

Activation Of Crude Oil Bio-Remediation In Contaminated Beach Water In Oman Using Shrimp Shell Waste

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Objectives

1. To assess the impact of using SSW as a seafood waste to increase microbial efficiency for crude oil biodegradation in the contaminated sea water
2. To assess and contrast the biodegradation effectiveness of crude oil with SSW and without
3. To Isolate and Identify the bacteria in crude oil using physiological and morphological tests, and identify cultures that are degrading using 16S rDNA sequencing.
4. To identify the optimum operational conditions and evaluate the statistical interactions between the different experimental factors and biodegradation efficiency .

Methods and Materials

Bacterial Culture Preparation Using Shrimp Shell Powder

Preparation of Shrimp Shell Powder (SSP)

- Collect shrimp shells from seafood processing waste
- Wash thoroughly with distilled water to remove salts, debris, and organic matter
- Boil the shells for 15-20 minutes to remove residual protein and fat
- Dry the shells in hot air oven at 50°C for 24 hours
- Grind the dried shells into fine powder using grinder
- Sieve the powder (opt).

Preparation of SSP-Based Nutrient Medium

- Obtain a pure culture and identified target broth
- Grow the bacteria in a standard broth for 3-5 days at optimal temperature

Inoculum of SSP Medium

- Aseptically inoculate the sterile SSP-based medium with 1-5 % (v/v) of prepared bacterianoculum
- Incubate at the optimal temperature (e.g., CFU/mL or under relevant parameters over time)

Bioremediation

- Add different volume of bacterial culture to contaminated water
- Incubated the samples for 7-30 days

Bioremediation

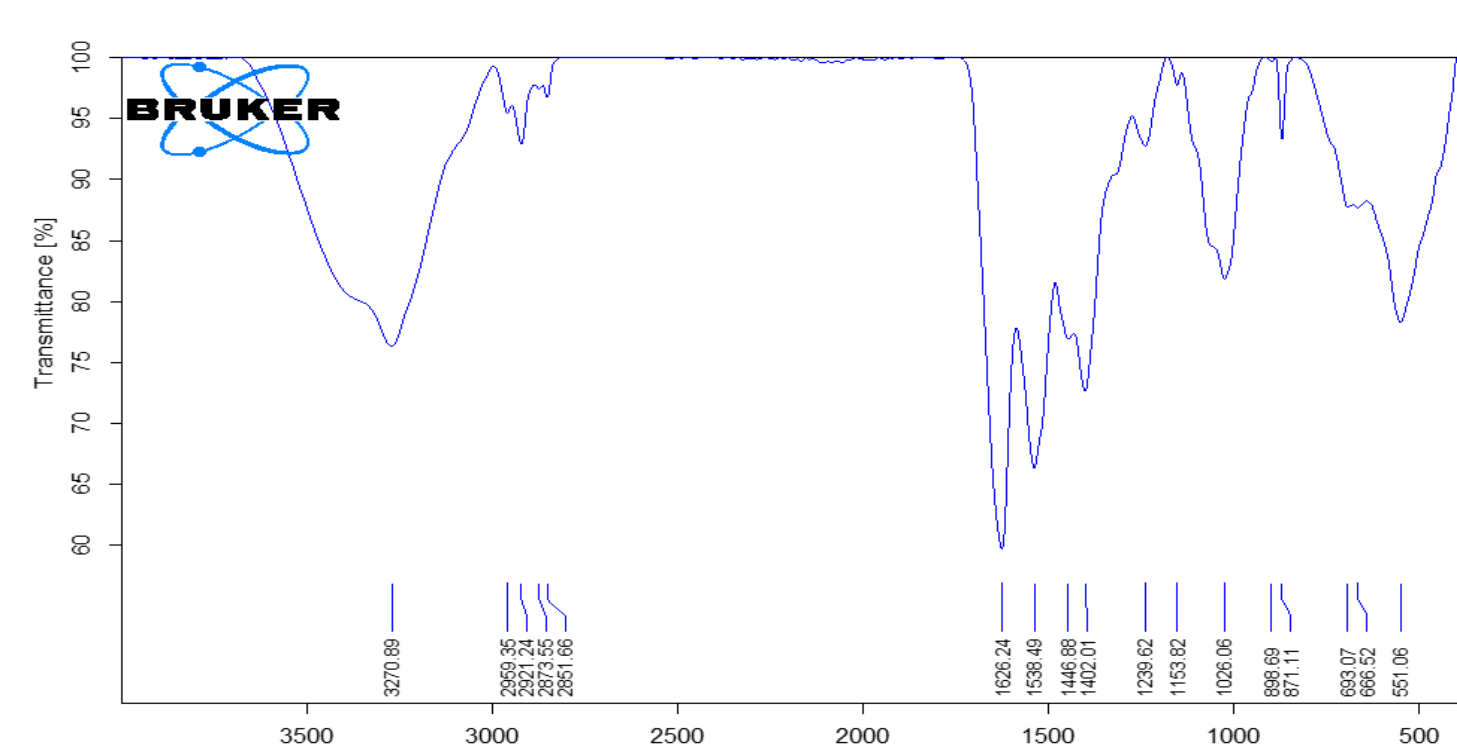


Figure 1. FTIR Analysis for Shrimp Shell

Table 1 Experimental design for the performance of Shrimp Bacterial Culture on TPH removal

Std	ID	Run	Space Type	Factor 1	Factor 2	Response 1
				A:SBC mL	B:Incubation time day	TPH Removal (%)
3	3	1	Factorial	1	30	63
5	5	2	Axial	1	17.5	52
13	9	3	Center	5.5	17.5	82
12	9	4	Center	5.5	17.5	82
7	7	5	Axial	5.5	5	57
4	4	6	Factorial	10	30	99
10	9	7	Center	5.5	17.5	81
8	8	8	Axial	5.5	30	93
6	6	9	Axial	10	17.5	96
9	9	10	Center	5.5	17.5	81
2	2	11	Factorial	10	5	76

Abstract

This study explores the use of shrimp shell powder (SSP), a seafood waste byproduct, as a sustainable nutrient medium for cultivating bacterial strains capable of degrading total petroleum hydrocarbons (TPH) in contaminated water. A five-stage process—ranging from SSP preparation to its application in bioremediation—was developed and evaluated. The SSP served as a dual source of carbon and nitrogen in the bacterial culture medium. Using a factorial experimental design, the effects of two critical factors—shrimp bacterial culture (SBC) volume and incubation time—on TPH removal efficiency were assessed. The highest TPH removal rate observed was 99%, demonstrating the feasibility of SSP-based microbial bioremediation. Analysis of variance (ANOVA) confirmed that both SBC volume and incubation time had statistically significant effects on TPH degradation ($p < 0.0001$), with a high model accuracy ($R^2 = 0.9828$). These findings highlight the potential of shrimp waste valorization for effective and eco-friendly environmental remediation.

Results

- Maximum TPH removal achieved: 99% at 10 mL SBC and 30 days incubation time.
- Most influential factors: Both SBC volume (A) and incubation time (B) were highly significant ($p < 0.0001$).
- Model performance: $R^2 = 0.9828$ Adjusted $R^2 = 0.9706$ Standard deviation = 3.10
- Optimal conditions for high removal ($\geq 93\%$) occurred between: SBC: 5.5–10 mL Incubation time: 17.5–30 days

Table 2 Analysis of Variance Average (ANOVA)

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significant
Model	3855.93	5	771.19	80.21	< 0.0001	significant
A-SBC	2320.67	1	2320.67	241.38	< 0.0001	
B-Incubation time	1176.00	1	1176.00	122.32	< 0.0001	
AB	1.0000	1	1.0000	0.1040	0.7565	
A ²	128.75	1	128.75	13.39	0.0081	
B ²	93.80	1	93.80	9.76	0.0168	
Residual	67.30	7	9.61			
Pure Error	1.20	4	0.3000			
Cor Total	3923.23	12				
Std. Dev.	3.10					
Mean	75.54					
R ²	0.9828					
Adjusted R ²	0.9706					

TPH Removal (%) = +81.38 +19.67 A +14.00 B - 0.5000AB -6.83 A² -5.83 B²

Where A: Shrimp Bacterial Culture (mL)
 B: Incubation time (day)

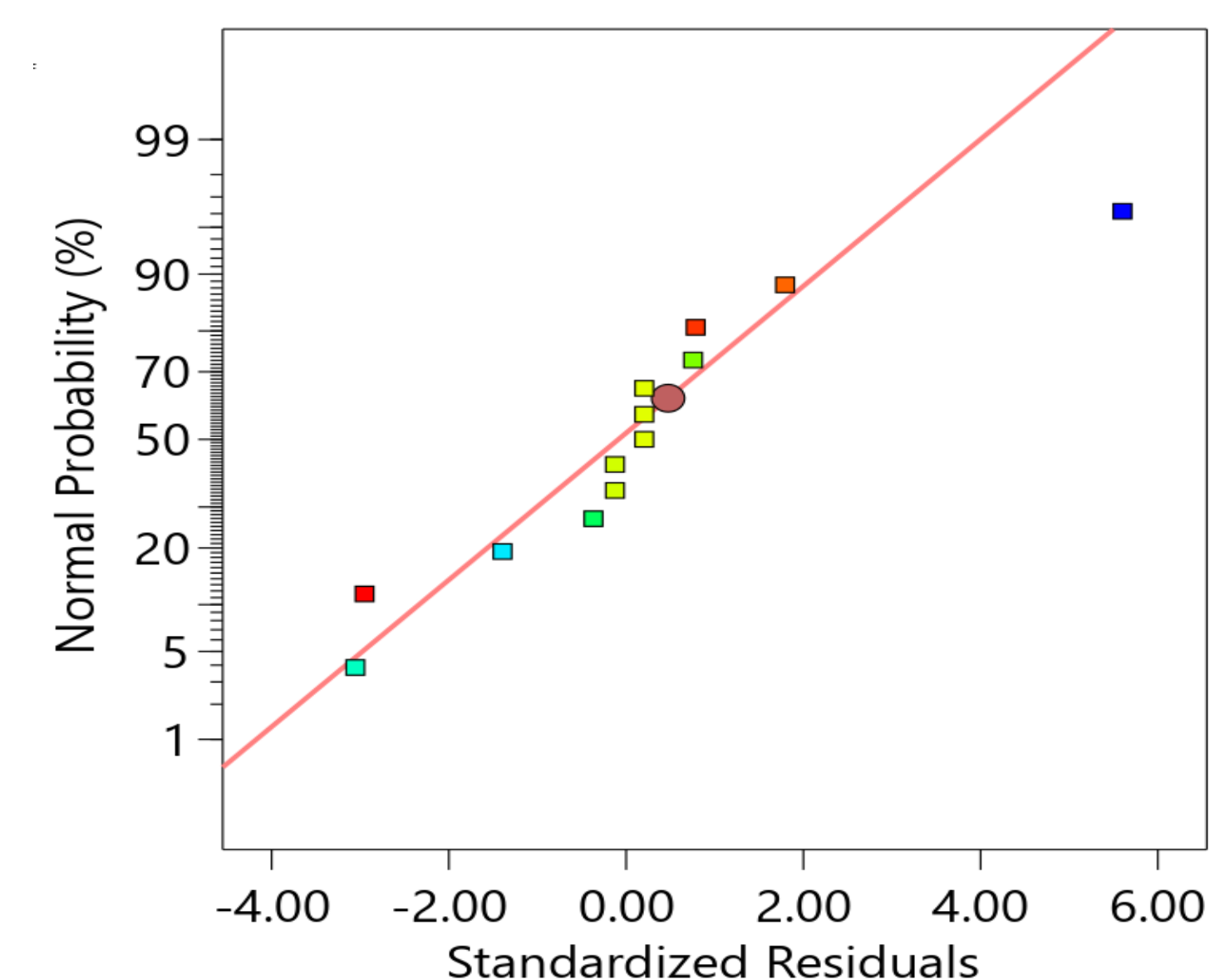


Figure 2. Normal Probability Plot for SBC performance of SBC in removing Crude oil

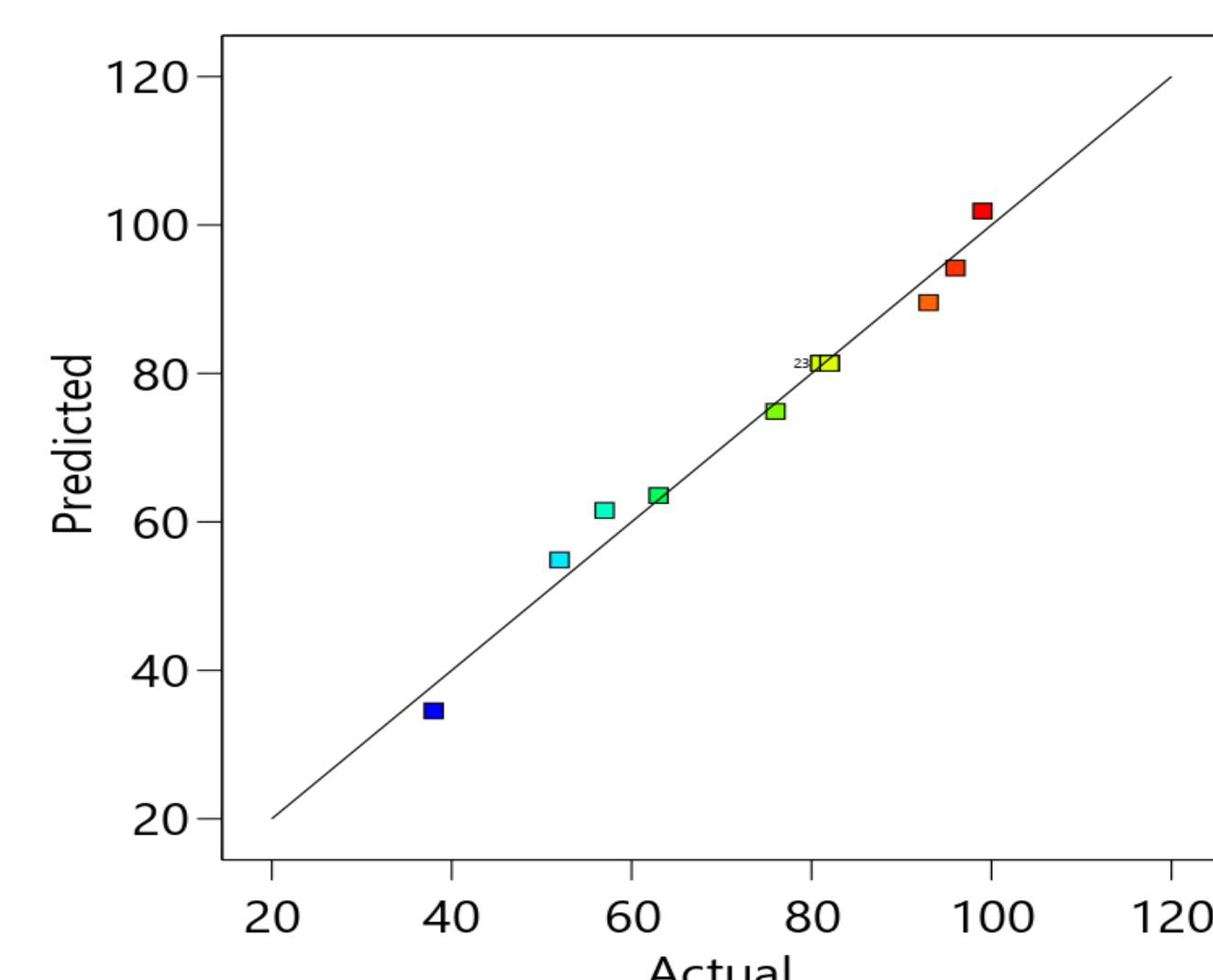


Figure 3. Predicted and actual for the performance of SBC in removing Crude oil .

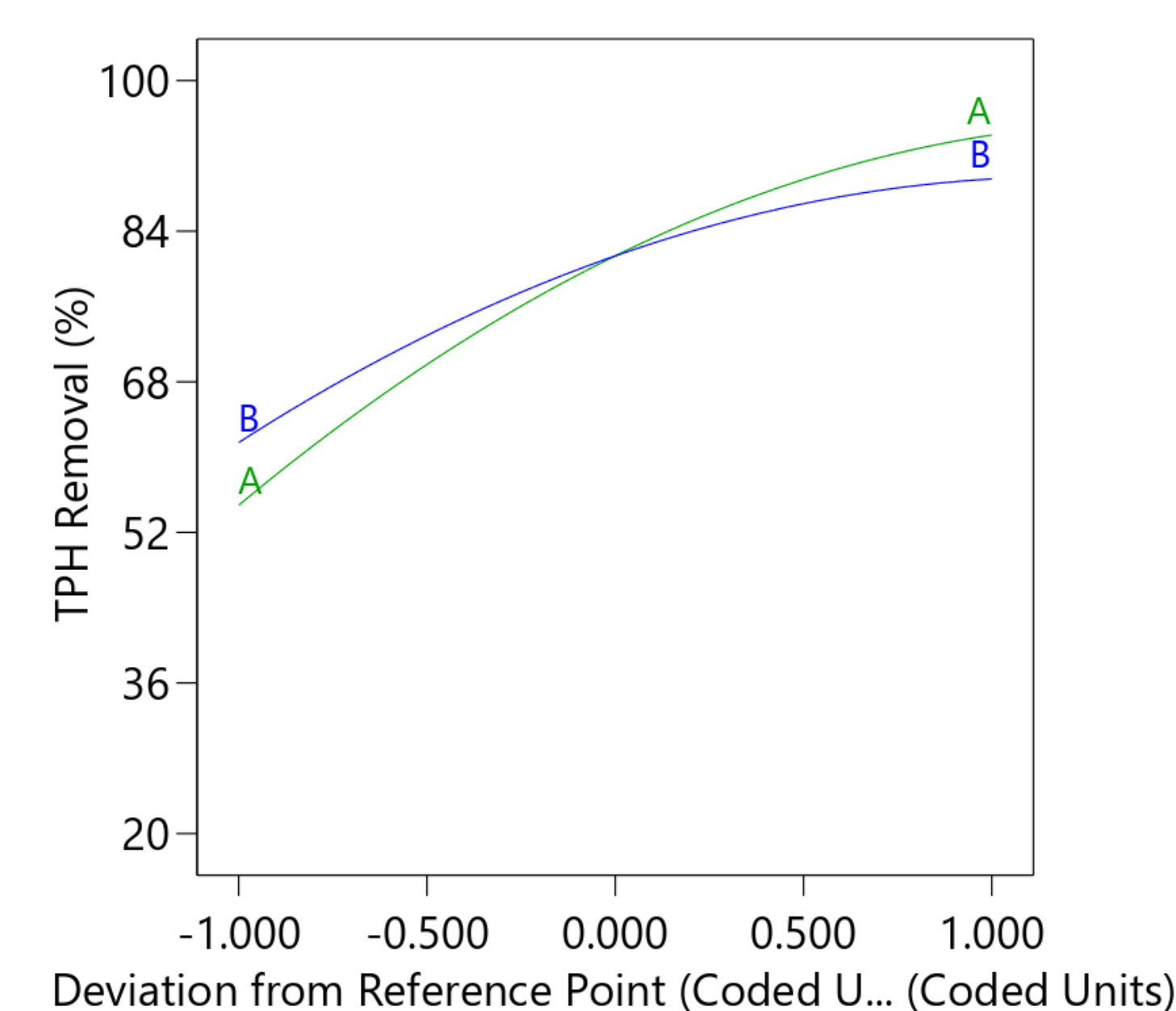


Figure 4. Perturbation Curve for the interaction of SBC And incubation time in removing Crude oil .

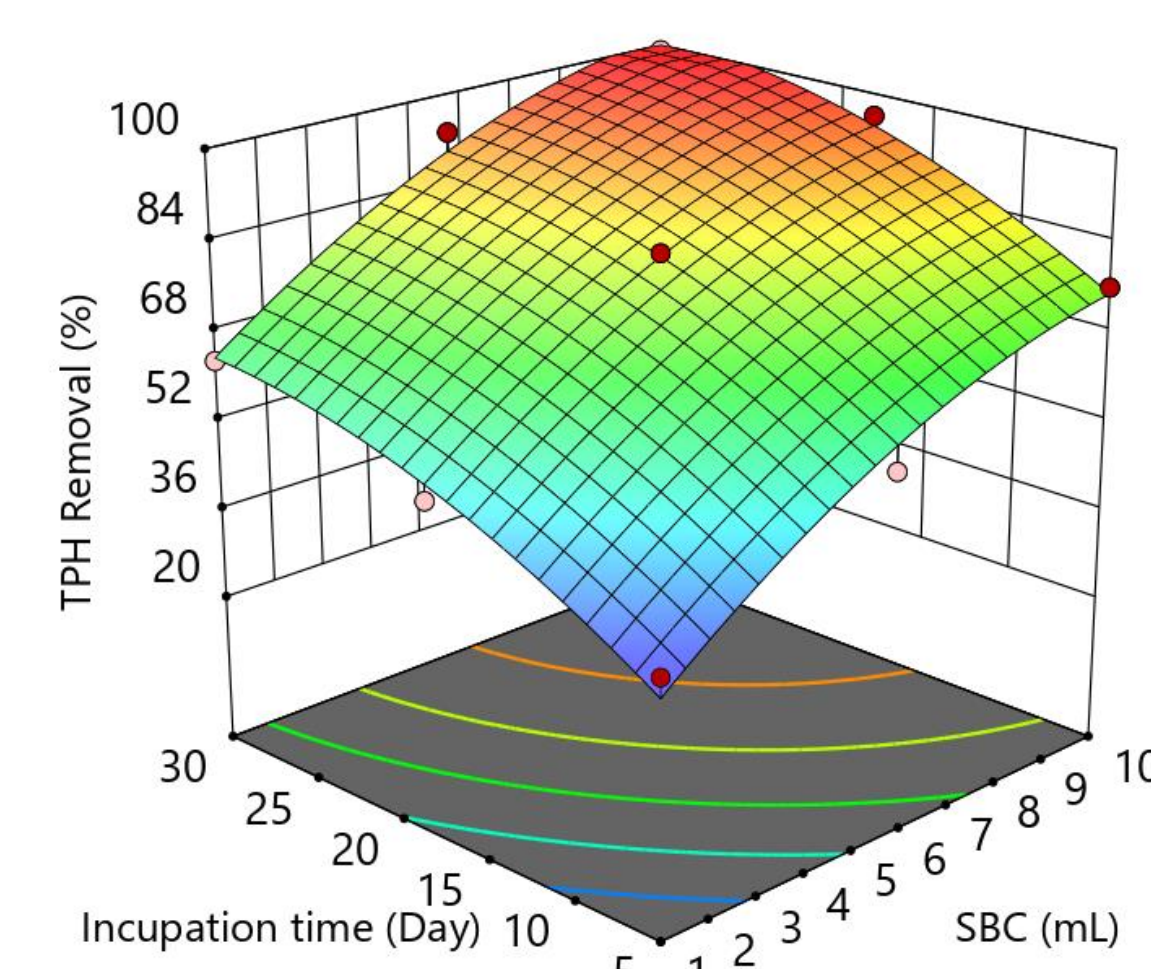


Figure 5. Response surface plot for the performance of SBC in removing Crude oil .

Conclusions

The study demonstrates that shrimp shell powder (SSP) can be effectively utilized as a low-cost, sustainable nutrient medium for cultivating bacterial strains capable of removing total petroleum hydrocarbons (TPH) from contaminated water. Both the volume of shrimp bacterial culture and the incubation time significantly influence bioremediation efficiency, with optimal conditions achieving up to 99% TPH removal. These findings support the potential of seafood waste valorization as an eco-friendly strategy for treating petroleum-contaminated environments.

References

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