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Improvement of lipopeptides production by *Bacillus methylotrophicus* DCS1 using Plackett-Burman design and response surface methodology

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Abstract
Lipopeptides from *Bacillus methylotrophicus* DCS1 are endowed with interesting properties. The composition of production medium and culture conditions were optimized by studying the effect of 14 variables in a 23 run Plackett-Burman design (PBD) to screen for key factors with the aim of improving lipopeptides yield and surface activity with low production costs. In PBD, four significant factors (Urea, $CaCl_2$, gruel and temperature) affecting the production, were choosed for further optimization and constructed via Box-Behnken design (BBD). Enhanced lipopeptides production was carried out using gruel as carbon source at a concentration of 40 g L^{-1} and urea as nitrogen source at a concentration of 5.5 g L^{-1}. The optimal conditions were 33 °C temperature, pH 7.5, an agitation of 175 rpm and 2.5% inoculum size. Utilizing the predicted optimized conditions, the maximum lipopeptides yield of 4.7 g L^{-1} was reached which accord well with the predicted value.

Keywords: Lipopeptides, Medium optimization, Plackett-Burman design, Box-Behnken design, Production yield, Surface tension

1. Introduction
Biosurfactants are amphiphilic biomolecules produced by various microorganisms [1]. Interest in biosurfactant production has noticeably raised over the past decade, although extensive production was impossible due to high total costs and low production yields. The limited use of biosurfactants in several biotechnology fields such as pharmaceutical, food industry and environmental field is due to their production in high cost culture media and using expensive substrates such as forms of pure sugars and amino acids. However, from an economic point of view, biosurfactants are uncompetitive with the synthetic surfactants. They can only substitute chemical surfactants if the cost of the culture medium compounds and the process is minimal [2]. In spite of the benefits and pertinent applicability of these bioactive compounds, the success of biosurfactants rely on the use of economical substrates, which account for the 10-30% of the overall costs [3, 4].
The key factor affecting the success of biosurfactant production is the establishment of an economical process that exploits low-cost materials and yields high productivity [5, 6]. The economic production of biosurfactants at large scale for new applications remains a challenge. In recent years, there has been an increase of agro-industrial by-products and agricultural wastes use as substrates for the economic biosurfactants synthesis, which would reduce pollution and entire costs [7, 8]. The food industries engender large quantities of organic residues that can be utilized for biosurfactants production. The benefits of using agro-industrial wastes are the reduction of their treatment cost and the benefit from the selling of the biosurfactant [9-13].
The industrial transformation of renewable resources to beneficial compounds got a lot of attention from the environmental standpoint. The actually available resources are oils, which are generated from oil processing and refinery at broad scale, such us lard, marine oils, soap stock and free fatty acids from the extraction of oil from seeds. A variety of by-products and organic wastes from agriculture and related industries like cellulose and lignocellulose could be utilized as substrates for biosurfactant synthesis [14].
The structural features and functional properties of the biosurfactant not only depends on the producer strain but also on the culture conditions, thus, the nature of the carbon and nitrogen

sources, C/N ratio and physico-chemical parameters such as temperature, pH and aeration affect the amount and the nature of biosurfactant synthesized [15]. Many techniques such as Response Surface Methodology (RSM) and various statistical approaches have been efficiently utilized in many studies to reduce the cost and time consumed in producing biosurfactant [16, 17].

The purpose of this study is to identify and optimize the significant parameters that affect the efficacity of lipopeptides DCS1 production in terms of high yield and low production costs. In this study, a new economic culture medium was elaborated using Plackett-Burman design and response surface methodology.

2. Materials and methods

2.1. Biosurfactant producing strain

B. methylotrophicus DCS1 strain was used in the present work as a biosurfactant producer; it was isolated from hydrocarbon contaminated soil in Sfax city, Tunisia. The strain was cultivated in Luria Bertani medium as described in our previous work [18].

2.2. Preliminary optimization of carbon and nitrogen sources

The carbon and nitrogen sources optimization was realized in a run of experiments changing one variable at a time, keeping other factors fixed at a specific set of conditions, aiming to obtain high lipopeptides productivity. The carbon sources used at a concentration of 10 g L^{-1} were: gruel, soluble starch, soybean meal, cuttlefish waste flour, head and viscera of sardinella flour, octopus waste flour, "belle de nuit" tuber flour, shrimp waste flour, barley flour, potato peelings, tuber flour, crab waste flour, triggerfish viscera flour and smooth emissole viscera flour, without a nitrogen source. Initial conditions: 1% (w/v) carbon substrate concentration; batch fermentation conditions: 35 mL of mineral salt medium with the following composition: 0.14 g L^{-1} KH_2PO_4, 2.0 g L^{-1} Na_2HPO_4, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 40 mg L^{-1} $CaCl_2$, 20 mg L^{-1} $FeSO_4$ $7H_2O$, 1.2 mg L^{-1} $MnSO_4$ H_2O, 1 mg L^{-1} $CuSO_4$ $5H_2O$, 2.32 mg L^{-1} $ZnSO_4$. Minerals and trace elements concentrations are chosen by referring to the literature. Physico-chemical parameters were pH 7.0, 150 rpm agitation and 72 h incubation time.

For determining the adequate concentration of the best carbon source, different concentrations were assessed (5, 10, 15, 20, 25 and 30 g L^{-1}), and for assessment of the most suitable nitrogen source for the production of biosurfactants, ammonium sulfate $(NH_4)_2SO_4$, ammonium chloride (NH_4Cl), urea ($CO(NH_2)_2$), yeast extract, casein peptone, sodium nitrate ($NaNO_3$) and triggerfish waste flour were used at a concentration of (1 g L^{-1}) with the optimum nature and concentration of carbon source. The effect of the concentration of the most appropriate nitrogen source was evaluated; the concentrations tested are 0, 1, 2, 3, 5, 7 and 10 g L^{-1}).

The composition of the culture medium described above was optimized in order to improve lipopeptides DCS1 yield and surface activity.

2.3. Measurement of surface tension

Surface tension measurements of the cell-free culture broth supernatants were carried out as described in our previous work [19].

2.4. Lipopeptides recovery

The lipopeptides recovery from culture media was carried out as described in our previous work [18]. Lipopeptides recovered were freeze dried and weighed.

2.5. Plackett-Burman Design (PBD)

For a process, the experimental designs are used to screen the parameters. A screening study can be described as a step allowing to quickly identify, in a large number of factors, those which are effectively influencing a process in a predetermined experimental domain. The well-known screening experiment designs are Hadamard [20] and Plackett-Burman, for which the number of simulations is close to the number of factors studied. These experimental designs are most often saturated and the mathematical model is without interactions [21].

PBD use orthogonal matrix pre-defined as a matrix multiple of 4 (8, 12, 16, 20,…). A matrix with N lines allows to study N-1 factors. In our study, we have 14 factors, so we have to use a matrix with minimum 15 lines. However 15 is not defined as a matrix in the PBD, therefore we have to choose the nearest matrix which is 20 that allows to study 19 factors. We have 5 fictive or dummy variables (15-19) and this matrix is with 3 center points to verify the reproducibility of the experience, so the matrix is with 23 runs.

In this work, 14 factors namely, gruel, urea, KH_2PO_4, $MgSO_4$, Na_2HPO_4, $CaCl_2$, $FeSO_4$, $MnSO_4$, $CuSO_4$ and $ZnSO_4$ concentrations, initial pH, agitation, temperature and inoculum size, were screened for their effects on reducing surface tension of the culture medium and on the yield of lipopeptides production. The ranges of input parameters to perform the experiments were fixed based on the literary data and on the preliminary optimization (single parameter optimization). For each factor, three different coded levels corresponded to low (-1), intermediate (0) and high (+1) levels. Table 1 presents the selected factors and their levels for experimental design.

In Plackett-Burman analysis, a total of 23 combinations of broth compounds and physico-chemical conditions were generated by the Design Expert Software (version 11.0), as represented in Table 2, to regress analysis of the chosen variables, and all these experiments/set-ups were realized in 250 mL conical flask containing 35 mL of sterile modified medium. The surface tension was measured and the crude lipopeptides yield has been determined for each experiment. Plackett-Burman design results gotten from different combinations of the above 14 variables were used to build a Pareto Chart in order to determine the significant variables to test them further in the Box-Bhenken analysis for the lipopeptides production.

2.6. Box-Behnken Design (BBD) optimization

BBD is a subset of RSM; it is an efficient statistical technique, used for analyzing process parameters, modeling and optimization. BBD establish relationship between multiple process outputs (Y) and many operating conditions (x_1, x_2, …., x_n) by using correlation expressed by the following equation: $\hat{Y} = f(x_1, x_2, …., x_n) + e = Y + e$

with $\hat{Y}$: predicted/theoretical calculated response, Y: experimental response and e: error

The relationship among responses Y1 (the surface tension, mN m^{-1}), Y2 (the lipopeptides yield, g L^{-1}) and the

independent variables can be estimated by the following quadratic model:

$$\hat{Y} = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i,j=1}^{n} \beta_{ij} x_i x_j$$

where $\hat{Y}$: is the predicted response, β_0 : the intercept term or the model constant, x_i and x_j : the input variables or the individual effects, β_i : The linear effects or the regression coefficient, β_{ii} : the squared effects and β_{ij} : is the interaction term, $x_i x_j$: the interaction effect, x_i^2 : the quadratic effect.

A four-factor Box-Behnken design was employed to optimize lipopeptides DCS1 production. The matrix is composed of a total 27 experiments using independent factors such as gruel concentration (A), urea concentration (B), temperature (C) and $CaCl_2$ concentration (D).

3. Results and discussion

A promising biosurfactants production by microorganisms depends considerably on the use of an inexpensive and abundant feedstock. The target in the commercial production of biosurfactants can be described as "optimum quality and quantity at minimum cost" [5]. It is very important to investigate the medium composition and growth cultivation conditions due to their influence on the type and concentration of the biosurfactants produced [22]. In this regard, the effect of various compounds of an economic culture medium and the effect of the physico-chemical conditions including temperature, agitation and pH were evaluated to fix the optimal conditions for a maximum lipopeptides production by *B. methylotrophicus* DCS1. Production was tracked by measuring the surface tension of the cell-free broth and determination of lipopeptides yield.

3.1. Preliminary optimization study

The influence of carbon and nitrogen sources on the surface tension and yield of production were investigated and changes in the pH values were determined (Fig. 1).

3.1.1. Optimization of the carbon source

The use of economic carbon sources, like food industry and agriculture by-products or waste, to synthesize biosurfactants appears to be an attractive and inexpensive alternative [14]. For the purpose of reducing the production cost of lipopeptides, 14 different carbon sources which are organic wastes and residues were studied for their effectiveness on lipopeptides DCS1 production.

The type of carbon source influenced the surface tension reduction and the lipopeptides yield. The surface tension measurements differed according to the compound used; gruel was the greatest substrate in reduction of surface tension from its initial value of 57 mN m^{-1} (uninoculated culture medium) to 29.6 mN m^{-1} with a reduction percentage of almost 40%. The second substrate, which reduces importantly the surface tension, is barley flour with a reduction percentage of almost 35% (from 53 to 34.5 mN m^{-1}). Geetha *et al*. [23]. and Hippolyte *et al*. [24]. reported that bacteria of the genus *Bacillus*, *Pseudomonas*, *Candida*, etc. are used for the biosurfactant synthesis from agro-industrial residues such as date molasses, cassava residues, orange peel, sugarcane bagasse, corn steep liquor, etc.

B. methylotrophicus DCS1 produces lipopeptides with different yields depending on the carbon source, but the yield obtained with each substrate is weak and less than 1 g L^{-1}.

The results obtained testing different gruel concentrations (from 5 to 30 g L^{-1}) show that lipopeptides yield increases with increasing concentration and the surface tension remains constant (30.5 mN m^{-1}) from 15 g L^{-1} gruel concentration (Fig. 1a). The gruel concentration selected to assess the effect of various nitrogen sources is of 20 g L^{-1} representing a good lipopeptides yield and an important reducing of surface tension.

3.1.2. Effect of nitrogen sources on lipopeptides synthesis

It has been proved that nitrogen sources play an essential function in the synthesis of biosurfactants by microorganisms [25]. From the best gruel concentration (20 g L^{-1}), the effect of the nitrogen source was tested for the most frequently used inorganic nitrogen sources.

It was found that the nature of nitrogen source influenced the surface tension and lipopeptides production by *B. methylotrophicus* DCS1 strain. Among the four nitrogen sources tested, it was observed that urea was the most adequate nitrogen sources in terms of both minimal surface tension (32.9 mN m^{-1}) and yield (1.14 g L^{-1}), followed by sodium nitrate ($NaNO_3$) with a surface tension of 32.4 mN m^{-1} and a yield of 0.85 g L^{-1} (Fig. 1b). Instead, there is no significant difference between sodium nitrate and urea; they are good sources of nitrogen for biosurfactants synthesis. Our findings are in agreement with those described by Elazzazy *et al*. [26] who showed that urea and $NaNO_3$ were the most suitable nitrogen source for biosurfactants production by *Virgibacillus salarius* (KSA-T) isolate.

Whereas, ammonium sulfate $(NH_4)_2SO_4$ did not show a good results with reduction of surface tension from 57 to 43.3 mN m^{-1} and with a yield of 0.17 g L^{-1} (Fig. 1b). This result is in accordance with that of Makkar and Cameotra[27] who reported that certain *Bacillus* strains did not use $(NH_4)_2SO_4$ for biosurfactants production or bacterium growth; although they are able to use potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$) or ammonium nitrate (NH_4NO_3).

In addition, ammonium chloride (NH_4Cl) was not an adequate nitrogen source for lipopeptides DCS1 production, with reduction of surface tension to a value of 45.3 mN m^{-1} and with a yield of 0.36 g L^{-1} (Fig. 1b). This result is in agreement with that of Elazzazy *et al*. [25] who demonstrated that NH_4Cl led to a substantial increase in the strain growth but not for biosurfactant production. However, our finding is in disagreement with that published by Jadhav *et al*. [28] who stated that NH_4Cl is the most suitable nitrogen source for biosurfactants production by *Oceanobacillus* sp. BRI 10.

Different concentrations of urea have been tested (Fig. 1c) to get an idea about the appropriate concentration range. Thus, urea was choosed for further experiments, taking into consideration its efficiency, availability and low cost. To conclude, the preliminary optimization study showed that gruel and urea are the best carbon and nitrogen sources, respectively and allowed us to choose the levels of the independent variables.

3.2. Determination of significant factors by PBD

The Pareto Chart in the form of a histogram are presented to determine the effects of variables, where absolute values are arranged from high to low (Fig. 2). The horizontal line in the Pareto Chart designates the minimum statistically

significance effect magnitude for 5% significance level, whereas the vertical column lengths are correlating to the degree of significance for each effect and any effect that surpasses the horizontal line is considered significant. Urea and $CaCl_2$ concentrations have a positive effect on surface tension, while gruel concentration has a negative effect. For the lipopeptide yield, the gruel concentration has a significant positive effect and the temperature has a negative effect (Fig. 2). All the other factors are non-significant, their effect on responses and the fixed values that will be used in BBD are presented in Table 3.

3.3. BBD optimization

3.3.1. Statistical analysis and model development

To study the effect of the significant factors (A: gruel, B: urea, C: temperature and D: $CaCl_2$ concentration) as well as the interactions between them, we established a BBD. The matrix and the corresponding system obtained responses are displayed in Table 4. Based on experimental data, the results were also analyzed through Analysis of Variance (ANOVA). The ANOVA analysis was performed to determine statistical significance and relevance of the model equation. The ANOVA analysis shows that the factors don't act significantly on the surface tension, so we will not take into account the optimization of surface tension. Whereas for lipopeptide yield, the ANOVA analysis shows that the model is valid and highly significant with a low p-value (< 0.05) and a high model F-value (21.44).

The experimental and theoretical values were supposed to have very satisfied correlation, thereby R^2 concurred well with R^2_{adj}. In fact, the coefficient of determination value (R^2 = 88.8%) signified that only 11% of the total variations were not explained by the model. While, the adjusted determination coefficient (R^2_{Adj} = 84.6%) was also very high to confirm the high significance of the model. Vigneshwaran *et al.* [29] reported a comparable result with R^2 value of 94.86% for the investigation of biosurfactant synthesis by *Brevibacillus* sp. AVN13.

Figure 3 illustrates the scattering between the data points and the diagonal line where an appropriate agreement and a correlation was settled between the actual data of the yield and the predicted values obtained by the model. Hence, the theoretical/mathematical model was confirmed to navigate in the defined space by BBD.

The following quadratic equation was established by multiple regression analysis on experimental results using Design Expert software (version 11.0).

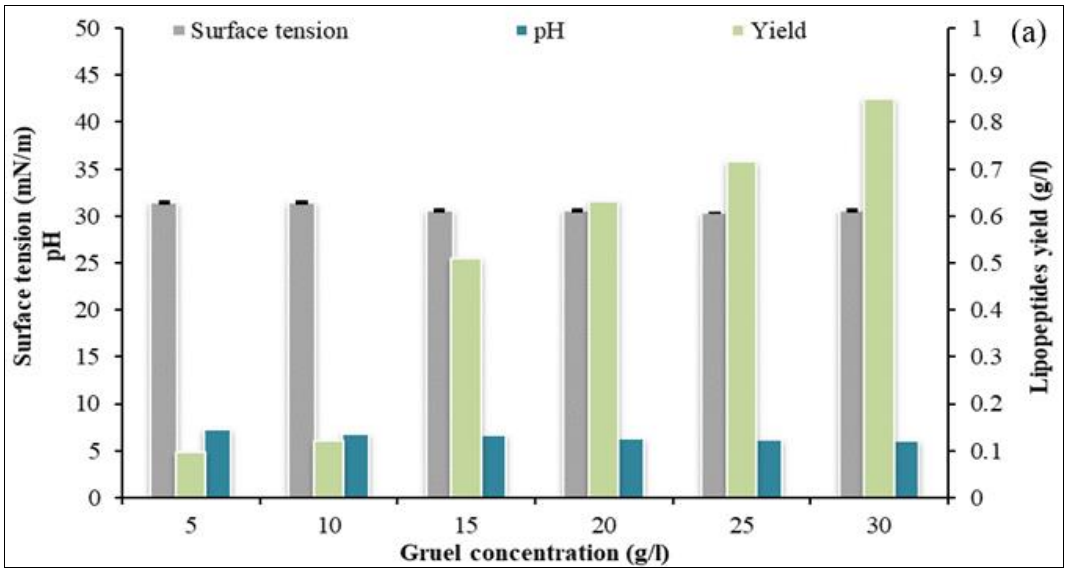
The final equation of the inverse of yield in terms of coded factors :

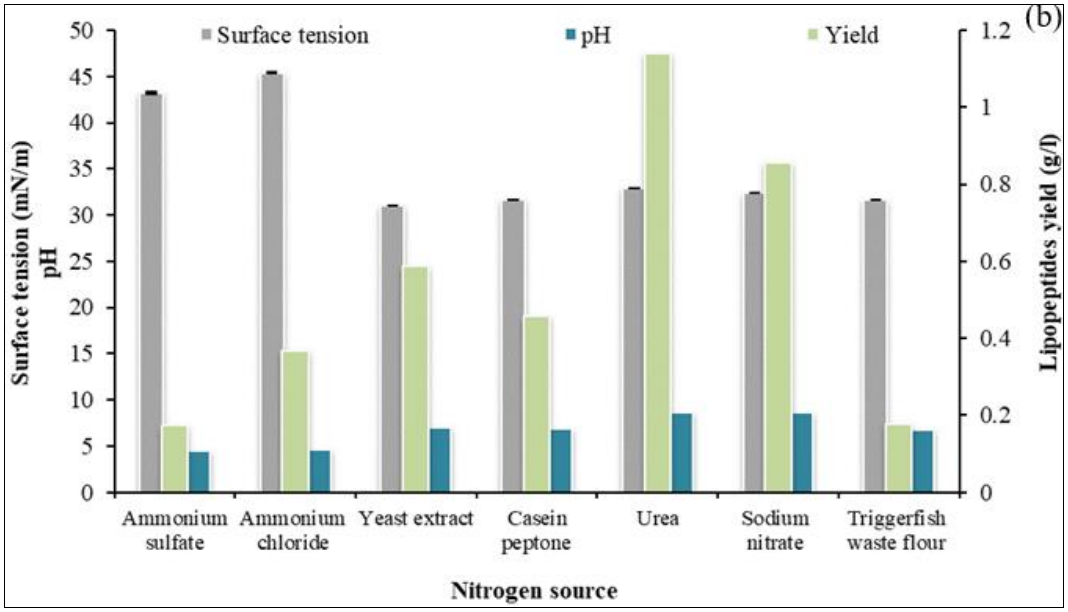
$$1.0/(\text{Yield}) = +0.402 - 5.255A + 0.750B + 2.143C - 2.190AB - 2.773AC + 5.367A^2 + 1{,}995C^2$$

The synergistic and antagonistic effect of the interaction terms were disclosed using positive and negative coefficients in the regression equation, respectively.

3.3.2. Interaction effects of input parameters on output responses

In RSM, the interaction effects of input parameters on output response were evaluated using the three-dimensional response surface plots. The interaction effect of gruel concentration and temperature on lipopeptides DCS1 yield is illustrated in Fig. 4 and the interaction effect of gruel and urea concentrations is shown in Fig. 5. The 3-D response surface plots show antagonistic effects between the different factors with a positive quadratic effect for gruel, a slightly negative quadratic effect for temperature and a slightly negative linear effect for urea (Fig. 4 and Fig. 5). The optimal composition of the culture medium and conditions were 40 g L^{-1} gruel concentration, 5.5 g L^{-1} urea concentration, a temperature of 33 °C and 0 mg L^{-1} $CaCl_2$ concentration, with a prediction of 4.715 g L^{-1} lipopeptides. Validation of the predicted results was executed by using the optimized conditions in experiments. The observed experimental lipopeptides yield was 4.717 g L^{-1} and the surface tension was reduced up to 32 mN m^{-1} from its initial value of 57 mN m^{-1}. The actual work allowed us to determine the culture components and physico-chemical conditions that lead to a four-fold increase in lipopeptides yield.





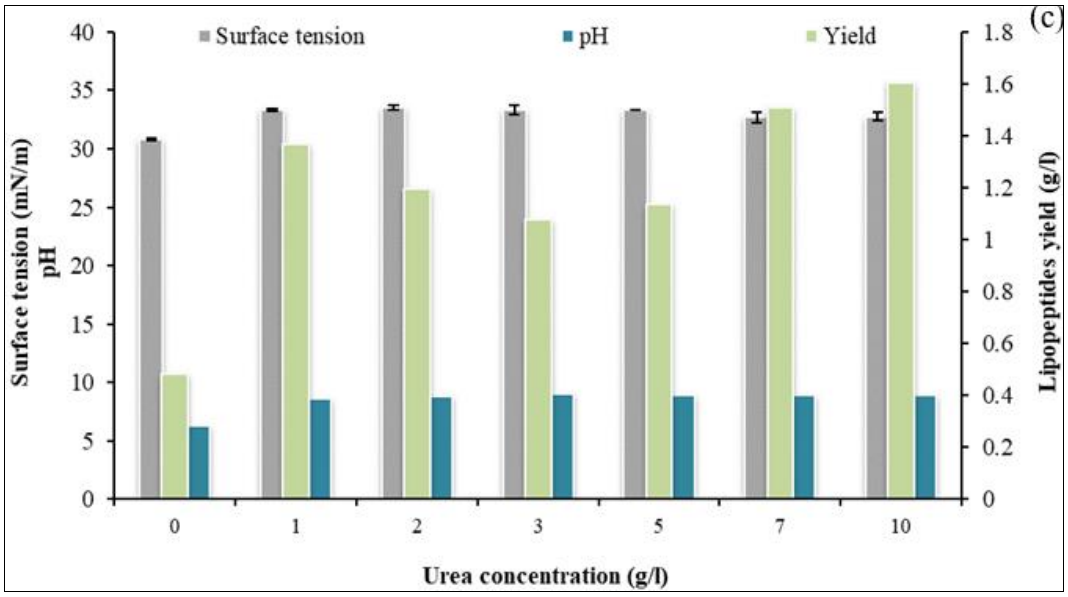
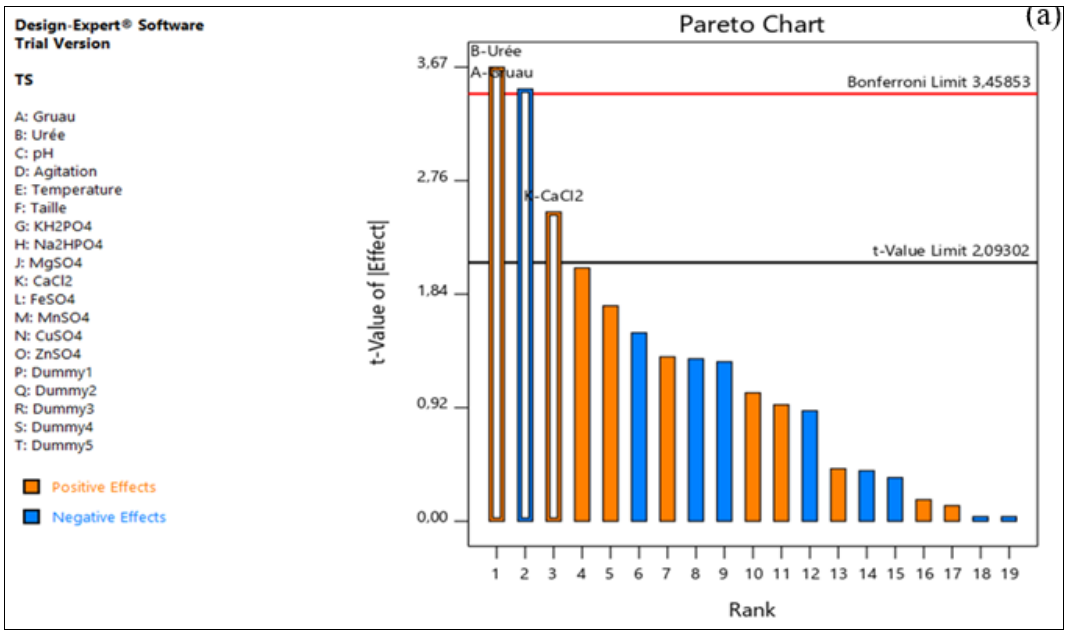


Fig 1: Effect of gruel concentration (a) nitrogen sources (b) and urea concentration (c) on lipopeptides DCS1 production



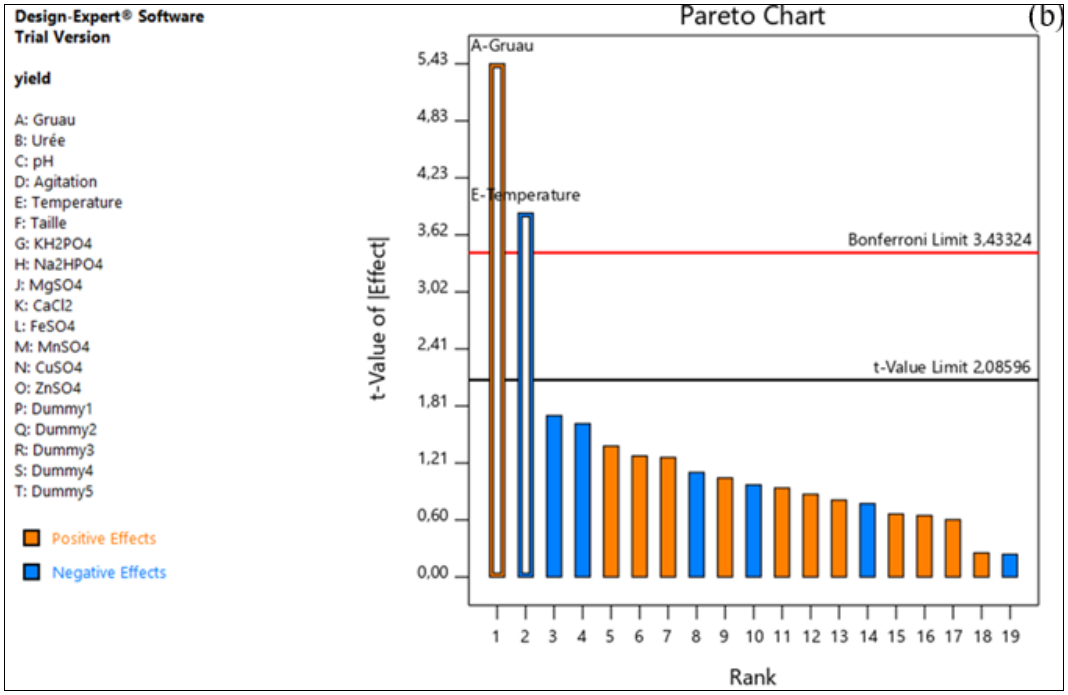


Fig 2: Pareto Chart of surface tension (a) and yield (b) responses

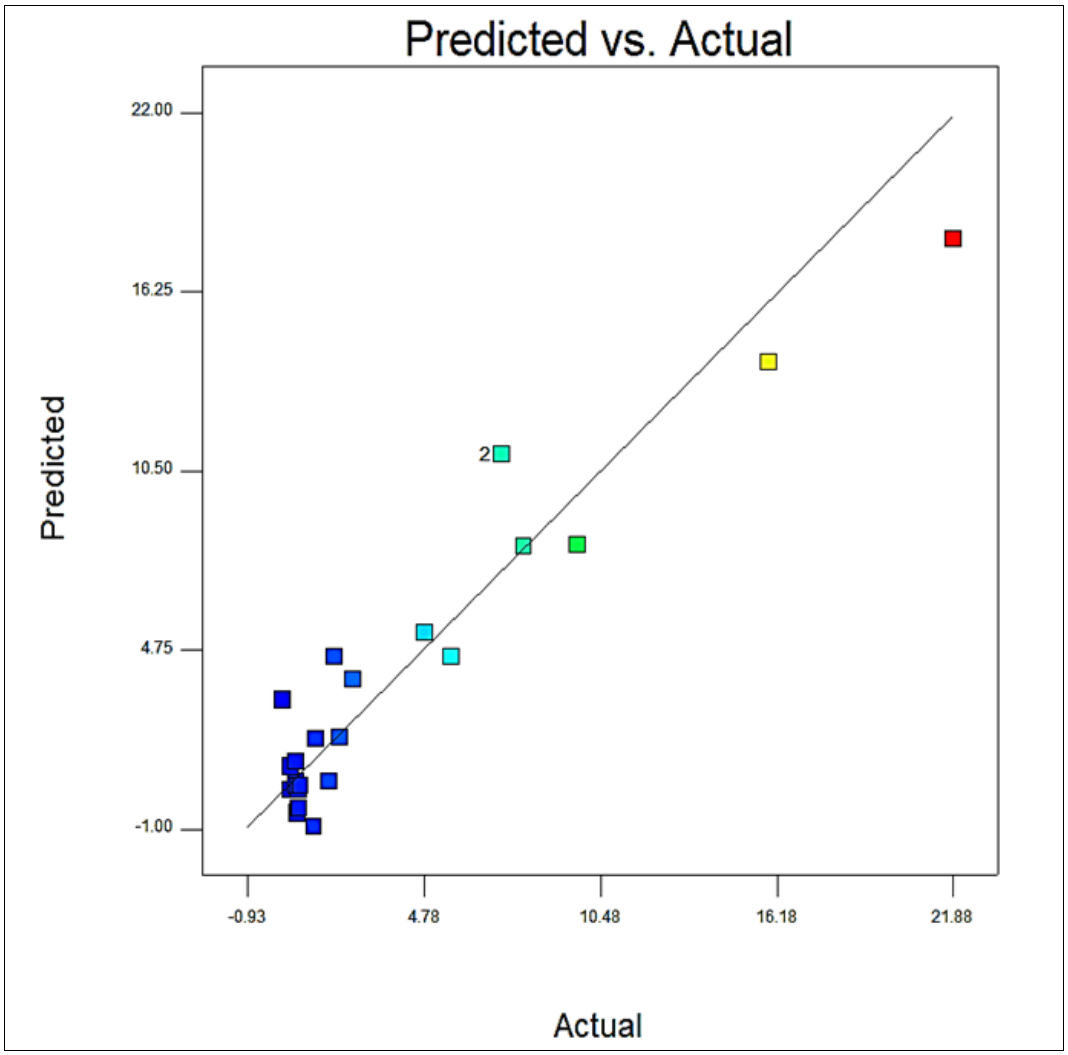


Fig 3: Regression plots showing performances of RSM model

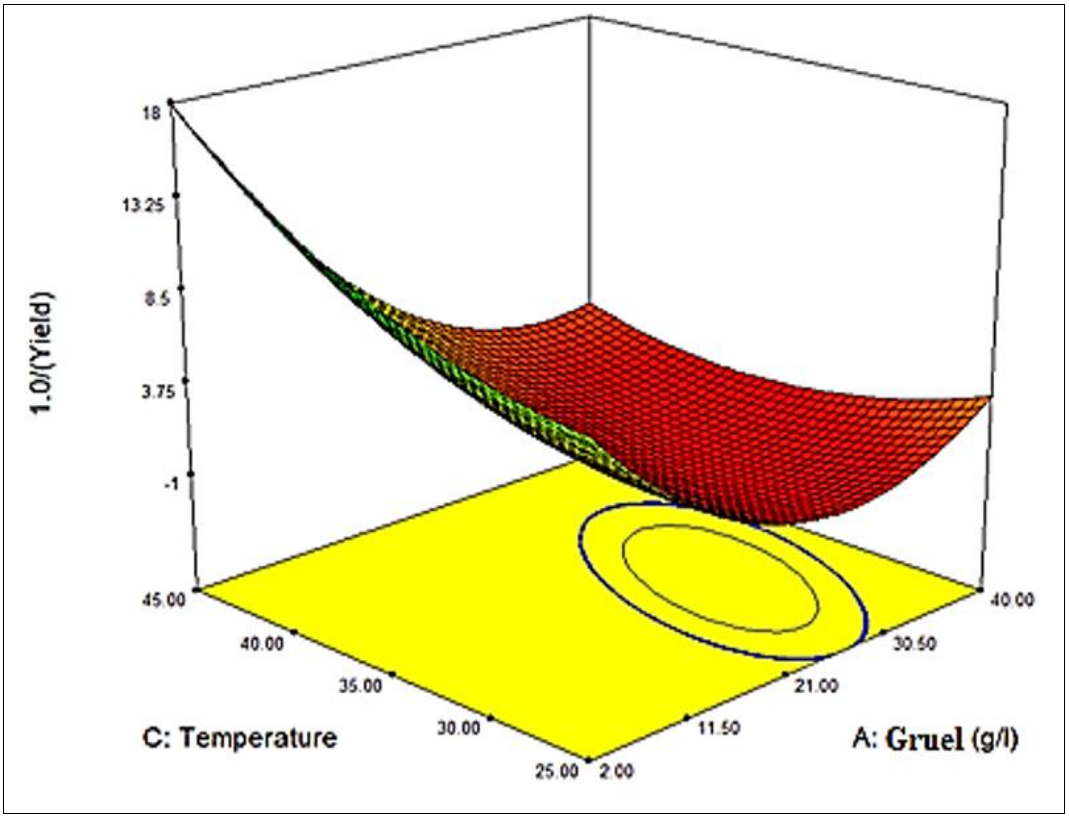


Fig 4: Three-dimensional response surface plots for lipopeptides DCS1 production showing the interactive effects of temperature and gruel concentration

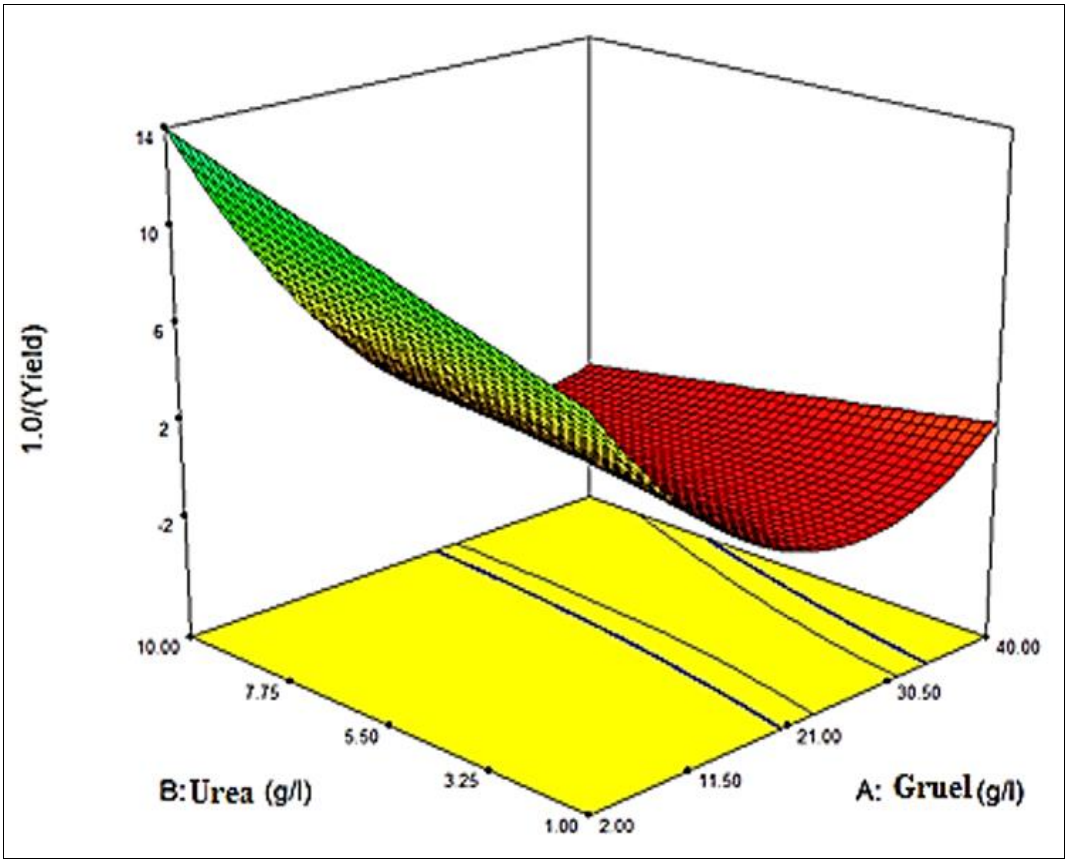


Fig 5: Three dimensional response surface plots for lipopeptides DCS1 production showing the interactive effects of urea and gruel concentrations

Table 1: Selected factors and levels for experimental design

Factor	Code	Unit	Levels		
			Low (-1)	Center (0)	High (+1)
Gruel	A	g/l	2	21	40
Urea	B	g/l	1	5.5	10
Initial pH	C	-	6	7.5	9
Agitation	D	rpm	100	175	250
Temperature	E	°C	25	35	45
Inoculum size	F	% (v/v)	1	2.5	4
KH_2PO_4	G	g/l	0	0.07	0.14
Na_2HPO_4	H	g/l	0	1	2
$MgSO_4$	J	g/l	0	0.25	0.5
$CaCl_2$	K	mg/l	0	20	40
$FeSO_4$	L	mg/l	0	10	20
$MnSO_4$	M	mg/l	0	0.6	1.2
$CuSO_4$	N	mg/l	0	0.5	1
$ZnSO_4$	O	mg/l	0	1.16	2.32

Table 2: Plackett-Burman experimental design matrix and the corresponding system obtained responses

Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10	Factor 11	Factor 12	Factor 13	Factor 14	Factor 15	Factor 16	Factor 17	Factor 18	Factor 19	Response 1	Response 2
		A	B	C	D	E	F	G	H	J	K	L	M	N	O	P : Dum1	Q : Dum2	R : Dum3	S : Dummy4	T:Dummy5	Surface tension	Yield
21	1	21	5.5	7.5	175	35	2.5	0.07	1	0.25	20	10	0.6	0.5	1.16	0	0	0	0	0	30.3	1.737
20	2	2	1	6	100	25	1	0	0	0	0	0	0	0	0	-1	-1	-1	-1	-1	30.1	0.131
3	3	40	1	9	250	25	1	0.14	2	0.5	40	0	1.2	0	2.32	-1	-1	-1	-1	1	29.7	2.548
19	4	40	1	6	250	45	4	0.14	0	005	0	20	0	0	0	-1	1	1	-1	1	30.1	1.2428
12	5	2	10	6	250	25	1	0	0	005	40	0	1.2	1	0	-1	1	1	1	1	54.6	0.077
14	6	40	10	6	250	25	4	0	0	0	0	20	1.2	0	2.32	1	-1	-1	1	1	30.2	2.862
16	7	40	10	9	250	25	4	0	2	0	0	0	0	1	2.32	-1	1	1	-1	-1	30.7	2.485
11	8	40	1	9	100	25	1	0	2	0.5	0	20	1.2	0	0	1	1	1	1	-1	29.6	3.5
1	9	40	10	6	100	45	4	0.14	2	0	40	0	1.2	0	0	-1	-1	1	1	-1	34.4	0.4
23	10	21	5.5	7.5	175	35	2.5	0.07	1	0.25	20	10	0.6	0.5	1.16	0	0	0	0	0	29.8	1.697
18	11	2	1	9	250	45	4	0	2	0	40	0	0	0	0	1	1	-1	1	1	34.7	0.037
4	12	40	10	6	250	45	1	0	2	0.5	40	20	0	1	0	1	-1	-1	-1	-1	30.9	0.714
22	13	21	5.5	7.5	175	35	2.5	0.07	1	0.25	20	10	0.6	0.5	1.16	0	0	0	0	0	30	2.394
5	14	2	10	9	100	45	4	0	0	0.5	40	20	1.2	0	2.32	-1	1	-1	-1	-1	54	0
10	15	2	10	6	100	25	1	0.14	2	0	40	20	0	0	2.32	1	1	1	-1	1	55.8	0.0028
9	16	40	1	6	100	25	4	0.14	0	0.5	40	0	0	1	2.32	1	1	-1	1	-1	29.4	2.037
17	17	2	10	9	250	45	1	0.14	0	0.5	0	0	0	0	2.32	1	-1	1	1	-1	45.9	0.0857
2	18	2	10	9	100	25	4	0.14	2	0.5	0	20	0	1	0	-1	-1	-1	1	1	33	0.1428
7	19	2	1	6	250	45	1	0.14	2	0	0	20	1.2	1	2.32	-1	1	-1	1	-1	30.5	0.034
6	20	2	1	9	250	25	4	0.14	0	0	40	20	1.2	1	0	1	-1	1	-1	-1	30.2	0.0885
13	21	40	1	9	100	45	1	0	0	0	40	20	0	1	2.32	-1	-1	1	1	1	35.8	0.1428
15	22	40	10	9	100	45	1	0.14	0	0	0	0	1.2	1	0	1	1	-1	-1	1	34.5	0.2028
8	23	2	1	6	100	45	4	0	2	0.5	0	0	1.2	1	2.32	1	-1	1	-1	1	33	0

Table 3: Effect of non-significant factors on responses and the fixed values

	Surface tension	Yield	Min	Max	Value
C-Initial pH	Negative	Positive	6	9	7.5
D-Agitation	Negative	Positive	100	250	175
F-Inoculum size	Negative	Positive	1	4	2.5
G-KH_2PO_4	Negative	Negative	0	0.14	0
H-Na_2HPO_4	Negative	Positive	0	2	1
J-$MgSO_4$	Positive	Positive	0	0.5	0.5
L-$FeSO_4$	Positive	Positive	0	20	20
M-$MnSO_4$	Positive	Positive	0	1.2	1.2
N-$CuSO_4$	Negative	Negative	0	1	0
O-$ZnSO_4$	Positive	Positive	0	2.32	2.32

Table 4 : Box-Behnken design matrix and the corresponding system experimental responses

Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2
		A : Gruel	B : Urea	C : Temperature	D : $CaCl_2$	Surface tension	Yield
10	1	40	5.5	35	0	34.3	1.622
3	2	2	10	35	20	38.2	0.0628
9	3	2	5.5	35	0	35.8	0.137
1	4	2	1	35	20	31.3	0.125
17	5	2	5.5	25	20	33.8	0.1028
14	6	21	10	25	20	32.55	2.177
25	7	21	5.5	35	20	32.55	1.5428
16	8	21	10	45	20	33.2	0.208
8	9	21	5.5	45	40	34	0.177
23	10	21	1	35	40	33.7	1.36
5	11	21	5.5	25	0	32.3	2.185
24	12	21	10	35	40	33.3	1.5914
27	13	21	5.5	35	20	32.65	1.274
26	14	21	5.5	35	20	32.3	1.38
15	15	21	1	45	20	32.8	0.4028
4	16	40	10	35	20	33.5	0.825
12	17	40	5.5	35	40	33	0.5857
22	18	21	10	35	0	33.2	1.605
21	19	21	1	35	0	33	1.41
19	20	2	5.5	45	20	36.8	0.0457
6	21	21	5.5	45	0	31.9	0.534
2	22	40	1	35	20	31.3	0.488
7	23	21	5.5	25	40	33.4	1.394
18	24	40	5.5	25	20	32.9	4.717
20	25	40	5.5	45	20	32.55	0.785
11	26	2	5.5	35	40	33.3	0.137
13	27	21	1	25	20	33.7	1.465

4. Conclusion

The critical factor affecting the success of biosurfactant production is the development of an effective process that uses inexpensive compounds and gives high yield. To improve biosurfactant production, economical culture medium components such as agriculture by-products or waste must be used. In this study, a low cost culture medium for production of potent lipopeptides by *B. methylotrophicus* DCS1 strain, based on the combination of industrial waste and cheap substrates, was successfully evaluated and seems to be very promising.

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6. References

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