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Optimization of Flocculation Parameters for Efficient Microalgal Biomass Harvesting: A Response Surface Methodology Approach

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Abstract: Efficient microalgal biomass harvesting is a major bottleneck in the commercial utilization of microalgae for biofuels, nutraceuticals, and wastewater treatment. This study optimizes the flocculation parameters for *Chlorella* sp. and *Monoraphidium* sp. using Response Surface Methodology (RSM) to enhance sedimentation efficiency while minimizing chemical usage. Growth kinetics analysis identified the optimal harvesting times for both species, after which flocculation efficiency was systematically evaluated under varying ferric chloride ($FeCl_3$) concentrations, pH levels, and salinity conditions. The optimized conditions for *Chlorella* sp. were $FeCl_3$ at 49.4 mg L^{-1}, pH 5.6, and salinity 18.2 ppt, achieving a flocculation efficiency of 93.6%. For *Monoraphidium* sp., the highest efficiency (89.2%) was obtained at $FeCl_3$ 66.1 mg L^{-1}, pH 5.6, and salinity 11.5 ppt. The RSM model exhibited strong predictive accuracy, with an R^2 value of 99.07% for *Chlorella* sp. and 94.83% for *Monoraphidium* sp., confirming its reliability in optimizing biomass recovery. Statistical analysis indicated that pH and flocculant concentration were the most influential factors governing flocculation efficiency. These findings demonstrate a cost-effective and scalable approach for improving microalgal harvesting, reducing energy-intensive processing, and enhancing the economic viability of microalgae-based bioproducts.

Keywords: *Chlorella* sp., *Monoraphidium* sp., Biomass recovery modeling, Bioremediation, Sustainable bioprocessing

Introduction

Microalgae are a versatile and sustainable biological resource with diverse applications in pharmaceuticals, biofuels, wastewater treatment, and food industry. Their rapid growth rate and high biochemical composition, including valuable lipids, proteins, and carbohydrates, make them an attractive candidate for biotechnological exploitation. Among the many species of microalgae, *Chlorella* sp. has gained significant attention for its pharmaceutical and nutraceutical potential, while *Monoraphidium* sp. has demonstrated promise in biofuel production and bioremediation applications [1, 2]. Despite their potential, large-scale commercialization of microalgae-based products is hindered by the inefficiency and high cost associated with biomass harvesting [3].

Conventional microalgae harvesting techniques, such as centrifugation, flotation, and sedimentation, present several challenges, including high energy consumption, low efficiency, and scalability constraints [4]. Flocculation has emerged as a promising alternative, offering a cost-effective and efficient approach for biomass recovery [5]. Among the commonly used flocculants, ferric chloride ($FeCl_3$) is particularly effective in aggregating microalgal cells and facilitating sedimentation. However, optimizing the flocculation process requires a systematic and empirical approach to maximize efficiency

while minimizing environmental and economic drawbacks [6].

The inefficiency of current large-scale microalgae harvesting methods underscores the urgent need for sustainable and cost-effective alternatives. This challenge is particularly significant for both commercial and environmental applications, including biofuel production, nutraceuticals, and wastewater remediation [3]. Enhancing harvesting efficiency not only improves the economic feasibility of microalgae-based industries but also supports the role of microalgae in environmental cleanup efforts. Addressing this challenge necessitates advanced optimization strategies to refine harvesting techniques and enhance their scalability [7, 8].

The primary objectives of this study are to evaluate the growth performance of *Chlorella* sp. and *Monoraphidium* sp. under controlled cultivation conditions, determine optimal growth and harvest times for efficient biomass accumulation, assess flocculation efficiency under varying conditions, and optimize the flocculation process using RSM to enhance sedimentation efficiency. By improving biomass recovery efficiency, this research contributes to the advancement of sustainable biotechnological applications, aligning with global sustainability goals. The integration of RSM in microalgae harvesting strategies not only enhances economic viability but also fosters environmentally responsible practices, making microalgae-based solutions more accessible and impactful for various industrial and environmental applications.

Materials and methods

Microalgae Cultivation and Growth Evaluation

The microalgae isolates used in this study - *Chlorella* sp. and *Monoraphidium* sp.- were obtained from the Mansoura Algal Culture Collection at the Algae Biotechnology & Water Quality Lab at Mansoura University. Cultures were cultivated in Bold's Basal Medium (BBM) [9] under controlled laboratory conditions, to ensure optimal growth, with a light intensity of 100 µmol photons m^{-2} s^{-1}, a 16:8-hour light-dark cycle, and a temperature of 25 ± 1°C. Aeration was continuously provided to ensure homogeneous mixing and prevent sedimentation using sterile air pumps fitted with 0.22 µm filters to prevent contamination [10].

Microalgal growth was monitored by measuring the optical density (OD) at 680 nm daily using a spectrophotometer, allowing for real-time assessment of culture density changes over time. The specific growth rate (µ) was calculated using the following equation [11, 12]:

$$\mu = \frac{\ln(a_2) - \ln(a_1)}{t_2 - t_1}$$

where (a_1) represents the initial culture absorbance at time (t_1), and (a_2) represents the culture absorbance at a later time (t_2). The difference between (t_2) and (t_1) represents the duration of the exponential growth phase.

In addition to OD measurement and specific growth rate calculation, dry biomass was quantified by harvesting microalgal cultures at the end of the incubation period, and biomass was collected via filtration using pre-weighed Whatman GF/C filters. The retained biomass was dried at 60°C in a humidity-controlled oven until a constant weight was achieved. The dry weight was calculated and expressed as mg L^{-1} [13].

To evaluate the biochemical composition of the microalgae, carbohydrate, lipid, and protein contents were analyzed using standard biochemical assays. Carbohydrate content was quantified using the phenol-sulfuric acid method [14], a widely used colorimetric assay that detects sugar derivatives based on their reaction with concentrated sulfuric acid and phenol. Lipid extraction was performed following the Bligh and Dyer method [15], which utilizes a chloroform-methanol-water mixture to effectively isolate total lipid content from microalgal cells. Protein concentration was determined using the Lowry method [16], a sensitive assay based on protein-copper interactions, with bovine serum albumin (BSA) used as a standard for calibration.

Flocculation Efficiency

Flocculation experiments were conducted in 150 mL sample cups containing 100 mL of microalgal culture. During the flocculation experimental runs, cultures with different flocculation parameters were continuously stirred using rpm-controlled motorized rods for 15 minutes at 100 rpm to promote interaction

homogeneous mixing, followed by a settling period of 30 minutes under static conditions. The supernatant was carefully withdrawn, and flocculation efficiency (FE) was assessed by measuring the reduction in OD at 680 nm then calculated according to the following equation

$$FE\ (\%) = \frac{a_0 - a_t}{a_0} \times 100$$

where a_0 indicates the Initial absorbance prior to sedimentation and absorbance after sedimentation time was denoted by a_t.

A factor-by-factor approach was employed to determine the optimal range of key physicochemical parameters influencing flocculation, including flocculant concentration, pH, and salinity. The tested range for flocculant concentration ($FeCl_3$) was from 10 to 100 mg L^{-1}, pH was from 3 to 12 and salinity was from 5 to 50 ppt NaCl.

Response Surface Model Optimization with Central Composite Design

The optimal parameters determined from the factor-by-factor analysis were subsequently used to construct the experimental matrix for the Central Composite Design in the Response Surface Methodology model. The experimental CCD matrix used in the optimization process is presented in Table (1).

The CCD experimental design consisted of 20 experimental runs, including factorial points, axial points, and center points, to assess both linear and quadratic effects of the independent variables. The mathematical model used to describe the flocculation efficiency (Y) as a function of the independent variables was expressed as follows [17, 18]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_I^2 + \sum_{i<j} \beta_{ij} X_i X_j + \varepsilon$$

Where the response variable (Y) represents the dependent variable being studied, while β_0 is the intercept of the model. The coefficients of the linear terms are denoted as β_i, whereas β_{ii} represents the coefficients of quadratic terms. Interaction effects between independent variables are captured by β_{ij}, with X_i and X_i representing the independent variables in the model. Lastly, ε accounts for the random error. Statistical analysis was performed to generate response surface plots, which visually represented the influence of each factor on flocculation performance. Following the identification of optimal conditions, confirmatory experiments were conducted to validate the accuracy of the predictive model. Flocculation efficiency was quantified by measuring the reduction in OD at 680 nm before and after sedimentation, providing a direct assessment of cell removal efficiency.

Statistical Analysis

Experimental data were analyzed and visualized using JMP 17.2 statistical software [19]. Student's T-test was conducted to determine significant differences between paired groups. To optimize flocculation efficiency and explore the interactions between variables, a Response Surface Methodology (RSM) approach was employed. The RSM analysis included effect estimation, ANOVA to evaluate model significance, and a prediction profiler to visualize and identify optimal parameter settings. A 95% confidence interval was applied to all experimental analyses. However, for the RSM model, a 90% confidence level was used to account for the method's sensitivity, ensuring robust and reliable predictions.

Table 1: Coded and actual level values of the experimental variables used for the CCD matrix.

Factor	Name	*Chlorella* sp.			*Monoraphidium* sp.		
		-1	0	1	-1	0	1
X_1	Flocculant (mg/L)	20	40	60	40	60	80
X_2	pH	4	6	8	3	5	7
X_3	Salinity (ppt)	15	25	35	0	10	20

Results and Discussion

Growth Kinetics and Evaluation

The optical density (OD) measurements at 680 nm for *Chlorella* sp. and *Monoraphidium* sp. demonstrated a typical sigmoidal growth curve (shown in Figure 1). For *Chlorella* sp., OD increased steadily until day 9 (1.742), after which the growth rate plateaued, indicating the onset of the stationary phase. Similarly, *Monoraphidium* sp. exhibited maximum OD around day 11 (1.398). These results suggest that the optimum harvesting time for *Chlorella* sp. is between days 8 and 9, whereas *Monoraphidium* sp. reaches its peak biomass around days 10 to 11.

The specific growth rate (μ) values illustrated in Figure (2) further support these findings, with *Chlorella* sp. exhibiting a higher growth rate (0.321 day^{-1}) compared to *Monoraphidium* sp. (0.228 day^{-1}). The higher growth rate of *Chlorella* sp. may be attributed to its adaptability to the culture conditions and faster nutrient uptake efficiency [20].

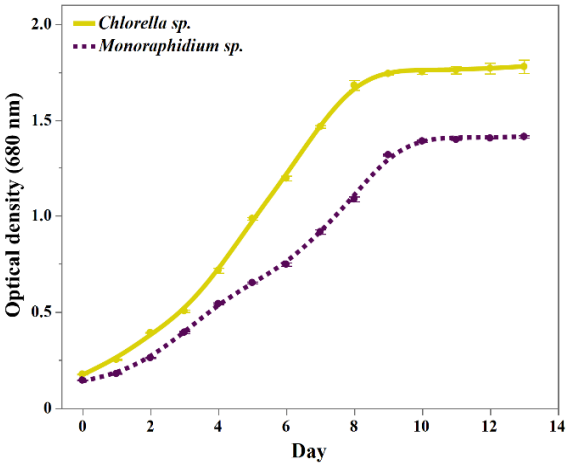

Figure 1: Optical density (680 nm) progression of *Chlorella* sp. and *Monoraphidium* sp. over time.

The dry biomass results in Figure (2) revealed that *Monoraphidium* sp. produced a significantly higher biomass yield (678.00 mg L^{-1}) compared to *Chlorella* sp. (439.33 mg L^{-1}). This difference could be due to variations in cellular morphology and metabolic pathways that influence biomass accumulation [20].

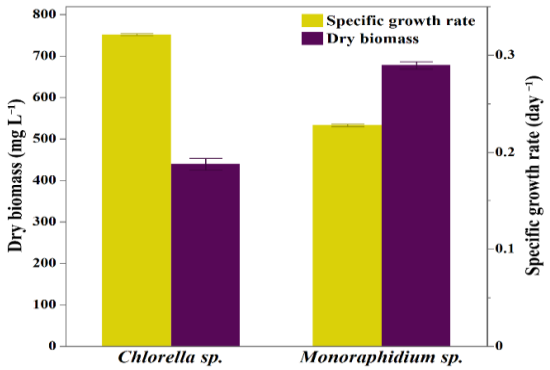

Figure 2: Specific growth rate and Dry biomass of *Chlorella* sp. and *Monoraphidium* sp.

In terms of biochemical composition (Figure 3), *Chlorella* sp. exhibited a notably higher protein content of 61.12 % compared to 22.79 % in *Monoraphidium*, while *Monoraphidium* sp. accumulated higher lipid (39.09 %) and carbohydrate (30.66 %) fractions which are higher than those in *Chlorella* sp. (12.21 % and 15.93 %, respectively). The superior protein content in *Chlorella* sp. makes it a promising candidate for applications in protein-rich feed and nutritional supplements, whereas *Monoraphidium* sp. appears more suitable for biofuel production due to its enhanced lipid accumulation [21, 22].

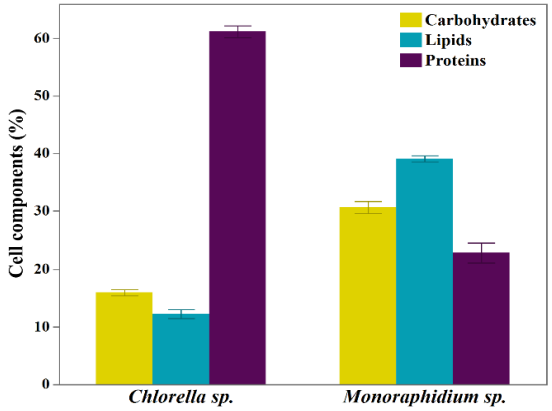

Figure 3: Comparative analysis of cellular composition (% of carbohydrates, lipids, and proteins) in *Chlorella* sp. and *Monoraphidium* sp.

Flocculation Efficiency

Figure (4) demonstrates how the flocculation efficiency (FE%) of *Chlorella* sp. and *Monoraphidium* sp. was analyzed across three parameters, i.e. flocculant concentration, pH, and salinity (Figure 4A, 4B and 4C, respectively).

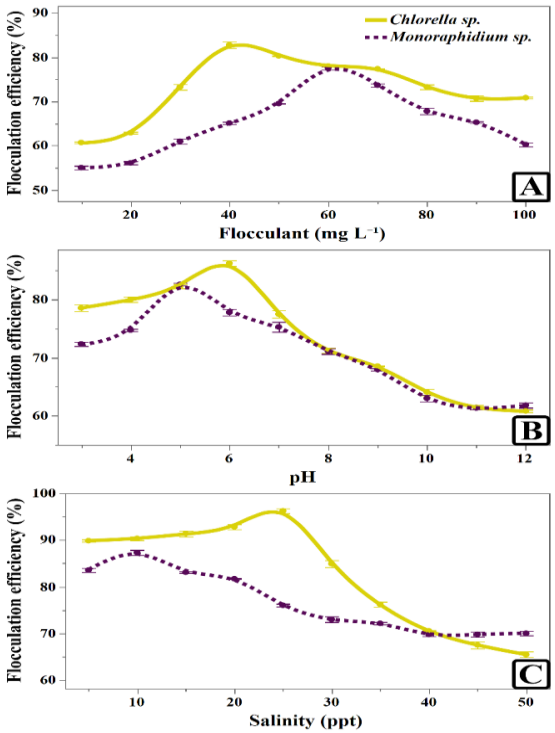

Figure 4: Flocculation efficiency trends for A) flocculant concentration, B) pH, and C) salinity.

For flocculant concentration, both species exhibited an increasing trend in FE up to an optimal point, beyond which efficiency declined. For *Chlorella* sp., the highest FE (82.78%) was observed at 40 mg L^{-1}, whereas *Monoraphidium* sp. peaked at 60 mg L^{-1} (77.58%). The decline in FE at higher concentrations may be attributed to charge neutralization saturation leading to particle restabilization. [23, 24].

Similarly, pH variation significantly influenced FE, with *Chlorella* sp. achieving the highest flocculation at pH 6 (86.2%) and *Monoraphidium* sp. at pH 5 (82.6%). Deviations from these optimal pH values resulted in decreased FE, likely due to changes in the surface charge of microalgal cells affecting coagulation efficiency [25]. Salinity also played a crucial role, with *Chlorella* sp. achieving maximum FE (96.24%) at 25 ppt and *Monoraphidium* sp. (87.32%) at 10 ppt. However, a decline was observed at higher salinity levels, suggesting that excessive ionic strength may hinder effective cell aggregation [26].

Response Surface Model Optimization

The central composite design (CCD) model summary in Table (2) demonstrated strong predictive capabilities for *Chlorella* sp., with an R^2 value of 99.07% and an adjusted R^2 of 98.03%, indicating a high correlation between experimental and predicted values. In contrast, *Monoraphidium* sp. exhibited a lower predictive power (R^2 = 94.83%, adjusted R^2 = 89.09%), with a relatively low predicted R^2 value (47.22%), suggesting some model limitations in accurately predicting experimental outcomes. The interactions between flocculant concentration, pH, and salinity in the RSM model presented in Figure (5) and Table (3) highlight the complex dynamics governing the flocculation process. The significant quadratic effects of each parameter (pH*pH, salinity*salinity, and flocculant*flocculant) observed in both *Chlorella* sp. and *Monoraphidium* sp. indicate a nonlinear relationship, where increasing beyond a certain optimal level results in diminished efficiency . For *Chlorella* sp., pH had the most substantial effect (F = 102.98, $p < 0.001$), suggesting its critical role in altering the surface charge of microalgal cells, thereby affecting electrostatic interactions with the flocculants [27, 28].

Table 2: CCD model predictability and validation summary for *Chlorella* sp. and *Monoraphidium* sp.

Microalgae	S	R^2	R^2 (adj)	R^2 (pred)
Chlorella sp.	0.61	99.07%	98.03%	92.13%
Monoraphidium sp.	1.74	94.83%	89.09%	47.22%

In contrast, flocculant concentration was the dominant factor for *Monoraphidium* sp. (F = 48.26, $p < 0.001$), indicating that its aggregation is more dependent on flocculant-driven charge neutralization and bridging mechanisms. The interaction between flocculant and pH, while not statistically significant for either species ($p > 0.05$), suggests that specific pH conditions may enhance or suppress the effectiveness of particular flocculants due to changes in solubility and aggregation behavior. However, the interaction between flocculant and salinity was significant for *Chlorella* sp. (F = 15.88, p = 0.003), indicating that increasing salinity initially promotes flocculation, likely by reducing electrostatic repulsion, but excessive levels may disrupt the process by destabilizing

cell aggregation [26, 29]. The lack of strong two-way interactions for *Monoraphidium* sp. ($p > 0.05$) suggests that its flocculation response is more independently influenced by individual factors rather than synergistic effects. The optimal conditions predicted by the RSM model -flocculant 49.4 mg L^{-1}, pH 5.6, and salinity 18.2 ppt for *Chlorella* sp., and flocculant 66.1 mg L^{-1}, pH 5.6, and salinity 11.5 ppt for *Monoraphidium* sp.- aligned well with the actual validated responses (93.6% and 89.2% efficiency, respectively), confirming the model's predictive reliability.

The regression equations demonstrate these interactions and offer insights into the observed trends as shown in the following second-order polynomial equations:

$$FE_{C.sp.}(\%) = 26.90 + 0.439X_1 + 16.66X_2 + 0.893X_3 - 0.00537X_1^2 - 1.356X_2^2 - 0.03673X_3^2 - 0.0211X_1 * X_2 + 0.01152X_1 * X_3 - 0.0225X_2 * X_3$$

$$FE_{M.sp.}(\%) = -62.0 + 3.091X_1 + 15.78X_2 + 1.088X_3 - 0.02421X_1^2 - 1.518X_2^2 - 0.0595X_3^2 + 0.0143X_1 * X_2 + 0.00258X_1 * X_3 + 0.0195X_2 * X_3$$

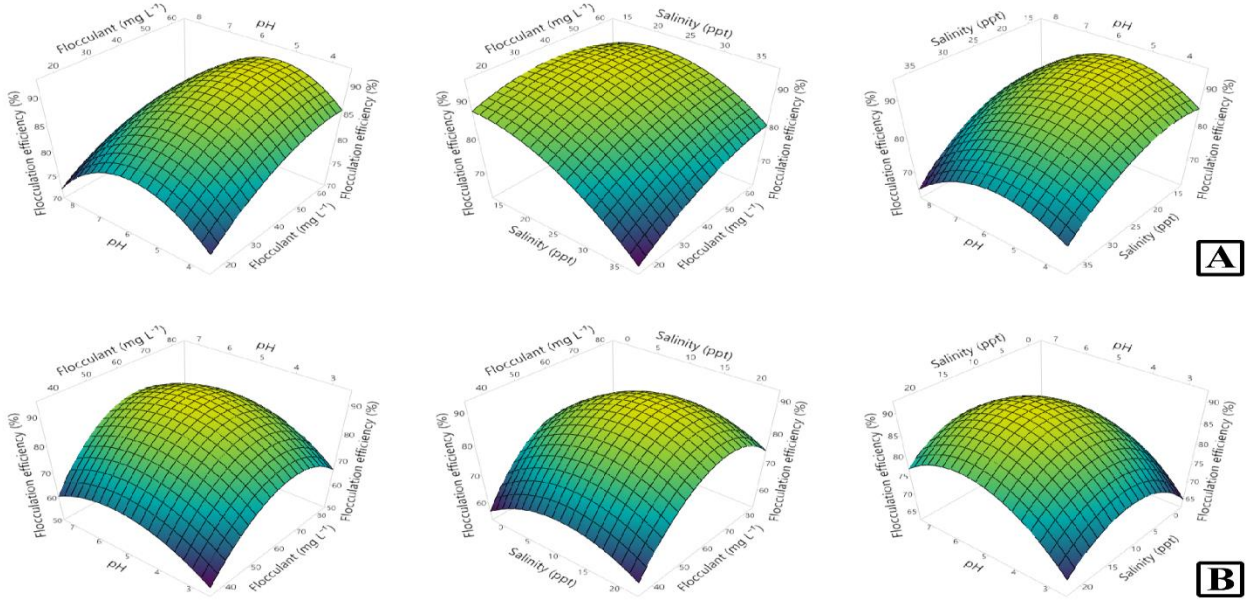

Figure 5: Response surface plots showing the interactive effects of flocculant concentration, pH, and salinity on flocculation efficiency for A) *Chlorella* sp. and B) *Monoraphidium* sp.

Table 3: Interaction effects of flocculant, pH, and salinity based on ANOVA results.

Source	DF	*Chlorella* sp.				*Monoraphidium* sp.			
		Adj SS [a]	Adj MS [b]	F-Value [c]	P-Value [d]	Adj SS	Adj MS	F-Value	P-Value
Model	10	358.954	35.8954	95.49	< 0.001*	502.74	50.273	16.52	< 0.001*
Blocks	1	2.44	2.4398	6.49	0.311*	2.89	2.89	0.95	0.355
Linear	3	38.829	12.9429	34.43	< 0.001*	155.57	51.855	17.04	< 0.001*
Flocculant (X_1)	1	3.452	3.4523	9.18	0.014*	146.86	146.86	48.26	< 0.001*
pH (X_2)	1	38.711	38.7111	102.98	< 0.001*	44.156	44.156	14.51	0.004*
Salinity (X_3)	1	3.16	3.1596	8.41	0.018*	7.444	7.444	2.45	0.152
Square	3	79.241	26.4136	70.27	< 0.001*	273.17	91.056	29.92	< 0.001*
X_1*X_1	1	8.572	8.5725	22.81	0.001*	174.23	174.23	57.25	< 0.001*
X_2*X_2	1	54.626	54.626	145.32	< 0.001*	68.444	68.444	22.49	0.001*
X_3*X_3	1	25.061	25.061	66.67	< 0.001*	65.655	65.655	21.57	0.001*
2-Way Interaction	3	6.996	2.3321	6.2	0.014*	0.837	0.279	0.09	0.963
X_1*X_2	1	0.8	0.8001	2.13	0.179*	0.366	0.366	0.12	0.737
X_1*X_3	1	5.969	5.9685	15.88	0.003*	0.3	0.3	0.1	0.761
X_2*X_3	1	0.228	0.2278	0.61	0.456	0.171	0.171	0.06	0.818
Error	9	3.383	0.3759			27.388	3.043		
Lack-of-Fit	5	3.373	0.6747	275.37	< 0.001*	27.378	5.476	2105.98	< 0.001*
Pure Error	4	0.01	0.0025			0.01	0.003		
Total	19	362.337				530.12			

[a]Adjusted sum of squares,

[b] Adjusted mean squares

[c] Fishers's exact test statistic value

[d] Probability of significance against the null hypothesis

* Significant at 90% level of confidence (P < 0.1)

Table 4: Comparison of predicted and actual validated flocculation efficiency values for optimized conditions.

Microalgae	Setting			Predicted Response	Actual Response
	Flocculant	pH	Salinity	Efficiency (%)	Efficiency (%)
Chlorella sp.	49.4	5.6	18.2	92.5	93.6
Monoraphidium sp.	66.1	5.6	11.5	90.4	89.2

The optimized CCD settings were experimentally validated (Table 4), demonstrating a strong agreement between predicted and actual flocculation efficiencies. For *Chlorella* sp., the predicted FE (92.5%) closely matched the actual response (93.6%). Similarly, *Monoraphidium* sp. exhibited a minor deviation between predicted (90.4%) and actual (89.2%) FE values, confirming the reliability of the model in optimizing flocculation conditions.

Conclusion

This study highlights the distinct growth, biochemical composition, and flocculation efficiency of *Chlorella* sp. and *Monoraphidium* sp., with Response Surface Methodology (RSM) playing a key role in optimizing biomass recovery. *Chlorella* sp. exhibited superior flocculation efficiency and high protein content, making it well-suited for large-scale harvesting, while *Monoraphidium* sp. demonstrated higher lipid accumulation and biomass yield, supporting its potential for biofuel production. The RSM-based optimization significantly improved flocculation performance, particularly for *Chlorella* sp., enhancing the efficiency of biomass recovery. These findings underscore the broader applicability of microalgal flocculation in sustainable biotechnology, whether for biomass harvesting or bioremediation in wastewater treatment.

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