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(RESEARCH ARTICLE)

Application of foaming capacity as response value in optimization of biosurfactant production

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Abstract

Equipment for determination of surface tension (ST) which is the response value often used during biosurfactant optimization are not readily available in many laboratories. Foaming capacity (FC) was thus investigated as response value during biosurfactant optimization. Thirteen variations of glycerol-mineral salts medium were used to culture *Pseudomonas* sp. for biosurfactant production and optimization. Variations in the medium were based on pH, carbon-nitrogen (C-N) and carbon-phosphorus (C-P) ratio. Inoculated media were incubated at ambient temperature with orbital shaking at 150 rpm for four days. FC of the media were then determined and fitted using a polynomial model so as to obtain a prediction profile. The prediction profile was used to determine the combination of C-N ratio, C-P ratio, and pH that will lead to the highest FC. This combination was used in another experimental run for biosurfactant production, and at the end the culture was screened for biosurfactant activity. The results obtained showed that foaming was achieved in selected experimental runs, and the model for the prediction profile was worked out to be $Y = -513.03 + 103.3804 X_1 + 2.1211 X_2 + 16.2848 X_3 – 0.1108X_1X_2 – 0.3656 X_1X_3 – 0.0339X_2X_3 – 6.1730 X_1^2 – 0.0099 X_2^2 – 0.4432 X_3^2$. From the prediction profiles it was seen that the highest FC (32.04%) was achievable at combination of pH 7.0, C-N 40, and C-P 13. Biosurfactant activity of the culture with optimized combination showed that ST reduced from 56.43 to 35.28 $mN.m^{-1}$. It is concluded that FC can be used in place of ST during biosurfactant optimization procedures.

Keywords: Foaming capacity; Surface tension; Biosurfactant; Optimization; Response surface methodology.

1. Introduction

Biosurfactants are surface active compounds synthesized by certain genera of bacteria and selected yeasts [1]. Industrial scale production of biosurfactant is economically challenging [2]. Researchers thus carry out product optimization so as to proffer procedures and processes that will minimize waste of substrates while ensuring maximum product yield. Optimization of Biosurfactant production involves coming up with a set of conditions that will lead to maximum or optimum yield of biosurfactant during production. This has been achieved with the use of Response surface methodology (RSM) with three to five variable mathematical designs/models such as the Box-Behnken design [3, 4]. In RSM all chosen variables are optimized simultaneously, thus eliminating the limitations associated with a single factor optimization process.

Biosurfactants are capable of lowering surface tension [5], and this property is most often used in screening for biosurfactant production. Surface tension is also the response value more often used when RSM is applied in biosurfactant optimization [4, 6, 7]. Surface tension determination during assessment for biosurfactant production is achieved using the Du-Nouy-Ring method and the capillary rise method [8, 9, 10]. In the Du-Nouy-ring method, an instrument called a tensiometer is used in measuring the surface tension [10]. In the capillary rise method a capillary tube is used in measuring the rise in height which is used alongside other parameters in calculating surface tension [11].

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Tensiometers and calibrated capillary tubes are equipment not readily available in many Microbiology laboratories in developing countries. There are however other methods that rely on simple apparatus that are readily available in any Microbiology laboratory that can be used in detecting and estimating biosurfactant production. They include the oil spread assay, emulsification capacity, and foaming capacity [12, 13, 14]. The values obtained from these methods are quantitative, and can be use as response values when applying RSM in biosurfactant optimization. The aim of this research work is thus to determine the effectiveness of using foaming capacity as a response value in the optimization of biosurfactant production.

2. Material and methods

2.1. Sourcing for a biosurfactant producing bacterium

Bacteria that produce biosurfactants include *Bacillus* spp., *Rhodococcus* spp., and *Pseudomonas* spp. [15, 16, 17]. *Pseudomonas* sp. was chosen and isolated for use as the biosurfactant producing bacterium.

Water samples were collected from fish ponds in the African Regional Aquaculture Centre (ARAC), Port Harcourt, Nigeria. The samples were collected with the aid of disinfected water bottles of about 500 ml capacities. *Pseudomonas* species were isolated from the water samples using nutrient agar supplemented with 60 $\mu g.ml^{-1}$ Chloramphenicol. The water samples and their 10^{-1} dilution were inoculated separately onto the agar media using the spread plate technique. Inoculated plates were incubated at ambient temperature (28 - 31 °C) for 48 h. After incubation, colonies that developed on the agar plates were checked for their ability to fluoresce under ultra violet (UV) light. Isolated colonies that fluoresced under UV light were sub-cultured in a confluent streaking manner onto nutrient agar plates. Stock cultures of the isolates were developed from the confluent streak plates by scoping the colonial materials with the aid of sterile spatula and transferring into sterile 20% glycerol in sterile bijou bottles. Stock cultures were stored in the refrigerator at 4°C. Colonies developed from the stock cultures were subjected to Gram staining, Methyl Red and Vogues-Proskauer test to confirm that the isolates belong to *Pseudomonas* species.

2.2. Culture media preparation for biosurfactant production and optimization

Glycerol-Mineral salts medium (Table 1a and Table 1b) were prepared for culturing the bacterium for biosurfactant production and optimization experiment. Thirteen different variations of the medium, with the thirteenth medium in triplicate, were prepared for the optimization experiment. The variations in the Glycerol-Mineral salts medium was based on three culture medium factors (pH, C-N ratio, C-P ratio), and on three levels (-1, 0, +1) for response surface analysis. The range of values of the selected culture medium factors and the chosen level of response surface analysis, based on the Box-Behnken design, is presented in Table 2. Based on the three selected factors with two extra centre points as required by the Response Surface Methodology, and with the aid of the Box-Behnken experimental design matrix (Table 3), a preparation of fifteen setups comprising thirteen different media with the thirteenth medium in triplicate were designed for the optimization experiment. In preparation of the thirteen variation of the Glycerol-Mineral salts medium with the thirteenth medium in triplicate, 100ml of the Glycerol-Mineral salts medium was dispensed into 15 conical flasks (250ml capacity) labeled 1-15, followed by the addition of the required amount of nitrate ($NaNO_3$) and phosphate (KH_2PO_4) salts (Table 4). The calculations used in achieving the required amount of nitrate and phosphate can be found in Peekate and Abu (2017). The pH of the media was then adjusted accordingly (Table 3) using 1.0M NaOH and a pocket size pH meter (Hannah instrument, UK). The media were then sterilized in an Autoclave at 121°C for 15 minutes.

Table 1a Composition of the glycerol-mineral salts medium for culturing the bacterium for biosurfactant production

Constituent	Concentration	Quantity
Glycerol (% v/v)	3	60 ml
KH_2PO_4	*	-
$MgSO_4.7H_2O$ (g.L-1)	0.4	0.8 g
NaCl (g.L-1)	1.0	2.0 g
$CaCl_2.2H_2O$ (g.L-1)	0.1	0.2 g
$NaNO_3$	*	-
TES (% v/v)	0.1	2 ml
Distilled water	-	1938 ml
Total volume		2,000 ml

TES: Trace elements solution; *the concentration was varied in order to achieve a particular C-N and C-P ratio.

Table 1b Composition of TES

Constituent	g per L distilled water
$MnSO_4.H_2O$	1.5
$FeSO_4.7H_2O$	0.5
$CuSO_4.5H_2O$	0.2
$Na_2MoO_4.2H_2O$	0.1
$ZnSO_4.7H_2O$	1.5
$H3BO_3$	0.3

Table 2 Culture medium factors and levels for response surface analysis

	Level		
Factor	**-1**	**0**	**+1**
pH	5.5	7.0	8.5
C-N ratio	20	40	60
C-P ratio	10	13	16

C-N: carbon-nitrogen, C-P: carbon-phosphorus

Table 3 Combination of the culture medium factors for experimental runs using the Box-Behnken experimental design matrix

Run number	.pH (X_1)	C-N (X_2)	C-P (X_3)
1	- 1 (5.5)	- 1 (20)	0 (13)
2	- 1 (5.5)	+ 1 (60)	0 (13)
3	+ 1 (8.5)	- 1 (20)	0 (13)
4	+ 1 (8.5)	+ 1 (60)	0 (13)
5	- 1 (5.5)	0 (40)	- 1 (10)
6	- 1 (5.5)	0 (40)	+ 1 (16)
7	+ 1 (8.5)	0 (40)	- 1 (10)
8	+ 1 (8.5)	0 (40)	+ 1 (16)
9	0 (7)	- 1 (20)	- 1 (10)
10	0 (7)	- 1 (20)	+ 1 (16)
11	0 (7)	+ 1 (60)	- 1 (10)
12	0 (7)	+ 1 (60)	+ 1 (16)
13	0 (7)	0 (40)	0 (13)
14	0 (7)	0 (40)	0 (13)
15	0 (7)	0 (40)	0 (13)

Adapted from Zhang and Dequan (2013), and Kumar *et al*. (2015).

Table 4 Amount of $NaNO_3$ and KH_2PO_4 salts added to the Glycerol-Mineral salts media for each experimental run to achieve their respective C-N and C-P ratio.

R/N	C	NN	N	C-N	KP	P	C-P
	g/100 ml	g/100 ml	g/100 ml	C/N	g/100 ml	g/100 ml	C/P
1	1.47	0.45	0.0735	20	0.50	0.1131	13
2	1.47	0.15	0.0245	60	0.50	0.1131	13
3	1.47	0.45	0.0735	20	0.50	0.1131	13
4	1.47	0.15	0.0245	60	0.50	0.1131	13
5	1.47	0.22	0.0368	40	0.64	0.1470	10
6	1.47	0.22	0.0368	40	0.40	0.0919	16
7	1.47	0.22	0.0368	40	0.64	0.1470	10
8	1.47	0.22	0.0368	40	0.40	0.0919	16
9	1.47	0.45	0.0735	20	0.64	0.1470	10
10	1.47	0.45	0.0735	20	0.40	0.0919	16
11	1.47	0.15	0.0245	60	0.64	0.1470	10
12	1.47	0.15	0.0245	60	0.40	0.0919	16
13	1.47	0.22	0.0368	40	0.50	0.1131	13
14	1.47	0.22	0.0368	40	0.50	0.1131	13
15	1.47	0.22	0.0368	40	0.50	0.1131	13

R/N: Run number, C: carbon concentration (the concentration of carbon in 100ml of medium containing 3% v/v glycerol is 1.47g/L), NN: $NaNO_3$ concentration, N: calculated nitrogen concentration, KP: KH_2PO_4 concentration, P: calculated phosphorus concentration.

2.3. Culturing for biosurfactant production and optimization

A broth culture of a selected confirmed *Pseudomonas* species was first prepared by transferring 1 ml of the stock culture into 200 ml sterile nutrient broth (Titan Biotech Ltd., Rajasthan, India) which was then incubated at 37 °C for 48h. After incubation, 1 ml of the broth culture was inoculated into the fifteen sterile preparations of the Glycerol-Mineral salts medium. After inoculation, the setups were incubated at ambient temperature (28 – 31 °C) for four days on a PSU-20i Multi-functional Orbital Shaker (Keison Products, UK) operated at 150rpm for 8h per working day. At the end of the incubation period, the foaming capacity of broth from the different setups was determined, and the different foaming capacities fitted using a generalized polynomial model (Eq. 1) for 3-factor design [4].

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{1,2} X_1X_2 + \beta_{1,3} X_1X_3 + \beta_{2,3} X_2X_3 + \beta_{1,1} X_1^2 + \beta_{2,2} X_2^2 + \beta_{3,3} X_3^2 \quad \text{... (Eq. 1)}$$

Where Y is the predicted foaming capacity; X_1, X_2, and X_3 represent the values for the three factors (pH, C-N ratio, and C-P ratio); β_0 is the value of fitted response at the centre point of the design; β_1, β_2, and β_3 are the linear coefficients; $\beta_{1,2}$, $\beta_{1,3}$, and $\beta_{2,3}$ are the interaction coefficients; and $\beta_{1,1}$, $\beta_{2,2}$, and $\beta_{3,3}$ are the quadratic coefficients.

2.4. Generating a prediction profile from the optimization experiment

Model fitting was achieved using second order polynomial regression equations. The principles of Matrices was applied, with the aid of Microsoft excel®, in resolving the equations so as to obtain a prediction profile. The prediction profile was used to determine combinations of the values of C-N ratio, C-P ratio, and pH that will lead to high foaming capacity (indicating maximum biosurfactant production). The most favourable combination was used in a new experimental run for biosurfactant production.

2.5. Culturing for biosurfactant production using optimized conditions

About 500ml of a culture medium having the most favourable combination of pH, C-N ratio, and C-P ratio that will lead to the highest foaming capacity as determined from the prediction profile was prepared. The medium was sterilized in

an Autoclave at 121°C for 15 minutes, allowed to cool, and inoculated with 5ml broth culture of the *Pseudomonas* sp. used in the optimization stage. On inoculation, the broth was incubated at ambient temperature (28 – 31 °C) for seven days on a PSU-20i Multi-functional Orbital Shaker (Keison Products, UK) operated at 150rpm for 8h per working day. At the end of the incubation period, the broth culture was screened for biosurfactant activity.

2.6. Screening for biosurfactant activity

2.6.1. Surface tension measurements

The capillary rise method was used in measuring the surface tension of the culture broth. A sterile capillary tube of about 0.2cm diameter was used to measure the rise in height of the broth culture. The rise in height was then used to calculate the surface tension with the aid of Equation 2 [11].

$\gamma(mN.m^{-1}) = ½\ rhdg$ … … … … … … … … (Eq. 2)

Where 'r' is the radius of the capillary tube in cm; 'h' is the rise in height in cm of the liquid; 'd' is the broth density in $g.cm^{-3}$; and 'g' is the acceleration due to gravity in $cm.s^{-2}$, i.e. 980 $cm.s^{-2}$.

2.6.2. Oil spread diameter

About 40ml of distilled water was poured into Petri dishes and oil films generated on the water surface by adding several drops of diesel oil. A drop of broth culture was placed in the centre of the oil films, and the diameter of the ensuing zone of clearance measured.

2.6.3. Drop collapse activity

Each well in a ceramic plate was coated with a drop of used engine oil. The well plate was incubated at 37°C for about 1h. After incubation, two drops of the culture broth was transferred into the different oil-coated wells. After 1 minute, the shapes of the drops were observed with the aid of a magnifying lens.

2.6.4. Foaming capacity

The foaming capacity of the broth culture was determined by transferring 10ml of the broth into 50ml graduated measuring cylinders, which was then vortex vigorously for 1 minute. The foaming height and the total height was measured, and used to calculate the foaming capacity (FC). The calculation was done using equation 3 [13].

FC (%) = (height of foam / total height) × 100 … … … … (Eq. 3)

3. Results and discussion

3.1. Identity of the fluorescent isolates

Selected isolated colonies that fluoresced under UV light were coded P1 – P7. Results generated from Gram staining reaction, Methyl Red and Vogues Proskauer (MR-VP) tests carried out on the isolates is presented in Table 5. From the Table it can be seen that all the fluorescent isolates are Gram negative rods, and isolates P1 – P4, and P6 are MR-VP negative. *Pseudomonas* species are Gram negative rods and MR-VP negative [18, 19, 20]. Also, selected species of *Pseudomonas* are usually fluorescent [20], and may exhibit some level of antibiotic resistance. For instance, *Pseudomonas aeruginosa* has been cited to be intrinsically resistant to chloramphenicol [21]. It should be noted that the fluorescent bacteria were isolated using nutrient agar supplemented with 60 $\mu g.ml^{-1}$ chloramphenicol. Bacterial isolates P1 – P4, and P6 are thus highly suspected to be species of *Pseudomonas*.

Table 5 Identification test results of the fluorescent isolates

ISC	GS	MPH	MR	VP	SO
P1	-ve	rods	-ve	-ve	*Pseudomonas* sp.
P2	-ve	rods	-ve	-ve	*Pseudomonas* sp.
P3	-ve	rods	-ve	-ve	*Pseudomonas* sp.
P4	-ve	rods	-ve	-ve	*Pseudomonas* sp.
P5	-ve	rods	-ve	+ve	*Vibrio* sp.
P6	-ve	rods	-ve	-ve	*Pseudomonas* sp.
P7	-ve	rods	-ve	+ve	*Vibrio* sp.

ISC: isolate code, GS: Gram staining, MPH: Microscopic morphology, MR: methyl red, VP: Voges-Proskauer, SO: suspected organism.

3.2. Foaming capacity of broth from the experimental runs

The foaming capacity of broth from the 15 experimental runs of the optimization experiment is presented in Table 6. From the Table it can be seen that foaming was achieved in run-numbers 2-4, and 7-15. Foam formation has been achieved with biosurfactant at very low concentration [22]. In another related study, stable foam formation of 8 – 165 minutes with foaming height of 11 – 35 mm has been achieved with a species of *Pseudomonas* during biosurfactant production [23]. The various foaming capacities achieved in selected experimental runs in this study are thus indications of different level of biosurfactant production in the different experimental runs.

Fitting of the foaming capacities and the different combinations of the factors using a generalized polynomial model (Equation 1) is presented in Table 7. In determining the coefficients(β_0, β_1, β_2, β_3, $\beta_{1,2}$, $\beta_{1,3}$, $\beta_{2,3}$, $\beta_{1,1}$, $\beta_{2,2}$, $\beta_{3,3}$) in the polynomial model, the matrix equation $\hat{\beta} = (X^T X)^{-1} X^T Y$ [24] was used. From the calculations the coefficients β_0, β_1, β_2, β_3, $\beta_{1,2}$, $\beta_{1,3}$, $\beta_{2,3}$, $\beta_{1,1}$, $\beta_{2,2}$, $\beta_{3,3}$ were deduced to be - 513.03, 103.3804, 2.12108, 16.2848, - 0.1108, - 0.3656, - 0.0339, - 6.1730, - 0.0099 and - 0.4432 respectively. The polynomial model is thus written as,

$$Y = -513.03 + 103.3804 X_1 + 2.1211 X_2 + 16.2848 X_3 - 0.1108 X_1X_2 - 0.3656 X_1X_3 - 0.0339 X_2X_3 - 6.1730 X_1^2 - 0.0099 X_2^2 - 0.4432 X_3^2 \quad \ldots \quad \ldots \quad \ldots \quad \text{(Eq. 4)}$$

The analysis of variance of the polynomial model (Table 8) indicated that at least one of the coefficients is significant. In other words a regression model exists between foaming capacity and one or more of the factors (pH, C-N, and C-P).

Table 6 Foaming capacity and pH of broth from the 15 experimental runs

R/N	TH (mm)	FH (mm)	FC (%)
1	0	0	0
2	20	2	10
3	24	6	25
4	23	5	21.7
5	20	0	0
6	20	0	0
7	19	6	31.58
8	16	4	25
9	16	2	12.5
10	20	6	30
11	18	4	22.22
12	19	6	31.58
13	18	6	33.33
14	17	5	29.41
15	18	6	33.33

R/N: run number, TH: total height, FH: foaming hight, FC: foaming capacity

Table 7 Culture medium factors and foaming capacity values fitted into the generalized model

	.pH	C-N	C-P							FC
	X_1	X_2	X_3	X_1*X_2	X_1*X_3	X_2*X_3	X_1^2	X_2^2	X_3^2	
[X]										Y
1	5.5	20	13	110	71.5	260	30.25	400	169	0
1	5.5	60	13	330	71.5	780	30.25	3600	169	10
1	8.5	20	13	170	110.5	260	72.25	400	169	25
1	8.5	60	13	510	110.5	780	72.25	3600	169	21.7
1	5.5	40	10	220	55	400	30.25	1600	100	0
1	5.5	40	16	220	88	640	30.25	1600	256	0
1	8.5	40	10	340	85	400	72.25	1600	100	31.58
1	8.5	40	16	340	136	640	72.25	1600	256	25
1	7	20	10	140	70	200	49	400	100	12.5
1	7	20	16	140	112	320	49	400	256	30
1	7	60	10	420	70	600	49	3600	100	22.22
1	7	60	16	420	112	960	49	3600	256	31.58
1	7	40	13	280	91	520	49	1600	169	33.33
1	7	40	13	280	91	520	49	1600	169	29.41
1	7	40	13	280	91	520	49	1600	169	33.33

FC: foaming capacity, [X]: design matrix.

Table 8 Summary of the ANOVA of the generalized model

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F statistics	P value
Regression	3	2021.989	673.9962	36.69	0.1
Error	11	202.0703	18.37		
Total	14	2224.0593			

The tabulated statistic $F_{\alpha,k,n-(k+1)}$, i.e. $F_{0.1,3,11}$ is 2.66

3.3. Generated prediction profile from the optimization experiment

Equation 4 was used in generating the prediction profiles (Table 9). The three dimensional (3-D) surface chart of the prediction profiles are presented in Figure 1a-1c. From Table 9 it is seen that the highest foaming capacity (32.04%), indicating optimum biosurfactant production, is achievable at the combination of pH 7.0, C-N 40, and C-P 13. In Peekate and Abu [25], where surface tension was used as the response value, an optimized combination of pH 5.5, C-N 20, and C-P 16 was achieved. The disparity between the two combinations could be attributed to a possible negative effect of acidic pH on foaming during biosurfactant production. Scrutinizing Table 6, it can be seen that the foaming capacity of run-numbers 1, 5, and 6 which are acidic experimental runs were 0%. Also, observing the 3-D surface chart of the prediction profiles it can be seen that at the acidic pH, the foaming capacity for the various combinations were below 10% (Fig. 1a), whereas at neutral (pH 7.0) and alkaline pH (pH 8.5) foaming capacities were up to 30% (Fig. 1b and 1c). It has been speculated that maximum foam volume depends on the pH of the production solution rather than the type of biosurfactant [26]. In other related studies, pH of 7.0, 7.8, and 8.0 has been shown to be an optimum factor for biosurfactant production [27, 28, 29]. The optimized combination of pH 7.0, C-N 40, and C-P 13 obtained in this study is thus a possible combination required for maximum biosurfactant yield.

Table 9 Foaming capacity prediction profile for different combinations of pH, C-N, and C-P.

X_2 (C-N)	X_3 (C-P)				
	10	**13**	**16**	**19**	**22**
At pH 5.5					
20	-13.26	-3.05	-0.82	-6.57	-20.29
40	-1.68	6.49	6.69	-1.1	-16.86
60	1.97	8.11	6.27	-3.54	-21.34
80	-2.3	1.81	-2.06	-13.91	-33.74
100	-14.48	-12.41	-18.32	-32.2	-54.06
At pH 7.0					
20	17.26	25.82	26.41	19.02	3.64
40	25.51	32.04	30.59	21.16	3.76
60	25.84	30.34	26.85	15.39	-4.05
80	18.25	20.71	15.19	1.7	-19.77
100	2.74	3.17	-4.38	-19.91	-43.42
At pH 8.5					
20	20	26.92	25.86	16.82	-0.2
40	24.93	29.81	26.72	15.64	-3.41
60	21.93	24.78	19.65	6.55	-14.54
80	11.02	11.83	4.67	-10.47	-33.59
100	-7.81	-9.03	-18.23	-35.4	-60.56

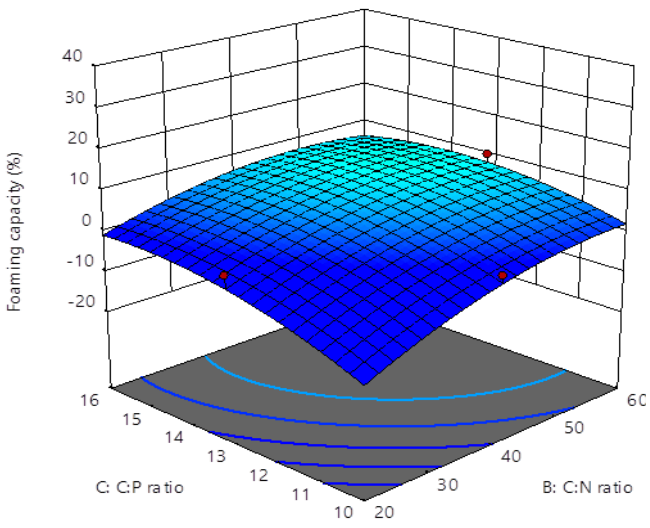

Figure 1a 3-D surface chart of the prediction profile for foaming capacity as a function of C-N and C-P ratio at pH 5.5.

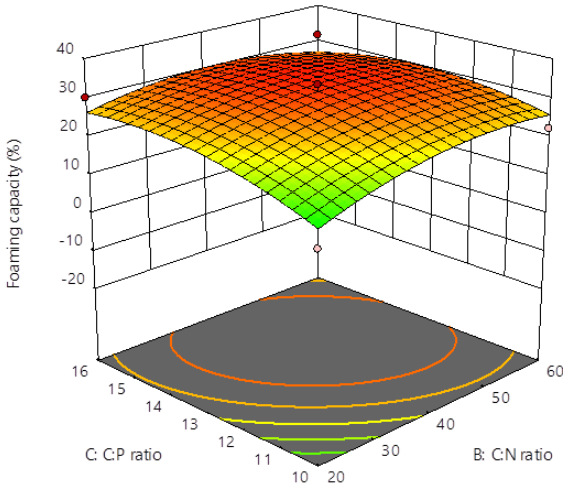

Figure 1b3-D surface chart of the prediction profile for foaming capacity as a function of C-N and C-P ratio at pH 7.0.

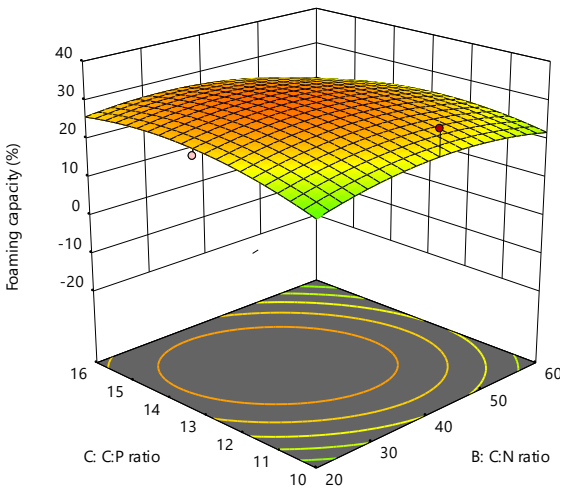

Figure 1c 3-D surface chart of the prediction profile for foaming capacity as a function of C-N and C-P ratio at pH 8.5.

3.4. Biosurfactant activity of broth from setup with optimized combination

The biosurfactant activity of broth from setup with optimized combination (pH 7.0 / C-N 40 / C-P 13) is presented in Table 10. From the Table it can be seen that the drop collapse activity of broth from the setup was positive, and the surface tension was reduced from 56.43 to 35.28 $mN.m^{-1}$. Surface tension values that have been obtained for culture broth of biosurfactant producing microorganisms range from 26.7 to 38.75 $mN.m^{-1}$ [30, 31]. The considerable reduction of surface tension obtained in this study is thus an indication that biosurfactant was produced.

Table 10 Biosurfactant activity of broth from setup with optimized conditions

Activity	B7	B0
Surface tension (mN.m-1)	35.28	56.43
Oil spread diameter (mm)	5	0
Drop collapse activity	+ ve	- ve

B7: Setup having combination of pH 7.0, C-N 40, and C-P 13 after 7 days of incubation, B0: Setup on day 0 before inoculation.

4. Conclusion

Foaming capacity, whose determination relies on readily available apparatus, was investigated in this study for use as response value during biosurfactant optimization through RSM. In using foaming capacity as the response value, optimized combination of pH 7.0, C-N 40, and C-P 13 was achieved where *Pseudomonas* sp. was used as the biosurfactant producing organism, and variants of a glycerol-mineral salts medium were used as the biosurfactant production medium. The optimized combination obtained in this study is a possible combination required for maximum biosurfactant yield as indicated by results obtained by other researchers. There is thus a possibility of using foaming capacity in place of surface tension as the response value during biosurfactant optimization through RSM. The strength of this possibility can be evaluated in future research works where foaming capacity will be used to re-assessed combinations obtained through the use of surface tension.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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