

Isolation, Identification and Selection of a Suitable Type of Bacteria for MEOR in the Egyptian Oil Fields

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Abstract

The aim of the present study is to, isolate and characterize, the different indigenous microbes that may found in some Egyptian crudes and to determine their suitable media and nutrient for use in microbial flooding test.

Three different crude oil samples (A, B and C) and one formation water sample (W) which are taken from three different Egyptian fields, that are located in the western desert and Gulf of Suez area were used to isolate and identify the different types of bacteria that may present in these samples. The results showed that, three different types of bacteria were found in the first two crude samples (sample A and B) while the third crude oil sample and the formation water sample were found to be free from the indigenous bacteria.

The identification and characterization process showed that the three types of the indigenous bacteria are the *Pseudomonas aeruginosa*, *Pseudomonas fluorescens biotype G* and the *Cellulosimicrobium celluans*. These three types together with another two different additional types of bacteria (*Bacillus circulans* and *Bacillus megaterium*) which were selected from agriculture, were tested with six different nutrient types, to select the best type of bacteria that will be suitable for using in the MEOR to increase the recovery of oil. The results of this study showed that the *Pseudomonas aeruginosa* is the best type of bacteria that may increase the oil recovery, in oil fields under study, as it produces biosurfactant when it is activated with the molasses solution, with concentration of 2 gm/lit, as a nutrient.

Introduction

Due to the fluctuation in oil prices, most of the Enhanced Oil Recovery (EOR) processes, and especially the ones typically recommended for light crude such as chemical methods or miscible gas injection processes have become economically unattractive. The oil industry currently is in dire need of a reasonable cost process that can both technically and economically be successful. This need increases more and more, especially in case of heavy crude oils, like those found in the old Egyptians fields in Western Desert and/or in the Gulf of Suez areas.

Microbial Enhanced Oil Recovery (MEOR) is a technology that uses micro-organisms to facilitate, increase or extend oil production from reservoir. The concept is known since more than 40 years; however, early proposals were poorly conceived and, in most cases, had no practical value. Recent studies have developed microbial biotechnology to resolve specific production problems in oil reservoirs. MEOR process involves the use of in-reservoir micro-organisms or specially selected natural bacteria which are capable of metabolizing hydrocarbons to produce organic solvents, like alcohols and aldehydes, fatty acids, surfactants and other biochemical that are known to be effective at encouraging oil mobility⁽¹⁾.

Research groups in many countries have conducted studies of enhancing oil recovery by means of various types of bacteria which produced different types of metabolism. These bacteria may be activated, either in-situ or ex-situ, to produce bioproducts or microbial mass which may affect enhanced oil recovery from the reservoir.

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The application of MEOR technology has a promising prospect since it is supported by; a) simple technology and equipment which are easy to operate, b) the process can be easily monitored, c) it is a low-cost process, d) the technology is capable of recovering the residual oil trapped in the reservoir rock⁽²⁾. A considerable amount of research effort was directed, at Cairo University to investigate the possibility of using bacteria, which is known as a low-cost material, to improve the application of the enhanced oil recovery processes in the old Egyptian fields.

The goals of the present research are to isolate and characterize the different indigenous microbes that may be found in some Egyptian crudes and formation water and to determine the best media and nutrient for the use in microbial flooding test.

Material used

- 1- Ringer solution that has the following composition: Sodium Chloride, 2.25g; Calcium Chloride, 0.12g; Potassium Chloride, 0.105g and Sodium Bicarbonate, 0.5g with final pH=7⁽³⁾ is used for dilating the samples of oil and formation water to be tested.
- 2- Nutrient broth agar has the following composition: Peptone 5g, Beef 1g, Yeast 2g, Glucose 10g, NaCl 5g, and agar 15 g. All these quantities were added to one liter of distilled water. This media was used to culturing the isolated bacteria.
- 3- Kinky media with the following composition was prepared with final pH \pm 7.2⁽³⁾: Protease peptone 20g, Agar 15g, Glycerol 10g, K₂SO₄ 10g and MgCl₂ 3.5g. All this countifies were added to one-liter distilled water. This media was used for culturing the *Pseudomonas* bacteria only.
- 4- Any type of bacteria needs three basic components to grow (nitrogen, carbon and salt). There for six different nutrients, having the compositions shown in Table 1, were used to activate the isolated bacteria with these different nutrients to determine which base is suitable for the bacteria to produce the biosurfactant. It is commonly known that, Molasses is a carbon base, Peptone, Yeast and Beef are a nitrogen base and NaCl is a salt base.
- 5- Three crude oil samples and one formation water sample which were withdrawn from three Egyptian oil fields, located in the Gulf of Suez and Western Desert areas were used to study the possibility of isolation of the indigenous bacteria. The physical properties of the crude oil samples are given in Table 2.
- 6- Two types of bacteria from agriculture were collected to test their applicability in MEOR. These two types were *Bacillus circulaus* (BC) and *Bacillus megaterium* (BM).

Experimental work

The protocol of the experimental work that have been done to isolate and identify the different types of bacteria which may be found in the crude oil samples can be explained as follows:

Sample coding

The four samples were kept closed until bacterial isolation procedure started, the three types of oil are nominated as A, B and C, and the water sample as W.

Bacteria isolation

All procedures were conducted at a constant temperature of 30 °C, and the isolation steps are as follows:

- 1- Dilation of samples, a part of every sample, of the crude oil and formation water, of volume 10 cm³ was taken and put in 90 cm³ of the Ringer solution.

2- Separation of various types:

- The streak plate method pour plate technique ⁽⁴⁾ was used to separate the different culture. Using the nutrient broth agar (15 g/liter) to fill the plates while working with.
- Several pre-cultures were carried out (4 times 24 hours between each) before plating the cultures.
- Bacteria from well-defined colonies were then transferred to fresh plates using a sterile needle.
- An isolate was considered to be a pure strain after three consecutive transfers without evidence of other microorganisms. The time between each two transfers is around 72 hours

3- Transfer to liquid media; smears from each colony were diluted with 9 ml sterilized Ringer solution. After sterilization the diluted solutions were prepared in Ringer solution.

4- Culture purity; the culture purity was verified by simple staining ⁽⁴⁾ and microscopic examination of cells at 100x magnification. Cellular morphology was also examined at this time.

5- Bacteria count by using the stand plate method ⁽⁴⁾.

6- The pure isolated culture of each bacteria type is transferred to a slant agar to keep them until start working with them.

Identification of bacteria type

Five basic tests (Gram stain, Spore stain, Acid fast stain, Oxygen requirements and Catalase test) were carried out to select the best type of kites of the stander Biolog test which will be performed for the bacterial identification.

Steps of the Slandered Biolog test

The following test procedures were carried out by using of the kites and the test of GN2 microplates ⁽⁵⁾:

- 1- A pure culture of isolates was grown on a Biolog universal growth 5% sheep blood agar plate.
- 2- The bacteria were swabbed from the surface of the agar plate and suspended to a specified density in GN inoculating fluid.
- 3- 150 µl of bacterial suspension was pipetted into each cell of the GN2 Microplate.
- 4- The Microplate was incubated at 30°C for 24 hours.
- 5- The oxidation of 95 different carbon sources was detected indirectly by observing reduction of tetrazolium dye with BIOLOG computer software.

Confirmation test

Some types of bacteria will give a special type of pigment with defined color when using Kinky media.

Measuring of the different fluid properties

The following is a general description of the procedures that were carried out to determine the effect of this bacteria types on the oil properties in order to determine their applicability in MEOR.

General procedure:

- 1- Clean and sterilize the graduated test tubes using the following sequence:

Wash them with detergent and tap water followed by distilled water then put them inside the oven with a temperature above 100 °C for 15 min for drying.

Cover the test tube with the cotton plug and put it inside the oven at a temperature of 160 °C for 60 min for sterilization.

- 2- Prepare the nutrient solution in a flask, then put it inside the Autoclave for 20 min to sterilize the nutrient.
- 3- Get an oil sample in a flask, then put it inside the Autoclave for 20 min to sterilize the oil sample.
- 4- Prepare the media which will be used to activate the bacteria and put it inside the Autoclave for 20 min to sterilize the media.
- 5- Transfer, under sterilized conditions, i.e. in the front of a flame, 3 cm³ from the old bacteria solution to the new solution which will be used after a time period (one or two days) to standard the age of bacteria.
- 5- In front of the flame, under sterilized condition, open the sterilized empty tube and fill it with 25 cm³ of sterilized oil, 22 cm³ of sterilized nutrient and 3 cm³ from the bacteria solution.
- 6- Seal off the test tubes very well using sterilized plug and shake it to get a good contact between aqueous nutrient solution and crude oil and to be sure that the bacteria was distributed all over the tube then close the tubes with cotton plugs.
- 7- The tubes were left for incubation during a time interval of one week in a specific temperature.
- 8- The aqueous and the oleic solutions volumes were recorded and the separation of the aqueous phase from the oleic phase, using pipette, was done then measure the fluid properties, of the aqueous and oleic phase, such as (viscosity, surface tension, pH and conductivity).

Result and discussions

Characterization of the isolate

12 pure cultures were isolated, 6 from crude oil A, 6 from crude oil B. For crude oil C and the formation water sample W, no colony was found in the plates.

From the observation of the shape of isolated bacteria in the plate and slant, it was found that all colonies in crude oil A and B have the following characterizations: round, granular, entire, flat, colorless, translucent, dull, bounded and have a normal size.

Identification of bacteria type

All of the isolated bacteria were found to be an aerobic, gram-negative, short rods, no spore was performed, and no resistance for acids. Only six isolates were chosen, from the above 12 isolates, according to the visual appearance of the isolated bacteria and by choosing only one type from the different types that have the same appearance. These six selected isolates were used to perform the stand Biolog test and the results are shown in the Table 3.

Only the three isolates A5 (*Pseudomonas aeruginosa*), B2 (*Pseudomonas fluorescens biotype G*) and B3 (*Cellulosimicrobium celluianus*) were chosen to be tested for the fluid properties determination as their percent of identification were found to be around 100%.

The resulted types of *Pseudomonas* were confirmed, as the *Pseudomonas aeruginosa* has a brown color and the *Pseudomonas fluorescens* has a fluorescent yellow color.

Fig. 1 shows the shape of these three types of bacteria in a plate with related conditions, in a slanted agar, liquid media and under microscope.

Two different types, in addition to the above three types, were collected to study their properties and their performance when activated with different nutrient. These two additional types were chosen to be gram positive and spore formed. Fig. 2 shows the shape of these two types in a slanted agar, Petri dish, liquid media and under microscope. These two types are referred to as BM and BC in the following.

Fluid properties

Selecting the best type of bacteria and its nutrient

The results of measuring the different properties of the aqueous and oleic phase are shown in Fig. 3 through Fig. 9. Such properties are: viscosity, pH, surface tension, conductivity and aqueous phase volume when the different types of bacteria were activated with the six different nutrients.

It is clear from Fig. 3 that, the lowest viscosity of the oleic phase was obtained when activate the bacteria A5 with the nutrient six. It is wears to mentioned that some type of bacteria with some type of nutrient change the type of the fluid (aqueous or oleic) to be non-Newtonian fluid which will be very danger to use as the viscosity will increase too much.

These results mean that the nutrient 6 is suitable, from viscosity point of view, to activate the bacteria of type A5 when it is used for Enhanced Oil Recovery processes. The activation of the bacteria A5 with this nutrient gives a pH value below 5, a minimum surface tension (around 48 dyne/cm) and the minimum aqueous phase volume is also obtained when using this nutrient as shown in Figs. 5,7 and 9. No significant change in the oleic phase surface tension was observed with the different types of the bacteria when added to different nutrients as shown Fig. 6. There is a clear change in the conductivity with all types of bacteria and nutrient used, as shown in Fig. 8.

The properties of aqueous solution only are not enough to make a good judgment on the ability of the bacteria to be suitable for the MEOR. This is because some types of bacteria decrease the surface tension, viscosity, pH and conductivity of the aqueous phase while on the other hand increase the viscosity of oil. So, the tests should made in the presence of the oil.

From the above results, it is clear that, the best type of bacteria is the type A5. That is why the work will continue only with this type of bacteria.

Effect of the presence of oil on the bacteria efficiency

The same properties were measured for oil with bacteria, without nutrient, and for oil with nutrient only in absence of bacteria to study the effect of presence of oil on the activation of bacteria.

Fig. 10 through Fig. 16 show the effect of bacteria A5 on the properties of the oil and nutrient. Fig. 10 proved that the bacteria A5 gave the lowest viscosity in case of using nutrient number 6. As shown in Fig. 10, the viscosity of the oil, in the presence of nutrient only without bacteria is found to be 78.27 cp, while its value in the presence of bacteria only without nutrient is decrease to 76.92 cp. This means that the bacteria use the oil as a carbon source during its activity. Moreover, in case of the presence of nutrient and bacteria A5 together with the oil the value of the viscosity decreased to 64.43 cp. This phenomenon is confirmed also by the decrease in the surface tension of the oleic phase as shown in Fig. 13. Fig. 11 shows that, the decreasing in the viscosity of the aqueous phase, in the presence of oil, is much more than its decrease when it is measured for the nutrient with bacteria only in the absence of oil. Fig. 12 shows a decreased in the pH in case of using the bacteria with nutrient. Fig. 14 shows that the lowest surface tension of the aqueous phase was obtained with nutrient number 6.

Fig. 15 shows a clear difference in the conductivity of the aqueous phase due to presence of oil and its absence. Fig. 16 shows that the minimum aqueous phase volume separation was obtained with nutrient number 6 also. These results indicate that the best nutrient that can be used, is the nutrient number 6. Observing the aqueous volume in the tubes, that it is progressing day by day for one week leads to the following result; the percent of the aqueous phase increases with time which means that the bacteria break the emulsion of the water and oil. This gives an indication for the bacteria A5 that; it is producing some type of demulsified and biosurfactant which reduce the interfacial tension between the oil and water, and this leads to break the formed emulsion.

Conclusions

- 1- The present study gives a developed methodology to:
 - Isolate, identify and characterize the different types of bacteria that may be found in any crude oil.
 - Select a specific type of bacteria suitable for MEOR, and the selection of the best nutrient.
- 2- The application of the above developed methodology on the rock and crude oil of some Egyptian fields shows that:
 - The formation water and the third type of the crude oil (samples W and C) were found to be free from the indigenous bacteria while the other two types of crude (samples A and B) were found to contain indigenous bacteria.
 - Three types of bacteria were isolated and successfully identified in these two cruds. These three types of bacteria are *Pseudomonas aeruginosa*, the *Pseudomonas fluorescens biotype-G* and the *Cellulosimicrobium cellulans*.
 - The best type of bacteria for MEOR project on the field under study, out from the 5 types used, is A5 (*Pseudomonas aeruginosa*) and the best nutrient type was found to be the number 6 (molasses + NaCl).
 - It is not enough to study the capability of any type of bacteria only with properties of the aqueous solutions such as viscosity, surface tension, pH, etc. but these properties should be measured in the presence of the oil.
 - The type of the bio-product obtained from the activation of bacteria is a function of its type and nutrient type used for activation.
 - Bacteria A5 uses the crude oil as an additional carbon source.
 - Bacteria A5 (*Pseudomonas aeruginosa*) with nutrient number 6 give some type of emulsifier and surfactant as a metabolize.

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TABLE 1- COMPOSITIONS OF THE DIFFERENT NUTRIENTS WHICH ARE USED FOR SELECTING THE BEST TYPE OF BACTERIA

Nutrient No.	Composition	Weight [gm/lit.]
1	NaCl	5
2	Peptone	5
	Yeast	2
3	Peptone	5
	Yeast	2
	NaCl	5
4	Molasses	2
5	Peptone	5
	Yeast	2
	NaCl	5
	Molasses	2
6	NaCl	5
	Molasses	2

TABLE 2- RESERVOIR CONDITIONS AND PHYSICAL PROPERTIES OF THE CRUDE OILS UNDER STUDY.

Properties	Value
Initial reservoir pressure, psi	3000 - 4200
Reservoir temperature, °F	180 - 200
Bubble point pressure, psi	1350 - 1950
GOR, SCF/STB	260 – 450
Oil Gravity, API	20 - 28
Pour point, °C	-3 - 3
Density	0.92 – 0.95

Table 3- Results of Biolog analysis

Isolate number	Result	Probability %
A4	<i>Pseudomonas aeruginosa</i>	100
A5	<i>Pseudomonas aeruginosa</i>	100
A6	<i>Pseudomonas aeruginosa</i>	100
B2	<i>Pseudomonas fluorescens</i> biotype G	100
B3	<i>Cellulosimicrobium celluans</i>	98
B5	<i>Pseudomonase fluorescens</i> biotype G	71

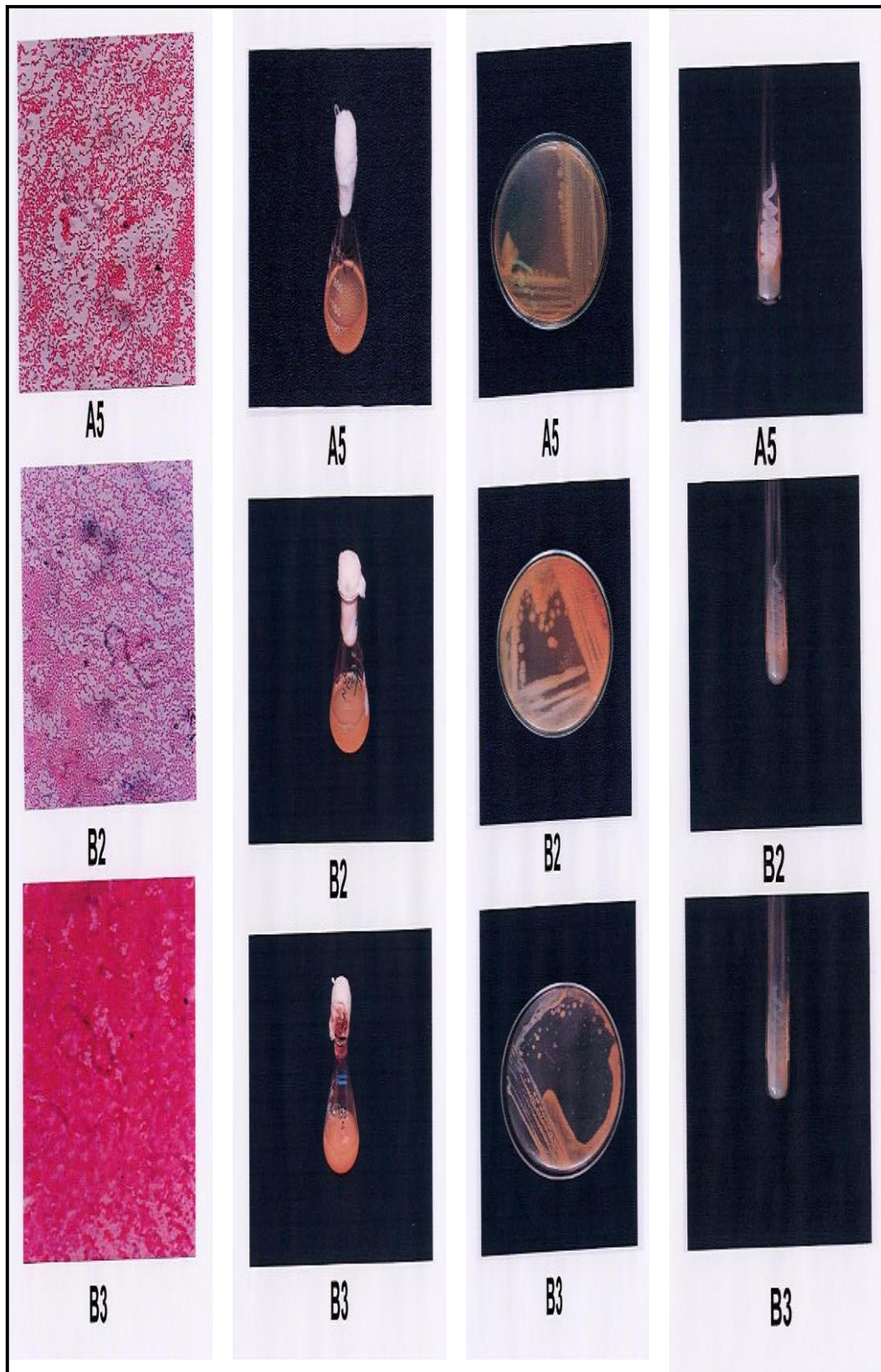


Fig. 1- The shape of the three isolated bacteria of type A5, B2 and B3 in a slanted agar Petri dish, liquid media and under microscope.

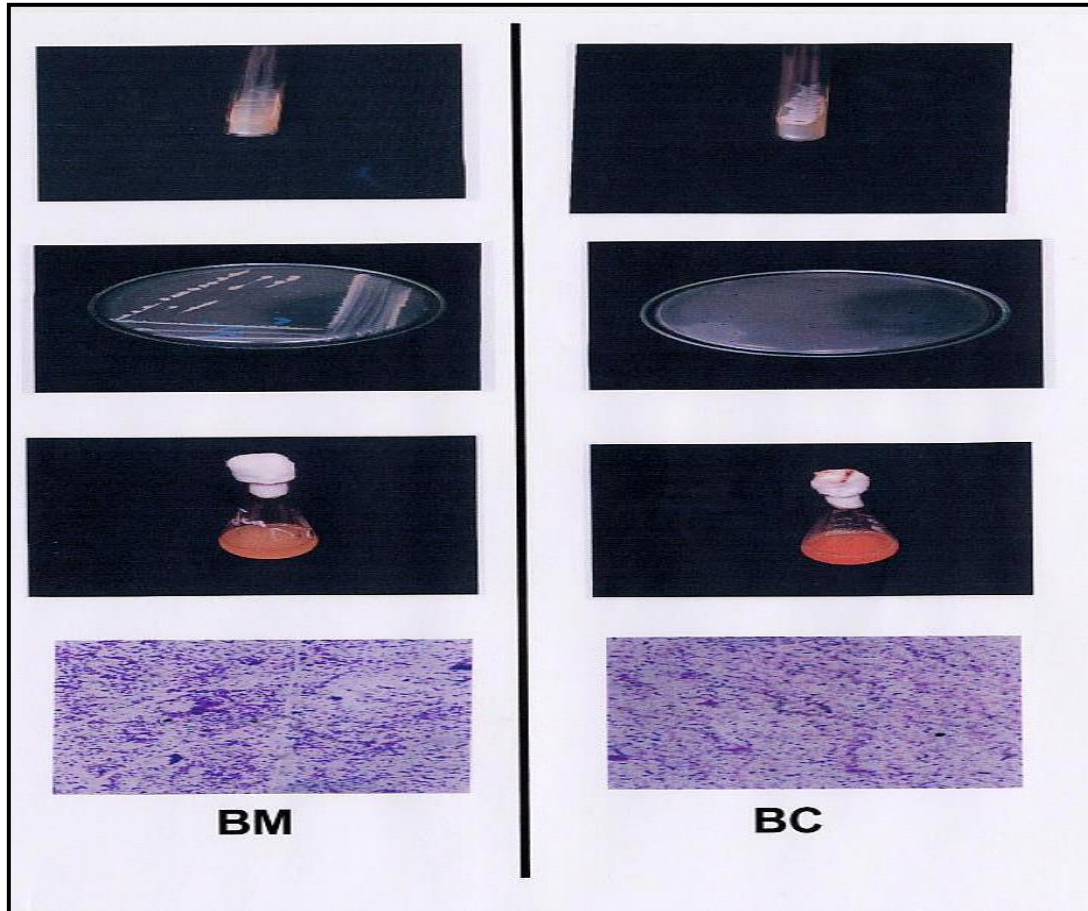


Fig. 2- The additional agriculture bacteria BM and BC in a slanted agar Petri dish, liquid media and under microscope.

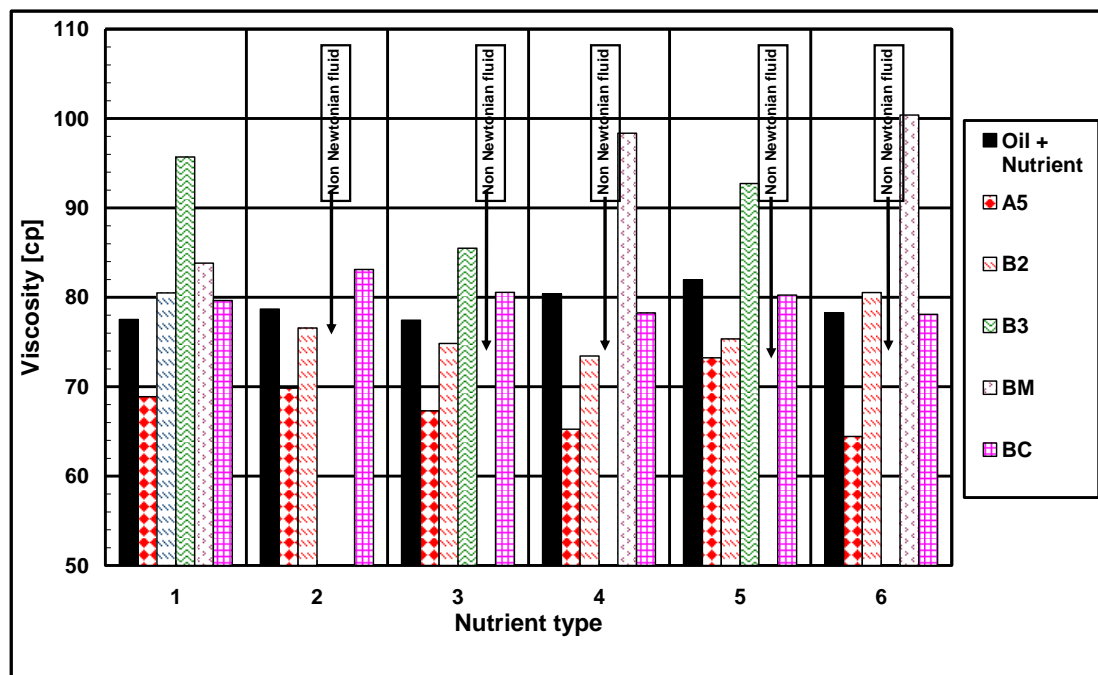


Fig. 3- Viscosity of the oleic phase in the presence of different types of bacteria when activated with the different types of nutrient at 30 °C.

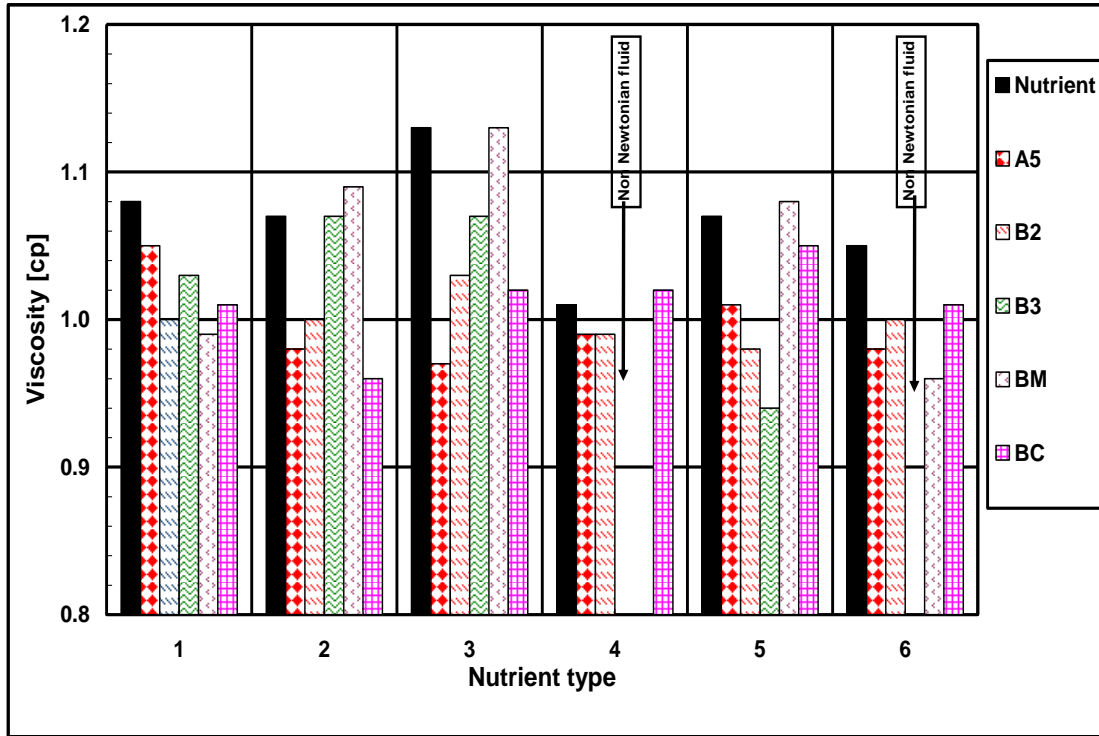


Fig. 4- Viscosity of the aqueous phase in the presence of different types of bacteria when activated with the different types of nutrient at 30 °C.

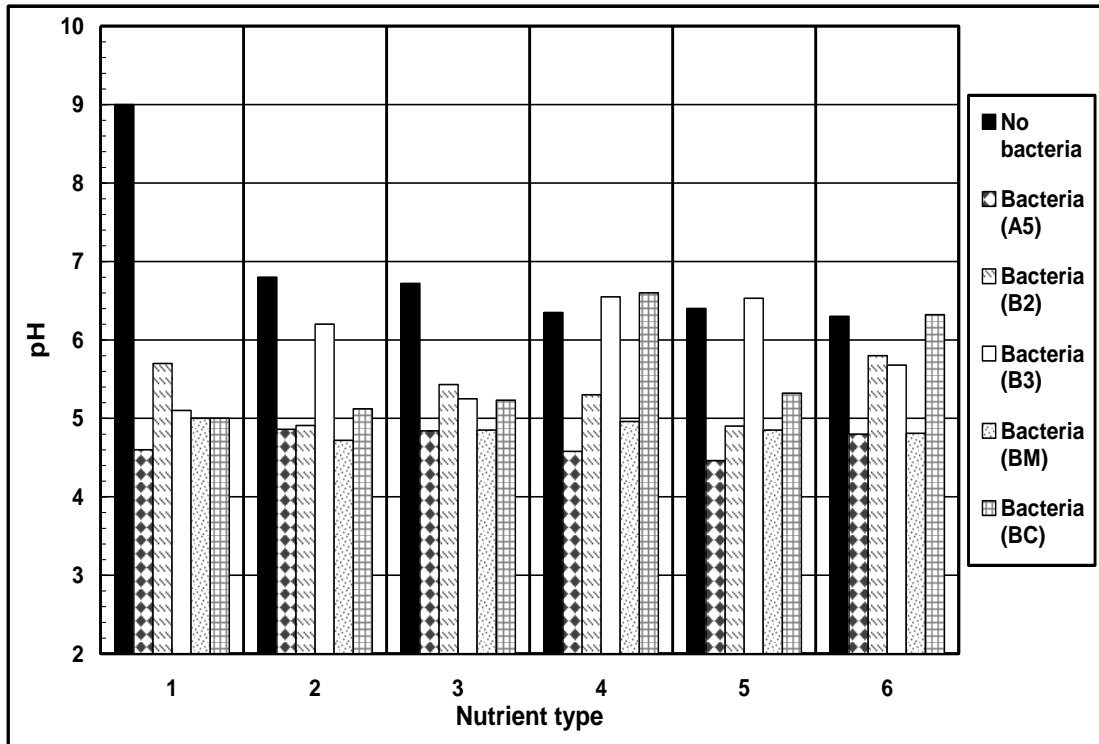


Fig. 5- The pH value of the aqueous phase in the presence of different types of bacteria when activated with the different types of nutrient at 30 °C.

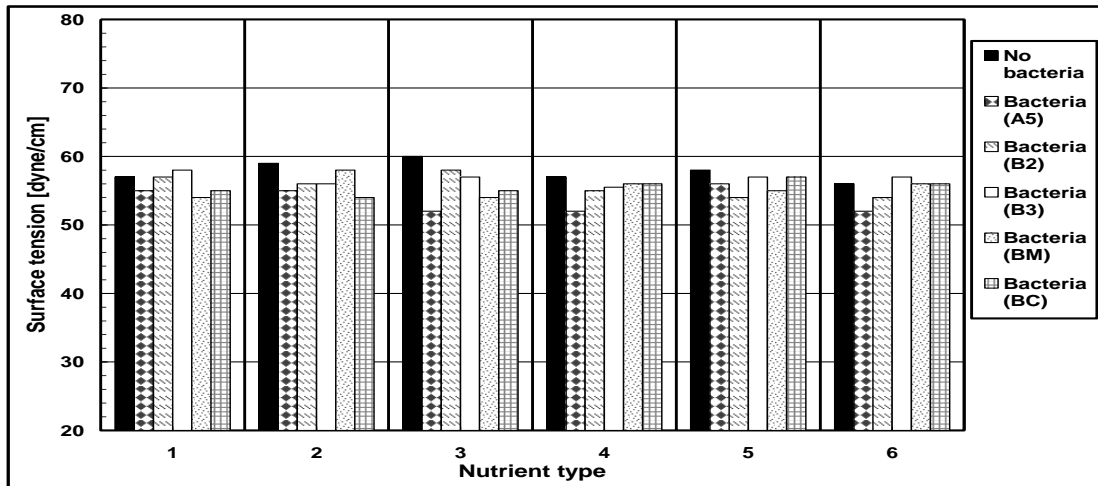


Fig. 6- Surface tension of the oleic phase in the present of difference types of bacteria when activated with the different types of nutrient at 30 °C.

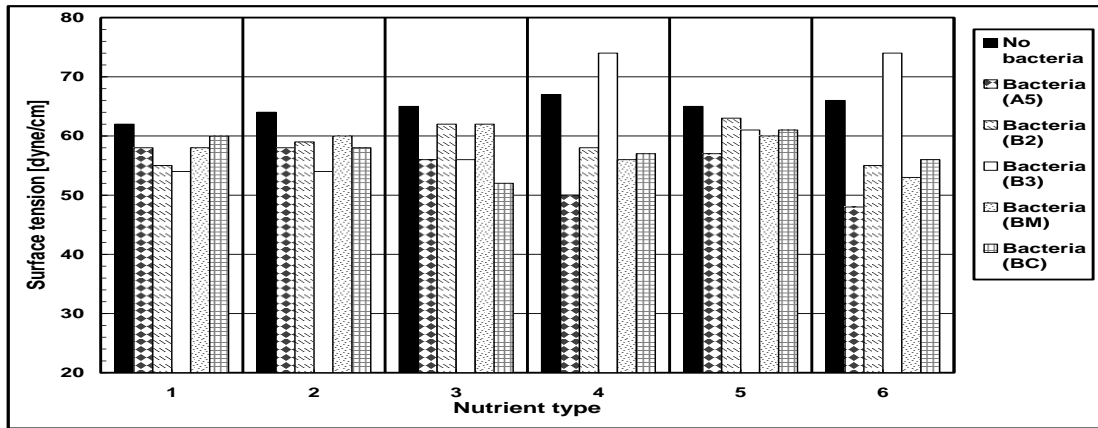


Fig. 7- Surface tension of the aqueous phase in the presence of different types of bacteria when activated with the different types of nutrient at 30 °C.

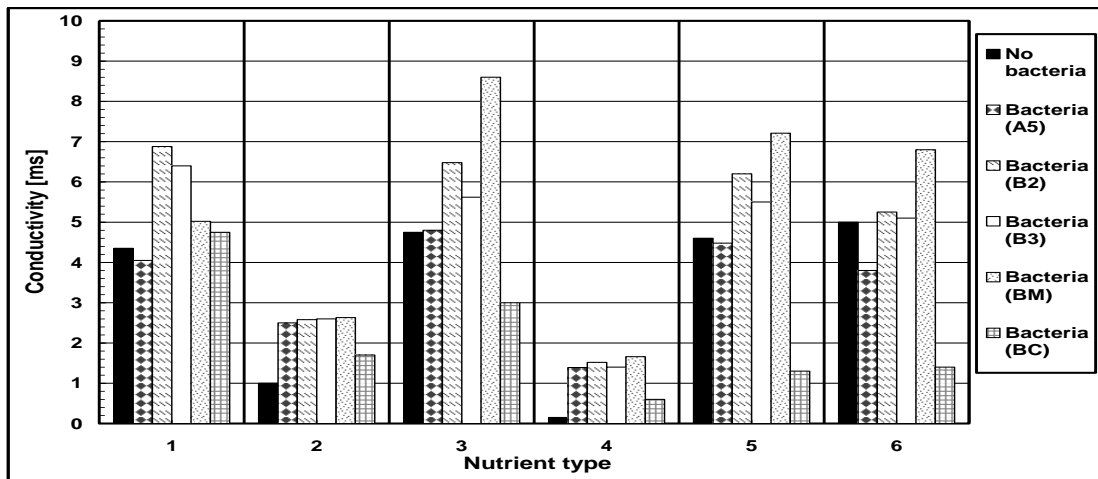


Fig. 8- Conductivity of the aqueous phase in the presence of different types of bacteria when activated with the different types of nutrient at 30 °C.

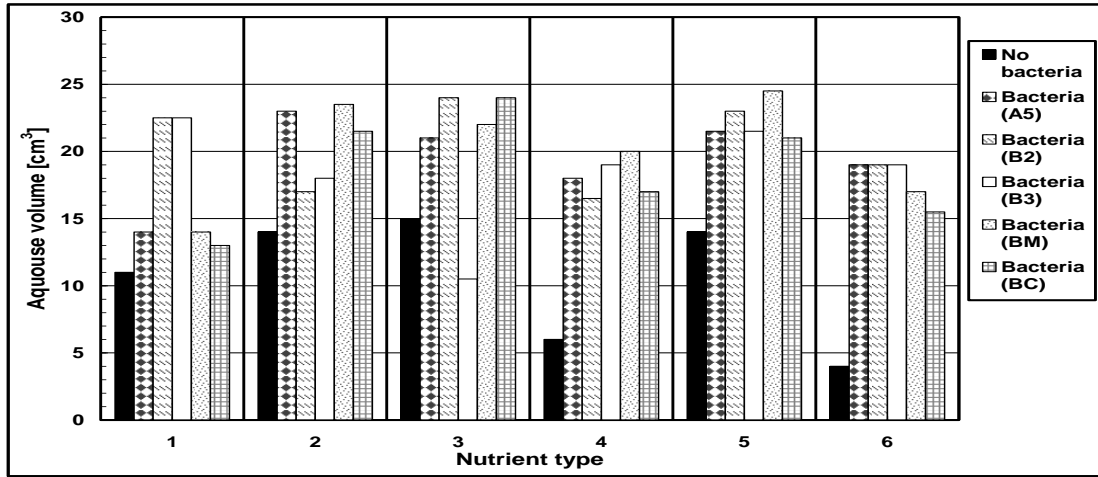


Fig. 9- Aqueous phase volume in the presence of different types of bacteria when activated with the different types of nutrient at 30 °C.

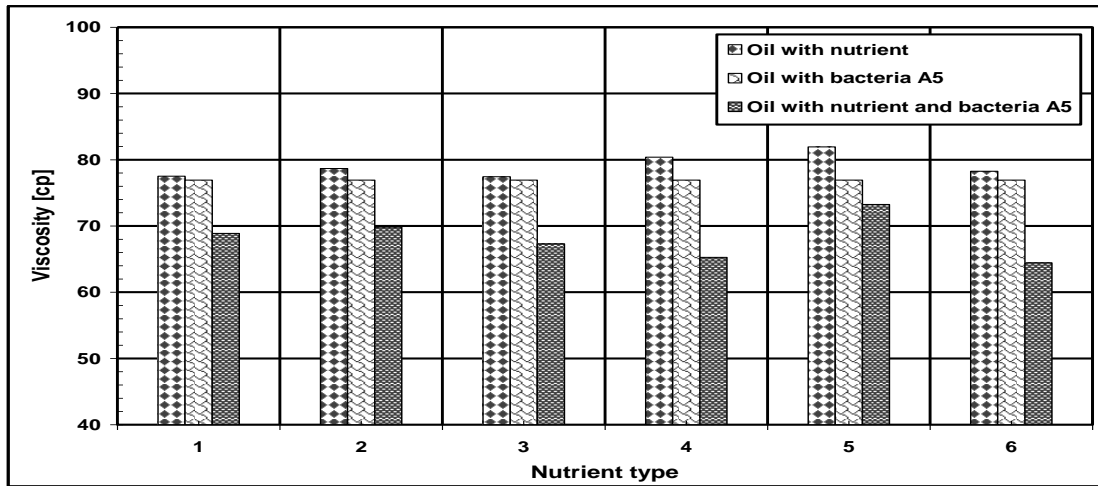


Fig. 10- Comparison of the viscosity in cases of the oil only, oil with nutrient and oil with nutrient in the presence of bacteria (oleic phase) for different nutrients at 30 °C .

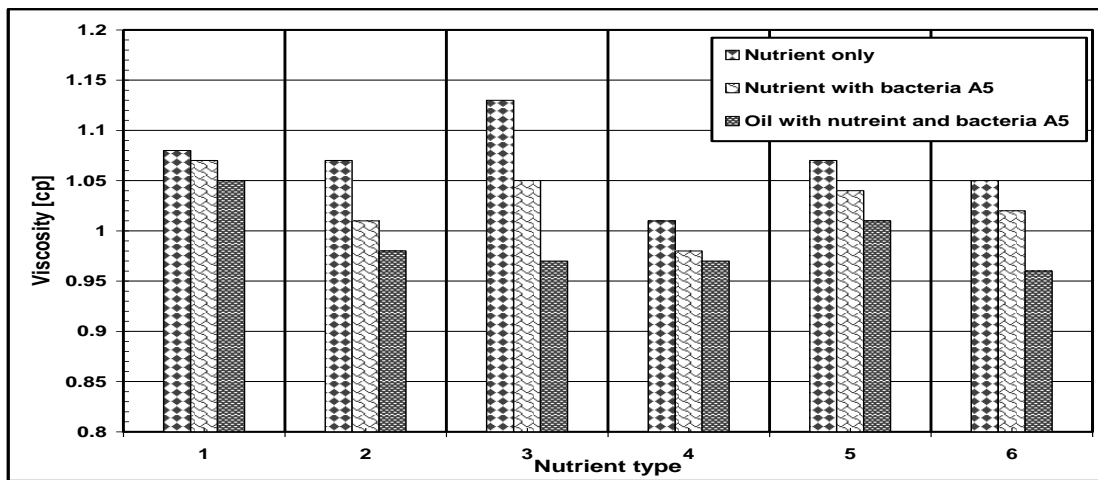


Fig. 11- Comparison of the viscosity in cases of the nutrient only, nutrient with bacteria A5 and oil with nutrient in the presence of bacteria A5 (aqueous phase) for different nutrients at 30 °C.

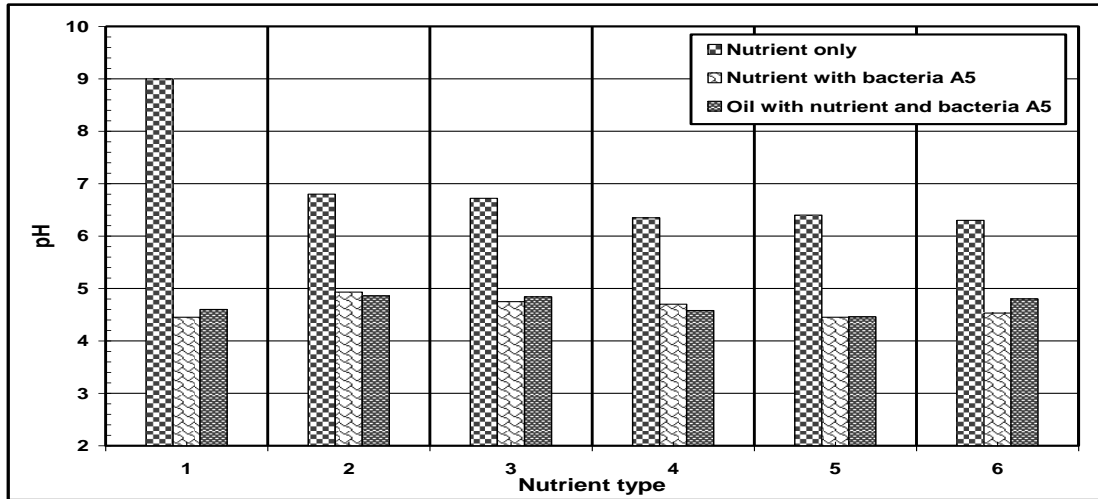


Fig. 12- Comparison of the pH in cases of the nutrient only, nutrient with bacteria A5 and oil with nutrient in the presence of bacteria A5 (aqueous phase) for different nutrients at 30 °C.

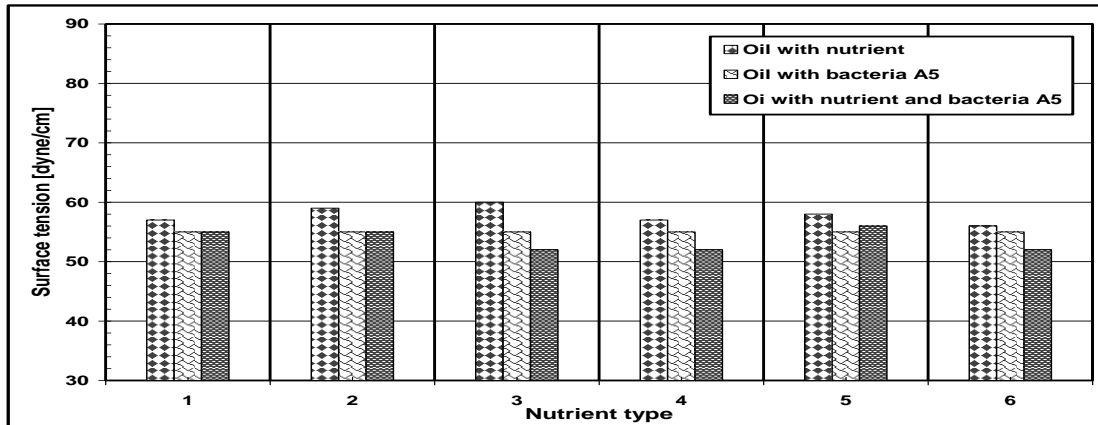


Fig 13- Comparison of the surface tension in cases of the oil only, oil with nutrient and oil with nutrient in the presence of bacteria (oleic phase) for different nutrients at 30 °C.

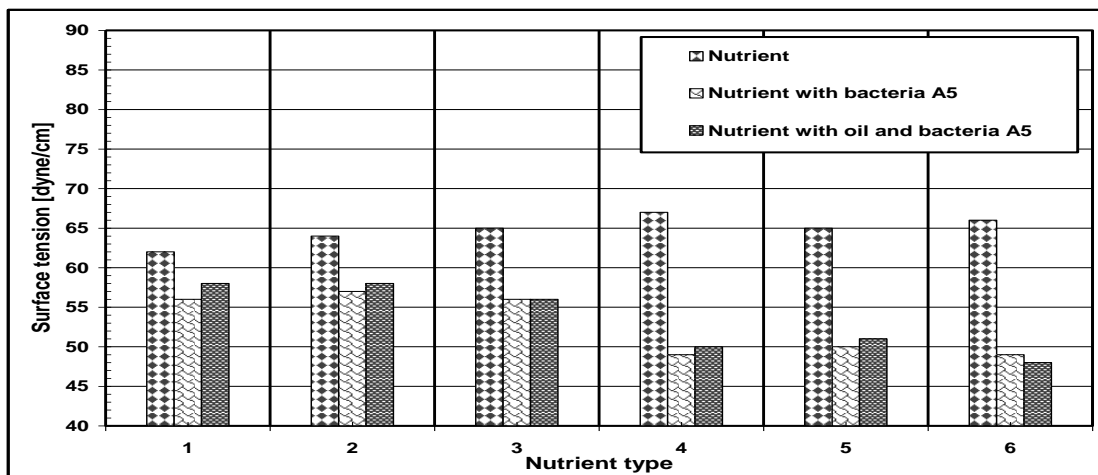


Fig. 14- Comparison of the surface tension in cases of the nutrient only, nutrient with bacteria A5 and oil with nutrient in the presence of bacteria A5 (aqueous phase) for different nutrients at 30 °C.

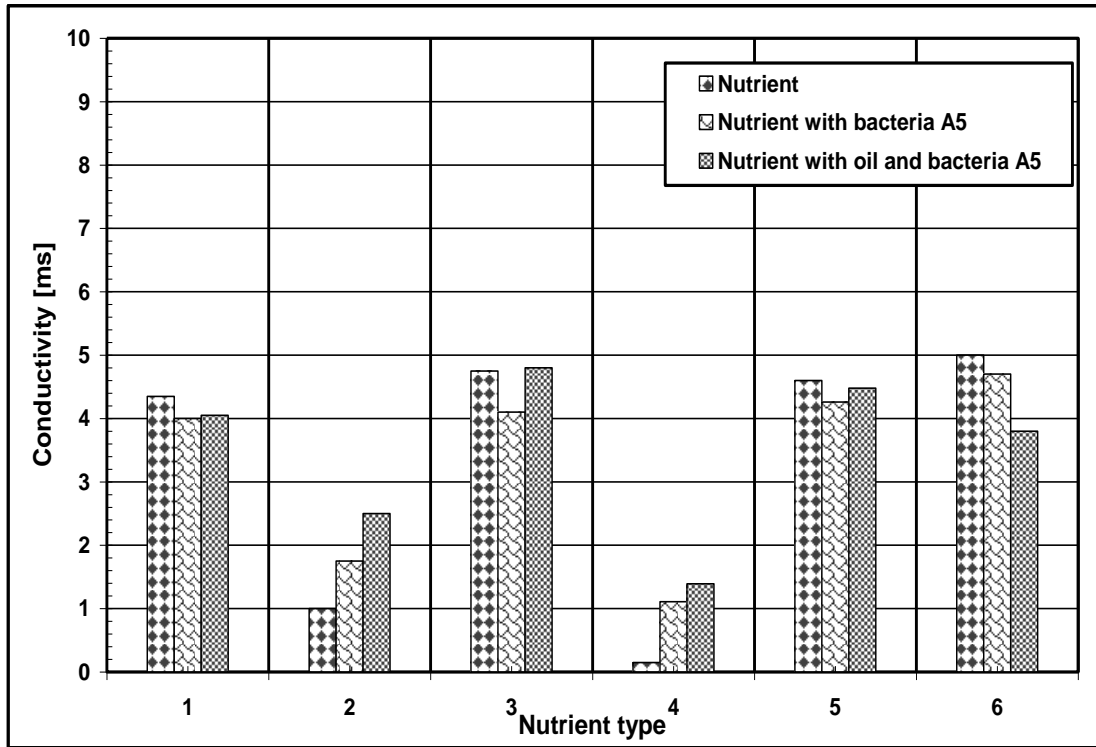


Fig. 15- Comparison of the conductivity in cases of the nutrient only, nutrient with bacteria A5 and oil with nutrient in the presence of bacteria A5 (aqueous phase) for different nutrients at 30 °C.

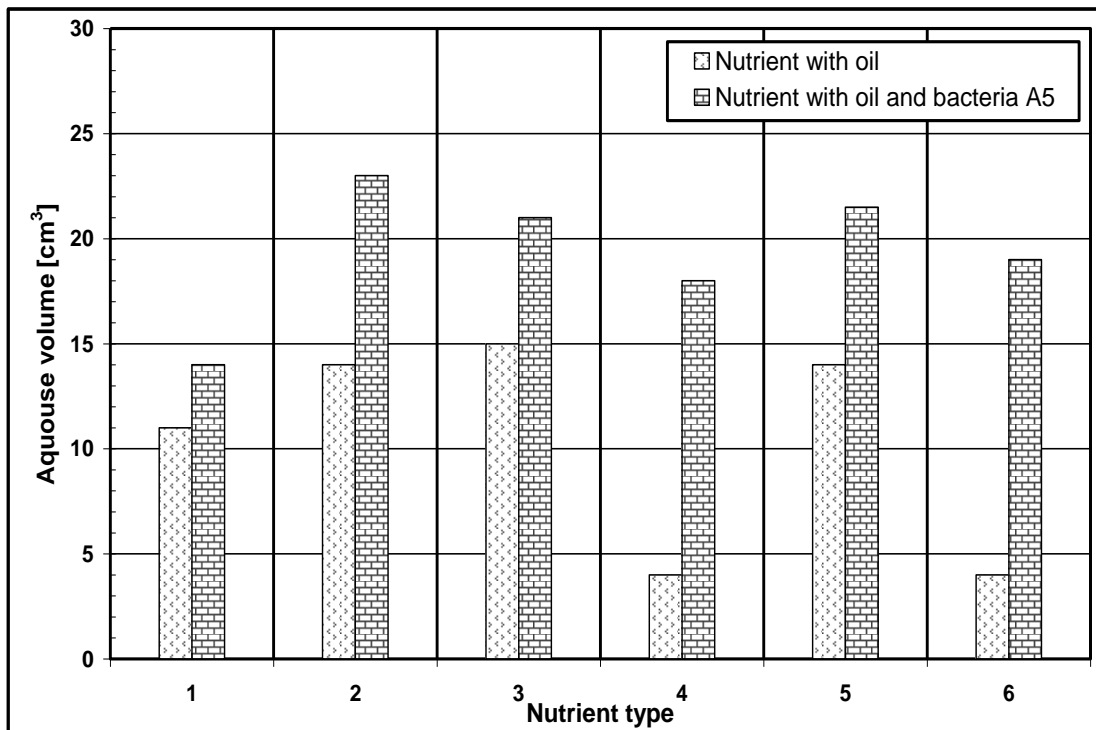


Fig. 16- Comparison of the aqueous phase volume in cases of the oil with nutrient and oil with nutrient in the presence of bacteria for different nutrients at 30 °C.