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# Application of Biosurfactant from *Candida sphaerica* UCP 0995 in Removal of Petroleum Derivative from Soil and Sea Water

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**Abstract:** The biosurfactant produced by *Candida sphaerica* in a fermentor containing 5% vegetal oil refinery waste and 2.5% was tested in the removal of motor oil from soils and seawater. In kinetic assays, the isolated biosurfactant removed more than 86% of the motor oil adsorbed to clay, silty and sandy soils at the critical micelle concentration (CMC). Static removal tests performed in glass columns demonstrated that the crude biosurfactant was able to remove 75% and 92% of the oil contained in clay and silty soil, respectively, whereas the isolated biosurfactant at its CMC removed 50% of the oil from sandy soil. In the washing of hydrophobic compound on a porous surface, the removal rate was 60%. The biosurfactant also proved to be efficient in detergency tests since the crude surfactant removed 41% of motor oil from contaminated cotton cloth. In tests carried out with seawater, the crude biosurfactant showed an oil spreading efficiency of 75% in both screening dispersion test and oil displacement efficiency methods. Regarding the swirling bottle test, the dispersion rate was 72% for the isolated biosurfactant at a concentration twice the CMC. The biosurfactant studied demonstrated potential for application as an adjuvant in biotechnological processes for environmental decontamination.

**Key words:** *Candida sphaerica*, biosurfactant, motor oil, bioremediation.

## 1. Introduction

Oil spills occur on a daily basis during cargo transportation or in the form of industrial spills of oil residue and petroleum byproducts. Petroleum is a hydrophobic hydrocarbon with negative effects on the structural and functional properties of the cell membranes of living organisms, offering considerable risk of contamination in both marine and terrestrial ecosystems [1].

The US Environmental Protection Agency [2] proposes different chemical, physical and biological technologies for the treatment of soils contaminated by oil-derived hydrocarbons.

One of the most investigated methods is bioremediation, which uses the natural degradation ability of plants and microorganisms to partially convert contaminants into less toxic compounds or completely convert such substances into carbon dioxide and water. While bioremediation is an effective, environmentally friendly method, the time and costs involved render this process unviable for the treatment of large amounts of waste [3].

Methods such as soil washing, used for the separation of contaminants without causing chemical harm to the soil, can increase the biodegradation rate [4]. The pollution of water and soil by oil-derived hydrocarbons and the disastrous consequences to the

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environment have motivated the development of environmentally friendly remediation technologies. Thus, the use of surfactant compounds emerges as an alternative to enhance the solubility of oils, allowing the desorption and solubilization of hydrocarbons and facilitating the assimilation of these compounds by microbial cells [5].

A number of microorganisms, such as fungi, yeasts and bacteria, feed on substances that are immiscible in water, producing and using a surface-active substance known as a biosurfactant [6].

Biosurfactants are amphiphilic compounds (with polar and non-polar moieties) comprised of glycolipids, phospholipids, lipopeptides and polymeric compounds, tend to separate at interfaces and reduced the surface tension [6].

Biosurfactants have a wide range of biotechnological applications in dairy, food, beverage, cosmetics, detergent, textile, paint, mining, petroleum, paper pulp and pharmaceutical industries [7-9]. Environmental imbalance, created by crude oils, hydrocarbons and toxic metals can be remediated by biosurfactant effectively as it forms stable emulsion, adsorbent or amalgam [10]. Biosurfactants are ecologically accepted, low toxic, biodegradable and effective in a wide range of extreme conditions including temperature, pH and salinity [6, 11] compared to chemical surfactants.

In this study, the low-cost biosurfactant produced by the yeast *Candida sphaerica* was evaluated in order to determine its potential as a remediation agent for the clean-up of oil spills. The biosurfactant was tested as an oil spill dispersant in seawater, in the removal of a hydrophobic pollutant adsorbed to soil through static and kinetic assays, as a detergent and a demulsifier.

## 2. Materials and Methods

### *2.1 Soils*

Three soils of different textures were used. The first was a sandy soil (88%) collected at the Itapirema Experimental Station of the Pernambuco Agronomic Institute in the city of Goiana, Pernambuco, Brazil. The second was a silty soil (43%) collected from the city of Abreu e Lima, Pernambuco. The third was a clay soil (60%) collected at the Alto reservoir in the neighborhood of Nova Descoberta in the city of Recife, Pernambuco.

### *2.2 Sea Water*

Sea water was collected near the Thermoelectric TERMOPE, located in the municipality of Cabo de Santo Agostinho, in Pernambuco state, Brazil. Water samples were collected and stored in plastic bottles of 5 L.

### *2.3 Percolating Fluids and Contaminant*

The biosurfactant was obtained in an optimized medium described in Ref. [12] and used in the removal tests. The following solutions were used for the movement of the hydrophobic contaminant adsorbed to the soil: distilled water (control); crude and isolated biosurfactant solutions. The biosurfactant was used in crude form (cell-free metabolic broth) and isolated form in solutions at the critical micelle concentration (CMC; 0.8 g/L) and at a concentration twice the CMC (0.16 g/L). Tween 20 (polyoxyethylene 20 sorbitan monooleate), which is a commercial surfactant, was used for comparative purposes at a concentration of 0.8 g/L in distilled water.

Engine lubricating oil (motor oil) was obtained from an automotive maintenance establishment in the city of Recife, Pernambuco, Brazil.

### *2.4 Preparation of Soils*

The soils were ground in a mortar to break up clods. Water was then added evenly to achieve 9.5% hygroscopic moisture. Cylindrical test specimens measuring 5 cm in diameter and 20 cm in length were molded for the column assays. The test specimens were molded with the least possible density to achieve a high degree of water conductivity.

*2.5 Removal of Motor Oil in Packed Columns through Static Assay*

Glass columns measuring 57 cm in height × 6 cm in diameter were initially filled with approximately 200 g of a mixture containing the soils and 20 g of engine lubricating oil. The surface was then inundated with 200 mL of the biosurfactant solutions under the action of gravity. Percolation of each biosurfactant solution was monitored in 5 min intervals for 24 h, when no further percolation of the solution was observed. Following the washing of the columns, the soil samples were washed with 20 mL of hexane for the removal of the residual oil. The solvent was rotoevaporated at 50 °C and the amount of oil removed was determined by gravimetry [13, 14].

*2.6 Removal of Motor Oil through Kinetic Assay*

The removal of motor oil from the contaminated soil was tested through the saturation of 50 g of soil with 5 g of motor oil. The laboratory-contaminated soil was placed in 250 mL Erlenmeyer flasks, to which 50 mL of the biosurfactant solution (crude, at the CMC and at a concentration twice the CMC) were added. The Erlenmeyer flasks were shaken at 200 rpm for 24 h at 28 ºC. The entire content was then centrifuged at 5,000 rpm for 1,200 s. Residual oil was determined through gravimetry [15].

*2.7 Washing of Hydrophobic Compound Adsorbed to Porous Surface*

The removal of motor oil adsorbed to rock was carried out by soaking the material in the contaminant until complete coverage and recording the volume spent. The material was then carefully placed in a 100 mL beaker with the aid of a pincers and submitted to washing with the cell-free metabolic broth (crude biosurfactant). After the culture process, the percentage of removal through washing was calculated. The amount of oil remaining on the washed solid was determined by gravimetry [16].

*2.8 Evaluation of Demulsification Performance*

A motor oil emulsion was prepared by mixing the oil and water at 1:1 ratio (v/v) with a digital mechanical mixer at 900 rpm for 15 min. The prepared emulsion left undisturbed within 24 h at 28 ºC. In a demulsification test, 2 mL of cell-free broth was added to a 20 mL graduated test tube containing 18 mL of the motor oil emulsion. The test tube was manually inverted 200 times to achieve complete mixing. The tubes were then left undisturbed at 28 ºC. The change in volume of the water phase was recorded at certain intervals. Demulsifying performance was evaluated by calculating the demulsification ratio as shown below.

Demulsification ratio = [(water volume (on the bottom))/(water volume in original emulsion + added culture volume)] × 100%.

Demulsifying speed was assessed by emulsion half-life (t1/2), which was the reaction time when 50% of the demulsification ratio was achieved. The demulsification ratio of the blanks (by dosing 2 mL of sterilized medium) was 0% within 24 h [17].

*2.9 Removal of Motor Oil from Contaminated Cotton Cloth*

Compatibility, stability and efficiency of the biosurfactant to remove oil with respect to commercially available detergents were also studied with a view to establish the potential of biosurfactant as a detergent additive. The synthetic surfactant Tween 20 and the crude biosurfactant were individually dissolved in water (1% w/v) and their efficiency to remove oil from an oil-contaminated cotton cloth was checked individually. For this, 3 g of motor oil was poured on a 25 cm × 25 cm cotton cloth and allowed to dry at 40 ºC for 24 h. To test the oil removal capability, each piece of cloth impregnated with oil was soaked in flasks containing 100 mL each of tap water (control), biosurfactant and synthetic surfactant solutions. The flasks were kept on a shaker at 28 ºC, 100 rpm for 60 min. The post-wash water

was used to measure the amount of oil removed from the cotton cloth by extracting it with hexane. The extraction process was repeated thrice; the hexane was recovered using a rotary evaporator and the residual motor oil was measured gravimetrically [18, 19].

*2.10 Screening Dispersion Test*

A quick comparative test method was used for visual determination of the dispersant effectiveness. The motor oil sample (0.100 μL) was carefully added to the surface of seawater (20 mL) and a 1 cm deep vortex was created by slow magnetic stirring. The dispersant mixture (5.0 μL) was added to the center of the vortex and the stirring rate was immediately increased, maintained at a maximum rate of 2000 rpm over a 60 s period and then stopped. The level of oil dispersion in the water was visually estimated after a one min rest. The classification A was attributed to the resulting brown-black mixture when all the oil was dispersed in the water leaving no slick at the surface, whereas the classification E was used to describe a total lack of dispersion, i.e., all the oil was returned to the surface a few seconds after the end of the stirring, leaving the aqueous phase nearly transparent. The letters B-D represent intermediate situations. All screen tests were performed at 28 ºC [20].

*2.11 Oil Displacement Test*

The oil displacement test was carried out slowly by dropping of 15 μL of motor oil onto the surface of 40 mL of distilled water layer contained in a Petri dish (15 cm in diameter) that spread all over the water surface area. This was followed with the addition of 10 μL of the crude biosurfactant onto the surface of the oil layer. The average value of the diameters of the clear zones of triplicate experiments was measured and recorded then calculated as percentage of the Petri dish diameter [21].

*2.12 Swirling Bottle Test*

A 1 L cylindrical open bottle (diameter–10 cm) with an outlet valve at the bottom to take samples was used in the dispersion experiment. Samples of 200 mL sea water were added to the bottle and 2 mL oil was gently added to the surface of water with a pipette. The crude or the isolated biosurfactant solution was dispensed at the center of the oil slick in the following proportions biosurfactant-to-oil ratio of 1:1, 1:2, 1:8 and 1:20 (v/v). The isolated biosurfactant was used at the CMC and at a concentration twice the CMC. The bottle was placed on an orbital shaker table at 28 ºC to induce a swirling motion in the water content of the bottle. Shaker speed was 150 rpm for a period of 10 min followed by 1 to 2 min settling time to let the bigger size droplets return to surface. Samples were taken at 0, 5 and 10 min. The first 2 mL of the sample was removed through the stopcock and discarded, and 30 mL of the sample was collected. This sample was extracted 3 times with hexane, once the biosurfactant is insoluble in hexane. The extract was adjusted. Efficacy was calculated by dividing the concentration of dispersed oil in the water, which was determined by analyzing the hexane extract, by the total concentration of oil, which depended on the total volume of oil added to the flask [19, 22].

## 3. Results and Discussion

Oil spills, no matter how large or small, have long been of concern to pollution control authorities. While no prevention of oil spills should be of the highest priority, there is always a risk of spill on land and water during its extraction, transport, refinery and use. Due to its destructive nature, once an area has been contaminated by oil, the whole character of the environment is changed [22].

In this work the authors have tested a biosurfactant as one of the practical responses to oil spills.

The physico-chemical analysis of water was obtained according to the Standard Methods for the Examination of Water and Wastewater [23]. The results obtained showed a salinity of 16.9 mg/L, a pH of 7.32, a total hardness of 100 mg/L and an alkalinity

of 138.46 mg/L.

The effect of percolating (crude, at the CMC and at a concentration twice the CMC, Tween 20 and distilled water) facing the chemical characteristics of the soils was analyzed. Table 1 shows the results of removal of motor oil on different soil types in packed columns.

Removals around 75% and 92% were obtained in the clay and silty soils, respectively, with the crude biosurfactant, while percentages removal between 30% and 50% were obtained for the isolated biosurfactant in the soils tested. It was also observed that the use of the isolated biosurfactant at its CMC is sufficient to reach the best removal percentages values.

The results obtained with the crude biosurfactant were satisfactory once the use of the cell-free metabolic broth means a reduction in the production costs of 30%-50%. The synthetic surfactant Tween 20 and the distilled water removed around 20% of the oil in the soils tested.

The results demonstrated that the texture and size of the soils particles influenced the action of percolating, since the removal percentages were different when compared in the three soils. In this context, a higher removal was observed in the silt and in the clay soils although they have lower permeability due to formation of macro-pores between the grains of sand, through which water and air circulate more easily, as happens with the sandy soil. It is likely that interaction occurred between the biosurfactant and the silty soil, since much of its classified particles in the silt fraction, of size between 0.05 mm and 0.002 mm, are very small and light and does not add as clays. Due to the amphipathic nature, the biosurfactant formed micellar aggregates that interacted with the contaminant, promoting greater oil removal.

Many researches described in the literature report the application of biosurfactants in the removal of hydrocarbons contained in packed columns, especially the ones produced by bacterium species [5].

The biosurfactant from *P. aeruginosa* removed 56% of the oil adsorbed in sand under extreme conditions [24]. Jain et al. [19] investigated the potential use of two biosurfactants in removing oil in glass columns compared to synthetic surfactants. The results showed the efficiency of biosurfactants produced by *B. subtilis* PT2 and *P. aeruginosa* SP4 in removing oil. They exhibited values of 68% and 57%, respectively, compared to the synthetic surfactants Tween 80 (52%), SDBS (51%) and Alfoterra 5PO-145 (55%).

The biosurfactant produced by *Rhodococcus* sp. TA-6 was able to remove 70% of oil in packed columns [3], while the biosurfactants produced by *Bacillus* species cultured in cheese way and molasses removed around 30% of the oil [25]. The crude biosurfactant produced by *P. aeruginosa* removed 54% of the oil contained in columns [26] and surfactin from *Bacillus* sp. removed 34%-62% of krosene [27].

Microbially produced biosurfactants were studied to enhance crude oil desorption and mobilization in model soil column systems. The ability of biosurfactants from *Rhodococcus ruber* to remove the oil from the soil core was 1.4-2.3 times greater than that of a synthetic surfactant of suitable properties, Tween 60. Biosurfactant was less adsorbed to the soil

**Table 1 Removal of motor oil adsorbed to different types of soils contained in packed columns in static assays by the biosurfactant from *C. sphaerica* UCP 0995 cultured in a medium containing 2.5% corn steep liquor and 5.0% vegetal oil refinery waste.**

| Soils | Removal of motor oil by percolating liquids (%) | | | | |
|---|---|---|---|---|---|
| | Distilled water | Tween 20 | Crude biossurfactant | Biosurfactant | |
| | | | | (CMC) | (2 × CMC) |
| Clay | 19.0 ± 0.5 | 18.0 ± 0.7 | 75.0 ± 0.6 | 33.0 ± 0.6 | 39.0 ± 0.7 |
| Silty | 24.0 ± 0.3 | 22.0 ± 0.4 | 92.0 ± 0.7 | 30.0 ± 0.4 | 34.0 ± 0.5 |
| Sandy | 23.0 ± 0.2 | 26.0 ± 0.3 | 50.0 ± 0.5 | 51.0 ± 0.3 | 52.0 ± 0.4 |

components than synthetic surfactant, thus rapidly penetrating through the soil column and effectively removing 65%-82% of crude oil [5].

The biosurfactants produced by *P. aeruginosa*, *B. subtilis* and *R. erythropolis* isolated from the formation water of Chinese petroleum reservoir were compared in oil recovery. Oil recovery experiments in physical simulation showed 7.2%-14.3% recovery of residual oil after water flooding when the biosurfactants of three strains were added [28].

Table 2 displays the results of the kinetic experiments of the removal of motor oil adsorbed to different types of soils contained in Erlenmeyer flasks. Removal rates of 86% and 88% were found with the solutions containing the biosurfactant at its CMC and at a concentration twice the CMC, respectively.

The particle size and thickness of soil samples and the concentration of the biosurfactant did not exerted effect on pollutant removal once the removal rates were similar between the soils. It can be observed that the surfactant at its CMC is suited for application in removal of motor oil adsorbed to soil.

The biosurfactant produced from *C. antarctica* cultured in waste residue removed 50% of the oil adsorbed in sand [29].

Urum et al. [4] found that an increase in the concentration of rhaminolipids from 0.004% and 0.55% enhanced the bioremediation process of oil-contaminated soils. In another study, however, high concentrations of a biosurfactant isolated from *P. aeruginosa* 57SJ were needed to remove pyrene adsorbed to soil [30].

Using a biosurfactant produced by *C. guilliermondii*, Coimbra et al. [31] found 89.82% removal rate of oil in beach sand. Using a biosurfactant produced by *Candida glabrata* at a concentration of 2.5%, Luna et al. [18] found an 84% removal rate of motor oil adsorbed to sand.

Moreover, Coimbra et al. [31] also achieved satisfactory results with 97% removal of petroleum contained in beach sand with the crude biosurfactant from *C. lipolytica*. Silva et al. [32] applied a biosurfactant produced by *P. aeruginosa* UCP0992 cultured in glycerol and found removal rates greater than 85% for diesel in sand. Results obtained by Costa et al. [33] for the biosurfactant from *P. aeruginosa* L2-1 at its CMC showed removal percentages of 90.7% of crude oil adsorbed in sand samples, whereas the crude and isolated biosurfactant (CMC) from *P. aeruginosa* UCP0992 removed 87%-95% diesel in sand [32].

A comparative study describing a method to evaluate the petroleum removal capacity in soil by two biosurfactants and a synthetic surfactant showed that the performance of the biosurfactants was better.

The rhamnolipids and the surfactin removed around 60% petroleum [34].

Few methods are appropriate for the cleaning of contaminated coral reefs, as such ecosystems are extremely delicate and difficult to access. The use of dispersants is an attractive method when a sensitive nearby ecosystem, such as a mangrove, is threatened by an oil spill. Indeed, mangroves are a target of chronic pollution and accidental oil spills, as these environments are generally in confined, low-energy areas [22].

**Table 2 Removal of motor oil adsorbed to different types of soils contained in Erlenmeyers flasks in kinetic assays by the biosurfactant from *C. sphaerica* UCP0995 cultured in a medium containing 2.5% corn steep liquor and 5.0% vegetal oil refinery waste.**

| Soils | Removal of motor oil by percolating liquids (%) | | |
|---|---|---|---|
| | Distilled water | Biosurfactant | |
| | | (CMC) | (2 × CMC) |
| Clay | 40.0 ± 0.6 | 86.0 ± 0.5 | 88.0 ± 0.3 |
| Silty | 40.2 ± 0.7 | 86.1 ± 0.7 | 88.0 ± 1.1 |
| Sandy | 40.0 ± 0.8 | 86.0 ± 1.2 | 88.0 ± 0.7 |

The washing tests were performed in porous surface with the crude biosurfactant, as the results were similar or better than those obtained with the isolated biosurfactant. The oil removal rate was approximately 60%, demonstrating the considerable potential of the biosurfactant from *C. sphaerica* as a dispersant in this situation. The washing process is illustrated in Fig. 1.

Every year, millions of tons of crude oil emulsions are generated by petroleum exploration [35]. Before being transported and refined, crude oil emulsions should be demulsified to reduce water content and recover crude oil because water and waterborne impurities present in the emulsion are corrosive to pipelines and containers. Moreover, excess water in the emulsion also increases transportation costs, as it increases the total volume to be transported. As a consequence of demulsification, a large amount of separated water is produced, which is either recharged into the stratum or discharged to the ambient environment [36]. Currently, chemical demulsifiers are widely used to break crude oil emulsions [37]. The crude biosurfactant from *C. glabarata* was tested as a biodemulsifier for the demulsification of a fresh motor oil emulsion. The results obtained showed that the emulsion half-life (t1/2) was achieved after 24 h, indicating that the biosurfactant is not appropriated for such application. Liu et al. [17] showed that the biodemulsifier-producing strain of *Alcaligenes* sp. S-XJ-1, isolated from petroleum-contaminated soil of the Karamay oilfield, exhibited excellent demulsifying ability.

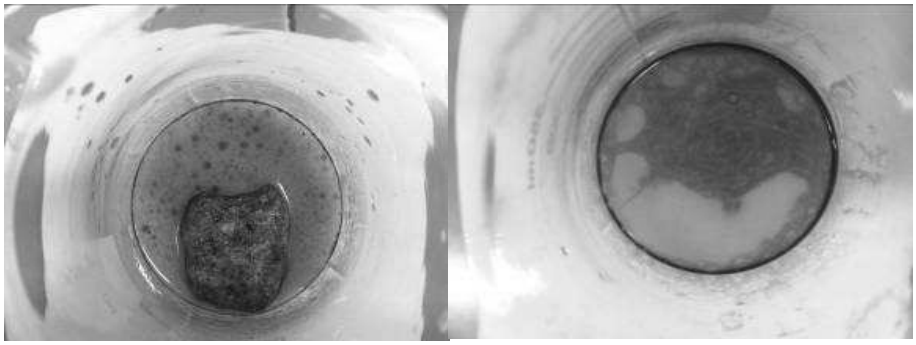
Biological surfactants have advantages over chemical surfactants as they are more efficient, effective and eco-friendly because they remove oil contaminants without modifying the chemical nature of soil by mobilization, due to the reduction of surface and interfacial tension [34].

The performance of crude biosurfactant was observed to be reasonable as it removed 41% oil compared to 22% oil removal by the commercially available surfactant Tween 20 from cotton cloth. The biosurfactant produced by *Klebsiella* cultivated in sucrose, on the other hand, showed excelent performance as it removed 100% of oil from cotton cloth [19].

Three methodologies were tested for the application of the biosurfactant as a dispersant agent.

The crude biosurfactant from *C. sphaerica* dispersed 75% oil in water after 1 min. It is important to say that the tests were conducted with the crude biosurfactant. So, it is expected to obtain better results with the use of the isolated biosurfactant. However, the use of the cell-free metabolic broth represents a considerable reduction in production costs of such compound, as discussed before.

According to Brochu et al. [20] bulky non-ionic surfactants have very particular property of dispersing oil in sea water. For example, Tween 81 and Tween 85 can disperse around 36% and 39% of oil in seawater, respectively, while the ionic Corexit 9527,



(A) (B)

**Fig. 1 Illustration of porous surface before (A) and during (B) washing process with crude biosurfactant from *C. sphaerica* UCP0995 cultured in medium containing 2.5% corn steep liquor and 5.0% vegetal oil refinery waste.**

Dispolen 34S and Dispolen 36S can not disperse any oil under the same conditions. The reason for this failure seems to be related to the presence of ionic surfactants or nonionic surfactants that are too water-soluble. According to Canevari [38], a formulation balanced for seawater would be too hydrophilic for fresh water. Problems can occur when oil dispersion is required in an estuarine environment where the salinity of water can range from 0% to 3.5% in a relatively limited area. In such an environment, Brochu ert al. [20] suggest a dispersant formulation containing mainly Tween 85 and/or Tween 81 which would free the oil spill-response team from the constraints of salinity variation. Although the biosurfactant produced by *C. glabrata* is anionic [12], the dispersion rate found in the work was very satisfactory under the conditions studied in the laboratory for a seawater sample with salinity of 1.6%. It seems that, with ionic surfactants, in addition to a favorable interfacial tension, the geometry and chemical composition of the molecules are important factors in the effectiveness of the dispersants, as suggested by Canevari [38].

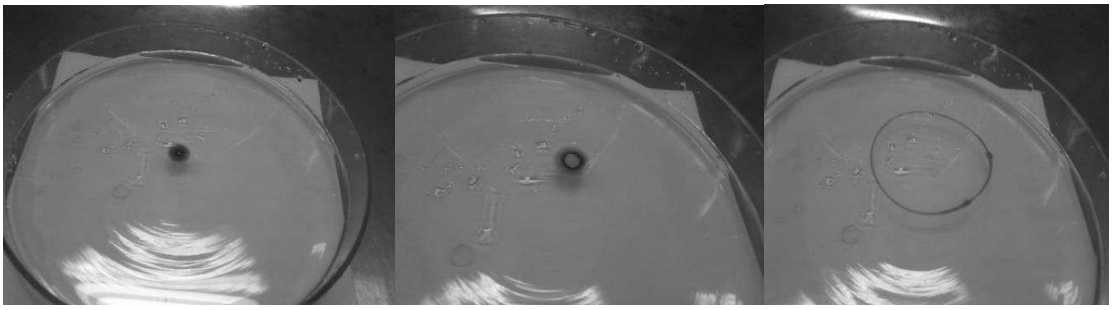
Fig. 2 illustrates the oil displacement efficiency of the biosurfactant from *C. sphaerica*. The results demonstrate an oil spreading efficiency of approximately 75%. Thus, this biomolecule exhibits considerable potential regarding the containment of oil spills in ocean environments.

According to Sitohy et al. [39] the isolated biosurfactant (0.1%) produced by *C. guilliermondii* NRRL Y-2075 cultivated in soy processing waste as substrate gave 100% oil displacement, while Triton X-100 gave 80% oil displacement and *B. subtilis* NRRL B-94 biosurfactant gave 57% oil displacement at the same concentration.

One of the oil spill remediation techniques is the application of dispersants to oil slicks. The dispersants used for this purpose are composed of complex mixtures of surfactants, solvents and additives. They enhance the rate of natural dispersion of oil and its removal from the contaminated surface. The application of dispersants minimizes the impact of oil spills on marine birds and mammals since it removes the oil from the water surface. In addition, the use of dispersants minimizes the impact of oil spills on sensitive resources on shorelines by reducing the amount of spilled oil. The increased surface area of oil as a result of its dispersion into small droplets is also expected to stimulate its biodegradation via the activity of naturally occurring microorganisms [40].

The effects of factors such as oil viscosity, mixing energy and temperature on the efficacy of a dispersant need to be evaluated. The solvent normally contained in dispersants acts as a solution for the surfactant components and serves as a carrier of the surfactants, enabling their penetration into an oil slick.

In this study the authors have tested the crude and the isolated biosurfactant without the addition of solvents or additives during 10 min after simulation of



(A) (B) (C)

**Fig. 2 Illustration of motor oil drop before (A), during (B) and after (C) dispersion due to the action of the crude biosurfactant from *C. sphaerica* UCP0995 cultured in a medium containing 2.5% corn steep liquor and 5.0% vegetal oil refinery waste.**

**Table 3 Evaluation of the biosurfactant from *C. sphaerica* UCP0995 cultured in a medium containing 2.5% corn steep liquor and 5.0% vegetal oil refinery waste as an oil spill dispersant.**

| Biosurfactant/oil ratio | Dispersion time (min) | Dispersion index (%) | | |
|---|---|---|---|---|
| | | Biosurfactant (CMC) | Biosurfactant (2 × CMC) | Crude biossurfactant |
| 1: 1 | 0 | 41.5 ± 0.6 | 72.0 ± 0.7 | 25.0 ± 0.2 |
| | 5 | 25.0 ± 0.7 | 58.0 ± 0.5 | 22.0 ± 0.5 |
| | 10 | 15.5 ± 0.4 | 47.0 ± 0.3 | 18.0 ± 0.1 |
| 1: 2 | 0 | 30.5 ± 0.5 | 39.0 ± 0.1 | 20.0 ± 0.5 |
| | 5 | 24.0 ± 0.3 | 34.0 ± 0.3 | 16.0 ± 0.6 |
| | 10 | 17.0 ± 0.1 | 23.5 ± 0.5 | 10.0 ± 0.4 |
| 1: 8 | 0 | 23.0 ± 0.1 | 31.7 ± 0.6 | 15.5 ± 0.3 |
| | 5 | 12.6 ± 0.6 | 26.4 ± 0.4 | 11.0 ± 0.1 |
| | 10 | 10.5 ± 0.4 | 18.3 ± 0.3 | 8.0 ± 0.7 |
| 1: 20 | 0 | 5.1 ± 0.1 | 7.0 ± 0.3 | 10.0 ± 0.2 |
| | 5 | 5.0 ± 0.1 | 5.0 ± 0.2 | 8.0 ± 0.5 |
| | 10 | 2.0 ± 0.3 | 5.0 ± 0.1 | 6.0 ± 0.3 |

an oil spill in a sea water sample (Table 3). It could be observed, for all the biosurfactant concentrations evaluated, that the time is an important factor since the best results were obtained immediately after the oil spill. It had been observed that the quick aggregation of the oil particles of on the water surface did not allow an efficient dispersion by the biosurfactant after the first minute. It was also observed that the lower the quantity of biosurfactant, the smaller the dispersion obtained. The best dispersion index was observed for a biosurfactant/oil ratio of 1:1 with a solution of the biosurfactant twice the CMC (72%), while the crude biosurfactant dispersed around 25% of the oil at the same condition. Other satisfactory dispersion percentages were obtained for the other conditions tested. The results obtained with this test demonstrate that the biosurfactant alone has potential for application as a dispersant. It is likely that the addition of additives will increase the efficiency of the biosurfactant.

Holakoo [22] showed that the commercial rhamnolipid JBR425 at 2% in saline applied at a dispersant oil-ratio of 1:2, 65% of crude oil in to the water without settling, but the percentage drop to 12.5% after 2 min of settling.

Sarubbo et al. [16] reported that the biosurfactant from *C. sphaerica* cultured in industrial residues showed high dispersing activity of motor oil, thus directing the oil spill in water, while the biosurfactant from *C. lipolytica* showed good oil emulsification activity, suggesting the ability of this last biosurfactant in solubilizing the oil and forming little droplets, thus facilitating the access of the hydrocarbon degrading microorganisms.

The dispersant/oil ratio is one of the critical factors influencing dispersant efficacy. Saeki et al. [41] studied the dispersion efficacy of the remediation agent JE1058BS that contains a biosurfactant produced by *Gordonia* sp. strain JE-1058. The efficacy values for 0.5%, 1%, 2%, and 3% JE1058BS/crude oil ratios were observed to be 33.4%, 70.7%, 79.5%, and 81.4%, respectively. This result indicated that a lower application ratio for oil spill remediation was sufficient.

It is important to say that the biosurfactant from *C. sphaerica* was used without the addition of solvents or dispersants which show the potential of this low-cost biosurfactant that would improve its efficacy if used in commercial formulations.

## 4. Conclusions

The biosurfactant produced by *Candida sphaerica* UCP0995 exhibited the capacity to remove a hydrophobic contaminant from soils under both static

and dynamic operating conditions, demonstrating its considerable potential as a low-cost adjuvant in industrial and environmental applications. This biosurfactant can also be used in microbial enhanced oil recovery and the cleaning of tanks as well as for the bioremediation of seawater contaminated with oil products. Tests for application of the biosurfactant as a detergent show the potential of the biosurfactant to be used in other industrial sectors.

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