Effectiveness of a bio-catalytic agent used in the bioremediation of crude oil-polluted seawater

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Published on April 22nd,2021

https://doi.org/10.1016/j.heliyon.2021.e06926

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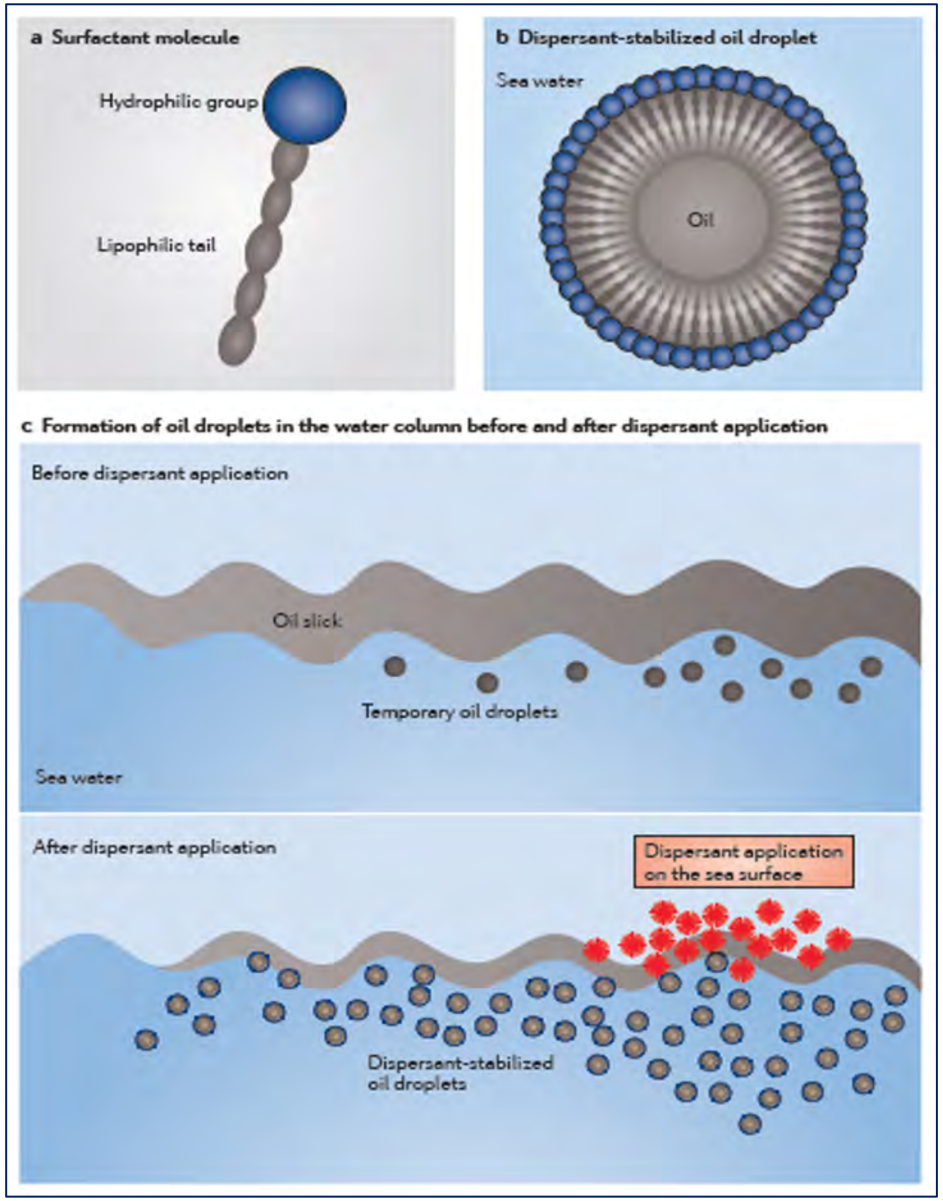

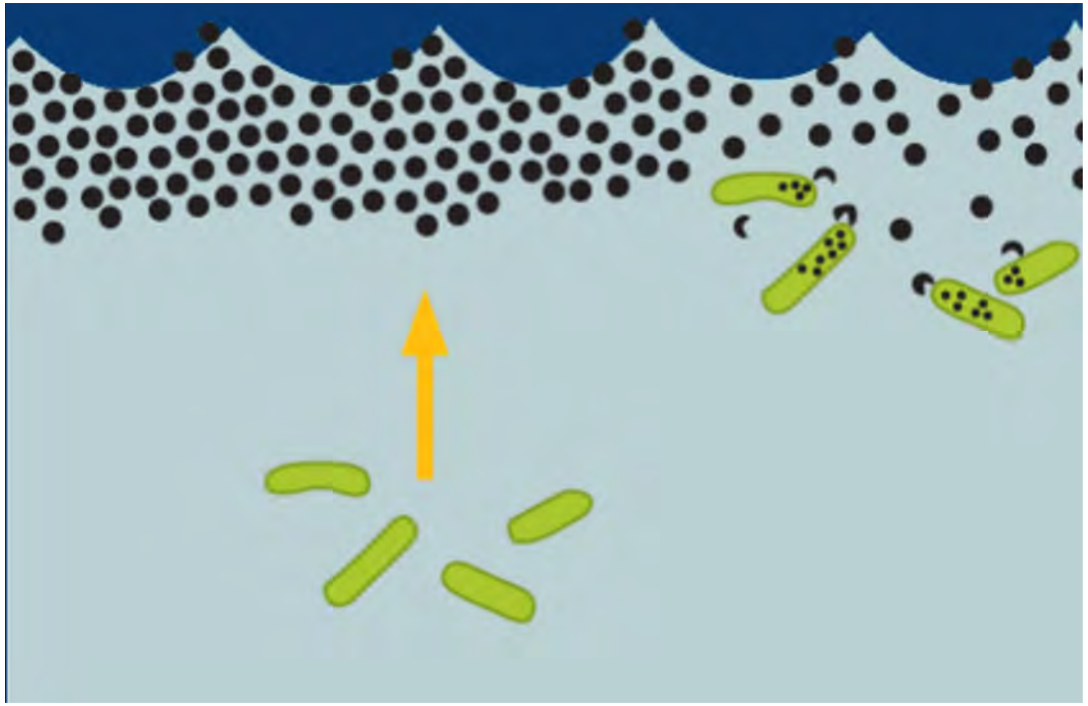

Figure 1. *Action Mechanism and efficiency of biosurfactants in a petroleum hydrocarbons-contaminated seawater medium.*
Source: Using dispersants after oil spills: impacts on the composition and activity of microbial communities. 2015

MATERIALS AND METHODS

Sample Preparation and Bioremediation Study

Table 1. Properties of the crude oil VASCONIA	
API gravity	24.27
Density (kg m^{-3}) @ 15°C	907.9
Sulfur Concentration(wt.%)	0.833
Reid Vapour Pressure (kPa)	21.99
Flash Point(°C)	0
Número de ácido total (mg KOH/g)	0.21
Viscosity @ 40°C(cSt)	22
Source:: Ecopetrol S.A.	

Table 2. Physicochemical Properties of the Seawater used.

Parameters	Unit	Value
pH	pH units	8.58
Turbidity	NTU	0
COD	$mgO_2\ L^{-1}$	98
DO	$mgO_2\ L^{-1}$	7.70
Salinity	g of salts kg^{-1} of water	25.8
Chlorides	mg $Cl^-\ L^{-1}$	15900
Ortophosphates	mg $P.PO_4\ L^{-1}$	0.102
Total Microbial Count	CFU /100mL	ND*

MATERIALS AND METHODS

Table 3. Samples used and their components

Sample	Components
A (Control)	Crude oil and seawater only (640 mg L^{-1})
B	Seawater, bio-catalytic agent solution (2167.39 mg L^{-1}), and crude oil (640 mg L^{-1})
C	Seawater, bio-catalytic agent solution (10000 mg L^{-1}) and crude oil (640 mg L^{-1}).
D (Control)	Seawater, bio-catalytic agent solution (2167.39 mg L^{-1}) and aeration system.
E	Seawater, bio-catalytic agent solution (2167.39 mg L^{-1}), crude oil (640 mg L^{-1}) and aeration system.
F	Seawater, bio-catalytic agent solution (10000 mg L^{-1}), crude oil (640 mg L^{-1}) and aeration system.

700 mL de of seawater, 0.5 mL of crude oil and 1.3 mL of bio-catalytic agent (0.216% v/v).

MATERIALS AND METHODS

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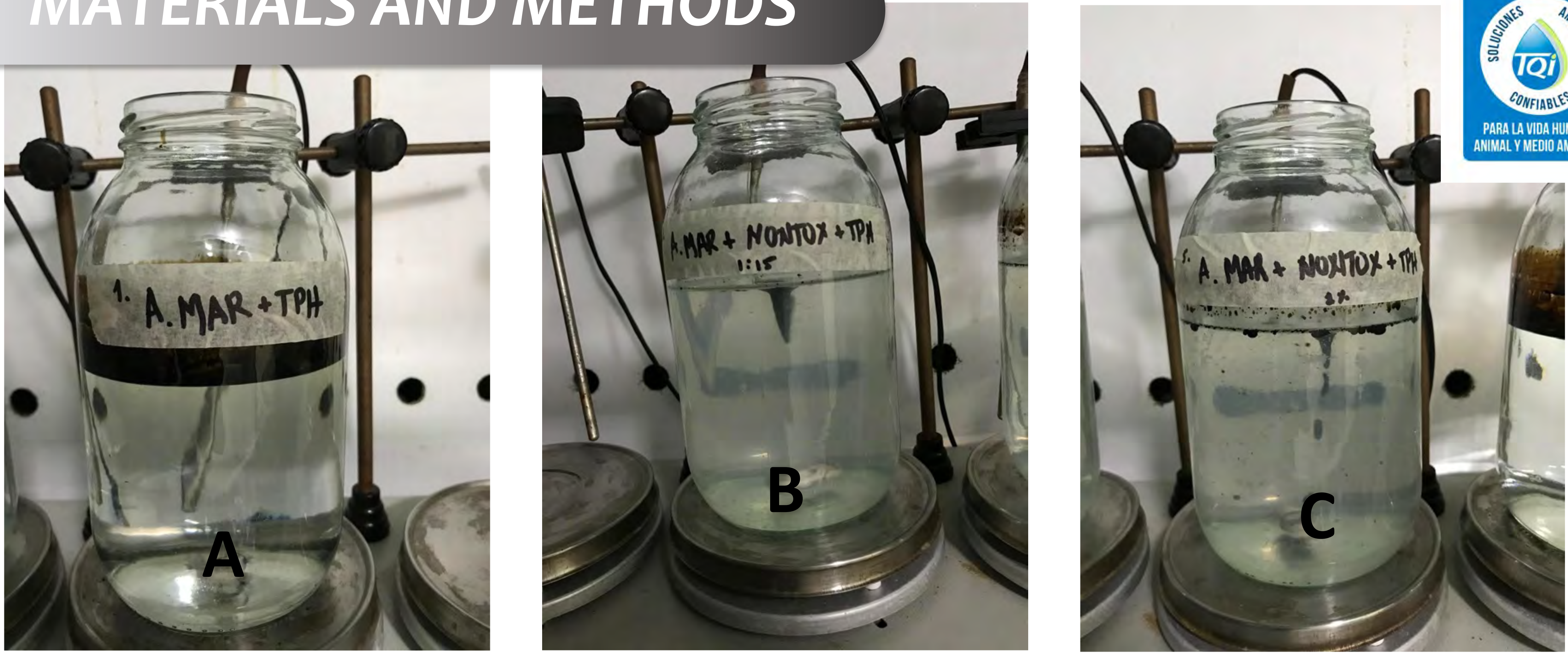
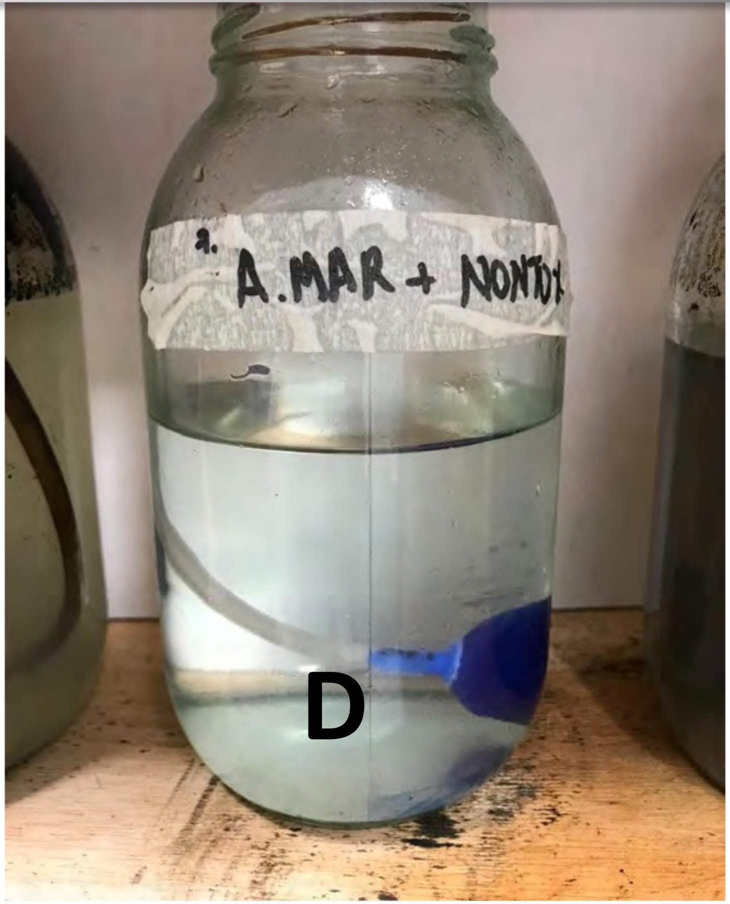
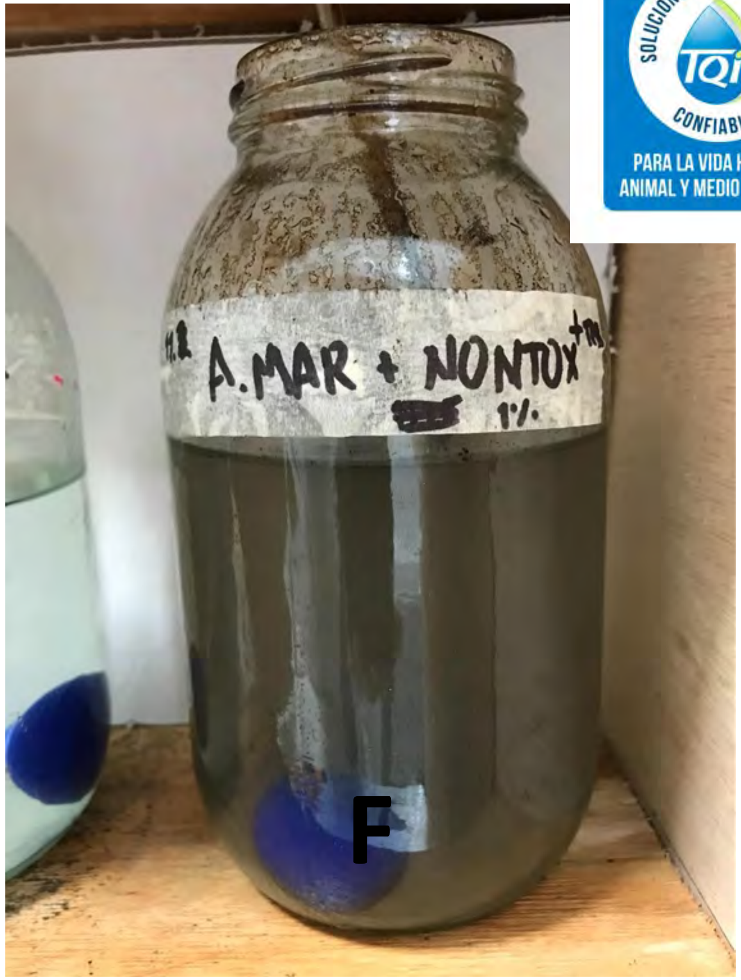


The samples labeled A, B, and C were subjected to an agitation system in a magnetic stirrer.

MATERIALS AND METHODS



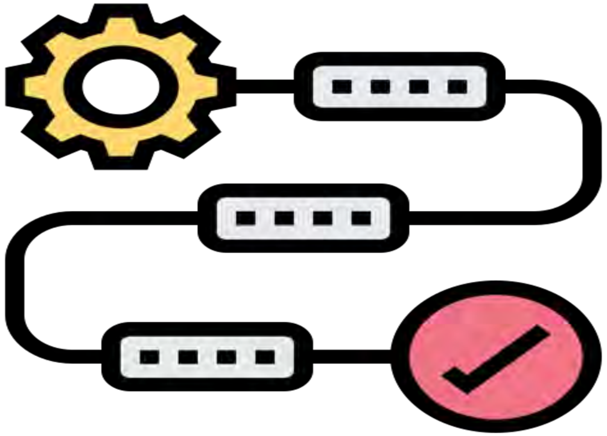


All the experimental set-up vessels were stored at 25 °C and average relative humidity of 64.5%.

The samples labeled D, E and F were agitated uninterruptedly for aeration and mixing to increase contact between the indigenous microbial consortium, nutrients, and contaminated water.

MATERIALS AND METHODS

Drop Collapse Test: A clean flat surface was used to carry out the experiment, and the holes in there were filled with 5 µL of vegetable oil and 5 µL of bio-catalyst solution were added to the oil surface.



Oil Spreading Assay:

5 mL of distilled water were poured into a 15 cm diameter Petri dish, followed by the addition of 100 µL of Bazu oil to the surface of the water to form a thin layer of oil.

About 10 µL of the bio-catalytic agent solution was added to the center of the formed oil layer, and the diameter of the cleaned area was measured.

MATERIALS AND METHODS

Emulsification Test: The test was realized by mixing 2 mL of kerosene with an equal volume of a bio-catalytic agent, which was previously stirred in a vortex type agitator for 2 min and left to stand for twenty-four (24) hours.

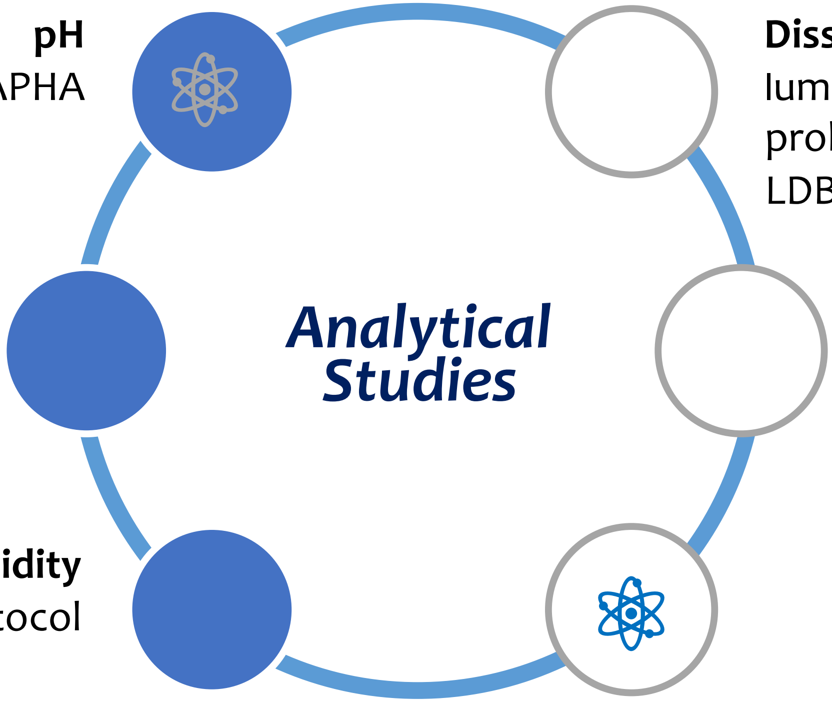
Critical Micelle Concentration(CMC):
This test required the preparation of different dilutions of a biocatalytic agent in distilled water. The changes in surface tension were measured in a digital tensiometer at a temperature of 25 °C, and distilled water of 96% purity was used as standard.

MATERIALS AND METHODS

pH
4500-H APHA

COD
5220D

Turbidity
2130 B protocol



Dissolved Oxigen(OD)
luminescence Oxygen probe (HACH® (LDO) LDBO101, model)

TPH
Gravimetric method: USEPA 1664A

All these physico-chemical properties were analyzed according to the *Standard Methods for examination of water and Wastewater*

RESULTS

Qualitative Characterization

Figure 2. *Drop Collapse Test Result. The drop did not retain its shape after 1 min of inspection.*

RESULTS

Qualitative Characterization

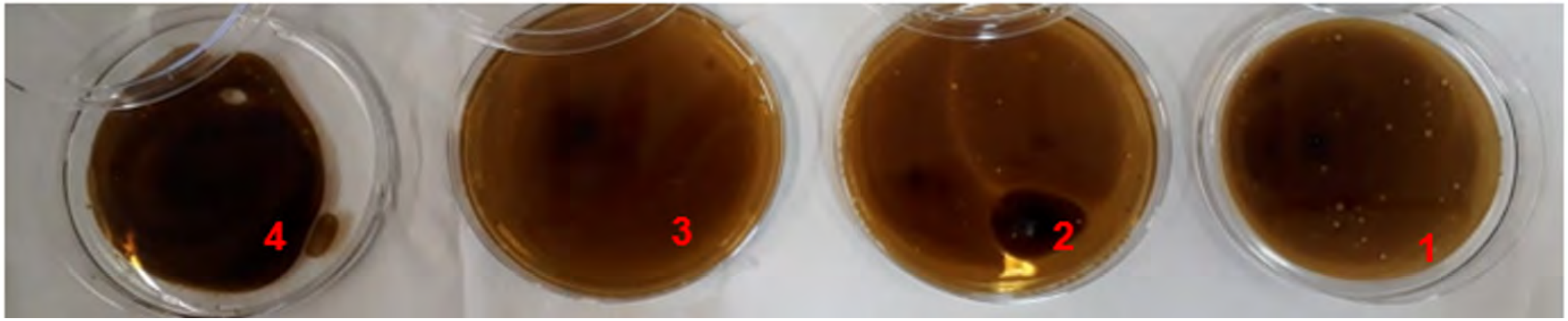


Figure 3. *Oil-Spreading assay before bio-catalytic solution application.*

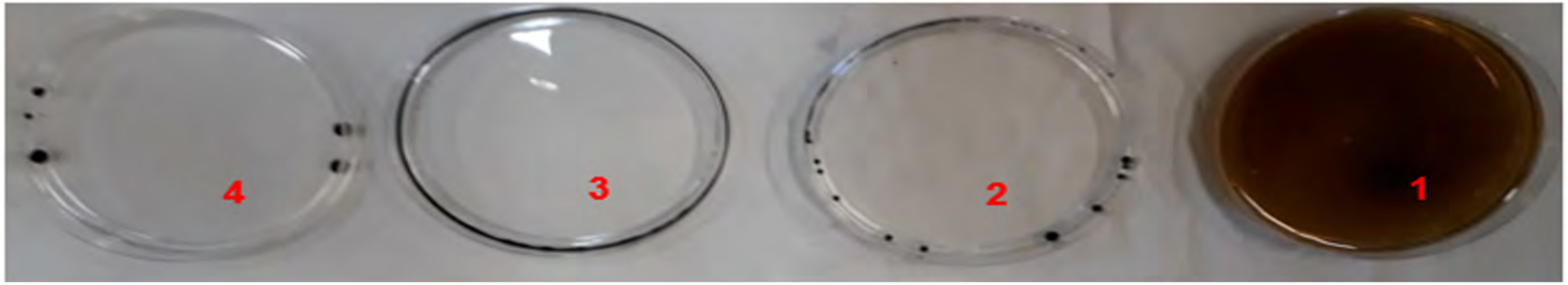


Figure 4. *Oil-Spreading assay after bio-catalytic solution application.*

(1) sample with crude oil and distilled water (negative control) ;(2), (3) y (4). sample with crude oil, distilled water and bio-catalytic solution.

Qualitative Assays

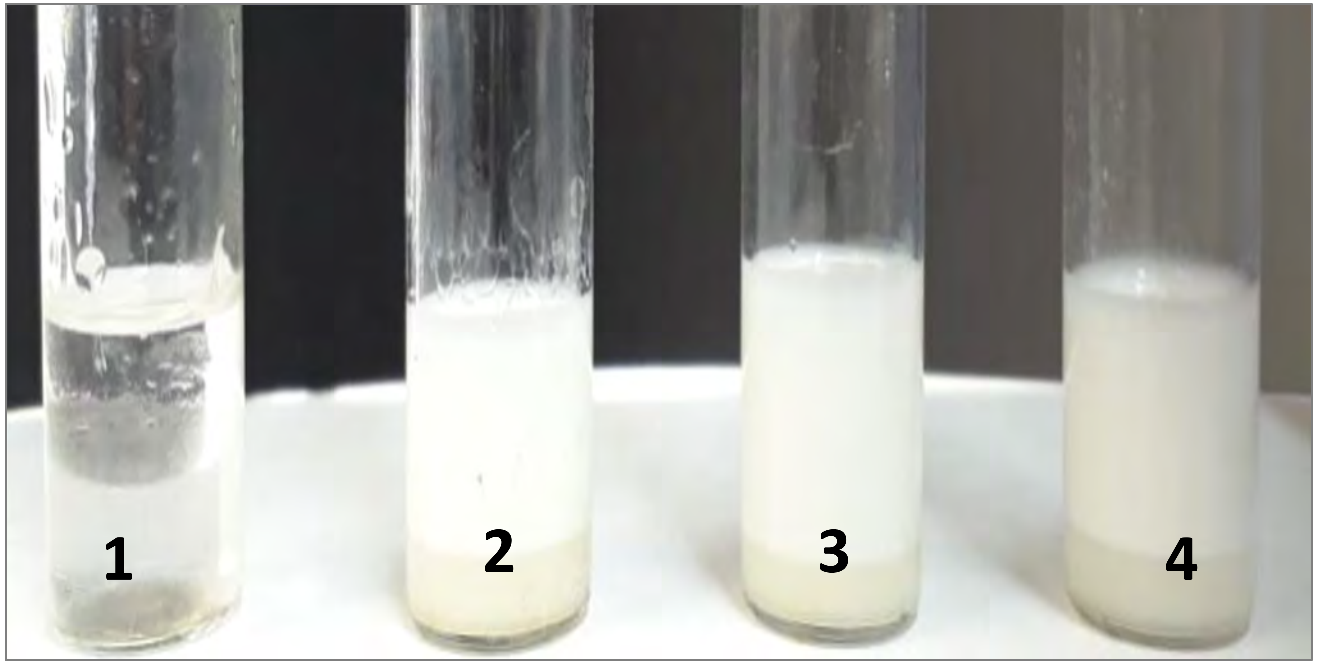


Figure 5. *Test-tube used to measure the emulsification index (E24), (1). The first test-tube is the control sample whose composition is only 2 mL kerosene.*
(2), (3), (4). These test-tubes contain a mixture of 2 mL kerosene with 2 mL of bio-catalytic agent solution previously stirred in a vortex type agitator for 2 min.

Average emulsification index: **74.47 % ±5.55**

Qualitative Assays

Table 4. *Surface Tension values measured at a given concentration of biocatalytic agent.*

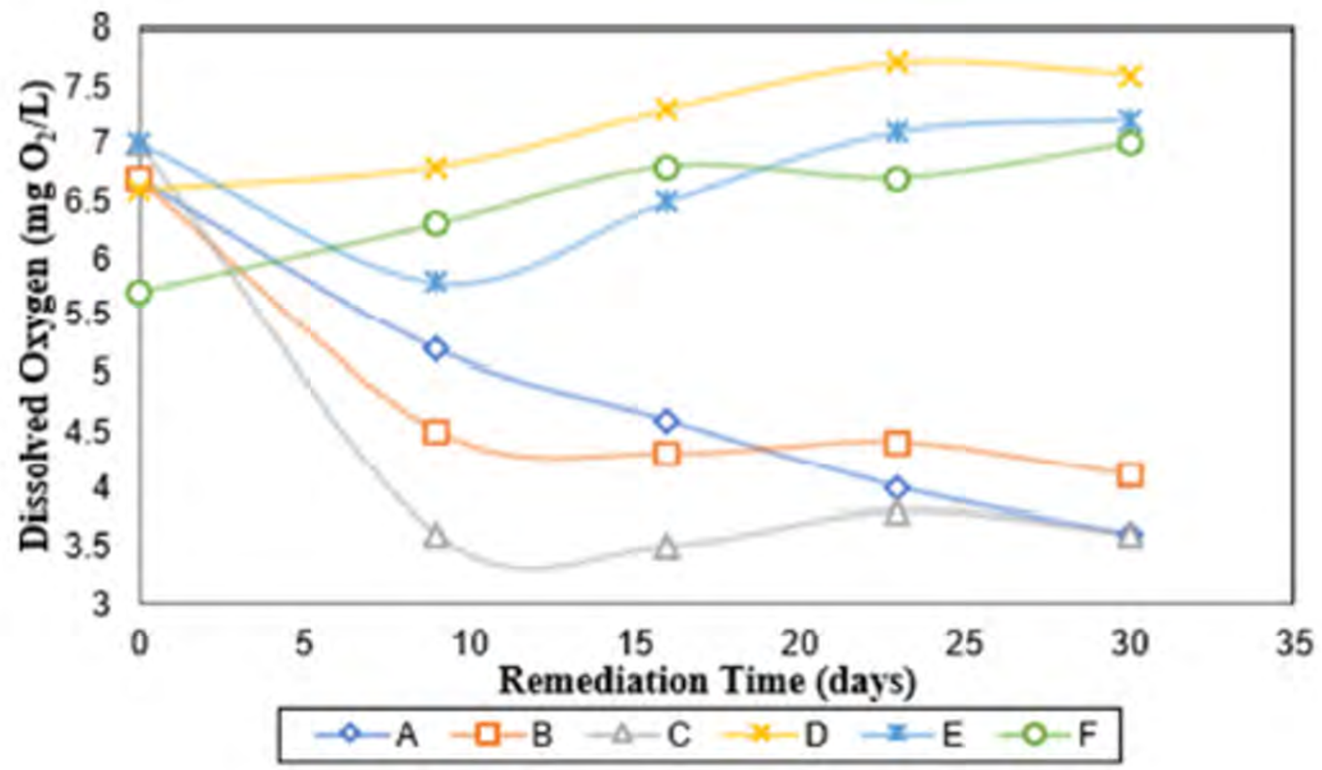
Bio-catalytic Agent Concentration (mg L^{-1})	Surface Tension (mN m^{-1})
540	30.0 ± 0.08
430	30.2 ± 0.09
140	29.8 ± 0.16
70	29.4 ± 0.25
60	29.6 ± 0.11
50	29.8 ± 0.41
40	29.0 ± 0.10
30	29.2 ± 0.64
20	29.8 ± 0.70
10	29.2 ± 1.05
5	29.4 ± 1.23
0 (Distilled water without bio-catalyst nor oil)	73.1 ± 0.95

Values below 40 mN/m and the concentration varies between 1 and 200 mg/L

Results

Physicochemical Properties Analysis

***Figure 6.* Variation of dissolved oxygen content with remediation time.**



It was appreciated that the DO increased with remediation time for the samples labeled D, E, and F, where the cracking of hydrocarbons was effective.

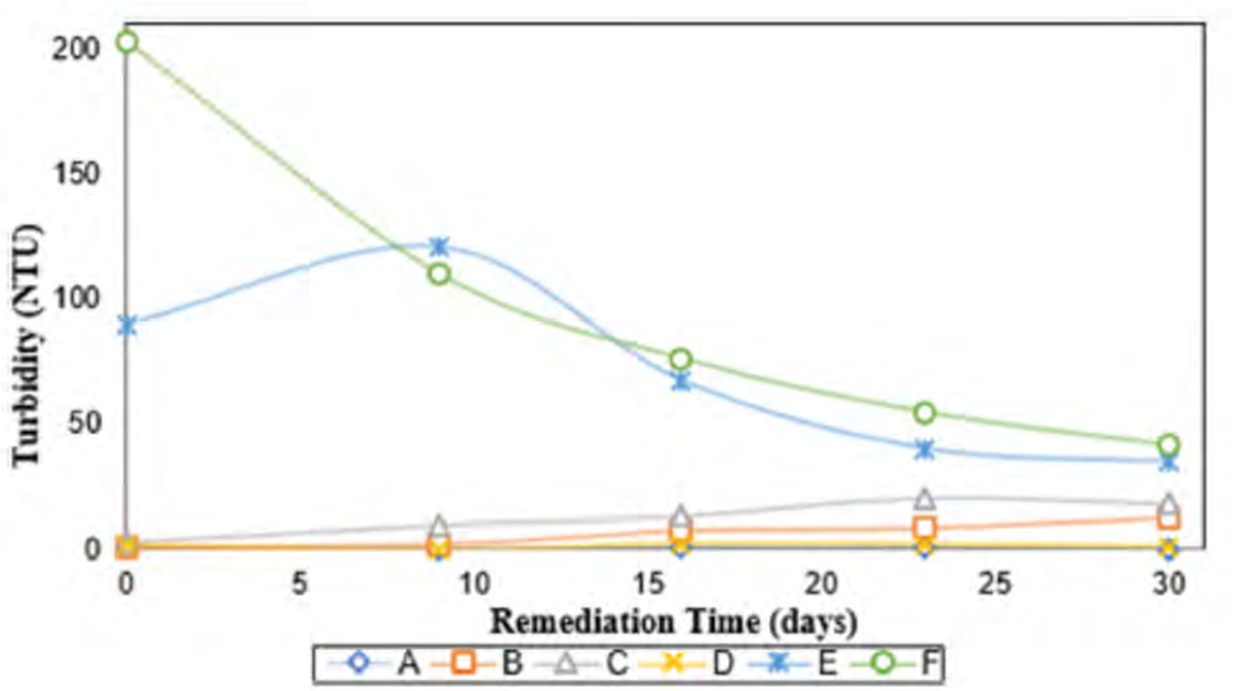
Bio-catalytic agent capacity to improve the Oxygen transfer and speed up the degradation of organic matter.

(A)Seawater with oil (640 mg L1); (B) Seawater with oil (640 mg L1) and biocatalytic agent solution (2167 mg L1); (C) Seawater with oil (640 mg L1)and bio-catalytic agent solution (10000 mg L1); (D)Seawater with bio-catalytic agent solution (2167.39 mg L1) and aeration system; (E) Seawater with oil (640 mg L1), bio-catalytic agent solution (2167 mg L1), and aeration system;(F) Seawater with oil (640 mg L1), bio-catalytic agent solution (10000 mg L1)and aeration system.

Results

Physicochemical Properties Analysis

Figure 7. Variation of turbidity with remediation time



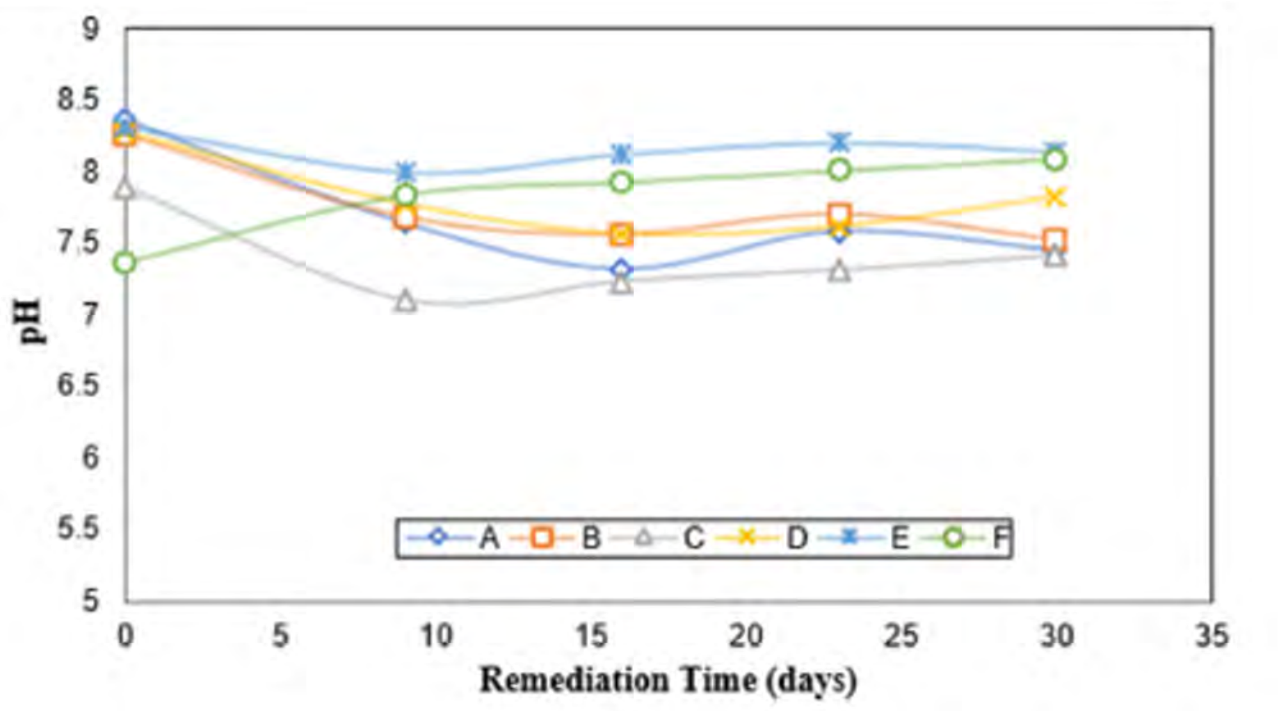
The samples labeled E and F achieved turbidity reduction values of 61.357% ± 0.053 and 79.623% ± 0.053, respectively.

(A) Seawater with oil(640 mg L1); (B) Seawater with oil (640 mg L1) and bio-catalytic agent solution(2167 mg L1); (C) Seawater with oil (640 mg L1) and bio-catalytic agent solution (10000 mg L1); (D) Seawater with bio-catalytic agent solution(2167.39 mg L-1) and aeration system; (E) Seawater with oil (640 mg L1), biocatalytic agent solution (2167 mg L1), and aeration system; (F) Seawater with oil (640 mg L1), bio-catalytic agent solution (10000 mg L1) and aeration system.

Results

Physicochemical Properties Analysis

Figure 8. Variation of pH with remediation time



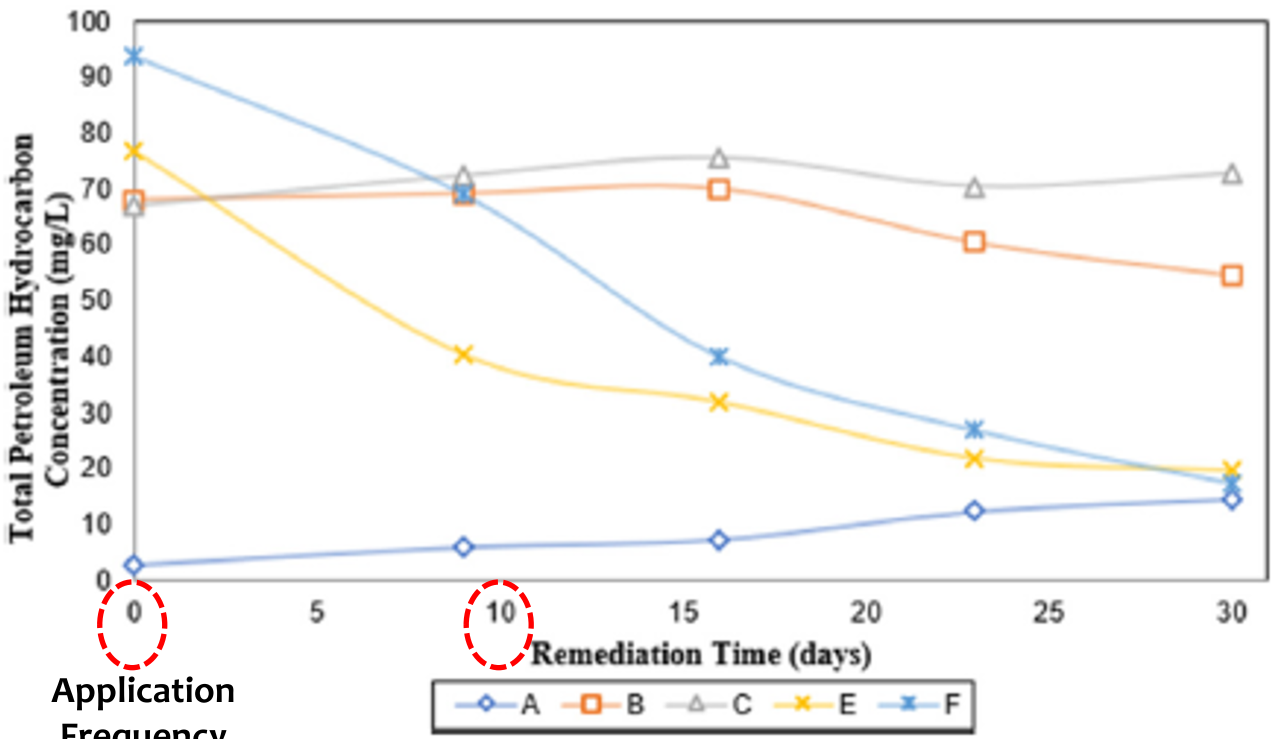
The pH of the sample labeled F exhibited a continuous rising in pH values, indicating that pollutant (petroleum hydrocarbons) in the water was decomposed to compounds that are more basic and less toxic

((A) Seawater with oil(640 mg L1); (B) Seawater with oil (640 mg L1) and bio-catalytic agent solution(2167 mg L1); (C) Seawater with oil (640 mg L1) and bio-catalytic agent solution (10000 mg L1); (D) Seawater with bio-catalytic agent solution(2167.39 mg L-1) and aeration system; (E) Seawater with oil (640 mg L1), biocatalytic agent solution (2167 mg L1), and aeration system; (F) Seawater with oil (640 mg L1), bio-catalytic agent solution (10000 mg L1) and aeration system.

Results

Physicochemical Properties Analysis

Figure 9. Variation of total petroleum hydrocarbon concentration with remediation time.



Application Frequency

The presence of a bio-catalyst solution and an aeration system generates the formation of micro-bubble. These micro-bubbles are the result of aggregates of surfactant molecules with a loose molecular packing, which provokes a more favorable Oxygen mass transfer into an aqueous medium.

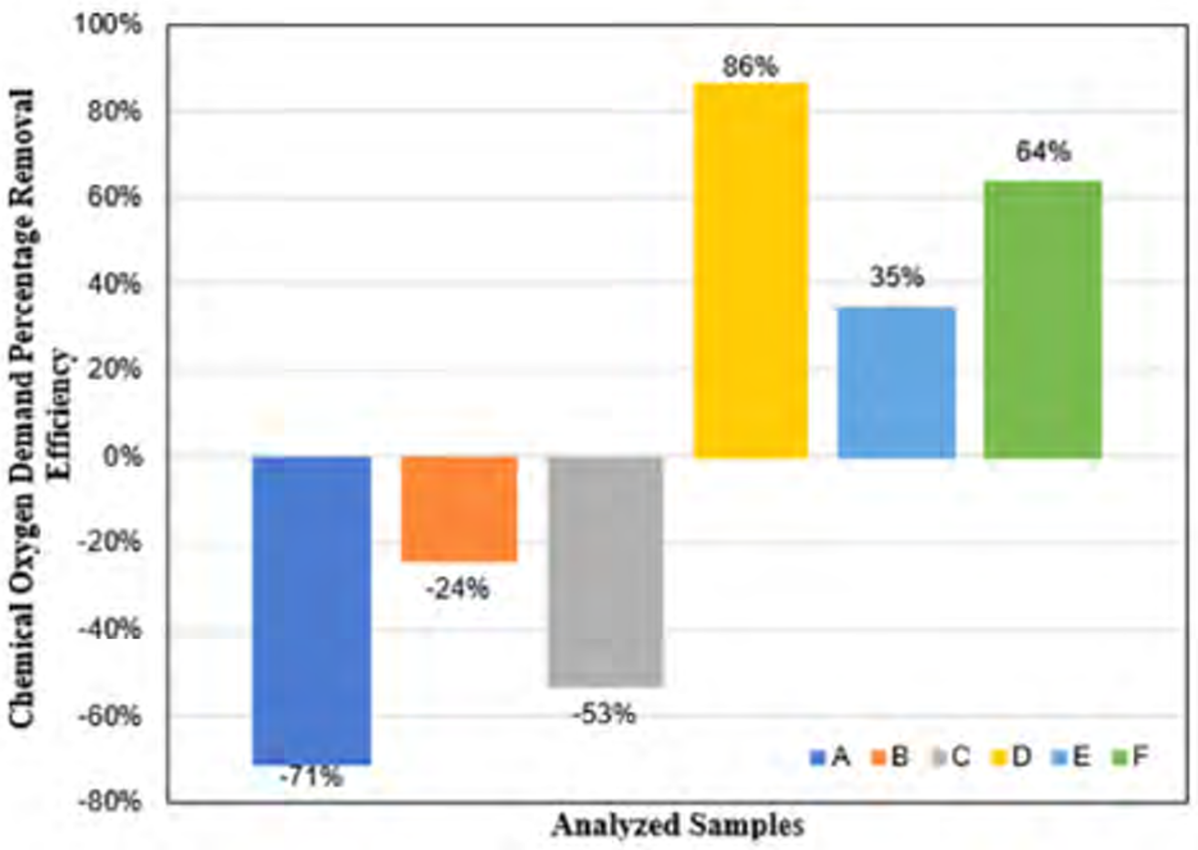
As we expected, the stimulated sample labeled F reached the highest %RTPH with a rough value of 81.537 ± 0.015, followed by 74.446 ± 0.015 corresponding to sample labeled E.

(A) Seawater with oil (640 mg L1); (B) Seawater with oil (640 mg L1) and bio-catalytic agent solution (2167 mg L1); (C) Seawater with oil (640 mg L1) and bio-catalytic agent solution (10000 mg L1); (E) Seawater with oil (640 mg L1), bio-catalytic agent solution (2167 mg L1), and aeration system;
(F) Seawater with oil (640 mg L1), bio-catalytic agent solution (10000 mg L1) and aeration system.

Resultados

Physicochemical Properties Analysis

Figure 10. Percentage of Chemical Oxygen Demand removal.



The highest %RCOD was 64.539 ± 0.125, and it was attained by sample labeled F, which had the most concentrated bio-catalyst solution, followed by sample labeled E which had a removal efficiency of 35.325% ± 0.125 for COD at the end of the remediation time.

((A) Seawater with oil (640 mg L1); (B) Seawater with oil (640 mg L1) and bio-catalytic agent solution (2167 mg L1); (C) Seawater with oil (640 mg L1) and bio-catalytic agent solution (10000 mg L1); (D)Seawater with bio-catalytic agent solution (2167.39 mg L-1) and aeration system; (E) Seawater with oil (640 mg L1), biocatalytic agent solution (2167 mg L1), and aeration system; (F) Seawater with oil (640 mg L1), bio-catalytic agent solution (10000 mg L1) and aeration system.

Remarks

The analyzed bio-catalyst showed positive outcomes for the drop-collapse test and oil-spreading assay.

The measurement of emulsification activity (E24) and Critical Micelle Concentration(CMC) displayed values of 74.47% and 40 mg/L, respectively.

The highest TPH removal efficiency was reached by the sample labeled F (81.537%), which contained 640 mg/L of petroleum and 10000 mg/L of biocatalyst solution.

Remarks

Agitated and aerated systems have an essential effect on the bioremediation process. TPH removal efficiencies for aerated samples were in a range of 70%–82%., while, those not subjected to an agitation system achieved only a near value of 20%.

Catabolic cooperation between groups of microorganisms is important during the bioremediation process, because sometimes the complete petroleum hydrocarbons degradation by an only microorganism is not possible.

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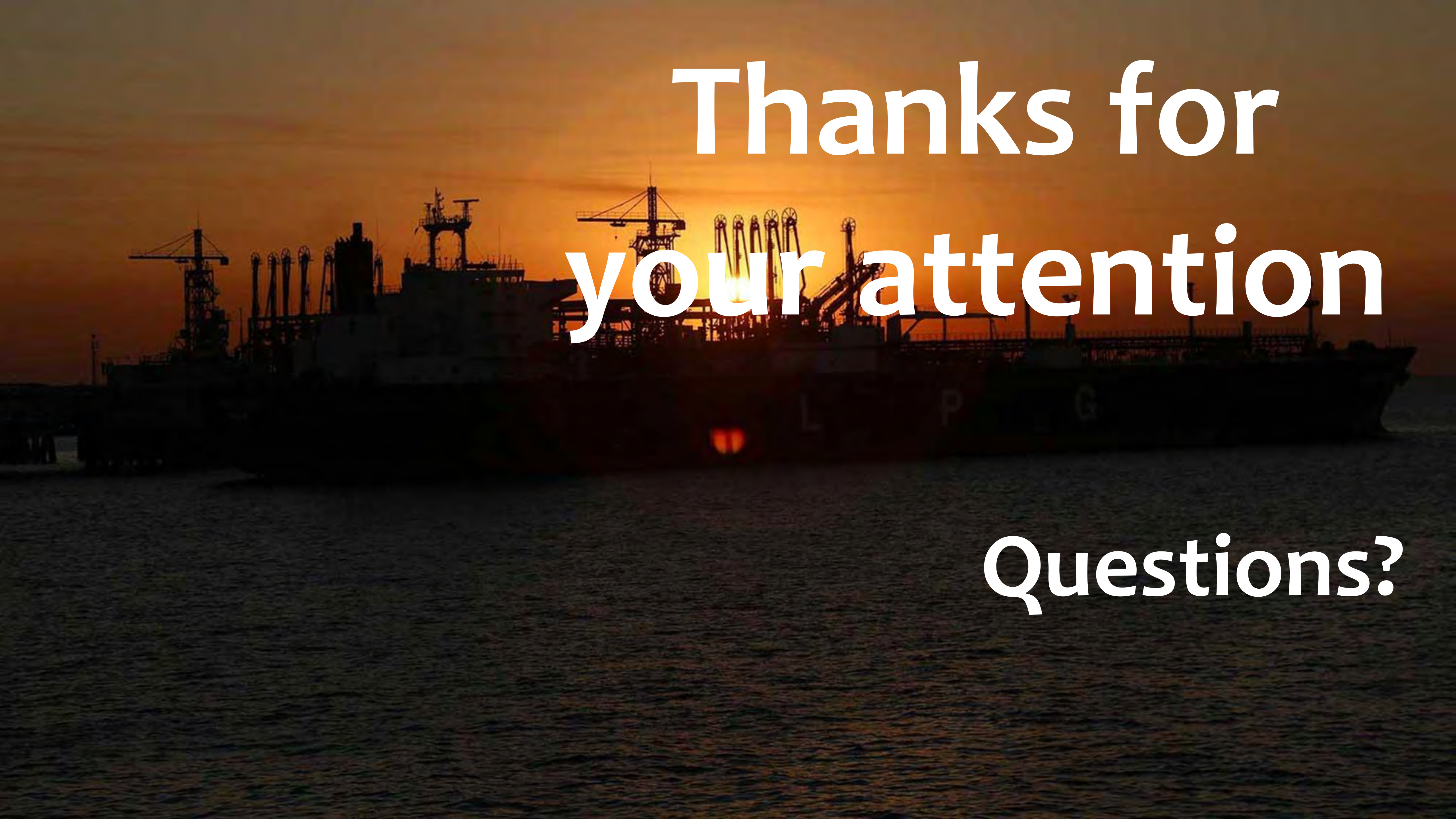
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Questions?

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