critical micelle concentration of 100 mg.L⁻¹ and a maximum bioremediation potential of 85.18%, as well as maximum values of 57.38% and 65.30% for STRR and EI₂₄ variables, respectively. Overall results pointed for a successful commercial application for the surfactin produced.

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1. Introduction

Biosurfactants are substances produced by microorganisms endowed with an amphipathic structure. The presence of hydrophilic and hydrophobic portions allows the biosurfactants to reduce the surface and interfacial tension of different compounds, that's why they are considered excellent foaming, dispersing and emulsifying agents as well as environmental applications such as bioremediation and improved oil recovery [1]. In comparison to synthetic surfactants, biosurfactants are an excellent alternative since they are more environmentally friendly due to their lower toxicity and greater biodegradability, as well as the maintenance of their specific activities under extreme conditions of temperature, pH, and salinity [2,3]. Due to its different properties, the versatility in the application of

biosurfactants has aroused the interest of different industry segments, such as the paint, food, cosmetics, detergents, textiles, agrochemical, and pharmaceutical industries [4].

Despite the possibility of being obtained from renewable sources, one of the main challenges for commercial use of biosurfactants is the onerous costs linked to the production process [5]. In order to significantly reduce this economic unfeasibility, other strategies have been studied and published in the literature, among which the use of alternative sources of abundantly available and low-cost nutrients to serve as a nutritional source for the producer microorganism [6,7]. More specifically, the substitution (total or partial) of expensive synthetic media for agro-industrial residue can contribute to the reduction of high production costs and, therefore, increase the competitiveness of biosurfactants against synthetic surfactants [8].

Corncob is a residue from the processing of corn. It is estimated that for every 100 kg of corn ear produced, around 18 kg are formed by the corncob [9]. With the world's largest producers the United States, China, and Brazil producing about 348.8, 219.6 and 93.5 million tons of corn, respectively, residue generation becomes a potential environmental problem [10]. The main constituents of corncob, as well as

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other lignocellulosic biomasses, are cellulose, hemicellulose, and lignin. Since these three components are strongly associated, providing the microorganism access to hemicellulose fractions requires the use of a pretreatment that breaks the lignocellulosic structure [11]. Xylans are one of the main polysaccharides present in hemicellulose, thus, the separation of hemicellulosic fraction from biomass offers the possibility of using this carbon source for xylan-fermenting microorganisms. Studies have shown that the bacterium *Bacillus subtilis* has the ability to metabolize xylans, which could make the use of rich fractions of hemicellulose corn cob liquor more suitable for surfactin production [12].

Lignocellulosic biomass is one of the most abundant, renewable and underutilized resource, been widely used in the field of bioprocesses as a cheap energy source for microbial fermentation, for example, as a substrate in alcohol or biosurfactant productions [13,14]. In this context, this work proposed the use of the hemicellulosic corn cob liquor, extracted by an alkaline pretreatment, as an alternative carbon source for the production of surfactin, which has not been reported yet. Despite the need of chemical reagents which infers on the operational costs, an alkaline pretreatment, unlike acid or hydrothermal processes, are known to act efficiently in the lignin solubilization process, reducing the degradation of carbohydrates of interest, such as cellulose and hemicellulose [15]. On the other hand, the use of differentiated pretreatment conditions may also favor the alkaline extraction of hemicellulose [16]. Furthermore, surfactin is a biosurfactant of the lipopeptide class, produced by the *B. subtilis* and recognized as one of the strongest and active biosurfactants available [17,18].

There are several factors that affect the quality and quantity of the biosurfactant produced, such as the source of carbon used and the growth conditions, such as pH, temperature, trace elements (K^+ , Mn^{2+} , Mg^{+2} and Fe^{2+}) and other nutrients [19,20]. The involvement of so many variables ends up fostering a growing number of scientific studies that aim to investigate the relationship of the factors mentioned above with the growth of the microbial cell, in order to promote significant improvements in the biosurfactant production pathways. For this, the experimental design emerges as an effective alternative in process optimization and evaluation of complex systems [21]. In fermentation processes, the advantages of statistical tools range from increased product reach and reduced process variability to greater conformity of output response and reduction both the time required for tests development and overall cost [22].

The current discussion is reinforced with projections that the total global market for biosurfactants will reach USD 5.52 billion by 2022, at a compound annual growth rate (CAGR) of 5.6% from 2017 to 2022 [23]. Biosurfactants will continue to be the focus of research around the world due to all their advantages in relation to synthetic surfactants. For the purpose of optimizing resources, processes and yields, studies can use statistical tools to determine optimal factor values instead of increasing the number of experiments to be performed.

2. Materials and methods

2.1. Microorganism and cultivation conditions

The strain of *Bacillus subtilis* ICF-PC used belongs to the Sergipe Microorganisms Culture Collection (CCMO/SE code: LMA-ICF-PC 001). The bacterium was maintained in nutrient agar tubes at a temperature of 4 °C. The composition of the concentrated salt solution, which will be diluted in the media, was elaborated by adapting the methodologies described in Cooper et al., [24] Sheppard and Cooper [25] and Marin et al. [26] containing (in $g.L^{-1}$): NH_4NO_3 100.0; KH_2PO_4 102.0; Na_2HPO_4 142.0; $FeSO_4$

$7H_2O$ 0.375; $MgSO_4 \cdot 7H_2O$ 4.93; $MnSO_4 \cdot 7H_2O$ 0.050; $CaCl_2 \cdot 2H_2O$ 0.250. For pre-inoculum, *B. subtilis* strain was conditioned in 2% peptone solution and incubated on a rotary shaker at 30 °C. After 18 h, 10 mL of the pre-inoculum were transferred to 90 mL of inoculum (1% glucose and 1% of mineral salt solution). The solution was incubated at 30 °C and 120 rpm for 24 h. After this period, 5 mL of inoculum and 95 mL of nutrient solution were added, varying the contents of hemicellulosic corn cob liquor (HCL), glucose, and mineral salt solution with final pH adjusted to 6.85, according to experimental design (Table 2). The fermentative media were kept under the same incubation conditions for 72 h. Afterward, the samples were centrifuged at 10,000 rpm for 20 min, obtaining a cell-free supernatant for further analysis.

2.2. Preparation and characterization of the substrate

The corn cob collected in the county of Poço Verde (state of Sergipe) was cut into small pieces and dried in an oven under a temperature of 45 °C for 24 h. Then, the dried material was subjected to a milling process carried out in a knife mill to be homogenized and stored at room temperature. The residue (*in natura*) was characterized by the modified method of Klason [27], which composition was $26.2 \pm 0.6\%$ cellulose, $25.3 \pm 1.1\%$ hemicellulose, and $34.7 \pm 1.5\%$ lignin.

2.2.1. Alkaline pretreatment and chemical composition of HCL

In order to extract the hemicellulosic fraction, the corn cob was subjected to an alkaline pretreatment under less aggressive conditions of temperature and alkali concentration. This process consisted of adding 10 g of the dry and ground sample into 100 mL of NaOH solution (0.75 mol.L^{-1}). The solution was heated and shaken for 2 h at 50 °C with an electric plate. After cooling and pH adjusted to 7.0 by the addition of acetic acid, the sample was filtered. For the determination of carbohydrates and organic acids, furfural and hydroxymethylfurfural, it was also used the modified Klason method [27]. The elemental composition (C, H, N, and O) was determined through a CHN analyzer (Thermo Finnigan FLASH EA 1112 Series). The oxygen content was calculated by difference, according to Sheng and Azevedo [28].

2.3. Optimization of carbon source and mineral salt solution

In order to evaluate the effects and interactions arising from the variation of the nutritional composition of the culture medium, a 2^3 full factorial design was performed for the following independent variables: concentration of HCL (X_1), concentration of glucose (X_2), and concentration of mineral salt solution (X_3). Each variable was evaluated in four coded levels, as presented in Table 2. A set of 18 experiments was performed by four replicates at the central point and six axial points. The interactions of the microorganism in different concentrations and constituents of the culture medium were analyzed by the statistic considering the results obtained for the following response variables: surface tension reduction rate and emulsification index. The STATISTICA software (version 13.2) was used for both regression analysis of experimental data and for surface response method.

2.4. Analytical methods

2.4.1. Concentration of cells

Cell concentration was performed by centrifuging 1 ml of the fermentation medium at 10,000 rpm for 15 min. After the supernatant was removed, the cell mass was resuspended in 1 mL of distilled water, vortexed, and diluted again in 4 mL of distilled water to measure the optical density at 610 nm using UV-M51 spectrophotometer (BEL Photonics, UV-VIS).

2.4.2. Surface tension reduction rate (STRR)

The surface tension measurements were determined using a tensiometer (Attension, Sigma 700/701) according to the Wilhelmy plate method. The analyses were performed on cell-free broth obtained after centrifugation of culture medium, with results expressed in $\text{mN}\cdot\text{m}^{-1}$. The surface tension reduction rate was determined from the Eq. (1) considering the values for surface tension of distilled water ($ST_{\text{H}_2\text{O}}$) and surface tension of biosurfactant (ST_{Bio})

$$\text{STRR} = [(ST_{\text{H}_2\text{O}} - ST_{\text{Bio}})/(ST_{\text{H}_2\text{O}})] \times 100 \quad (1)$$

2.4.3. Emulsification index (EI_{24})

The emulsification index was determined through the method proposed by Cooper and Goldenberg [29]. A mixture of 4 ml of the cell-free supernatant added to 6 ml of the kerosene was homogenized in vortex for 2 min. After 24 h at room temperature, the height of the emulsion layer (H_e) and the total height of the liquid in the column (H_t) were measured. The emulsification index was calculated from Eq. (2).

$$\% EI_{24} = H_e/H_t \times 100 \quad (2)$$

2.4.4. Semipurified surfactin extraction

The cell-free supernatant was subjected to acid precipitation by adjusting the pH to 2.0 with addition of HCl (6 N), maintaining the mixture standing for 24 h at 4 °C. The solution was then centrifuged at 5000 rpm for 25 min to separate the phases and the precipitate obtained was suspended in 1 ml of distilled water, vortexed, and subjected to oven drying at 50 °C to constant weight (24 h). For the purification, the crude surfactin was redissolved with 4 mL of distilled water and adjusted to pH 7.0 with 1 M NaOH, again being oven dried to constant weight [30]. The yield of the semi-purified surfactin was expressed in $\text{g}\cdot\text{L}^{-1}$.

2.4.5. Critical micellar concentration (CMC)

The CMC was determined by plotting the surface tension as a function of biosurfactant concentration. It was realized a serial dilution from a 1 $\text{mg}\cdot\text{mL}^{-1}$ biosurfactant sample. The CMC value corresponds to the central point of the curve inflection in this graph [31].

2.4.6. Bioremediation potential (BP)

BP was evaluated according to the methodology described by Mnif et al. [32] and Marin et al. [26]. Samples of 10 g of sand were contaminated with 1 g of commercial diesel oil. For three days the mixture was maintained at room temperature. After this period, a sample of 5 g of contaminated sand was transferred to a 150 mL Erlenmeyer flask containing 20 mL of biosurfactant, which concentrations were over CMC values. The mixture was shaken at 150 rpm and 30 °C during 24 h to decant the washed solution. This process was repeated twice and the remaining diesel was extracted with two washings of 10 mL dichloromethane, dried, and weighted.

3. Results and discussion

Under the conditions applied, the use of an alkaline pretreatment promoted an efficient fractionation of the residue with the extraction of hemicelluloses (polysaccharide rich in xylose), since the hemicellulose content of liquor (48.8%) was higher than that determined for the corncob residue (25.3%), according to results presented in Table 1. Khan et al. [33] observed that the yield of biosurfactant production from *B. subtilis* was improved in fermentative media where pentoses, such as xylose and arabinose,

Table 1

Chemical composition of hemicellulosic corncob liquor (HCL).

Chemical composition (%)	Hemicellulosic corncob liquor
Carbon	0.78
Hydrogen	10.57
Nitrogen	0.71
Oxygen	87.95
Cellulose	9.8 ± 0.6
Hydroxymethylfurfural	0
Hemicellulose	48.8 ± 1.2
Furfural	0
Soluble Lignin	13.5 ± 0.6

Table 2

Experimental range and levels of the independent variables used in the 2³ full factorial design for the evaluation of carbon sources and the mineral salt solution.

Real Variables (%)	Levels of the experimental design				
	-1.68	-1	0	+1	+1.68
Hemicellulosic corncob liquor (X_1)	0	8.16	20.16	32.16	40.32
Glucose (X_2)	0	1.02	2.52	4.02	5.04
Mineral salt solution (X_3)	0	0.41	1.01	1.61	2.02

were added. In this context, it is suggested that the corncob use could promote induction of biosurfactant production.

Compared with the results obtained by other pre-treatments, the hemicellulose content of 48.8% was superior to that found by Benko et al. [34], which used microwave-assisted heat treatment and extracted 22.8% of polysaccharides hemicellulose-based from corn fiber at a temperature of 210 °C. On the other hand, the content found was lower than that reported by Aguilar-Reynosa et al. [35], which recovered 66.9% of xylan in the hemicellulosic hydrolysate of the corn stover by means of a conduction-convection heating system at the temperature of 180 °C. Although it is a chemical pretreatment, in the present study softer operating conditions were used in order not to degrade the hemicellulose and, in particular, the remaining lignocellulosic fractions, which can happen in treatments with higher temperatures. These fractions can be used in a future biorefinery view.

The content of HCL, glucose, and mineral salt solution as carbon and nutrient sources are fundamental for the functioning of the cellular metabolism of the producing microorganism and, therefore, for the structure and properties of the produced biosurfactant [36]. Table 3 presents the results of the 2³ full factorial design performed to determine the role of each dependent variable in relation to the optimization of surfactin activity.

In the experiments performed for different cultures, parameters such as cell concentration and semipurified surfactin were also monitored. After 72 h of fermentation, the highest concentration of cells obtained corresponded to 4.54 $\text{g}\cdot\text{L}^{-1}$. Regarding the concentration of semipurified surfactin, the maximum value observed was 3.95 $\text{g}\cdot\text{L}^{-1}$, higher than those found in studies such as Gudiña et al. [8], which obtained a concentration of 1.3 $\text{g}\cdot\text{L}^{-1}$ with the use of corn straw liquor for the production of surfactin and Kumar et al. [37] which reached a concentration of 1.8 $\text{g}\cdot\text{L}^{-1}$ of semipurified surfactin using *Bacillus licheniformis*. Being always close to neutrality, the pH reading indicated a variation of 6.31–7.87 between the samples, oscillation already expected taking into account the difference in the composition of the culture media.

3.1. Statistical analysis of experimental data and model validation

The experimental results for the two studied responses were modeled as second order polynomial equations considering the

Table 3

Matrix of the experimental design of the coded independent variables in conjunction with the results for carbon sources and mineral salt solution evaluation.

Run	Coded variables			Response variables	
	X ₁	X ₂	X ₃	STRR	% El ₂₄
1	-1	-1	-1	38.46	9.13
2	1	-1	-1	40.22	8.30
3	-1	1	-1	40.04	6.51
4	1	1	-1	30.93	10.53
5	-1	-1	1	41.62	36.01
6	1	-1	1	38.84	10.01
7	-1	1	1	41.96	50.41
8	1	1	1	32.14	12.98
9	-1.68	0	0	38.18	29.77
10	1.68	0	0	38.05	8.25
11	0	-1.68	0	43.12	35.87
12	0	1.68	0	45.20	61.54
13	0	0	-1.68	42.30	28.35
14	0	0	1.68	46.16	64.25
15	0	0	0	47.15	64.38
16	0	0	0	47.91	47.05
17	0	0	0	44.48	57.90
18	0	0	0	44.32	62.95

X₁: Hemicellulosic corn cob liquor (HCL); X₂: Glucose; X₃: Mineral salt solution. STRR: Surface tension reduction rate; El₂₄: Emulsification index in kerosene.

significant factors, as can be verified at the Eqs. (3) and (4)

$$STRR = 46.16 - 3.63X_1^2 \quad (3)$$

$$\% El_{24} = 59.01 - 17.92X_1^2 + 9.91X_3 \quad (4)$$

Fisher's test (F) determined the value of the probability (p) that should be less than 0.05 (regression performed with 95% confidence) so that null hypothesis is rejected, indicating the dependence of the biosurfactant produced in relation to the variables studied. The statistical significance of the proposed models was verified by the p-value and the regression coefficients presented in Table 4. The percentage of variation explained was determined by the coefficient R², whereupon the model for the

Table 4

Regression coefficients and p-values corresponding to the biosurfactant production considering the responses of surface tension reduction rate (STRR) and emulsification index in kerosene (El₂₄) under different test conditions.

Variables	Regression coefficient	Standard error	p-value	Significance
STRR				
Average	46.16	1.76	0.0000	*
X ₁	-1.48	0.96	0.1610	NS
X ₁ ²	-3.63	1.00	0.0065	*
X ₂	-0.77	0.96	0.4416	NS
X ₂ ²	-1.49	0.99	0.1732	NS
X ₃	0.83	0.96	0.4087	NS
X ₃ ²	-1.46	1.00	0.1795	NS
X ₁ ·X ₂	-2.24	1.25	0.1110	NS
X ₁ ·X ₃	-0.65	1.25	0.6148	NS
X ₂ ·X ₃	0.17	1.25	0.8958	NS
El₂₄				
Average	59.01	6.72	<0.0001	*
X ₁	-7.06	3.64	0.0884	NS
X ₁ ²	-17.92	3.79	0.0015	*
X ₂	4.40	3.64	0.2611	NS
X ₂ ²	-7.39	3.79	0.0867	NS
X ₃	9.91	3.64	0.0262	*
X ₃ ²	-8.25	3.79	0.0612	NS
X ₁ ·X ₂	-0.82	4.76	0.8672	NS
X ₁ ·X ₃	-8.32	4.76	0.1182	NS
X ₂ ·X ₃	2.22	4.76	0.6531	NS

NS: not significant.
* significant at the 95% level.

surface tension reduction rate could explain 72.91% of the variability in response, with a p-value statistically significant at the 5% level for the mean (p = 0.0000) and a predicted value of 46.48%. It was obtained a positive effect for the mean (an increase in the content of the substrate employed leads to an increase in the response studied) while the quadratic liquor (p = 0.0065) presented a negative effect (a smaller quantity leads to a better response) as shown in Eq. (3).

For the emulsification index in kerosene, the explained percentage of variation was 83.48% with a predicted value of 65.74%. The p-value was statistically significant at the 5% level for the mean (p < 0.0001), as well as for the variables quadratic liquor (p = 0.0015) and linear salt solution (p = 0.0262). While the quadratic liquor obtained a negative effect, the linear salt solution had a positive effect (Eq. 4), and therefore, the higher amount of mineral salts used in the preparation of the culture medium was responsible for a higher emulsification index. Therefore, when lower levels of mineral salt solution were used in assays 1–4 present in Table 3, a number of lower values were obtained for El₂₄.

An analysis of variance (ANOVA) was used to verify the linear interaction between the factors and quadratic models from pure error. The lack-of-fit demonstrates a possible failure to represent experimental domain data that is not included in the regression. However, the proposed models are considered adequate due to the insignificance of the lack-of-fit (95% confidence level) (Table 5). Thus, the models are effective to describe the relationship between the conditions established for the variables and the responses studied.

3.2. Graphical analysis of response surface models

As already shown in Eq. (3), the response to STRR is related to an interaction next to the quadratic liquor variable. This interaction was investigated by plotting the response surfaces in three-dimensional space, with the vertical axis corresponding to the dependent variable as a function of the two horizontal axes representing the independent variables. As documented, it is known that surfactin can decrease the surface tension of water from 72 to 27 mN·m⁻¹, which consists in an STRR of 62.5% [38]. Therefore, the higher the values for the surface tension reduction rate better the biosurfactant performance, which makes the prediction of this factor extremely important for the analysis of the surfactin produced [39].

Fig. 1a shows the response surfaces for the surface tension reduction rate as a function of the concentrations of HCL, and glucose, presenting variables coded with values in the region of the central point, tending the region of the surface of lower concentration. Fig. 1b represents the response surface for the surface tension reduction rate as a function of the HCL and mineral salt solution concentrations. It was verified that the ideal range was close to the point central to the liquor, tending to a lower

Table 5

Lack of fit and pure error obtained from the analysis of variance (ANOVA) for surface tension reduction rate (STRR) and emulsification index (El₂₄) responses.

Source of variations	SS	df	MS	F-value	p-value	Significance
STRR						
lack-of-fit	90.2100	5	18.0420	5.3498	0.0990	NS
pure error	10.1174	3	3.3725			
total	368.9639					
El₂₄						
lack-of-fit	1271.546	5	254.309	4.1233	0.1365	NS
pure error	185.028	3	61.676			
total	8764.755					

SS: Sum of squares; df: Degree of freedom; MS: Mean square. NS: not significant at the 95% level.

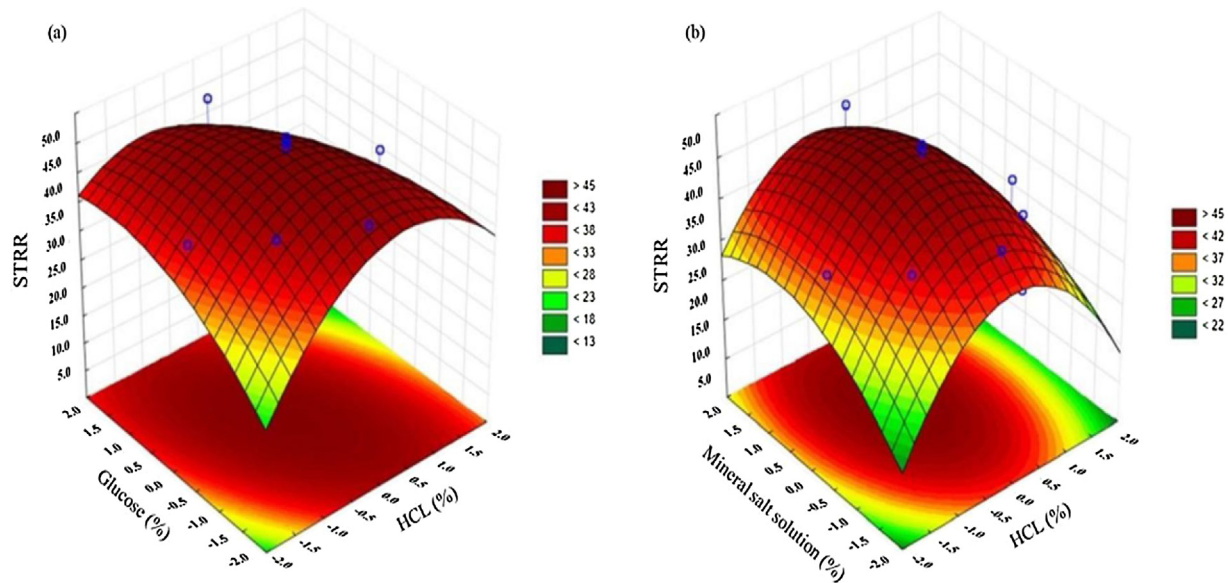


Fig. 1. Three-dimensional response surface for surface tension reduction rate as a function of interaction between factors: (a) HCL and glucose and (b) HCL and mineral salt solution.

content, while in the same region for the mineral salt solution the tendency was to the maximum of its content.

Thus, by establishing a parallel between Fig. 1 and the results described in Table 3, it is possible to notice that the biosurfactant from experiment number 16 presented the highest value for STRR response, corresponding to 47.91%. This is consistent with the results described by authors who also made use of *B. subtilis* for the production of biosurfactant, but in different culture media [8,40].

The proposed mathematical model for the surface tension reduction rate suggests an optimized concentration with coded variables of -0.21 for HCL, -0.09 for glucose, and +0.33 for the mineral salts solution (in accordance with the response surfaces of Fig. 1), with actual concentrations correspondent to 15.9% of HCL, 2.39% of glucose, and 1.21% of the mineral salt solution. This composition was used again for optimized production of biosurfactant B-STRR subsequently.

The response surface graph for the emulsification index as a function of both HCL and glucose concentrations is set in Fig. 2a.

The optimized condition was visualized around the center point, tending towards a lower concentration of liquor and higher glucose. Fig. 2b shows the response surface for the emulsification index as a function of the concentration of HCL and the concentration of the mineral salt solution, whose graphic analysis indicated that the ideal region comprised the intermediations of the central point, with tendency to a lower concentration for the liquor in this region, as well as a lower concentration of mineral salt solution an area above the central point, being in agreement with the fact that the run number 15 obtained the best result for the EI₂₄, equivalent to 64.38%. Since an oil/water ratio of 6:4 was used, it is known that the oil phase constituted 60% of the total volume, so, for EI₂₄ values equal or greater than 60, a complete emulsification of the oil phase occurred [38]. Moreover, in the run number 9 the absence of liquor and the use of sugar as the only feedstock providing carbon resulted in low values for EI₂₄ and STRR responses, especially when compared with those presented by experiments 15 and 16, which had liquor and sugar in their compositions.

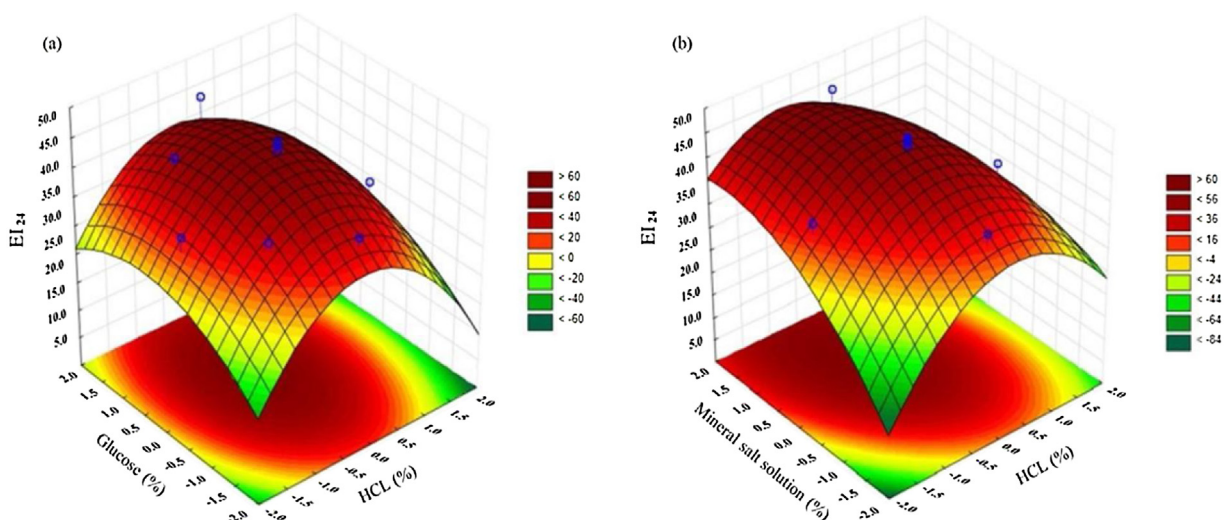


Fig. 2. Three-dimensional response surface for emulsification index as a function of the interaction between factors (a) glucose and HCL and (b) mineral salt solution and HCL.

Table 6

Values of the surface tension reduction rate, emulsification index and bioremediation potential for the optimized biosurfactants B-STRR, B-El₂₄, Glucose 4% and Tween 80.

Analyses	Experiments			
	B-STRR	B-El ₂₄	Glucose 4%	Tween 80
Surface tension reduction rate (%)	57.10	57.38	52.88	52.42
Emulsification index (%)	34.12	65.30	5.59	0.00
Bioremediation potential (%)	85.18	71.16	85.66	91.55

Thus, the response surfaces showed in Fig. 2 presented an optimized condition described by the mathematical model with coded variables of -0.41 for HCL, +0.45 for glucose, and +0.87 for the mineral salt solution, results corresponding to a real concentration of 15.24%, 3.20%, and 1.53%, respectively, for the emulsification index, conditions applied in the elaboration of another optimized biosurfactant (B-El₂₄).

3.3. Optimization of biosurfactant production

As two optimized compositions for the biosurfactant production have been established, two culture media were prepared again in order to evaluate the quality of surfactin produced at this work. To do so, a comparison was made between these new results against those obtained using a standard 4% glucose medium, under the same conditions of temperature, agitation and time of fermentation already known, and the synthetic surfactant polysorbate 80 (Tween 80). Table 6 shows the behavior of the

biosurfactant according to the tests of surface tension reduction rate, emulsification index, and bioremediation potential.

As verified in Table 6, the best results for STRR were obtained by the biosurfactants B-STRR (57.10%) and B-El₂₄ (57.38%), which was higher than the 46.48% value predicted by the model. The results obtained for both were superior to glucose 4% and to the chemical surfactant Tween 80, highlighting the effectiveness of the product elaborated in the laboratory from alternative substrates. Promoting a comparison between the literature and the presented results, it was verified that they were superior to those reported by Abdel-Mawgoud et al. [38], who used molasses as a substrate for *B. subtilis* and obtained a percentage of surface tension reduction of 48.57%. The reduction of surface tension obtained was also higher than the percentage of 43.62% and 39.22% determined by Pornsunthorn-tawee et al. [41] for *Bacillus subtilis* PT2 and *Pseudomonas aeruginosa* SP4, respectively; in which the authors made use of nutrient broth with palm oil as a source of carbon.

The ability to form and stabilize an emulsion is a precept used to verify if the microorganism is producing biosurfactant [42]. Several factors can influence the emulsifying properties of biosurfactant, such as organic and aqueous phase composition, emulsion-stabilizing nature, temperature, and the presence of fine particulates [43]. The emulsification index reached by the optimized biosurfactants B-STRR (34.12%) and B-El₂₄ (65.30%) were higher than those determined by the biosurfactant produced from glucose 4% and the synthetic surfactant (Table 6). The variations found for this response may be correlated to the different concentrations of the substrates used to obtain microbiological surfactant. The more expressive experimental value was obtained by the biosurfactant

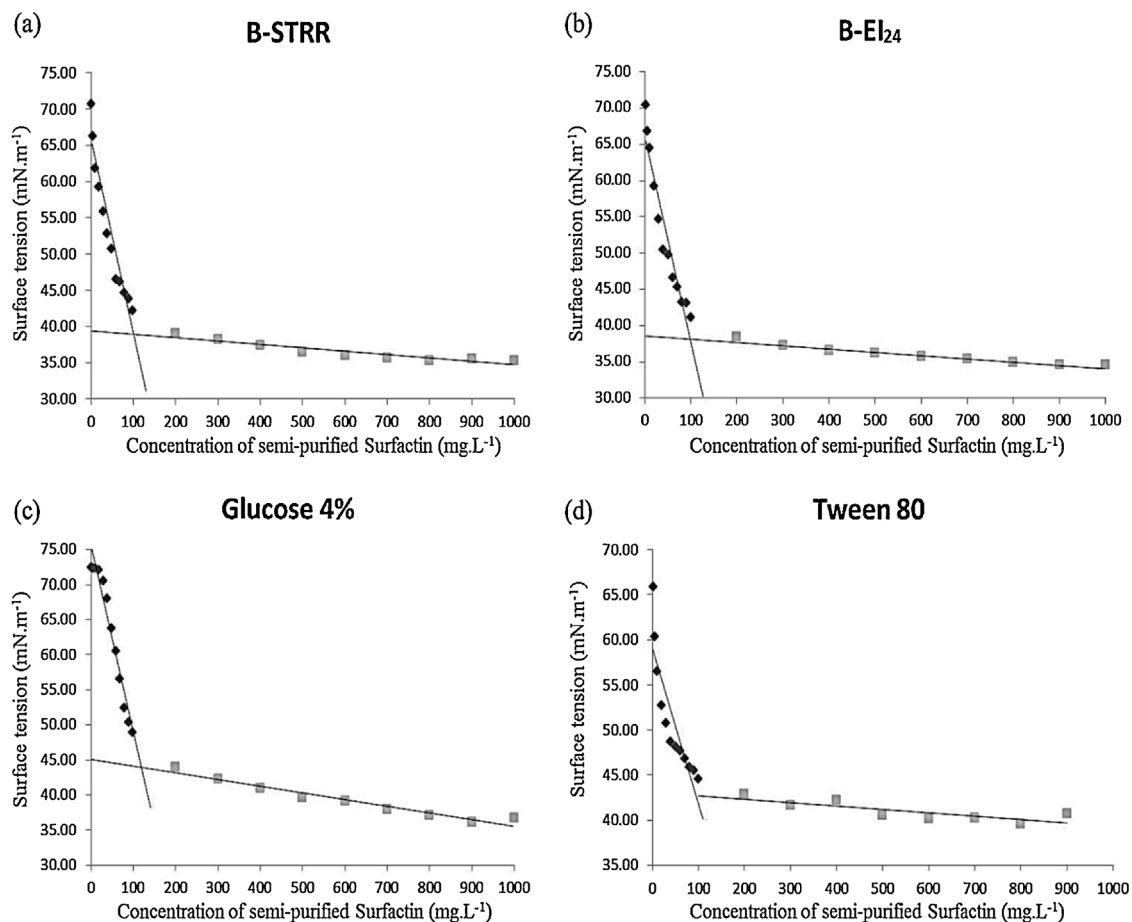


Fig. 3. Plot of surface tension as a function of surfactin concentration to determine the value of CMC of the following emulsifier agents: (a) B-RTS, (b) B-El₂₄, (c) Glucose 4% and (d) surfactant Tween 80.

B-El₂₄ which was close to the predicted one (65.30% versus 65.74%, respectively). This can be considered satisfactory according to the criterion that establishes that the emulsifier agent has the ability to maintain at least 50% of the original emulsion volume after 24 h of its formation [44]. The result determined for B-El₂₄ was higher than the 58% found by Liu et al. [45] but lower than that found by Oliveira et al. [42], an emulsification index for surfactin correspondent to 67%.

To evaluate the potential of bioremediation in contaminated sand, it is necessary to determine the value of the critical micellar concentration, which is the parameter used to predict the efficiency of the biosurfactant by measuring the concentration value necessary to obtain a significant reduction in the water surface tension. Thus, the CMC is defined from the inflection point of the surface tension curve versus concentration of surfactin [46].

Based on the inflection point provided by each of the plotted curves, the measured CMC corresponded to approximately 100 mg.L⁻¹ for the surfactants B-STRR, B-El₂₄ and Tween 80 (Fig. 3a,b,e,d), with the exception of the biosurfactant produced from glucose 4%, which CMC value was 120 mg.L⁻¹ (Fig. 3c). The lower the CMC more efficient the surfactant and therefore more favorable they are in economic terms in the use of industrial processes. Bognolo [47] did a survey of the CMC range for different synthetic surfactants, whose values were kept on a scale of 0.7 to 2900 mg.L⁻¹. This oscillation is linked to the difference in the composition of the surfactants. The surfactin produced in the present work presented a better CMC than those reported by the author for the synthetic surfactants linear alkylbenzene sulfonate and sodium lauryl ether sulfate, which presented values of 590 and 2,000–2,900 mg.L⁻¹, respectively. In contrast, the results found in these trials were higher than other data already documented, since surfactin can reach a CMC value up to 11 mg.L⁻¹. In addition to the composition, this difference can also be explained by the strong influence that acyl chain length of surfactin exerts along with its ability to form micelles [48].

Treating contaminated soils is not considered an easy task as pollutants, that may be toxic, mutagenic or carcinogenic, are often strongly bound to soil particles [49]. The application of surfactants to contaminated soil and water at concentrations above the critical micelle concentration can potentially reduce interfacial tension, increase solubility, and facilitate biodegradation [50]. Therefore, to evaluate the biosurfactants with respect to the bioremediation potential in sand contaminated with commercial diesel oil, a biosurfactant concentration of 200 mg.L⁻¹ was used. As shown in Table 6, the values remained in a range between 71.16 and 91.55%, which the optimized biosurfactants (B-STRR and B-El₂₄) had a lower value than the commercial surfactant Tween 80. This may be related to the dependence that the biodegradation of hydrocarbons in contaminated soils presents in relation to the environmental conditions and the types of hydrocarbons in contaminated soil [51,52]. Nevertheless, the biosurfactants developed in the present study demonstrated a very satisfactory effect on the bioremediation of the sand/diesel oil system, especially the optimized surfactin B-STRR, which exhibited a bioremediation potential of 85.18%.

4. Conclusions

The hemicellulosic liquor extracted from corncob by an alkaline treatment presented a high potential as a sustainable and economic substrate for growth of *B. subtilis*, which proves the relevance of the proposal to take advantage of the C5 fraction from the hemicellulose of vegetal biomass. In addition to the type of carbon source, the production of biosurfactants is also influenced by the concentration of the substrate, so the initial studies of surfactin production performed by a design of experiment allowed

the identification and elaboration of two optimized biosurfactants: B-STRR and B-El₂₄. These bioproducts were submitted to experiments like surface tension reduction rate and emulsification index in kerosene, which results put them as quality emulsifier agents according to literature parameters. The optimized biosurfactants showed an excellent ability for bioremediation of soils contaminated with diesel oil, which encourages their commercial application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to CAPES, CNPq, FAPITEC/SE and Northeastern Biotechnology Network for supporting the development of Brazilian science.

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