ISSN: 2978-7270

Journal of Oil, Petroleum and Natural Gas Research

SCIENTIFIC Research and Community

Research Article

Open Access

Microbial Purification of Oil and Gas Production Water at the Petrochad (mangara) Limited Badila Field

Brahim Bakimbil[1]*, Niraka blaise[2], Samba Koukouare Prosper[3], Fadimatou Hamadou[4] and Djouldé Darman Roger[5]

[1]Hydrocarbon Exploitation Department of the National Higher Institute of Petroleum of Mao, Chad

[2]National Advanced School of Engineering, University of Maroua, PO Box: 46 Maroua (Cameroon)

[3]Research Professor at the National Higher Institute of Petroleum in Mao, Chad

[4]Student (Cheick Anta Diop University Dakar)

[5]Department of Sustainable Agriculture, Faculty of Sciences, University of Garoua, Cameroon

ABSTRACT

RPG14, RPG18 and RPG20 isolates selected at the end of a screening test were subjected to optimization of physicochemical and nutritional parameters. As a result, a 3-liter extraction for each culture medium was launched. The optimal yield after 20 days of incubation is estimated for RPG14 is 68.62 g/l, for RPG18 is 60.42 g/l and for RPG20 is 69.85 g/l. Five graduated tubes containing 150 ml each of oil and gas production water and therefore 25, 30 and 50 ml of supernatant of each isolates RPG14, RPG18 and RPG20 are added to each tube. The whole is placed on an electric centrifuge heater type MPW-260RH and rotates at 300 rpm for 15 minutes. At each centrifugation under a temperature (55, 70 and 75°C), the tubes are passed to a mini-wastewater decanter type 6016-SIMOP. The volume of the oil layer in the tube is read after 20min, 40min and 60min of decantation. The purification rate is calculated. The results reveal that biosurfactants can sanitize the EPP 100%. However, the quantity of biosurfactants does not have an influence on the purification. However, the longer the residence time of biosurfactants in the EPP, the higher the purification rate. The purified EPP is collected and subjected to a physicochemical analysis, these waters comply with the IFC 2007, WHO and FAO discharge standards.

***Corresponding author**

Brahim Bakimbil, Hydrocarbon Exploitation Department of the National Higher Institute of Petroleum of Mao, Chad.

Received: April 19, 2025; **Accepted:** April 23, 2025; **Published:** May 03, 2025

Keywords: Production water, biosurfactants, purification, Environment

Introduction

Excessive water production in the Petrochad field is one of the major problems in terms of oil droplet entrainment and those in solution. [1]. It affects oil recovery and requires high treatment and disposal costs [2]. Oils are held in solution by surface tensions that remain high [3]. A significant amount remains in the form of fine droplets in solution which constitutes a stable inverse emulsion, the continuous phase of which is water. These waters are regularly discharged into the environment or buried underground [4]. This way of managing them causes irreversible and considerable impacts on the environment despite the physicochemical treatment inflicted. One of the most effective methods is biological treatment.

The objective of this work is to submit theisolatesRPG14, RPG18 and RPG20 were selected following a screening test, to be optimized by physicochemical and nutritional parameters to increase the ability to lower the surface tensions that persist in keeping the oils in solution. Subsequently, an extraction of the biosurfactants was carried out, taken and added to a quantity of raw production water under the action of temperature in order to release the oils still remaining in solution. A physicochemical analysis of the purified water under the action ofisolates RPG14, RPG18, RPG20, are carried out and compared to the EPPG discharge standards on the Badila field.

Materials and Methods

Location of the Study Site

The Petrochad (Mangara) Limited field consists of two sites (Mangara and Badila). These two fields are located in the Doba Basin south ofthe capital N'Djamena. This field was discovered in 1978 by the American company CONOCO and put into operation in 2013 by the company Griffiths International Energy (GIE). The Badila field is located mainly in the department of Nya Pendé. It is located between 08° 20' 25.25'' North latitude and 16° 19' 40.32'' East longitude, in the southwest of Chad. In other words, it is located approximately 430 km southwest of N'Djamena, and 60 km from the city of Moundou, the economic capital of Chad. This field is located in the province ofLogone Oriental, capital of Doba, borders the Central African Republic and the Republic of Cameroon. The map below shows the location of the Badila Field, which houses all the oil processing and shipping facilities for the Mangara project, Petrochad site.

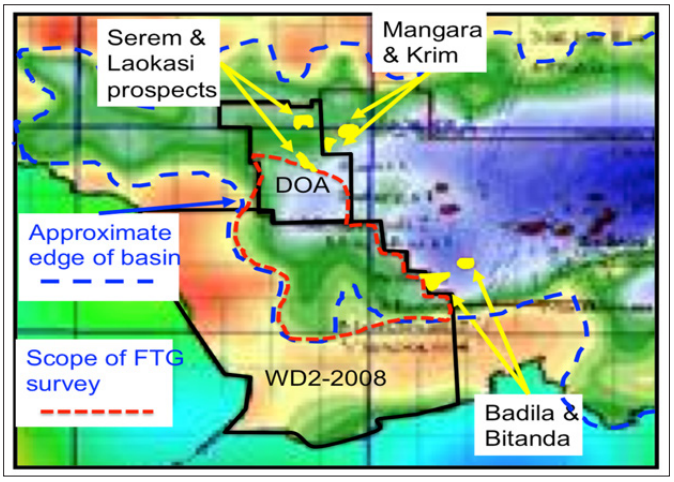

Figure 1: Map of The Study Site

Samples: Thirty-seven (37) samples from the EPPG and quagmires were taken in May and June 2022. However, the samples were taken with a spatula, taking the usual precautions for disinfecting the tools (flaming with 90° ethyl alcohol) to avoid any risk of contamination of the samples which were collected in sterile plastic containers.

Cultivation and Isolation

The approach followed for the isolation of Haloanaerobium is that of it is ensured by a preliminary enrichment, where 10 ml of the waters are introduced into 90 ml of the liquid Sehgal-Gibbons (SG) medium, contained in a 250 ml Erlenmeyer flask. The mixture is then stirred for 30 minutes to obtain a good dilaceration of the particles and incubated at 37 ° C for 15 days. Then, a dilution series is carried out, where 100 µl of the dilutions 10-1 up to 10-3, are inoculated on the surface on the solid culture medium SG [5].The Petri dishes are incubated at 37°C for 15 to 20 days in plastic bags, in order to avoid rapid drying of the culture medium and crystallization of its salts.

Purification and Conservation of Isolates

The isolates are first purified by successive subcultures of well-separated and macroscopically distinct colonies on the solid culture medium SG. Once purified, each isolate is designated by a code number, which consists of the three letters RPG, followed by a serial number. The preservation of the microorganisms thus designated is done by several methods depending on the purpose. Generally, two preservation techniques have been carried out, one is for short-term preservation, it most often consists of subcultures on agar slants with storage at 4 ° C, and the culture will be subcultured every 03 to 06 months. However, the second technique is for long-term preservation, where the purified isolates are transferred into sterile 1.5 ml Eppendorf microtubes, containing 20% glycerol, storage is done at -10 ° C.

Screening of Biosurfactant-Producing Isolates

For screening, ten isolates (RPG11, RPG12, RPG13, RPG14, RPG15, RPG16, RPG17, RPG18, RPG19, RPG20) were selected from each sampling site. Biosurfactant-producing isolates were selected using the following four methods: Drop collapse assay, Oil spreading assay, Emulsion stability (ES%) test, and Surface tension measurement. The experiments were performed in three replicates.

Drop Collapse Test

This test is based on the destabilization of an oil droplet by surfactants. It consists of using a 96-well microplate, each containing 100µl of an oily phase. However, the oils that were tested are: sunflower oil, olive oil, mineral oil, car oil and diesel. These oils were equilibrated for one hour at room temperature. 10µl of the culture of each of the isolates to be tested is added to the wells, observation is made after 1 min using a binocular microscope. If the liquid does not contain surfactants (biosurfactants), the polar water molecules repel from the hydrophobic surface and the drop remains stable. If the liquid contains surfactants, the drop spreads because the interfacial force or tension between the aqueous phase and the oil phase is reduced. The results were interpreted as follows: from "+" to "++++" corresponding to partial or total diffusion on the oil surface. Droplets that gave a rounded shape were marked as "-" indicating the absence of biosurfactant production [6- 8].

Oil Dispersion Test

In the oil dispersion test, 50 ml of synthetic seawater was added to the surface of a glass Petri dish of (90 × 15 mm) dimension, plus a volume of 20 µl of crude oil or mineral oil, making a thin layer on the water surface. 10 µl of the culture was added on the oil surface, the tests were carried out due to three repetitions for each sample [9-11]

Emulsification Test

This test consists of mixing 2 ml of the culture with 2 ml of diesel in a test tube (15×125 mm). The mixture was stirred for 4 minutes and left to stand. The emulsion volume (EV%) and emulsion stability (ES%) were measured as follows:

$$EV\% = \frac{\text{Hauteurdel'emulsion (mm)} \times \text{surface (mm2)}}{\text{Volume du liquide total (mm3)}} \times 100$$

$$ES\% = \frac{\text{EV\% à temps 24h}}{\text{EV\% à temps 0h}} \times 100$$

The emulsions formed by the bacterial cultures were compared with those formed by a 1% solution of a synthetic surfactant (SDS) as a positive control and by the sterile culture medium as a negative control [12-13]. A criterion cited to confirm the production of biosurfactants is the ability to maintain at least 50% of the initial volume of the emulsion after 24 hours of its formation [14-15].

Measurement of Surface And Interfacial Tensio n: Surface tension measurements of the bacterial cell-free supernatants were determined using a tensiometer (TD1C LAUDA). The reported values are the average of three measurements. Samples of 50 ml were collected at 24-h intervals and centrifuged at (10,000 × g for 25 min) at room temperature. The criterion used for the selection of biosurfactant-producing isolates was the reduction of the surface tension of the medium over time below 40 mN.m-1[16].

Optimization of Physicochemical Conditions

LThe production of biosurfactants can be influenced by culture conditions such as temperature, pH, agitation speeds and salinity levels (NaCl and MgSO4.7H2O).

Effect of pH

LpH is an important factor, it can limit or promote the production of biosurfactants. For this, we tested several pH values: 6, 6.5, 7, 7.5 and 8 (the incubation temperature is 37°C with shaking at 120 rpm).

Procedure: The experimental conditions for isolate RPG14 are: NaCl (250 g/l), agitation of (120 rpm) and temperature of (37°C)

for 13 days of incubation. The experimental conditions for isolate RPG18 and RPG20 are: NaCl (250 g/l), agitation of (120 rpm) and temperature of (37°C) for 13 days of incubation.

Effect of Temperature

In order to determine the optimal temperature for the synthesis of biosurfactants, we propose to test the following temperatures: 27, 30, 37, 40 and 45°C (optimized pH and stirring at 120 rpm).

Procedure: The experimental conditions for isolate RPG14 are: pH (7), agitation (120 rpm) and NaCl (250 g/l) for 13 days of incubation. The experimental conditions for isolate RPG18 and RPG20 are: pH (7.5), agitation (120 rpm) and NaCl (250 g/l) for 13 days of incubation.

Effect of Agitation

The agThe aim of this process is to ensure mass transfer between the three phases: liquid, consisting of the culture medium; solid (the cells); and gas. Therefore, we tested three stirring speeds: 50, 100 and 150 rpm (optimized temperature and pH).

Procedure: The experimental conditions for isolate RPG14 are: pH (7), T (37°C) and NaCl (250 g/l) for 13 days of incubation. The experimental conditions for isolates RPG18 and RPG20 are: pH (7.5), T (45°C) and NaCl (250 g/l) for 13 days of incubation.

Effect of NaCl (%)

Different NaCl concentrations were tested (8%, 15%, 20%, 25% and 30%), the optimized values of temperature, pH, and agitation were taken into consideration.

Procedure: The experimental conditions for isolate RPG14 are: pH (7), agitation of (150 rpm) and temperature of (37°C) for 13 days of incubation. The experimental conditions for isolates RPG18 and RPG20 are: pH (7.5), agitation of (150 rpm) and temperature of (45°C) for 13 days of incubation.

Effect of MgSO4, 7H2O (M)

Different concentrations of MgSO4, 7H2O, were tested (0.005M, 0.01M, 0.05M,
0.1M, 0.2M and 0.3M), the optimized values of temperature, pH, stirring and NaCl concentration are taken into consideration.

Procedure: The experimental conditions for isolate RPG14 are: NaCl (8%), pH (7), shaking (150 rpm) and temperature (37°C) for 13 days of incubation. The experimental conditions for isolates RPG18 and RPG20 are: NaCl (25%), pH (7.5), shaking (150 rpm) and temperature (45°C) for 13 days of incubation.

Optimization of Nutritional Conditions

Effect of Carbon Source

Cultures were carried out on liquid SG medium, in the presence of carbon sources: monosaccharide (glucose), disaccharide (lactose), polymer (starch) and organic acid (sodium citrate). Each substrate was used separately as the sole carbon source at a rate of 4 g/l. In addition, diesel and glycerol at a concentration of 4% (v/v). The substrate inducing the best production rate was tested at different concentrations. Carbohydrates were sterilized by filtration through a millipore membrane (0.45µm) and aseptically added to the culture medium.

Variation of Concentrations Indiesel

The experimental conditions for isolate RPG14 and RPG18 are: NaCl (8%), MgSO4, 7H2O (0.005 M), pH (7), shaking (150 rpm) and temperature (37°C) for 13 days of incubation. The experimental conditions for isolate RPG20 are: NaCl (25%), MgSO4, 7H2O (0.1 M), pH (7.5), shaking (150 rpm) and temperature (45°C) for 13 days of incubation. The letters represent the significant differences between the different concentrations of diesel.

Variation of Csodium Citrate Concentrations

Experimental conditions for the isolateRPG14 and RPG18 are: NaCl (8%), MgSO4, 7H2O (0.005 M), pH (7), shaking (150 rpm) and temperature (37°C) for 13 days of incubation. The experimental conditions for isolate RPG20 are: NaCl (25%), MgSO4, 7H2O (0.1 M), pH (7.5), shaking (150 rpm) and temperature (45°C) for 13 days of incubation.

Variation of The Cglycerol Concentration

The experimental conditions for isolate RPG14 and RPG18 are: NaCl (8%), MgSO4, 7H2O (0.005 M), pH (7), shaking (150 rpm) and temperature (37°C) for 13 days of incubation. The experimental conditions for isolate RPG20 are: NaCl (25%), MgSO4, 7H2O (0.1 M), pH (7.5), shaking (150 rpm) and temperature (45°C) for 13 days of incubation.

Effect of Nitrogen Source

THEThe study of the effect of different nitrogen sources on the production of biosurfactants is carried out by cultures on liquid SG media, in the presence of various nitrogen sources due to 7g/l of sodium nitrate (NaNO3), ammonium sulfate (SO4(NH4)2), yeast extract and urea (CH4N2O) separately. The substrate inducing the best production rate is tested at different concentrations.

Mesure of The Parameters

PFor both physicochemical and nutritional parameters, after 13 days of incubation, the culture was centrifuged at 8000×g at 4°C for 30 minutes. The supernatant was collected and the surface tension was read using a tensiometer, the results were expressed as mN.m-1. The surface tension produced by the biosurfactants was also expressed as a percentage reduction in surface tension, calculated using the following equation:

$$\%\text{RTS} = \left[1 - \left(\frac{TS_{apres}}{TS_{avant}}\right)\right] \times 100$$

OrTS_{avant} is the surface tension measured before inoculation of the isolate
And is the surface tension measured after inoculation of the isolate (Ainon et al., 2013)$TS_{après}$

Extraction of Biosurfactants

The biosurfactant extraction was carried out according to the method described by Smyth et al. (2010). The supernatants of the RPG14, RPG18 and RPG20 bacterial cultures were obtained after centrifugation at 8000 rpm for 15 min and acidified to pH = 4 with HCl solution (4.8 N). Then, the extraction was carried out with ethyl acetate (v/v) repeated three times. The organic phase was dried in the presence of magnesium sulfate (MgSO4) (0.1 G per 100 ml of solvent) and obtained by evaporation of the extraction solvent using a rotovapor (BUCHR R-114) at 45 °C. A partially purified biosurfactant was recovered, weighed and expressed in (g/l). The yield of biosurfactant production is represented by the average of two independent experiments.±standard deviation.

Production Water Purification Rate After Action of Isolates

Five graduated tubes containing 150ml each of oil and gas production water and therefore 25, 30 and 50ml of supernatant

of each isolate RPG14, RPG18 and RPG20 are added to each tube. The whole is placed on an electric centrifuge heater type MPW-260RH and rotates at 300rpm for 15min. At each centrifugation under a temperature (55, 70 and 75°C), the tubes are transmitted to a mini-wastewater decanter type 6016-SIMOP, the volume of the oil layer in the tube is read after 20min, 40min and 60min. During these three periods, the purification rate is calculated according to the formula:

$$\text{Taux de décantation}(\%) = \left[1 - \left(\frac{V_{huile}}{V_{total}}\right)\right] \times 100$$

OrV $_{huile}$ is the volume of oil measured decantation
And is the volume of raw production water measured before settlingV_{total}

Oil Extraction and Analysis of Decanted Water:
In the 6016-SIMOP mini wastewater decanter, the decanted water is extracted and stored in sterilized tubes at 4°C. Samples are then taken and analyzed by spectrometry.

Sampling And Physicochemical Analyses
The samples taken were first subjected to a physicochemical analysis, so the pH and electrical conductivity were measured by the HANNA type multi parameter, the sodium, potassium, magnesium, sulfate, chloride, ammonium, total hydrocarbon, phenol, barium, manganese ions are analyzed by flame spectrometer and the DR 2400 spectrophotometer.

Principle: The DR 2400 is a simple and comfortable photometer. Its spectral range is between 400 and 880 nm with an operating temperature range of 0 to 40°C. When a monochromatic light beam of wavelength λ of intensity Io passes through a solution to be analyzed, it undergoes absorption and comes out with a weakened intensity I. This decrease in intensity is due to the absorption of one or more frequencies by the medium crossed. From the proportion of light intensity absorbed by the solution, the concentration C (mg/l) of the absorbing substance can be deduced by the Beer-Lamber relationship according to the expression: D = log = alc Where a is the molar absorption coefficient; it depends on the nature of the absorbing substance, the wavelength. L is the optical path of the radiation through the solution. C: is the concentration of the solution. The inorganic components of the water sample are placed in the presence of special reagents. The intensity of the color produced is measured. This is a measure of the concentration of inorganic ions to be analyzed. For each test, a blank analysis is carried out with distilled water and the reagents $\frac{I_0}{I}$.

The Flame Spectrophotometer: Potassium and sodium ions were analyzed by this device.
Principle: This method uses the property of neutral atoms to absorb a quantum of energy at a certain wavelength. The flame photometer "BWB-XP" is an instrument for the simultaneous determination of 5 elements: Na, K, Ca, Li and Ba in clear water. The photometer "BWB-XP" uses a low-temperature flame using a mixture of air and fuel (propane or butane).

Results And Discussion
Optimization Of Physicochemical Parameters
Table 1 presents the summaries of the optimization of the physicochemical parameters of biosurfactant production by RPG14, RPG18 and RPG20 isolates. Parameters such as pH, temperature, agitation and mineral salts showed very appreciable reductions in surface tensions with high percentages of reductions. They are extremely important for the yield and characteristics of biosurfactants. Obtaining large quantities of biosurfactants required the optimization of parameters such as temperature (45, 50 and 55°C), pH (7.5 and 8), aeration and agitation speeds (100 to 150 rpm). Previous work has shown that most optimal biosurfactant production is carried out at temperatures of 25 to 30°C [17-21]. Our results are close [22].

who argued that the best production occurred when the pH was 8. There best value of biosurfactant production is 45.5 g/l, was obtained when the air flow rate was 1 v/v and the dissolved oxygen concentration was maintained at 50% of saturation [23-24].

Table 1: Summary of the Optimization of Physicochemical Parameters of Biosurfactant Production by Rpg14, Rpg18 And Rpg20 Isolates

Condition of culture	Culture condi-tions (Before op-timization)	Culture condi-tions (After op-timization) RPG14	Culture condi-tions (After op-timization) RPG18	Culture condi-tions (After op-timization) RPG20
Mineral salts	NaCl = 250g/l MgSO4.7H2O = 200g/l	NaCl = 150g/l MgSO4.7H2O = 0.009MF	NaCl = 250g/l MgSO4.7H2O = 0.09 MF	NaCl = 200g/l MgSO4.7H2O = 0.05 M
Ph	7.2	7.5	8	8.0
Agitation (rpm)	120	150	150	100
Temperature (°C)	37	50	55	45
TS (mN/m)	TSRPG14 = 23.7 TSRPG18 = 22.45 TSRPG20=22.75	TS=18.92±0.2 TS=10.68±0.6 TS=7.77±.6 TS=10.75±0.6 TS=11.30±0.01	TS=9.31± 0.2 TS=10.37±0.8 TS=7.94±0.6 TS=6.94±0.6 TS=11.60±0.7	TS=11.60±0.2 TS=6.15±0.03 TS=21.14± 0.6 TS=5.96±0.6 TS=4.40±0.7
%RTS	%RTSRPG14 = 57.65 %RTSRPG18 = 59.89 %RTSRPG20=59.35	%RTS=70.5± 0.6 %RTS=70.5± 0.6 %RTS =71.02±0.1 %RTS=71.02±0.7 %RTS=71.02±0.01	%RTS=70.03±0.8 %RTS=70.03±0.8 %RTS =62.3±0.9 %RTS=75.3±0.7 %RTS = 71.03±0.01	%RTS=70± 0.03 %RTS=70± 0.03 %RTS =62.4±0.1 %RTS=81.01±0.6 %RTS = 81.01±0.7

Optimization of Nutritional Parameters

Table 2 presents Summary of optimization of nutritional parameters of biosurfactant production by isolates RPG14, RPG18 and RPG20 The composition and emulsifying activity of biosurfactants does not only depend on the producing strain, but also on the culture conditions including the nature of carbon and nitrogen sources, as well as the C:N ratio [25-26]. However, the quality and quantity of biosurfactants produced are affected and influenced by the nature of the carbon substrate: diesel (5%), sodium citrate (5.5%) and glycerol (5.5%) produced very low surface tensions with high percentages of reductions. Varieties of nitrogen sources were used that allowed to obtain the optimal production of biosurfactants, in the form of urea (5.5%), yeast extracts (5.5%), ammonium sulfates (5%). For carbon sources these results allowed to sufficiently reduce the surface tensions half as much as those obtained before optimization with high percentages of reductions of more than 70%. For nitrogen sources of all three isolates, yeast extract seems basically the best culture medium with high percentages of reduction of more than 80%. Culture medium sources of saline origin are better producers of biosurfactant [27]. These sources also give them a power of resistance to degradation [28-29].

Painting 2: Summary of the Optimization of Nutritional Parameters of Biosurfactant Production by Rpg14, Rpg18 And Rpg20 Isolates

Condition of culture	Culture conditions (Before optimiza-tion)	Culture condi-tions (After op-timization) RPG14	Culture condi-tions (After op-timization) RPG18	Culture condi-tions (After op-timization) RPG20
Carbon source	Na citrate = 3g/l	Diesel = 5% Na citrate = 5.5% Glycerol = 5.5%	Diesel = 5% Na citrate = 5.5% Glycerol = 5.5%	Diesel = 5% Citrate Na=5.5% Glycerol = 5.5%
TS mN/m	TSRPG14 = 23.7 TSRPG18 = 22.45 TSRPG20=22.75	TS=6.24± 0.6 TS=9.35±0.6 TS=9.35±0.6	TS=5.72±0.6 TS=6.89±0.6 TS=6.89± 0.5	TS=5.90± 0.6 TS=10.06±0.6 TS=10.06±0.5
%RTS	%RTSRPG14 = 57.65 %RTSRPG18 = 59.89 %RTSRPG20=59.35	%RTS =70.29± 0.6 %RTS=71.67±0.6 %RTS=71.67±0.6	%RTS =69.92±0.6 %RTS=70.03±0.6 %RTS=70.03±0.6	%RTS =70± 0.6 %RTS=64.09±0.6 %RTS=64.09±0.6
Nitrogen source	E. Yeast = 8.5%	E. Yeast = 5.5% Urea = 5.5% (NH4)2SO45%	E. Yeast = 4.5% Urea = 4.5% (NH4)2SO45.5%	E. Yeast = 4.5% Urea = 4.5% (NH4)2SO45.5%
TS mN/m	TSRPG14 = 23.7 TSRPG18 = 22.45 TSRPG20=22.75	TS=6.50±0.6 TS=7.35±0.6 TS=5.93±0.5	TS=2.22±0.5 TS=4.25±0.5 TS=6.89± 0.5	TS=2.45±0.5 TS=2.95±0.5 TS=10.06±0.5
%RTS	%RTSRPG14 = 57.65 %RTSRPG18 = 59.89 %RTSRPG20=59.35	%RTS=80.30±0.5 %RTS=76.29±0.5 %RTS=74.69±0.5	%RTS=91.29±0.5 %RTS=85.34±0.5 %RTS=80.86±0.6	%RTS=88.39±0.5 %RTS=86.65±0.5 %RTS=68.57±0.5

Extraction of Biosurfactants from Rpg14, Rpg20 And Rpg20 Isolates

Once the optimization of the biosurfactant production conditions was completed, including physicochemical factors (temperature, pH, agitation, NaCl and MgSO4,7H2O) and nutritional factors (carbon source and nitrogen source); an extraction of a volume of 3 liters of culture medium was started under optimized conditions for each isolate, the objective of which is to recover the largest possible amount of biosurfactants. Several incubation periods were tested. Only after 20 days of incubation, an extraction of biosurfactants was carried out according to the extraction protocol mentioned above. The extract was recovered and then concentrated with complete evaporation. The amount of biosurfactants that was produced by the isolates RPG14, RPG18 and RPG20 was weighed using the analytical balance. The optimal yield is estimated for RPG14 to be 68.62 g/l, for RPG18 to be 60.42 g/l, and for RPG20 to be 69.85 g/l. Beyond 20 days, biosurfactant production becomes increasingly low. Similar work has shown that at periods as short as 20 days, production is excellent [30]. It can also be very poor with the activity of microorganisms [31].

Table 4: Results of Optimal Extraction of Biosurfactants from Rpg14, Rpg18 And Rpg20 Isolates at Different Incubation Periods

Incubation (days) /(4°C)	Yield (g/l)		
	RPG14	RPG18	RPG20
15	46.32	45.80	46.20
20	68.62	60.42	69.85
25	57.65	57.12	57.25
30	48.23	47.68	48.75

Eppg Purification Rate under the Influence of Biosurfactant Variation and Temperature.

Tables 4, 5 and 6 reproduce the results of the tests carried out on Badila EPPG, i.e. the oil in solution at a density of 38°API. With the different concentrations of biodemulsifiers, the temperature underwent a variation. The results show a preponderant influence of the isolates with the variation of the temperature on the settling time of oil and gas production water. We varied the concentration of the biodemulsifiers under different temperatures to determine the settling rate in 20 min, 40 min and 60 min.

Purificationunder the action of Biosurfactants Produced by the Rpg14 Isolate

Table 4 shows the results of the reproduction of the tests carried out on the oil production waters of the Petrochad (Mangara) Limited field in Badila. We varied the concentration of the supernatant of the RPG14 isolates according to the temperature, the volume of the oil was measured after 20, 40 and 60 min. As a result, the rate of the decanted water is calculated by the method described above. In this table 4, it was found that at the same temperature of 55°C, with variation of the biosurfactant concentration of the RPG14 isolate from 50 to 25 mg/l, the decantation rate varied in accordance with the retention time. However, the amount of biodemulsifiers did not have an influence on the separation. The same test was repeated with a temperature of 70°C with the inverse of the variation of the RPG14 supernatant content, the same result was observed. But at a temperature of 75°C with a concentration of 30mg/l, the settling rate evolved in accordance with the retention time over all three periods. Previous work has shown that the longer the residence time of the biosurfactants in the solution, the better the separation [33-34]. Oils in solution leave the liquid center as the surface tension decreases [35].

Table 5: Results of Purification Tests Carried out on Oil and Gas Production Waters using Isolate Rpg18

Temperature (°C)	Supernatant of isolate RPG18 (mg/l)	Water purification rate		
		20 min	40 min	60 min
55	50	95%	98%	94%
55	25	96%	98%	99%
70	50	99%	99%	99%
70	25	99%	98%	99%
75	30	95%	99%	100%

Purificationunder the action of Biosurfactants Produced by the Rpg20 Isolate

Regarding Table 6, we note that at the same temperature of 55°C, with variation of the RPG20 isolate content from 50 to 25 mg/l, the settling rate varied in accordance with the retention time, but the amount of demulsifiers always remains without influence on the separation. The same test was repeated with a temperature of 70°C with the inverse of the variation of the RPG20 isolate content, the same result was observed. However, at a temperature of 75°C with a concentration of 30 mg/l, the settling rate underwent an increase in all three periods with a jump after 60 min.

Generally speaking, for all three isolates, depending on the temperature variation, the settling rate is a function of residence time. The longer the time, the more complete the settling [38-39]. However, the amount of supernatant has no influence on the settling rate [40]. By keeping the temperature constant at 55°C and varying the volume of biodemulsifiers from 50 to 25 ml after 40 min of decantation the purification percentage decreased from 85% to 80%, this confirms the influence of temperature on the reduction of surface tension and consequently on purification [41].

Table 6: Results of Purification Tests carried out on Oil And Gas Production Waters using Rpg20 Isolate

Temperature (°C)	RPG20 isolate supernatant (mg/l)	Water purification rate		
		20 min	40 min	60 min
55	50	38%	85%	99%
55	25	35%	80%	99%
70	50	94%	95%	97%
70	25	91%	92%	100%
75	30	94%	95%	100%

Physicochemical Qualities of Oil and Gas Production Waters after the Action of Biosurfactants Produced by Isolates.

Before purification using biosurfactants produced by isolates RPG14, RPG18 and RPG20, these waters collected in tubes underwent a physicochemical analysis before and after purification. After these analyses, a purification efficiency (E) was estimated for each parameter.

Physicochemical Qualities of Eppgs After Action of Biosurfactants Produced by Isolate Rpg14

According to Table 7 and the illustration in Figure 2, almost all the concentration of oils and metals was drastically reduced after mixing the produced water and the RPG14 isolate. These reductions went from simple to quarter in all four tubes. In tube 1 the action of the RPG14 isolate allowed us to observe that the lowest reduction in concentration was noted for total hydrocarbons which were eliminated at the height of 82%. In tube 2, the low elimination was noted at a height of 81% in total hydrocarbons while manganese was eliminated at 92.7% in the same tube. In tube 3, phenol was eliminated at 77.7%, while manganese was eliminated at 91.7%. In tube 4, barium is removed at 85.8% while total hydrocarbons are removed at a rate of 89.7% and finally in tube 5 we observe total hydrocarbons which are removed at 83.4% while manganese has been removed at 94.5%. Figure 23 illustrates the efficiency of the reduction of the constituents in the EPPG by the RPG14 isolate in the tubes. Overall, total hydrocarbons were not reduced to the same level as the other parameters. This is related to the residence time of the isolates in the EPPG [42]. While other parameters are strongly eliminated like the case of barium. This elimination will considerably reduce the phenomenon of tartar [43].

Table 7: Results of The Physicochemical Quality of Eppg before and after Purification By Rpg14 Isolates

Tubes	Reduction rate of oil and metal concentrations in EPPG after purification by RPG14 isolate (mg/l)											
	Total hydrocarbons			Phenols			Barium			Manganese		
	Here before	Co after	E(%)	Here before	Co after	E(%)	Here before	Co after	E(%)	Herebefore	Co after	E(%)
1	28	5.23	82	2.1	0.09	95.8	2.1	0.09	95.8	3.32	0.13	96
2	32	6.67	81	2.3	0.2	91.4	2.3	0.2	91.4	3.54	0.26	92.7
3	32	4.67	87.5	1.8	0.4	77.7	1.8	0.4	77.8	2.57	0.22	91.5
4	29	3.45	89.7	2.0	0.07	96.8	2.0	0.07	85.8	3.01	0.36	88.1
5	30	5.34	83.4	2.1	0.3	85.8	2.1	0.3	85.8	3.26	0.18	94.5

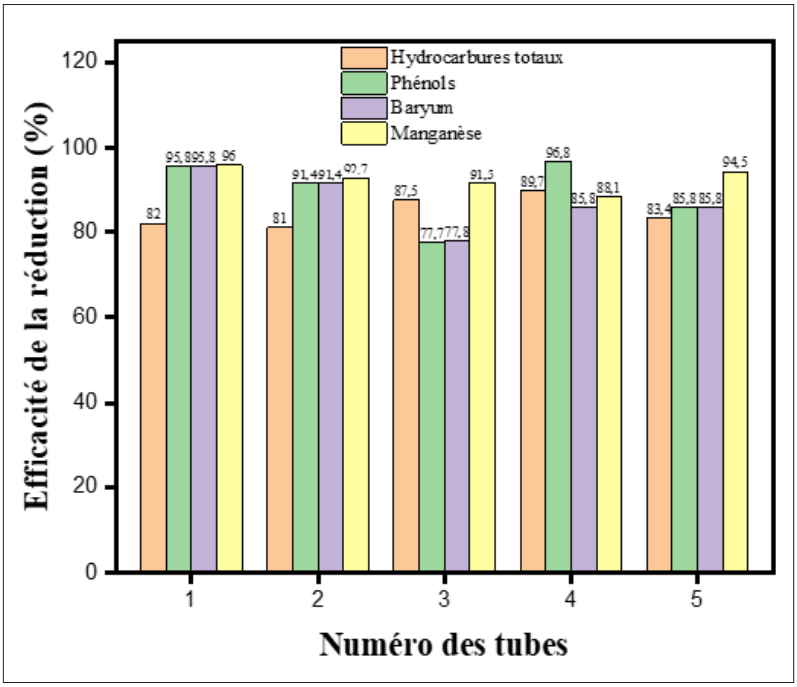

Figure 2: Efficacy of Reduction of Constituents in Eppg by Rpg14 Isolate in Tubes

3-5-2- Physicochemical qualities of EPPGs after action of biosurfactants produced by the RPG18 isolate

Table 8 shows the different concentrations measured before and after the action of the RPG18 isolate. In tube 1, total hydrocarbons were slightly eliminated at 75.90% while phenol was eliminated at 96.82%. In tube 2, phenol was eliminated at 78.26% while barium was at a 93.04% reduction rate. In tube 3, phenol remained reduced at 78.95% while manganese was eliminated at 90.98%. In tube 4, total hydrocarbons were eliminated at 88.45%. In tube 5, total hydrocarbons were eliminated at 77% while manganese was at a 93.87% reduction rate. Figure 24illustrates the efficiency of the reduction of constituents in EPPG by the RPG18 isolate in the tubes. At all levels, it can be seen that phenol and heavy metals are significantly removed compared to total hydrocarbon concentrations.

Table 8: Results of the Physicochemical Analysis of Eppg before and after Purification by Rpg18 Isolates

Tubes	Reduction rate of oil concentrations in EPPG after purification by RPG18 isolates (mg/l)											
	Total hydrocarbons			Phenol			Barium			Manganese		
	Herebefore	Co after	E(%)	Herebefore	Co after	E (%)	Herebefore	Co after	E (%)	Herebefore	Co after	E (%)
1	30	7.23	75.90	2.2	0.07	96.82	2.2	0.08	96.36	3.26	0.16	95.09
2	31	6.11	80.29	2.3	0.5	78.26	2.3	0.16	93.04	3.54	0.26	92.66
3	32	5.45	82.97	1.9	0.4	78.95	1.9	0.31	83.68	2.55	0.23	90.98
4	29	3.35	88.45	2.0	0.05	97.50	2.0	0.05	97.50	3.12	0.26	91.67
5	31	7.13	77.00	2.2	0.4	81.82	2.2	0.42	80.91	3.26	0.20	93.87

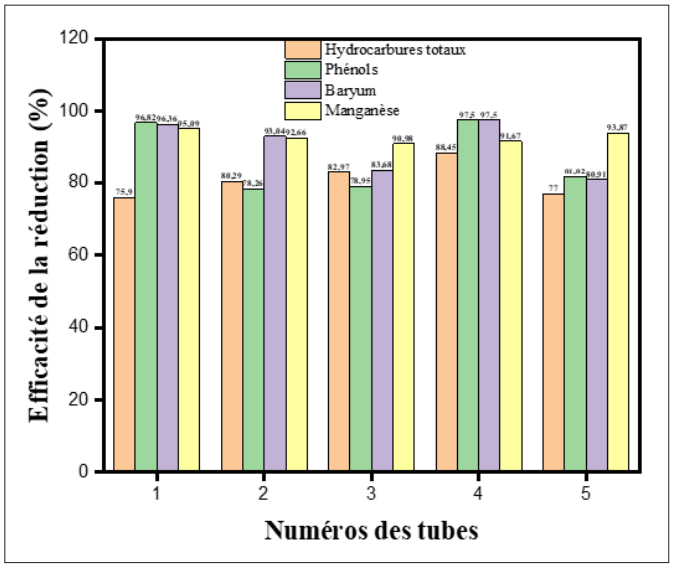

Figure 3: Efficacy of Reduction of Constituents in Eppg by Rpg18 Isolate in Tubes

Physicochemical Qualities of Eppgs after action of Biosurfactants Produced by the Rpg20 Isolate

Table 9 shows the different concentrations measured before and after the action of the RPG20 isolate. In tube 1, total hydrocarbons were slightly eliminated at 80.47% while phenol was eliminated at 98.70%. In tube 2, total hydrocarbons were eliminated at 73.66% while phenol was at a 98.70% reduction rate. In tube 3, total hydrocarbons remained reduced at 84.18% while phenol was eliminated at 98.00%. In tube 4, total hydrocarbons were eliminated at 88.04% while phenol was eliminated at 97.62%. In tube 5, total hydrocarbons were eliminated at 77.06% while manganese was at a 93.56% reduction rate. Figure 25illustrates theefficiency of the reduction of constituents in EPPG by the RPG20 isolate in the tubes. The same observation was observed as in the previous case, where phenol was strongly eliminated followed by heavy metals.

Table 9: Results of the physicochemical analysis of EPPG before and after decantation by RPG20 isolates

Tubes	Reduction rate of oil concentrations in EPPG after decantation by RPG20 isolates (mg/l)											
	Total hydrocarbons			Phenols			Barium			Manganese		
	Here before	Co after	E (%)	Here before	Co after	E(%)	Here before	Co after	E(%)	Here before	Co after	E(%)
1	32	6.25	80.47	2.3	0.03	98.70	13	0.83	93.62	4.26	0.22	94.84
2	32	8.43	73.66	2.3	0.04	98.26	10	0.44	95.60	3.34	0.23	93.11
3	33	5.22	84.18	2.0	0.04	98.00	11	1.45	86.82	2.83	0.23	91.87
4	28	3.35	88.04	2.1	0.05	97.62	14	1.65	88.21	3.42	0.26	92.40
5	31	7.11	77.06	2.2	0.4	81.82	12	1.46	87.83	3.26	0.21	93.56

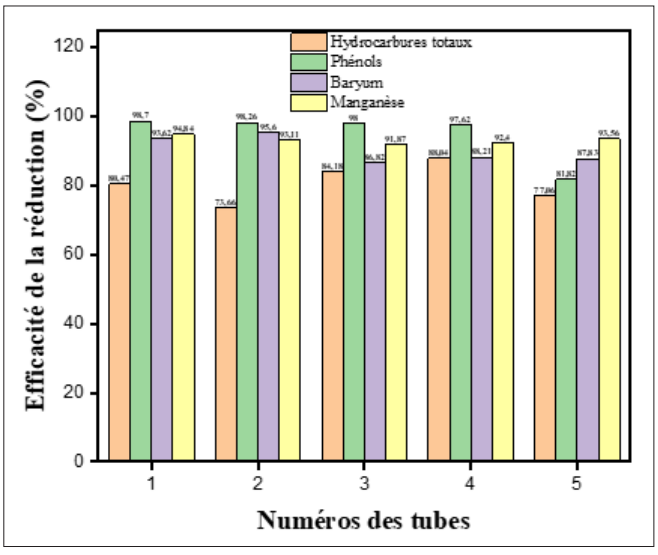

Figure 4: Efficacy of Reduction of Constituents in Eppg By Rpg20 Isolate in Tubes

Quality of Oil and Gas Production Waters after the action of Isolates

After purification with RPG14, RPG18 and RPG20 isolates, the EPPGs in the lower part of the tubes are collected, analyzed and compared to international guidelines for the discharge of oil and gas production water. Compliance with these guidelines results in environmentally friendly discharge.

Qualities of Eppgs after Action of Rpg14 Isolate

The results of the analyses and the standards used are recorded in Table 10 below. The measured total hydrocarbon contents of the water extracted from the tubes are all below the discharge standards (limit of quantification). The phenol contents quantified in tubes 1 and 2 are below the IFC 2007, WHO and FAO standards. While that quantified in tube 3 is slightly above the WHO and FAO guidelines. The barium contents fully comply with the IFC 2007, WHO and FAO standards and are all below 0.7 mg/l. The manganese contents do not comply with the WHO guidelines. In Figure 26, when compared to the WHO standards for drinking water - Risks to human and animal health, oil and gas production waters after the action of the RPG14 isolate (even if they meet the IFC 2007 and FAO standards for discharges) are not intended for human or animal consumption. They present a high health risk. However, they can be irrigated properly in agricultural plots and are not dangerous for plants[44-47].

Table 10: Results of the Comparison with The Standards of Rejections after Decantation of Eppg by Rpg14 Isolates.

	Comparison with standards for discharges of concentrations of oils and heavy metals remaining in EPPGs(mg/l)					
Parameters (mg/l)	**Tube 1**	**Tube 2**	**Tube 3**	**IFC 2007**	**WHO**	**FAO**
Total Hydrocarbons	5.23	6.67	4.67	10	15	15
Total phenols	0.09	0.2	0.4	0.5	0.3	0.3
Barium	0.09	0.2	0.4	≤0,7	0.7	0.7
Manganese	0.13	0.26	0.22	0.5	0.1	<2

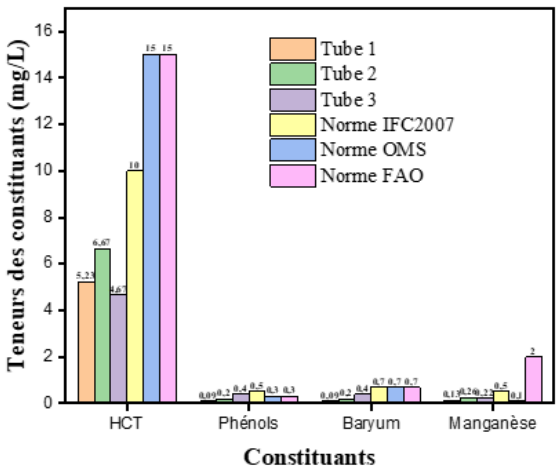

Figure5: Qualities of Eppgs After Action of Rpg14 Isolate and Comparison with Rejection Standards.

Qualities of Eppgs After Action of Rpg18 Isolate

The results of the analyses and the standards used are recorded in Table 20 and illustrated by the figure27 which presents the qualities of EPPGs after action of the RPG18 isolate and comparison with rejection standards. The measured levels of total hydrocarbons, phenol and barium in the water extracted from the tubes are all below the discharge standards (limit of quantification). Except for the manganese levels, which are slightly higher than the WHO guidelines; however, they comply with the IFC2007 and FAO standards.

Table 11: Results of The Comparison With The Standards of Rejections after Decantation Of Eppg By Rpg18 Isolates.

	Comparison with standards for discharges of concentrations of oils and heavy metals remaining in EPPGs(mg/l)					
Parameters (mg/l)	**Tube 1**	**Tube 2**	**Tube 3**	**IFC 2007**	**WHO**	**FAO**
Total Hydrocarbons	7.23	6.11	5.45	10	15	15
Total phenols	0.07	0.5	0.4	0.5	0.3	0.3
Barium	0.08	0.16	0.31	≤0,7	0.7	0.7
Manganese	0.16	0.26	0.23	0.5	0.1	<2

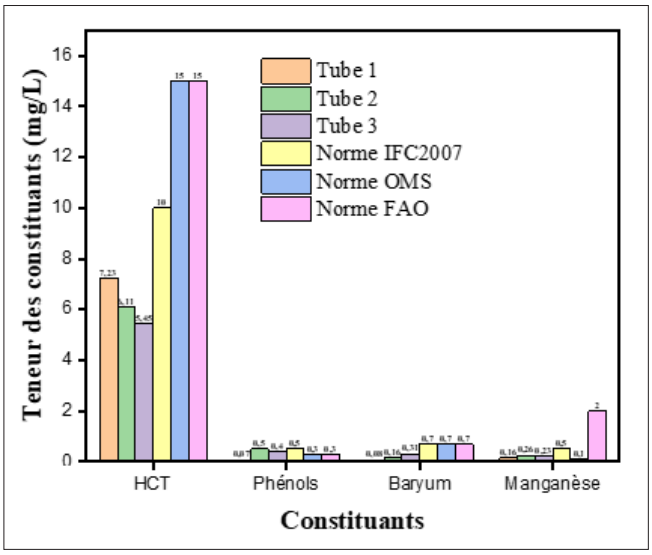

Figure 6: Qualities of Eppgs after Action of Rpg18 Isolate and Comparison With Rejection Standards

Qualities of Eppg After Action of Rpg20 Isolate

The results established in Table 21 and illustrated by Figure 28 are edifying. The same observation was noted for isolate RPG20 where manganese was not completely consumed to the WHO standard. While all other contents respect the rejection standards.

Table 12: Results of the Comparison with the Standards of Rejections after Decantation of Eppg by Rpg20 Isolates.

	Comparison with standards for discharges of concentrations of oils and heavy metals remaining in EPPGs(mg/l)					
Parameters (mg/l)	**Tube 1**	**Tube 2**	**Tube 3**	**IFC 2007**	**WHO**	**FAO**
Total Hydrocarbons	6.25	8.43	5.22	10	15	15
Total phenols	0.03	0.04	0.4	0.5	0.3	0.3
Barium	0.83	0.44	1.45	≤0,7	0.7	0.7
Manganese	0.22	0.23	0.23	0.5	0.1	<2

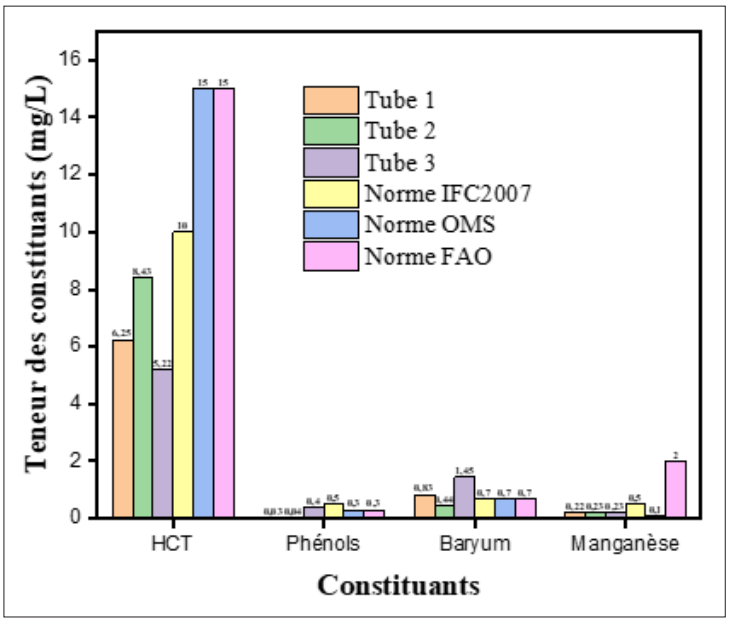

Figure7: Qualities of Eppgs after Action of Rpg20 Isolate and Comparison with Rejection Standards

.....?

Conclusion:

The biosurfactants extracted during the optimization of the physicochemical and nutritional parameters of the isolates RPG14, RPG18 and RPG20 were used to purify the oil and gas production waters in the Petrochad (Mangara) Limited field. An optimal amount of biosurfactants from these three isolates was extracted after 20 days of incubation. The optimal yield is estimated for RPG14 is 18.62 g/l, for RPG18 is 20.42 g/l and for RPG20 is 19.85 g/l. The volumes of each type of biosurfactants were sampled and injected into the oil production waters under the effect of temperature variation. Reductions in the concentrations of total hydrocarbons, phenols and heavy metals were observed and compared to the discharge standards.

References

1. Morin Crini N, Winterton P, Trunfio G, Torri G, Louvard N, et al. (2017) Chapter IVChemical parameters of water and industrial discharges. In Contaminated industrial water: Regulations, chemical and biological parameters & innovative purification processes 103-144.
2. Almoustapha O, Millogo Rasolodimby J (2006) Biogas and compost production from eichhornia crassipes, (mart) solms-laub (pontederiaceae) for sustainable development in Sahelian Africa. VertigO - the electronic journal in environmental sciences 7: 2.
3. Pilenko T (2011) Deep offshore hydrocarbon production. Annales des Mines - Responsibility and environment 64: 17-23.
4. Ochando Pulido JM, Vuppala S, García-López AI, Martínez-Férez A (2024) A focus on anaerobic digestion and co-digestion strategies for energy recovery and digest valorization from olive-oil mill solid and liquid by-products. Separation and Purification Technology 333: 125827.
5. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E et al (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science (New York, NY) 313: 1137-1140.
6. Ashish, Debnath (Das) M (2018) Application of biosurfactant produced by an adaptive strain of C.tropicalis MTCC230 in microbial enhanced oil recovery (MEOR) and removal of motor oil from contaminated sand and water. Journal of Petroleum Science and Engineering 170: 40-48.
7. Gaur S, Kaur M, Kalra R, Rene ER, Goel M (2024) Application of microbial resources in biorefineries: Current trends and future prospects. Heliyon 28615.
8. Loganathan R, Selvaduray KR, Nesaretnam K, Radhakrishnan AK (2010) Health promoting effects of phytonutrients found in palm oil. Malaysian Journal of Nutrition 16: 309-322.
9. Morikawa M, Hirata Y, Imanaka T (2000) A study on the structure-function relationship of lipopeptide biosurfactants. Biochimica Et Biophysica Acta 1488: 211-218.
10. De Souza Monteiro A, Domingues VS, Souza MV, Lula I, Gonçalves DB (2012) Bioconversion of biodiesel refinery waste in the bioemulsifier by Trichosporon mycotoxinivorans CLA2. Biotechnology for Biofuels 5: 29.
11. Rathour RK Ahuja V, Bhatia RK, Bhatt AK (2018) Biobutanol: New era of biofuels. International Journal of Energy Research 42: 4532-4545.
12. Aboelkhair H, Diaz P, Attia A (2022) Biosurfactant production using Egyptian oil fields indigenous bacteria for microbial enhanced oil recovery. Journal of Petroleum Science and Engineering 208: 109601.
13. Gieg LM, Jack TR, Foght JM (2011) Biological souring and mitigation in oil reservoirs. Applied Microbiology and Biotechnology 92: 263-282.
14. Speight JG, El-Gendy NS (2018) Chapter 4 Microbial Enhanced Oil Recovery. In JG Speight & NS El-Gendy (Eds.), Introduction to Petroleum Biotechnology 103-130.
15. Youssef N, Elshahed MS, McInerney MJ (2009) Chapter 6 Microbial Processes in Oil Fields: Culprits, Problems, and Opportunities. In Advances in Applied Microbiology 66: 141-251.
16. Alkan H, Mukherjee S, Kögler F (2023) Chapter 8 Microbial enhanced oil recovery. In Q. Wang (Ed.) Recovery Improvement 3: 427-531.
17. Zhou S, Peng S, Li Z, Zhang D, Zhu Y, et al. (2022) Characterization of microbial communities and functions in shale gas wastewaters and sludge: Implications for pretreatment. Journal of Hazardous Materials, 424, 127649.
18. Borden RC (2007) Competitor bioremediation of perchlorate and 1,1,1-trichloroethane in an emulsified oil barrier. Journal of Contaminant Hydrology 94: 13-33.
19. Gong J, Sun X, Xu L, Lu H (2017) Contribution of thermogenic organic matter to the formation of biogenic gas hydrate: Evidence from geochemical and microbial characteristics of hydrate-containing sediments in the Taixinan Basin, South China Sea. Marine and Petroleum Geology 80: 432-449.
20. Okoro C, Smith S, Chiejina L, Lumactud R, An D (2014) Comparison of microbial communities involved in souring and corrosion in offshore and onshore oil production facilities in Nigeria. Journal of Industrial Microbiology & Biotechnology 41: 665-678.
21. Orphan VJ, Taylor LT, Hafenbradl D, Delong EF (2000) Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. Applied and Environmental Microbiology 66: 700-711.
22. Shi H, Gao W, Zheng Y, Yang L, Han B, et al. (2023) Distribution and abundance of oil-degrading bacteria in seawater of the Yellow Sea and Bohai Sea, China. Science of The Total Environment 902: 166038.
23. Durand M, Touchette D, Chen YJ, Magnuson E, Wasserscheid J (2023) Effects of marine diesel on microbial diversity and activity in high Arctic beach sediments. Marine Pollution Bulletin 194: 115226.
24. Sawale SD, Kulkarni AA (2022) Current technical advancement in biogas production and Indian status. In Advanced Biofuel Technologies 501-532.
25. Cao X, Gao X, Zheng K, Wu S, Wu Y (2022) Efficient pollutants removal and microbial flexibility under high-salt gradient of an oilfield wastewater treatment system. The Science of the Total Environment 823: 153619.
26. Majumder D, Maity JP, Tseng MJ, Nimje VR, Chen HR, et al. (2014) Electricity generation and wastewater treatment of oil refinery in microbial fuel cells using Pseudomonas putida. International Journal of Molecular Sciences 15: 16772-16786.
27. Deng S, Wang B, Zhang W, Su S, Dong H (2021) Elucidate microbial characteristics in a full-scale treatment plant for offshore oil produced wastewater. PloS One 16: e0255836.
28. Patel AK, Chauhan AS, Kumar P, Michaud P, Gupta VK (2022) Emerging prospects of microbial production of omega fatty acids: Recent updates. Bioresource Technology 360: 127534.
29. Wang L, Liu J, Li Y, Liu Z, Zhang L (2023) Elemental sulfur-driven autotrophic denitrification process for effective removal of nitrate in mariculture wastewater: Performance, kinetics and microbial community. Chemosphere 337: 139354.
30. BR (2023) Energy-related wastewater contamination alters microbial communities of sediment, water, and amphibian skin. Science of The Total Environment, 880: 163160.

31. Oldenburg TBP, Larter SR, Adams JJ, Clements M, Hubert C, et al. (2009) Methods for recovery of microorganisms and intact microbial polar lipids from oil-water mixtures: Laboratory experiments and natural well-head fluids. Analytical Chemistry 81: 4130-4136.
32. Emmel B, Bjørkvik B, Frøyen TL, Cerasi P, Stroisz A (2023) Evaluating the hydrogen storage potential of shut down oil and gas fields along the Norwegian continental shelf. International Journal of Hydrogen Energy 48: 24385-24400.
33. Nazina TN, Shestakova NM, Pavlova NK, Tatarkin YV, Ivoilov VS (2013) Functional and phylogenetic microbial diversity in formation waters of a low-temperature carbonate petroleum reservoir. International Biodeterioration & Biodegradation 81: 71-81.
34. Ortmann AC, Cobanli SE, Wohlgeschaffen G, Poon HY, Ryther C, et al. (2023) Factors that affect water column hydrocarbon concentrations have minor impacts on microbial responses following simulated diesel fuel spills. Marine Pollution Bulletin 194: 115358.
35. Procida G, Ceccon L (2006) Gas chromatographic determination of free fatty acids in olive mill waste waters. Analytica Chimica Acta 561: 103-106.
36. Fida TT, Chen C, Okpala G, Voordouw G (2016) Implications of Limited Thermophilicity of Nitrite Reduction for Control of Sulfide Production in Oil Reservoirs. Applied and Environmental Microbiology 82: 4190-4199.
37. Mouser PJ, Borton M, Darrah TH, Hartsock A, Wrighton KC (2016) Hydraulic fracturing offers view of microbial life in the deep terrestrial subsurface. FEMS Microbiology Ecology 92.
38. Mateo JJ, Maicas S (2015) Valorization of winery and oil mill wastes by microbial technologies. Food Research International 73: 13-25.
39. Taher F, Mahfouz A (2022) Valuation of wastewater treatment and biodiesel production from microalgae by direct transesterification method. Egyptian Journal of Chemistry.
40. Leng L, Yang L, Chen J, Hu Y, Li H, et al. (2021) Valorization of the aqueous phase produced from wet and dry thermochemical processing biomass: A review. Journal of Cleaner Production 294: 126238.
41. Fiala K, Thongjarad A, Leesing R (2024) Valorization of durian peel as a carbon feedstock for a sustainable production of heterogeneous base catalyst, single cell oil and yeast-based biodiesel. Carbon Resources Conversion 7: 100224.
42. Tsakona S, Skiadaresis AG, Kopsahelis N, Chatzifragkou A, Papanikolaou S, et al. (2016) Valorization of side streams from wheat milling and confectionery industries for consolidated production and extraction of microbial lipids. Food Chemistry 198: 85-92.
43. Campa MF, Techtmann SM, Ladd MP, Yan J, Patterson M (2019) Surface Water Microbial Community Response to the Biocide 2,2-Dibromo-3-Nitrilopropionamide, Used in Unconventional Oil and Gas Extraction. Applied and Environmental Microbiology 85: e01336-19.
44. Koffi YB, Ahoussi KE, Kouassi AM, Biemi J (2014) Mining, oil and gas resources of Côte d'Ivoire and the problem of water resource pollution and flooding. Geo-Eco-Trop 38: 119-136.
45. Liu JF, Lu YW, Zhou L, Li W, Hou ZW, et al. (2020) Simultaneous detection of transcribed functional assA gene and the corresponding metabolites of linear alkanes (C4, C5, and C7) in production water of a low-temperature oil reservoir. Science of The Total Environment 746: 141290.
46. Zhao F, Zhou JD, Ma F, Shi RJ, Han SQ, et al. (2016) Simultaneous inhibition of sulfate-reducing bacteria, removal of H2S and production of rhamnolipid by recombinant Pseudomonas stutzeri Rhl: Applications for microbial enhanced oil recovery. Bioresource Technology 207: 24-30.
47. Zhu X, Wang Q (2022) Chapter 10 Microbial control. In (Ed.), Flow Assurance 1: 709-773.