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Application of Response Surface Methodology in the Formulation of an Eco-friendly Degreaser using Rhamnolipid Biosurfactant

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ABSTRACT

Formulating a bio-based degreaser emulsion can be challenging because it requires maximizing the performance to be as good as a commercial chemical-based product without compromising the cost and effect on the environment. This study aimed to develop an eco-friendly degreaser using natural Rhamnolipid biosurfactant through response surface methodology (RSM), which was then validated and assessed compared to chemical-based commercial products. The Plackett–Burman design (PBD) and Box–Behnken design (BBD) were used to screen and optimize factors for the formulation. From the PBD's result, the amount of Rhamnolipid, with other environmentally friendly ingredients of D-limonene, sodium citrate, and calcium carbonate, were shortlisted as significant factors in the formulation. Next, the BBD optimization study revealed that only rhamnolipid and sodium citrate significantly affected the two-way interaction of the factor on the result of the surface tension and oil displacement test (ODT). In the validation study, the experimental value was very close to the rate predicted by the model for the response surface tension and ODT (24.14 mN/m and 241 mm, respectively). A comparison of the performance with commercial products proved that this formulation was comparable to that commercial product. Overall, this study revealed that the effective eco-friendly degreaser formulation was successfully produced using a minimal amount of rhamnolipid biosurfactant with a response surface methodology as a tool.

1. Introduction

Degreasers based on hazardous chemical formulations are used globally in automotive, electronics, aircraft, and consumer goods. There is a growing demand for biologically based degreasers due to the environmental protection concerns related to the Sustainable Development Goals (SDG) [1]. Increasing consumer awareness has also become a driver for developing an eco-friendly degreaser that uses safe, water-based, and naturally sourced ingredients [2].

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In a bio degreaser formulation, the biosurfactant plays a major role by blending the constituent ingredients. Hence, the selection of suitable surfactants is required for a degreaser to be effective [3]. Rhamnolipid is frequently referred to as the best biosurfactant because of its superior surface activity, which makes it suitable for immediate application in various industries. Furthermore, due to their ability to reduce the water surface tension to as low as 30 mN/m, rhamnolipids have significant potential for cleaning applications [4,5]. Therefore, numerous studies have been published on the effectiveness of rhamnolipids and have confirmed their efficacy in applications such as detergent formulation, ultrafiltration membrane cleaning, whiteboard cleaner and cleaning diesel from contaminated soil [4,6-11]. However, a report on the use of rhamnolipids in oil cleaning is still lacking.

Despite many publications on rhamnolipid cleaning applications, most studies were conducted using the one-factor-at-a-time (OFAT) approach. Process and formula (product) optimization using the design of experiment (DOE) approach is an improvement over the conventional OFAT approach, with a more accurate prediction of the optimal level for increased efficiency [12,13]. Because OFAT cannot determine the interactions between factors, it cannot guarantee that a process or formula is optimal [14]. Moreover, random experiments require several trials and have errors with little or no statistical testing [15]. In addition, DOE techniques are the most reliable because they may achieve process or formulation goals with fewer experiments, reducing the experimental time required [16]. Response surface methodology (RSM) is one of the most widely used DOE techniques involving statistical and mathematical methods for screening and optimizing processes [17]. Plackett–Burman design (PBD) and Box–Behnken design (BBD) regular screening and optimization are used in RSM.

PBD is a fractional factorial design, a screening design in which each factor is analyzed at two levels. By comparing the differences between each factor to investigate the main effect and determine the significant factors, many factors can be eliminated to avoid wasting test resources in a later phase of the optimization phase by using too many factors or less important factors [18]. PBD can be used to find crucial factors with the minimum number of experimental runs and an exceptionally high degree of accuracy. The main effects are independent, and interactions between factors are not a concern at this screening stage [14].

Typically, after the screening phase, the most influential factors on processes or formulations are determined and used in the optimization phase with a more complicated design. At this stage, interactions between factors in higher-order terms, such as quadratic or cubic, are studied [19]. BBD is an RSM design that requires only three levels, represented by the values +1, 0, and −1. BBD was developed by merging two-level factorial designs with incomplete block designs [14].

Since the RSM approach, particularly the PBD and BBD, has been widely used in product optimization, it was adopted in this study [20-22]. This study aimed to determine factors that have a significant effect on the formulation of eco-friendly degreaser using the PBD, followed by optimizing the formulation by BBD. The optimized eco-friendly degreaser formulation is then validated by comparing the predicted and experimental values and assessing their performance compared to commercial products available on the market.

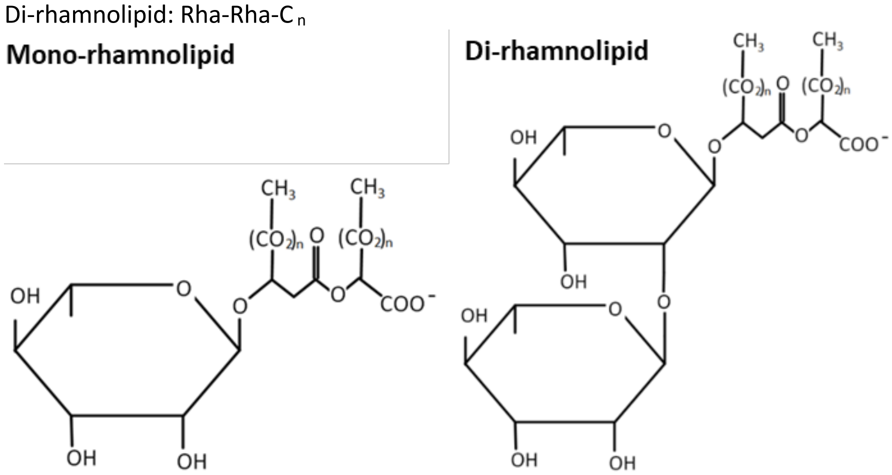
2. Materials and Methods
2.1 Materials

Rhamnolipid biosurfactant (Cas No.: 869062-42-0, 90% purity), D-limonene (Cas No.: 5989-27-5, 97% purity), calcium carbonate (Cas No.: 471-34-1), and orange oil (Cas No.: 8008-57-9) was purchased from Sigma-Aldrich. Sodium citrate (Cas No.: 6132-04-3) was purchased from Eva Chem. The D-limonene was diluted to 10% concentration. All the materials were chosen to analyze the individual variable effect on the formulation.

2.2 Properties of the Rhamnolipid Biosurfactant

Rhamnolipids are selected for use in an eco-friendly degreaser formulation due to their microemulsion formation capacity and absorbent ability. During the cleaning process, rhamnolipids will absorb or penetrate oil–water interfacial tension and then reduce the solution surface tension until the critical micelle concentration (CMC) for trapping the oil forming the micelle is reached [23]. Thus, it is vital to select an appropriate biosurfactant when formulating an efficient eco-friendly degreaser. The physical and chemical characteristics of rhamnolipids are shown in Table 1 [4,23-31].

Table 1
Physical and chemical properties of rhamnolipids [4,23-31]

	Rhamnolipid
Formula	Fatty acid tail (8, 10, 12 and 14 carbons) and one or two rhamnose moiety Mono-rhamnolipid: Rha-C_n Di-rhamnolipid: Rha-Rha-C_n
Structure	
Main origin	*Pseudomonas aeruginosa*
Molecular weight	504 – 649 g/mol
Appearance	Yellowish powder
Odor	Fruity
Solubility	Soluble in water and organic solvents
Surface tension in aqueous solution	As low as 30 mN/m
Biodegradability	Readily degraded agent
Toxicity	Very low toxicity

2.3 Formulation of Eco-friendly Degreaser

Each ingredient (A: rhamnolipid, B: D-limonene, C: sodium citrate, D: calcium carbonate, and E: orange oil) was weighed in grams and diluted with distilled water to a total of 100 g. The eco degreaser formulation was mixed with a magnetic stirrer for 10 min and allowed to stand for 24 h before analysis. The response tests were randomized to reduce the effects of unexplained variability in real responses due to extrinsic factors.

2.4 Screening of Formulation by Plackett–Burman Design (PBD)

The statistical design used in this study was a PBD with 12 runs to screen for significant factors and to identify the parameters affecting the formulation [25,26]. Five numerical factors were evaluated: rhamnolipid (surfactant), D-limonene (solvent), sodium citrate (builder), calcium carbonate (pH adjuster), and orange oil (flavor). The effect of each factor on response was determined by comparing the mean difference between two levels (low (−1) and high (+1)) (Table 2).

Table 2
Codes, factors, units, and levels for screening in the PBD

Codes	Factors	Units	Level	
			Low (-1)	High (+1)
A	Rhamnolipid	g	0.1	1
B	D-limonene	g	0.1	30
C	Sodium citrate	g	0.1	10
D	Calcium carbonate	g	0	0.01
E	Orange oil	g	0	0.01

Each component was mixed (in gram) and diluted with distilled water to a total of 100 g solution.

2.5 Optimization of Formulation by Box–Behnken Design (BBD)

Further investigation of the effect of the major factors from PBD was conducted using a response surface approach via a BBD with 27 runs to investigate both the individual and mutual interactions of four factors: rhamnolipid (surfactant), D-limonene (solvent), sodium citrate (builder), and calcium carbonate (pH adjuster) [32]. The number of nonsignificant factors determined from PBD was fixed in the formulation. The effect of each factor on the response was determined by comparing the mean difference at three levels, low (−1), center (0), and high (+1) (Table 3). This BBD investigated the quadratic relationships and interactions between the factors and responses, enabling a comprehensive definition of nonlinear relationships in the formulation [33].

Table 3
Codes, factors, units, and levels for optimization in the BBD

Codes	Factors	Units	Fix value	Level		
				Low (-1)	Center (0)	High (+1)
A	Rhamnolipid	g	-	0.1	0.55	1
B	D-limonene	g	-	0.1	15.05	30
C	Sodium citrate	g	-	0.1	5.05	10
D	Calcium carbonate	g	-	0	0.005	0.01
E	Orange oil	g	0.01	-	-	-

The statistically significant terms were determined by analysis of variance (ANOVA) with a significance level of $p<0.05$. The R^2 and R^2 Adj values were assigned to determine how well the model explained the variability of the dependent variables. The lack of fit parameter was examined for all models to ensure its non-significance, indicating model validity [21].

2.6 Performance Analysis of Eco-friendly Degreaser Formulation
2.6.1 pH values

The pH values of the test solution were determined by using a pH meter (SevenExcellence Multiparameter, Mettler Toledo). All experiments were conducted at room temperature.

2.6.2 Oil Dispersion Test (ODT)

The surface activity was determined experimentally using the oil dispersion test (ODT) [34]. This procedure began by pouring 250 mL of saltwater into a 300 mm circular tray. Next, the motor oil was slowly added to the middle surface of the circular tray, followed by the test solution, which was added dropwise. The ratio of motor oil to the test solution was 1:1. Then, the clear zone at the center of the circular tray was measured. All experiments were conducted at room temperature.

2.6.3 Surface tension

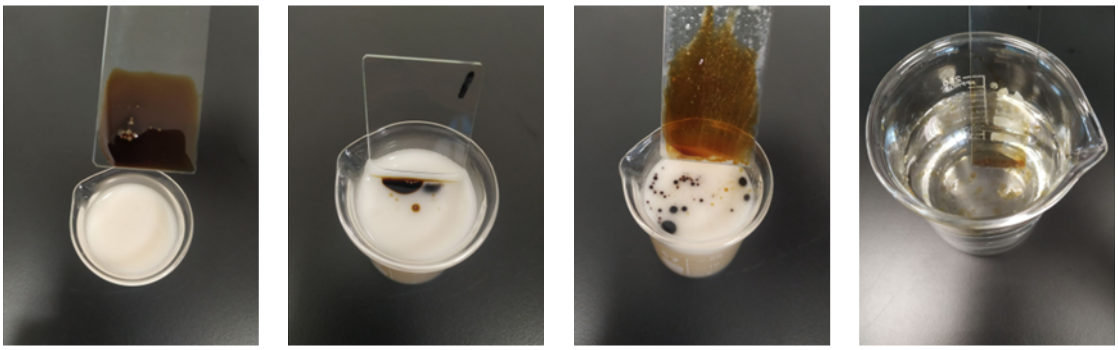
Surface tension was calculated with a tensiometer (Model No: 59780-90) Cole-Parmer, USA) using the capillary rise method [35]. The height difference that a test solution reaches in a capillary is proportional to the surface tension and is determined by Eq. (1), where γ is the surface tension, h is the distance between menisci, r is the radius of the capillary, d is the sample density, and g is gravity. All experiments were conducted at room temperature.

$$\gamma = 1/2(\mathrm{h})(\mathrm{r})(\mathrm{d})(\mathrm{g}) \tag{1}$$

2.6.4 Removal of oil

The oil removal study used glass slides [36]. The glass slides were uniformly covered with 100 µL of motor oil. The contaminated part of the glass slides was submerged in the test solution for 1 min before it was dipped in distilled water to remove any excess test solution. The glass slides were then dried for 30 min at 40 °C in a forced-air oven and weighed. The percentage of oil removal was calculated using Eq. (2), where Mc is the mass of the contaminated glass slides, Mw is the mass of the glass slides after washing and drying, and Mi is the initial mass of the glass slides. Figure 1 illustrates the process of cleaning oil from glass slides.

$$\text{Oil removal (\%)} = \frac{\mathrm{Mc-Mw}}{\mathrm{Mc-Mi}} \mathrm{x}100 \tag{2}$$



(a) Covered uniformly with heavy oil (b) Submerged into the test solution (c) After submerged into test solution (d) After dipped into distilled water

Fig. 1. Cleaning oil from glass slides

2.6.5 Phytotoxicity test

The phytotoxicity test was conducted by measuring the seed germination and root elongation of tomato seeds (*Solanum lycopersicum*) prepared in sterile Petri dishes (diameter:15 cm) containing

Whatman no. 1 filter paper [36,37]. The test solutions were diluted to a 1:10 ratio with distilled water. The tomato seeds (20 seeds) were initially pre-treated for 15 min with 0.5% sodium hypochlorite (NaClO). Then, the tomato seeds were transferred to the Petri dishes, 40.0 ml of test solution was added, and the seeds were incubated for 7 days at 27 °C. The control study was conducted with distilled water only. The incubation process was performed in a dark room. The relative seed germination, relative root length (≥5mm), and germination index (GI) were determined from Eq. (3) to Eq. (5). The classification was based on the GI of the formulations. Only formulations with a GI of > 80% were classified as non-toxic.

$$\text{Relative seed germination (\%)} = \frac{\text{number of seed germination in test sample}}{\text{number of seed germination in control sample}} \text{x}100 \tag{3}$$

$$\text{Relative root length (\%)} = \frac{\text{mean root length in test sample}}{\text{mean root length in control sample}} \text{x}100 \tag{4}$$

$$\text{Germination Index (GI)} = \frac{\text{\% germination}}{\text{\% root growth}} \text{x}100 \tag{5}$$

2.7 Statistical Analysis and Model Validation

Experimental design and data analysis were conducted using the Minitab® 21.1 statistical software package (Minitab Inc., State College, PA, USA). The significant effects were determined using ANOVA at $p<0.05$. The optimized eco-friendly degreaser response was designed to minimize the surface tension and maximize the ODT. The validity of the model was determined by performing the experiment with the most suitable experimental parameters generated by the software, and the errors were calculated using Eq. (6). The model was considered valid if the relative error was less than 15% [38].

$$R\text{elative error (\%)} = \frac{\text{Predicted value} - \text{Experimental value}}{\text{Predicted value}} \text{x}100 \tag{6}$$

2.8 Performance Evaluation by Comparison with the Commercial Product

The performance of the prepared optimized eco-friendly degreaser was compared to three commercial degreaser products in terms of pH, surface tension, ODT, removal of oil test, and phytotoxicity test.

3. Results and Discussion

3.1 Screening of Significant Factors Using PBD

The PBD was chosen to identify the significant factors in developing the eco-friendly degreaser formulation. This design identifies the main effects based on the outcomes of tests performed according to a matrix where high and low levels of factors are investigated [21]. This PBD generated 12 formulations (PBD-1 to PBD-12); the findings are shown in Table 4. The minimum and maximum values for pH, surface tension and ODT were 8.863 and 10.089, 22.7274 and 32.3205 mN/m, and 32 and 235 mm, respectively.

Table 4
PBD experimental design and response

Formulation	A	B	C	D	E	pH	Surface tension, (mN/m)	Oil displacement test (ODT), (mm)
PBD-1	1	30	0.1	0.01	0	9.623	25.1181	207
PBD-2	1	0.1	10	0.01	0	9.394	25.0785	150
PBD-3	0.1	30	0.1	0	0	9.653	29.8006	55
PBD-4	0.1	0.1	0.1	0.01	0.01	9.536	32.3205	32
PBD-5	1	30	0.1	0.01	0.01	9.647	25.1974	235
PBD-6	0.1	0.1	0.1	0	0	8.949	31.6508	75
PBD-7	0.1	0.1	10	0.01	0.01	10.089	27.6525	35
PBD-8	1	0.1	10	0	0	9.212	23.6752	150
PBD-9	1	0.1	0.1	0	0.01	8.863	27.7524	160
PBD-10	0.1	30	10	0	0.01	9.303	25.2035	33
PBD-11	0.1	30	10	0.01	0	10.082	24.1988	45
PBD-12	1	30	10	0	0.01	9.284	22.7274	128

The Pareto chart indicates the factors that significantly affected the process or formulation in terms of the standard effect of each item [39]. The Pareto chart in Figure 2(a) shows that only D significantly influenced the pH at a p-value 0.007. Figure 2(b) shows that the parameters with a significant effect on surface tension were C > A > B at p-value 0.000, 0.000, and 0.002, respectively. Meanwhile, Figure 2(c) shows that the significant effect parameters in relation to ODT were A > C, at p-values 0.000 and 0.037, respectively. E had no significant effect on any response variables. A more complex model is required to examine significant two-way interactions between factors in terms of higher order [19].

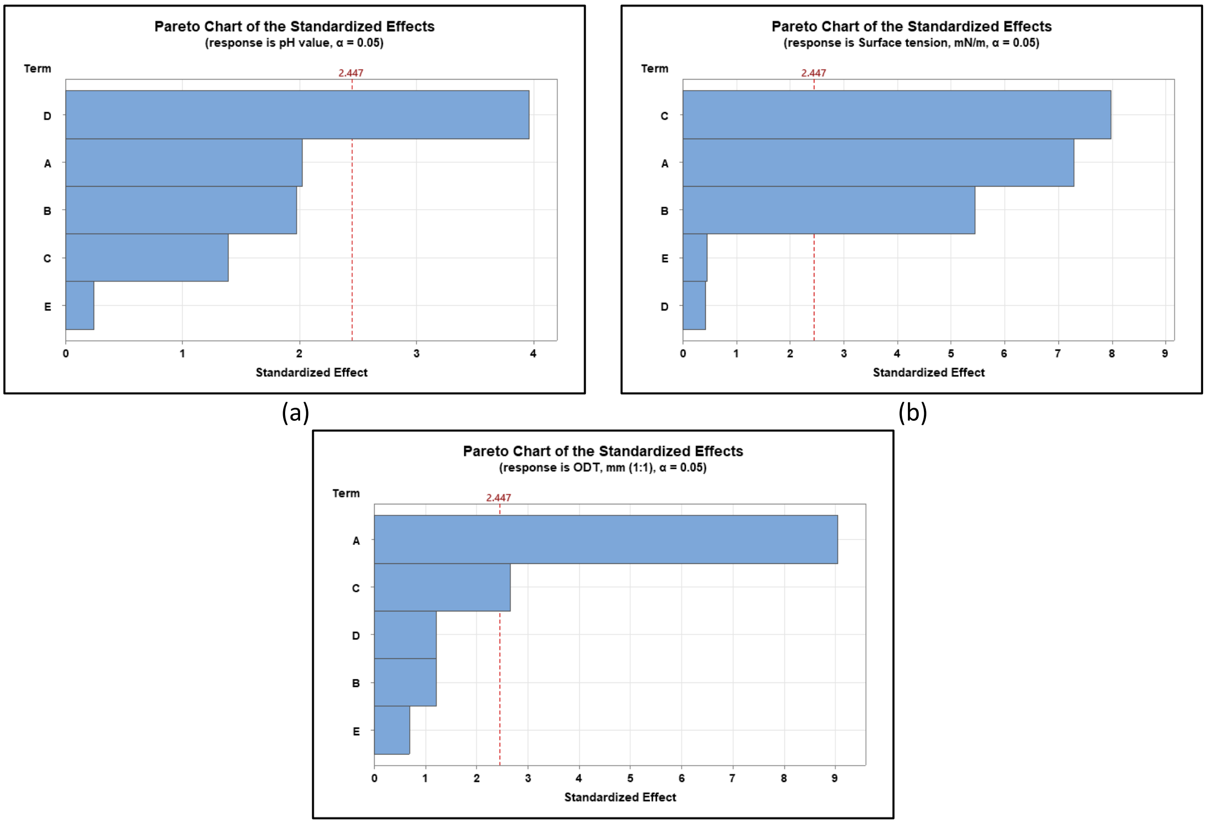


Fig. 2. Pareto chart of (a) pH, (b) Surface tension, and (c) ODT for screening of factors using PBD

The fit of the model was determined using the coefficients of determination R^2 and R^2 Adj. Overall, the R^2 values for all three responses (pH, surface tension, and ODT) were greater than 80%; they were 81.08%, 96.08%, and 93.90%, respectively. Then, the coefficients of determination R^2 Adj for responses (pH, surface tension, and ODT) were 65.31%, 92.81%, and 88.82%, respectively. The higher these coefficients, the more closely the model fits the data [40]. The screening of the eco-friendly degreaser formulation revealed that four parameters (rhamnolipid, D-limonene, sodium citrate, and calcium carbonate) had a significant effect. Hence, further optimization studies for this factor were conducted using BBD.

3.2 Statistical Analysis of Eco-friendly Degreaser Formulation Using Box–Behnken Design

In BBD, 27 runs were conducted to optimize the formulation by taking the four critical factors obtained from the PBD with dependent responses by the eco-friendly degreaser formulation. Table 5 presents response data for all experimental BBD formulations with the minimum surface tension and maximum ODT achieved on BBD-9 (24.5387 mN/m) and BBD-27 (247 mm), respectively.

Table 5
BBD experimental design and response

Formulation	A	B	C	D	Surface tension, (mN/m)	Oil displacement test (ODT), (mm)
BBD-1	0.55	30.00	0.10	0.005	25.6554	177
BBD-2	0.10	30.00	5.05	0.005	26.4210	49
BBD-3	0.10	15.05	0.10	0.005	30.4840	78
BBD-4	0.55	15.05	5.05	0.005	25.1223	97
BBD-5	0.55	15.05	0.10	0.010	28.4822	150
BBD-6	0.55	0.10	5.05	0.010	26.7680	108
BBD-7	1.00	15.05	5.05	0.010	25.4731	145
BBD-8	0.55	0.10	10.00	0.005	26.3142	100
BBD-9	0.55	15.05	10.00	0.010	24.5387	108
BBD-10	1.00	30.00	5.05	0.005	25.0612	150
BBD-11	0.10	15.05	10.00	0.005	25.8483	38
BBD-12	0.55	15.05	0.10	0.000	26.9315	168
BBD-13	0.55	30.00	5.05	0.010	25.0944	92
BBD-14	0.55	15.05	5.05	0.005	26.4982	92
BBD-15	1.00	15.05	5.05	0.000	25.6173	142
BBD-16	0.55	0.10	0.10	0.005	30.0820	173
BBD-17	0.10	0.10	5.05	0.005	27.6319	40
BBD-18	0.55	30.00	5.05	0.000	25.2109	93
BBD-19	1.00	15.05	10.00	0.005	24.9205	150
BBD-20	0.10	15.05	5.05	0.000	26.2795	42
BBD-21	0.55	30.00	10.00	0.005	24.7612	103
BBD-22	0.55	0.10	5.05	0.000	27.5730	113
BBD-23	1.00	0.10	5.05	0.005	25.1871	150
BBD-24	0.55	15.05	5.05	0.005	25.7287	103
BBD-25	0.55	15.05	10.00	0.000	25.9215	97
BBD-26	0.10	15.05	5.05	0.010	26.4563	43
BBD-27	1.00	15.05	0.10	0.005	25.7524	247

3.3 Effect of Factors on Surface Tension

Table 6 shows the ANOVA table for surface tension which tested the fit of the model. The corresponding model was significant for explaining experimental values (p-value 0.001). A nonsignificant value for the lack of fit indicated that the model was valid for this study [41]. Comparing the F-values of factors demonstrated that the order of influence was C > B > A > D. The prediction of model quality was examined by assessing the coefficient of determination (R^2) and the adjusted coefficient of determination (R^2 Adj). In general, the greater the value of these coefficients, the more closely the model matches the data [42]. In this study, the R^2 and the R^2 Adj values for surface tension were 89.39% and 77.00%, respectively. These results demonstrated a strong correlation between the experimental data and the values predicted by the model. Hence, the surface tension value can be predicted and obtained by applying regression analysis to the uncoded values (Eq. (7)).

$$Y_{Surface\ tension} = 32.04 - 5.44\ \mathrm{A} - 0.1764\ \mathrm{B} - 0.782\ \mathrm{C} + 103\ \mathrm{D} + 0.73\ \mathrm{AA} + 0.00101\ \mathrm{BB} + 0.0293\ \mathrm{CC} + 1962\ \mathrm{DD} + 0.0403\ \mathrm{AB} + 0.427\ \mathrm{AC} - 36\ \mathrm{AD} + 0.00971\ \mathrm{BC} + 2.30\ \mathrm{BD} - 29.6\ \mathrm{CD} \quad (7)$$

The ANOVA test indicates the factors that significantly influence surface tension in the linear and quadratic terms and the interaction between factors. Those factors have linear, quadratic, or direct (C > B > A > C*C) influences or indirect (A*C) influences. This analysis revealed that the amount of sodium citrate in the eco-friendly degreaser formulation has the most significant effect. By lowering the surface tension, sodium citrate lowers the surfactant needed in cleaning formulations and enhances the cleaning action [32]. This can be seen in the linear term (p-value 0.000, F-value 37.36). The amount of D-limonene and rhamnolipids showed the second most influence on the measured response (p-value 0.001, F-value 21.16 and p-value 0.001, F-value 20.27, respectively). D-limonene is commonly used to decrease the viscosity of surfactants, dilute cleaning products, and regulate their concentration [43]. A decrease in surface tension resulting from increased rhamnolipid percentage indicates a high level of cleaning efficiency and surface activity [44].

Table 6
Analysis of variance (ANOVA) of the response variables for surface tension optimization

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	51.2828	3.6631	7.22	0.001
A- Rhamnolipid	1	10.2851	10.2851	20.27	0.001
B- D-limonene	1	10.7392	10.7392	21.16	0.001
C- Sodium citrate	1	18.9585	18.9585	37.36	0.000
D- Calcium carbonate	1	0.0433	0.0433	0.09	0.775
A*A	1	0.1157	0.1157	0.23	0.642
B*B	1	0.2719	0.2719	0.54	0.478
C*C	1	2.7435	2.7435	5.41	0.038
D*D	1	0.0128	0.0128	0.03	0.876
A*B	1	0.2943	0.2943	0.58	0.461
A*C	1	3.6173	3.6173	7.13	0.020
A*D	1	0.0257	0.0257	0.05	0.826
B*C	1	2.0642	2.0642	4.07	0.067
B*D	1	0.1185	0.1185	0.23	0.638
C*D	1	2.1515	2.1515	4.24	0.062
Error	12	6.0891	0.5074		
Lack-of-Fit	10	5.1382	0.5138	1.08	0.572
Pure Error	2	0.9509	0.4754		
Total	26	57.3719			

The contour plot was used to better visualize the effects of different proportions of the factors with significant interaction (A*C), which had an interaction p-value 0.020, on the response surface tension (Figure 3). The proportion of hold values (D-limonene and calcium carbonate) were constant at 15.05 and 0.005 g, respectively. The surface tension of an effective eco-friendly degreaser product should be low [45]. The significant effect of the rhamnolipid and sodium citrate is critical in reducing the response of surface tension. At 1 g sodium citrate, when the amount of rhamnolipid was increased (0.2 to 1 g), the surface tension of the eco-friendly degreaser reduced from 29 to 26 mN/m.

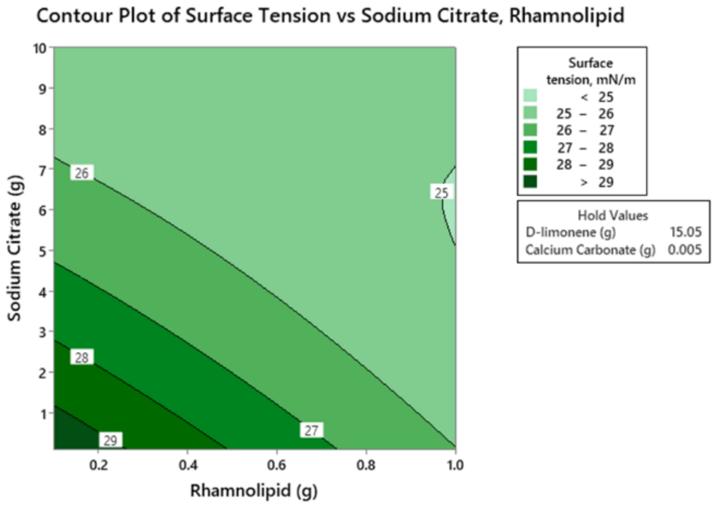


Fig. 3. Illustrated contour plot showing the relationship of rhamnolipid and sodium citrate to surface tension response

3.4 Effect of Factors on ODT

Table 7 shows the ANOVA table for the ODT response; the corresponding model was significant in explaining experimental values (p-value 0.000). A nonsignificant value for the lack of fit indicated that the model was valid for this study [41]. The comparison of the F-values of the factors demonstrated that the order of influence was C > A > B > D. The prediction of model quality was further investigated by evaluating the coefficient of determination (R^2) and the adjusted coefficient of determination (R^2 Adj). The R^2 and R^2 Adj values for ODT were 97.67% and 94.95%, respectively, indicating a very strong correlation between the experimental value and the value predicted by the model. Therefore, the value of ODT can be predicted and obtained with regression analysis of the uncoded values (Eq. (8)).

$$Y_{ODT} = 85.8 + 187.8\,A - 0.660\,B - 19.18\,C - 1613\,D - 20.7\,AA + 0.0228\,BB + 1.439\,CC - 31111\,DD - 0.334\,AB - 6.40\,AC + 148\,AD - 0.0023\,BC + 11.1\,BD + 303\,CD \quad (8)$$

The ANOVA test identifies the factors that significantly influence the ODT in the linear and quadratic terms, and the interactions between factors. Those factors have linear or quadratic direct influences (A > C > C*C) or indirect (A*C) influences. This ANOVA table reveals that the rhamnolipid amount in the eco-friendly degreaser formulation has the most significant effect on the ODT. This can be seen in the linear term (p-value 0.000, F-value 320.10). The amount of sodium citrate had the second greatest influence on the measured response (p-value 0.000, F-value 104.95).

Table 7
ANOVA of the response variable for oil displacement test (ODT) optimization

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	62926.8	4494.8	35.92	0.000
A- Rhamnolipid	1	40059.3	40059.3	320.10	0.000
B- D-limonene	1	34.5	34.5	0.28	0.609
C- Sodium citrate	1	13134.1	13134.1	104.95	0.000
D- Calcium carbonate	1	6.3	6.3	0.05	0.827
A*A	1	93.8	93.8	0.75	0.404
B*B	1	138.6	138.6	1.11	0.313
C*C	1	6632.2	6632.2	53.00	0.000
D*D	1	3.2	3.2	0.03	0.875
A*B	1	20.3	20.3	0.16	0.695
A*C	1	812.2	812.2	6.49	0.026
A*D	1	0.4	0.4	0.00	0.953
B*C	1	0.1	0.1	0.00	0.977
B*D	1	2.8	2.8	0.02	0.884
C*D	1	225.0	225.0	1.80	0.205
Error	12	1501.8	125.1		
Lack-of-Fit	10	1433.2	143.3	4.18	0.208
Pure Error	2	68.5	34.3		
Total	26	64428.6			

Figure 4 illustrates the contour plot for evaluating the effects of proportions of the factor with significant interaction (A*C), which had an interaction p-value of 0.026, on the response of ODT. The proportion of hold values (D-limonene and calcium carbonate) were constant at 15.05 and 0.005 g, respectively. The ODT of an effective eco-friendly degreaser product should be high. The amount of rhamnolipid and sodium citrate had a significant effect in increasing the response of ODT. At 1 g sodium citrate, when rhamnolipid increased (0.2 to 1 g), the ODT of the eco-friendly degreaser increased from 50 to 200 mm. The need for sodium citrate as a builder is significant because it enhances or maintains the cleaning effectiveness of the rhamnolipid [46]. The analysis of both responses (surface tension and ODT) had the same result: rhamnolipid and sodium citrate interaction is crucial to the formulation of the eco-friendly degreaser.

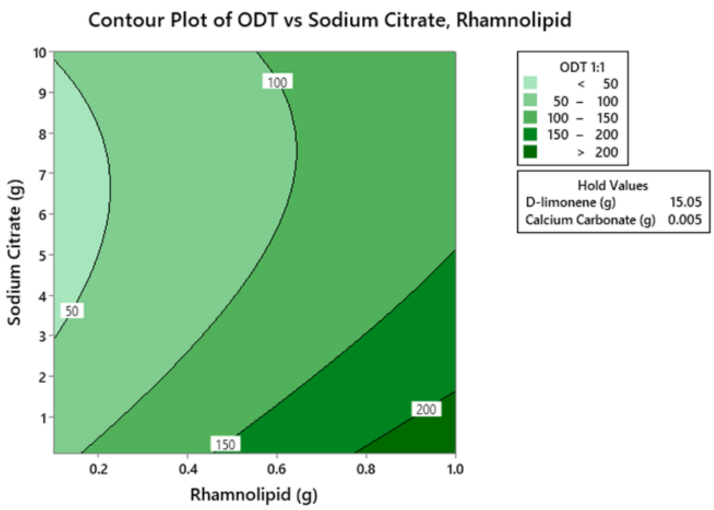


Fig. 4. Illustrated contour plot showing the relationship of rhamnolipid and sodium citrate to response ODT

3.5 Optimization and Validation of Eco-friendly Degreaser

To optimize all responses with varying objectives, a response optimizer technique based on the desirability function and a graphical optimization strategy based on the overlay plot was applied [47]. To achieve the optimal balance between the two responses, a composite desirability (D) with values ranging from 0 (unacceptable response value) to 1 (desirable value) was used to optimize both responses simultaneously. An optimization graphic was created using this regression model to determine the ideal composition for the eco-friendly degreaser (Figure 5). The eco-friendly degreaser formulation was optimized by the multi-response optimizer option in Minitab software based on specific criteria values. The responses for surface tension were minimized, while the responses for the ODT were maximized. The eco-friendly degreaser optimal values for rhamnolipid, D-limonene, sodium citrate, orange oil, and water were calculated as 1.00, 30.00, 0.10, 0.01, and 68.89 g, respectively. The composite desirability was 0.9855, suggesting that this formulation produced reliable outcomes for all responses.

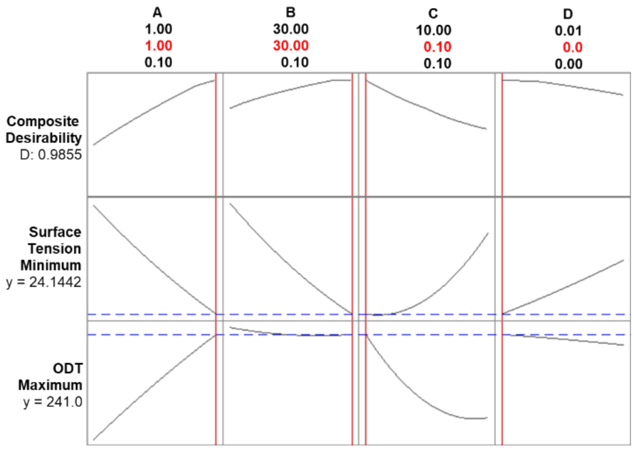


Fig. 5. Response optimizer for minimum surface tension and maximum ODT

The predicted values of surface tension (24.14 mN/m) and ODT (241 mm) were verified experimentally. The % relative error obtained for surface tension and ODT was below 15% at 0.35% and 0.97%, respectively (Table 8). Low relative errors indicate a reasonable agreement between the predicted and experimental values, proving the validity of the model and ascertaining the effects of factors [38].

Table 8
Predicted and experimental values for the optimized formulation

Sample	Surface tension, mN/m			ODT, mm		
	Experimental value	Predicted value	Error (%)	Experimental value	Predicted value	Error (%)
S1	24.07	24.14	0.29	240	241	0.41
S2	24.32	24.14	0.75	243	241	0.83
S3	24.14	24.14	0.00	245	241	1.66
	Mean Error		0.35	Mean Error		0.97

3.6 A Comparative Study to Assess Eco-friendly Degreaser Performance

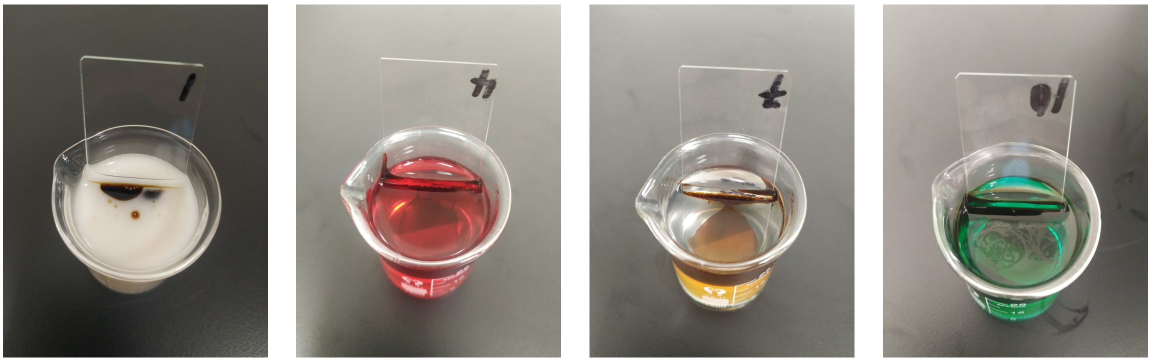
This study assessed the performance of eco-friendly degreaser and compared it to three commercial products (Table 9). Five responses were chosen and compared: pH, surface tension, ODT, removal of oil, and phytotoxicity. The pH of the optimized eco-friendly degreaser was mildly alkaline (8.84±0.005) compared to the commercial product, which was strongly alkaline (11.32±0.010 to 13.88±0.010). Strongly alkaline degreasers are very corrosive and result in rust on the surfaces of metal equipment or machinery [48]. However, cleaning products with extreme pH values (pH>11.5) are very likely to be corrosive to the eyes and skin [49].

Table 9
Comparison to commercial products

Evaluation	Optimized Eco-friendly degreaser	Commercial A	Commercial B	Commercial C
pH	8.84±0.005	13.88±0.010	13.73±0.089	11.32±0.010
Surface tension, mN/m	24.18±0.127	23.44±0.097	22.78±0.094	24.97±0.063
ODT, mm	243±2.517	239±1.155	221±3.606	72±2.887
Removal of oil (%)	58.09±0.986	67.49±0.541	71.39±0.704	51.18±0.920
Phytotoxicity test	Non-toxic	Toxic	Toxic	Toxic

The surface tension and ODT for the optimized eco-friendly degreaser and the three commercial products were observed and calculated. The surface tension response of the optimized eco-friendly degreaser was comparable to that of the commercial products (commercial B < commercial A < optimized eco-friendly degreaser < commercial C). Surface tension values as low as 24.18±0.127 for optimized eco-friendly degreaser indicated a good quality product for oil cleaning [3]. Moreover, for the ODT response, the optimized eco-friendly degreaser dispersed oil much more effectively than the commercial products (commercial C < commercial B < commercial A < optimized eco-friendly degreaser). The larger the ODT diameter for the dispersion test, the greater the oil dispersion capacity, reflecting a high surface activity. The capacity of oil to spread depends on the decrease in water–oil interfacial tension caused by the interaction of the degreaser and the oil [50].

Figure 6 shows the removal of oil from glass slides. The effect of the optimized eco-friendly degreaser formula was comparable to the other chemical-based commercial products (commercial C < optimized eco-friendly degreaser < commercial A < commercial B). Determining the percentage of oil removed from the glass slide examined the ideal builder performance (minimum proportion of builder for maximum oil removal) to obtain a lower final builder composition with maximum cleaning efficiency [36]. This result shows that the combination of biosurfactant and builder in this study (rhamnolipid and sodium citrate) yielded a highly effective eco-friendly degreaser formulation for removing oil. The role of Rhamnolipids was proven significant in the oil removal as mentioned in Idika *et al.,* [11]. The mechanism for the oils removal with rhamnolipid improves the removal of non-aqueous phase liquids (NAPLs) by decreasing their surface/interfacial tension at air-water and water-oil interface [11]. The builder addition in the formulation supported the performance due to their ability on enhance the cleaning efficiency [51].

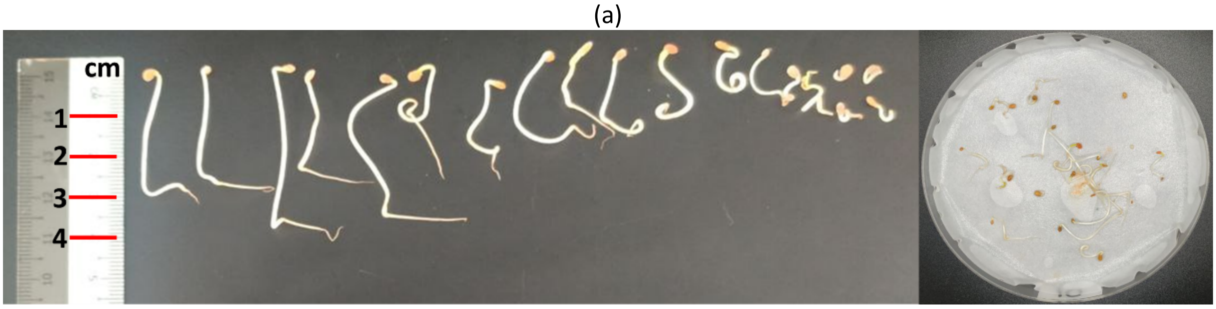


(a) (b) (c) (d)

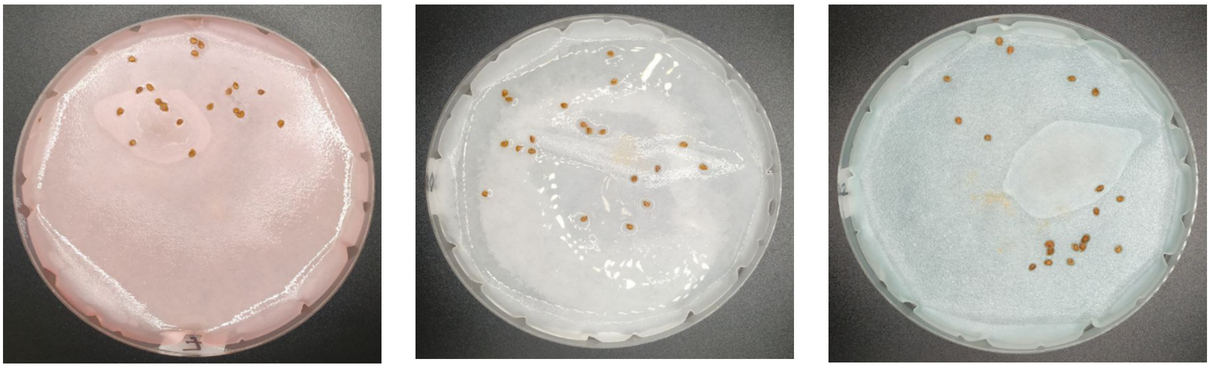
Fig. 6. Removal of oil from glass slides (a) Optimized formulation, (b) Commercial A, (c) Commercial B, and (d) Commercial C

The GI is one of the most prevalent methods for determining the phytotoxicity of a substance or product. This index relies on the sensitivity of plant seed germination to toxic substances [52]. Figure 7 shows the findings of phytotoxicity tests using the vegetable species *S. lycopersicum* to compare the eco-friendly degreaser formulation to commercially available products. The optimized eco-friendly degreaser had a 100% germination rate compared to the control, whereas there was no germination for any commercial products. This demonstrates that the optimized eco-friendly degreaser was non-toxic, safe to use, and had strong competitive potential. Substituting surfactants derived from chemical sources with those derived from renewable sources is a viable strategy for enabling safer and less toxic formulations, producing an environmentally preferable solution [53].

(a)



(b)



(c) (d) (e)

Fig. 7. Phytotoxicity test (a) Optimized eco-friendly degreaser, (b) Control, (c) Commercial A, (d) Commercial B, and (e) Commercial C

4. Conclusion

PBD and BBD models were successfully applied in formulating the eco-friendly degreaser with Rhamnolipids biosurfactant and a combination of a selected range of environmentally friendly ingredients. The formulation has been well-validated and found as effective as chemical-based commercial products. Rhamnolipid biosurfactant was a significant factor in the formulations and exhibited efficient oil dispersion and removal performance. Rhamnolipid was shown to be highly promising as a biosurfactant choice in oil degreasing formulation, which can be commercialized in the future.

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