

RESEARCH

Open Access



Optimization of biosurfactant production by *Pseudomonas aeruginosa* strain Pa using rubber tree seed oil as sole carbon source

Yapi Joel Angba^{1,2*}, Alpha Ousmane Touré^{2,3}, Koutouan Désiré Martial Abro¹, Mahamane Nassirou Amadou Kiari¹, Allali Patrick Drogui⁴ and Kouassi Benjamin Yao¹

*Correspondence:

Yapi Joel Angba

yapi.angba21@inphb.ci

¹African Center of Excellence for the Valorization of Waste Into High Value-Added Products (CEA-VALOPRO), Institut National Polytechnique Félix HOUPOUËT-BOIGNY (INP-HB), Yamoussoukro, Côte d'Ivoire

²Department of Chemical Engineering and Applied Biology, Cheick Anta Diop University of Dakar, Ecole Supérieure Polytechnique (ESP), Dakar-Fann, Senegal

³College of Engineering, School of Chemical, Biological and Environment Engineering, Oregon State University, Corvallis, OR, USA

⁴Water, Land and Environment Department, Institut National de la Recherche Scientifique (INRS), University of Quebec, Quebec, QC, Canada

Abstract

Biosurfactants (BS) are highly emulsifying, biodegradable, non- or low-toxic, stable and multifunctional molecules. However, high production costs and low yields limit their large-scale production and use. Consequently, using low-cost substrates (waste) and optimizing production conditions are necessary to reduce production costs and increase the yield of biosurfactants. This study aimed to optimize the conditions for the production of BS by *Pseudomonas aeruginosa* Pa using rubber tree seed oil RO (*Hevea brasiliensis*), a cheap and available substrate, as the sole carbon source. Factors significantly influencing biosurfactant production were screened using a Plackett–Burman design (PBD) and response was based on the emulsification index. The selected factors were optimized using the response surface methodology (RSM) through a Box–Behnken design (BBD). The biosurfactant produced under the optimized conditions was extracted by the coupled method of acid precipitation and organic solvent extraction using different solvents. PBD results showed that the initial pH of the production medium, NaCl concentration and rubber tree seed oil concentration significantly influenced BS production. Optimal levels of these factors were obtained for a pH of 8.7, a NaCl concentration of 0.072% and a rubber tree seed oil concentration of 6.91%. Under optimized culture conditions, the emulsification index of the biosurfactant produced reached $92.15 \pm 0.89\%$. Rubber tree seed oil showed a BS production capacity superior to commercial carbon sources (conventional sources). Diethyl ether was chosen as a suitable solvent for extracting biosurfactant from the cell-free supernatant. This study showed that the use of rubber tree seed oil, an agro-industrial waste product, is efficient and guarantees the economic feasibility and sustainability of biosurfactant production.

Keywords Biosurfactant, *Pseudomonas aeruginosa*, Plackett–Burman design, Box–Behnken design, Rubber tree seed oil



1 Introduction

Biosurfactants (BS) are natural surface-active compounds that are receiving particular attention due to their high biodegradability, low toxicity, efficiency and stability under extreme environmental conditions [1]. In addition, biosurfactants are multifunctional compounds that can be used in various applications, including environmental remediation, agriculture, biofilm formation, quorum sensing, textiles, pharmaceuticals, cosmetics and the food, petroleum and petrochemical industries [2–4].

Biosurfactants are produced by microorganisms (bacteria, fungi, yeasts). The microorganisms identified as biosurfactant producers belong to the genera *Pseudomonas*, *Clostridium*, *Brevibacterium*, *Rhodococcus*, *Acinetobacter*, *Thiobacillus*, *Bacillus*, *Leuconostoc*, *Lactobacillus*, *Enterobacter*, *Saccharomyces*, *Aspergillus*, *Ustilago*, *Penicillium*, *Corynebacterium*, *Citrobacter*, *Candida*, *Paenibacillus* sp., *Serratia*, *Rhizopus* [5–9]. However, *Pseudomonas* and *Bacillus* strains remain the most widely used in biosurfactant production due to their higher production capacity, with around 37% and 34% utilization rates, respectively. *Serratia* and *Rhizopus* strains are the least used to produce BS [10]. Bacteria are the main producers of biosurfactants.

However, commercial production of biosurfactants by bacteria is low due to high production costs associated with low yields [11]. Therefore, using renewable wastes as substrates and optimizing media composition and culture conditions are necessary to reduce production costs and increase biosurfactant yield. It has been reported that around 50% of the total production cost is linked to the cost of substrates [12]. In this sense, various substrates such as brewery waste, orange peel, olive oil, sawdust, wastewater from cassava processing, starch, mango kernel oil and sugarcane molasses have been suggested to make biosurfactant production profitable [13–19]. Previous studies have shown that oily substrates are more efficient for the production of biosurfactants [20]. Therefore, rubber tree seed oil (*Hevea brasiliensis*), an inexpensive and inedible substrate, was preferred for biosurfactant production in this study.

Hevea brasiliensis, commonly known as Hevea tree (Family: Euphorbiaceae), is a tree of Amazonian origin, cultivated mainly for its latex and found in Southeast Asia, the South Pacific, Central and West Africa and tropical America [21, 22]. An estimated 14.3 million hectares (ha) of rubber trees are planted worldwide, including 0.6 million in Côte d'Ivoire [23]. The rubber tree produces large quantities of by-products, notably seeds. Average seed yield varies from 70 to 500 kg/ha per year [24]. In Côte d'Ivoire, rubber tree seeds are used mainly as propagation material. With only 10% of seeds used for nurseries, between 75,000 and 100,000 tons of rubber tree seeds are not valorized and remain in the fields as agricultural waste, either germinating naturally or rotting [25, 26]. Mature rubber tree seeds contain between 40 and 52.5% by weight of kernels, and dried kernels contain between 35 and 50% by weight of oil [27]. This makes rubber tree seeds an important source of oil for biosurfactant production.

In addition, to improve the yield of biosurfactants produced from cheap oily substrates, several optimization strategies, such as the 'one variable at a time' optimization method and statistical models (Plackett–Burman design, response surface methodology) have been suggested. The initial step in a study aimed at optimizing BS production by a microorganism may be the selection of significant nutritional and environmental components. The Plackett–Burman design (PBD) can be used to select these main influencing factors from a large number of process variables [28]. However, it does not give

an optimal value for each variable, and further optimization is required [29]. Response surface methodology (RSM) is a statistical approach to the design of experiments to evaluate the individual and interactive effects of independent variables on response and determine optimal conditions [30]. The Box-Behnken design (BBD) is a type of response surface design. The BBD is useful for non-sequential one-shot experiments and provides a good estimate of the first- and second-order coefficients. The BBD has no axial points; in the BBD, all design points lie within the safety zone. Thus, the reduced number of design points makes it less expensive to implement [2, 31]. The conventional 'one variable at a time' optimization method involves varying one parameter at a time and holding the others constant. Unlike RSM, this approach is costly, time-consuming, and does not reveal the interaction effect of the various parameters [32].

The present study aims to improve biosurfactant production in a cost-effective manner in order to promote the commercial production of natural surfactants. RSM using BBD was applied to optimize biosurfactant production using rubber tree seed oil as the sole carbon source.

The novelty of this work lies mainly in the use of rubber tree seed oil, an abundant and inexpensive non-edible oil, as a carbon substrate for the optimization of biosurfactant production, which has never been done to our knowledge.

2 Materials and methods

2.1 Plant material

Rubber tree seed oil (*Hevea brasiliensis*) was used as a carbon substrate in this study. The seeds of *Hevea brasiliensis* were collected from rubber tree plantations in Adonkoi (5° 24' 50" N, 4° 12' 36" W) in the Abidjan district of southern Côte d'Ivoire during the period of August to September 2022. According to local regulations in Côte d'Ivoire, no specific permits, permissions or licenses were required for the collection of fallen seeds in rubber plantations.

2.2 Microorganism and growth media

The *Pseudomonas aeruginosa* strain Pa used in this study was obtained from the Laboratoire Bio-24 (Dakar, Senegal). The strain was reactivated on nutrient agar and incubated at 30 °C for 24 h before use. The *Pseudomonas aeruginosa* strain is shown in Fig. 1.

Nutrient broth (NB) and mineral salt medium (MSM) were used for microbial growth. The slightly modified mineral salt medium (MSM) proposed in previous studies had the following compositions, in g/L of distilled water: glucose (10); Na₂HPO₄ (6); KH₂PO₄ (3); NaNO₃ (1); MgSO₄·7H₂O (0.24); CaCl₂·2H₂O (0.1) [33].

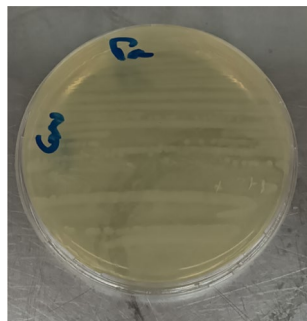


Fig. 1 *Pseudomonas aeruginosa* strain Pa

2.3 Extraction and characterization of rubber tree seed oil

Rubber tree seeds were collected and crushed, then the kernel and shell were separated by manual hulling. The healthy kernels were trilled and dried. The oil was extracted from the dry kernels using a screw press. The crude oil obtained was used without prior treatment. The rubber tree seeds treatment process is summarized in Fig. 2. The oil was characterized by determining its fatty acid and mineral composition. The fatty acid composition of the oil was determined using the high-performance liquid chromatography (HPLC) technique, while the mineral composition was determined using the atomic adsorption technique.

2.4 Optimization of biosurfactant production

2.4.1 Pre-inoculum preparation

A 24-h-old *Pseudomonas aeruginosa* strain Pa from nutrient agar was activated in Nutrient broth (NB). Five loops of the bacterial isolate were inoculated into a 100 mL flask containing 50 mL sterile NB and incubated at 35 ± 1 °C for 24 h with 150 rpm agitation on a rotary shaker.

2.4.2 Inoculum preparation

A 5% size of bacterial pre-inoculum was inoculated into 50 mL of sterile MSM. The medium was incubated under the same conditions as before (35 ± 1 °C, 24 h, 150 rpm). The next day, the absorbance of the culture was measured as optical density (OD) at 600 nm to give an OD of between 1.6 and 1.7.

2.4.3 Plackett–Burman design (PBD)

A Plackett–Burman design (PBD) was applied to determine the environmental components and conditions influencing biosurfactant production by *Pseudomonas aeruginosa* using rubber tree seed oil (RO) as a carbon source. The PBD uses experimental orthogonal Hadamard matrices composed of low-level (-1) and high-level (+1) input variables [28, 34]. The number of experiments that make up these matrices is a multiple of four. This design makes it possible to examine N-1 factors with only N experiments. For this study, six factors and their respective levels were selected from the literature to assess their effect on biosurfactant production across a total of eight trials. The six factors and their (-1) and (+1) levels are presented in Table 1. BS production was carried out in 50 mL of MSM with rubber tree seed oil as the sole carbon source. Media containing the desired factor combinations were prepared and autoclaved at 120 °C for 15 min. Rubber tree seed oil was sterilized separately, cooled to room temperature and added to the media. The different production media were inoculated with 1 or 5% bacterial inoculum and incubated for 24 or 96 h, depending on the experimental design, at 35 ± 1 °C with 150 rpm agitation. The Plackett–Burman experimental design is shown in Table 2.



Fig. 2 Rubber tree seed treatment process

Table 1 Factors screened by PBD and their corresponding levels

Variables	Coded symbol	Coded levels	
		− 1	+ 1
Initial pH	X_1	5	9
Incubation time (hours)	X_2	24	96
NaCl concentration (% w/v)	X_3	0	2.5
RO concentration (% v/v)	X_4	3	7
NaNO ₃ concentration (g/L)	X_5	1.5	3.5
Inoculum size (% v/v)	X_6	1	5

Table 2 Plackett–Burman experimental design with coded and real values

Run	X_1	X_2	X_3	X_4	X_5	X_6
1	1(9)	1(96)	− 1(0)	1(7)	− 1(1.5)	− 1(1)
2	− 1(5)	1(96)	1(2.5)	− 1(3)	1(3.5)	− 1(1)
3	− 1(5)	− 1(24)	1(2.5)	1(7)	− 1(1.5)	1(5)
4	1(9)	− 1(24)	1(2.5)	1(7)	1(3.5)	− 1(1)
5	− 1(5)	1(96)	− 1(0)	1(7)	1(3.5)	1(5)
6	1(9)	− 1(24)	− 1(0)	− 1(3)	1(3.5)	1(5)
7	1(9)	1(96)	1(2.5)	− 1(3)	− 1(1.5)	1(5)
8	− 1(5)	− 1(24)	− 1(0)	− 1(3)	− 1(1.5)	− 1(1)

The emulsification index (%E₂₄) of the cell-free supernatant was taken as the response. The emulsification index is a highly reliable and accurate method for assessing biosurfactant production [35]. NemrodW software (Trial version 2017) was used to analyze the responses obtained (calculation of coefficients and standard deviation). The coefficients of the factors were determined as the difference between the mean of the measurements taken at the high (+) and low (−) levels of the factors. These coefficients indicate the main effects of the factors studied [36]. If the absolute value of a variable's coefficient is more than twice its standard deviation, it is considered to have a significant influence on the response (95% significance level) [34, 37].

2.4.4 Response surface methodology (RSM)

The biosurfactant production was optimized using Response Surface Methodology (RSM) with a Box-Behnken design. This methodology makes it possible to obtain the maximum amount of information in a minimum number of experimental trials [31]. It allows experiments to be planned efficiently, models to be constructed, the effect of factors to be evaluated and the optimum conditions for a multivariate system to be determined [38]. To this end, the influences of three factors, namely the Initial pH of the medium (X_1), NaCl concentration (X_2) and rubber tree seed oil concentration (X_3) on the emulsification index were studied. The independent variables were evaluated at three levels (− 1, 0, + 1). Experimental design and statistical analysis of the data were performed using Design Expert software (Version 13). Thus, 17 experiments were carried out according to the experimental design. The independent variables and their levels are presented in Table 3.

Table 4 shows the Box-Behnken matrix with the coded variables (− 1, 0, + 1) and the real variables.

Biosurfactant production was carried out in 100 mL Erlenmeyer flasks containing 50 mL MSM with rubber tree seed oil as the sole carbon source. Rubber tree seed oil was autoclaved separately at 120 °C for 15 min (then cooled to room temperature) before

Table 3 Factors selected for optimization and their levels

Variables	Coded symbol	Coded levels		
		− 1	0	+ 1
Initial pH	X ₁	5	7	9
NaCl concentration (%)	X ₂	0	1.25	2.5
RO concentration (%)	X ₃	3	5	7

Table 4 Experimental Box – Behnken matrix

Run	Coded levels			Actual levels		
	X ₁	X ₂	X ₃	pH	Conc.NaCl (%)	Conc.RO (%)
1	− 1	− 1	− 1	5	0	5
2	+ 1	− 1	− 1	9	0	5
3	− 1	+ 1	− 1	5	2.5	5
4	+ 1	+ 1	− 1	9	2.5	5
5	− 1	− 1	+ 1	5	1.25	3
6	+ 1	− 1	+ 1	9	1.25	3
7	− 1	+ 1	+ 1	5	1.25	7
8	+ 1	+ 1	+ 1	9	1.25	7
9	0	− 1	− 1	7	0	3
10	0	+ 1	− 1	7	2.5	3
11	0	− 1	+ 1	7	0	7
12	0	+ 1	+ 1	7	2.5	7
13	0	0	0	7	1.25	5
14	0	0	0	7	1.25	5
15	0	0	0	7	1.25	5
16	0	0	0	7	1.25	5
17	0	0	0	7	1.25	5

Conc., Concentration

being added to the medium. Media prepared according to the Box-Behnken design were inoculated with 1% of the bacterial inoculum and incubated at 35 ± 1 °C for 96 h with 150 rpm agitation on a rotary shaker. The initial pH of MSM was adjusted using 1 M NaOH or 1 M HCl. At the end of incubation, the emulsification index (% E₂₄) was measured in duplicate to assess the efficiency of biosurfactant production.

The development of a regression model is necessary to predict the value of the emulsification index at any point in the experimental range selected. The goodness of fit of the regression model was examined by the regression coefficients (R^2) and the coefficient of variation (CV). The significance of the effects of the factors and their interactions was assessed using an analysis of variance (ANOVA) based on the Fisher test, the F value, and the *p* value.

An equation for the model was established to analyze the correlation between the variables (X_i) and the response (Y). Equation (1) gives the second-degree equation of the quadratic model generated.

$$Y = b_0 + \sum_i b_i X_i + \sum_{ij} b_{ij} X_i X_j + \sum_{ii} b_{ii} X_i^2 \quad (1)$$

With Y represents the predicted response (Emulsification index); b_0 , the value of the average coefficient; b_i , the effect of the factors X_i and b_{ij} the effect of the interactions of factors i and j , b_{ii} the quadratic effects and X_i and X_j the coded variables.

Optimal values for the independent variables were obtained using the 'numerical optimization' function of the Design Expert software (Version 13). The validation of the model obtained was verified experimentally under optimal conditions.

2.4.5 Determination of the emulsification index (%E₂₄)

At the end of incubation, the medium was centrifuged at 3000 rpm for 40 min and the cell-free supernatant obtained was used to determine the emulsification index as reported by Rawat et al. [39]. An equal volume of engine oil and cell-free supernatant (2 mL/2 mL) was transferred to a test tube. The tube was vigorously shaken for 2 min using a vortex (ZX3, VELP Scientifica). The tube was left to stand for 24 h at room temperature. The ratio between the height of the emulsified layer and the total solution was measured and then multiplied by 100 to obtain the emulsification index of the test sample according to Eq. 2.

$$\%E_{24} = \frac{\text{height of emulsified layer after 24 hours}}{\text{total height}} \times 100 \quad (2)$$

2.5 Influence of various carbon sources on the production of BS

The influence of different carbon sources on biosurfactant production was studied using various commercial carbon sources (conventional sources) as a replacement for rubber tree seed oil (RO). Glucose, mannitol, glycerol, fructose and sucrose were tested individually. A control using no carbon source was carried out.

2.6 Evaluation of various solvents for the recovery of biosurfactant produced by *Pseudomonas aeruginosa* strain Pa under optimized culture conditions

Di-ethyl ether, ethyl acetate, ethyl acetate–methanol (2:1), ethyl acetate–methanol (3:1), chloroform–methanol (1:1), chloroform–methanol (2:1) and dichloromethane were tested for biosurfactant extraction using the coupled acid precipitation and organic solvent extraction method described by Rastogi et al. [17] with a few modifications. After incubation under optimal conditions, the broth was centrifuged at 3000 rpm for 40 min. The supernatant obtained was then acidified with 1 M HCl to pH 2 and stored overnight at 4 °C to allow precipitation of the biosurfactants. Liquid–liquid extraction was carried out with a solvent-supernatant mixture (1:1) in a separating funnel, shaken vigorously for 5 min and left at room temperature for 24 h. The organic layer was then separated and collected in a beaker. The collected layer was allowed to evaporate and then dried at 80 °C overnight. The dry weight of the extracted crude biosurfactant was obtained gravimetrically, recorded and expressed as g/L.

3 Results and discussion

3.1 Characteristics of rubber tree seed oil

The fatty acid composition of rubber tree seed oil is shown in Table 5. The results are expressed as a percentage of the total area (% At).

HPLC analysis revealed the presence of several fatty acids in the oil. The oil contains mainly oleic acid (46.38%), stearidonic acid (22.24%), linoleic acid (9.12%) and palmitic acid (5.74%). This oil contains 90.23% unsaturated fatty acids and 9.77% saturated fatty acids. Previous studies have observed similar unsaturated fatty acid contents ranging

from 90.1 to 92.6% for vegetable oils [40]. Although the fatty acid composition of this rubber tree seed oil differs from the trend in fatty acid profiles documented in some studies [41, 42], rubber tree seed oil remains a mixture of various fatty acids. In previous reports, it was observed that a mixture of fatty acids gave a higher biosurfactant yield compared to individual fatty acids [18]. Other studies have reported that oils rich in oleic acid improved biosurfactant yield [43].

The results of the oil's mineral composition show that it contains phosphorus (9.146 mg/100 g), potassium (7.945 mg/100 g), sodium (7.789 mg/100 g), calcium (3.382 mg/100 g), magnesium (0.775 mg/100 g), iodine (0.715 µg/100 g), selenium (0.695 mg/100 g), zinc (0.099 mg/100 g), iron (0.0197 mg/100 g), copper (0.0095 mg/100 g) and manganese (0.001 mg/100 g). Minerals are good sources for bacterial growth and biosurfactant production [44]. Microorganisms that produce biosurfactants are strongly influenced by metal cations [9]. Previous studies have shown that the interaction between K^+ and Mg^{2+} is beneficial for biosurfactant production [45]. K^+ is essential for promoting biosurfactant production. Exponential cell growth was also observed in the presence of K^+ . Magnesium is important for maintaining correct cell division [12]. Gudiña et al. have shown that iron, manganese and magnesium act as cofactors for enzymes involved in biosurfactant synthesis [46].

From the above, it appears that rubber tree seed oil is an ideal substrate for microbial growth and biosurfactant production. In the rest of the study, biosurfactant production was therefore optimized using rubber tree seed oil as a carbon substrate.

3.2 Statistical analysis of the Plackett–Burman Design (PBD) for the selection of factors influencing biosurfactant production

The PBD was used to screen for various factors affecting biosurfactant production by *Pseudomonas aeruginosa*. The results obtained correspond to the average of three replicates for each test and range from 20.69 to 79.54%, with the highest value in test 5, followed by tests 8 (76.90%) and 1 (73.22%) (Table 6). Based on the responses obtained, it can be concluded that the experimental zone chosen is sensitive to variations in the various factors selected.

The coefficients of the polynomial model resulting from the Hadamard matrix and their common standard deviation are presented in Table 7.

Table 5 Fatty acid composition of rubber tree seed oil

Fatty acids	Codes	%At
Lauric acid	C12:0	0.77
Myristic acid	C14:0	0.59
Palmitic acid	C16:0	5.74
Palmitoleic acid	C16:1	3.04
Stearic acid	C18:0	0.11
Oleic acid	C18:1	46.38
Linoleic acid	C18:2	9.12
Linolenic acid	C18:3	1.39
Stearidonic acid	C18:4	22.24
Arachidic acid	C20:0	2.55
Eicosenoic acid	C20:1	0.44
Arachidonic acid	C20:4	3.19
Eicosapentaenoic acid	C20:5	3.09
Docosahexaenoic acid	C22:6	1.34

Table 6 Hadamard matrix and corresponding responses

Tests	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	Emulsification index (%)
1	9	96	0	7	1.5	1	73.22
2	5	96	2.5	3	3.5	1	55.92
3	5	24	2.5	7	1.5	5	69.65
4	9	24	2.5	7	3.5	1	35.00
5	5	96	0	7	3.5	5	79.54
6	9	24	0	3	3.5	5	42.40
7	9	96	2.5	3	1.5	5	20.69
8	5	24	0	3	1.5	1	76.90

Table 7 PBD regression analysis for emulsification index

Noun	Coefficient	F. Inflation	Standard deviation	Signification
b0	56.665		3.632	*
b1	−13.837	1.00	3.632	*
b2	0.677	1.00	3.632	ns
b3	−11.350	1.00	3.632	*
b4	7.687	1.00	3.632	*
b5	−3.450	1.00	3.632	ns
b6	−3.595	1.00	3.632	ns

b0: the average effect; bi: the effect of the Xi factors used in the PBD experiments

* Significant; ns: not significant

Comparing the absolute value of the coefficients with their standard deviation, we find that three of the six factors were statistically significant in biosurfactant production, namely pH, sodium chloride concentration and rubber tree seed oil concentration. Non-significant factors should be fixed based on the signs of their coefficients for subsequent studies, as it has been reported that a variable with a negative concentration effect would have a greater impact at lower concentrations, while a factor with a positive concentration effect would have a better impact at higher concentrations [47]. Consequently, the incubation time factor should be set at its highest level (+ 1), and the NaNO₃ concentration and inoculum size should be set at their lowest levels (− 1), i.e. 96 h, 1.5 g/L and 1%, respectively, to further maximize biosurfactant production. Previous studies have shown that low inoculum levels are favourable for biosurfactant production [48, 49]. Other studies have also reported that limiting nitrogen concentration improves biosurfactant production [50].

3.3 Optimization of biosurfactant production using response surface methodology

3.3.1 Results of the Box-Behnken design experiment matrix

The BBD was used to optimize biosurfactant production using the three selected independent variables: initial pH, NaCl concentration and rubber tree seed oil concentration. The experimental matrix with the results is presented in Table 8. The experimental results show that the emulsification index values obtained vary from 18.18 to 92.06%. This variation in results suggests the effects of different factors studied on system response, hence the relevance of PBD results.

3.3.2 Development of a mathematical regression model of the emulsification index

Statistical analysis of the models shows that the quadratic model is the best-fitting model for describing the variation of the emulsification index as a function of pH, NaCl

concentration and rubber tree seed oil concentration. The values of R^2 and adjusted R^2 are 0.9995 and 0.9988, showing a correlation between the observed and predicted values [51, 52]. The points distributed very close to the regression line suggest this good correlation (Fig. 3a). Also, the very low coefficient of variation (CV 1.07%) indicates a high degree of precision and reliability of the experimental data [53]. Figure 3b shows a very random distribution of residual plots with no trend, which also confirms the model's suitability [51].

Regression analysis was used to establish a relationship between the emulsification index and the three independent variables. The suggested model equation is:

$$Y(\%) = 56.832 - 7.51X_1 - 22.1662X_2 - 3.47625X_3 - 15.24X_1X_2 - 3.405X_1X_3 - 5.1325X_2X_3 + 7.29275X_1^2 - 1.33475X_2^2 + 7.01525X_3^2 \quad (3)$$

where Y is the response (Emulsification Index) and X_1 , X_2 and X_3 , the coded values of the independent factors Initial pH, NaCl concentration and rubber tree seed oil concentration, respectively.

3.3.3 Analysis of variance (ANOVA) of the suggested quadratic model for the emulsification index

The ANOVA results obtained (Table 9) show an F value of 1471.44 ($F \geq 0.05$), which implies that the model is significant [54]. There is only a 0.01% chance that such a large F -value is due to noise. A good model must have a significant regression [55]. The F -value for the lack of fit of 2.77 implies that the lack of fit is not significant compared with the pure error. There is a 17.53% chance that such a large lack-of-fit F -value is due to noise. The results also show that all the terms in the model are significant at the 95% level (p -value < 0.05) [51]. These results indicate that pH, NaCl concentration and rubber tree seed oil concentration all have significant effects on biosurfactant production by *Pseudomonas aeruginosa* strain *Pa*. This confirms the relevance of the choice of variables. NaCl concentration was the most important factor in increasing the emulsification index. The interaction between pH and NaCl concentration was the strongest.

Table 8 BBD experimental design and response

Tests	X_1	X_2 %	X_3 %	Emulsification index (%)	
				Experimental values	Predicted values
1	5	0	5	76.92 ± 1.05	77.17
2	9	0	5	92.06 ± 0.46	92.69
3	5	2.5	5	64.00 ± 0.00	63.37
4	9	2.5	5	18.18 ± 0.24	17.69
5	5	1.25	3	78.72 ± 0.46	78.75
6	9	1.25	3	70.83 ± 1.54	70.48
7	5	1.25	7	78.26 ± 0.00	78.61
8	9	1.25	7	56.75 ± 0.19	56.72
9	7	0	3	83.33 ± 0.83	83.05
10	7	2.5	3	48.33 ± 0.77	48.92
11	7	0	7	86.96 ± 0.00	86.36
12	7	2.5	7	31.43 ± 0.00	31.71
13	7	1.25	5	56.91 ± 0.83	56.83
14	7	1.25	5	56.52 ± 1.07	56.83
15	7	1.25	5	56.52 ± 1.07	56.83
16	7	1.25	5	57.69 ± 0.00	56.83
17	7	1.25	5	56.52 ± 1.07	56.83

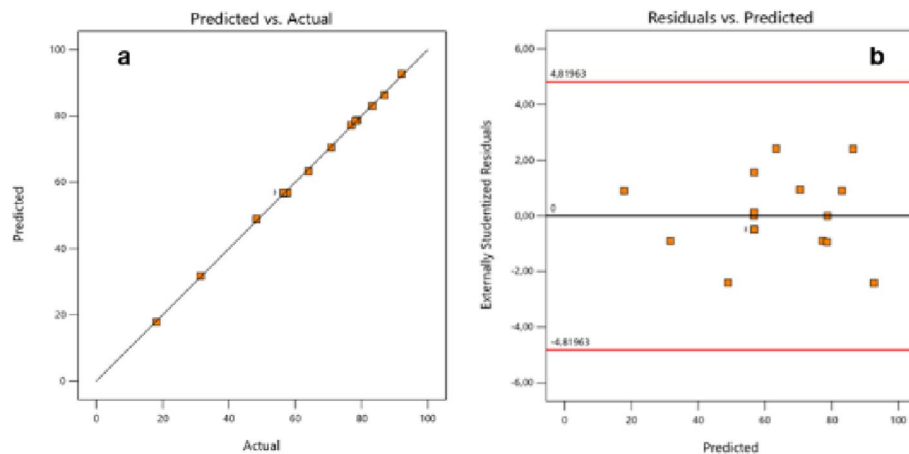


Fig. 3 Correlation between observed and predicted values of emulsification index (a), residual based on predicted response (b)

Table 9 Analysis of variance for emulsification index

Source	Sum of squares	df	Mean square	F-value	p-value	Significance
Model	6015.71	9	668.41	1471.44	< 0.0001	Significant
A – pH	451.20	1	451.20	993.27	< 0.0001	
B – NaCl	3930.74	1	3930.74	8653.11	< 0.0001	
C – RO	96.67	1	96.67	212.82	< 0.0001	
AB	929.03	1	929.03	2045.16	< 0.0001	
AC	46.38	1	46.38	102.09	< 0.0001	
BC	105.37	1	105.37	231.96	< 0.0001	
A ²	223.93	1	223.93	512.97	< 0.0001	
B ²	7.50	1	7.50	16.51	0.0048	
C ²	207.22	1	207.22	456.16	< 0.0001	
Residual	3.18	7	0.4543			
Lack of fit	2.15	3	0.7152	2.77	0.1753	Not significant
Pure error	1.03	4	0.2586			
Cor total	6018.89	16				

3.3.4 Influence of the factors studied on the emulsification index

Response surface plots were used to interpret the experimental results much more easily. 3D curves were plotted as a function of any two variables to study the effect of the interactions of the independent variables on the system response. The remaining variable was held at its central value.

Figure 4a shows the effect of the interaction of pH and NaCl concentration on the emulsification index, keeping the concentration of rubber tree seed oil constant. The results show that the emulsification index is strongly influenced by the pH/NaCl concentration interaction. The emulsification index decreased with NaCl concentration for all the pH values studied. This variation is greater for higher pH values. For NaCl concentrations below 0.5%, the emulsification index increases with pH, while for higher NaCl concentrations, the emulsification index decreases with pH. Maximum emulsification index values were observed at NaCl concentrations below 0.5% and pH above 7.

Figure 4b shows the effect of the interaction of NaCl concentration and rubber tree seed oil concentration on the emulsification index, keeping the pH constant. The figure shows that the emulsification index varies considerably with the NaCl concentration, whatever the rubber tree seed oil concentration used, and slightly with the oil

concentration. The emulsification index decreases with NaCl concentration. Maximum emulsification index values are observed at NaCl concentrations below 0.5%. These results show that the *Pseudomonas aeruginosa* strain Pa was not capable of metabolism under high salinity conditions. Previous studies have reported that limiting concentrations of magnesium, calcium, potassium, sodium salts and trace elements resulted in higher yields of biosurfactants produced by *Pseudomonas aeruginosa* [56]. In previous studies, high biosurfactant production by *Pseudomonas aeruginosa* RS29 was obtained at salinity below 0.8% and alkaline pH between 7 and 8 [57].

Figure 4c shows the effect of the interaction between pH and the concentration of rubber tree seed oil on the emulsification index, keeping the NaCl concentration constant. The results obtained show a slight variation in the emulsification index with the pH/rubber tree seed oil concentration interaction.

3.3.5 Determining the optimum conditions for the production of BS

The biosurfactant production process was optimized using the Desirability Function Approach. The results showed that optimal conditions for biosurfactant production corresponded to a pH of 8.7, a NaCl concentration of 0.072% and an oil concentration of 6.91% ($\approx 7\%$). The predicted emulsification index under these conditions was 92.492%.

3.3.6 Model validation

To validate the model developed, three cultures of the *Pseudomonas aeruginosa* strain Pa were carried out under optimal conditions. The experimental response of the

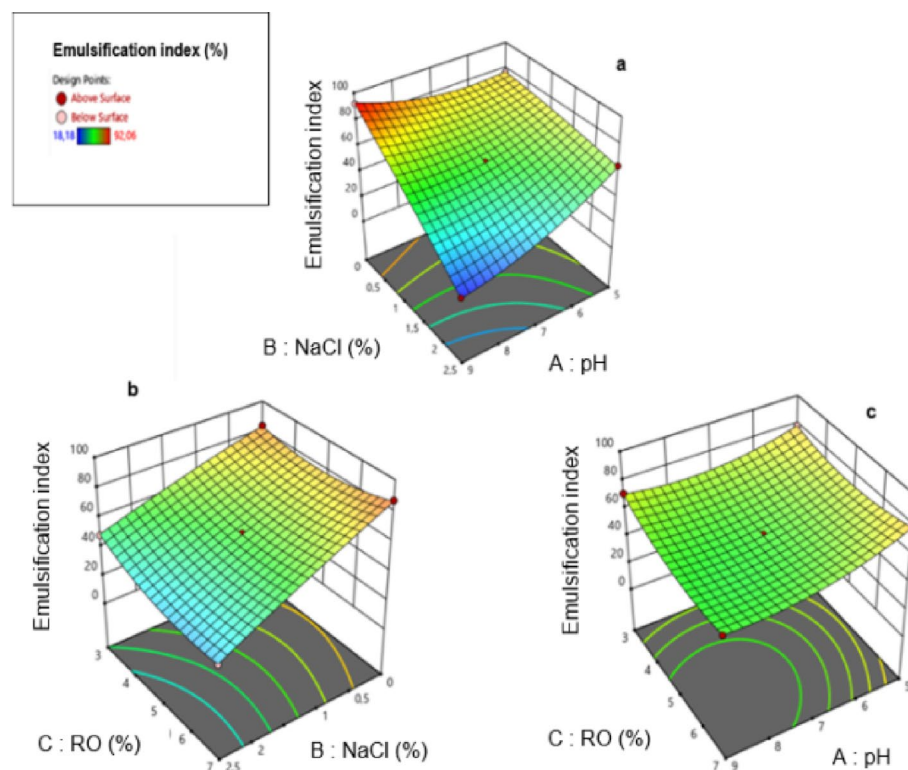


Fig. 4 Graph in (3D) showing the effect of pH and NaCl concentration on the emulsification index (a), the effect of rubber tree seed oil concentration and NaCl concentration on the emulsification index (b), and the effect of rubber tree seed oil concentration and pH on the emulsification index (c)

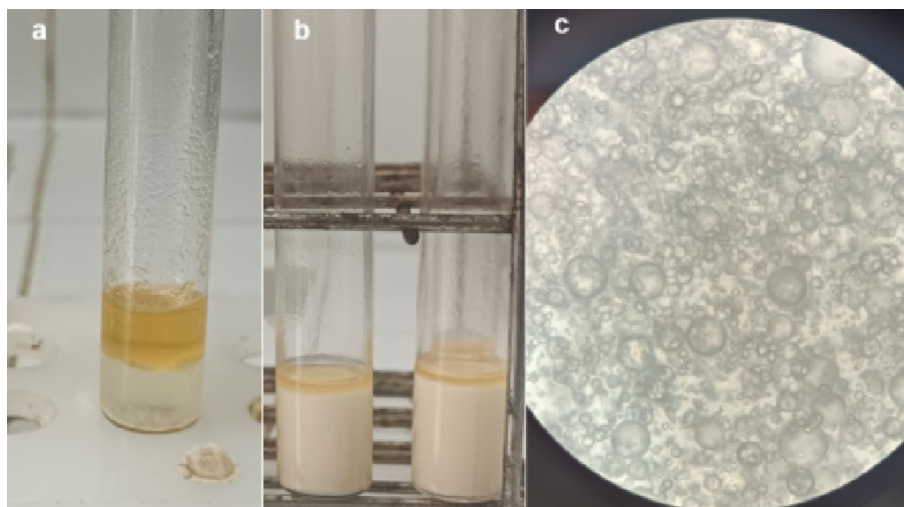


Fig. 5 Emulsification test; **a** control (engine oil+distilled water), **b** emulsion obtained under optimized conditions, **c** emulsion observed using an optical microscope

Table 10 Optimal conditions and model validation

pH	NaCl (%)	RO (%)	%E ₂₄		Relative error (%)
			Predicted	Experimental	
8.7	0.072	6.91	92.492	92.15	0.37

emulsification index obtained was $92.15 \pm 0.89\%$. The emulsion obtained under optimum conditions is shown in Fig. 5.

The experimental response was found to agree with the predicted response of 92.492%. The percentage error between the experimental and predicted responses was 0.37%. This low error confirms the accuracy of the model and the reproducibility of the experiments [58]. This means that the model developed is valid and can be used to predict the value of the emulsification index at any point in the experimental field. The validation of the model is given in Table 10.

By comparing the results with others obtained, the current results can be considered interesting, with some works showing higher emulsification index values and others lower. Bezza and Chirwa produced a biosurfactant from a strain of *Bacillus subtilis* grown on mineral salts and glycerol with a motor oil emulsifying activity equal to 76% [59]. Ehrhardt et al. synthesized a biosurfactant from pineapple processing residues enriched with 3% glycerol, giving a motor oil emulsification capacity of up to 62.5% [60]. Pardhi et al. produced a biosurfactant using a *Pseudomonas guguanensis* D30 strain capable of emulsifying motor oil to 80% [47]. Batista et al. obtained emulsification indices for biosurfactants produced by a strain of *Candida tropicalis* in different media, ranging from 85.51 to 94.06% against motor oil [61].

3.4 Effect of various commercial carbon sources on biosurfactant production under optimal conditions

To assess the effectiveness of rubber tree seed oil as a carbon substrate for biosurfactant production, various commercial carbon sources were tested for comparison. The results obtained (Fig. 6) show that the emulsification index is strongly influenced by the carbon source. The emulsification index of the cell-free supernatant was 92.15%,

79.70%, 66.67%, 63%, 63%, 30% and 12.5% for rubber tree seed oil (RO), glucose, glycerol, fructose, sucrose, mannitol and control, respectively. Comparison of the results obtained shows that rubber tree seed oil could constitute an alternative carbon source to commercial carbon sources for improving biosurfactant production by *Pseudomonas aeruginosa* strain Pa. This would reduce biosurfactant production costs. Studies have reported that the emulsifying activity of a biosurfactant produced by *Pseudomonas fluorescens* was higher in media containing vegetable oils as substrates [62]. The vegetable oil (olive oil) used in biosurfactant production by a strain of *Pseudomonas fluorescens* gave a higher emulsification index than glucose and hexadecane [63]. Previous studies have also shown that vegetable oil was a better carbon substrate than carbohydrates for biosurfactant production by *Pseudomonas* strains [18]. Vegetable oil is one of the first substrates reported for high yields of biosurfactants [64].

3.5 Extracted biosurfactant

This study tested different solvent systems to extract biosurfactant from the cell-free supernatant of *Pseudomonas aeruginosa* grown under optimal conditions with rubber tree seed oil as the sole carbon source.

The biosurfactant extracted by all the solvents tested was a viscous, sticky, oily, yellowish-brown product. This appearance is consistent with that described in the literature [65–67]. However, other reports mention biosurfactants as a white powder [68]. The common appearance of the BS extracted by these different solvents could be explained by the fact that the appearance of the biosurfactants produced can differ mainly from one microorganism to another. In this study, various solvents were used to extract biosurfactants produced by the same organism (*Pseudomonas aeruginosa* strain Pa). The colorless appearance of the biosurfactant turning into a white powder described above was produced by *S. bombicola* ATCC 22214.

The different biosurfactant yields obtained using different extraction solvents are shown in Table 11. Among the different solvent systems used, diethyl ether and ethyl

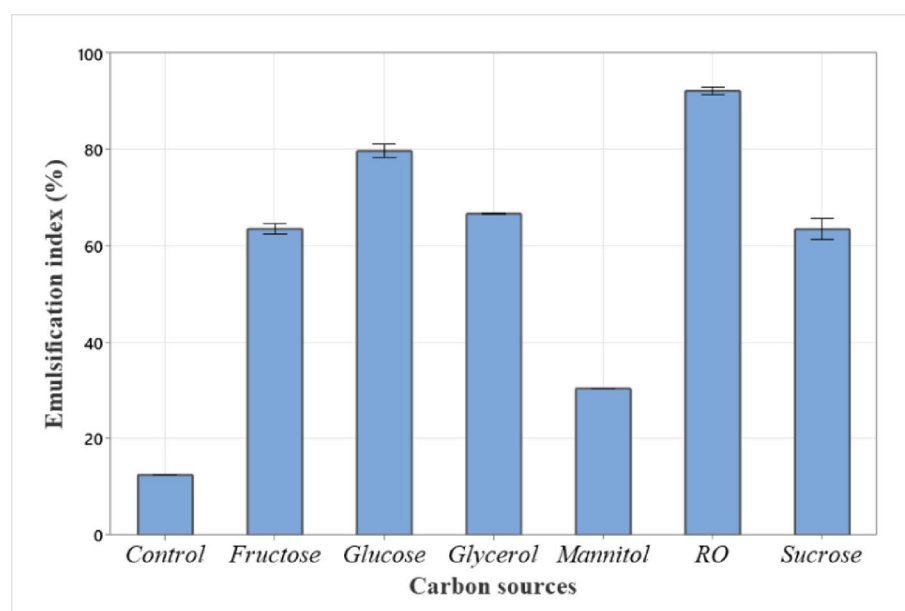


Fig. 6 Effect of different carbon sources on biosurfactant production

Table 11 Quantity of biosurfactant extracted using different solvent systems

Extraction solvent	Biosurfactant extracted (g/L)
Diethyl ether	10.37 ± 0.51 ^a
Ethyl acetate – methanol (2:1)	10.22 ± 0.17 ^a
Ethyl acetate – methanol (3:1)	6.83 ± 0.24 ^b
Chloroform – methanol (1:1)	6.50 ± 0.70 ^b
Chloroform – methanol (2:1)	6.50 ± 0.00 ^b
Dichloromethane	3.00 ± 0.00 ^c
Ethyl acetate	6.50 ± 0.70 ^b

Values are means ± standard error of the means. Means not sharing the same letters are significantly different

acetate–methanol (2:1) proved to be the best solvents for extracting the biosurfactant produced by *Pseudomonas aeruginosa* with more than 10 g of biosurfactant extracted per liter of cell-free supernatant followed by ethyl acetate, ethyl acetate–methanol (3:1), chloroform–methanol (1:1) and chloroform–methanol (2:1) with over 6 g/L while dichloromethane gave the lowest yield of 3 g/L. In this study, diethyl ether was chosen for biosurfactant extraction as it has been reported in previous work that this type of solvent is cheap, less toxic, readily available and can be used to significantly reduce biosurfactant extraction costs and minimize environmental risks [69].

4 Conclusion

This study aimed to optimize biosurfactant production by *Pseudomonas aeruginosa* strain Pa using rubber tree seed oil (*Hevea brasiliensis*) as the sole carbon source. The factors significantly influencing biosurfactant production selected using the Plackett–Burman design were pH, NaCl concentration and rubber tree seed oil concentration. These three selected variables were optimized by response surface methodology using the Box–Behnken design. Optimal factor levels were obtained for an initial pH of 8.7, a NaCl concentration of 0.072% and a rubber tree seed oil concentration of 6.91%. The emulsification index observed under these conditions was 92.15%. Diethyl ether was used to extract the maximum amount of biosurfactant from the cell-free supernatant of *Pseudomonas aeruginosa*. The present study illustrated the use of rubber tree seed oil as an efficient carbon source to replace commercial carbon sources for biosurfactant production by *Pseudomonas aeruginosa*.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Y.J.A., A.O.T., K.D. M.A., M.N.A.K., A.P.D. and K.B.Y. The first draft of the manuscript was written by Y.J.A., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This study was funded by the African Center of Excellence for Waste-to-Value (CEA-VALOPRO) and by the European Union through the RESING intra-African academic mobility project.

Data availability

Data can be made available on request.

Declarations

Ethics approval and consent to participate

The collection of plant material complied with all applicable local and national guidelines. No permissions or licenses were required for the collection of *Hevea brasiliensis* seeds from plantations.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Received: 30 March 2025 / Accepted: 21 July 2025

Published online: 01 September 2025

References

1. Olasanmi IO, Thring RW. The role of biosurfactants in the continued drive for environmental sustainability. *Sustainability*. 2018. <https://doi.org/10.3390/su10124817>.
2. Ozdal M, Gurkok S, Ozdal OG. Optimization of rhamnolipid production by *Pseudomonas aeruginosa* OG1 using waste frying oil and chicken feather peptone. *Biotech*. 2017. <https://doi.org/10.1007/s13205-017-0774-x>.
3. Johnson P, Trybala A, Starov V, Pinfield VJ. Effect of synthetic surfactants on the environment and the potential for substitution by biosurfactants. *Adv Coll Interface Sci*. 2020;288:102340. <https://doi.org/10.1016/j.cis.2020.102340>.
4. Jimoh AA, Lin J. Biosurfactant: a new frontier for greener technology and environmental sustainability. *Ecotoxicol Environ Saf*. 2019;184:109607. <https://doi.org/10.1016/j.ecoenv.2019.109607>.
5. Clements T, Ndlovu T, Khan S, Khan W. Biosurfactants produced by *Serratia species*: classification, biosynthesis, production and application. *Appl Microbiol Biotechnol*. 2019;103(2):589–602. <https://doi.org/10.1007/s00253-018-9520-5>.
6. Inamuddin, I., Ahamed, M.I., Lichtfouse, E.: *Water pollution and remediation: photocatalysis*. vol. 57. in *Environmental Chemistry for a Sustainable World*. vol. 57. Springer International Publishing. (2021). <https://doi.org/10.1007/978-3-030-54723-3>
7. Magalhães ERB, Silva FL, Sousa MADSB, Santos ESD. Use of different agroindustrial waste and produced water for biosurfactant production. *Biosci Biotechnol Res Asia*. 2018;15(1):17–26. <https://doi.org/10.13005/bbra/2604>.
8. Pele MA, et al. Conversion of renewable substrates for biosurfactant production by *Rhizopus arrhizus* UCP 1607 and enhancing the removal of diesel oil from marine soil. *Electron J Biotechnol*. 2015;38:40–8. <https://doi.org/10.1016/j.ejbt.2015.12.003>.
9. Singh P, Patil Y, Rale V. Biosurfactant production: emerging trends and promising strategies. *J Appl Microbiol*. 2019;126(1):2–13. <https://doi.org/10.1111/jam.14057>.
10. Eras-Muñoz E, Farré A, Sánchez A, Font X, Gea T. Microbial biosurfactants: a review of recent environmental applications. *Bioengineered*. 2022;13(5):12365–91. <https://doi.org/10.1080/21655979.2022.2074621>.
11. Sharma R, Singh J, Verma N. Optimization of rhamnolipid production from *Pseudomonas aeruginosa* PBS towards application for microbial enhanced oil recovery. *3 Biotech*. 2018. <https://doi.org/10.1007/s13205-017-1022-0>.
12. Vieira IMM, et al. Potential of pineapple peel in the alternative composition of culture media for biosurfactant production. *Environ Sci Pollut Res*. 2021;28(48):68957–71. <https://doi.org/10.1007/s11356-021-15393-1>.
13. de Oliveira Schmidt VK, et al. Cassava wastewater valorization for the production of biosurfactants: surfactin, rhamnolipids, and mannosylerythritol lipids. *World J Microbiol Biotechnol*. 2022;39(2):65. <https://doi.org/10.1007/s11274-022-03510-2>.
14. Domínguez Rivera A, Martínez Urbina MA, López y López VE. Advances on research in the use of agro-industrial waste in biosurfactant production. *World J Microbiol Biotechnol*. 2019;35(10):155.
15. Sarubbo LA, et al. Biosurfactants: production, properties, applications, trends, and general perspectives. *Biochem Eng J*. 2022;181:108377. <https://doi.org/10.1016/j.bej.2022.108377>.
16. Moshtagh B, Hawboldt K, Zhang B. Optimization of biosurfactant production by *Bacillus Subtilis* N3–1P using the brewery waste as the carbon source. *Environ Technol*. 2019;40(25):3371–80. <https://doi.org/10.1080/09593330.2018.1473502>.
17. Stogti S, Tiwari S, Ratna S, Kumar R. Utilization of agro-industrial waste for biosurfactant production under submerged fermentation and its synergistic application in biosorption of Pb²⁺. *Bioresour Technol Rep*. 2021;15:100706. <https://doi.org/10.1016/j.biteb.2021.100706>.
18. Sathi Reddy K, Yahya Khan M, Archana K, Gopal Reddy M, Hameeda B. Utilization of mango kernel oil for the rhamnolipid production by *Pseudomonas aeruginosa* DR1 towards its application as biocontrol agent. *Bioresour Technol*. 2016;221:291–9. <https://doi.org/10.1016/j.biortech.2016.09.041>.
19. Kumar AP, Janardhan A, Radha S, Viswanath B, Narasimha G. Statistical approach to optimize production of biosurfactant by *Pseudomonas aeruginosa* 2297. *3 Biotech*. 2015;5(1):71–9. <https://doi.org/10.1007/s13205-014-0203-3>.
20. Nasir MS, Mohd Yahya AR, Md Noh NA. Agro-industrial wastes as potential substrates for rhamnolipid production by *Pseudomonas aeruginosa* USM-AR2. *Trop Life Sci Res*. 2024;35(1):33–47. <https://doi.org/10.21315/tlsr.2024.35.1.3>.
21. Verheye, W.: *Growth and production of rubber* (2010)
22. Priyadarshan PM. Refinements to Hevea rubber breeding. *Tree Genet Genomes*. 2017;13(1):20. <https://doi.org/10.1007/s11295-017-1101-8>.
23. Penot, E., Thaler, P., Nouvellon, Y., Chambon, B., Sainte-Beuve, J.: Hévée. Etat des lieux sur la déforestation et les standards de durabilité, Rapport d'étude pour le CST Forêt de l'AFD (2020)
24. Onoji SE, Iyuke SE, Igbafe AI, Nkazi DB. Rubber seed oil: a potential renewable source of biodiesel for sustainable development in sub-Saharan Africa. *Energy Convers Manage*. 2016;110:125–34. <https://doi.org/10.1016/j.enconman.2015.12.002>.
25. Soualiho B, Yao J-CN, John KK, Losseyini K, Augustin AA. Evaluation of the chemical and biological properties of oil extracted from detoxified rubber tree (*Hevea Brasiliensis*) kernels. *Am J BioSci*. 2022. <https://doi.org/10.11648/j.ajbio.20221006.16>.
26. Akossi MJC, Kouassi KE, Abollé A, Ouedraogo WKI, Yao KB. Transesterification of crude rubber oil catalyzed by lipase extract powder of germinated rubber kernels for biodiesel production. *Energies*. 2025. <https://doi.org/10.3390/en18051252>.
27. Gimbin J, et al. Biodiesel production from rubber seed oil using a limestone based catalyst. *AMPC*. 2012;02(04):138–41. <https://doi.org/10.4236/ampc.2012.248036>.
28. Praharyawan S, Susilaningih D, Syamsu K. Statistical screening of medium components by plackett-burman experimental design for biosurfactant production by Indonesia *Indigenous Bacillus* SPDSW17. *Asian J Microbiol Biotechnol Environ Exp Sci*. 2013;15:805–13.
29. Kumar PN, Swapna TH, Khan MY, Reddy G, Hameeda B. Statistical optimization of antifungal iturin A production from *Bacillus amyloliquefaciens* RHNK22 using agro-industrial wastes. *Saudi J Biol Sci*. 2017. <https://doi.org/10.1016/j.sjbs.2015.09.014>.
30. Tiwary M, Dubey AK. Characterization of biosurfactant produced by a novel strain of *Pseudomonas aeruginosa*, Isolate ADMT1. *J Surfact Detergents*. 2018;21(1):113–25. <https://doi.org/10.1002/jsde.12021>.
31. Ebadipour N, Lotfabad TB, Yaghmaei S, RoostaAzad R. Optimization of low-cost biosurfactant production from agricultural residues through response surface methodology. *Prep Biochem Biotechnol*. 2016;46(1):30–8. <https://doi.org/10.1080/10826068.2014.979204>.
32. Jawan R, et al. Evaluation of the estimation capability of response surface methodology and artificial neural network for the optimization of bacteriocin-like inhibitory substances production by *Lactococcus lactis* Gh1. *Microorganisms*. 2021;9(3):579. <https://doi.org/10.3390/microorganisms9030579>.

33. Samyannu M, Achary A. Utilization of agro-industry residue for rhamnolipid production by *P. aeruginosa* AMB AS7 and its application in chromium removal. *Appl Biochem Biotechnol*. 2017;183(1):70–90. <https://doi.org/10.1007/s12010-017-2431-6>.
34. Ouattara LY, et al. Optimization of the autoclave-assisted alkaline delignification of cocoa (*Theobroma cacao*) pod husks using KOH to maximize reducing sugars. *BioResources*. 2022;17(1):826.
35. Ali F, et al. Production optimization, stability and oil emulsifying potential of biosurfactants from selected bacteria isolated from oil-contaminated sites. *Royal Soc Open Sci*. 2021;8(10):211003. <https://doi.org/10.1098/rsos.211003>.
36. Mnif I, Chaabouni-Ellouze S, Ghribi D. Optimization of the nutritional parameters for enhanced production of *B. subtilis* SPB1 biosurfactant in submerged culture using response surface methodology. *Biotechnol Res Int*. 2012. <https://doi.org/10.1155/2012/795430>.
37. Amadou Kiari M, et al. Process conditions optimization of plant waste-derived microporous activated carbon using a full factorial design and genetic algorithm. *J Mater Environ Sci*. 2022;13:884–99.
38. Sharon AB, Ahuekwe EF, Nzubechi EG, Oziegbe O, Oniha M. Statistical optimization strategies on waste substrates for solving high-cost challenges in biosurfactants production: a review. *IOP Conf Ser Earth Environ Sci*. 2023;1197(1):012004. <https://doi.org/10.1088/1755-1315/1197/1/012004>.
39. Rawat G, et al. Exploring the potential of novel *Bacillus* sp. G6: isolation, characterization, and optimization of biosurfactant production from oil contaminated soil. *J Mol Liquids*. 2024;396:124013. <https://doi.org/10.1016/j.molliq.2024.124013>.
40. Kostik V, Memeti S, Bauer B. fatty acid composition of edible oils and fats. *J Hyg Eng Des*. 2013;4:112–6.
41. Koffi EN, Niamketchi LG, Koffi Guéi JF, Konan MK, Anin L. Chemical analysis and antifungal activity of rubber seed oil (*Hevea brasiliensis*) from the Southern region of Cote d'Ivoire. *J Mater Environ Sci*. 2022;13(4):382–90.
42. Onoji SE, Iyuke SE, Igbafe AI. *Hevea brasiliensis* (Rubber Seed) oil: extraction, characterization, and kinetics of thermo-oxidative degradation using classical chemical methods. *Energy Fuels*. 2016;30(12):10555–67. <https://doi.org/10.1021/acs.energyfuels.6b02267>.
43. Makkar RS, Cameotra SS. Effects of various nutritional supplements on biosurfactant production by a strain of *Bacillus subtilis* at 45°C. *J Surfact & Detergents*. 2002;5(1):11–7. <https://doi.org/10.1007/s11743-002-0199-8>.
44. Santos D, Rufino R, Luna J, Santos V, Sarubbo L. Biosurfactants: multifunctional biomolecules of the 21st century. *IJMS*. 2016;17(3):401. <https://doi.org/10.3390/ijms17030401>.
45. Wei Y-H, Lai C-C, Chang J-S. Using Taguchi experimental design methods to optimize trace element composition for enhanced surfactin production by *Bacillus subtilis* ATCC 21332. *Process Biochem*. 2007;42(1):40–5. <https://doi.org/10.1016/j.procbio.2006.07.025>.
46. Gudiña EJ, Fernandez EC, Rodrigues AI, Teixeira JA, Rodrigues LR. Biosurfactant production by *Bacillus subtilis* using corn steep liquor as culture medium. *Front Microbiol*. 2015. <https://doi.org/10.3389/fmicb.2015.00059>.
47. Pardhi D, Panchal R, Rajput K. Screening of biosurfactant producing bacteria and optimization of production conditions for *Pseudomonas guguanensis* D30. *Biosci Biotechnol Res Commun*. 2020;13(1):170–9.
48. Sharma R, Singh J, Verma N. Optimization of rhamnolipid production from *Pseudomonas aeruginosa* PBS towards application for microbial enhanced oil recovery. *3 Biotech*. 2018;8(1):20. <https://doi.org/10.1007/s13205-017-1022-0>.
49. Umar, A., Zafar, A., Wali, H., Siddique, M.P., Malik, Z.A. AHMED, S.: Surfactin-like biosurfactant production and optimization by *Bacillus Subtilis* SNW3 product characterization and its influence on seed development and plant growth. (2021); <https://doi.org/10.21203/rs.3.rs-550205/v1>
50. Deepika KV, Raghuram M, Bramhachari PV. Rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* strain KVD-HR42 isolated from oil contaminated mangrove sediments ». *AJMR*. 2017;11(6):218–31. <https://doi.org/10.5897/AJMR2015.7881>.
51. Amadou Kiari MN, et al. Adsorption kinetics, thermodynamics, modeling and optimization of bisphenol A on activated carbon based on *Hyphaene Thebaica* shells. *Case Stud Chem Environ Eng*. 2024;10:100903. <https://doi.org/10.1016/j.csee.2024.100903>.
52. Deepika KV, Kalam S, Perali R, Podile A, Chari B. Optimization of rhamnolipid biosurfactant production by mangrove sediment bacterium *Pseudomonas aeruginosa* KVD-HR42 using response surface methodology. *Biocatal Agric Biotechnol*. 2015. <https://doi.org/10.1016/j.bcab.2015.11.006>.
53. Almeida DG, Soares Da Silva RDCF, Luna JM, Rufino RD, Santos VA, Sarubbo LA. Response surface methodology for optimizing the production of biosurfactant by *Candida tropicalis* on industrial waste substrates. *Front Microbiol*. 2017. <https://doi.org/10.3389/fmicb.2017.00157>.
54. Rauf MA, Marzouki N, Körbahti BK. Photolytic decolorization of *Rose Bengal* by UV/H₂O₂ and data optimization using response surface method. *J Hazard Mater*. 2008;159(2–3):602–9. <https://doi.org/10.1016/j.jhazmat.2008.02.098>.
55. Ouattara LY, et al. Optimization of the autoclave-assisted alkaline delignification of cocoa (*Theobroma cacao*) pod husks using KOH to maximize reducing sugars. *BioRes*. 2021;17(1):826–48. <https://doi.org/10.15376/biores.17.1.826-848>.
56. Rismani E, Fooladi J, Por GH. Biosurfactant production in batch culture by a *Bacillus licheniformis* isolated from the Persian Gulf. *Pakistan J Biol Sci*. 2006;9(13):2498–502. <https://doi.org/10.3923/pjbs.2006.2498.2502>.
57. Saikia RR, Deka S, Deka M, Banat IM. Isolation of biosurfactant-producing *Pseudomonas aeruginosa* RS29 from oil-contaminated soil and evaluation of different nitrogen sources in biosurfactant production. *Ann Microbiol*. 2012;62(2):753–63. <https://doi.org/10.1007/s13213-011-0315-5>.
58. Diop, A., Faye M., Diedhiou, D., Diop C.M.: Optimization and modelling of the preparation of activated carbons from neem (*Azadirachta indica* A. Juss) seed hulls based on Response Surface Methodology (RSM). vol. 11 (2023)
59. Bezza FA, Chirwa MN. Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by *Bacillus subtilis* CN2. *Biochem Eng J*. 2015;101:168–78. <https://doi.org/10.1016/j.bej.2015.05.007>.
60. Ehrhardt D, Secato J, et al. Biosurfactant production by *Bacillus subtilis* using the residue from processing of pineapple, enriched with glycerol, as substrate. *Chem Eng Trans*. 2015;43:277–82. <https://doi.org/10.3303/CET1543047>.
61. Batista RM, Rufino RD, Luna JM, de Souza JEG, Sarubbo LA. Effect of medium components on the production of a biosurfactant from *Candida tropicalis* applied to the removal of hydrophobic contaminants in soil. *Water Environ Res*. 2010;82(5):418–25. <https://doi.org/10.2175/106143009X12487095237279>.
62. Stojmenova E, Vasileva-Tonkova E, Sotirova A, Galabova D, Lalchev Z. Evaluation of different carbon sources for growth and biosurfactant production by *Pseudomonas fluorescens* isolated from wastewaters. *Zeitschrift für Naturforschung C*. 2009;64(1–2):96–102. <https://doi.org/10.1515/znc-2009-1-216>.

63. Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi A. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. *Desalination*. 2009;223(1):143–51. <https://doi.org/10.1016/j.desal.2007.01.198>.
64. Adetunji AI, Olaniran AO. Production and potential biotechnological applications of microbial surfactants: an overview. *Saudi J Biol Sci*. 2021;28(1):669–79. <https://doi.org/10.1016/j.sjbs.2020.10.058>.
65. Kashif A, et al. Current advances in the classification, production, properties and applications of microbial biosurfactants—A critical review. *Adv Colloid Interface Sci*. 2022;306:102718. <https://doi.org/10.1016/j.cis.2022.102718>.
66. Makkar RS, Cameotra SS, Banat IM. Advances in utilization of renewable substrates for biosurfactant production. *AMB Express*. 2011;1(1):5. <https://doi.org/10.1186/2191-0855-1-5>.
67. Soares da Silva RDCF, et al. Production and characterization of a new biosurfactant from *Pseudomonas cepacia* grown in low-cost fermentative medium and its application in the oil industry. *Biocatal Agric Biotechnol*. 2017. <https://doi.org/10.1016/j.bcab.2017.09.004>.
68. Eras-Muñoz E, Gea T, Font X. Carbon and nitrogen optimization in solid-state fermentation for sustainable sophorolipid production using industrial waste. *Front Bioeng Biotechnol*. 2023. <https://doi.org/10.3389/fbioe.2023.1252733>.
69. Gandhimathi R, Seghal Kiran G, Hema TA, Selvin J, Rajeetha Raviji T, Shanmughapriya S. Production and characterization of lipopeptide biosurfactant by a sponge-associated marine *actinomyces Nocardopsis alba* MSA10. *Bioprocess Biosyst Eng*. 2009;32(6):825–35. <https://doi.org/10.1007/s00449-009-0309-x>.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.