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Comparative Performance of Three Algae-Derived Green Coagulants for Total Hydrocarbon Removal from Industrial Wastewater

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ABSTRACT

Total petroleum hydrocarbons (TPH) in industrial wastewater represent a significant environmental challenge, requiring efficient remediation techniques. This study evaluates the effectiveness of three algal species *Oscillatoria* sp., *Oedogonium* sp., and mixed diatoms as natural bio-coagulants for TPH removal. The process was optimized through Response Surface Methodology (RSM) based on a Central Composite Design (CCD) to assess the influence of pH, coagulant dosage, and contact time. The experimental data indicated that *Oscillatoria* sp. achieved a maximum removal efficiency of 99.7% at pH 7, a dosage of 1000 mg/L, and a mixing speed of 50 rpm for 15 min. *Oedogonium* sp. reached 93.5% removal under alkaline conditions (pH 9, 50 rpm, 22.5 min), while diatoms showed an efficiency of 86.8% at pH 7 (30 rpm, 22.5 min). The reduction in TPH levels was confirmed via GC-FID analysis. Statistical analysis showed that while the models for *Oscillatoria* sp. and diatoms were significant, the *Oedogonium* sp. model exhibited a lack-of-fit, potentially due to the complex nature of the wastewater matrix. FTIR analysis identified hydroxyl, carboxyl, and amino groups as the functional groups responsible for the bio-coagulation process. These results indicate that the studied algal biomass can serve as promising bio-coagulants for the preliminary treatment of oil-contaminated wastewater.

INTRODUCTION

Hydrocarbons are complex organic compounds composed of carbon and hydrogen atoms, existing in diverse structural forms such as straight chains, branched chains, or rings **(Badia & Burres, 2010)**. In environmental pollution research, total petroleum hydrocarbons (TPH) are identified as major contaminants in industrial wastewater. The discharge of TPH into aquatic ecosystems poses significant threats to marine life and human health due to its toxicity and environmental persistence. TPH enters the environment through natural phenomena—such as volcanic eruptions and hydrothermal reactions—as well as anthropogenic activities, including oil spills, industrial processes, and the combustion of fossil fuels **(Chen *et al.*, 2013; Lindén & Palsson, 2013; Kong *et al.*, 2015**).

Highly toxic petroleum hydrocarbons significantly impact human health **(Ordinioha & Birisibe, 2013)**. Beyond carcinogenicity, these compounds may induce hormonal imbalances affecting reproduction and growth **(Aguilera *et al.*, 2010)**. Consequently, developing efficient treatment methods is essential. Coagulation is a key chemical process that removes suspended solids by altering the electrostatic charges of particles. During this process, coagulants neutralize the charges of suspended particles, colloids, or oily substances **(Salih, 2023)**. Flocculation follows as a mixing stage to increase particle size, facilitating sedimentation (**Bratby, 2006**).

While chemical coagulants, such as aluminum sulfate (alum), are widely used due to their low cost, they exhibit several disadvantages. These include high sensitivity to pH and temperature, the requirement for high dosages, and the production of substantial amounts of sludge **(Tzoupanos & Zouboulis, 2008)**. Furthermore, residual aluminum is a neurotoxic element associated with health risks **(Huang *et al.*, 2014)**. Therefore, organic coagulants derived from biological sources have gained attention as promising alternatives due to their availability and potential as sustainable materials (**Dotto *et al.*, 2019**).

Recently, algal bioactive compounds have emerged as significant tools in environmental applications, including wastewater treatment **(Bilal *et al.*, 2018)**. Functional groups on the algal cell wall—such as carboxyl, hydroxyl, and phosphate groups—interact with the environment and serve as binding sites for pollutants **(Spain *et al.*, 2021)**. Understanding these properties is crucial for optimizing biosorption mechanisms. This study focuses on using specific algae species as natural coagulants for TPH removal. Given that performance depends on surface charge, this research investigates the impact of pH-targeted adjustment of the aqueous phase on the coagulation efficiency of *Oscillatoria* sp., *Oedogonium* sp., and mixed diatoms.

MATERIALS AND METHODS

1. Algae Collection and Identification

Local algae samples were collected during different periods of 2024 from specific aquatic habitats in Basrah, southern Iraq. *Oscillatoria* sp. was collected in September 2024 from the Karma 1 Water Project (30.56° N,47.75°E). *Oedogonium* sp. was obtained in November 2024 from the Water Injection Station in Abu Skhair (30.60°N,47.68°E), while the diatom community (dominated by *Nitzschia* sp. and *Navicula* sp.) was collected in December 2024 from a stagnant pond in a local garden. The samples were identified using a light microscopem as shown in Fig. (1). This taxonomic identification was carried out under supervision and with the expert assistance of the research supervisor, Asst. Prof.Dr. Maitham Abdullah Al-Shaheen, at the Department of Ecology College of Science, University of Basrah. The identification followed the taxonomic keys and reference manuals provided by **Prescott (1984)** and **Al-Shaheen (2019)**. For verification and documentation, voucher specimens were preserved in 4% formalin, and other representative samples were kept as dried samples; all these materials are deposited in the specialized laboratories of the Department of Ecology under the internal voucher codes: OSC-9-2024 for *Oscillatoria* sp., ODE-11-2024 for *Oedogonium* sp., and DIA-12-2024 for mixed diatoms.

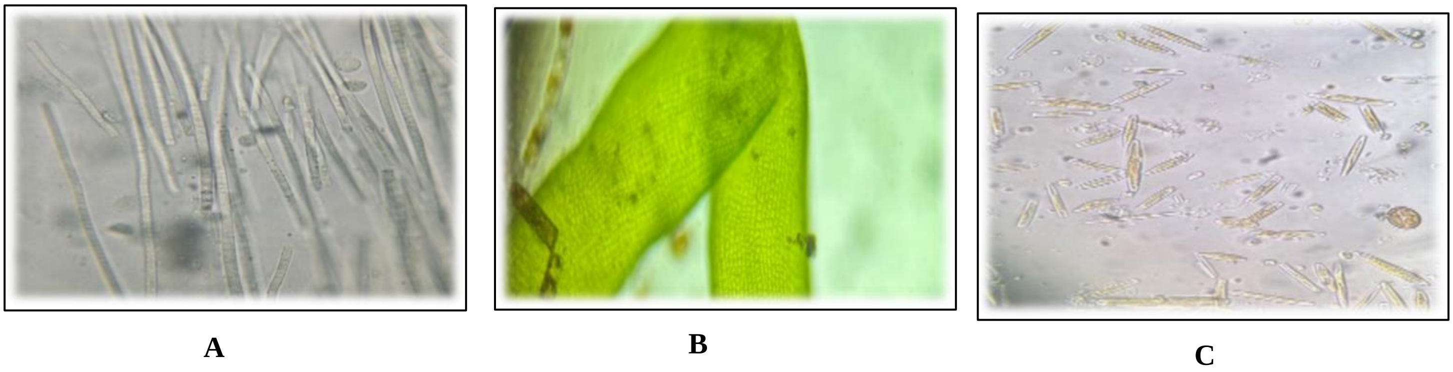

Fig. 1. Microscopic images of the selected algae species used in the study. (A) *Osillatoria* sp., (B) *Oedogonium* sp., (C) mixed diatoms.

2. Preparation of Algal Coagulant Extract and pH Conditioning

After identification, selected algae were washed with distilled water. The biomass was then spread in thin layers and dried under direct sunlight for 3 to 5 days until a constant weight was achieved and the samples became brittle. The dried biomass was then crushed into a fine powder using an electric blender and sieved to a particle size of < 150 µm and stored in a dry container (**Ahmad *et al.*, 2021**).

To prepare the green coagulants extract, 100 ml of distilled water was first adjusted to the target pH levels (5,7, and 9) using 0.1 M NaOH and HCl. Subsequently,10 g of each algal powder was added to the pH-conditioned water and mixed using a magnetic stirrer hotplate for 30 minutes at room temperature to ensure the homogeneity **(Benalia *et al.*, 2021)**. The final aqueous extract was filtered using filter paper (0.33 mm ZELPA Belgium paper), and then further using a centrifuge to ensure complete extraction of the active compounds.

3. Experimental Design and Statiscal Optimization

Response surface methodology (RSM) based on a (CCD) was employed using Design-Expert software (version13) to optimize operating conditions. The design variables included the pH-conditioned extraction (5-9), slow mixing speed (10-50 rpm), and slow mixing time (15-30 min). A total of 17 experimental runs were generated as shown in Table (1), incorporating replicated center points to ensure the validity and reliability of the results. All experiments were conducted in triplicate, except for the center points (replicated runs) generated by the software. The optimal doses for each coagulant were pre-determined through preliminary runs as 500 mg/L for diatoms, 1000 mg/L for *Oscillatoria* sp, and 3000 mg/L for *Oedogonium* sp. These specific doses were selected to ensure a fair comparison based on the maximum capacity of each species. Furthermore, the rapid mixing speed and time were fixed at 180 rpm for 5 minutes, and the settling time was set at 30 minutes. These parameters were kept constant based on the findings of preliminary experiments to ensure consistent conditions throughout the optimization process.

Table 1. Central composite design (CCD) matrix and the observed responses for wastewater treatment.

Run	**pH (C)**	**Slow mix (rpm) (A)**	**Time (min) (B)**	**Removal (%) Diatoms**	**Removal (%) *Oedogonium* sp.**	**Removal (%) *Oscillatoria* sp.**
1[1]	7	30	22.5	%85.53	%87.06	%86.88
2	7	10	15	%81.79	%87.275	%89.02
3[1]	7	30	22.5	%86.8	%87.06	%87.09
4	5	10	22.5	%84.3	%52.578	%88.07
5	7	50	15	%75.59	%45.599	%99.75
6	7	10	30	%77.25	%88.266	%86.99
7	5	50	22.5	%53.5	%79.917	%85.52
8	9	30	30	%77.42	%89.047	%61.84
9	9	30	15	%68.97	%80.738	%88.91
10	7	50	30	%32.53	%82.547	%77.77
11	9	50	22.5	%21.22	%93.556	%86.91
12	5	30	30	%50	%90.272	%51.03
13[1]	7	30	22.5	%84.4	%87.06	%84.9
14	7	30	22.5	%86.01	%88.871	%85.87
15	9	10	22.5	%45.93	%87.111	%80.67
16[1]	7	30	22.5	%84.65	%87.06	%86.31
17	5	30	15	%69.81	%85.896	%83.22

[1] Runs 1,3,13,14, and 16 represent the center point replicate.

4. Collection of Contaminated Water Samples from Shatt al-Basrah gas-fired power plant

Wastewater samples were collected in March 2025 from the effluent discharge points of the Shatt al-Basrah gas-fired power plant. The samples were gathered using high-density polyethylene (HDPE) containers (5-liter capacity). Prior to sampling, these containers were thoroughly cleaned to prevent contamination. After collection, the containers were sealed, labeled, and transported in a chilled cooler box to the laboratory. All samples were stored at 4 °C and were thoroughly homogenized before each experimental run to ensure consistent characteristics throughout the study.

5. TPH Extraction and GC-FID Analysis

The concentration of TPH in the water samples was determined using the liquid-liquid extraction method. A 100 mL volume of the wastewater sample was mixed with 100 mL of chloroform (1:1 ratio) in a separating funnel. The mixture was shaken slowly and allowed to stand for 15 minutes to ensure complete phase separation. The bottom organic layer, containing the extracted hydrocarbons, was collected and evaporated at room temperature to remove the solvent **(Chaillan *et al.*, 2004)**. The residue was re-dissolved in 10 mL of n-hexane and transferred into air-tight glass vials for GC-FID analysis.

Analysis was performed using an Agilent 7890A Gas Chromatograph equipped with a Flame Ionization Detector (FID) and an HP-5 capillary column (30 m × 0.25 mm × 0.25 µm). The operational conditions were as follows: 5 µL of the extract was injected in split mode (25:1) at an inlet temperature of 280 °C.

The column flow was maintained at 1.5 mL/min using Nitrogen (99.99% purity) as the carrier gas. The oven temperature program started at an initial temperature of 40 °C (held for 2 min), then increased at a heating rate of 25 °C/min to a final temperature of 310 °C, where it was held for 13 min, resulting in a total analysis time of 25.8 minutes. The FID detector temperature was maintained at 320 °C. For quantification, an external standard calibration was conducted according to the ASTM D5037 standard method using mixture of alkanes (C_8 to C_{40}) at 50 µg/mL, the calibration curve exhibited high linearity $R^2 > 0.99$, confirming the reliability of the results. The limit of Detection (LOD) and limit of Quantification (LOQ) were determined to be 0.05 µg/mL and 0.15 µg/mL, respectively. Quality assurance (QA/QC) procedures included the analysis of procedural blanks, recovery efficiencies (which ranged between 92% and 98%), and replicate injection performed in triplicates (n = 3) to ensure precision and monitor for any potential contamination. The TPH removal efficiency was calculated using the following equation:

$$\textbf{Removal } \% = \left[\frac{C_0 - C_t}{C_0}\right] \times 100$$

Where:

C_0: The initial concentration of TPH in the wastewater (mg/L).

C_t: The residual concentration of TPH after treatment (mg/L).

RESULTS AND DISCUSSION

Design Expert software was used to simulate optimal conditions for coagulation and flocculation processes in order to achieve the best results. Table (2) shows the range and levels of the experimental input variables.

Table 2. Conditions, ranges, and levels of input factors for response surface modeling (RSM).

Factor	**Unit**	**Minimum**	**Maximum**	**Coded low**	**Central (0)**	**Coded High**
pH	---	5	9	-1↔5	7	+1↔9
Slow mixing speed	Rpm	10	50	-1↔10	30rpm	+1↔50
Slow mixing time	Min	15	30	-1↔15	22.5	+1↔30

1. Application of Algae-Derived Flocculants to Treat Contaminated Water at a Power Plant

Algae-derived flocculants were applied to the wastewater from the Shatt al-Basra gas-fired power plant to evaluate the flocculants effectiveness in reducing pollutants. Table (3) shows the characteristics of the power plant's wastewater.

Table 3. Physical and chemical characteristics of the Shatt al-Basrah power plant.

Parameter	**Concentration** [1]
Total hydrocarbons	73.064 mg/L±1.15
TSS	140 mg/L±2.5
Turbidity	335.66 NTU±5.2
EC	28184 µs / cm±150
COD	221.2 mg/L±3.4

[1] Values are mean of triplicates ±SD.

2. The Effect of pH, Mixing Rate, and Mixing Time on Total Hydrocarbons

The results obtained from the response surface methodology of TPH is directly influenced by extraction conditions of the algal coagulants. This indicates that the chemical nature and functional quality of the extracted coagulants are highly dependent on the pH of the medium used during extraction process. *Oscillatoria* sp. achieved its peak removal efficiency (99.75%) in pH7. The findings of the current study are in alignment with the research by **Mensah-Akutteh (2022)** using RSM, which indicate that a natural medium is among the most favorable conditions for coagulation and flocculation processes. This is attributed to the enhancement of charge neutralization and floc formation, which consequently improves the removal efficiency of pollutants such as TPH. Furthermore, **Ugboma *et al.* (2023)** demonstrated that *Oscillatoria* sp. possesses a significant capacity to degrade petroleum compounds and improve water quality, achieving removal rates of 83.5%. while diatoms and *Oedogonium* sp. coagulants showed technical efficiency in this study, *Oscillatoria* sp. proved to be good choice for treating hydrocarbon-contaminated water. The exceptional performance of *Oscillatoria* sp. can be explained by its action mechanism — physical trapping, acting as a biological net to capture hydrocarbon compounds. The bridging mechanism is facilitated by the polysaccharides identified in the FTIR analysis, which promote the formation of stable and heavy flocs through inter-particle bridging.

The results of the analysis of variance (ANOVA) for the TPH OSC response model, as shown in Table (4), indicated that the slow mixing time was the most influential factor, showing a highly significant effect with a *p*-value of 0.0001. Conversely, the pH factor was the least influential, with a non-significant effect (*p* - value = 0.433). The coefficient of determination R^2 0.887, and adjusted R^2 were 0.742, while the predicted R^2 value was -0.7811 as presented in Table (5). Based on the TPH removal analysis, the coded model equation is as follows:

$$TPHOSC = 86.45 + 0.6500A - 10.41B + 1.31C - 4.99AB + 2.20AC \\ + 1.28BC + 8.11A^2 - 5.94B^2 - 9.02C^2$$

Table 4. Analysis of ANOVA for the quadratic model of TPH removal by *Oscillatoria* sp.

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	***P*-value**	
Model	1749.92	9	194.44	6.12	0.0130	significant
A-slow mixing speed	3.38	1	3.38	0.1064	0.7538	
B-Slow mixing time	866.74	1	866.74	27.29	0.0012	
C-pH	13.76	1	13.76	0.4331	0.5315	
AB	99.50	1	99.50	3.13	0.1200	
AC	19.32	1	19.32	0.6082	0.4610	
BC	6.55	1	6.55	0.2064	0.6634	
A^2	276.76	1	276.76	8.71	0.0213	
B^2	148.31	1	148.31	4.67	0.0675	
C^2	342.95	1	342.95	10.80	0.0134	
Residual	222.32	7	31.76			
Lack of Fit	219.25	3	73.08	95.38	0.0004	significant
Pure Error	3.06	4	0.7662			
Cor Total	1972.23	16				

Table 5. Fit statistics and model validation parameters for TPH removal by *Oscillatoria* sp.

Std. Dev.	5.64	**R^2**	0.8873
Mean	82.99	**Adjusted R^2**	0.7423
C.V. %	6.79	**Predicted R^2**	-0.7811
		Adeq Precision	10.6839

The 3D response surface plots (Fig. 2) illustrate the cumulative effect of the independent variables on the TPH removal percentage, providing a visual confirmation of the statistical results obtained in the ANOVA table.

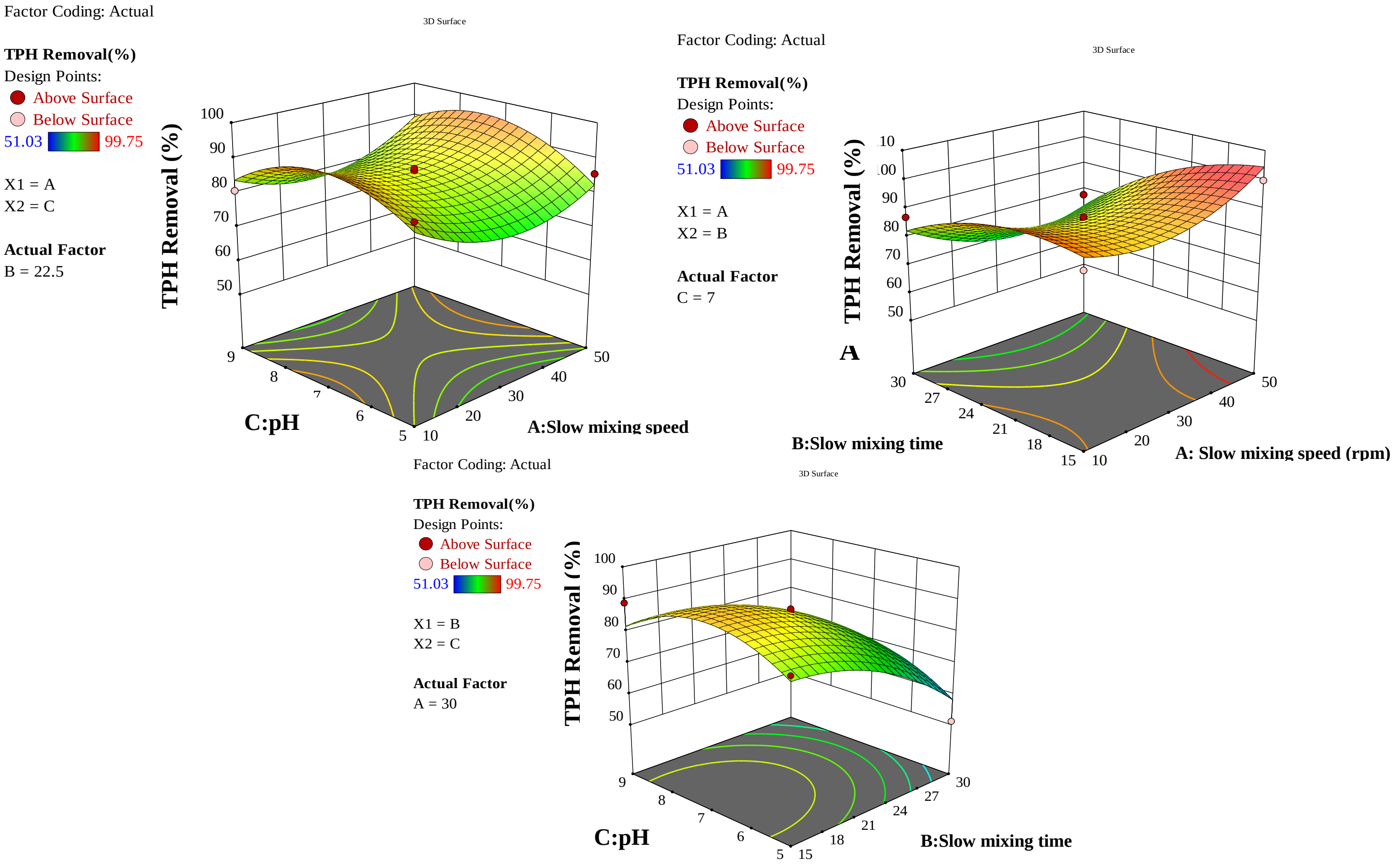

Fig. 2. 3D response surface plots for TPH removal efficiency (%) by *Ocsillatoria* sp. as a function of: (A) slow mixing speed and time at fixed pH 7; (B) slow mixing speed and pH at fixed time 22.5 min; and (C) slow mixing time and pH at fixed speed 30 rpm.

As for the *Oedogonium* sp. bio-coagulant, it demonstrated high removal efficiencies in most experiments, reaching a maximum removal rate of 93.56% in an alkaline medium, the primary mechanism for the reduction of total hydrocarbons (TPH) is attributed to the association of particles with the physical fraction removed during the coagulation and flocculation processes. These results are consistent with previous studies on the treatment of polycyclic aromatic hydrocarbons (PAHs) in industrially contaminated water, such as the study by **Nowacka *et al.* (2016)**. The efficiency can also be attributed to the ability of green algae to remove hydrocarbons through adsorption and the binding of pollutants to the functional groups on the algal cell walls, which are common mechanisms utilized by green algae for pollutant removal **(Danouche *et al.*, 2012)**. Furthermore, the alkaline medium facilitated the dissolution of proteins and other substances from the algal cell walls into the water, thereby enhancing the effectiveness of the coagulant agent.

The results of the analysis of variance (ANOVA) for the TPH response model, as shown in Table (6), indicate that the slow mixing time was the most influential factor on hydrocarbon removal. However, it showed a statistically non-significant effect with a *p*-value of 0.186. Conversely, the slow mixing speed was the least influential factor, also with a non-significant effect (*p*-value = 0.713).The coefficient of determination R^2 0.207 and the adjusted R^2were 0.024, while the predicted R^2 value was -0.611, as presented in Table (7). Based on the total hydrocarbon equation the coded equation is as follows:

$$\boldsymbol{TPH = 82.35 - 1.70A + 6.33B + 5.22C}$$

The RSM model for *Oedogonium* sp. showed limited predictive significance, which could be attributed to the complex nature of the bio-coagulant's active groups or interactions within the treatment medium.

Table 6. Analysis of ANOVA for the quadratic model of TPH removal by *Oedogonium* sp.

Source	Sum of Squares	df	Mean Square	F-value	*P*-value	
Model	561.80	3	187.27	1.14	0.3709	not significant
A-slow mixing speed(rpm)	23.16	1	23.16	0.1405	0.7138	
B-slow mixing time(min)	320.35	1	320.35	1.94	0.1867	
C-ph	218.29	1	218.29	1.32	0.2705	
Residual	2142.71	13	164.82			
Lack of Fit	2140.08	9	237.79	362.51	< 0.0001	significant
Pure Error	2.62	4	0.6559			
Cor Total	2704.50	16				

As indicated by the statistical parameters discussed above and summarized in Table (6), the polynomial model for *Oedogonium* sp. exhibits limited predictive capacity. Consequently, this specific model cannot be utilized for predictive optimization or process control. The poor fit suggests that the coagulation behavior of *Oedogonium* sp. does not follow a simple second-order polynomial pathway under the tested parameters, likely due to uncontrolled interactions with alkaline-extracted proteins. Future studies should explore alternative design spaces or model simplification for this species.

Table 7. Fit statistics and model validation parameters for TPH removal by *Oedogonium* sp.

Std. Dev.	12.84	**R^2**	0.2077
Mean	82.35	**Adjusted R^2**	0.0249
C.V. %	15.59	**Predicted R^2**	-0.6116
		Adeq Precision	3.7099

The 3D response surface plots (Fig. 3) illustrate the cumulative effect of the independent variables on the TPH removal percentage, providing a visual confirmation of the statistical results obtained in the ANOVA table.

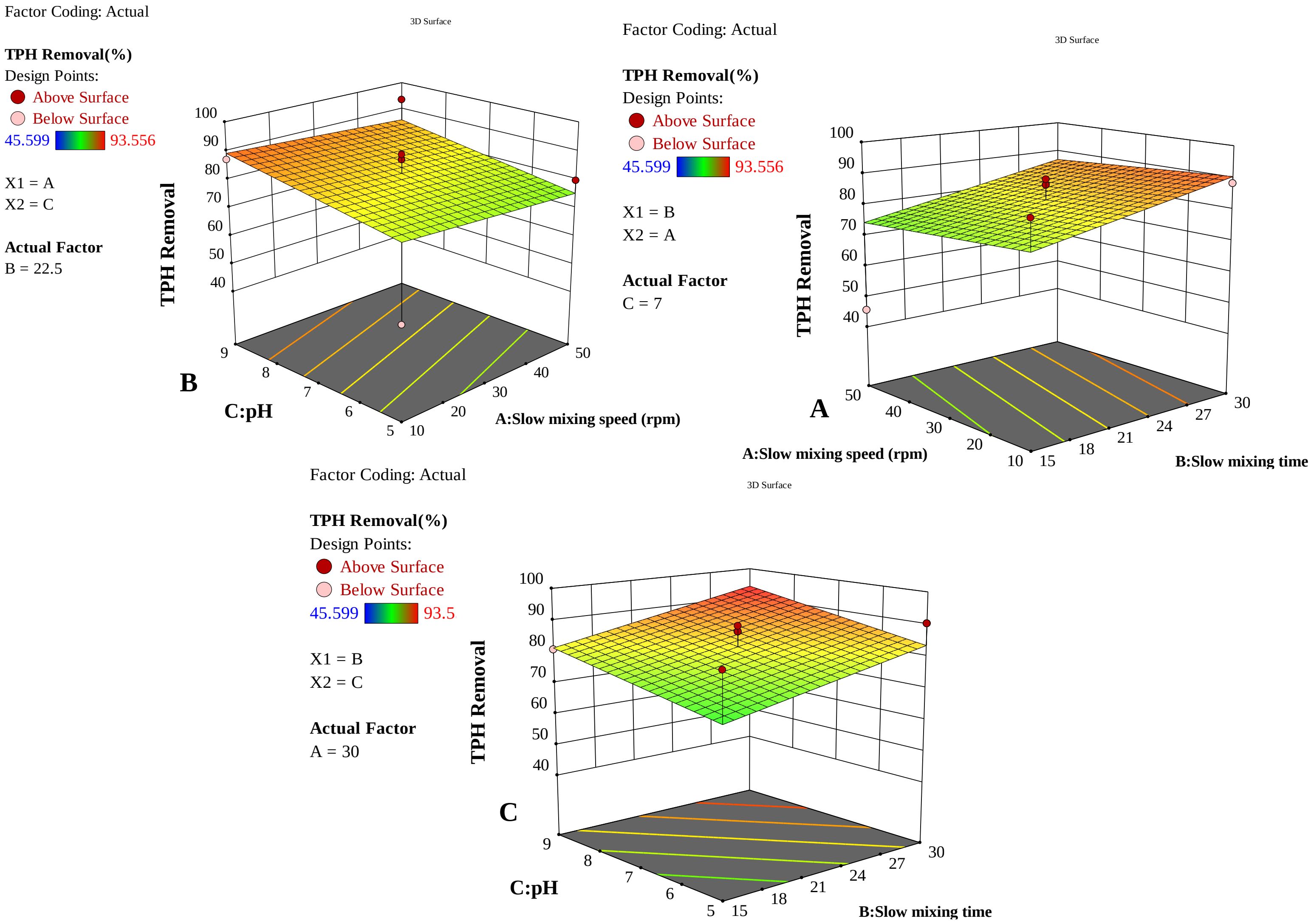

Fig. 3. 3D response surface plots for TPH removal efficiency (%) by *Oedogonium* sp. as a function of: (A) slow mixing speed and time at fixed pH 7; (B) slow mixing speed and pH at fixed time 22.5 min; and (C) slow mixing time and pH at fixed speed 30 rpm.

Meanwhile, the diatoms recorded a maximum removal efficiency of 86.8% at pH 7. This high performance is attributed to the secretion of extracellular polymeric substance (EPS), which acts as bio-surfactant. These substances effectively influence the solubility and interaction of hydrocarbons within the medium **(Paniagua-Michel & Banat 2024)**. Consequently, this study demonstrates the potential of utilizing diatoms as an efficient agent for hydrocarbon reduction, as evidenced by the current findings.

The results of the analysis of variance (ANOVA) for the TPH response model, as shown in Table (8), indicate that the slow mixing speed was the most influential factor, showing a significant effect with an F-value of 8.43 and a *p*-value < 0.05. In contrast, mixing time and pH did not show a significant effect, with *p*-values of 0.545 and 0.066, respectively. This demonstrates that slow mixing speed is the primary influencing factor. The coefficient of determination R^2 0.83 and the adjusted R^2 were 0.634, while the predicted R^2 value was -1.553, as presented in Table (9). Based on the TPH removal, the coded equation is as follows:

$$\boldsymbol{TPH = 85.49 - 12.49 * A - 2.73B - 9.33C - 9.63AB - 0.1075AC - 2.21BC - 22.46A^2 + 3.67 * B^2 - 13.43C^2}$$

Table 8. Analysis of ANOVA for the quadratic model of TPH removal by diatoms.

Source	Sum of Squares	df	Mean Square	F-value	*P*-value	
Model	5432.30	9	603.59	4.08	0.0386	significant
A-slow mixing speed(rpm)	1247.75	1	1247.75	8.43	0.0229	
B-slow mixing time(min)	59.79	1	59.79	0.4040	0.5452	
C-ph	696.39	1	696.39	4.71	0.0667	
AB	370.95	1	370.95	2.51	0.1574	
AC	0.0462	1	0.0462	0.0003	0.9864	
BC	19.49	1	19.49	0.1317	0.7274	
A^2	2123.72	1	2123.72	14.35	0.0068	
B^2	59.42	1	59.42	0.4015	0.5464	
C^2	758.98	1	758.98	5.13	0.0579	
Residual	1035.84	7	147.98			
Lack of Fit	1032.01	3	344.00	360.02	< 0.0001	significant
Pure Error	3.82	4	0.9555			
Cor Total	6468.14	16				

Table 9. Fit statistics and model validation parameters for TPH removal by diatoms.

Std. Dev.	12.16	***R²***	0.8399
Mean	70.37	**Adjusted *R²***	0.6340
C.V. %	17.29	**Predicted *R²***	-1.5538
		Adeq Precision	6.2696

The 3D response surface plots (Fig. 4) illustrate the cumulative effect of the independent variables on the TPH removal percentage, providing a visual confirmation of the statistical results obtained in the ANOVA table.

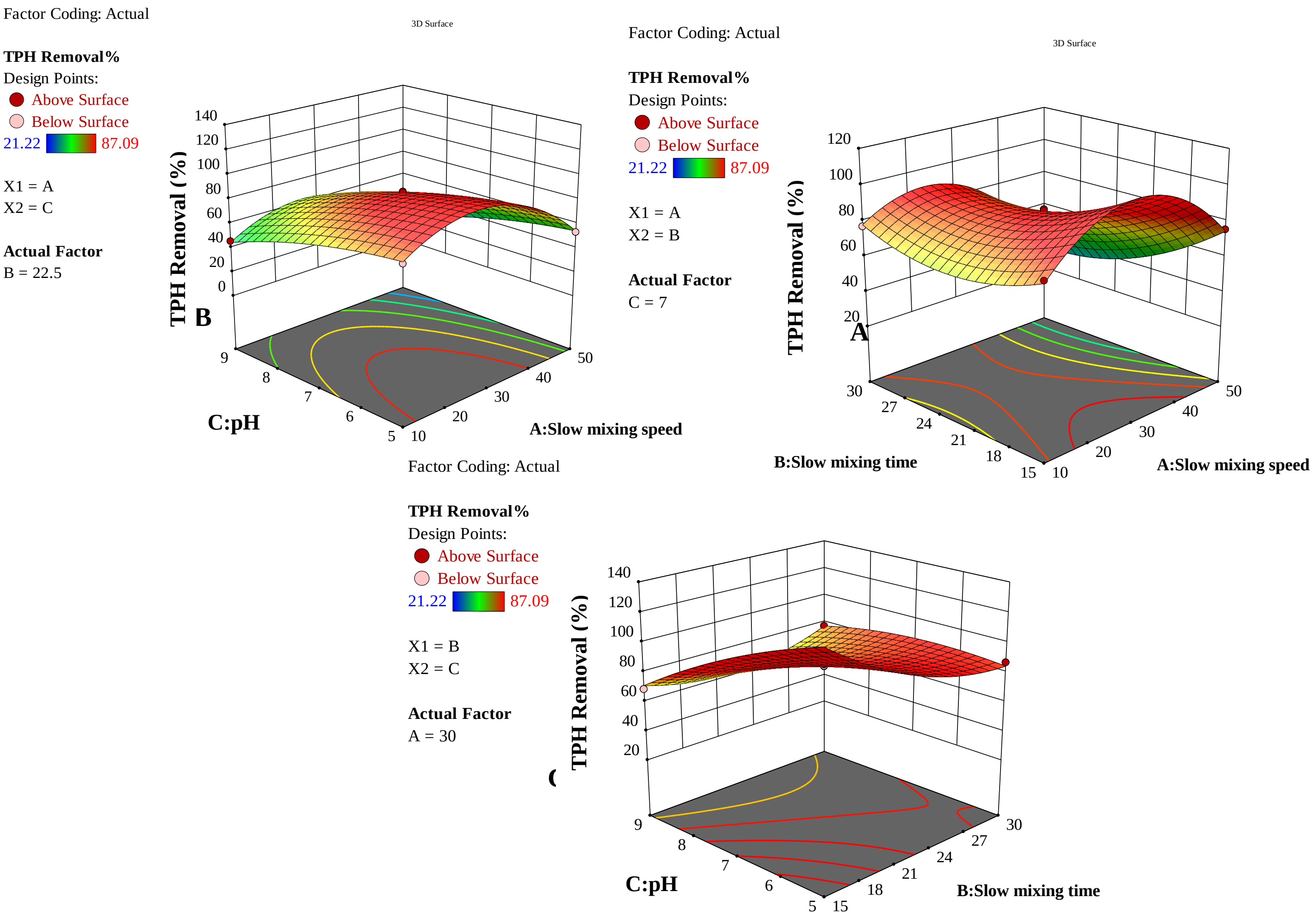

Fig. 4. 3D response surface plots for TPH removal efficiency (%) by *Diatoms* as a function of: (A) slow mixing speed and time at fixed pH 7; (B) slow mixing speed and pH at fixed time 22.5 min; and (C) slow mixing time and pH at fixed speed 30 rpm.

The observed variations in the R^2 values and the non-significance of certain p -values in some models can be attributed to several practical factors. Primarily, this study utilized real wastewater collected directly from the power plant rather than synthetic solutions prepared in a controlled laboratory environment. Real wastewater contains a complex matrix of diverse pollutants and ions that can interfere with the coagulation-flocculation process, making it challenging to achieve ideal statistical fits. Furthermore, the use of algae as a bio-coagulant introduces inherent biological variability. As biological materials, algal cells and their associated functional groups naturally exhibit variations that can influence the consistency of the experimental outcomes. The occurrence of negative predicted R^2 values further indicates the models' high sensitivity to these minor fluctuations within the complex wastewater matrix. Despite these statistical limitations, the practical laboratory results consistently demonstrated the high efficiency of these algal species in removing hydrocarbons. This confirms their robustness and potential as sustainable alternatives for industrial wastewater treatment applications, FTIR analysis and interaction mechanism with hydrocarbons. To further understand the mechanism of hydrocarbon removal, FTIR analysis was conducted to identify the active functional groups responsible for the coagulation process.

3. Characterization of *Oscillatoria* sp. Using FTIR Analysis

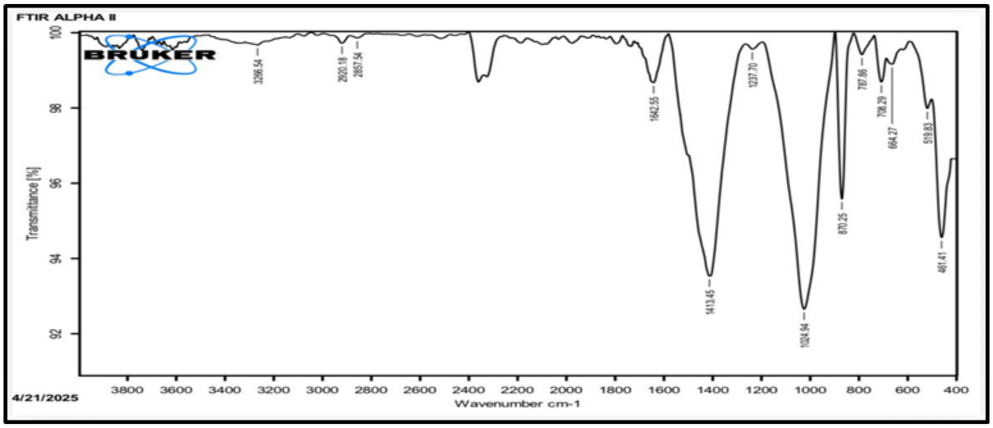

Fig. 5. Characterization of *Oscillatoria* sp. using FTIR analysis.

The functional groups of *Oscillatoria* sp. were identified by comparing the obtained FTIR absorption bands with standard IR spectrum tables provided by Sigma-Aldrich, as shown in Fig. (5) and Table (10).

Table 10. FTIR peak assignments for *Osillatoria* sp. Based on standard IR spectrum tables provided by Sigma-Aldrich.

Peak Frequency	Standard Range	Functional Group Assignment
3266.54	3200-3550	O-H Group Alcohol
2920.18	2850-3000	C-H Group Alkanes
2857.54	2850--2970	C-H Group Alkanes
1642.45	1600-1680	C=C Amide l
1413.45	1350-1470	C-H bending
1237.70	1000-1300	C-O Ester
1024.94	1000-1250	C-N Polysaccharides
870.25	675-900	C-H bending
664.27	600-700	C-H bending

The removal of hydrocarbons may be attributed to the presence of polysaccharides, indicated by the C-O and C-N peaks at 1237 and 1024 plays a vital role in hydrocarbon removal. These carbohydrates act as natural polymeric bridges that trap oil droplets, while the C-H groups at 2920 strengthen the bond with hydrocarbons, leading to higher removal efficiency.

4. Characterization of *Oedogonium* sp. Using FTIR Analysis

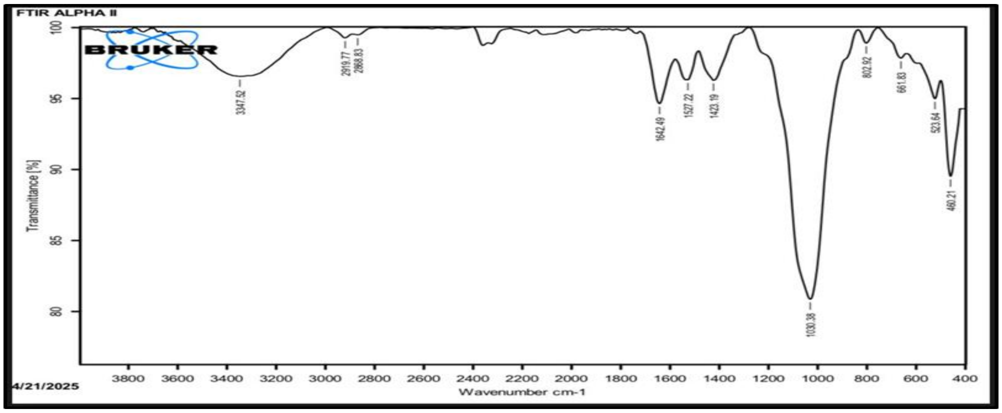

Fig. 6. Characterization of *Oedogonium* sp. using FTIR analysis.

The functional groups of the *Oedogonium* sp. were identified by comparing the obtained FTIR absorption bands with standard IR spectrum tables provided by Sigma-Aldrich (Fig. 6 & Table 11).

Table 11. FTIR peak assignments for *Oedogonium* sp. based on standard IR spectrum tables provided by Sigma-Aldrich.

Peak Frequency	Standard Range	Functional Group Assignment
3347.52	3200-3550	O-H Group Alcohol
2919.77	28500-3000	CH2-CH3
2868.83	2840-2870	CH2-CH3
1642.49	1600-1680	C=O
1527.22	1500-1550	C=O
1423.19	1350-1470	C-H
1030.38	1000-1100	C-O-C Stretching
802.92	750-850	C-H
661.83	600-700	C=C
523.64	500-600	C-Cl
460.21	400-500	M-O

The removal efficiency of hydrocarbons by *Oedogonium* sp. bio-coagulant may be attributed to the synergistic effect of its functional groups. The presence of polysaccharides and cellulose components, confirmed by the C-O-C stretching at 1030, provides a robust polymeric framework that acts as a bridge to entrap oil droplets. Furthermore, the hydrophobic nature of the algal surface, indicated by the strong C-H stretching peaks at 2919 and 2868 enhances the physical adsorption of non-polar hydrocarbon molecules. Additionally, the amide groups at 1642 and hydroxyl groups at 3347 provide active sites for charge neutralization, facilitating the aggregation and subsequent settling of hydrocarbon contaminants from the treated water.

5.Characterization of Diatoms Using FTIR Analysis

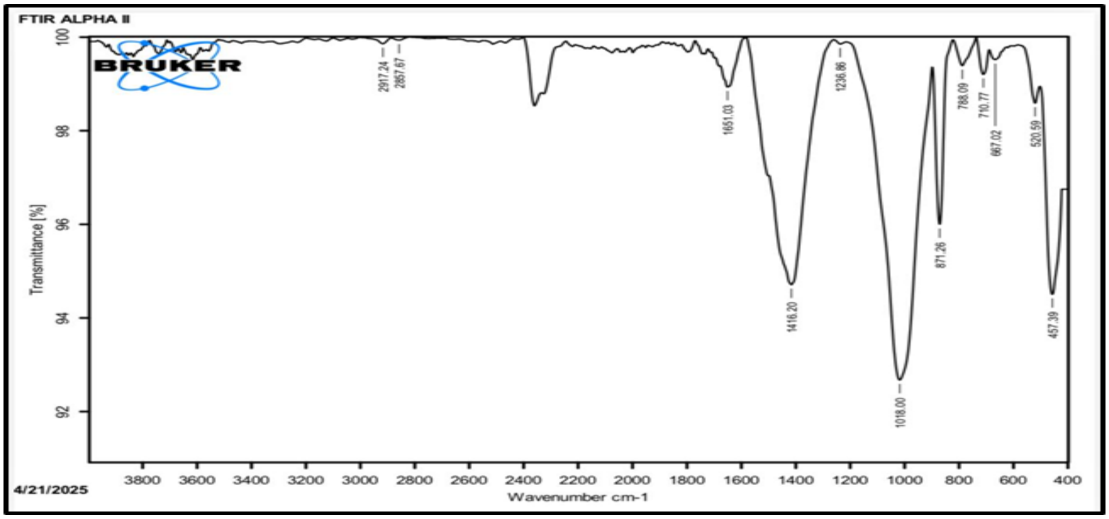

Fig. 7. Characterization of diatoms using FTIR analysis.

The functional groups of the *Oedogonium* sp. were identified by comparing the obtained FTIR absorption bands with standard IR spectrum tables provided by Sigma-Aldrich, as shown in Fig. (7) and Table (12).

Table 12. FTIR peak assignments for diatoms, based on standard IR spectrum tables provided by Sigma-Aldrich.

Peak Frequency	Standard Range	Functional Group Assignment
2917.24	2850-3000	C-H
2857.67	2840-2870	C-H
1651.03	1600-1680	C=C
1416.20	1350-1470	S=O
1018	950-1100	Si-O-Si silica
871.26	800-900	C-O or Si-O
710.77	700-800	Si-O
788.09	700-800	Si-O

457.39	450-550	Si-O-Si
520.59	450-550	Si-O-Si

The removal of hydrocarbons may be attributed to the silica-rich structure of the Diatom mix, where the Si-O-Si and Si-O groups at 1018 and 710 provide a porous surface for physical adsorption. Additionally, the C-H and CH2 groups at 2917 and 2857 enhance the hydrophobic affinity, allowing the coagulant to effectively trap and stabilize oil droplets within the treated water.

6. GC-FID Analysis of TPH Removal

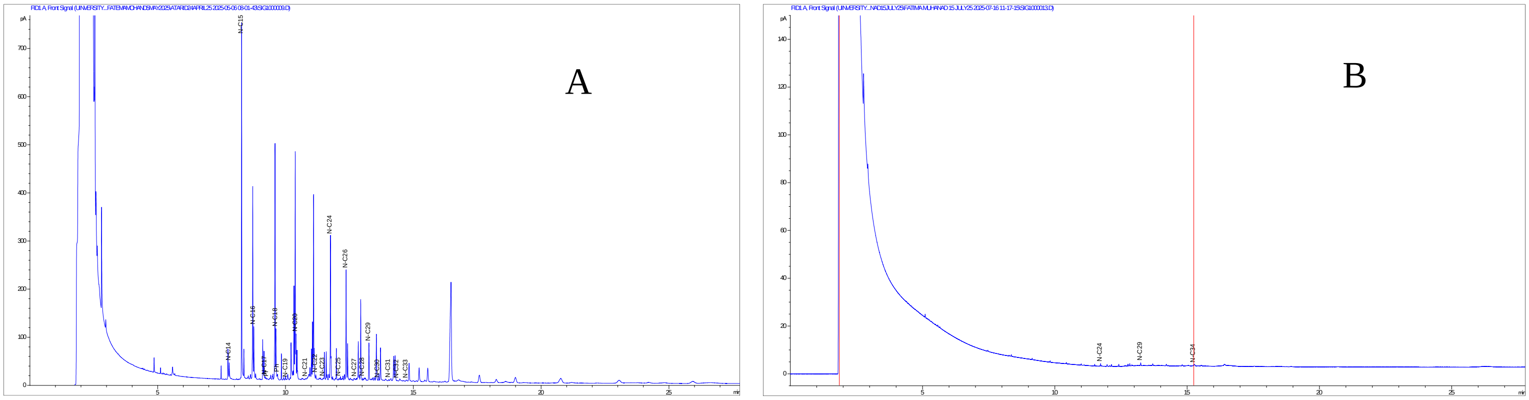

Fig. 8. GC-FID chromatograms of oily wastewater (A) before treatment (control) and (B) after treatment with *Oscillatoria* sp. bio-coagulant.

The efficiency of TPH removal by *Oscillatoria* sp. was further confirmed through GC-FID analysis. As illustrated in Fig. (8), a clear comparison between the control sample (A) and the treated sample (B) reveals a significant reduction in the intensity and number of hydrocarbon peaks. In the control chromatogram, numerous sharp peaks representing various TPH fractions are observed. However, after the bio-coagulation process, most of these peaks either diminished or completely disappeared, indicating that the *Oscillatoria* sp. biomass successfully captured and removed the majority of the petroleum compounds from the wastewater.

7. Model Validation and Performance Confirmation

As shown in Fig. (9) and Table (13), the model predicted the optimal conditions for each algae species with a desirability of 1.000.

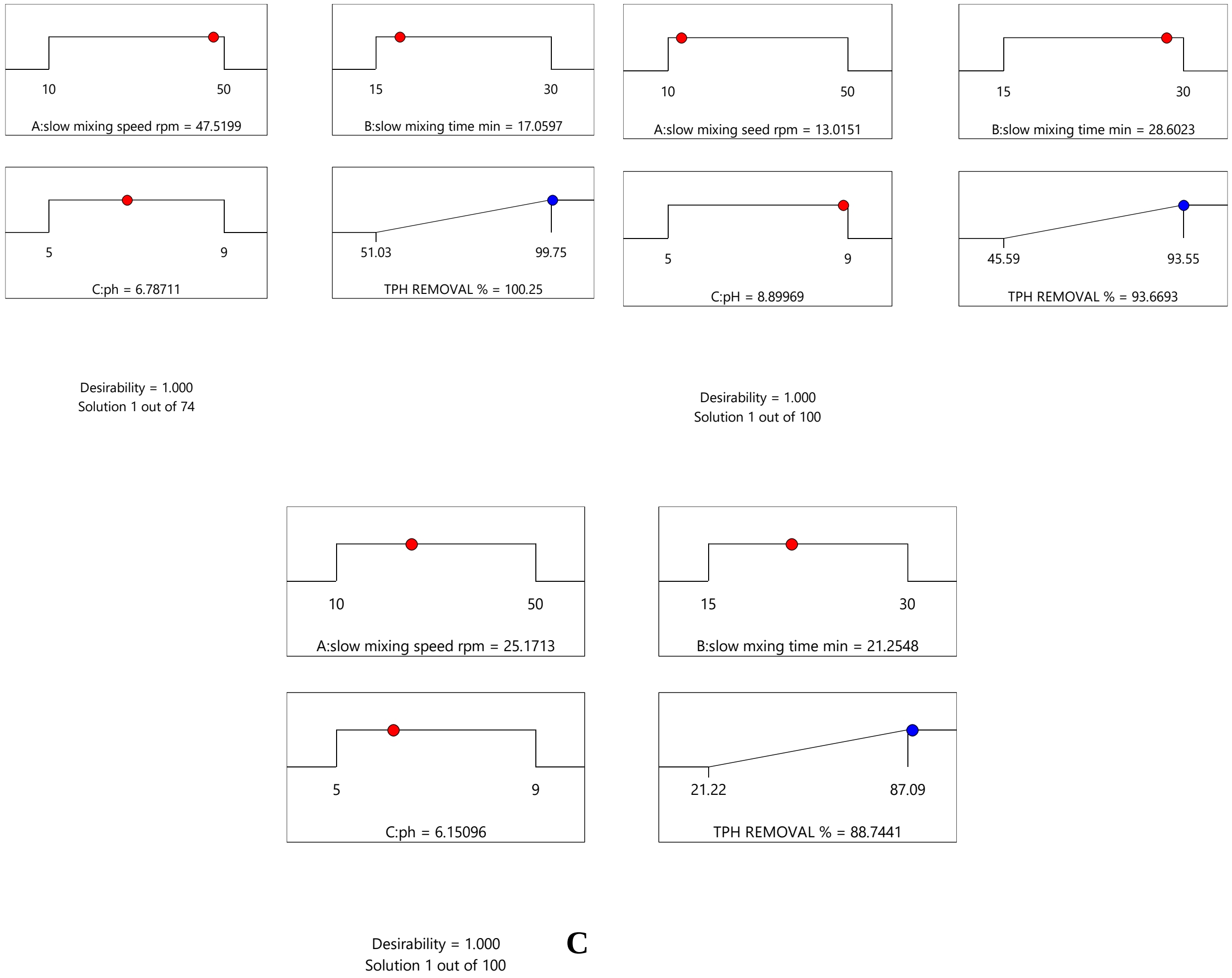

Fig. 9. Optimization ramps for maximum TPH removal by (A) *Oscillatoria* sp., (B) *Oedogonium* sp., and (C) mixed diatoms.

To validate the statistical reliability of the RSM models, independent validation experiments were conducted at the predicted optimal conditions for each coagulant. As shown in Table (13), the observed TPH removal efficiencies showed a strong correlation with the predicted values for *Oscillatoria* sp., and mixed diatoms, with percentage errors of 0.49% and 3.07%, respectively. The experimental values are presented as the mean of triplicate measurements ± standard deviation. Regarding *Oedogonium* sp., the observed removal efficiency was 88.26% compared to a predicted value of 93.66%, resulting in a percentage error of 5.76. This slightly higher deviation aligns with the previously noted lack-of-fit and negative R^2 values, for this specific model. Therefore, while the *Oedogonium* sp. model effectively illustrate the general trend of factor interaction regarding its absolute predictive precision. The overall findings demonstrate that these algal species serve as promising natural coagulants for wastewater treatment, providing efficient TPH removal under the optimized conditions.

Table 13. Comparison between RSM predicted and experimental results for model validation.

Algal species	Slow mixing speed rpm	Slow mixing time min	pH	Predicted Removal(%)	Observed Removal (%)	Error
Oscillatoria sp.	47.520	17.060	6.787	100.25%	99.75% ±0.22%	0.49
Oedogonium sp.	13.015	28.602	8.900	93.66%	88.26% ±1.15%	5.76
Diatoms	25.171	21.255	6.151	88.74%	86.01% ±0.85	3.07

CONCLUSION

This study demonstrated the high efficiency of natural bio-coagulants extracted from *Oedogonium* sp., *Oscillatoria* sp., and diatom mix in removing hydrocarbons from industrial wastewater. Based on the Central Composite Design (CCD) results, the optimum removal efficiency was found to be highly dependent on the pH and species type. *Oscillatoria* sp. achieved its maximum removal of 99.75% at neutral pH 7, while *Oedogonium* sp. showed its best performance with 93.55% removal at alkaline pH 9. The Diatom mix reached a peak removal of 86.8% at pH 7. The FTIR analysis revealed that the cause of this removal may be attributed to key functional groups, specifically hydroxyl (3266 cm^{-1}), amide (1642 cm^{-1}), and aliphatic C-H groups (2920 and 2857 cm^{-1}), which facilitated pollutants entrapment through hydrophobic interactions and charge neutralization. Additionally, the silica (Si-O-Si) structure in diatoms (at 1018 cm^{-1}) provided a porous surface for physical adsorption. These findings suggest that algal-based coagulants effective alternatives for wastewater treatment, though further toxicity and sludge stability tests are recommended for future large-scale applications.

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