

# Production, Characterization and Application of a New Biosurfactant Derived from Egyptian Sunflower Seeds

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**Abstract:** A new biosurfactant (SR122) was obtained for the first time from the seeds of sunflower plant growing locally in Egypt. This bioproduct was recovered from the seeds using simple and cost effective method that gave higher biosurfactant yield (20-25% w/w). The new product (SR 122) may be an alternative to the microbial and synthetic surfactants because it is non toxic (as it was originated from safe natural sources- the plant), cost-effective, produced in higher yield (as compared to the microbial biosurfactant) and may acquire public acceptance. The high surface activity of SR 122 (as measured by the ODA method, surface tension reduction and E24 method), the non-toxicity and tolerance to wide range of harsh condition of temperatures (up to 121°C), pH values (2-11) and different NaCl concentrations (2% -25% w/v) make this new product a promising candidate for the application in many industries and for the protection of the environment. On a large laboratory scale SR122 was applied for cleaning oil storage tank. During the cleaning process more than 95% of the oily sludge was recovered and the viscosity of the recovered oil was decreased allowing the oily sludge to be easily floated on the top of the biosurfactant as a distinct phase. This product SR 122 was offered for a patent no: 1289/2014

**Keywords:** New biosurfactant, Sunflower seed meal, Application, Cleaning oil storage vassels, Bioremediation, Patent.

## 1. Introduction

Surfactant are one of two main groups, chemically synthesized surfactants and biosurfactants (which are directly derived from natural sources such as plants, microorganisms and animals).

Biosurfactants have the advantages over the chemically synthesized surfactants due to their biodegradability, low or no toxicity, low irritability, compatibility with human skin and ecologically acceptable. They are termed "environmental friendly" and "green products" (Banat et al, 2000).

Many environmental and agriculture activities are important areas for biosurfactant applications. They play important roles in soil remediation, oil recovery and plant pathogen elimination. They are also used in detergents, paints, coatings, cosmetics pharmaceutical and food industries (Mukherjee et al, 2006).

In recent years, researches in the area of biosurfactants has expanded due to their use in different industrial activities. The development of such research represent the important role of these biological compounds for the protection of the environment (Khopode et al, 2012).

A variety of biosurfactants are derived from plants (termed phytogenic biosurfactants) such as saponins, lecithin, soyprotein and soybean oil. Saponins are widely distributed among plants. The most significant source of dietary saponins are legume plants such as soybeans, broad beans, peanuts, kidney beans and lenticells (Xu et al, 2011; Oakenfull, 1981).

Saponins have emulsifying and foam properties and are used in medicine, beverages, confectionary, cosmetics

and pharmaceuticals. They also have antimicrobial and insecticidal activities (Venken et al, 2007).

Lecithin is a low molecular weight natural surfactant found in both plants and animals. It is derieved from soybean seeds due to its abundance and low cost. Soylecithin is used or emulsifier, antioxidant, stabilizer, lubricant, wetting agent and nurritional supplement (Li, 2006).

Although the microbial biosurfactants have many advantages, they are of limited global industrial production due to the expensive raw materials, low production yield and high purification cost necessary for food, cosmetic and pharmaceutical applications. Also the industrial application of biosurfacants is limited by a lack of public acceptance of the biosurfactant- producing microorganisms (Zhang et al, 2012; Xu et al, 2011).

For the above reasons surfactant of plant origin are alternative for both the chemically synthesized surfactants and the microbial biosufactants in many applications. They gain public acceptance as they derived from safe natural source- the plants. At the same time they are cost effective and may be derived in high quantities.

To the best of our knowledge, no studies are available describing any surface active compounds derived from sunflower plants especially their seeds. Accordingly we are the first investigators studying the extraction, characterization and certain applications of this type of phytogenic biosurfatance (here known as SR 122).

## 2. Materials and Methods

### (1) Extraction of the biosurfactant SR 122.

The biological product SR 122 was extracted from the seed meal of sunflower plant by the solvent ethylacetate. The solvent was evaporated leaving behind a yellowish oily

product. This product was diluted with distilled water or tap water (pH 8.5- 9) to a level of 1/25-1/400 (i.e. 4 to 0.25% w/v, forming stable milky solution.

## **(2) Measurement of the surface activity of SR 122**

### **2-1 Oil displacement test**

This method was carried out according to Techaoei et al (2011). The method is sensitive, in which only small amount of sample is required to measure the activity of the product. The size of the developed oil displacement circle area reflects the activity of the biosurfactant. The larger the size, the higher the activity (Hamza et al, 2013). This method is carried out as follows:

- Fifty ml sea water or distilled water was introduced into large petri dish (15 cm diam) 40 ul of light crude oil or motor oil was spread on the surface of the water.
- 20 ul of each dilution of the bioproduct SR 122 was placed on the oil film in the petridish.
- The diameter of clear zone circle was measured, and then the area of the clear circle was calculated and expressed as ODA cm<sup>2</sup> (oil displacement area)

### **2-2 The 12 wells porcelain method**

This method was developed by Diab and Diab (2014), "work not published" as follows:

- 12 wells porcelain plates were used. Each clean well was coated by a film of oil.
- 10 ul of each dilution of SR 122 was added to each oil-coated well.
- After one minute the developed clear zones and/or the collapse of the oil were observed.

This method is sensitive and requires small amount of the product. The developed expel oil clear zone and/or the collapse of the oil are clearly observed and recorded (Fig. 1).

### **2-3 Emulsification index (E24).**

This method was carried out according to Tabatabee et al (2005) and Techaoei et al (2011) as follows:

- a) In a screw capped tube, 4 ml of dilution 1/150 (w/v) of the bioproduct SR 122 was added to 4 ml of each of the following:
  - Crude oil mixed with kerosene (1:1)
  - Crude oil
  - Kerosene
  - Used motor oil
  - Paraffin oil
  - Corn oil
  - Soybean oil
  - Sunflower oil
  - Olive oil
- b) The tubes were vortexed at high speed for two minutes. The mixture was left to settle for 24h, after which the E24 was calculated as follows:

$$E_{24} = \frac{\text{The height of the emulsion layer}}{\text{The total height of the mixture}} \times 100$$

The ability of a biosurfactants to form stable emulsion with vegetable oils suggests potential application in food

and pharmaceutical industries, while the ability to form stable emulsion with petroleum hydrocarbons suggests valuable applications in petroleum industries. An emulsion is considered stable if its volume after 24h corresponds to 50% or more (Lima et al, 2011). Higher E24 more than 50% suggests potential application of the bioproduct (Gnanamani et al., 2010).

### **2-4 Measurement of the surface tension:**

The surface tension of the extracted bioproduct and its dilutions 1/100, 1/150 and 1/300 (1% , 0.7% and 0.3% w/v) were measured by a tensiometer. For the calibration of the tensiometer, the surface tension of pure water that was used for making the different dilutions of the product was measured.

## **(3) Stability of the Biosurfactant SR 122**

### **3-1 Thermostability Test**

This method was carried out according to Techaoei et al (2011) and Haddad et al (2009) as follows:

Ten ml portions of SR122 solution (0.7% w/v) were exposed to various temperatures (50-121c°) for 20 min. allowed to cool at room temperature and the activity of the product was measured by using the ODA method.

### **3-2 Effect of different pH values**

The biosurfactant SR 122 solution (0.7% w/v) was adjusted to different pH values (2-11). The activity of the biosurfactant SR 122 was measured at each pH value by using the ODA method.

### **3-3 Effect of salinity:**

The effect of NaCl concentrations was carried out by adding different concentration of NaCl (0% - 25% w/v) to the SR 122 solution and allowed to stand for 30 min after which the activity in each concentration was measured by the ODA method.

## **(4) Application of the biosurfactant SR 122 for cleaning Oil-contaminated Vessels**

Different dilutions of the biosurfactant SR 122 (1/100, 1/150, 1/200 and 1/300) were used for cleaning oil-contaminated vessels using the method described by Diab and El-Din (2013) as follows:

- Five tubes were filled with crude petroleum oil and left stagnant for 96h.
- The tubes were emptied leaving their walls and bottoms contaminated with the oil.
- A known volume (10 ml) of each dilution of SR 122 was gently introduced into each tube. The remaining one tube received water only as control
- The results were observed and recorded within 2-30 min.

On a large laboratory scale SR 122 at dilution 1/100 was applied for cleaning a microcosm representing oil-storage tank. The microcosm was represented by a tank (50 x 30 x 30 cm). In this tank crude oil was stored for a period of 24 month.

- At the end of the storage period, the tank was emptied, leaving its bottom covered with the oily sludge.
- Three liters of the biosurfactant (1/100 w/v) were introduced into the tank. The tank was located on a shaker operated at 120 rpm, for a period of 96h.
- The results were observed and recorded periodically.

### 3. Results and Discussion

The new bioproduct SR 122 was extracted from the seeds of the Egyptian sunflower plant in the form of a yellowish oily material. This oily product was able to reduce the surface tension to  $42 \text{ mNm}^{-1}$ , had oil displacement area of  $39.4 \text{ cm}^2$  and gave milky solution when it was diluted with water (pH 8.5 -9). (this SR 122 bioproduct was offered for a patent No 1289/2014). Different dilutions of 1/10 to 1/400 (w/v) of this bioproduct were prepared and tested for their surface activities by using the 12 well porcelain plate (Fig 1) and the oil displacement area (ODA) methods. All of the dilutions tested showed positive interfacial tension reduction for the two methods (Table 1, Figs 1-3). It must be noted that the 12 well porcelain plate method is based on the reduction of the interfacial tension between petroleum oil and solid surfaces. While the ODA method is based on the reduction of the interfacial tension between oil and water. The higher ODA  $\text{cm}^2$  values were recorded with dilutions 1/50, 1/100 and 1/150 ( $153.8 \pm 3.2$ ,  $171.9 \pm 0.0$  and  $165.1 \pm 3.1$  respectively). A notable distinction character of SR 122 is that increased ODA values were observed with the increase of dilution rates from 1/10 ( $95.0 \text{ cm}^2$ ) to 1/200 ( $103.8 \pm 305 \text{ cm}^2$ ) with optimum values at 1/100 ( $171.9 \pm 0.0 \text{ cm}^2$ ) and 1/150 ( $165.1 \pm 3.1 \text{ cm}^2$ ).

This character may be used to distinguish biosurfactant of plant origin from those of microbial origin. Periera et al (2013) demonstrated that surface tension increased with decreasing the concentration of the biosurfactants produced by three strains of *Bacillus subtilis*. They found that the non-diluted biosurfactants reduced surface tension to  $28.1\text{-}29.4 \text{ mNm}^{-1}$ , and 1/10 dilution reduced surface tension to  $32.8\text{-}35.0 \text{ mNm}^{-1}$  while dilutions of 1/100 reduced the surface tension to  $46.2\text{-}70 \text{ mNm}^{-1}$ . Results of the surface tension reduction show that the higher surface tension reduction was achieved by dilution 1/100 ( $29 \text{ mNm}$ ) followed by dilution 1/150 ( $37.0 \text{ mNm}$ ) and dilution 1/200 ( $51 \text{ mNm}$ ).

As we mentioned before, the highest ODA values were recorded when dilutions 1/50, 1/100 and 1/150 of SR 122 were examined ( $153.8 \pm 3.2$ ,  $171.9 \pm 0.0$  and  $165.1 \pm 3.1 \text{ cm}^2$  respectively). Referring to Table (2) our ODA values represent the highest values when compared to those recorded by other biosurfactants. In this table the highest ODA values recorded were  $143.2 \text{ cm}^2$  and  $110.6 \text{ cm}^2$  by *Pseudomonas aeruginosa* SCMU/O6 (Techaoei et al, 2011). A part from these values, the table also show that no ODA values higher than  $69.43 \text{ cm}^2$  were recorded (Thaniyvarm et al, 2008). Accordingly, it is the first time to find a biological surfactant, especially of plant origin that is able to produce these highest ODA values especially when diluted to 1/50 – 1/150.

Hamza et al (2013) screened 20 bacteria for biosurfactant production by using the oil spread method, microplate method and drop collapse method. They found that 45% of the bacterial isolates were positive for the oil spread method (oil displacement area ODA). They recorded the results as follows:

+ : Clear zone diameter of 5-9 mm ( $0.2\text{-}0.6 \text{ cm}^2$  ODA)

++ : Clear zone diameter of 10-15 mm ( $0.8\text{-}1.8 \text{ cm}^2$  ODA)

+++ : Clear zone diameter of >15- <21 mm ( $>1.8\text{-} <3.4 \text{ cm}^2$  ODA)

++++ : Clear zone diameter of >21-<30mm ( $>3.46\text{-} <7.1 \text{ cm}^2$  ODA)

Tambekar et al (2013) screened 14 bacteria for biosurfactant production using the oil spread method, the drop collapse method and B-haemolysis test. They considered 5 mm and 10 mm diameter clear zones as positive biosurfactant production by the oil spread method. They recorded 13 bacterial strain (92.9%) as positive biosurfactant producers by using the oil spread method. Techaoei et al (2011) isolated 25 bacterial isolates from garage sites, all of the 25 bacterial strains were biosurfactant producer when they were tested by the oil displacement area (ODA) method. They recorded this results as follows:

+ : Oil displacement area (ODA) of  $0.1\text{-}3.14 \text{ cm}^2$

++ : Oil displacement area (ODA) of  $3.4\text{-}12.57 \text{ cm}^2$

+++ : Oil displacement area (ODA) of  $12.57\text{-}28.28 \text{ cm}^2$

++++ : Oil displacement area (ODA) of  $>28.28 \text{ cm}^2$

The excellent interfacial reduction character of the bioproduct SR 122 as indicated by the oil displacement area and the 12 wells porcelain plate methods give this product a potential application for enhanced oil recovery from reservoirs due to the reduction of the interfacial tension between oil and water and the reduction of the capillary force between oil and the reservoir rocks which lead to increase mobilization of the oil in the reservoir (Periera et al, 2013).

The results of the emulsification activity of SR 122 as measured by the emulsification index (E24) are found in Tables (3 and 4) and illustrated in Figs (4 and 5). These results show that all of the different dilution of SR 122 tested (1/100-1/400) were able to emulsify crude oil- kerosene mixture and soybean oil but with different E24 values. Dilutions 1/100 and 1/150 were promising dilutions as they gave higher E24 values against crude oil-kerosene mixture ( $72.1 \pm 1.0\%$  and  $77.4 \pm 0.9\%$  respectively), and against soybean oil ( $64.6 \pm 1.2\%$  and  $60.5 \pm 0.7\%$  respectively). On the other hand dilution 1/200 and 1/300 gave E24 values of more than 66% against crude oil kerosene mixture and more than 57% against soybean, while dilution of 1/400 was able to produce more than 50% E 24 value against both crude oil-kerosene mixture and soybean oil. From the above results dilution 1/150 was selected and tested against 9 different oil (Table 4, Fig 6). The results obtained show that the maximum E24 value was 100% against crude oil followed by  $77.4 \pm 0.9\%$  (against crude oil-kerosene mixture) and  $76.0 \pm 1.0\%$  against used motor oil. The least E24 was  $15.0 \pm 0.5\%$  against kerosene.

As for the E24 of SR 122 against vegetable oils (Table 4), the higher value was  $60.5 \pm 0.7\%$  against soybean oil, followed by  $59.3 \pm 0.9\%$  against olive oil and corn oil.

Willumsen and Kosaric (1997) and Lima et al (2011) proposed that an emulsification character is considered stable if its E24 corresponds to 50%. Techaoei et al (2011) reported that one of the desirable characteristics of a biosurfactant is emulsification properties. Aniyanwu et al (2011) reported that the ability of a biosurfactant to emulsify hydrocarbon- water mixture has been demonstrated to enhance the biodegradation of the

hydrocarbons and is potentially useful in enhanced oil recovery. The ability of the biosurfactant to form stable emulsion with different hydrocarbons and vegetable oils gives to the biosurfactant many advantages in petroleum and pharmaceutical industries.

The application of biosurfactants in several fields of industries depends on their stability at wide range of temperatures, pH values and salinity (Khopade et al, 2012).

Results of thermostability of SR 122 showed that this product was able to maintain its surface activity (as measured by the ODA method) unaffected in a wide range of temperature even after heating at the autoclave temperature of 121°C for 20 minutes. This character gives this new bioproduct SR 122 a potential use in food, cosmetics and pharmaceutical industries, where heating to achieve sterility is required (Abouseoud et al, 2008). The thermostability characteristic also increases the potential application of the new bioproduct SR 122 in a broader perspective including at conditions where high temperature prevail as in biological enhanced oil recover (Khopade et al, 2012).

Results of the stability of SR 122 at different pH values show variation of its surface activity with the variation of pH values (2-11) as indicated by the variation of ODA values (Table 5). At pH 8, the product showed its optimum surface activity ( $142.0 \pm 2.8$  ODA  $\text{cm}^2$ ). This was followed by pH 7 ( $136.5 \pm 2.1$  ODA  $\text{cm}^2$ ) and pH 9 ( $124.0 \pm 5.1$  ODA  $\text{cm}^2$ ). These result show that the highest surface activity was achieved at slightly alkaline pH as well as the neutral pH values, although at the alkaline pH 9 high ODA value of  $124.0 \pm 5.7$   $\text{cm}^2$  was recorded. The results also show that SR122 was able to retain  $96.2 \pm 1.6\%$  and  $87.3 \pm 4.0\%$  of its activity at pH 7 and pH 9 respectively ( this is relative to the optimum pH 8). At the acidic pH values (2-5), it retained  $53.5 \pm 0.6\%$  to  $64.8 \pm 2.0\%$  of its activity, while at the higher pH values 10 and 11 it retained  $79.6 \pm 3.0$  and  $22.8 \pm 1.6\%$  respectively.

As for the stability of this new SR 122 in the presence of different of NaCl (0-25%), the results (Table 6) show that the surface activity of this product (as tested by the ODA method) differ according to the differences in NaCl concentrations. It retained  $99.1 \pm 2.2\%$  -  $84.9 \pm 1.4\%$  of its activities at 2-10% NaCl. At 15% and 20% NaCl concentrations, it retained  $74.1 \pm 1.8\%$  and  $72.8 \pm 0.0\%$  respectively of its activity ( this is relative to 0.0% NaCl). These results confirm the stability of SR122 at wide range of NaCl concentrations (2-20% w/w). On the other hand it was able to retain  $57.1 \pm 2.1\%$  of its activity in presence of 25% NaCl (w/v).

Oil reservoirs are one of the harsh environments, where temperature can range from 20 to 90°C, normal salinity to hyper saline and wide range of pH values (Al-Bahry et al, 2012). The new product SR122 meets those condition as it showed surface activity and stability over wide range of environmental factor. Accordingly, it represents a promising candidate for enhanced oil recovery and for using at commercial scale.

In the present work the potential characteristics of the new bioproduct SR 122 can be summarized in the following points.

- Has high emulsification activity as indicated by the higher E24 values against petroleum oil; petroleum oil- kerosene mixture and used motor oil (100%, 77.4% and 76.0 respectively). At the same time it has promising E24 values against vegetable oils (54.5% - 60.5%).
- Has the ability to reduce the surface tension to 29.0 mN/m at a dilution of 1/100.
- Highly reduces the interfacial tension between oil and water and between oil and solid surface as indicated from the higher ODA values and the results of the 12 wells porcelain plate method.
- It showed surface activity at wide range of temperature (0-121°C), pH values (2-11) and NaCl concentrations (2% - 25% w/v).
- Non-toxic and cost-effective because it is of plant origin.
- It can be considered as an alternative to both microbial biosurfactants and the chemically synthesized surfactants.

All of the above characters give this new product (SR122) the potential uses in petroleum, pharmaceutical, cosmetics and food industries. It may be useful for cleaning oil- storage tanks, recovery of oil from oily sludge, washing oil-contaminated soils, enhanced oil recovery from reservoirs and enhancement of bioremediation processes.

In the present work the efficiency of SR 122 for cleaning oil-contaminated vessels was examined in the laboratory. The result show that after 2-3 min of the addition of 1/100 and 1/150 dilutions of SR 122 solution, most of the oil was removed from the bottom and the wall of the tubes and floated over the SR 122 solution as a distinct phase (Fig 7, b-c). On the other hand large amount of oil was also recovered 10 min after the addition of 1/200 dilution of SR 122 solution (Fig 7, d), while very small amount of the oil was removed after 20 min of the addition of the diluted solution 1/300 (Fig. 7, e). When water was used as control, no recovery of oil was observed even after 30 min (Fig 7, a).

On a large laboratory scale, SR122 (dil. 1/100) was applied for cleaning a microcosm representing an oil storage tank (50 x 30 x 30 cm). Crude oil was stored in this tank for a period of more than 24 month, then the tank was emptied leaving the oily sludge covering the bottom (Fig. 8, a). 3 liters of the biosurfactant was introduced into the tank. (Fig. 8, b) which was left on a shaker operated at 120 rpm for 96h at room temperature. The results were observed and could be summarized in the following points:

- During 5-60 min of the addition of SR 122 solution, the oily material was gradually recovered and floated on the top of the liquid phase (Fig 8a-8d).
- At the end of 96 h= more than 95% oil was recovered. The recovered oil floated as distinct phase covering nearly all the surface of SR 122 solution, leaving the bottom of the tank nearly clean.
- When about 3L of water was added to the content of the tank, mixed and transferred to a new clean tank, the recovered oil was evenly spreaded over the aqueous phase. From this result it is of interest to observe a modification and reduction in the viscosity of the recovered oil. This may facilitate the pumping of this oil.

- At this point, the volume of the recovered oil could be measured and the cost of the operation can be estimated (this will be carried out further in a separate work).

The above results clearly show that the new product SR122 represents a good candidate for cleaning and recovery of oil from oil-contaminated vessels, oil-storage tanks, tank cars, oil tanks, pipelines and other containers used to transport or store crude oil. Such a clean process represents economically non-hazardous and cost-effective strategy as compared to other cleaning methods.

In the present work, when SR 122 was used for the cleaning process, the oil was mobilized without forming a suspension, this is through replacement technique, in which the oil was released from the system due to reduction in the interfacial tension, and then the oil was floated and remained a distinct phase. Oil will undergo displacement if the interfacial tension between oil solid is highly reduced to overcome the capillary force (Urum, 2004).

#### 4. Acknowledgements

This study has been conducted in the laboratories of the faculty of Biotechnology at October University for Modern Sciences and Arts (MSA). The authors acknowledge the top management of MSA, Professor Nawal El-Degwi, Professor Khayri Abdelhamid for their assistance and encouragement. We would like to thank the faculty members and the lab technician in the Faculty of Biotechnology at MSA.

**Table 1:** Surface activity of the different dilutions (W /v) / concentrations (g/L) of the bioproduct SR 122 as measured by the ODA method, n= 3, ± standard deviation

Dilutions (w/v)	Concentrations (g/L)	Diameter clear zone (m m)	ODA (cm <sup>2</sup> )
1/10	100.0	110.0 ± 0.0	95.0 ± 0.0
1/25	40.0	124.5 ± 0.7	121.7 ± 1.4
1/50	20.0	140.0 ± 1.0	153.8 ± 3.2
1/100	10.0	148.0 ± 1.0	171.9 ± 3.0
1/150	6.6	145.0 ± 1.5	165.1 ± 3.1
1/200	5.0	115.0 ± 1.0	103.8 ± 2.5
1/300	3.3	106.3 ± 2.1	89.1 ± 3.5
1/400	2.3	75.0 ± 1.5	44.2 ± 1.7

**Table 2 :** ODA values and/or diameter clear zone (m m) of some biosurfactant as recorded by different authors

Microorganisms	ODA Cm <sup>2</sup>	References
<i>Bacillus subtilis</i> SH20	63.6 ± 3.4	Diab and Eldin (2013)
<i>Bacillus subtilis</i> SH20	50.2 ± 2.1	
<i>Pseudomonas aeruginosa</i> SH 29	63.0 ± 2.8	
<i>Pseudomonas aeruginosa</i>	0.95 (11 mm)	Chioma <i>et al</i> (2013)
<i>Bacillus subtilis</i>	0.40 (7 mm)	
<i>Pseudomonas aeruginosa</i> UKMP14T	0.6 (9 mm)	Hamza <i>et al</i> (2013)
<i>Rhodococcus sp.</i>	0.6 (9 mm)	
<i>Pseudomonas oeruginosa</i> L1	7.1 (30 mm)	Tambekar and Gadakh (2013)
<i>P. aeruginosa</i> PVG1	9.6 (35 mm)	
<i>P. aeruginosa</i> PVG1	23.1 (55 mm)	
<i>P. aeruginosa</i> G2	63.3 (90 mm)	
<i>Stenotrophomonas mulliphila</i>	12.52 (40 mm)	
<i>Acinetobacter sp</i> MW1	2.45	Dhail <i>et al</i> (2012)
<i>Pseudomonas sp</i> MW2	3.14	
<i>Pseudomonas sp</i> JN 2	2.55	
<i>Bacillus sp</i> JN1	2.55	
<i>Glucobacter sp</i> MS3	2.30	
<i>Arthrobacter sp</i> M51	2.22	
<i>Bacillus subtilis</i> N/o	28.2 (60 mm)	El-Sersey (2012)
<i>Pseudomonas aeruginosa</i> Y5	23.74 (55 mm)	Zhang <i>et al</i> (2012)
<i>Pseudomonas aeruginosa</i> Y 17	26.40 (58 mm)	
<i>Pseudomonas aeruginosa</i> SCMU106	143.2 with (NH <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> 110.6 with NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> 11.17 with Na No 3 10.83 with (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 9.8 with NH <sub>4</sub> No <sub>3</sub>	Techaoei <i>et al</i> (2011)
<i>Bacillus sp</i> B2	6.15 (28 mm)	Violate <i>et al</i> (2011)
<i>Bacillus sp</i> 0517	7.1 (30 mm)	
<i>Bacillus subtilis</i> BS 3	3.14 (20 mm)	Priya and Usharani (2009)
<i>Pseudomonas aeruginosa</i> P3	3.8 (22mm)	
<i>Picia anomals</i>	69.43	Thaniyavarn <i>et al</i> (2008)
<i>Myroides sp</i> SMI	3.14	Mancerat and phetrong (2007)
<i>Vibrio paraheamolyticus</i> SM2	1.13	
<i>Bacillus subtilis</i> SM3	2.55	
<i>Micrococcus luteus</i> SM4	1.13	
<i>Acinetobacter antiratus</i> SM 6	2.55	
<i>Vibrio parcheamolyticus</i> SM7	1.13	
<i>Bacillus pumiless</i> SM8	2.55	
Unidentified SP SM 5	2.01	

**Table 3:** Emulsification index (E 24) of the different dilutions/ concentrations of SR 122 using crude oil-kerosene mixture and soybean oil

Dilution (w/v)	Conc. g/l	E24 (%)	
		Crude oil + kerosene	Soybean oil
1/100	10	72.1 ± 1.0	64.6 ± 1.2
1/150	7	77.4 ± 0.9	60.5 ± 0.7
1/200	5	67.5 ± 1.1	57.8 ± 0.3
1/300	3.3	66.9 ± 0.3	57.2 ± 0.6
1/400	2.5	51.0 ± 1.4	56.5 ± 1.4

**Table 4:** Emulsification index (E24) of the biosurfactant SR122 against different oils when 1/150 dilution was used. n= 2, ± : standard directions

Oils	E24 (%)
Crude oil (CO)	100 ± 0.0
Crude oil Kerosene (COK)	77.4 ± 0.9
Kerosene (K)	15.0 ± 0.5
Used motor oil (UO)	76.0 ± 1.0
Paraffin oil (P)	57.7 ± 1.2
Soybean Oil (SO)	60.5 ± 0.7
Olive oil (OL.O)	59.3 ± 0.9
Sunflower oil (SnO)	54.5 ± 0.4
Corn Oil (Cn O)	59.3 ± 1.1

**Table 5:** Stability of SR 122 at different pH values. Retained activity (%) is given relative to the activity at pH 8 (the optimum value) n = 2, ± = standard deviation

pH values	Activity (ODA cm <sup>2</sup> )	Retained activity (%)
2	76.0 ± 2.8	53.5 ± 0.6
3	81.0 ± 1.4	57.0 ± 1.0
5	92.0 ± 2.8	64.8 ± 2.0
7	136.5 ± 2.1	96.2 ± 1.6
8	142.0 ± 0.0	100.0 ± 0.0
9	124.0 ± 5.1	87.3 ± 4.0
10	113.0 ± 4.2	79.6 ± 3.0
11	32.5 ± 2.1	22.2 ± 1.6

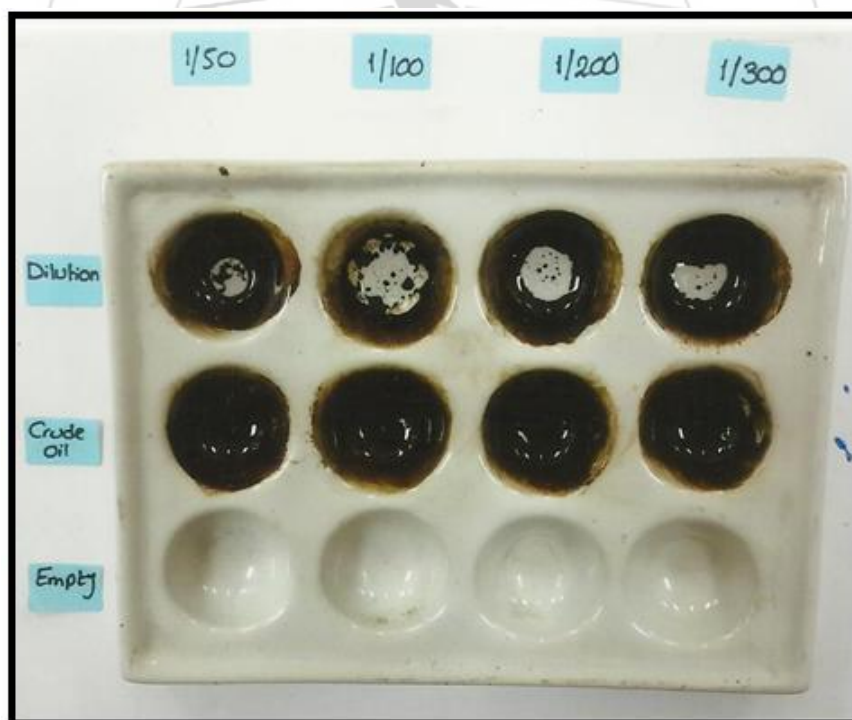
**Table 6:** Stability of SR122 at different NaCl concentrations Retained activity (%) is given relative to the activity at 0% NaCl n= 2, ± : standard deviation

NaCl concentrations (% w/v)	Activity (ODA cm <sup>2</sup> )	Retained activity (%)
0	162.0 ± 0.0	100.0 ± 0.0
2	160.5 ± 3.5	99.1 ± 2.2
3	156.5 ± 2.1	96.6 ± 1.2
5	147.0 ± 2.8	90.7 ± 1.2
10	137.5 ± 2.1	84.9 ± 1.4
15	120.0 ± 2.8	74.1 ± 1.8
20	118.0 ± 0.0	77.8 ± 0.0
25	92.5 ± 3.5	57.1 ± 2.1

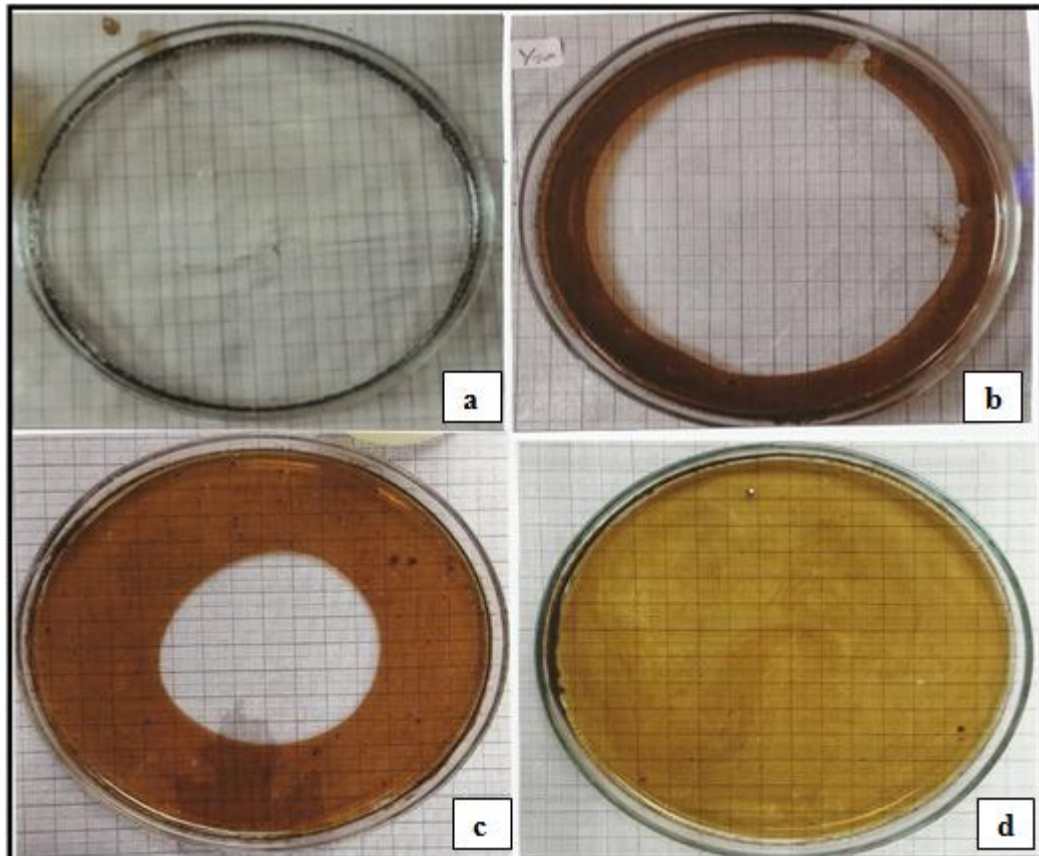
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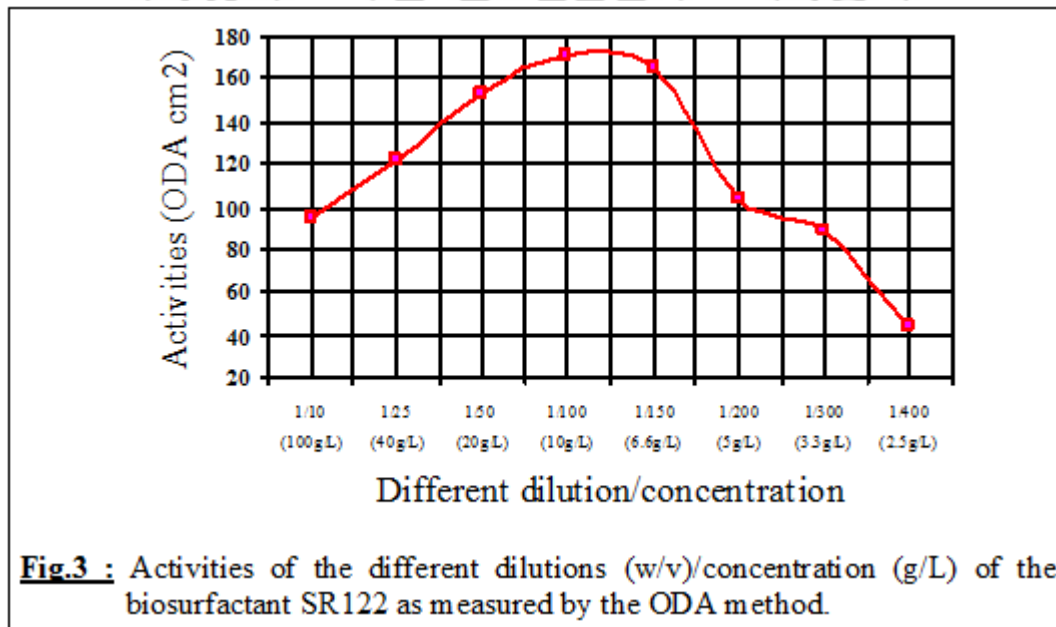
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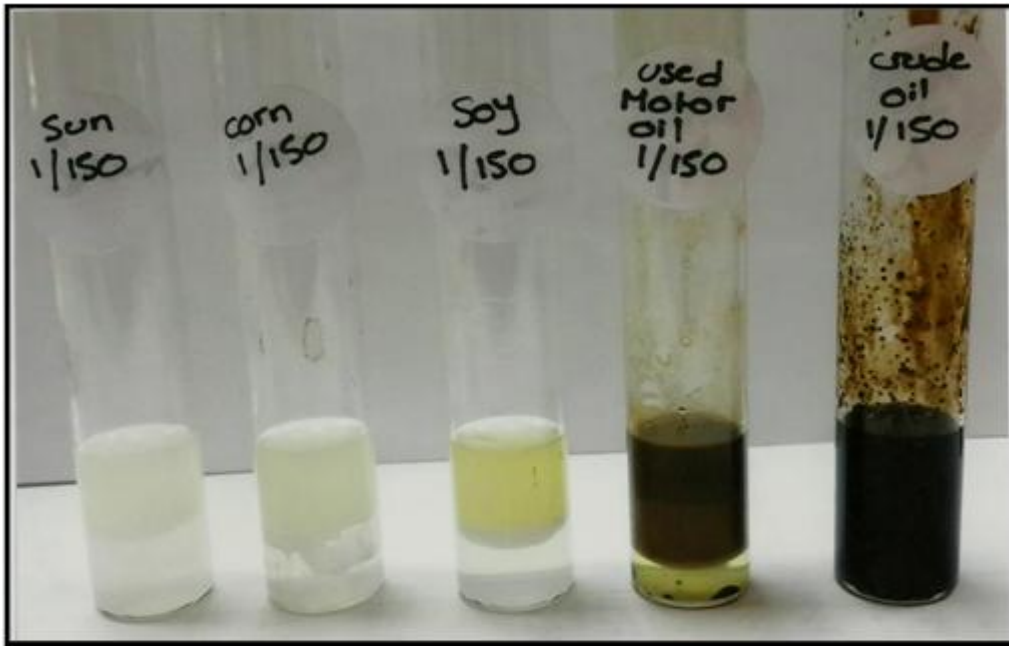
**Figure 1:** Screening the surface activity of the different concentrations of SR122 using the porcelain plate method. Visible clear zones indicate positive surface activity.



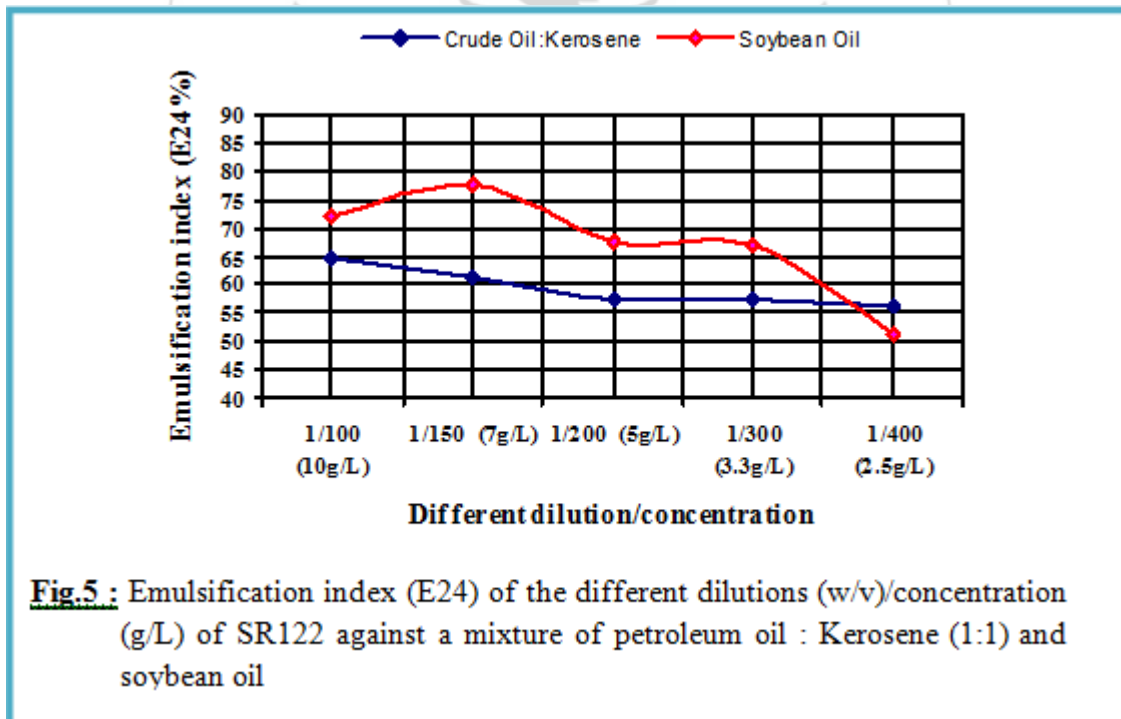
**Figure 2:**  
 Surface activity of the product SR122 at different concentrations using the ODA method:  
 a - Dilution 1/100 showing ODA of 171.0 cm<sup>2</sup>.  
 b - Dilution 1/200 showing ODA of 103 cm<sup>2</sup>.  
 c - Dilution of 1/300 showing 44 cm<sup>2</sup> ODA.  
 d - Oil spreader layer on the water surface prior the addition of SR122.



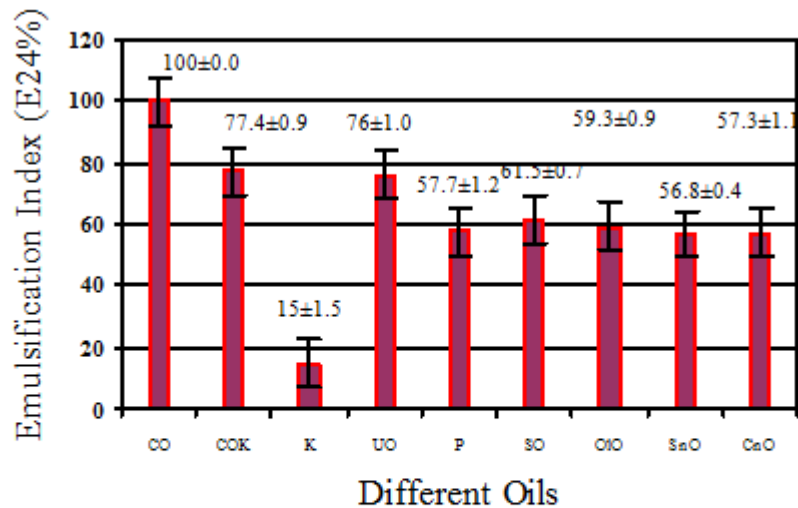




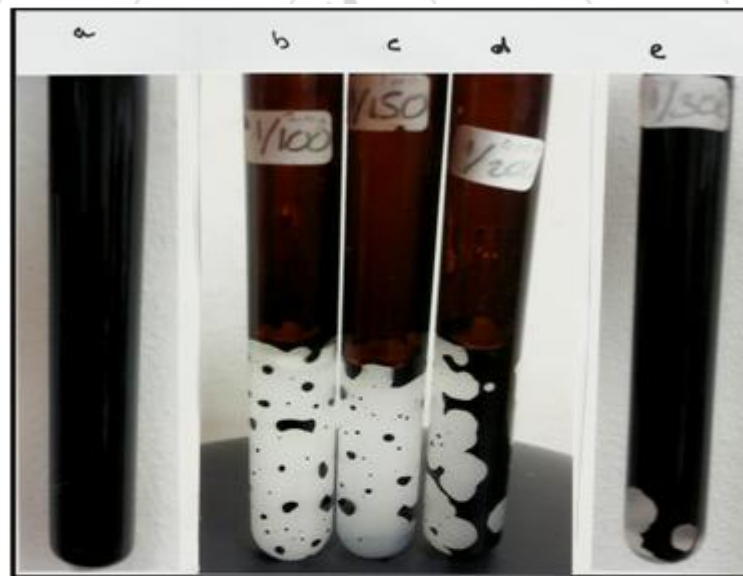
**Figure 4:**  
 Emulsification index (E24) of SR122 with different oils:  
 Sunflower oil, corn oil, soybean oil, used motor oil and  
 crude petroleum oil.



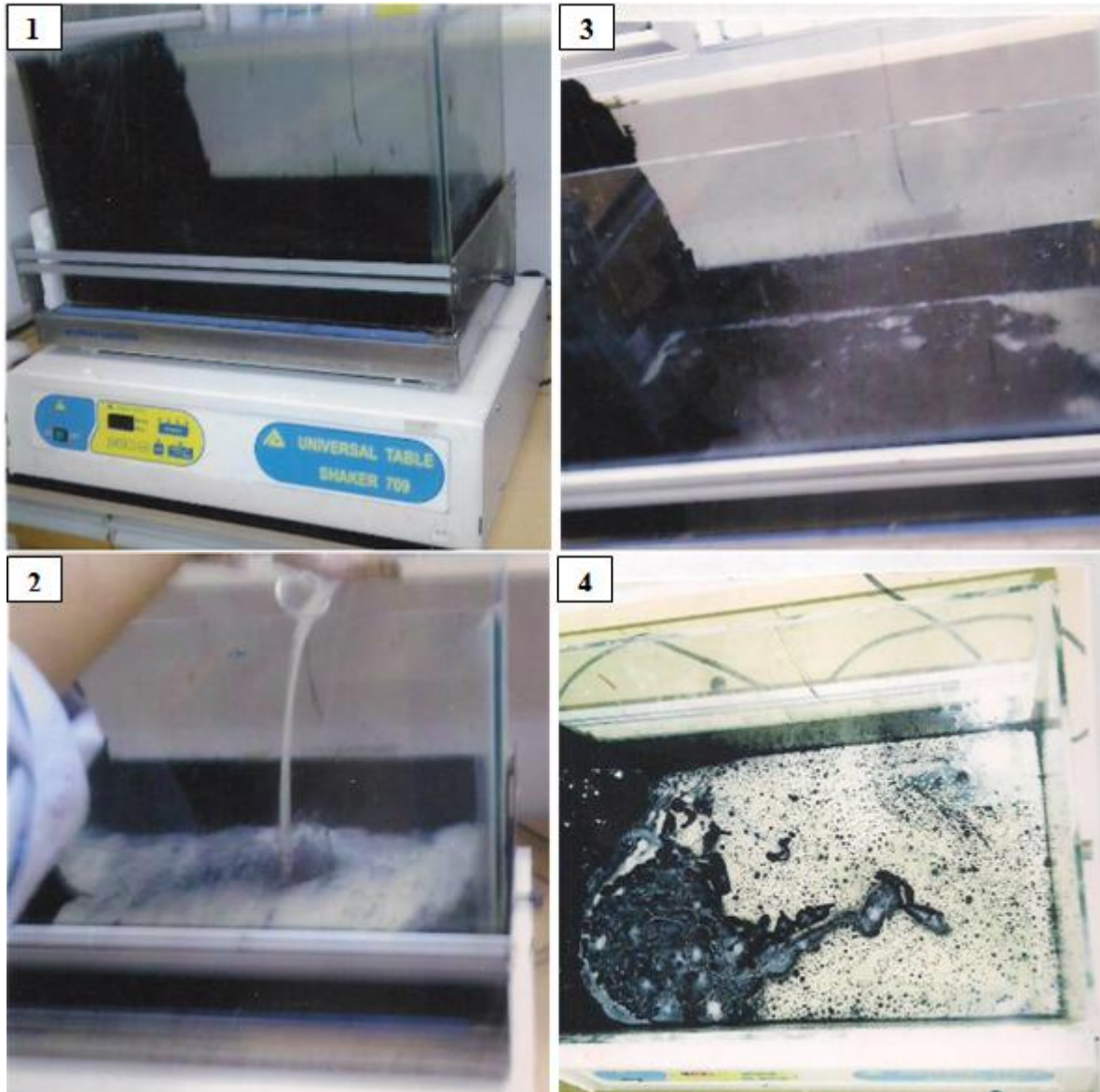
**Fig.5 :** Emulsification index (E24) of the different dilutions (w/v)/concentration (g/L) of SR122 against a mixture of petroleum oil : Kerosene (1:1) and soybean oil



**Fig.6 :** Emulsification index (E24%) of the SR122 bioproduct against crude oil (CO), crude oil : kerosene (COK), kerosene (K), used motor oil (UO), paraffin oil (P), soybean oil (SO), olive oil (OIO), sunflower oil (SnO), and corn pil (CnO)



**Figure 7:** Application of SR122 for cleaning oil - Contaminated tubes (pipes).  
 a- Control tube with water after 30 min of starting the experiment.  
 b- two minutes after the addition of 1/100 dilution of SR122.  
 c - Two minutes after the addition of 1/150 dilution of SR122.  
 d - Ten minutes after the addition of 1/200 dilution of SR122.  
 e - 20 minutes after the addition of 1/300 of SR122.



**Figure 8:** Application of the product SR122 for cleaning an oil storage tank (Open tank).

1. The tank after removing the 24 month storage oil and putting the tank on a shaker. Parts of the walls are covered with a layer of oil, and oily sludge was formed in the bottom of the tank.
2. Introducing the product SR122 into the tank, and left on a shaker operated at 120 rpm.
3. After 20 minutes of the addition of SR122 (Top view).
4. After 60 minutes of the addition of SR122 (Side view).