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# Application of the Biosurfactant Produced by *Candida Sphaerica* as a Bioremediation Agent

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Environmental pollution caused by petroleum and its derivatives, such as diesel fuel, heavy oil, fuel residues, mineral oil and engine oil, is an issue of importance regarding both economic development and ecological restoration. Considerable amounts of petroleum products contaminate groundwater and soil as a consequence of leaks and spills from petroleum refinery processes, oil transportation and storage tanks. While contamination is caused by accidents in some cases, it is often the result of negligent disposal. Biosurfactants have received considerable attention in the field of environmental remediation processes. These substances influence such processes due to their efficacy as dispersion and remediation agents as well as their environmentally friendly characteristics, such as low toxicity and high biodegradability. Thus, this study investigated the potential application of a biosurfactant for enhanced removal capability of motor oil from contaminated sand and water, under laboratory conditions. The biosurfactant was produced by the yeast *Candida sphaerica* grown in distilled water supplemented with 9% ground nut oil refinery residue and 9% of corn steep liquor were used with their producer microorganism in the remediation of motor oil contained in sand and sea water. Sand oil bioremediation experiments were carried out for 90 days, while in sea water the period was 30 days. The results showed that the addition of the biosurfactant increased the degradation of the oil in the sand to 90%. With regard to the removal of oil on seawater, it was observed that removal percentages were around 85%. In this way, the biosurfactants produced, besides being obtained from low cost substrates, demonstrated efficiency in the removal of oils in sand and water, allowing the substitution of chemical treatment agents by environmental friendly agents.

## 1. Introduction

Oil spill has been a worldwide challenge in the modern society, which not only causes substantial economic loss, but also poses serious threats to the environmental and human health (Wang et al., 2018). Petroleum-based compounds are highly pollutant when released into the environment, constituting considerable public health and environmental problems due to the contamination of soil and water (Almeida et al., 2017; Cardona et al., 2019). Soil the and water that is accidentally contaminated with petroleum hydrocarbons can be remediated by physical, chemical, or biological methods. However, new trends in soil and water restoration avoid introducing synthetic chemicals (Luna et al., 2017). One of the promising techniques to restore contaminated environments is the utilization of bioremediation is recognized as the most preferred measures on removal of oil because they are generally cost effective and environmentally friendly (Jimoh and Lin, 2019; Wang et al., 2019).
Bioremediation played an important role in the cleaning of the spillage of 41 million liters of oil by the oil tanker Exxon Valdez in the Gulf of Alaska in 1989, giving rise to the development of this technology and demonstrating that there are good reasons to believe in the effective application of this method for the treatment of future oil spills under appropriate circumstances. While it was difficult to evaluate the effects of

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treatment due to the heterogeneity of the contamination, other studies have demonstrated the importance of the use of surfactants to enhance the biodegradation of oil (Luna et al., 2018).
Surfactants are used to increase the solubility of oil and enable bioremediation (Silva et al., 2018). The biosurfactants are environmentally compatible, have lower toxicity and can be released into the environment without resulting in further damage from residues. Furthermore, can be synthesized from renewable feedstocks such as industrial wastes and by-products especially, such as vegetable oil, distillery and milk product residues (Olasanmi and Thring, 2019, WSilva et al., 2018). Biosurfactants are amphipathic active surface molecules that are produced by a large variety of microorganisms and have the capacity to reduce the surface and interfacial tensions of solutions (Silva et al., 2018). Characteristics such as detergency, emulsification, dispersion, wetting action and solubilization confer considerable versatility to these biomolecules, making them potential candidates for the replacement of synthetic surfactants, which are more toxic (Nogueira Felix et al., 2019).
Therefore, the development of economic processes for biosurfactant production is key to reducing costs and increasing competitiveness (Almeida et al., 2017). Thus, this study investigated the potential application of a biosurfactant for enhanced removal capability of motor oil from contaminated sand and water, under laboratory conditions.

## 2. Materials and methods

### 2.1 Microorganism

*Candida sphaerica* (UCP 0995) was obtained from the culture collection of the Catholic University of Pernambuco, Brazil. The microorganism was maintained at 5 ºC on Yeast Mold Agar (YMA) slants containing (w/v): yeast extract (0.3 %), malt extract (0.3 %), tryptone (0.5 %), D-glucose (1.0 %) and agar (5.0 %).

### 2.2 Sand

Standard sand samples NBR 7214 (ABNT, 2015) were used in the experiments. The sand has Particle size 0.15 to 0.30 mm, Water 0.2 %, Specific density 2.620 g/cm$^3$ and Organic matter 100 ppm.

### 2.3 Substrates

Two types of industrial waste were used as substrates to produce the biosurfactant. Corn steep liquor was purchased from Corn Products of Brazil (municipality of Cabo de Santo Agostinho, Pernambuco, Brazil) and Ground nut oil refinery residue, provided by ASA LTDA in the city of state Recife-PE.

### 2.4 Growth Conditions

The biosurfactant production conditions used in this work were previously established according to Luna et al. (2015). The inoculum of *C. sphaerica* was prepared by transferring cells grown on aslant with 50mL of yeast mold broth (YMB). The seed culture was incubated for 24h at 28 ºC and agitated at 200rpm. The basal medium was composed 9.0% ground nut oil refinery residue and 9.0% corn steep liquor dissolved in distilled water. The medium was sterilized by autoclaving at 121°C for 20min. The final pH of the medium was 6.0. The inoculum (1.0%, v/v) was added to the cool medium at the amount of $10^4$ cells/mL. Cultivation was carried out in Erlenmeyer flasks at 30 °C with shaking at 200 rpm for 144 h.

### 2.5 Evaluation of biodegradation capacity of oil adhered to sand

Samples of 10 g of sand contaminated with motor oil were added to 100 mL of potable water and the mixture was enriched with 1 mL of sugarcane molasses acquired from a local sugar plant.The mixture was sterilized with fluent steam, next, solutions of the isolated biosurfactant at the CMC and 2 × CMC and 15% of the producing microorganism (15% of the inoculum containing 108 cells/mL of the yeast) previously cultured in its preparation medium (YMB) were added. The mixtures were incubated at 150 rpm for 90 days at 28ºC. One percent molasses was added to the mixtures every 15 days of the experiment four times (15, 30, 45 and 60 days). Samples (5 mL) were removed every 15 days (15, 30, 45, 60, 75 and 90 days) for the determination of the percentage of motor oil removed from the sand, totaling seven samples. The percentage of oil degradation was calculated by the amount of oil removed (determined by gravimetry) (Joshi et al., 2008).

### 2.6 Application of biosurfactant for remediation of contaminated seawater

The motor oil biodegradation experiments were performed in 250-mL Erlenmeyer flasks containing 50 mL of seawater and 1% motor oil. The medium was sterilized and then inoculated with 5% of inoculum of the biosurfactant-producing yeast. The experiments were conducted under three different conditions: 1) seawater + motor oil + *C. sphaerica* UCP 0995; 2) seawater + motor oil + *C. sphaerica* UCP 0995 + biosurfactant at the critical micelle concentration (CMC: 0.2 g/L); and 3) seawater + motor oil + *C. sphaerica* UCP 0995 + biosurfactant at two times the CMC (0.4 g/L). The flasks were incubated in a rotary shaker at 150 rpm for 30 days. Samples were removed for analysis every ten days (total: three samples).

*Table 1: Mixtures formulated for motor oil removal from sand.*

| Mixtures | Composition |
|---|---|
| Control | Contaminated sand + sugarcane molasses |
| Condition 1 | Contaminated sand + sugarcane molasses + *C. sphaerica* |
| Condition 2 | Contaminated sand + sugarcane molasses + biosurfactant from *C. sphaerica* at CMC + *C. sphaerica* |
| Condition 3 | Contaminated sand + sugarcane molasses + biosurfactant from *C. sphaerica* at 2 × CMC + *C. sphaerica* |

*Table 2: Formulated mixtures for biodegradation experiments of motor oil in seawater.*

| Mixtures | Composition |
|---|---|
| Control | Seawater + motor oil |
| Condition 1 | Seawater + motor oil + *C. sphaerica* |
| Condition 2 | Seawater + motor oil + *C. sphaerica* + biosurfactant at CMC |
| Condition 3 | Seawater + motor oil + *C. sphaerica* + biosurfactant at 2 × CMC |

## 3. Results and Discussion

### 3.1 Removal of hydrophobic contaminant from sand by surfactants in kinetic assay

Figure 1 presents the results of the removal of motor oil adsorbed to sand by the biosurfactant produced by *Candida sphaerica* in the kinetic assay.

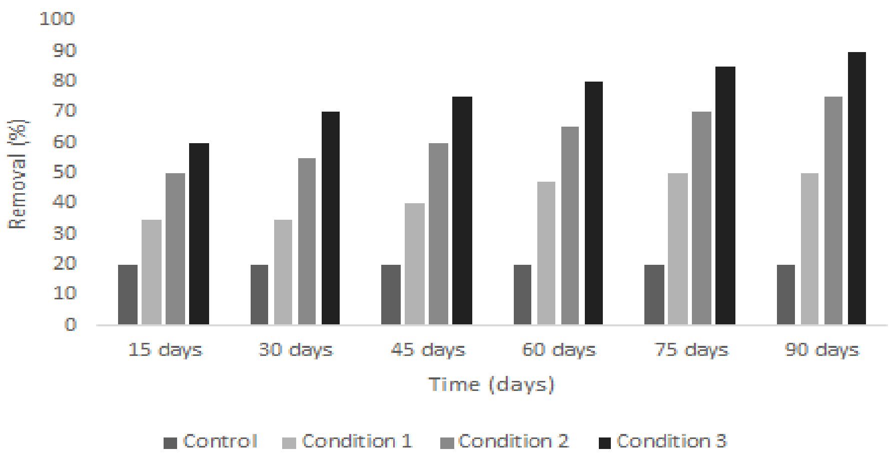


*Figure 1: Removal of oil adsorbed to sand by bioremediation process using biosurfactant produced by Candida sphaerica.*

The addition of biosurfactant increased the oil removal rate compared to the condition without biosurfactant. The highest removal rates (90%) were achieved at the 90-day evaluation with the biosurfactant at a concentration of 2 × CMC. Thus, the concentration of the isolated biosurfactant exerted an influence on the removal rate, with an increase in the solubilization of the oil in the aqueous phase by the biosurfactant above the CMC. According to (Silva et al., 2018), two mechanisms are associated with oil removal from soil: mobilization and solubilization. Mobilization occurs at concentrations below the CMC and the phenomena

associated with this mechanism include the reduction of surface and interfacial tensions; the surfactant monomers increase the angle of interaction between the soil and hydrophobic contaminant, enabling the separation of the contaminant from the soil particles and the consequent displacement of the oil. Solubilization occurs at concentrations above the CMC; the contaminant is partitioned in the center of the surfactant micelles (Chaprão et al., 2015). The addition of biosurfactant to the biostimulation method has positive effects on the desorption of hydrophobic organic compounds adsorbed to soil and the increase in the solubility of these compounds, especially when biosurfactants are used at concentrations above the CMC (Nitschke and Pastore 2002). In a study conducted by (Chaprão et al., 2015), biosurfactants from *Candida sphaerica* and *Bacillus sp*. achieved oil removal rates of 70 and 80%, respectively. An isolated biosurfactant from *C. glabrata* achieved an 84% motor oil removal rate (de Luna et al., 2009).
Moreover, (Santos et al. 2017a) demonstrated the considerable capacity of a biosurfactant produced by C. lipolytica regarding the removal of motor oil and petroleum adsorbed to sand. Using a biosurfactant produced by *P. cepacian*, (Soares da Silva et al. 2017) found removal rates greater than 70%, with maximum removal (96%) achieved when the isolated biosurfactant was used at a concentration of 2 × CMC.
In this study, it can also be observed that the concentration of isolated biosurfactant influenced the percentage of removal, demonstrating the increase of oil solubilization capacity in the aqueous phase by Candida sphaerica biosurfactant above CMC. Therefore the bioremediation technique in hydrocarbon contaminated (Figure 2) soils demonstrates a positive role of biosurfactants in pollutant biodegradation (Mao et al., 2015).

*Figure 2: oil adsorbed on sand by the bioremediation process using the biosurfactant produced by C. sphaerica by kinetic assay.*

### 3.2 Application of biosurfactant for remediation of contaminated seawater

Figure 3 presents the results of the bioremediation experiments involving oil in seawater in a 30-day period. The increase in time (days) and biosurfactant concentration were favorable to the increase in the percentage of oil degradation.

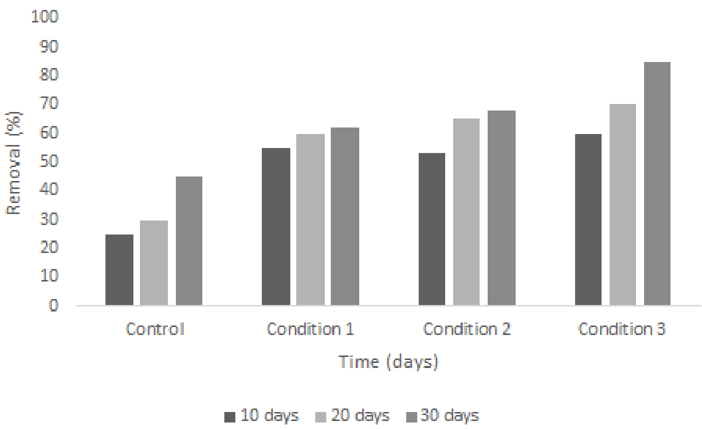


*Figure 3: Removal of oil contaminant from seawater by bioremediation process using biosurfactant produced by Candida sphaerica.*

The best result was achieved at 30 days, with 85% oil removal when the biosurfactant was added at 2 × CMC. However, lower concentrations of biosurfactant (Conditions 1 and 2) also achieved good results, with the removal rate increasing over time. Santos et al., 2017, report the promising effect of a biosurfactant produced by *C. lipolytica* regarding the growth of autochthonous microorganisms in seawater and the enhancement of the biodegradation of motor oil at concentrations of ½ CMC, CMC and 2 × CMC over a 30-day period. Dispersion is related to both the interfacial tension and the surfactant concentration, and is different from displacement in that the displacement process is only related to the interfacial tension between aqueous and hydrophobic phases and no emulsion form (Sobrinho et al., 2013).The dispersant capacity of a biosurfactant is of extreme importance in the treatment of marine environments contaminated with hydrocarbons. This characteristic facilitates the access of autochthonous microorganisms to the pollutant, potentiating the bioremediation process (Luna et al., 2013). In the present study, the isolated biosurfactant from *Candida sphaerica* promoted the accelerated growth of these microorganisms throughout the 30 days of culture and served as a solubilizing agent for motor oil, thereby facilitating its biodegradation (Figure 4).

*Figure 4: oil adsorbed to seawater by bioremediation process using the biosurfactant produced by C. sphaerica.*

## 4. Conclusions

This paper described the production of a low cost biosurfactant and demonstrated its applicability in the bioremediation of contaminated environments with petroleum products. In the kinetic assays, the motor oil removal rate from soil was 90% in a period of 90 days. In the tests performed with contaminated seawater, the oil removal rate was 85%. The results demonstrate that the biosurfactant produced by *C. sphaerica* has promising properties as a bioremediating agent for soil and water contaminated with hydrophobic compounds.

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