Journal of Scientific & Industrial Research
Vol. 81, June 2022, pp. 613-621

Synthesis and Optimization of Rhamnolipids from Tree Borne Oils and Fats for Cosmetics Applications

Anurag Bapat[1], Ashwini Meshram[1], Subhalaxmi Pradhan[2] and Chandu S Madankar[1]*

[1]Department of Oils, Oleochemicals and Surfactants Technology, Institute of Chemical Technology, Mumbai 400 019, India

[2]Department of Basic Sciences, School of Basic and Applied Sciences, Galgotias University, UP, India

Received 29 March 2022; revised 27 April 2022; accepted 27 April 2022

Rhamnolipids are bio-surfactants with strong surface active characteristics. These are potential additives for cosmetic formulations. The production of these molecules is possible through various routes, however, their synthesis from renewable sources using bio-agents can be environment friendly and economical. Studies on bacteria are in focus for the development of rhamnolipid bio-surfactant. *Pseudomonas aeruginosa* is one of the bacteria being studied in depth because of its efficiency to form rhamnolipids from a range of carbon, nitrogen, substrate sources, such as tree borne oils, modified fats and blends. The rhamnolipid synthesis process was improved using response surface approach. The produced rhamnolipid has been used for preparing a cosmetic formulation. The product has shown good stability. The current study's findings indicated that the use of mathematical modeling is extremely successful in determining optimum reaction conditions and increasing the yield of a specific bio-process.

Keywords: Modified fat blends, *Pseudomonas aeruginosa*, Rhamnolipids, Surfactant

Introduction

The term surfactant refers to a surface active agent. Surfactant is a chemical grade that has the potential to be used in a wide variety of applications including detergents, paints, paper products, medicines, cosmetics, petroleum, and food and water purification.[1] It is an amphipathic molecule with both hydrophilic and hydrophobic (mainly hydrocarbon) moieties that partition preferentially at the interface of liquid phases with varying degrees of polarity and hydrogen bonding, such as e.g. oil/water or air/water interfaces. It is divided into two categories: synthetic surfactants and bio-surfactants. Synthetic surfactants, which are formed by organic chemical processes and are therefore not biodegradable, remain in the soil and groundwater as well as in agricultural products.[2] As a result, they have a negative influence on the environment and humans. Because of its flexible character, such as low toxicity, high reactivity at multiple temperatures, and most importantly, biodegradability and environmental compatibility, microbial surfactants have progressively grown.

Bio-surfactants are classified based on the types of bio-surfactant producing microbial species and the chemical structure of their products and have applications in crude oil recovery, health-care food processing, and bio-remediation of contaminated-site.[3] Bio-surfactants comprise of lipopeptides and lipoproteins, glycolipids, phospholipids, and polymeric surfactants. The most commonly isolated and studied forms of bio-surfactant are glycolipids, which are composed of carbohydrates and long-chain aliphatic acids. Given the properties listed above, rhamnolipid is one of the best instances of glycolipid.

Rhamnolipids are physicochemically and biologically advantageous secondary metabolites. The high cost of manufacturing, however, is the principal impediment to widespread use of bio-surfactants. The use of low cost renewable substrates from a range of industries, including agriculture (sugar, molasses, plant oil, oil wastes), distillery wastes, animal fat, and the oil industry, food processing industry (used cooking oil) has been proposed as a potential method to lessen the bio-surfactant manufacturing costs.[4,5] Besides cost, rhamnolipid synthesis has a number of other difficulties, including poor cellular yield, high cost of raw materials, costly downstream processing, and a lack of information on specific reaction conditions.

Rhamnolipids, the most explored bio-surfactant is synthesized mainly by *Pseudomonas aeruginosa*. These microbial species can be located in a range

*Author for Correspondence
E-mail: chandumadankar@gmail.com

of settings, including soil, water, and plants, and they can also infect humans. Under the appropriate circumstances, this bacterium produces the glycolipid bio-surfactant rhamnolipid.[6] The synthesis of rhamnolipid from *pseudomonas aeruginosa* is aided by a number of reaction variables, including carbon and nitrogen feed, temperature, pH, salinity, carbon/nitrogen ratio, and so on.[7] Bacterial growth and substrate pattern have the greatest impact on rhamnolipid synthesis. As a result, optimising and maintaining the growth conditions that affect biosurfactant production have become critical components in the development of a cost-effective bioprocess.

Several research utilizing various optimization methodologies have been undertaken to optimise process parameters for rhamnolipid synthesis, with response surface methodology (RSM) being the most promising method for process parameter optimization in bio-surfactant production.[8] RSM is a significant statistical approach for improving the efficiency of complicated operations. It can cut down on the number of trials required to assess numerous factors and their interactions. Box-Behnken design is a form of response surface design that has been utilized in a variety of studies. RSM is an excellent approach for this investigation since it offers a statistical model that can be utilized to determine the link between the improved parameters.[9]

Rhamnolipids, one of the popular bio-surfactant are generally synthesized on tree borne oils, modified fats and blends, and waste cooking oil or chicken as a carbon source, but, large-scale production for most microbial surface-active agents has not achieved a suitable economical level due to their poor yields. Furthermore, downstream processing to recover and purify microbial surfactants requires a high-cost input.[10,11] These challenges can be resolved by using waste material as a growth substrate for producing bio-surfactants. As a result, the objective of this research is to use tree borne oil as a substrate to improve the production of rhamnolipid bio-surfactant and to use RSM to determine the optimal conditions. By studying the components in various proportions and concentrations, a quadratic model of response surface methodology was developed.

Material & Methods

Raw Materials

All of the materials used in the investigation were of analytical grade. Tree borne oils was procured from local market. Industrial grade Hexane (CAS No.110-54-3), methanol, Ethyl acetate were purchased from MSDS Fine Chemicals Ltd. Mumbai. Other chemical such as glycerol (AR grade 99% pure) was purchased from the department of oils, oleochemicals & surfactant technology, ICT Mumbai. Culture media, trace elements, mineral salts, yeast extract were purchased from Hi-media laboratory Mumbai.

Micro-Organism

Pseudomonas aeruginosa MTCC 9027 strain was purchased from the Microbial type culture collection (MTCC), Chandigarh India. For antimicrobial testing *Bacillus subtilis* stain were purchased from the Microbial type culture collection (MTCC) Chandigarh, India.

Cultivation Conditions

Pseudomonas aeruginosa MTCC 9027 was preserved on nutrient agar slants. A loopful of culture cells were scraped from the nutrient agar slant and added to 50 ml inoculum medium with the following composition (g/L): peptone: 5.0, sodium chloride: 5.0 Yeast extract: 1.5, pH:7.4, HM Peptone B:1.5, pH: 7.4 for a 7-day incubation period. *Pseudomonas aeruginosa* was cultivated at 30°C and 150 rpm.

For the development of the isolates, the following components (g/L) were used: $NaNO_3$ 4.0, NaCl 1.0, KCl 1.0, $CaCl_2.2H_2O$ 0.1, KH_2PO_4 3.0, $Na_2HPO_4.12H_2O$ 3.0, $MgSO_4$ 2.0, $FeSO_4.7H_2O$ 0.001. Trace element stock solution was prepared with the composition (g/L): $FeCl_3.6H_2O$ 0.08, $ZnSO_4.7H_2O$ 0.75, $CuSO_4.5H_2O$ 0.075, $MnSO_4.H_2O$ 0.75, H_3BO_3 0.15, $Na_2MOO_4.2H_2O$ 0.05.[(12)]

Before autoclaving, the pH of the medium was adjusted to 6.8. Autoclaved tree borne oil was used as a hydrophobic carbon substrate along with glycerol as hydrophilic carbon source and yeast extract as nitrogen source. Shake flask incubation was performed in 250 mL Erlenmeyer flasks with 50 mL mineral salt medium and 5% tree borne oil at 30°C for 120 rpm. Batches were stopped once the 7-day incubation period was completed, and samples were ready to measure the rhamnolipid content.

Biomass Measurement

The biomass was extracted from a 2 mL broth solution by centrifugation at 78000 rpm for 20 minutes. The sample's dry weight was determined after drying at 100°C until it reached a consistent weight. [13]

Selection of Optimal Carbon and Nitrogen Source

In order to use low cost industrial by-products and agricultural wastes as cost-effective alternative substrates for microbial growth and rhamnolipid production, tree borne oil was used as hydrophobic source of carbon and energy. Yeast extract was used as nitrogen source. The culture conditions were the same as previously stated.

Statistics

Design Expert (version 13) software was used to examine all the data from the RSM test in order to determine the most optimal production media composition for RSM modeling of rhamnolipid generating medium composition. All of the tests were carried out in triplicate. ANOVA was used to assess the statistical significance of the findings.[14]

Rhamnolipid Content

One mL of solution was centrifuged for 15 minutes at 8000 rpm. The cell-free supernatant was centrifuged after being washed with ethyl acetate.

The ethyl acetate washings were collected and diluted to a concentration of 10 ml. One mL of ethyl acetate extract was placed in a stoppered test tube and evaporated with a rotary evaporator.[15]

Rhamnolipid Recovery

After 7 days, the pH was adjusted to 8.0 (using 10M NaOH) and the biomass was extracted by centrifugation for 20 minutes at 7800 rpm. The pH of the supernatant was adjusted to 2 (using 6N H_2SO_4), and it was kept at 4°C overnight. The very next day, an identical volume of chloroform-methanol (2:1) combination was added. The resulting suspension was agitated for 10 minutes in a rotary shaker to remove the organic phase.[16] The extraction procedure was conducted for second time. A rotary evaporator was used to concentrate the derived rhamnolipid from pooled organic phases.

Analytical Detection and Quantification of Crude Rhamnolipid

TLC was used to examine the production of mono and di-rhamnolipid using silica gel plates and a mobile phase of chloroform/methanol/water (65:15:2, v/v/v). The sample was applied to the TLC plate with a glass capillary, and the plate was vertically placed in a TLC chamber containing the mobile phase. Following plate development, the spots were visible by spraying methanol/H_2SO_4 (50:50 v/v) over the plate and heating it at 140°C.[17] The Rf values of the individual spots were obtained by dividing the distance run from the spot by the distance run from the solvent front as shown in Fig. 1.

Results and Discussion

Analysis of Substrate tree borne oil

The chromatogram analysis of the substrate tree borne oil was performed as shown in Fig. 2.

The fatty acid profile of the tree borne oil showed about 79% of Palmitic acid content. These components were identified by comparison of their retention times.[18]

Analysis of Rhamnolipid Bio-surfactant by IR Spectroscopy

According to the FTIR graph as shown in Fig. 3, the wide band at region A (3390.60 cm^{-1}) suggest the existence of hydroxyl group (-OH) free stretch owing to hydrogen bonding. The existence of aliphatic CH_3, CH_2, and C-H bond stretching is indicated by the wide band detected in regions B and D (2923.69–2854.13 and 1460.73–1375.04 cm^{-1}). Furthermore, bands identified at 1740.36 cm^{-1} of region C show the existence of carbonyl (C=O) stretching, while the other two peaks observed between E (1118.11–

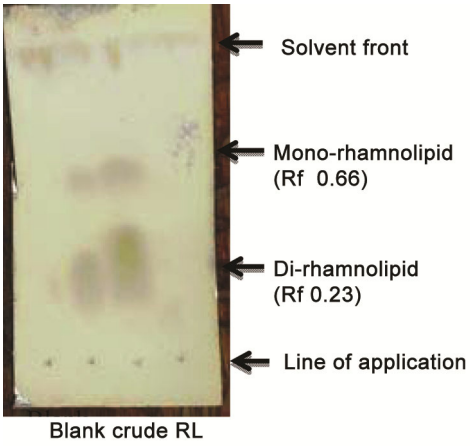

Fig. 1 — TLC of crude rhamnolipid synthesized by P. Aeruginosa with developing solvent

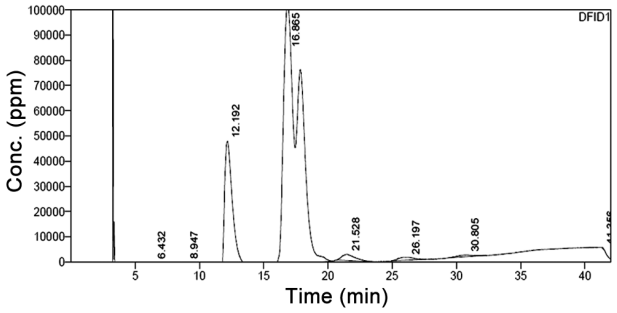

Fig. 2 — Gas Chromatogram of fat/oil

1095.55 cm^{-1}) area indicate the presence of C-O-C bond stretching, which is a typical of ester functional group. Moving on, pyranyl isorption band detected in region F at 846.32 cm^{-1} and β-pyranyl II sorption band seen in region G at 721.41 cm^{-1} which indicates the existence of di-rhamnolipid in the composition.[19]

Response Surface Methodology (RSM) Model

The whole experimental design for this study was developed utilizing response surface methodology (RSM) to optimize various factors in tribological behavior using Minitab Software. Response surface approach is a statistical model design that aids in the determination of numerous co-relationships between process parameters and tribological criteria, as well as the investigation of the influence of these process parameters on the linked responses Montgomery.[20] This Technique is based on the use of a factorial design in which the major influence of the component that causes variation in response is created by a change in the quantity of the factor investigated, while the others are held constant. The quadratic model was used to examine the data. Every factor in the experiment was assessed at three distinct levels (−1, 0, 1), as well as the lowest and maximum ranges of the different parameters at various concentrations. There were a total of 17 trials in which three variables were changed. These tests were carried out in 250 ml flasks containing production medium (100 ml mineral salt medium) with varying concentrations of parameters as well as 2% inoculum. Other variables like pH, temperature, agitation, and incubation time were held constant. The responses were calculated using a second-order polynomial equation. Using the multiple regression approach, the data was fed into the equation. Although the reaction contains three changeable factors, studying each one individually takes a long time. To understand how the parameters interact, one experimental approach called Response Surface Methodology can be used.[21]

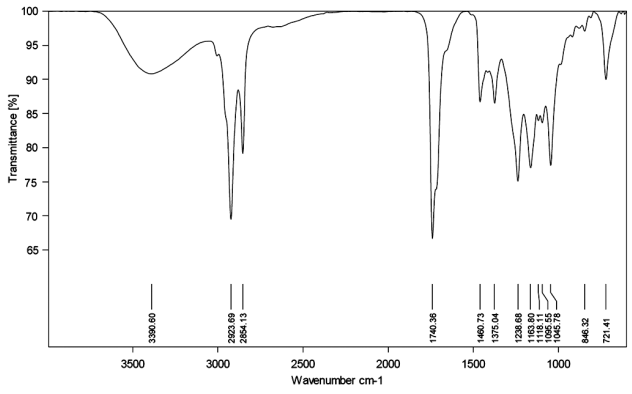

Fig. 3 — IR Spectroscopy of Rhamnolipid Bio-surfactant

The 3^K Factorial Design and Statistical Data

Statistical experimental design is a popular way for assessing the influence and interactions of individual or different factors in a given process. Using Design Expert, a Central Composite Design (CCD).[11] was created for three selected process factors, namely Glycerol, tree borne fat/oil, and Yeast extract, in the current study. With 17 trial runs, the Design Expert Software created a full length rhamnolipid factorial design. As a response, rhamnolipid concentrations (g/l) were used. The data collected from the trials was analyzed with Design Expert 13, and multiple regressions were used to assess the influence of process factors. Box-Behnken Design of RSM is used to optimize the effect of the individual as well as the interaction of the input parameters on the above mentioned output variables.

The input parameters are coded into two levels as shown in Table 1.

The following design was created using Design-Expert 13 software based on the inputs stated in the design overview, as shown in Table 2. The experiment featured 17 experimental runs, with 5 of them at the design's center.

Statistical Modeling

A statistical model was built using the experimental data from Table 2. To find correlations between inputs and outputs, several models such as linear, 2FI, and quadratic were tried and evaluated using analysis of variance (ANOVA).[22] In comparison to the other investigated models, ANOVA revealed that the quadratic model matches the experimental data quite well for all of the above-mentioned output parameters. Model Equation of Yield of rhamnolipid is shown below.

Table 1 — RSM Design summary for rhamnolipid bio-surfactant

Factor coded	Name	Low actual	High actual	Low coded	High coded
A	Tree borne fat/oil, g/l	1	5	−1	1
B	Glycerol, g/l	5	11	−1	1
C	Yeast extract, g/l	1	7	−1	1

Table 2 — Design of experiment using RSM

Standard No.	Tree borne fat g/l	Glycerol g/l	Yeast Extract g/l	Response yield g/l
1	3	8	4	7.3
2	1	8	7	9.3
3	3	8	4	7.4
4	5	11	4	7.4
5	3	8	4	7.2
6	5	8	7	7.6
7	1	11	4	12
8	3	11	1	5.5
9	1	8	1	8.4
10	5	8	1	9
11	3	8	4	7.3
12	5	5	4	13
13	1	5	4	9.15
14	3	11	7	6.3
15	3	5	7	6.9
16	3	8	4	7.4
17	3	5	1	7.7

Table 3 — Fit Summary

Source	Std Dev.	R^2	Adjusted R^2	Predicted R^2	—
Linear	2.03	0.0743	−0.1393	−0.8775	—
2FI	1.84	0.4159	0.0655	−1.7505	—
Quadratic	0.1034	0.9987	0.9970	0.9863	**Suggested**
Cubic	0.0837	0.9995	0.9981	—	Aliased

Table 4 — AVOVA table for response with yield

Source	Sum of squares	Df	Mean Square	F-value	*p*-value
Model	57.92	9	6.44	601.65	<0.0001
A- Tree borne fat/oil	0.4278	1	0.4278	40	0.0004
B- Glycerol	3.85	1	3.85	359.96	<0.0001
C- Yeast extract	0.0313	1	0.0313	2.92	0.1312
AB	17.85	1	17.85	1668.84	<0.0001
AC	1.32	1	1.32	123.64	<0.0001
BC	0.6400	1	0.6400	59.83	0.0001
A^2	26.77	1	26.77	2502.24	<0.0001
B^2	1.26	1	1.26	117.46	<0.0001
C^2	6.75	1	6.75	631.16	<0.0001
Residual	0.0749	7	0.0107	—	—
Lack of Fit	0.0469	3	0.0156	2.23	0.2268
Pure Error	0.0280	4	0.0070	—	—
Cor Total	57.99	16	—	—	—

$$\text{Yield} = 8.72858 - 0.6975 \times A - 0.323889 \times B + 1.03667 \times C - 0.352083 \times A \times B - 0.0958333 \times A \times C + 0.0444444 \times B \times C + 0.630313 \times A^2 + 0.0606944 \times B^2 - 0.140694 \times C^2$$

The R-square value shows the percentage of total variation in proposed model as in Table 3. As a result, the R-square value of 0.9987 for the quadratic yield model supports the experimental data's fit to the developed quadratic model. The adjusted R-square value of 0.9970 indicates the experimental data and projected values are in good agreement. The anticipated R-square value of 0.9863, on the other hand, suggests that the quadratic model may be utilized to estimate yield.

Analysis of Variance of Yield

Analysis of variance, as previously stated, describes the variance within the data of an output response including independent input factors and their interactions in Table 4. As a result, the greater the F-Value for an input parameter, the larger the response variable variation related to the given input parameter. The model F-value of 601.65 indicates the model is significant, with a 0.01% probability such a huge F-value is due to noise.

A, B, C, AB, AC, B^2, C^2 significantly contribute to the variation in the yield of product; where A represents tree borne fat/oil, B represents glycerol, and C represents yeast extract; when comparing the

F-value, the terms connected with tree borne fat load have the greatest F-value. The F-value of AB, i.e. tree borne fat/oil glycerol, is 1345.63. The word connected with combining carbon and nitrogen sources has a large F-vale. A lower lack of fit F-value of 2.79 and P-value 0.1721, showing a 17.21% likelihood of error, also implies that the model fits well. Although ANOVA is adequate to show the important role of input factors and their interactions in the variance of the response, the response graphs must be studied to understand how the input variables impact the response.

Effect of Input Variables and their Interactions on Rhamnolipids Yield

Effect of Amount of the Tree Borne Fat/Oil

The influence of tree borne fat/oil on yield at various glycerol levels is verified. No substantial increase in yield is found when the tree borne fat/oil level rises. However, in the instance of yeast extract, the influence of tree borne fat/oil on yield at various amounts of yeast extract has been checked. It has been discovered that the compatibility between tree borne fat/oil and yeast extract does not perform well; there is a 0.5% difference in yield when the quantity of yeast extract is increased. Thus, it is seen that the yield of product increases with tree borne fat/oil, although it has a lesser effect with yeast extract.[23]

Effect of the Amount of Glycerol

The yield of the product produced is lowest when the glycerol content is lowest. And highest yield is obtained when the glycerol content is highest. It is easy to assume from this correlation that yield is directly proportional to glycerol content, but only to a certain extent. The research depicts the influence of glycerol on yield at various amounts of tree borne fat/oil and yeast extract. It has been discovered that as the amount of glycerol included in the product improves, so does its yield. At lower and greater levels of tree borne fat/oil, the yield trend is symmetrical. However, because yeast extract is incompatible with glycerol, it yields less.

Effect of the Yeast Extract

There is influence of yeast extract on product yield. At both the low and high end of the scale, the yield is significantly affected by the amount of yeast extract used. Since the term's F-value is 2.92, examining the input variables individually is insufficient. As a result, the impact of the interaction between the input factors on the yield must be analyzed.

Interaction Between Tree Borne Fat/oil & Glycerol

The yields as a result of the interaction between the tree borne oil and the amount of glycerol are shown in Fig. 4. When either or both variables are raised, the tree borne oil and the amount of glycerol work together to improve the yield of the product, resulting an increase in yield. A larger amount of fat/oil, combined with a higher amount of glycerol, results in a higher yield. When tree borne oil is raised from 1.0 to 5.0%, there is a gradual rise in yield. Similarly, increasing the quantity of glycerol in AB from 5.0 to 11.0% w/w resulted in a higher yield. As a result, the interaction between the terms A and B, which represent fat/oil and glycerol quantity, is substantial, with an F-value of 1668.5.

Interaction between Tree Borne Oil & Yeast Extract

The yield is shown as a function of the interaction between the amount of tree borne oil and the amount of yeast extract (Fig. 5). Fat/oil serves as a substrate for a higher output, while yeast extract serves as a nitrogen source. The 3D image shows the effect of different quantities of tree borne frying oil and yeast extract. It has been revealed that as the amount of tree borne oil added to the product increases, the yield increases as well. However, in the case of tree borne oil and yeast extract compatibility, the graph trend is symmetrical. As a result, it produces less. As can be

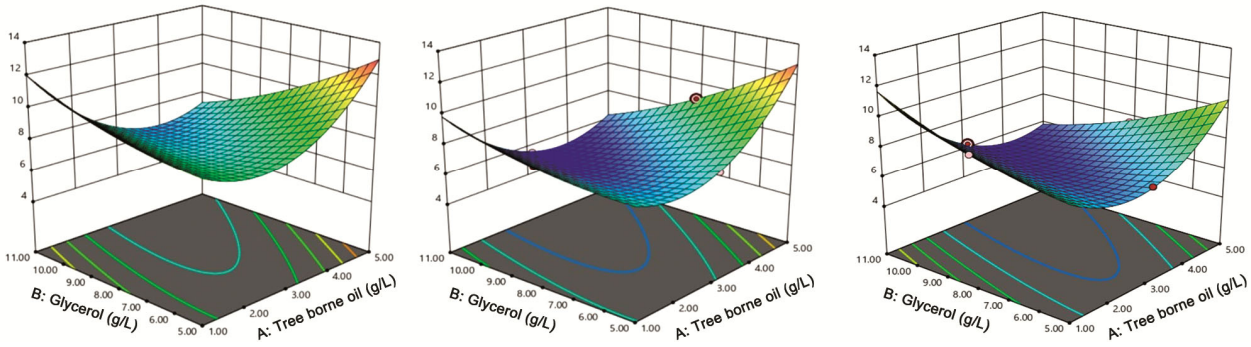

Fig. 4 — Effect of fat/oil and glycerol on the yield

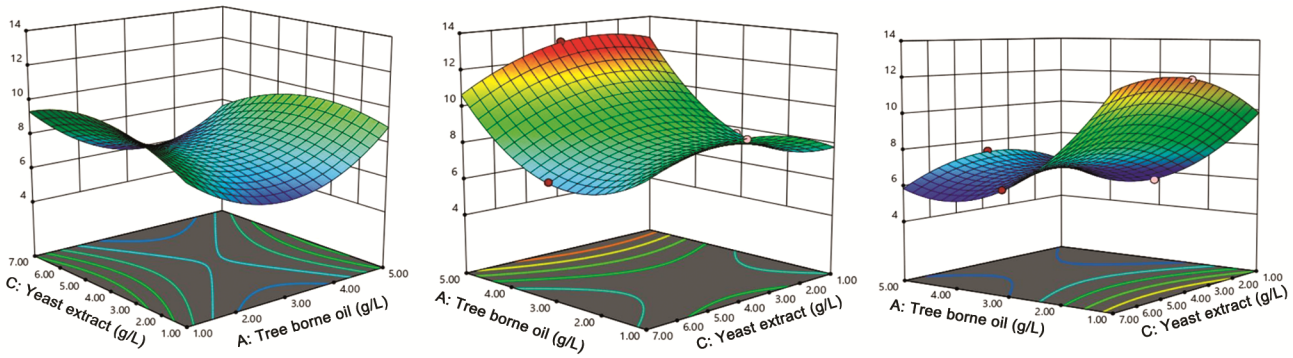

Fig. 5 — Effect of fat/oil and yeast extract on the yield

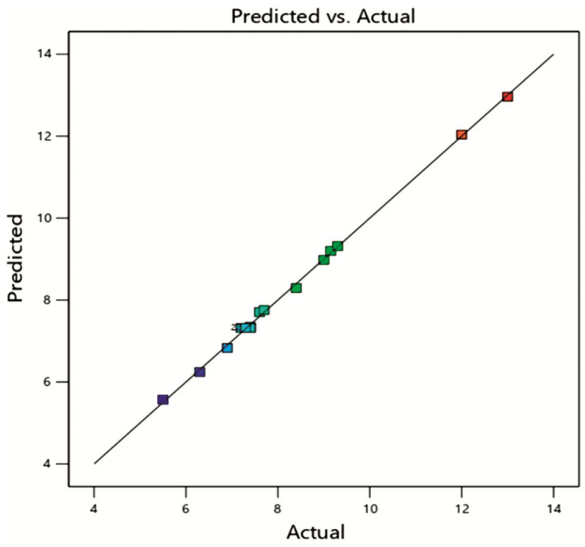

Fig. 6 — Comparison between predicted and the actual value of the respective model

Fig. 7 — Antimicrobial activity

observed, tree borne oil boosts product output, albeit it has a reduced influence with interaction between tree borne oil and yeast.

Model Accuracy

The importance of model accuracy testing has been mentioned earlier, and Fig. 6 shows a comparison of the anticipated values generated by the models and the actual value achieved experimentally. The projected values exhibit a cluster around the line of the actual values in the created model, which is the yield model. This proves that the model's predictions match the data from the experiment. Given the high R^2 and modified R^2 values, as well as the fair match between the actual and projected values, the prediction based on this model may be considered trustworthy. As a result, the optimization tool may be used to investigate bio-surfactant creation in more depth.

Antimicrobial Activity of Rhamnolipid

Nitschke *et al*. found antimicrobial activity of rhamnolipid against a variety of bacteria and fungi. Rhamnolipid have an immediate effect on the bacterial cell surface. Rhamnolipid had no growth inhibitory impact on gram-negative *E. coli* in our investigation, and no identifiable zone of inhibition was detected in Fig. 7. Gram-positive bacteria *B. subtilis* on the other hand, demonstrated positive growth inhibition in the presence of rhamnolipid.[24] The illustration depicts a discrete zone of inhibition. This study found that rhamnolipid has antibacterial activity against gram-positive bacteria. It has been shown that antibacterial activity against gram-positive bacteria was more powerful than antimicrobial activity against gram-negative bacteria (Fig. 7).

Oil Displacement Test

The oil displacement method is quick and simple, requires no specialized equipment and need only a modest amount of sample. This test is done to determine if the product has surfactant activity and

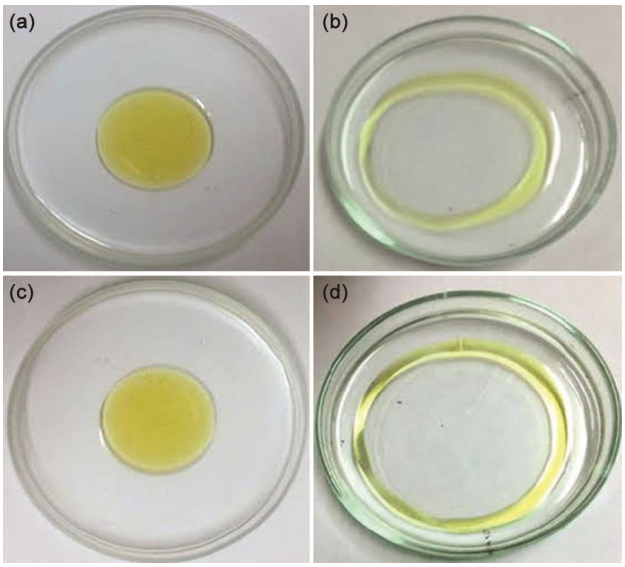

Fig. 8 — Oil displacement test

Table 5 — Formulation of anti-aging cream

Ingredient	% wt
Mangiferin	3
Stearic acid	5
Cetyl alcohol	6
Liquid paraffin	6.6
Glycerin	5
Methylparaben	0.05
Rhamnolipid	3.5
Propylene glycol	30
Distilled water	40.85

there direct relationship between sample diameter and rhamnolipid surfactant concentration (Fig. 8). The oil drop when placed without surfactant in petri dish remain particulate due to the hydrophobic nature of the oil surface, which induces droplet aggregation.[25]

Cosmetics Formulation using Rhamnolipid and its stability

Rhamnolipids are biocompatible and ideal for use in cosmetics, personal skincare and pharmaceutical formulations. Several studies demonstrated that the degree of toxicity of microbial biosurfactants is dose-dependent, and several researchers have reported that biosurfactants such as rhamnolipids are less toxic to mammalian cells compared to their chemical counterparts, therefore, confirming their safety for use among all biosurfactants, glycolipids are the most studied in cosmetic and personal care formulations.[26, 27] The anti-aging cream formulation was developed using rhamnolipids as shown in Table 5 and found to have good consistency.

The effectiveness of rhamnolipids as surfactant and the stability of the prepared formulation were observed in terms of creaming index, pH, colour, visual aspect, phase separation, and sedimentation. It performed well in terms of all of these parameters showing that rhamnolipids posess great emulsifying properties in the anti-aging cream formulation and helps in achieving the product with greater stability and sustainability.

Conclusions

The response surface methodology is used to optimize yield of rhamnolipids by utilizing tree borne oil and *P. aeruginosa*. The main focus of this study was to increase the rhamnolipid production and optimizing the set of experimental trials using RSM. It has been found out that response surface methodology offers several advantages like fewer experiments are needed to study the effects of all parameters and an optimal combination of all variables can be discovered. Interactions of all the parameters of reaction was discussed and optimized. The prepared cream using the biosurfactant rhamnolipid confirms its utility in formulation of anti-aging cream which has replaced the conventional surfactants reducing environmental impact. This study provides a future prospect for the large-scale production of environmentally friendly rhamnolipids using tree borne oil which can be used in cosmetic formulations.

Authors have declared no conflict of interest

References

1 Varjani S J & Upasani V N, Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactants, *Bioresour Technol*, **232** (2017) 389–397.

2 Desai J D & Banat I M, Microbial production of surfactants and their commercial potential, *Microbiol Mol Biol Rev*, **61(1)** (1997) 47–64.

3 Fenibo E O, Ijoma G N, Selvarajan R & Chikere C B, Microbial surfactants: The next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation, *Microorganisms*, **7(11)** (2019) 1–29.

4 Pacwa-Płociniczak M, Płaza G A, Piotrowska-Seget Z & Cameotra S S, Environmental applications of biosurfactants: Recent advances, *Int J Mol Sci*, **12(1)** (2011) 633–654.

5 Makkar R S, Cameotra S S & Banat I M, Advances in utilization of renewable substrates for biosurfactant production, *AMB Express*, **1(1)** (2011) 1–19.

6 Nikolova C & Gutierrez T, Biosurfactants and their applications in the oil and gas Industry: Current state of knowledge and future perspectives, *Front Bioeng Biotechnol*, **9** (2021) 1–19.

7 Thakur P, Saini N K, Thakur V K, Gupta V K, Saini R V & Saini A K, Rhamnolipid the glycolipid biosurfactant: Emerging trends and promising strategies in the field of biotechnology and biomedicine, *Microb Cell Factories*, **20(1)** (2021) 1–19.

8 Banat I M, Satpute S K, Cameotra S S, Patil R & Nyayanit N V, Cost effective technologies and renewable substrates for biosurfactants' production, *Front Microbiol*, **5** (2014) 1–18.

9 Randhawa K K S & Rahman P K S M, Rhamnolipid biosurfactants-past, present, and future scenario of global market, *Front Microbiol*, **5** (2014) 1–7.

10 Nalini S & Parthasarathi R, Optimization of rhamnolipid biosurfactant production from Serratia rubidaea SNAU02 under solid-state fermentation and its biocontrol efficacy against Fusarium wilt of eggplant, *Ann Agrar Sci*, **16(2)** (2018) 108–115.

11 Bezerra M A, Santelli R E, Oliveira E P, Villar L S & Escaleira L A, Response surface methodology (RSM) as a tool for optimization in analytical chemistry, *Talanta*, **76(5)** (2008) 965–977.

12 Kaskatepe B & Yildiz S, Rhamnolipid biosurfactants produced by pseudomonas Species, *Braz Arch Biol Technol*, **59** (2016) 1–16.

13 Jadhav J V, Anbu P, Yadav S, Pratap A P & Kale S B, Sunflower Acid oil-based production of rhamnolipid using pseudomonas aeruginosa and its application in liquid detergents, *J Surfactants Deterg*, **22(3)** (2019) 463–476.

14 Xiangsheng Z & Lu D, Response surface analyses of rhamnolipid production by Pseudomonas aeruginosa strain with two response values, *Afr J Microbiol Res*, **7(22)** (2013) 2757–2763.

15 Pratap A, Wadekar S, Kale S, Lali A & Bhowmick D N, Non-traditional oils as newer feedstock for rhamnolipids production by *Pseudomonas aeruginosa* (ATCC 10145), *J Am Oil Chem Soc*, **88(12)** (2011) 1935–1943.

16 Diab A M, Ibrahim S & Abdulla H M, Safe application and preservation efficacy of low-toxic rhamnolipids produced from *P. aeruginosa* for cosmetics and personal care formulation, *Egypt J Microbiol*, **55(1)** (2020) 57–70.

17 Jadhav J, Dutta S, Kale S & Pratap A, Fermentative production of rhamnolipid and purification by adsorption chromatography, *Prep Biochem Biotechnol*, **48(3)** (2018) 234–241.

18 Zayed M A, Abd El-Kareem, M S M & Zaky N H S, Gas chromatography-mass spectrometry studies of waste vegetable mixed and pure used oils and its biodiesel products, *J Pharm Appl Chem*, **3(2)** (2017) 109–116.

19 Kiefer J, Radzuan M N & Winterburn J, Infrared spectroscopy for studying structure and aging effects in rhamnolipid biosurfactants, *Appl Sci*, **7(5)** (2017) 1–7.

20 Peng X, Yang G, Shi Y, Zhou Y, Zhang M & Li S, Box–Behnken design based statistical modeling for the extraction and physicochemical properties of pectin from sunflower heads and the comparison with commercial low-methoxyl pectin, *Sci Rep*, **10(1)** (2020) 3595.

21 Sakinah M H, Amirruddin A K, Kadirgama K, Ramasamy D, Rahman M M & Noor M M, The application of response surface methodology in the investigation of the tribological behavior of palm cooking oil blended in engine oil, *Adv Tribol*, **2016** (2016) 1–11.

22 Thani N S M, Ghazi R M & Ismail N, Pengoptimuman kaedah gerak balas permukaan untuk penyingkiran minyak menggunakan kulit pisang sebagai penjerap-bio, *Malaysian J Anal Sci*, **21(5)** (2017) 1101–1110.

23 Madankar C S & Nair A, Studies on extraction, microencapsulation and potential applications of Ginger Oleoresin, *J Sci Ind Res* **80** (2021) 685–691.

24 Nitschke M, Costa S G, Contiero J, Structure and applications of a rhamnolipid surfactant produced in soybean oil waste, *Appl Biochem Biotechnol*, **160(7)** (2010) 2066–2074.

25 Shamaa S & Bahjat S, Detection of Rhamnolipid Production in Pseudomonas aeruginosa, *J Phys Conf Ser*, **1294(6)** (2019) 1–10.

26 Lourith N & Kanlayavattanakul M, Natural surfactants used in cosmetics: glycolipids, *Int J Cosmet Sci*, **31** (2009) 255–261.

27 Piljac T & Piljac G, Use of rhamnolipids in wound healing, treating burn shock, atherosclerosis, organ transplants, depression, schizophrenia and cosmetics, Patent WO1999043334 A1, 1999