ISSN: 2754-4915

Journal of Material Sciences & Manufacturing Research

SCIENTIFIC Research and Community

Research Article

Open Access

Evaluation of Microbial Performance for Oil Recovery in Oil and Gas Production Waters in Badila-Chad

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ABSTRACT

37 samples from EPPG and swamps of produced waters were collected, analyzed, cultured and isolated to produce biosurfactants capable of reducing surface and interfacial tensions measured in oil production waters in the Petrochad (Mangara) Limited field in Badila. Only 10 isolates selected and codified, showed good biosurfactant production capacities. All these 10 isolates were subjected to screening tests (drop collapse, oil dispersion, emulsion stability and surface tension measurement). Three best isolates showed very good biosurfactant production capacities and consequently better reduction of surface tension (RPG14 = 23.7mN / m, RPG18 = 22.45mN / m, RPG20 = 22.75mN / m) and interfacial tension (RPG14 = 14.35 ±0.5 mN/m, RPG18= 15.35 ±0.5 mN/m, RPG20= 15.45 ±0.5 mN/m) compared to those measured in the field between 2014 and 2023.

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Received: February 20, 2025; **Accepted:** February 24, 2025; **Published:** April 29, 2025

Keywords: Produced Water, Isolation, Screening, Surface Tension, Interfacial Tension, Badila, Chad

Introduction

Produced water is water produced simultaneously with oil and gas from a deposit initially as crude oil. [1]. The latter is then separated on the surface into water-oil-gas by a separation unit designed for this purpose [2]. The separated product takes specific directions or the production water is led into a settling tank and then to the storage tank by appropriate pipes [3]. However, these waters are not directly usable because of their high oil, ion and metal contents. The presence of the latter is linked to the high values of surface and interfacial tensions at the molecular level [4]. We proposed to explore the production of biosurfactants by mesophiles, isolated from oil production waters and swamps from the Petrochad field in Badila. The choice of these production waters and swamps is justified by its saline composition and by the geological conditions of the reservoirs of the fields of Petrochad (Mangara) Limited. The purpose of this work is to isolate mesophilic bacteria from oil and gas production waters from the different oil fields of the Doba basin, capable of producing biosurfactants capable of stabilizing the oil/water emulsion, drastically reducing the surface and interfacial tension between the phases of the fluids compared to the tensions measured before and at the outlet of the central treatment plant.

Materials and Methods

Location of the Study Site

This study was carried out on the Badila field in the Donia canton, Donia commune, located in the South about 500 km from the city of N'Djamena (Chad). Donia is in the Nya-Pendé department, Logone Oriental province which covers an area of 23,802 km 2 subdivided into 06 departments and 23 communes. Logone Oriental borders the CAR and the Republic of Cameroon. The particularity of the Badila field is characterized by a reduced number of producing wells with a high flow rate. Figure 1 shows the mapping of the study area. The climate of the study area is of the Sudano-Sahelian type, dominated by wooded savannah vegetation where temperatures range between 10 and 45 ° C. The rainy season extends from April to October. Speaking about the pedological and geological profile of the study area, it is important to mention that Badila is a complex structure with elements of faults and compression folding oriented NE. The NW-SE fault that defines the oil trap is a reactivated basement fault with a similar orientation to other Lower Cretaceous basement faults. The basement structure is typical of the Doba Basin, a series of half-grabens separated by horsts. The Badila basement structure conforms to a NE-SW extension. The soils that developed in this area of Mangara and Badila can be grouped into three categories, namely hydromorphic soils or vertisols along the valleys and depressions, ferruginous and shallow soils on sulphurous curasse and young soils on recent alluvium found along the axis of the watercourses.

Citation: Brahim Bakimbil, Niraka blaise, Samba Koukouare Prosper, Fadimatou Houmarou, Djoulde Darman Roger (2024) Evaluation of Microbial Performance for Oil Recovery in Oil and Gas Production Waters in Badila-Chad. Journal of Material Sciences & Manufacturing Research. SRC/JMSMR-233.
DOI: doi.org/10.47363/JMSMR/2025(6)195

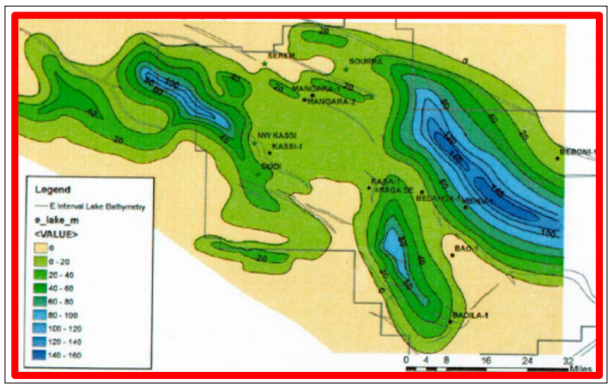

Figure 1: Location of Study Area Horizons E Center of the Basin (Glencore)

Data Collection and Sampling

Data Collection: We recorded the surface and interfacial tensions of the emulsions at the inlet and outlet of the central treatment plant as a function of pressure and temperature between 2014 and 2023. Data collection followed the method of Duchesne.

Sampling: Thirty-seven (37) samples from the EPPG and quagmires were taken in May and June 2022. However, the samples are made by spécial syringes at the factory out let and by plastic cups in the retention basins. This, taking the précautions of use of désinfection of the tools (flaming with alcohol)ethylic to 9 0 °) to avoid all risk contamination of samples who have was collected in the bottles sterile plastic.

Sampling and Physicochemical Analyses

The samples taken first underwent a physicochemical analysis, so the pH and electrical conductivity were measured by the HANNA type multi parameter, the sodium, potassium, magnesium, sulfate, chloride, ammonium ions are analyzed by the DR 2400 spectrophotometer

Principle: The DR 2400 is a simple and comfortable photometer. Its spectral range is between 400 and 880 nm with a temperature range of use from 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, we can deduce the concentration C (mg/l) of the absorbing substance by the Beer-Lamber relationship according to the expression: D = log I_O/I= 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 brought into contact with special reagents. The intensity of the colour produced is measured. This is a measure of the concentration of inorganic ions to be analysed. For each test, a blank analysis is carried out with distilled water and the reagents.

Culture and Isolation: The analyzed samples are then cultured. The approach followed for the isolation of Haloana erobium is that it consists of enriching the samples beforehand, where 10 ml of oil production water is introduced into 90 ml of liquid Sehgal-Gibbons (SG) medium, contained in a 250 ml Erlenmeyer flask [5] . The mixture is then shaken for 40 minutes to obtain good homogenization of the particles and then incubated at 37 ° C for 20 days. Then, a dilution series is carried out, in which 100 µl of the dilutions 10 -1 to 10 -3 are inoculated on the surface of the solid SG culture medium. The Petri dishes are incubated at 37°C for 20 to 25 days in plastic bags, in order to avoid rapid drying of the culture medium and crystallization of its salts.

Purification of Isolates: All isolates are first purified by successive subcultures of well-separated and visibly distinct colonies on the solid culture medium SG. Once purified, a code number was assigned to each isolate, which consists of three letters RPG, followed by a sequence number.

Conservation of Isolates: The conservation of isolates thus designated is done by the short-term isolate conservation method, it most often consists of carrying out subcultures on agar slants with conservation at 4°C, and the culture will be sub cultured every 03 to 06 months.

Screening of Biosurfactant-Producing Isolates

For screening, ten isolates (RPG11, RPG12, RPG13, RPG14, RPG15, RPG16, RPG17, RPG18, RPG19, RPG 20) were selected from each sampling site. The biosurfactant-producing isolates were selected using the following four methods: drop collapse test, oil dispersion test, emulsion stability (ES) test, surface and interfacial tension measurement. The experiments were performed in three replicates.

Droplet 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 100 µl of an oil 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. 10 µl 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 force or interfacial 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. Drops that gave a rounded shape were marked as "-" indicating the absence of biosurfactant production [6,7].

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 performed due to three replicates for each sample [8,9] .

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 allowed to stand. The emulsion volume (EV%) and emulsion stability (ES%) were measured as follows:

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

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

Emulsions formed by bacterial cultures were compared to those formed by a 1% solution of a synthetic surfactant (SDS) as a positive control and by sterile culture medium as a negative control [10] . 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 h of its formation [11].

Surface and Interfacial Tension Measurement: Surface tension measurements of supernatants free of bacterial cells 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 time intervals and centrifuged at (10,000 × g for 25 min) at room temperature. The criterion used for the selection of biosurfactant-producing isolates is the reduction of the surface tension of the medium over time below 40 mN.m-1 [12] .

Statistical Analysis of the Correlation Between the Different Tests: A general rank correlation test, according to Spearman, was conducted to determine the correlation between the four methods used for screening biosurfactant-producing strains. This Spearman correlation coefficient, rs varied between -1 (strongly negative correlation) to +1 (strongly positive correlation) [13] .

Results and Discussion

Sample Collection Results: The oil production water samples were collected from Doba basin fields of Petrochad (Mangara) limited field at Badila. The samples were collected from the outlet of free water separator (FKWO) and from the retention ponds (main, percolation and irrigation), produced water sloughs, drilling mud sloughs in 50ml sterilized tubes. The samples were immediately stored at 4°C to preserve the microbial consortium of the oil and gas produced water samples until use.

Table 1: Results of EPPG Sample Collection at Petrochad (Mangara) Limited Badila Oil and Gas Field

Collection locations	UCT exit	Retention basins	Production water quagmires	Drilling mud quagmires
Number of samples	04	16	10	07
Sample numbers	RP11, RP12, RP13, RP14	BR11, BR12, BR 13,… . BR26	BP11, BP12, BP13…….. BP20	BF11, BF12, BF13,…… BF17

Physicochemical Analyses: The purpose of these analyses is to confirm the salinity of the sampling environment and access the saline characteristics of oil and gas production waters. In Table 2, the pH of all samples is oriented towards an acid-base trend. This is the extreme environment where mesophiles obtain comfortable adhesion [14]. While the electrical conductivity values are high, confirming the salinity status of the samples taken [15]. The contents of all ions are high and confirm the identity and especially the origin of the production waters. The waters from the Upper and Lower Cretaceous of the Doba basin are moderately saline. These are waters from the non-cemented sandstone reservoir and are closely linked to the different salt deposits [5]. Some salts are even injected as substrate to microorganisms during secondary recovery and reproduced with the production water [16]. These different samples from the Petrochad (Mangara) Limited field no longer need to be abundantly enriched with salt before being isolated.

Table 2: Physicochemical Quality of Oil Production Waters

Collection locations			Mineral salts (mg/L)						
	Ph	Conductivity ds /m	Na+	K+	Ca+	cl-	So4+	Mg+	NH4+
From UCT Entry to Exit	9.3	3	402	677.5	712	342	409	325	235
Retention basins	9	3.2	345	587	580	304	367	318	302
Production water quagmires	8.9	2.9	328	770.4	498	367	367	243	180
Drilling mud quagmires	9.7	1.9	314.5	532	476	298	285	276	176

Isolation of Haloana Eerobium

Haloana Erobium has summer isolated on the medium Sehgal - Gibbons (SG), for one pH of 7.2 and incubated at 37 ° C for 15 days. This environment turned out to be very interesting For isolation And there selection of the Haloana and Erobium, has leave of four places of sampling of oil production water from the Badila field. Isolation has ended obtaining 37 isolates after a re-picking successive of their colonies on gel, the ten isolats have shown themselves to be very interesting and promising. These latter present below shape of the colonies of small size, convex, regular, transparent. Then colores at fur and to measure of the elaboration carrots and sometimes white. They are renamed by three letters and two numbers as follows : RPG11, RPG12, RPG13, RPG14, RPG15, RPG16, RPG17, RPG18, RPG19, RPG20. These colonies have a appearance sticky, which gives them existence and presence composed glycolipidic or glycoproteins. It is for this reason they were choosen for the scree ning of active strains, view that the researched biomolecules can be of interest of this nature biochemical. Table 3 presents the results of the selection of strains at each site after the isolation of Haloana erobium The latter are recoded for better distinction. The isolates are distributed according to their origin to allow orientation and identify their origins. This orientation provides an overview of the microbial activity of the environment. The coding according to the origin of the isolate establishes a distinction between the isolates and above all an interpretation of the bacterial activity [17] . Out of (07) samples taken at the exit of the plant, only one (RPG11) was very active. While in the retention basin five isolates (see Table 3) are active. The isolation of the swamp from the production water has three isolates, but only one in the drilling mud swamps. It must be recognized that the active presence of microorganisms in the completion mud is linked to the continuous injection of nitrogen during the start-up of the production wells after completion.

Table 3: Results of Strain Selection after Isolation of Haloana Erobium

Collection sites	UCT exit	Retention basins	Production water quagmires	Drilling mud quagmires
Number of isolates retained	01	05	03	01
Coding of isolates	RPG11	RPG12, RPG13, RPG14, RPG15, RPG16	RPG17, RPG18, RPG19	RPG20

Strain Screening Productrice of Biosurfactants

In order to determine the capacity of the different isolates to produce biosurfactants, we carried out a screening of the ten isolate it all, using it the technical nextes : Test of the collapse of there drop ; Test of dispersion of oil ; Test of stability of the emulsion (E S %), Measurement test of there super voltage.

The results obtained has leave death tests of screening are mentioned in the painting and illustrated by s figures . The choice of these technics among of the tens of the technical existing , is justified by their benefits of which there simplicity , The weak cost , the implant has been raficee the use of equipment relatively commane Who East accessible in almost all the labs de microbiology . Also, the difficulties who have enamelled the screening works are mainly related to there sensitivity of the tensio meter used For the measure of superficial tension .

Test for Drop Collapse

The drop collapse tests were carried out on five (05) types of fluids, each containing one of the ten isolates selected. The results are recorded in Table 3. It appears (according to the scoring system chosen for this purpose) that the results range from (+ : partial spreading of isolates on the surface of the oil) has (++++ : complete spreading of isolates on the surface of the oil) of the gort you are rounded have summer branded as a result negative (-) indiquant the absence of the production of bio surface factors . The SG (S e h g a l - Gibbons) environment sterilized as A control negative (-) And a solution of S D S has 1 % East used as a witness positive (++++) » . Tests carried out with sunflower oil show that isolate RPG20 is the one that causes the best spreading of biosurfactants on the surface of the drop. With isolates RPG18 and RPG14 there is an average spreading while with the other isolates, a low spreading is observed, except for isolate RPG19 where there is almost no spreading of biosurfactants. In view of these findings, it goes without saying that isolate RPG20 is more suitable for microbiological treatment of water polluted by sunflower oil. Isolate RPG19 is strongly discouraged. If this was the case with sunflower oil, it is important to lift the veil on the tests carried out with olive oil.

As for olive oil, the observation is that the RPG14 isolate is the one that leads to the maximum production of biosurfactants, therefore it is more recommended for the microbiological elimination of traces of olive oil. The RPG12 isolate, for its part, does not promote the formation of any biosurfactants, therefore, it is not recommended in the logic of the intended objective. The RPG18 and RPG20 isolates lead to an average production of biosurfactants. The other isolates ultimately produce biosurfactants but weakly. Now that the tests with olive oil have been shed light, what about mineral oil ?

With mineral oil, the first observation (important in view of the objective) is that all isolates are active in terms of the production of biosurfactants. Contrary to what was observed with sunflower and olive oils, two types of isolates (RPG18 and RPG20) lead to maximum production of biosurfactants instead of just one type. The other isolates come out with average and low production of biosurfactants. The first isolates (RPG18 and RPG20) are therefore those to be recommended for the elimination of traces of mineral oil in the logic of the method studied. having reviewed the tests with mineral oil, it is important to see what happens with engine oil and diesel.

Engine oil and diesel were the best in terms of the number of isolates that produced maximum biosurfactants. Unlike the first three fluids previously mentioned (where one or two types of isolates were recorded producing maximum biosurfactants), engine oil and diesel seem to be more easily naturalizable since three types of isolates (RPG14, RPG18 and RPG20) were found to have high potential in the production of biosurfactants. Apart from the RPG11 isolate that produced zero yield in terms of biosurfactants, the other isolates with these last two fluids were crowned by an average production of biosurfactants. If a low type of isolates leads to a maximum production of biosurfactants with sunflower, olive and mineral oils this could be explained by the diversity and complexity of the constituent molecules of these oils. Indeed, they are each made up of a range of free fatty acids and triglycerides [18]. These molecules are not only gigantic (triglycerides) but sometimes contain multiple bonds and high energy bonds, making lysis by microorganisms difficult. In addition, these molecules are made up of polar bonds (CO, CN, etc.) and are therefore hydrophilic (good affinity with water), which makes them difficult to eliminate since the emulsion is not obvious. With engine oil and diesel, on the contrary, the ease with which RPG14, RPG18 and RPG20 isolates produce biosurfactants can be explained by the simplicity of the molecules constituting these fluids. Indeed, diesel and engine oil are essentially made up of hydrocarbons (CxHy) [19]. The predominant bonds are therefore those of the carbon-carbon and carbon-hydrogen type, weakly or not polarized , which gives the compounds that contain them hydrophobic properties, i.e. having no affinity with water [20]. The breaking of these bonds, which should lead to the fragmentation of the molecules and, by ricochet, the emulsion, thus becomes easy for microorganisms. Similar results have been reported by previous work [21,22].

Table 4: Results of the Test of the Collapse of there Drop

Isolates	Test oils				
	Sunflower oil	Olive oil	Mineral oil	Engine oil	Diesel
RPG11	+	+	++	-	++
RPG12	+	-	++	++	++
RPG13	++	++	+	+	++
RPG14	+++	++++	+++	++++	++++
RPG15	+	+	++	+	++
RPG16	++	++	+	++	++
RPG17	+	+	++	++	++
RPG18	+++	+++	++++	++++	++++
RPG19	-	+	++	+	++
RPG20	++++	+++	++++	++++	++++
Control (+)	++++	++++	++++	++++	++++
Control (-)	-	-	-	-	-

The results of our experience indicates that the three isolats RPG14, RPG18 and RPG20 are positive, he had a very good activity by report At control positive (S DS has 1%). So that the others isolate it all, present of the results negative see you later or less positifs (RPG11, RPG12, RPG13, RPG15, RPG16, RPG17 and RPG19) ; and n taking and consideration the degree of the collapse of there drop visually And the nature of there phase today the user. The best of them results are obtained with THE g a soil, engine oil And mineral oil than olive oil and sunflower oil. In this to experience, we have used the bacterial culture as source of biosurfactants. For the isolates that produce of the biosurfac ts extracellular , he y had a good one activity observed . This result included THE strains microbiome having a hy drop hob icity cell high , but not a production of the biosurfacing agents . THE results get us In this test were similar to results reported p a r [10] . The strains with more dix answers positi ves are summer considered as producers of bio surfactants. For A better their screening, the dix isolats have were exhibited at other confirmative tests.

Having reviewed the behaviors of the different types of isolates according to the oïl used, Eich isolate has the ability to produce or not biosurfactants and then to cause the collapse or not of the drop. This being the case, it is important to lift the veil on the possibility of the drop to be dispersed. Table 4 represents the results of the different diameters of the spread according to the period of the spread of the isolates RPG14, RPG18 and RPG20 selected during the drop collapse test. During this test there was a monitoring of the movement of the drops of the supernatant of the three selected isolates producing biosurfactants or the diameter of each spread was measured according to time;

Table 5: Results of Different Periods of Spreading of RPG14, RPG18 and RPG20 Isolates in the Drop Collapse Test

Tests	Dimensions	Isolates					
		RPG14		RPG18		RPG20	
Test 1	Spread period (min)	1	10	1	10	1	10
	Spread diameter (mm)	9	15	11	14	9	16
Test 2	Spreading period (min)	10	20	10	20	10	20
	Spread diameter (min)	25	40	25	38	12	45
Test 3	Spreading period (min)	20	30	20	30	20	30
	Spread diameter (mm)	45	65	40	60	46	63

Oil Dispersion Test

We found that the test of dispersion oil was and concordance with THE test results of the collapse from the a drop. However, the isolates who have presented of the results positive ifs In the latter test, were Also positive for the test of dispersion of the oil. These results have confirmed the capacity of production biosurfactants by the isolats RPG14, RPG18 and RPG20.

In this context, the isolates selected from the oil-in-water dispersion test is also one of the tests that serves as a prelude to the production of biosurfactants. This test is well requested to understand more about the phenomenon of surface tension. This technique consists of introducing a volume of 10µl of supernatant, recovered by centrifugation in a Petri dish containing a volume of 50ml of distilled water added to a volume of 1ml of crude oil. A positive reading of the presence of biosurfactants in the supernatant is reflected by the appearance of a clear zone on a brown background.

The results obtained showed that isolates RPG14, RPG18 and RPG20 are highly biosurfactant producers with diameters of the clear zones formed of 60 mm, 55 mm and 65 mm. Except for isolate RPG11 which is very weakly non-producer of biosurfactants with a clear zone diameter of 11 mm. This observation suggests that the three isolates are of major industrial interest, as they are capable of producing molecules with emulsifying activity. Isolates RPG13, RPG16 and RPG17 have clear zones with average diameters of 35 mm, 30 mm and 35 mm and therefore average producers of biosurfactants. While RPG12, RPG16 and RPG19 have clear zones with small diameters of 23 mm, 24 mm and 21 mm, therefore weak producers of biosurfactants.

Table 6: Oil Dispersion Test Results

Test	Results	
	Light area on a brown background, biosurfactant producer at a diameter in the light area	
Supernatant: 10µl + Distilled water: 50 ml + Crude oil: 1ml	RPG14 RPG18 RPG20	Isolates
	60 55 65	Diameter (mm)
	RPG13 RPG16RPG17	
	35 30 35	
	RPG11 RPG12 RPG19	
	23 24 21	

Figure 4 illustrates oil displacement diameters of different supernatant on the water surface. Surface of there area of shift of oil on the phase what is it proportional to the concentration of the biosur factants [8]. However, in this test, it there is no had not a estimate of there c on centration of the biosurfactants by report has the activity of dispersion of oil, but he it's a g it of a test of presupposition For check the presence or the absence of biosurfactants products by the ten strains . According to there figure 4, on can consider that the isolats RPG14, RPG18 and RPG20 , have a activity d i s p e rs a nt close of this l l e of control positive (SDS to 1%), So that the others isolats : RPG11, RPG12, RPG13, RPG15, RPG16, RPG17 , and RPG19 does not are not assets . Of the similar results for the test of the collapse from the drop and the test of dispersion oil have was reported by Youssef And al . However, these results suggerent that the you cheque of dispersion oil east more is sensitive to the fact that the method of the collapse of the drop for the detection of biosurfactants.

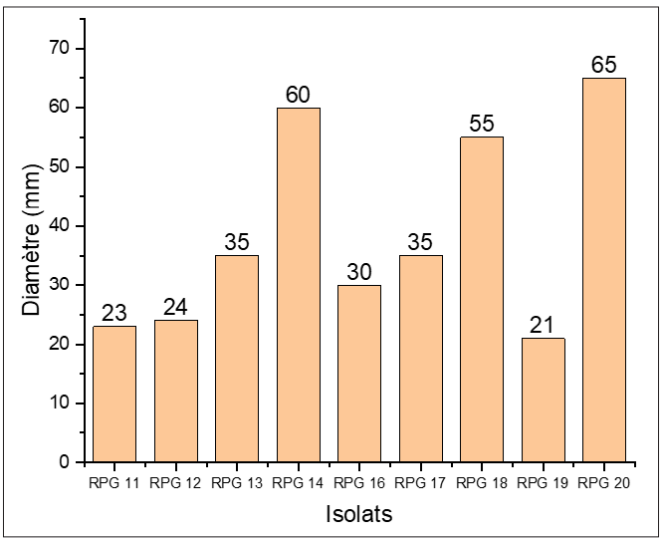

Figure 2: Illustrations of the Displacement Diameters of the Oil of the Different Supernatant on the Water Surface

Test of Stability of Emulsion

In order to acess the extent to which the drop (substrate) can be easily degraded by microorganisms, it would be necessary to have an idea of the stability of its fragments after dispersion. For this purpose, it was considered good form to study the stability of the drop. Figure 2 presents the results of the study. From this figure, it emerges that the isolates RPG 14 (80 ±0.5%), RPG 18 (70 ±0.5%) and RPG 20 (54.38 ±0.5%) are those that exhibit very good stability starting from isolate RPG 14 to isolate RPG 20. The low percentage of emulsion stability obtained with isolate RPG 20 compared to the other isolates can be explained by the fact that the sampling was done at the level of an irrigation basin. Indeed, the latter is located downstream of the main and percolation basins. In these two basins, the organic matter is drastically reduced before reaching the terminal basin (irrigation basin). Since the fluids in this terminal basin are weakly loaded with organic matter, the quantity of microorganisms there will be relatively low than that which would be found in the basins upstream. This being the case, the microbial activity leading to the emulsion and later its stability is thus limited. As for the RPG 14 and RPG 18 isolates, the stability of the emulsion being very high can be explained by the fact that the sampling was done not only in the main basins (higher pollutant load compared to the irrigation basin) but also the samples were taken on the peripheries of the basins where, in addition to the contribution of plant detritus from the plants located near the sites, there is a large accumulation of oils (due to the natural phenomenon of centrifugation) escaping from the treatment units. A high quantity of organic matter automatically leads to excessive proliferation of microorganisms. These, during catabolism reactions, will cause oxidation-reduction reactions leading to the formation of electrical charges on the surface of the substrates (oils). The same substrates, experiencing the same types of reactions under the same conditions, will cause the same types of overall surface charges and consequently electrostatic repulsions between the dispersed particles [23]. These repulsions will only have the effect of keeping the dispersed particles away from each other, hence the stability of the emulsion over time.

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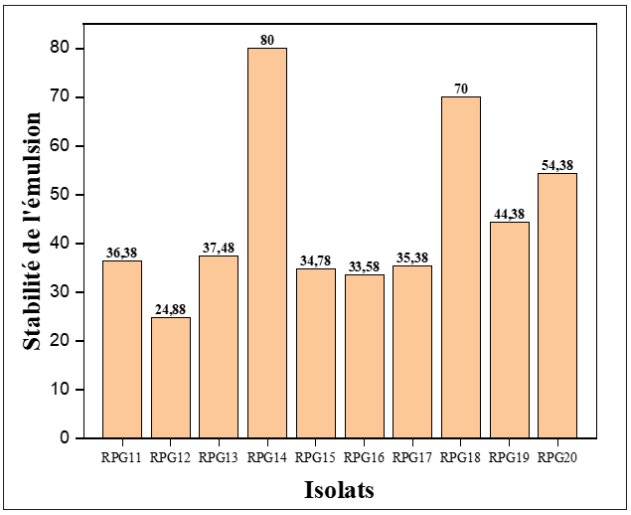

Figure 3: Illustration of the Results of the Aemulsion Stability Test (ES%) of the Isolates

Direct Measurement test of Surface and Interfacial Tension

In order to understand the surface tension of the ten isolates (from RPG 11 to RPG 20), direct measurements were carried out in the laboratory just after the samples were collected. Figure 3 presents the results of these measurements. The assessment was made in accordance with the Bodour method and Maier (1998) who states that the surface tension is good for considering oil recovery when it is less than 40 mN/m. Based on this method, we can say that isolates RPG 14, RPG 18 and RPG 20 are those that can easily lend themselves to oil recovery since the surface tensions recorded were respectively 23.70 ±0.5 mN/m , 22.45 ±0.5 mN/m and 22.75 ±0.5 mN/m . These low surface tension values are in line with the droplet collapse, dispersion and emulsion stability tests because when the emulsion is large, the surface tension is the lowest possible [25]. Previous work has reported that the lower the surface tensions, the more easily the oils in solution are released [26] .

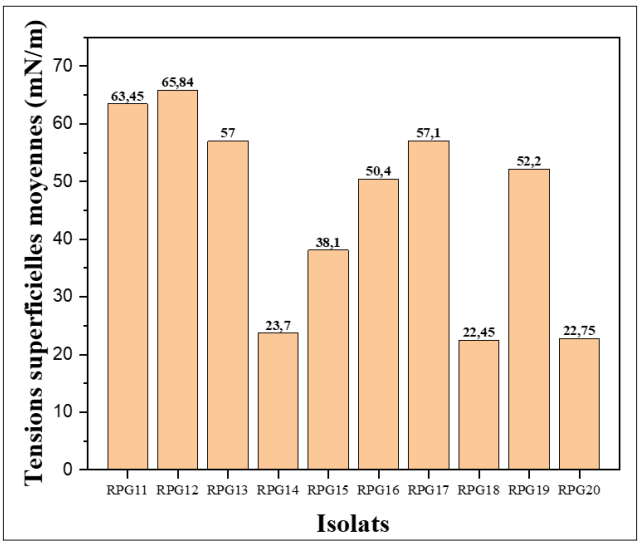

Figure 4: Illustration of the Surface Tension Measurement Test Results

Statistical Analyses of the Correlation between the Different Tests

Table 7 presents the variations in the correlation coefficient between the four methods used to detect biosurfactant production, Spearman's rho (r s = - 0.661) reveals a strong correlation negative between the test of the stability of the emulsion And the test of the m easurement of the tension super f icie l l e . A correlation negative plus weak (r s = - 0.636) has been d e t e c t e d between the test of dispersion oil and THE test of the voltage measurement super f i c i e l l e . However , he there was it a weak correlation negative (r s = 0.) between there method of the collapse of there drop And there tension superficial . The test of dispersion oil and the test of the stability of the emulsion are very strongly correlated with a rho (r s = 0. 321) . However, weak correlations positives (r s = 0.86 4) et (rs = 0.288) have summer observed between the technique of the collapse of there drop And THE test of disper if on of the oil And between the e test of the collapse of the g out t e and the e test of stability of the emulsion.

Table 6: Correlation Statistics between the Different Methods

SPEARMAN CORRELATION COEFFICIENT ρ_s(				
Test	**EG**	**DH**	**WHETHER**	**MTS**
Drop collapse (EG)	1			
Oil dispersion (OD)	0.864	1		
Emulsion stability (ES)	0.822	0 . 321	1	
Surface tension measurement	-0.807	- 0.636	- 0.636	1

The interest croissant to biosurfac ts has led to development of a mu l t i tude of methods for screening of strains producers of the biosurfacing agents . A combination differentes methods East appried for a screening successful . In the after presentation study them three iso l a ts RPG14, RPG18 And RPG20 who have summer isolated has leave EPPG enriched with salt from the Mangara and Badila fields (Chad), have showed good ability has produce the s bio surfactants , in reducing there tension superficial to the - below of 40 mN/ m with a stability of the emulsion greater than 5 0 % , who the rends new potential candidates for the production of biosurfactants . These biosurfactants drastically reduce surface tensions and quantitatively release the oils retained in the EPPGs.

Microbial Reduction of Surface Tension

Table 8 presents the results of the microbial reduction of surface tension at the outlet of the Badila central treatment plant. In view of this table, the reduction of surface tension at the outlet of the plant is proportional to the tensions generated at the outlet. The increase in these tensions is linked to the inefficiency of the demulsifiers injected for separation. The RPG14 isolate produces biosurfactants capable of drastically reducing surface tensions. The surface tension reduction percentages of 2020, 2015 and 2021 illustrate this (Figure 4). The surface tension reduction percentages are very high, this will lead to better release of oils in oil and gas production waters [27] . The RPG18 isolate also produces biosurfactants capable of reducing surface tensions and the years 2020, 2015 and 2021 are an illustration of this (Figure 5). In these same years, the RPG18 isolate was more efficient than the RPG14 isolate. The same observation is made for the RPG20 isolate (Figure 6). Overall, a reduction of more than 50% in surface tensions at the exit of the plant was observed. But if the biosurfactants were injected at the entrance to the plant, the percentage of reduction would be even less significant because of the resilience of the fluids and the restrictions of the equipment linked to the passage of the fluid [28] . The properties of the fluid can also influence the reduction of surface tension [29] .

Table 7: Results of Microbial Reduction of Surface Tension at the Outlet of the Badila Central Treatment Plant

Years	Surface tension (mN/m)								
	RPG14			RPG18			RPG20		
	TS before	TS after	RTS (%)	TS before	TS after	RTS (%)	TS before	TS after	RTS (%)
2014	52.15 ±0.5	23.70 ±0.5	54.55 ±0.5	52.15 ±0.5	22,45± 0,5	56,95± 0,5	52,15± 0,5	22,75± 0,5	56,38± 0,5
2015	64,22± 0,5	23,70± 0,5	63,10± 0,5	64,22± 0,5	22,45± 0,5	65,04± 0,5	64,22± 0,5	22,75± 0,5	64,57± 0,5
2016	55,71± 0,5	23,70± 0,5	57,46± 0,5	55,71± 0,5	22,45± 0,5	59,70± 0,5	55,71± 0,5	22,75± 0,5	59,16± 0,5
2017	55,72± 0,5	23,70± 0,5	57,47± 0,5	55,72± 0,5	22,45± 0,5	59,71± 0,5	55,72± 0,5	22,75± 0,5	59,17± 0,5
2018	55,72± 0,5	23,70± 0,5	57,47± 0,5	55,72± 0,5	22,45± 0,5	59,71± 0,5	55,72± 0,5	22,75± 0,5	59,17± 0,5
2019	52,28± 0,5	23,70± 0,5	54,67± 0,5	52,28± 0,5	22,45± 0,5	57,06± 0,5	52,28± 0,5	22,75± 0,5	56,48± 0,5
2020	64,22± 0,5	23,70± 0,5	63,10± 0,5	64,22± 0,5	22,45± 0,5	65,04± 0,5	64,22± 0,5	22,75± 0,5	64,57± 0,5
2021	62,15± 0,5	23,70± 0,5	61,87± 0,5	62,15± 0,5	22,45± 0,5	63,88± 0,5	62,15± 0,5	22,75± 0,5	63,40± 0,5
2022	53,6± 0,5	23,70± 0,5	55,78± 0,5	53,6± 0,5	22,45± 0,5	58,12± 0,5	53,6± 0,5	22,75± 0,5	57,56± 0,5
2023	48,44± 0,5	23,70± 0,5	51,07± 0,5	48,44± 0,5	22,45± 0,5	53,65± 0,5	48,44± 0,5	22,75± 0,5	53.03 ±0.5
Totals	56.42 ±0.5	23.70 ±0.5	57.65 ±0.5	56.42 ±0.5	22.45 ±0.5	59.89 ±0.5	56.42 ±0.5	22.75 ±0.5	59.35 ±0.5

Microbial Reduction of Interfacial Tension

Table 9 presents the results of microbial reduction of interfacial tension at the outlet of the Badila central treatment plant. In view of the table, the three isolates showed excellent interfacial tension reduction capacity. Figures 4, 5 and 6 illustrate the high reductions in interfacial tensions. This can be explained by a high release of dispersed oils [30]. The biosurfactants produced by the three isolates lower interfacial tensions much more than surface tensions. This can be explained by the fact that surface tensions are established at the molecular level while interfacial tensions are established between molecules, whose low breaking strength [31] .

Table 8: Results of Microbial Reduction of Interfacial Tension at the Outlet of the Badila Central Treatment Plant

Years	Interfacial tension (mN/m)								
	RPG14			RPG18			RPG20		
	TI before	TI after	RTI (%)	TI before	TI after	RTI (%)	TI before	TI after	RTI (%)
2014	66 ±0.5	14.35 ±0.5	78.26 ±0.5	66 ±0.5	15,35± 0,5	76,74± 0,5	66± 0,5	15,45± 0,5	76,59± 0,5
2015	64± 0,5	14,35± 0,5	77,58± 0,5	64± 0,5	15,35± 0,5	76,02± 0,5	64± 0,5	15,45± 0,5	75,86± 0,5
2016	57± 0,5	14,35± 0,5	74,82± 0,5	57± 0,5	15,35± 0,5	73,07± 0,5	57± 0,5	15,45± 0,5	72,89± 0,5
2017	45± 0,5	14,35± 0,5	68,11± 0,5	45± 0,5	15,35± 0,5	65,89± 0,5	45± 0,5	15,45± 0,5	65,67± 0,5
2018	69± 0,5	14,35± 0,5	79,20± 0,5	69± 0,5	15,35± 0,5	77,75± 0,5	69± 0,5	15,45± 0,5	77,61± 0,5
2019	52± 0,5	14,35± 0,5	72,40± 0,5	52± 0,5	15,35± 0,5	70,48± 0,5	52± 0,5	15,45± 0,5	70,29± 0,5
2020	47± 0,5	14,35± 0,5	69,47± 0,5	47± 0,5	15,35± 0,5	67,34± 0,5	47± 0,5	15,45± 0,5	67,13± 0,5
2021	62± 0,5	14,35± 0,5	76,85± 0,5	62± 0,5	15,35± 0,5	75,24± 0,5	62± 0,5	15,45± 0,5	75,08± 0,5
2022	70± 0,5	14,35± 0,5	79,50± 0,5	70± 0,5	15,35± 0,5	78,07± 0,5	70± 0,5	15,45± 0,5	77,93± 0,5
2023	66± 0,5	14,35± 0,5	78,26± 0,5	66± 0,5	15,35± 0,5	76,74± 0,5	66± 0,5	15.45 ±0.5	76.59 ±0.5
Totals	59.11 ±0.5	14.35 ±0.5	75.13 ±0.5	59.11 ±0.5	15.35 ±0.5	73.40 ±0.5	59.11 ±0.5	15.45 ±0.5	73.23 ±0.5

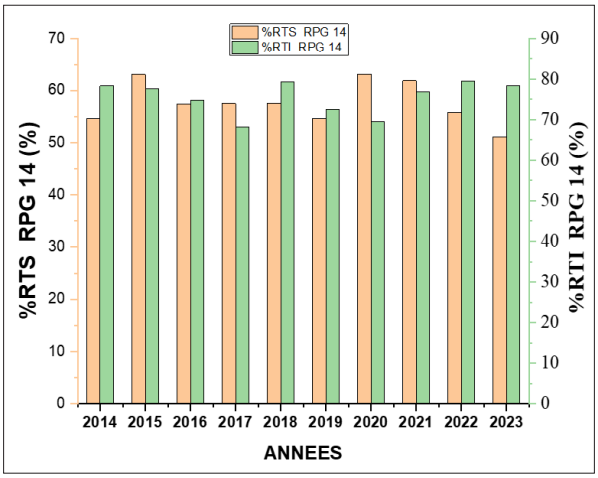

Figure 5: Illustration of the Percentage Reduction in Surface and Interfacial Tension of Isolate RPG14

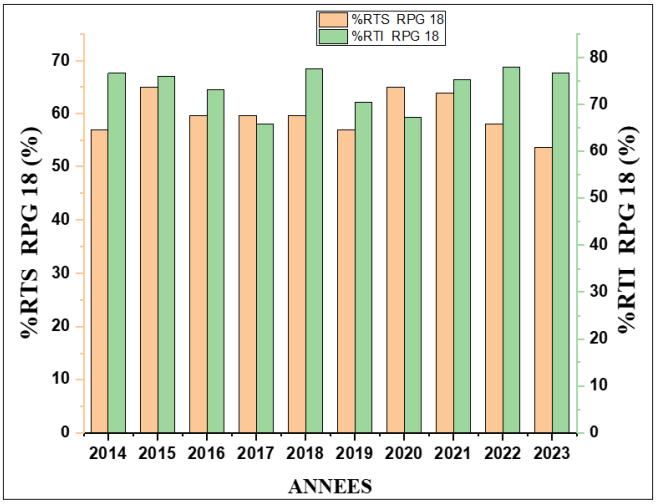

Figure 6: Illustration of the Percentage Reduction in Surface and Interfacial Tension of Isolate RPG18

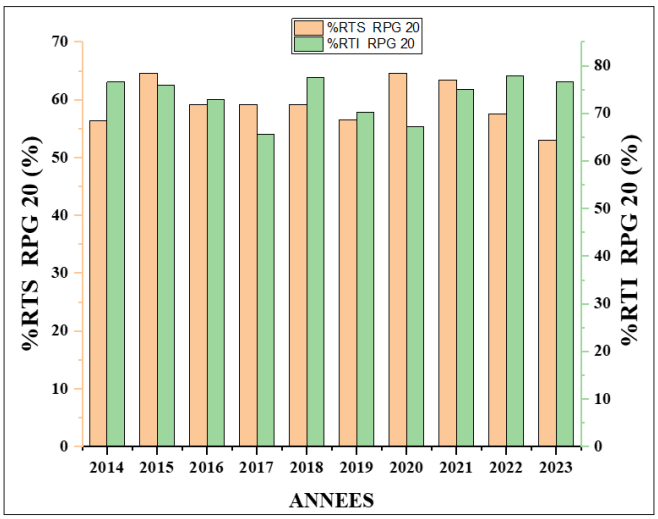

Figure 7: Illustration of the Percentage Reduction in Surface and Interfacial Tension of the RPG20 Isolate

Conclusion

Oil and gas production waters contain bacterial strains in surface conditions. The thirty-seven samples yielded ten biosurfactant-producing isolates, and three best isolates (RPG14, RPG18, and RPG20) were selected after a screening test. The three isolates exhibited better surface and interfacial tension reduction abilities than the remaining seven (RPG11, RPG12, RPG13, RPG15, RPG16, RPG17, and RPG19). Unlike the surface and interfacial tensions generated at the outlet of the treatment plant after injection of chemical demulsifiers such as VX Champion, the biosurfactants produced by the isolates can reduce surface tensions by more than 60% and interfacial tensions by more than 70% [32].

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