Revista Tecnológica - Universidade Estadual de Maringá - ISSN 1517-8048
DOI: 10.4025/revtecnol.v30i1.55243

OPTIMIZATION TRENDS IN TOTAL LIPOPEPTIDE PRODUCTION BY *Bacillus velezensis* 0G REVEALED A SUSTAINABLE SUBMERGED FERMENTATION METHOD USING SWEET POTATO PEELS

TENDÊNCIAS DE OTIMIZAÇÃO NA PRODUÇÃO DE LIPOPEPÍDEOS TOTAIS POR Bacillus velezensis 0G REVELARAM UM MÉTODO DE FERMENTAÇÃO SUBMERSO SUSTENTÁVEL USANDO CASCAS DE BATATA DOCE

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Abstract: Lipopeptide of the surfactin family are very potent biosurfactants with important applications for environmental remediation and chemical industries. In the present work, the complex production of lipopeptide (mostly surfactins) by Bacillus velezensis 0G, was evaluated under different growth conditions using factorial design 2^3 with central and axial points. All experiments were carried out based on sustainable submerged fermentation containing sweet potato peels. The response surface trends demonstrate a maximum biosurfactant production at inoculum volume of 32 mL; combined broth volume of 46 mL, and incubation time of 24h. These observations were based on the analysis of supernatant by HPLC.

Keywords: surfactin; multivariate optimization; response surface; Bacillus velezensis 0G

Resumo: Os lipopeptídeos da família surfactina são biossurfactantes muito potentes, com importantes aplicações para remediação ambiental e indústrias químicas. No presente trabalho, a produção complexa de lipopeptídeo (principalmente surfactinas) por Bacillus velezensis 0G foi avaliada sob diferentes condições de crescimento, utilizando-se o planejamento fatorial 2^3 com pontos centrais e axiais. Todas as experiências foram realizadas com base em fermentação submersa sustentável contendo cascas de batata-doce. As tendências da superfície de resposta demonstram uma produção máxima de biossurfactante em volumes de inóculo de 32 mL; volume combinado de caldo de 46mL e tempo de incubação de 24h. Estas observações foram baseadas na análise do sobrenadante por HPLC.

Palavras-chave: surfactina; otimização multivariada; superfície de resposta; Bacillus velezensis 0G

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DOI: 10.4025/revtecnol.v30i1.55243

1 Introduction

Lipopeptide of the surfactin family are very potent biosurfactants, with detergent action on biological membranes (JEMIL *et al.*, 2017). Some of the main biological functions of lipopeptide are: bactericidal, fungicidal, antiviral, and antitumor (GARG; PRIYANKA; CHATTERJEE, 2018), with important applications for environmental remediation and chemical industries(SYED; CHINTHALA, 2015; VECINO *et al.*, 2015). Lipopeptide biosurfactant molecules are produced by fermentative processes, characterized by low productivity and expensive downstream processing(DE *et al.*, 2015). Surfactants produced by different strains of *Bacillus*, in addition to being obtained by less aggressive processes from the environmental point of view, showed significant effects on the biodegradation of aliphatic and aromatic hydrocarbons (DE *et al.*, 2015; SOUSA *et al.*, 2014).

The submerged fermentation process consists of introducing the microorganism into a liquid medium as inoculum. In this process, fermenters contain the growth medium, providing controlled agitation, aeration, pH levels, temperature and dissolved oxygen concentration, among other parameters(BAKRI; MEKAEEL; KOREIH, 2011). In this regard, chemometrics applied to microorganisms permitted a huge advance in metabolomics, so the nutrients and therefore the production of biomolecules, either from primary or secondary metabolism, can be optimized (BEZERRA *et al.*, 2008; PEREIRA *et al.*, 2013; PRADO *et al.*, 2019; VAN DER GREEF; SMILDE, 2005). In order to minimize overall time, resources and waste, multivariate optimization tools are applied, such as experimental designs and response surface modeling (RAZA; AHMAD; KAMAL, 2014; TEÓFILO; FERREIRA, 2006).

Besides, considering the NEXUS approach for cities, where general efforts should focus on sustainable use of water, food and energy to ensure that there will be enough resources for future generations (DE AMORIM *et al.*, 2018; KUMAR; SAROJ, 2014), a major concern about reuse of organic waste is part of those efforts to enhance sustainability of industrial processes, in order to generate value-added products (LIN *et al.*, 2018). There has been a growing demand in recent decades for the use of agro-industrial waste, now called residual biomass (PELIZER; PONTIERI; MORAES, 2007). Particularly, sweet potato peel reportedly can be reused for metabolites production, ethanol and sugar (AKOETEY *et al.*, 2017).

In this work, we have established the conditions to optimize *Bacillus velezensis* 0G submerged fermentation, using sweet potato skins (organic waste) as carbon source. A multivariate optimization was applied with 2^3 factorial design with center point and axial points and response surface modeling. The three parameters studied were inoculum amount, broth volume and incubation time, optimized to increase surfactin production. The amount of surfactin produced was given by surface tension measurements, emulsification and HPCL analysis.

2 Material and Methods

2.1 Reagents and Equipment

All chemicals are of analytical grade and were used without further purification. The following reagents were used: Peptone, Sodium Chloride (NaCl), Glucose ($C_6H_{12}O_6$), Monopotassium Phosphate (KH_2PO_4), Sodium Hydroxide (NaOH), Diamonium Phosphate ((NH_4) $2HPO_4$), Disodium Phosphate (Na_2HPO_4), Calcium Chloride ($CaCl_2$) $MgSO_4$), Manganese Sulphate ($MnSO_4$) and Iron (I) Sulphate ($FeSO_4$), 70% Surfactin (Lipofabrik), 1M HCl, Bromotimol Blue (Neon), Cetylpyridinium Chloride (Synth), MOPS (3-

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morpholinopropanesulfonic acid), Potato peel, Acetonitrile and Trifluoroacetic acid. The strain *Bacillus velezensis* 0G (Guaíra) was used throughout the experiments (ETCHEGARAY *et al.*, 2017). The equipment used: autoclave (Chimis), analytical balance (Hettich), automatic pipette (Thermo Scientific), blender, greenhouse (Fanem), laminar flow chamber (Veco), incubator (Marconi), tensiometer (Cole-Parmer), centrifuge (Hettich universal 320R), microplate spectrophotometer (Titertek-Berthold - Crocodile mini workstation). High Pressure Liquid Chromatography (HPLC) Perkin Elmer, Flexar model, with column Pursuit C18 4.0x125 mm 5µm.

Phosphate buffer pH 8 was used (MORITA; ASSUMPÇÃO, 2007). All solutions were prepared with deionized water (> 18 MΩ cm, Milli-Q, Millipore). To ensure sterile conditions in liquid media, solid substrates the materials used are being autoclaved at 121 °C and 1.0 atm for 20 min before any experiment.

Non-contact mode topographic AFM images were obtained with a Thermo Microscope AutoProbe CP-Research. The ultra-low spring constant of its silicon nitride (Si_3N_4) cantilever (ca. 0.03 N m^{-1}), the use of small curvature tips (ca. 5 nm radius), and the scan rate of 1 to 4 Hz, allowed us to scan the of *Bacillus* structures without promoting any physical damage.

2.2 Cultivation of *Bacillus velezensis. Pre-inoculum*

A pre-inoculum of *Bacillus velezensis* 0G was cultivated in modified Landy medium (LANDY *et al.*, 1948). Following the proportion indicated on Table 1, a final volume of 2 mL was placed in 10 mL Erlenmeyers (covered with cotton, gauze, aluminum foil and autoclaved). Stock solutions were prepared in non-sterile medium and then -sterilized. Each Erlenmeyer received 500 µL of a glycerol stock of *Bacillus velezensis* OG, and then incubated with shaking (250 rpm) for 24h at 37 °C. The resulting broth was used as inoculum.

Table 1. Stock solutions for 10mL Landy's medium.

Components	**Concentration g/L**	**Volume (µL)**
Glycerol	375	160
Glucose	375	160
Arginine	100	100
Glutamic acid (fonte de N)	100	200
Yeast Extract	20	100
Micronutrient solution	30.1	100
KH_2PO_4	20	200
MOPS	600	100
Deionized water	1000	880

Source: Authors

2.3 Growth of *Bacillus velezensis OG* in base medium- parameter inoculum

The base medium, without potato peel, was prepared in a 250 mL Erlenmeyer and autoclaved. The proportion was (w/v) for 100 mL solution was: 0.76% peptone, 0.06% sodium chloride and 3% glucose, in sodium phosphate buffer, pH 8.0. Then 1 mL of pre-inoculum was inoculated into the medium. The Erlenmeyers with the inoculum solution, containing *Bacillus velezensis* were placed in the shaker at a temperature of 37 ° C and 250 rpm.

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2.4 Sweet Potato Peel Broth – parameter broth

The base medium inoculated with *B. velezensis* was mixed with the broth, a substrate made of sweet potato peel (0,12g/mL) in pH 8.0 phosphate buffer, enriched with 5mL of glycerol (40g L^{-1}) and 5 mL of the micronutrient solution [$(NH_4)_2HPO_4$ (0.39 g L^{-1}), Na_2HPO_4 (5.67g L^{-1}), KH_2PO_4 (4.08 g L^{-1}), $CaCl_2.2H_2O$ (0.001 g L^{-1}), $Mg_2SO_4.7H_2O$ (0.197 g L^{-1}), $Mn_2SO_4.H_2O$ (0.002 g L^{-1}) and $FeSO_4.7H_2O$ (0.015 g L^{-1})]), in different amounts, according to the experimental design.

2.5 Factorial design 2^3 with central and axial points for screening the best conditions for total lipopeptide production using *Bacillus velezensis* 0G by submerged fermentation

The factorial design of experiments 2^3 *with central and axial points* was performed. Table 2 presents the factors, or variables to be studied: amount of *broth*, amount of *inoculum* and *incubation time*. There are also levels that in the upper row of the table are coded from -1.68 to +1.68. In the table rows the values of these levels are decoded into experimental values.

Table 2. Levels and variables studied in factorial design 2^3 with central and axial points.

Variables \ Levels	**-1.682 (Axial point)**	**-1(Lower Level)**	**0(central point)**	**+1(Upper Level)**	**+1.682(Axial Point)**
Broth	43 mL	50 mL	60 mL	70 mL	77 mL
Amount of inoculum	6.4 mL	13.5 mL	20 mL	31.5 mL	33.6 mL
Incubation time	18 hours	24 hours	48 hours	72 hours	88 hours

Source: Authors

Table 3 shows the matrix signals for a 2^3 factorial design with center and axial points, where 19 trials were generated. The assays 1 to 14 were done in duplicate with their respective control (i.e. with all fermentation elements minus the inoculum addition). Assays 15-19 are the replicates of the central point.

Table 3. Signal level matrix for 2^3 factorial design with center and axial points.

Assay	Broth	Amount of inoculum	Incubation Time (hours)
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.682	0	0

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10	+1.682	0	0
11	0	-1.682	0
12	0	+1.682	0
13	0	0	-1.682
14	0	0	+1.682
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0

Source: Authors

The set of assays was performed in 3 batches, randomly chosen, in duplicate. After completion of the assigned *Bacillus* incubation time, the solutions were transferred to 50 mL Falcon® flasks and centrifuged at 9000 rpm for 10 min. The biomass was separated from the supernatant and stored. Supernatant were used for HPLC, emulsification and surface tension measurements.

2.6 Emulsification Index Measurements

Following the methodology of Cooper and Goldenberg (COOPER; GOLDENBERG, 1987) 6mL of toluene hydrocarbon and 4mL of supernatants from each assay were distributed in test tubes. Each tube was placed in vortex for 2 minutes. They were allowed to stand at room temperature and the emulsion´s height and total liquid height were measured after 24 hours from the moment of homogenization.

2.7 Determination of surface tension

For surface tension determination, the Cole-Parmer tensiometer, Surface Tensiomat® 21 was used. The equipment consists of a steel wire attached to a movable gear that enables its controlled twisting. At half the length of the wire is perpendicularly attached an aluminum rod that follows the twisting movement performed. At the other end of the rod is the measuring element. It is a platinum ring. Thus when used, with the application of a torsional force, the ring submerged in the liquid rises parallel to the liquid surface, until the meniscus rupture, indicating the value on display the surface tension.

2.8 HPLC measurements and data analysis

For HPCL measurements several runs were performed in a reverse phase C18 column, with isocratic mobile phase made of acetonitrile:water:trifluroacetic acid (80%:20%:0.1%). Total volume of 20mL was injected for each assay, keeping the supernatant sample: mobile phase proportion to 1:1. The run lasted 15 minutes: the first 10 minutes performed at 1 mL/min and the last five minutes at 1.5 mL/min.

HPLC curves were obtained using Chromera software. As the signal required treatment, baseline removal and peak area integration were performed using Origin™. Statistics were calculated using Excel (MS Office). Results analysis and response surface plots were built in Design Expert® version11 software.

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3 Results and Discussion

Usually, biosurfactant production is indirectly quantified. Methods based on measuring changes in the surface properties of biosurfactant water solutions have been validated and utilized. Thus surface tension measurements (SVENSSON; GUDMUNDSSON; ELIASSON, 1996), emulsification, foaming (PETKOVA *et al.*, 2020) and turbidometric methods (MUKHERJEE; DAS; SEN, 2009). However, these methods can only be used as semi-quantitative techniques. In an attempt to optimize direct biosurfactant analysis with HPLC, Biniarz made an interesting study employing solvent modification for the organic samples (BINIARZ; ŁUKASZEWICZ, 2017), which was adapted to this study. Following are the results with indirect and direct lipopeptide production quantification.

3.1. AFM topographic characterization image of *Bacillus velezensis.*

The growth of *Bacillus* was visualized by AFM measurements of residual biomass, as indicated in Figure 1.

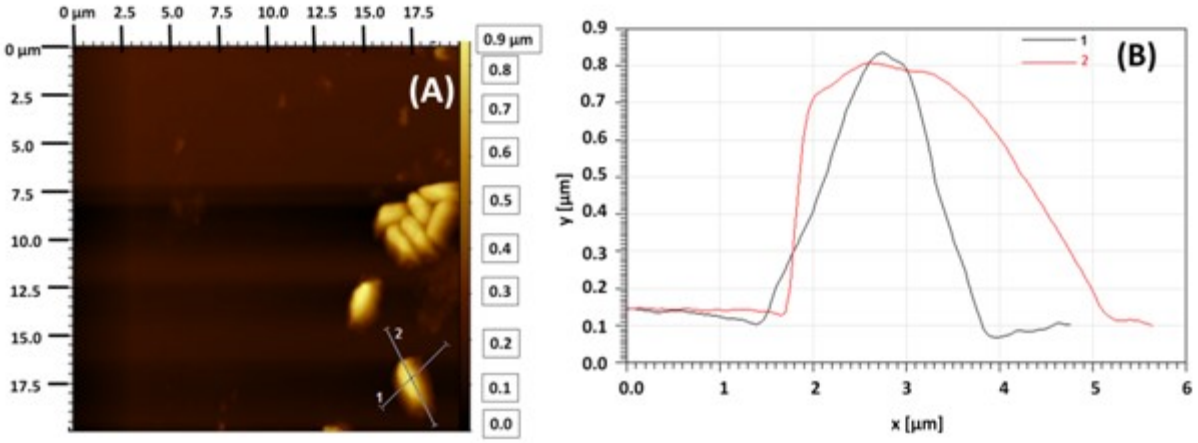

Figure 1: (A) (20 µm x 20 µm) Non-contact mode AFM topographic image of a group of *Bacillus velezensis* 0G on gold. **(B)** The maximum height measured for one unit is 0.7 µm and the FWHM for lines "1" and "2" are 1.30 µm and 2.50 µm, respectively.

The *Bacillus* unit observed on gold substrate has a maximum height of 0.7 µm and the FWHM for lines "1" and "2" are 1.30 µm and 2.50 µm, respectively.

3.2 Results of emulsification index (24h) and surface tension

Table 4 shows the 24-hour emulsification values (E24) and the surface tension of the duplicates of the 19 trials, central points and axial points of factorial design. Unfortunately the software could not build a reliable response surface, indicating that these indirect surfactin concentration measurements were not exact.

3.3 HPLC Analytical Curve and Sample Analysis.

The analytical curve was built for lipopeptide using Figure 2. It exhibits the absorbance profile (wavelength 512 nm) for several standard solutions (70% Surfactin), after removing the baseline from original signal.

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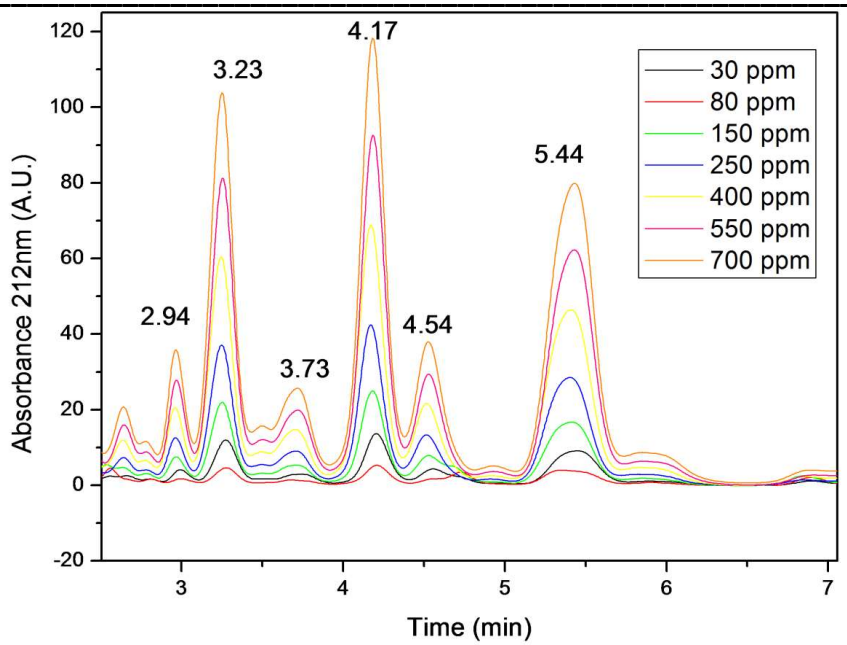

Figure 2. Graph absorbance (A.U) vs. Retention time (min) for several standard surfactin concentrations, varying from 30 ppm to 700 ppm (purity 70%).

There is a linear behavior of absorbance vs. retention times for t= 2.94 min, 3.23 min, 3.73 min, 4.17 min, 4.54 min and 5.44 min. But for the analyzed samples, there are contributions to total concentration only for the retention times of 2.94min and 3.23min, as the remaining peaks at longer retention times had no significant height, comparing to the peaks of standard surfactin used. The analytical curves for RT 2.94 min and RT 3.23 min are shown in figures 3 and 4, respectively:

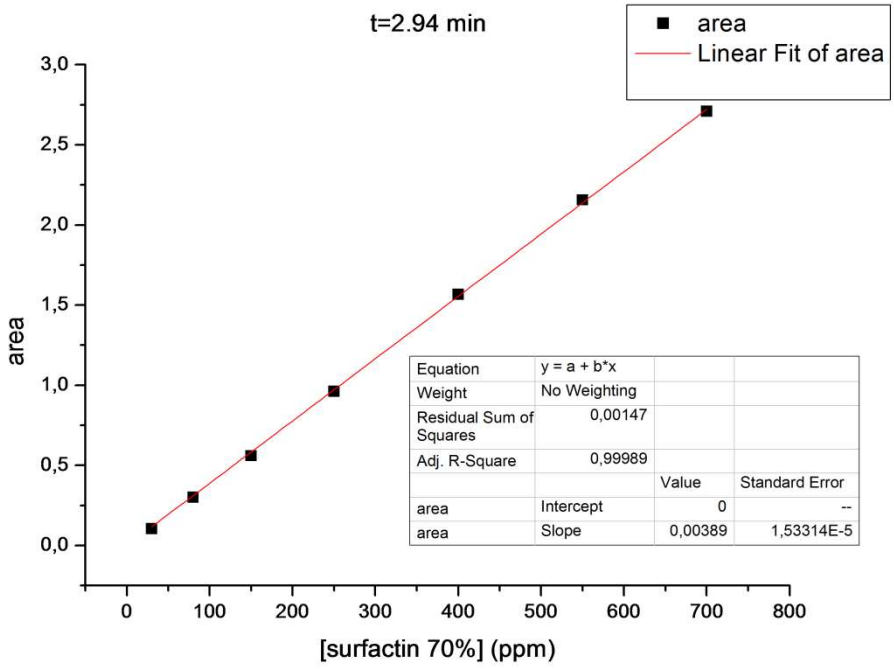

Figure 3. Analytical curve for time 2.94 min. The linear equation is Area = 0.00389 x Lipopeptide Concentration (ppm), R^2= 0.9989.

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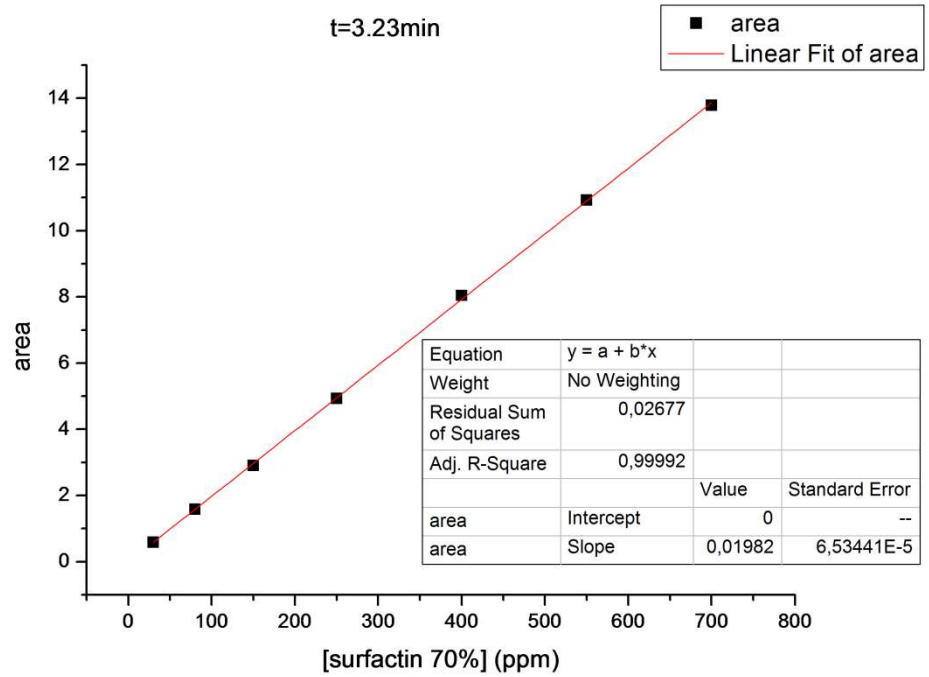

Figure 4. Analytical curve for time 3.23 min. The linear equation is Area = 0.01982 x Lipopeptide Concentration (ppm). $R^2 = 0.9992$.

Figure 5 shows the Absorbance (A.U.) vs. Retention time (min) for all samples. The peak area calculated for each sample was summed over all times, so the total lipopeptide production was obtained.

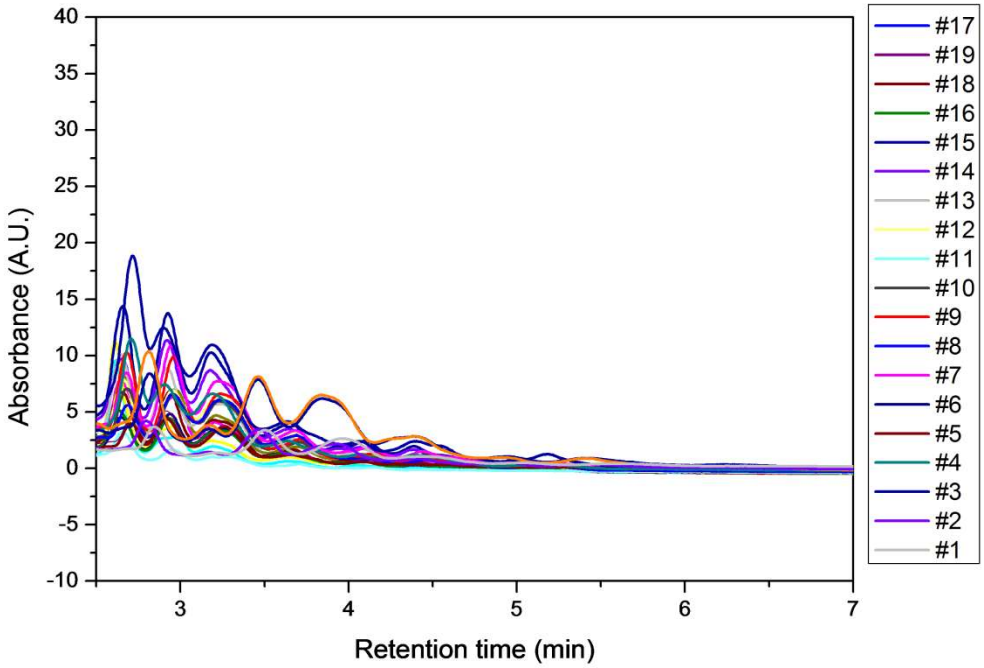

Figure 5. Absorbance (A.U.) vs. Retention time (min) for supernatant samples obtained from design of experiments.

Table 4 shows the calculated concentration for all samples through HPLC.

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Table 4. Results of surface tension, 24h emulsification index and concentration of surfactin produced.

#Assay	Parameter			Results		
	Broth	**Amount of inoculum**	**Incubation time**	**Emulsification Index E24 (%) – mean value**	**Surface Tension (mN/m)**	**Surfactin (mg/L)HPLC**
1	-1	-1	-1	6.55	47	190.06
				10.08	50	301.12
2	1	-1	-1	15.09	48.5	209.30
				14.81	50.5	273.92
3	-1	1	-1	9.09	47.75	418.30
				10.00	50.5	404.32
4	1	1	-1	20.00	47	120,40
				11,11	46,25	141,78
5	-1	-1	1	15,57	48	129,64
				13.64	46.5	135.75
6	1	-1	1	12.19	47.5	217.80
				9.43	50.25	153.40
7	-1	1	1	16.67	46	431.94
				22.45	48	507.64
8	1	1	1	15.45	48.25	213.18
				22.43	49.75	232.74
9	-1.682	0	0	17.59	47.75	402.06
				18.52	50	417.22
10	+1.682	0	0	13.91	47.5	218.98
				13.89	47	285.84
11	0	-1.682	0	10.99	50.75	56.44
				12.02	50.5	60.30
12	0	+1.682	0	12.96	48.75	217.7
				18.18	48.5	212.10
13	0	0	-1.682	17.74	49.75	348.38
				18.71	50	309.74
14	0	0	+1.682	11.01	48.5	411.74
				12.83	49.5	432.7
15	0	0	0	10.91	47	509
						517
16	0	0	0	13.89	47.5	162
						161
17	0	0	0	20.00	48.25	334
						310

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18	0	0	0	9.26	48	281 296
19	0	0	0	12.83	47.75	192 183

Source: Authors

3.4 Response Surface

The appropriateness of a given experimental technique for an application of interest can be evaluated by multivariate analysis, as it is a powerful tool to turn data into information (BEEBE; KOWALSKI, 1987). In this case, the production of lipopeptides, mostly surfactin, evaluated by both indirect and direct concentration measurements gave us a surprising result for the indirect concentration measurement techniques: both surface tension and emulsification results could not be used for response surface modeling. Those analyses generated non-reproducible results. Surface tension gave no trends for an optimum cultivation, while emulsification, being performed by observing the foam height, presented some poor results for quantitative discussions. A possible explanation for such difficulty is given by Petkova *et al*. Both foaming and emulsification process involving ionic surfactants are very sensitive to lower surface coverage, and simpler characteristics, such as total surfactant concentration, which do not account for the dynamic surface properties of the surfactants, cannot be used to explain the results of foaming or emulsification (PETKOVA *et al.*, 2020).

On the other hand, the direct concentration measurement by HPLC could provide enough information for the response surface plot. It was modeled to find the best conditions for fermentation, that is, the best amount of inoculum, broth and incubation time in order to provide the higher total lipopeptide production. A Quartic model could be calculated, as shown in table 5.

Table 5: ANOVA for Quartic model. Response1: Surfactin Concentration.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.764E+05	14	19744.78	146.55	<0.0001	significant
A-Broth	15045.92	1	15045.92	111.67	0.0001	
B-Amount of inoculum	20944.90	1	20944.90	155.45	<0.0001	
C- Incubation time	6584.93	1	6584.93	48.87	0.0009	
AB	47232.01	1	47232.01	350.56	<0.0001	
AC	800.80	1	800.80	5.94	0.0588	
BC	13688.51	1	13688.51	101.60	0.0002	
A^2	4282.68	1	4282.68	31.79	0.0024	
B^2	48501.05	1	48501.05	359.98	<0.0001	
C^2	13301.51	1	13301.51	98.72	0.0002	
ABC	466.35	1	466.35	3.46	0.1219	
A^2B	463.65	1	463.65	3.44	0.1227	
A^2C	4750.64	1	4750.64	35.26	0.0019	

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AB^2	951.99	1	951.99	7.07	0.0450
AC^2	0.0000	0			
B^2C	0.0000	0			
BC^2	0.0000	0			
A^3	0.0000	0			
B^3	0.0000	0			
C^3	0.0000	0			
A^2B^2	1648.52	1	1648.52	12.24	0.0173
A^2BC	0.0000	0			
A^2C^2	0.0000	0			
AB^2C	0.0000	0			
ABC^2	0.0000	0			
B^2C^2	0.0000	0			
A^3B	0.0000	0			
A^3C	0.0000	0			
AB^3	0.0000	0			
AC^3	0.0000	0			
B^3C	0.0000	0			
BC^3	0.0000	0			
A^2B^2	1648.52	1	1648.52	12.24	0.0173
A^2BC	0.0000	0			
A^2C^2	0.0000	0			
AB^2C	0.0000	0			
ABC^2	0.0000	0			
B^2C^2	0.0000	0			
A^3B	0.0000	0			
A^3C	0.0000	0			
AB^3	0.0000	0			
AC^3	0.0000	0			
B^3C	0.0000	0			
BC^3	0.0000	0			
A^4	0.0000	0			
B^4	0.0000	0			
C^4	0.0000	0			
Pure Error	673.67	5	134.73		
Cor Total	2.771E+05	19			

Source: Authors (2019)

Figures 6 to 9 show the response surfaces (2D and 3D) plots obtained in this study, based on HPLC data.

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DOI: 10.4025/revtecnol.v30i1.55243

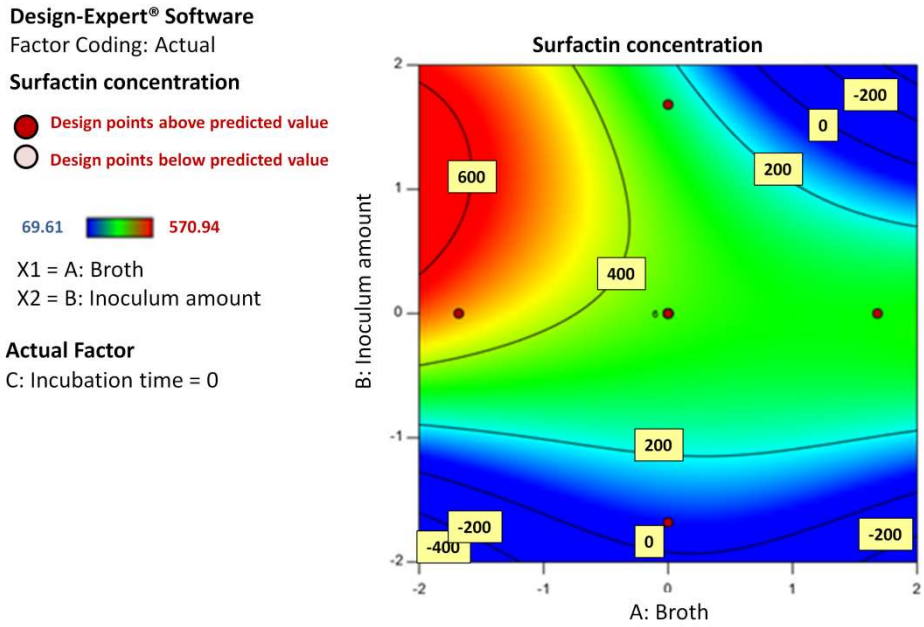

Figure 6. Response surface between Broth (X1) and Inoculum amount (X2). Red area shows the ideal region around -1.32 (X1) and +1.23 (X2).

Figure 6 shows the response surface between "Amount of inoculum" and "quantity of Broth". In this graph it is possible to observe trends in optimum lipopeptide production Warmer colors (orange, red) indicate higher lipopeptide production. Considering this, in this graph was pointed as being an ideal the region around -1.32 (X1) and +1.23 (X2).

Figure 7 shows the response surface between incubation time and amount of broth. Surprisingly the optimum condition for total lipopeptide concentration is given for both A) small incubation time and small amount of broth or B) great incubation time and greater amount of broth. But due to data given from figure 6, only the option A) can be considered -1.64 (X3).

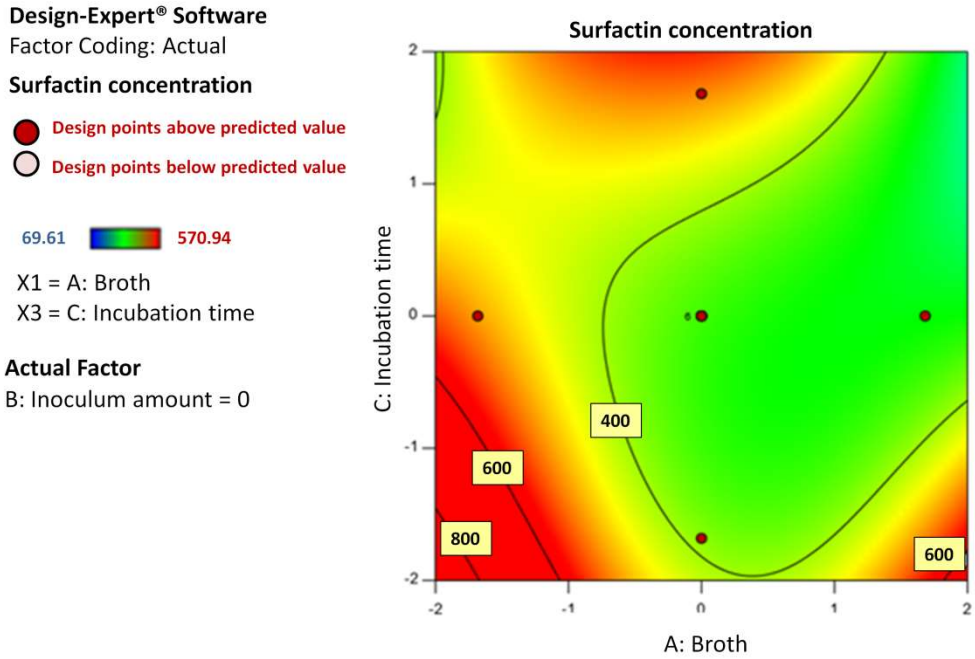

Figure 7. Response surface between Broth (X1) and Incubation time (X3). The best level for these combined factors indicates X1 of -1.51 and X3 of -1.64.

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The 3D plots are given in figure 8 and 9. The 3D response curve for the Amount of inoculum vs. quantity of Broth can be compared to Figure 9, showing the 3D response curve for the incubation time and Broth. Incubation time is an important parameter, but as the concavity of the surface in figure 9 is smaller than the one observed in Figure 8, so amount of inoculum plays a major role in the fermentation process.

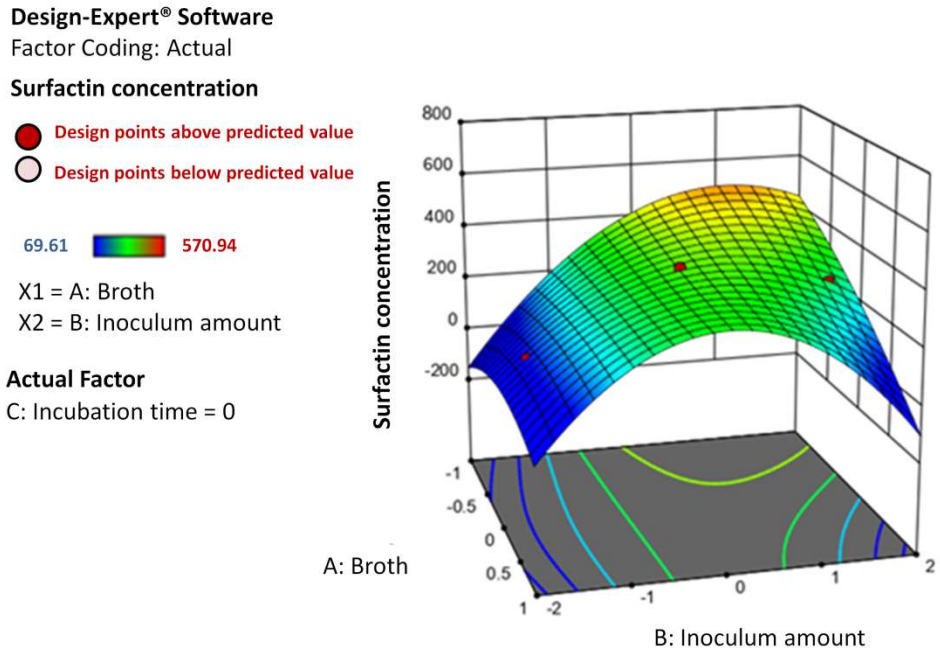

Figure 8. 3D response surface between broth (X1) and Inoculum amount (X2). The best levels for these combined factors -1.32 (X1) and +1.23 (X2).

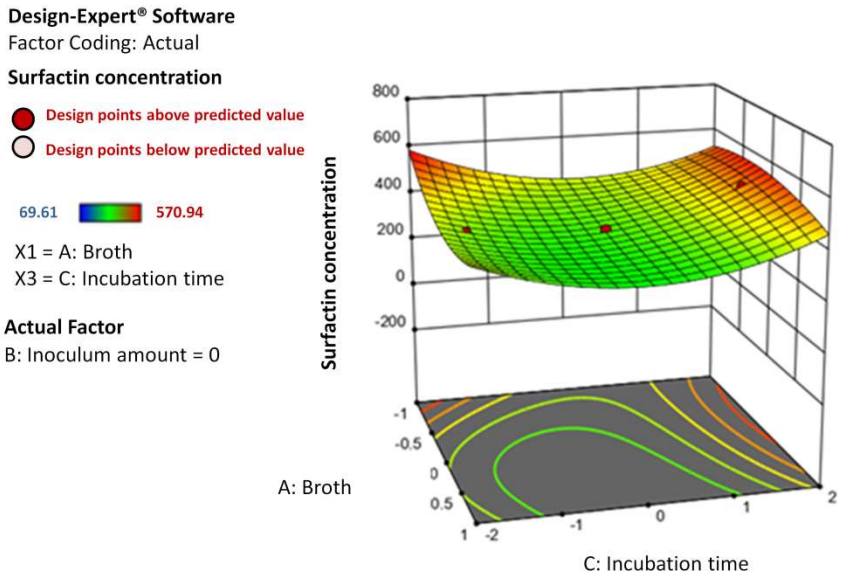

Figure 9. 3D response surface between Broth (X1) and Incubation time (X3). The best levels for these combined factors were -1.51 (X1) and -1.64 (X3).

A combined analysis of the 2 factor graphs and the 3D plots on each response surface, made it possible to identify some trends for best parameter levels: for the amount of broth near -1.30, for the inoculum amount around +1.23 and incubation time about -1.64. Decoding these factor values into experimental values, it can be concluded that the best parameters for

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the range studied in this work for the production of total lipopeptide from *Bacillus velezensis* are: 46 mL of broth (-1.30) with 32mL inoculum (+1.23) and 24-hour incubation time (-1.64). This result shows that aeration plays a main role on *Bacillus* cultivation, thus corroborating the study of Hmidet *et al*.(HMIDET *et al.*, 2017).

From the previous data, one can disguise that the challenge to optimize conditions for *Bacillus* biotechnological production of lipopeptide is to find a proper experimental technique to determine reliable lipopeptide concentration for each assay. In our study, surface tension and emulsification E24 were not exact and poorly qualitative (only indicating some surfactant activity). For modeling reliable values, we needed HPLC technique, with is sophisticated and more expensive. Same conclusions were addressed by Biniarz and Lukaszewicz (BINIARZ; ŁUKASZEWICZ, 2017). Further studies with colorimetric assays should be considered.

4. Conclusions

By using multivariate optimization it was possible to study the variation of different factors simultaneously to optimize *Bacillus velezensis* 0G submerged fermentation. The three parameters studied were inoculum amount, broth volume and incubation time, optimized to increase surfactin production. The response surface trends demonstrate a maximum biosurfactant production at inoculum volume of 32 mL; combined broth volume of 46 mL, and incubation time of 24h. The maximum amount of surfactin produced was 500 mg/L. From all experimental methods to determine the total lipopeptide concentration, HPLC measurements were accurate and reproducible enough to assure a semi-quantitative analysis, but surface tension and emulsification index analysis could be used for qualitative rather than quantitative analysis. Sustainability was obtained in submerged fermentation employing organic residues (sweet potato peel) as one of the carbon sources.

Conflict of interest statement

All authors declare they have no conflict of interests.

Acknowledgements

The authors would like to thank Mr. J. R. de Castro for technical support. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. – Scholarship PNPD (Gomes, W.E.) and PUC-Campinas provided scientific initiation scholarship to B.M.R. Part of the equipment used in this work was acquired by a grant from Fapesp (2013/20570-6).

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