Properties and Performance of the *Pseudomonas aeruginosa* Bacteria Under High Salinity and Temperature

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Abstract

Utilization of surfactants for Enhanced Oil Recovery (EOR) is an accepted technique with high potential from the technical point of view. However, technology application is frequently limited by environmental effect from the high formation salinity and high temperature. The activity of the bacteria, which produce the biosurfactant as a metabolism could be significantly improved through the use of a suitable media and a suitable nutrient using an optimization technique.

In a previous work, the authors succeeded to isolate and identify three types of bacteria, from three different oil cruds which were collected from three different Egyptian fields. The study showed that the best suitable type for the MEOR, among the three isolated types, is the *Pseudomonas aeruginosa.*

The objectives of this paper are: (1) to study the effect of the environmental conditions that may affect the activity of bacteria, (2) to select the most suitable media for the bacteria to give maximum activity and (3) to study the effect of salinity and temperature on the performance of this bacteria.

The results showed that, the best environmental conditions, at which this bacterium gives its maximum activity are: incubation time of 24 hours, at temperature of 35 ^{O}C, salinity 20,000 ppm and pH value equal to 7. The results showed also that at temperature 30 ^{O}C, and salinity 50,000 ppm, with suitable nutrient, the used type of bacteria can decrease: the surface tension of the aqueous solution up to 28 dyne/cm instead of 72 dyne/cm, the interfacial tension between the oleic phase and aqueous phase to 18 dyne/cm instead of 58 dyne/cm and also decrease the original viscosity of the oil by 2 cp. At higher temperature of 90 ^{O}C and salinity of 100,000 ppm the used bacteria still active but its efficiency decreased very match and became very low.

Introduction

The fermentative (microbiological) production of value-added chemicals from biomass derived substrates is the historical essence of industrial biotechnology. Surfactants are a distinct class of EOR chemicals that could be economically produced using biological methods. Application of biosurfactants for EOR generally falls into the category of Microbial Enhanced Oil Recovery (MEOR).

The application of MEOR can be divided into two distinct processes that are: 1) the beneficial and deliberate introduction of microorganisms into oil bearing formations, or 2) tending to the nutritional requirements of beneficial organisms that are already present in the oil-bearing formations [(1)].

The idea in both cases is to produce surface active compounds which are amphiphilic molecules with both hydrophilic and hydrophobic regions causing them to aggregate at interface between fluids with different polarities such as water and hydrocarbons [(2)] These biomolecules may also decrease the interfacial and the surface tension [(3)].

Recent studies in both Cairo and King Saudi Universities [(4-10)] were performed to investigate the effect of biochemicals from microorganisms, originally present in the cruds oil, on the interfacial forces, phase variation of oleic/aqueous systems and rock

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wettability. In some of these studies [4,6,7], it was found that the interfacial and the surface tension were markedly affected by the nutrient type and its concentration. These effects depend on the temperature at which the tests were carried out. In another studies [9-10], two Egyptian cruds were used, one of them contained bacteria of *Clostridium* type and the other contained *Bacillus* type. The investigators found that, for each crude oil, the phase variation and the interfacial tension was affected not only by the bacterial nutrient type and concentration but also by salinity, temperature and time of contact between the crude oil and nutrient used, and the type of crude oil.
Also some recent studies [11-25], pilot tests and field trials which were conducted during last ten years, were found to be limited to the conditions of using the bacteria and it was recommended to be at the low temperature and salinity.
The authors of the present study [26], in a previous work, studied the isolation of bacteria from three different Egyptian oil fields and succeeded to isolate and identify three types which are *Pseudomonas aeruginosa, Pseudomonas fluerescens biotype G* and the *Celluiosimicrobium celluians*. The best type, suitable for MEOR from these three isolated types, was found to be *Pseudomonas aeruginosa*. Nutrient with Molasses base was found to be the best type of nutrient for the *Pseudomonas aeruginosa* but the activity of this type of bacteria is largely affected by the temperature and salinity of the solution used and it needs a further research to investigate the real effect of the temperature and salinities on the activity of the *Pseudomonas aeruginosa*.
The aim of the present research is to study the effect of temperature and salinity on the performance of the *Pseudomonas aeruginosa* and its ability to produce the biosurfactant.

Experimental work

Materials used

Bacteria

The bacteria used in this study is the *Pseudomonas aeruginosa*, which was isolated (in the pervious study of the authors) from an Egyptian crude oil that is different from the crude that is used in the present study. This bacteria has the following characteristics: round, granular, flat, colorless, translucent, an aerobic, gram-negative, short rods, no spore performed and not resist to acid [26].

Media

Two types of media were selected to incubate the bacteria, the first is the basic media (Nutrient growth media [27]) as it was found in the literature that it is suitable for the activation of the most types of bacteria. The composition of this media is given in table 1. The second type is the modified media [27] which was selected specially for the used bacteria *Pseudomonas aeruginosa* to get a higher growth. The composition of this modified type is given in Table 2.

Nutrients

As proved before (in the pervious study of the authors) the best nutrient type was the nutrient number 6[26] with the composition of Molasses (2 gm/lit) and NaCl (5 gm/lit) and to study the effect of adding phosphate and nitrite salts to this nutrient, four new nutrients (named 7,8,9 and 10) were prepared with different compositions as given in Table 3.

Brine water

Two brine solutions were used, the first is the formation water (with salinity of 100,000 ppm) and the second one is the sea water (with salinity of 40,000 ppm). Both

of them were obtained from an oil field, which located in the Gulf of Suez area, Egypt. The compositions of these two solutions are given in table 4.

Oil

A crude oil, obtained from the same oil field, is used and tested, to be sure that it is free from any indigenous bacteria [26].

Experimental procedure

In order to determination of the properties of bacteria in the absence of oil, the selected bacteria were exposed to different environmental conditions in the absence of the reservoir oil to judge its activity at the different environmental conditions and to determine the following:

The growth curve of the used bacteria was determined using the following steps:

1- Prepare one liter from the nutrient growth media with a final pH value equal to 7.
2- Separate every 25 cm of this nutrient in a different tube.
3- Add 0.1 cm^3 from bacteria solution to every tube and incubate the tubes under temperature of 30^OC.
4- Measure the optical density for the first tube after 2 hrs then for the second tube after 4 hour and so on up to 36 hours.
5- Repeat step 4 but incubate the bacteria under shaking conditions with 150 ppm.

To study the effect of salinity on the activity of the used bacteria the following steps were performed:

1- Prepare 0.8 liter from the nutrient growth media with a final pH value equal to 7.
2- Separate every 100 cm^3 from this nutrient in eight different flasks and change the salinity inside each flask by adding a different quantity of NaCl salt in each flask to adjust the salinity of the solutions in the eight flasks as to become: 0, 500, 10000, 20000, 40000, 60000, 80000 and 100000 ppm respectively.
3- Add 0.1 cm^3 from the bacteria solution to each of the eight flasks and incubate them at constant temperature of 30^OC.
4- Measure the optical density for each flask after 24 hours.

To study the effect of temperature on the bacteria activity the following steps were performed:

1- Prepare one liter from the nutrient growth media with a final pH value equal to 7.
2- Separate every 100 cm^3 in ten different flasks.
3- Add 0.1 cm^3 from bacteria solution to each one of the ten flasks and incubate every flask at different temperature of (25, 30, 35, 40, 45, 50, 55, 60, 65 and 70^OC).
4- Measure the optical density for each flask after 24 hours.

To study the effect of salinity the following steps were performed:

1- Prepare 0.8 liter from the nutrient growth media with a final pH value equal to 7.
2- Separate every 100 cm^3 in eight different flasks then adjusting the pH by adding some quantity of NaOH to raise the pH value to 8,9,10,11,12 and adding the acid of HCl to lower it to 6,5,4.
3- Add 0.1 cm^3 from the bacteria solution to every flask and incubate the flask under temperature of 30^OC.

4- Measure the optical density for each flask after 24 hours.

General procedure for the determination of the physical properties of the aqueous and oleic solutions:

1- Clean and sterilize the graduated test tubes using the following sequence:
   - Wash each tube with detergent and tap water followed by distilled water then put it inside the oven at a temperature higher than 100 ^{O}C for 15 min for drying.
   - Cover each test tube with the cotton plug and put it inside the oven at temperature of 160 ^{O}C for 60 min for sterilization.

2- Prepare the solution of each nutrient in a flask then put it inside the Autoclave for 20 min to sterilize the nutrients.

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 and put it inside the Autoclave for 20 min for the sterilization.

5- Transfer 3 cm^3, in front of a flame, (under sterilized condition) from the used bacteria solution to the new solution, which will be used after a time period, (one or two days) to standardize the age of bacteria.

6- In front of the flame (under sterilized condition) open the sterilized empty tube and put inside it, 25 cm^3 of sterilized oil, 22 cm^3 of the selected sterilized nutrient and 3 cm^3 from the bacteria solution.

7- Seal off the test tubes very well using a sterilized plug and shake it to get a good contact between the aqueous nutrient solution and the crude oil in order to be sure that the bacteria is distributed all over the tube, then close the tubes with a cotton plugs.

8- The tubes should be left for incubation during a time interval of one week at constant temperature of 30 ^{O}C.

9- The aqueous and the oleic phase volumes, in each tube, were recorded and the separation of the aqueous phase from the oleic phase using pipit was done, then measure separately the fluid properties of each phase such as: viscosity, surface tension, pH and conductivity.

These general procedures were used when doing the experiments that were done to: 1) select the best media and nutrient for the used bacteria, 2) study the effect of the salinity and the temperature on the performance of the used bacteria.

The criteria for judgment the performance of the bacteria under study, with different media and nutrient at different salinities and temperatures, are that the best performance of the bacteria could be obtained under the following conditions: 1) the viscosity of the oleic phase should be at its minimum level, 2) the surface tension, the pH, and the conductivity of the aqueous phase should be also be at their minimum level.

Selection of the best suitable media for the used bacteria

The basic media, which used in this study, is a suitable media for the most types of bacteria. But for the used bacteria, a specific media which will be called here a "modified media" is used for the activation of this bacteria. This modified media should contain, in its composition, all the components of salts which may increase the activity of the used bacteria. The used bacteria is an aerobic, so it needs some salts having oxygen to activate it, therefor the phosphate and nitrite salts were added to the nutrient number 6 and this was the idea by which the four new nutrients solution were

prepared, from nutrient number 7 through nutrient number 10 as their compositions were given in table 4 in comparison with the composition of the nutrient number 6. In order to determine which one of the two media is more suitable for the activity of the used bacteria, the performance of the bacteria is determined by measuring its effects on the different properties of the aqueous and the oleic phase when the bacteria is incubated in each one of the two media under study and by using each one of the five available nutrients solutions and this is done as follows:

1- Prepare 0.25 liter of the basic media (nutrient growth) in a flask and another 0.25 liter from the modified media solution in another flask.
2- Transfer 3 cm^3 from the bacteria solution to each one of the two media, and lift the flask for incubation 24 hrs at 30 ^OC. This to be sure that all the bacteria are in the same age when starting the experimental work.
3- Prepare 50 cm from every nutrient (6, 7, 8, 9 and 10).
4- Prepare 5 tubes and put inside each tube 22 cm of the selected nutrient solution (one nutrient solution for each tube), 3 cm^3 from the basic media solution and 25 cm^3 of oil.
5- Prepare another 5 tubes and put inside each tube 22 cm^3 of the selected nutrient solution (one nutrient solution for each tube), 3 cm^3 from the modified media solution and 25 cm^3 of oil.
6- Lift the ten tubes for one week for incubation at 30 ^OC.
7- The aqueous and oleic phase volumes, in the tube, were recorded and the separation of the aqueous phase from the oleic phase using pipit was done, then measuring separately the fluid properties of each phase such as viscosity, surface tension, pH and conductivity.

Determination of the effect of the salinity and temperature on the performance of the used bacteria, in the presence of modified media, oil and the best nutrient

In the previous section the effect of increasing the salinity and temperature, on the performance of the used bacteria were studied in case of incubating the bacteria under study in the basic media. As the results of the above experimental work showed that the performance of used bacteria could be improved when the bacteria is incubated with another media and nutrient. So, to complete the study of the performance of the used bacteria, the effects of increasing the salinity (as a value and a composition) and the temperature, on the performance of the used bacteria should be determined again but, in this time, when the bacteria is incubated in the modified media and with the new best nutrient in the presence of oil. In order to achieve this goal, the following steps were done:

The effects of salinity value and composition were determined using the following steps:

1- Prepare one liter of the modified media solution in a flask.
2- Transfer 3 cm^3 from the bacteria solution to the modified media and lift it 24 hours for incubation under temperature 30^OC.
3- Prepare 3 liter from nutrient number 8.
4- Take one liter from nutrient number 8 and divided it equally into 5 flasks and change the salinity of each flask using NaCL only to be 20000, 40000, 60000, 80000 and 100000 ppm respectively.
5- Take another one liter from nutrient number 8 and subdivided it equally into 5 flasks and change the salinity of each flask using the same ratio of

the salt that are found in the composition of sea water, to be 20000, 40000, 60000, 80000 and 100000 ppm respectively.

6- Take the last liter from nutrient number 8 and subdivided it equally into 5 flasks and change the salinity of each flask, using the same ratio of the salts that are found in the composition of formation water, to be 20000, 40000, 60000, 80000 and 100000 ppm respectively.

Now we have 15 flasks which contain 15 different samples of nutrient number 8. The only different between these nutrients samples is the value of the salinity and its composition.

7- Prepare 15 test tubes, put inside each tube 22 cm^3 of certain nutrient sample (one for each tube), 3 cm^3 from the modified media and 25 cm^3 of oil.
8- Lift the 15 tubes for one week for incubation at 30 ^{O}C.
9- The aqueous and oleic phase volumes, in the tube, were recorded and the separation of the aqueous phase from the oleic phase using pipit was done, then measuring separately the fluid properties of each phase such as viscosity, surface tension, pH and conductivity.

The effect of the temperature increase was determined using the following steps:

1- Prepare one liter of the modified media solution in a flask.
2- Transfer 3 cm^3 from the bacteria solution to the modified media and lift it 24 hours for incubation under temperature 30^{O}C.
3- Prepare one liter from nutrient number 8 then increasing its salinity to 40,0000 ppm as sea water condition. (This is due to the most saline solution that the bacteria will be exposed to it is the sea water).
4- Prepare 4 test tubes put inside each tube 22 cm^3 of the nutrient number 8, 3 cm^3 from the modified media solution and 25 cm^3 of oil.
5- Lift the four tubes for one week for incubation under different temperature at 30, 50, 70, 90 ^{O}C respectively.
6- The aqueous and oleic phase volumes, in the tube, were recorded and the separation of the aqueous phase from the oleic phase using pipit was done, then measuring separately the fluid properties of each phase such as viscosity, surface tension, pH and conductivity.

Results and discussions

Properties of bacteria in the absence of oil

Growth curve at static and shaking conditions

The growth curves of the used bacteria, at the static condition and at shaking with 150 rpm, are shown in Fig. 1. It is clear from this figure that the best growth time is reached after 22 to 24 hours of the start of incubation in both static and shaking conditions. It can easily observe from fig.1, that the highest density of used bacteria, at the static conditions, was reached after 22 hours of incubation time while for the chalking conditions it is reached after 24 hours. So, due to this result, all the measurement for the pH, salinity and temperature were record at the time of 24 hrs after the incubation starting time.

Effect of salinity on the bacteria activity

Fig. 2 gives the change of bacteria density with the salinity of the solution of incubation, it is clear from Fig. 2 that the bacteria density increases with the increase of the salinity up to 20,000 ppm then after that it decreases with the further increase in salinity. So, it can easily conclude, from Fig. 2, that the optimum value of salinity, at which the used bacteria live with a maximum activity, is 20,000 ppm. After this

optimum value of the salinity the activity of this type of bacteria decreases gradually as the salinity increase. It is also clear from this figure that there is a little growth of used bacteria at higher salinity value (the density is around 0.22 at 100,000 ppm salinity) which mean that at this higher salinity the used bacteria can live but with very low activity.

Effect of temperature on the activity of the bacteria

Fig. 3 shows the effect of increasing the temperature of incubation, on the used bacteria activity. It can be observed from Fig. 3 that the optimum temperature for the growth and activity of the used bacteria is 35 ^{O}C. After this optimum temperature, as the temperature increases the efficiency of bacteria decrease. It is also seen that at 70 ^{O}C bacteria still growth but with very low activity as its density reaches 0.12 at this temperature.

Effect of pH on the activity of the bacteria

Fig. 4 gives the affect of the pH value, of the incubation media, on the activity of the used bacteria. It is clear from Fig. 4 that the best value for pH, which gives more growth, is between 7 and 8.

As a conclusion, from the figure 1 through figure 4, the optimum environmental conditions that make the used bacteria gives its maximum efficiency to produce its metabolism are: 24 hours of incubation time in a media has salinity of 20,000 ppm, pH value between 7 and 8, and at a temperature of 35^{O}C. As these optimum conditions change the efficiency of the used bacteria and its activity decrease. It is important to keep in mined that the bacteria under study, Pseudomonas *aeruginosa* can live at temperature 70^{O}C and salinity of 100,000 ppm but with very low activity level.

Comparison between the basic and modified media, with different nutrient, on the performance of the used bacteria

Table -5 shows the comparison between the values of the different physical properties, of the aqueous and oleic phases, which measured when the bacteria is activated with the basic media and its corresponding values when the bacteria is activated with the modified media. These different properties, of the aqueous and oleic phase, which represent a good criterion for the judgment of the performance of the bacteria are the viscosity, the pH value, the conductivity and the surface tension. It is clear from table 5 that, regardless the nutrient type used, the performance of the bacteria with the modified media is much better, for the use in the MEOR projects, than its performance with the basic media. As it always gives, with the modified media and any nutrient types, a lower viscosity of oleic and aqueous phase, a more suitable pH value and conductivity and it gives a much lower surface tension value for the aqueous phase. It can also easily conclude, from table 5, that the performance of the used bacteria is affected, to a great extent, by the type of nutrient which used with the modified media. The best performance could be obtained when using nutrient 8 with the modified media. The used bacteria, with this nutrient and the modified media decreases the viscosity of the oleic phase to 61.05 cp which is lower than the viscosity of the crude oil itself (62.2 cp) this result means that:

1- The used bacteria use the crude oil as an additional carbon source for its activity and this is done by creaking the heavy components of the crude which resulted in a decrease in its viscosity.
2- It is better for the used bacteria to live in presence of nitrite salts than any other salts like phosphate salt, that is why it gives the lowest viscosity and surface tension with nutrient 8 which is the only nutrient that contain a nitrite

salts only with NaCl and it does not contain a phosphate salt (as given in table 4).

Finally, it is important to mention that the presence of used bacteria with nutrient 8 and the modified media decreases the interfacial tension between the oil and the nutrient solution to 18 dyne/cm instead of 58 dyne/cm in case of the absence of the used bacteria. The used bacteria with nutrient 8 decreases the surface tension of the aqueous phase to 32 dyne/cm instead of 45 dyne/cm when it is activated with nutrient 8 but in the basic media.

Study of the salinity and the temperature on the performance of the used bacteria when it is incubated in the modified media with its best nutrient and in the presence of oil

Effect of different water salinities and its composition

Figures 5 through 8 show the effects of increasing the salinity, of the nutrient used, on the different properties of the oleic and aqueous phase such as viscosity, pH value, surface tension and the conductivity. It is clear from these figures that the salinity range from 40,000 ppm to 60,000 ppm is the best salinity range for the performance of the used bacteria with the all types of brine solution compositions such as NaCl equivalent, sea water composition and formation water composition. It also shows that the performance of the used bacteria is greatly affected not only by the values of the salinity of the solution used but also by the composition of this salinity. Figure 5 shows that for all values of the salinities, the viscosity of the oleic phase decreases much more, when using solution have a salt composition similar to the that of formation water, than when it used with solution as that of the sea water or solution of NaCl equivalent. Fig. 6 shows that the pH values are at its best reasonable values when the salinity compositions of the solutions used are like that of the formation water. Fig. 7 also confirms this result as the surface tension of the aqueous phase is at its lowest level when using solution have a salinity composition as that of the formation water for all the salinity values. Finally, for the data that are given in table 3, as a conclusion, the behavior of the bacteria, in the presence of the formation water, is much better than its behavior in the sea water and hence it is better for the used bacteria to live in an environment that has

1- Lower concentration ratio of NaCl compared to the total salinity.
2- Higher concentration ratio of $CaCl_2$ compared to the total salinity.
3- $MgCl_2$ concentration ratio to the total salinity until a certain value below its concentration in the sea water.
4- Lower concentration ratio of the $CaSO_4$ compared to the total salinity.

It is wealthy to mention that the performance of bacteria is changing due to the presence of oil. Recalling that in the previous section the results of studying the effect of salinity on the performance of the used bacteria in absent of oil showed that the maximum activity was obtained at salinity of 20,000 ppm (Fig. 2) while the results obtained in Figures 5 through 8 shows that the best performance of the used bacteria in the presence of oil was obtained at salinity between 40,000 to 60,000 ppm. This means that the presence of oil increases the capability of the used bacteria to resist the high salinity and this represent another prove for the previous conclusion concerning that the used bacteria use the oil as another carbon source for its nutrient.

Effect of temperature increase of the performance of the used bacteria

The effects of increasing the temperature on the activity of the used bacteria, is shown in Figures 9 through 12. It is clear, from these figures that:

The best temperature, at which the used bacteria gives its maximum performance, is 30 ^{O}C while the increase in the temperature above 30 ^{O}C, decreases the efficiency of the bacteria. As at temperature 30 ^{O}C the viscosity of the oleic phase was minimum (Fig. 9) and this viscosity increases as the temperature increase. This phenomenon was confirmed in figures 10, 11 and 12 as at this temperature the pH value is at its most suitable value, the surface tension and the conductivity are at their lowest possible level. It is clear from the same figures that the bacteria still have a little activity at 90 ^{O}C as it still gives a lower viscosity in the oleic solutions than that of the oil and nutrient without bacteria (Fig. 9). Also, it gives a lower surface tension 56 dyne/cm than that of the case without bacteria which was 64 dyne/cm. Fig. 10 shows that the pH decreases at 90 ^{O}C to 4.8 instead of 7 in the case of bacteria absent. This mean bacterium still active at this higher temperature of 90^{O}C as it produces acids and surfactant which lower the pH value of the aqueous phase and the viscosity of the oleic phase, but this activity is very low due to high temperature.
For the last tube, in which the incubation of the bacteria is made with the salinity of formation water of 100,000 ppm and at temperature of 90^{O}C, it gives the following results: it does not decrease the viscosity of the oleic phase, it decrease the surface tension of the aqueous phase to 58 dyne/cm only and the pH value of the aqueous phase decreases to 4.3 (not a suitable value for activation of the bacteria).
Now for all the evaluation of the characterization of used bacteria, it is observed that it can live at temperature of 90 ^{O}C and salinity of 100,000 ppm but its activity is very low.

Conclusions

- The present study gives a developed methodology to:
  - Select of the best media and nutrient for the activity of the used bacteria which was isolated from an Egyptian field.
  - Determine the optimum environmental conditions of: the incubation time, the salinity of the nutrient used, the temperature and the pH value.
- Based on the experimental works of the present study, the following conclusions can be obtained:
  - The used bacteria use the crude oil as an additional carbon source. So, it is not enough to study the capability of any type of bacteria only with aqueous solutions but should be tested in the presence of the oil.
  - At temperature 30 ^{O}C the used bacteria succeeded to decrease the surface tension of the aqueous phase from 72 to 28 dyne/cm and that of the oil from 53 to 50 dyne/cm. The interfacial tension was decreased from 58 to 18 dyne/cm and the oil viscosity was decreased below its original viscosity by around 2 cp.
  - The activity of the bacteria decreases very much when the temperature and the salinity increase. As it does not decrease the viscosity of the oleic phase, it decrease the surface tension of the aqueous phase to 58 dyne/cm only not to be 28 dyne/cm, the value that obtain at 30^{O}C and 50,000 ppm, and the pH value of the aqueous phase decreases to 4.88 (not suitable value for activation of the bacteria) when the used bacteria is incubated at salinity 100,000 ppm (sea water) and temperature 90^{O}C.
  - The composition of formation water salinity is more suitable for the activity of used bacteria than the composition of sea water salinity or NaCl equivalent.

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27- Ronald M. Atlas "Microbiological Media" Second Edition, Copyright 1997, International standard book number 0-8493-2638-9.

TABLE 1 - THE COMPOSITION OF THE BASIC MEDIA ([27])

Media No.	Composition	Weight [gm/lit.]
Basic media	Peptone	5
	Yeast	2
	NaCl	5
	Glucose	10
	Beef	2

TABLE 2- THE COMPOSITION OF THE MODIFIED MEDIA ([27])

Media No.	Composition	Weight [gm/lit.]
Modified media	Proteose peptone	20
	Glycerol	10
	K_2HPO_4	10
	$MgCl_2.6H_2O$	1.4

TABLE 3- DIFFERENT TYPE OF NUTRIENT

Nutrient No.	Composition	Weight [gm/lit.]
6	Molasses	2
	NaCl	5
7	Molasses	2
	NaCl	5
	K_2HPO_4	10
8	Molasses	2
	NaCl	5
	KNO_3	10
9	Molasses	2
	NaCl	5
	KNO_3	5
	K_2HPO_4	5
10	Molasses	2
	NaCl	5
	$(NH_4)_2. HPO_4$	10

TABLE 4– CHEMICAL COMPOSITION OF THE SEA WATER AND THE FORMATION WATER FOR THE FIELD UNDER STUDY.

Components	Sea water	Formation water
NaCl [ppm]	28,500	50,000
$CaCl_2$ [ppm]	1,500	42,000
$MgCl_2$ [ppm]	3,000	8,000
$CaSO_4$ [ppm]	7,000	0
Total salinity [ppm]	**40,000**	**100,000**

TABLE 5- COMPARISON BETWEEN THE EFFECT OF THE BASIC AND THE MODIFIED MEDIA ON THE PERFORMANCE OF THE USED BACTERIA AFTER INCUBATION FOR ONE WEEK AT 30 ^{O}C.

Nutrient No.	μ (Oleic) cp		μ (aqueous) cp		pH (aqueous)		Conductivity (aqueous) ms		Surface tension (aqueous) dyne/cm	
	Basic	Modified	Basic	Modified	Basic	Modified	Basic	Modified	Basic	Modified
6	63.7	62.8	0.95	0.92	5.2	6	3.6	3.2	48	45
7	N/A	65.95	N/A	0.96	N/A	6.86	N/A	7.75	N/A	55
8	63.5	61.05	0.92	0.9	6.7	7.1	8.7	8	45	32
9	N/A	64.98	N/A	0.98	N/A	6.7	N/A	8.5	N/A	40
10	64.2	63.45	0.95	0.94	6.49	6.8	10.5	9.41	50	35

N/A = not available

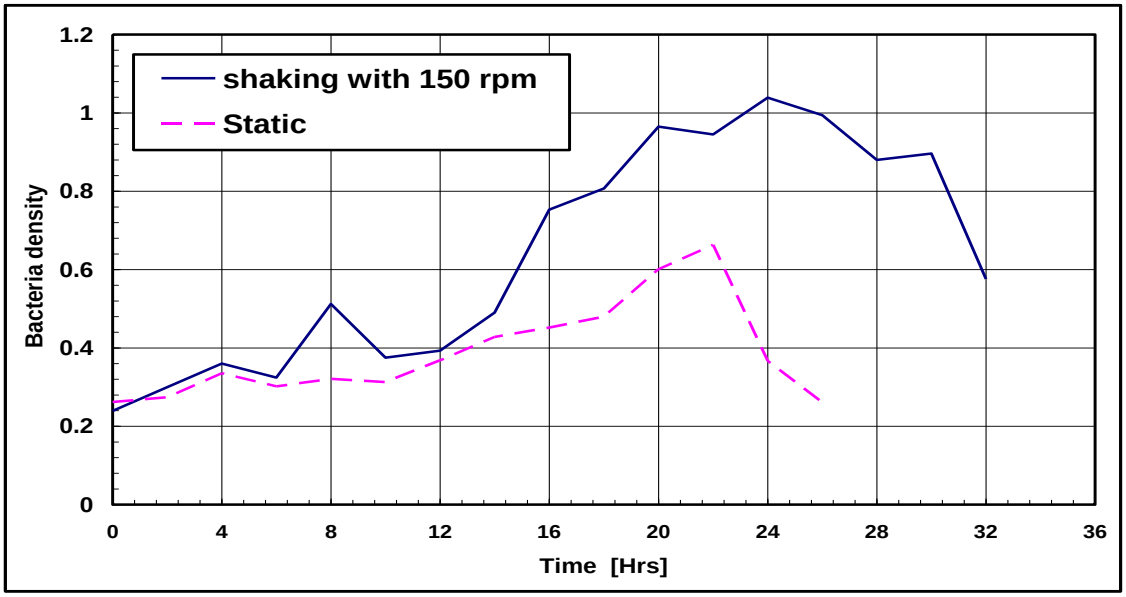

Fig. 1- Density of used bacteria growth with time in static and shaking conditions at 30 ^{O}C.

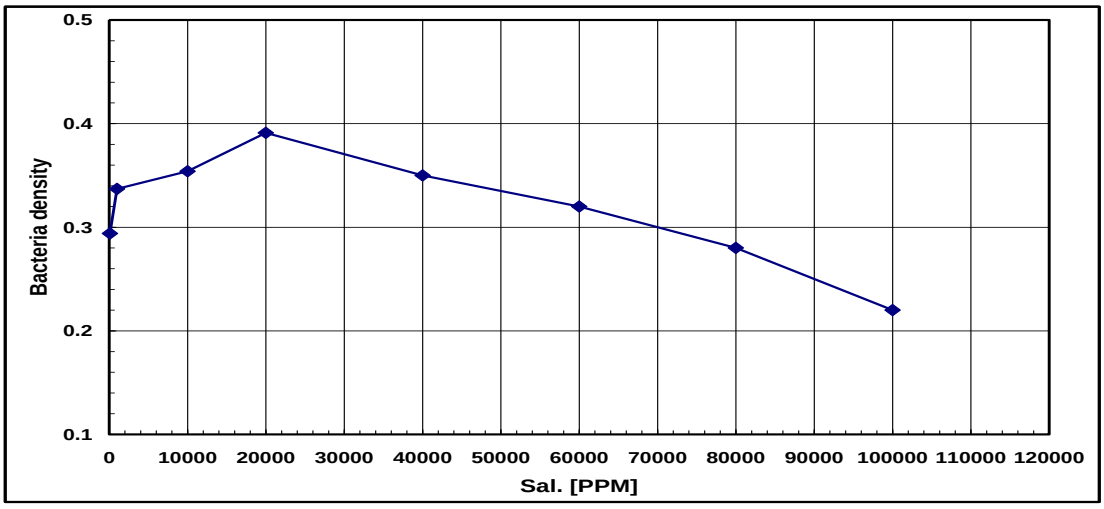

Fig. 2- Effect of salinity on the used bacteria growth at 30 ^{O}C.

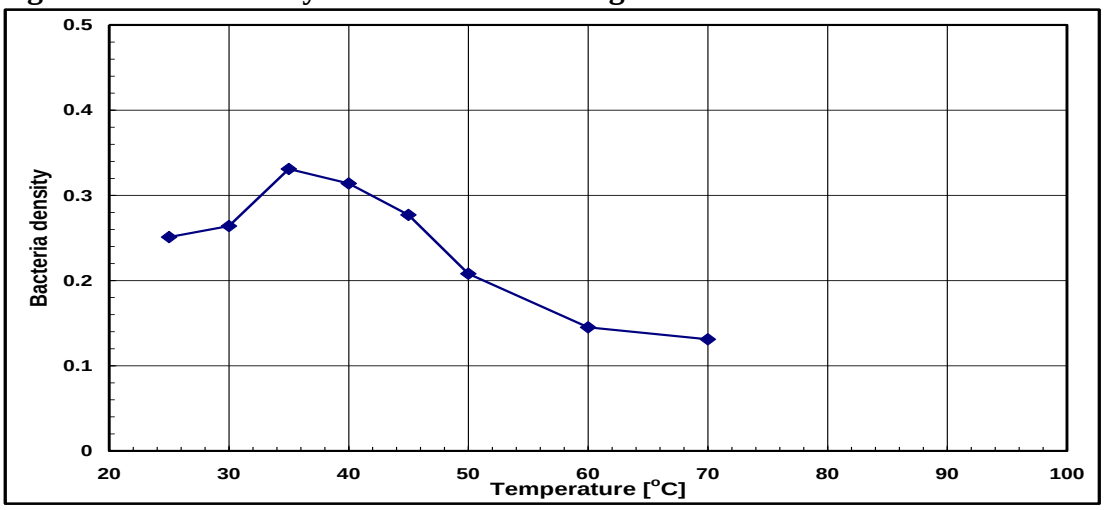

Fig. 3- Effect of temperature on the used bacterial growth.

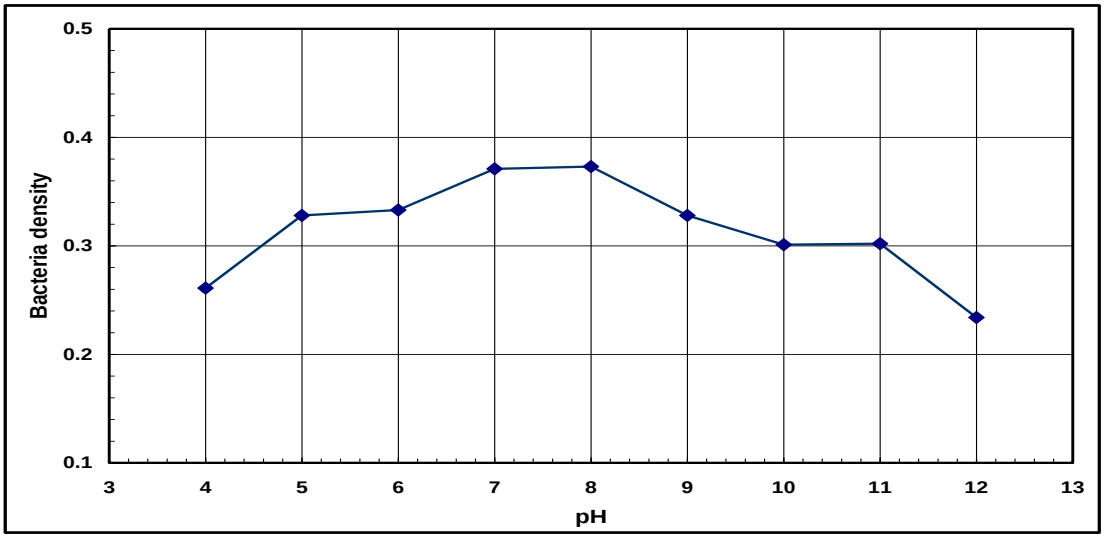

Fig. 4- Effect of pH on the used bacterial growth at 30 ^{O}C.

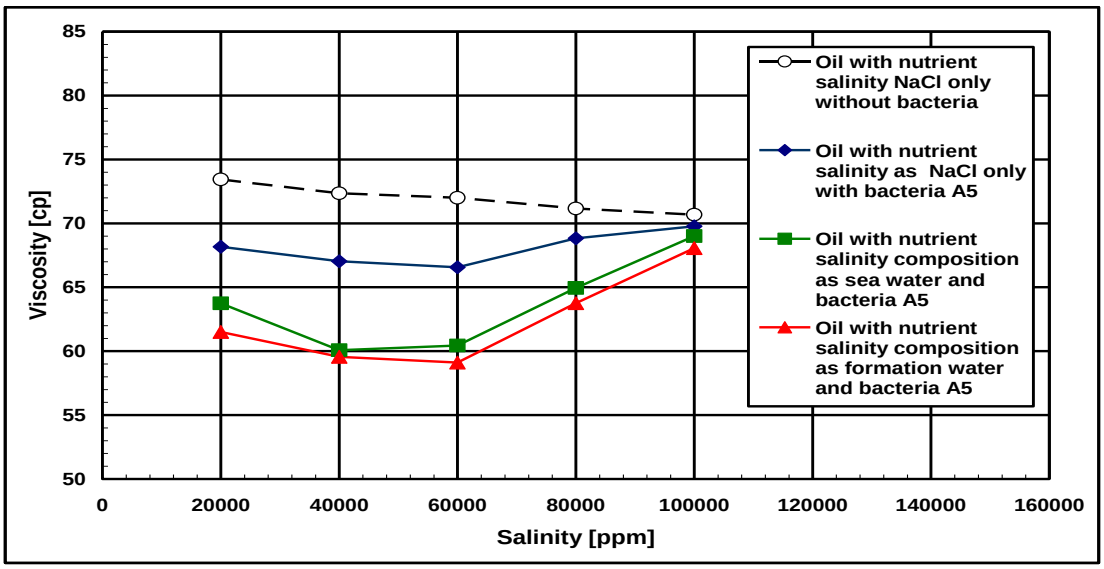

Fig. 5- Effect of different salinities and composition on the viscosity of the oleic phase after incubation at 30 ^{O}C.

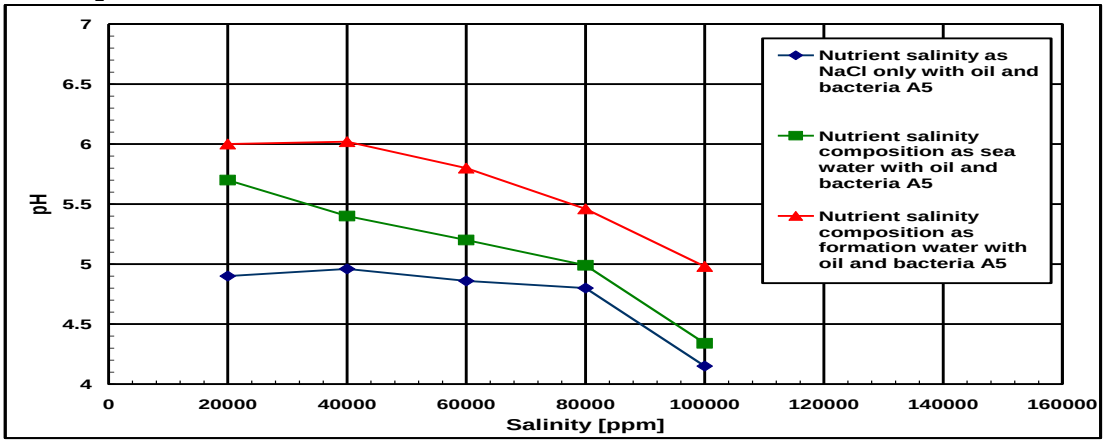

Fig. 6- Effect of different salinities and composition on the pH of the aqueous phase after incubation at 30 ^{O}C.

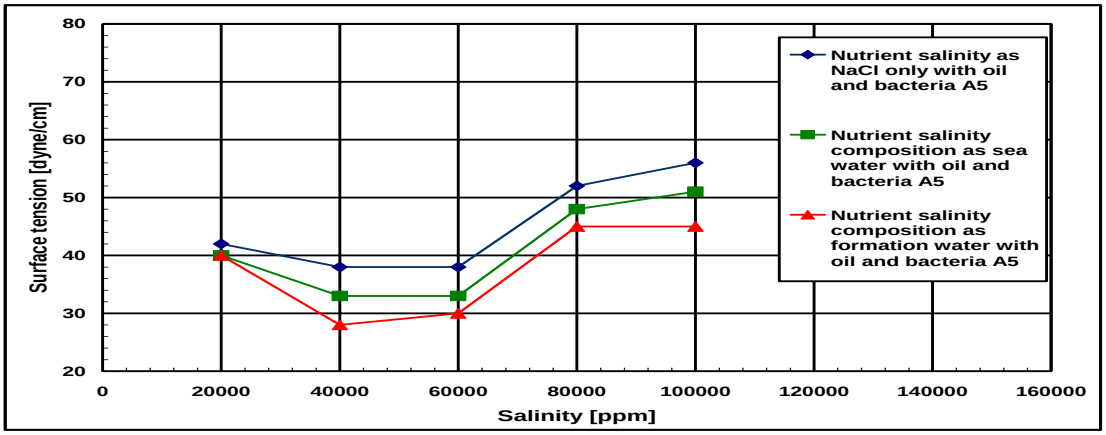

Fig. 7- Effect of different salinities and composition on the surface tension of the aqueous phase after incubation at 30 ^{O}C.

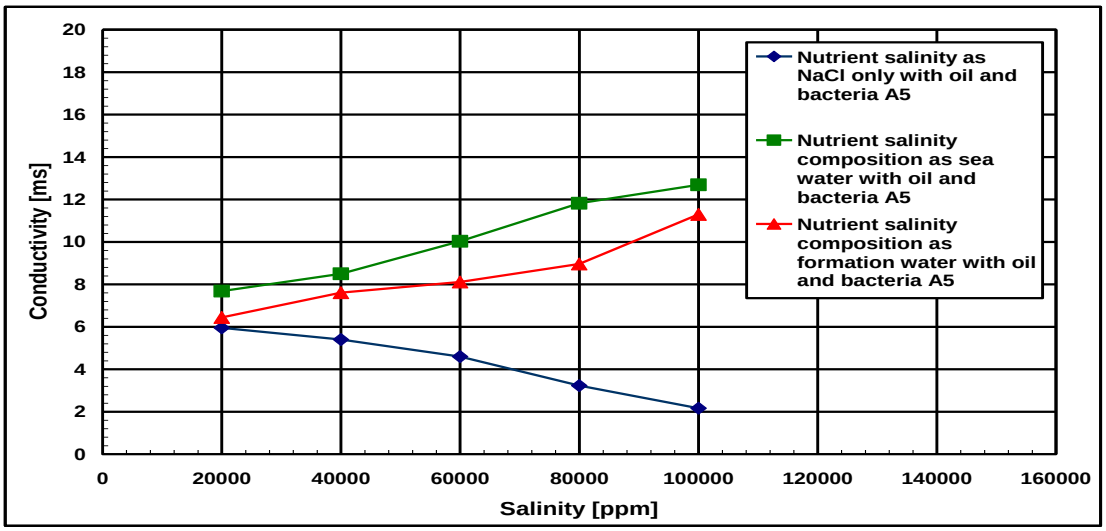

Fig. 8- Effect of different salinities and composition on the conductivity of the aqueous phase after incubation at 30 ^{O}C.

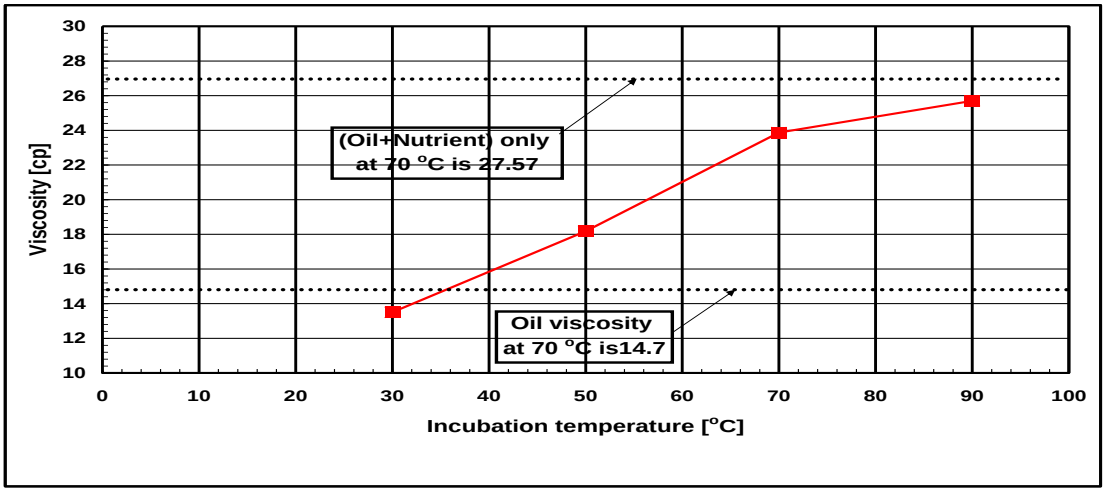

Fig. 9- Effect of different incubation temperatures on the viscosity of the oleic phase (sea water salinity 40000 ppm with different cations).

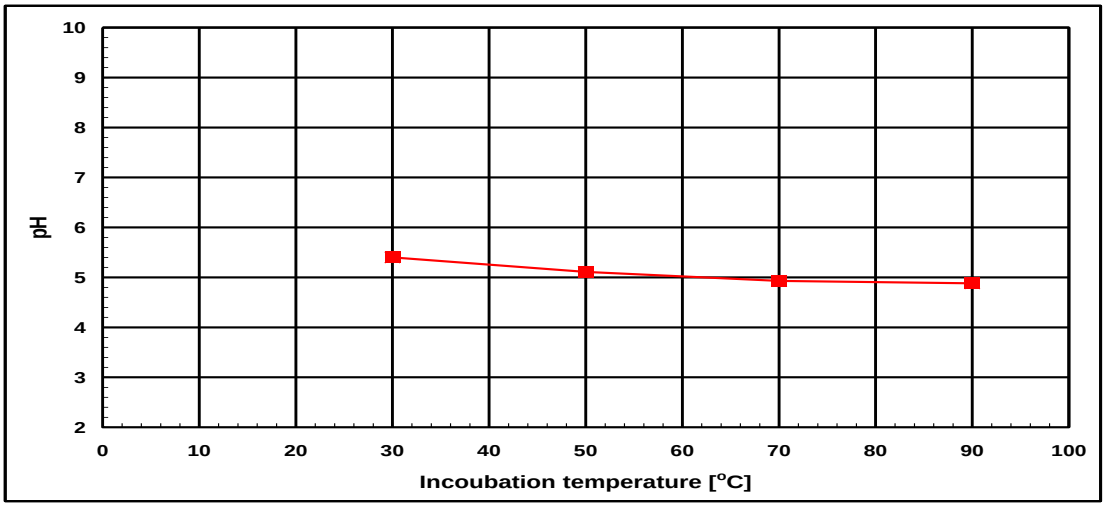

Fig. 10- Effect of different incubation temperatures on the pH of the aqueous phase (sea water salinity 40000 ppm with different cations).

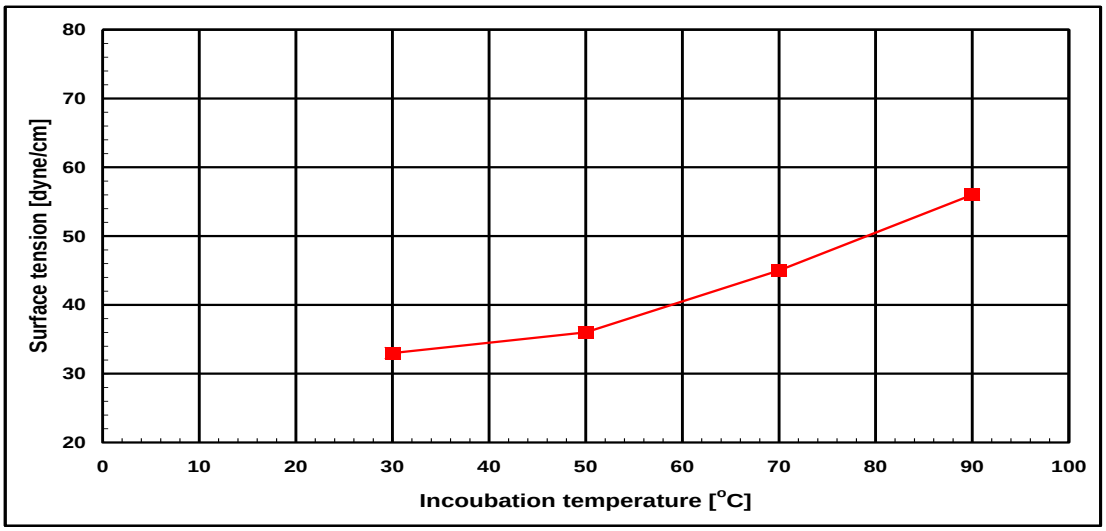

Fig. 11- Effect of different incubation temperatures on the surface tension of the aqueous phase (sea water salinity 40000 ppm with different cations).

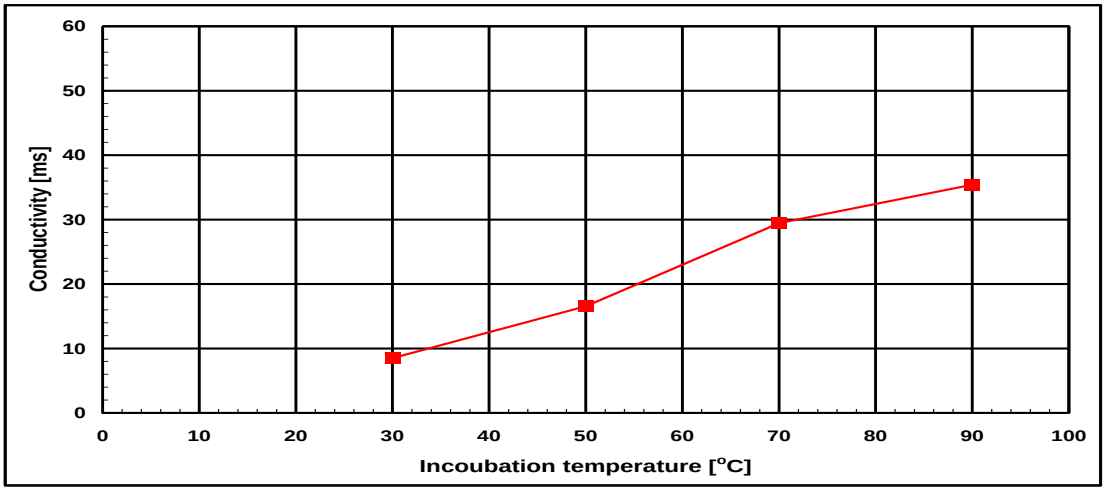

Fig. 12- Effect of different incubation temperatures on the conductivity of the aqueous phase (sea water salinity 40000 ppm with different cations).