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Application of *Candida sphaerica* Biosurfactant for Enhanced Removal of Motor Oil from Contaminated Sand and Seawater

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Environmental pollution caused by petroleum and its derivatives, such as diesel fuel, heavy oil, gasoline, 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, at 28°C during 144h under 200rpm. Bioremediation tests were conducted to examine the effectiveness of the biosurfactant and its isolated microbial producing species in the removal of oil contaminated soil and seawater. The results showed that the presence of the biosurfactant increased the removal rates, acting as an efficient enhancer for hydrocarbon biodegradation.The performance of biosurfactant was excellent, removing 95% of oil at concentration of twice the CMC value. With regard to the removal of oil on seawater, it was observed that removal percentages were around 85%. The biosurfactant was also effective in recovery of up to 80 % motor oil from the walls of beakers and in oil displacement 100% in seawater at twice CMC. These results indicate the potential value of the biosurfactant for application in the oil industry, especially in enhanced oil recovery, tank cleaning and in bioremediation of spills at seas and soils.

1. Introduction

Oil spill accidents result in significant contamination of the ocean and shoreline environments. It is estimated that 0.08%-0.4% of the total worldwide production of petroleum eventually reaches the oceans. Such incidents have intensified attempts to develop procedures and technologies for combating oil pollution in the environment. Soil 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. Among the remediation techniques available for contaminated sites, bioremediation is regarded as environmentally friendly because it preserves the soil structure, requires little energy input, and involves the complete destruction or immobilization of the contaminants, although the efficiency of biodegradation of oil pollutants is often limited by their poor water solubility (Santos et al., 2016). One of the approaches to enhance biodegradation of oils is to use biosurfactants, which could increase solubility of oils in water to enhance the bioavailability of the hydrophobic substrates, leading to higher oil degradation rates.

Surface-active compounds of biological origin have attracted much attention and their popularity seems to steadily increase during recent years. This fact may be attributed to an evolved approach towards industrial production, which favors both environmental awareness and sustainability through use of renewable resources (Bachmann et al., 2014; Sarubbo et al., 2015a). The numerous advantages of biosurfactants compared to their synthetic counterparts are yet another reason why these compounds seem so promising. While biosurfactants are generally equally effective in terms of solubilization and emulsification, they are also considered to be biodegradable, less toxic, and thus by far, more environmentally friendly than synthetic surfactants. Since these molecules may be obtained from waste materials, their production also seems to be feasible in terms of economic justification (Banat, 2010). All these relevant traits contribute to a high applicability of biosurfactants, which currently stems to several branches of industry. The much extolled environmental friendliness combined with the ability to solubilize hydrophobic compounds may well explain why biosurfactants have also been recognized as excellent agents for improving bioremediation of contaminated environments (Sarubbo et al., 2015b). First and foremost, biosurfactants tend to interact with poorly soluble contaminants and improve their transfer into the aqueous phase. This allows for mobilization of recalcitrant pollutants which have been embedded in the soil matrix and their subsequent removal. The presence of biosurfactants may also lead to a potential enhancement of biodegradation efficiency. In this concept, the biosurfactant molecules act as mediators, which increase the mass transfer rate by making hydrophobic pollutants more bioavailable for microorganisms (Silva et al., 2014; Luna et al., 2015). Thus, environmental and economic issues have motivated the completion of this study that presents biosurfactant production by the yeast *Candida sphaerica* using a low-cost medium supplemented. The application of the biosurfactant in the environment was also investigated.

2. Materials and Methods

2.1 Materials

Two types of industrial waste were used as substrates to produce biosurfactants. Ground-nut oil refinery residue was obtained from ASA LTDA, Recife-PE, Brazil, and corn steep liquor from Corn Products do Brasil, Cabo de Santo Agostinho-PE, Brazil. Seawater 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. Motor oil (15 cSt) was obtained from an automotive maintenance establishment in the city of Recife, Pernambuco, Brazil. We call motor oil to the lubricating oil after use.

2.2 Sand

Standard sand samples NBR 7214 (ABNT, 1982) 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 Micro-organism

Candida sphaerica UCP 0995 was obtained from the culture collection of the Catholic University of Pernambuco, Brazil. The micro-organism 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 %). Transfers were made to fresh agar slants each month to maintain viability.

2.4 Production of biosurfactant

The production of biosurfactant was performed in distilled water based medium with 9 % of refinery residue of soybean oil and 9 % of corn steep liquor. For inoculation, the flasks were allowed to cool down to room temperature (27 °C) before transferring 1 % (v/v) primary inocula of the cell suspension into the production media. The cultures were incubated in a rotary shaker for 144 h at 200 rpm. There was no adjustment of pH during cultivation.

2.5 Application of the biosurfactant in hydrophobic contaminant cleaning test

As a means to check the cleaning ability of the biosurfactant, the inner walls of a set of beakers were coated with motor oil. To remove the adhered oil, 50.0 ml of the cell-free broth or wash solutions containing the isolated biosurfactant at 1/2CMC, at CMC and 2XCMC was added to each beaker, vortexed for 1 min, and allowed to stand for 6 h (Pruthi and Cameotra, 2000).

2.6 Application of the biosurfactant in hydrophobic contaminant spreading

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 cell-free broth or aqueous solutions containing the isolated surfactant at 1/2CMC, CMC and 2XCMC 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 (Saeki et al., 2009).

2.7 Application of biosurfactant in removal of motor oil from sand

Samples of the contaminated sand (10g) were added to 100 mL drink water and the mixture was enriched with 1mLof sugar cane molasses. This mixture constituted the indigenous consortium. The isolated biosurfactant at 1/2 the CMC, the full CMC and twice the CMC were added and/or1% of its microbial producing specie, previously cultivated in nutrient broth were added and the medium kept in a rotary shaker at 150 rpm, and 28°C at during 90 days according to Table1. The experiments were performed in triplicate using 125 ml erlenmeyers flasks. Samples of 5mLwere removed after 5, 30, 45, 60, 75 e 90 days (Joo et al., 2008).

Table 1: Formulated mixtures for motor oil biodegradation experiments in sand.

Experiments	**Composition**
Set 1	Contaminated sand + sugar cane molasses (control)
Set 2	Contaminated sand + sugar cane molasses + *C. sphaerica* cells
Set 3	Contaminated sand + sugar cane molasses + *C. sphaerica* biosurfactant (0.0125%)+ *C. sphaerica* cells
Set 4	Contaminated sand + sugar cane molasses + *C. sphaerica* biosurfactant (0.025%)+ *C. sphaerica* cells
Set 5	Contaminated sand + sugar cane molasses + *C. sphaerica* biosurfactant (0.05%)+ *C. sphaerica* cells

2.8 Application of biosurfactant in removal of motor oil from seawater

Bioremediation tests were performed 125 ml Erlenmeyer flasks were filled with 50ml of fresh seawater obtained from the Suape Petrochemical Complex, state of Pernambuco, Brazil. 1.0% of motor oil and the isolated biosurfactant at 1/2 the CMC, the full CMC and twice the CMC were added and/or1% of its microbial producing specie, previously cultivated in nutrient broth were added and the medium kept in a rotary shaker at 150 rpm, and 28°C at during 30 days according to Table 2. Samples of 5mL were removed after 10, 20 e 30 days (Lai et al., 2009).

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

Experiments	**Composition**
Set 1	Contaminated Seawater + motor oil (Control)
Set 2	Seawater + motor oil+ *C. sphaerica* cells
Set 3	Seawater + motor oil + *C. sphaerica* biosurfactant (0.0125%)
Set 4	Seawater + motor oil + *C. sphaerica* biosurfactant (0.025%)
Set 5	Seawater+ motor oil + *C. sphaerica* + biossurfactante (0.05%)

2.9 Total motor oil biodegradation rate in sand or seawater

The samples were drawn for estimation of motor oil degradation by gravimetric analysis. The residual motor oil was extracted in a preweighed beaker with hexane in a separating funnel. Extraction was repeated twice to ensure complete extraction. After extraction, hexane was evaporated in a hot air oven at 68–70°C, the beaker was cooled down and weighed.

The % degradation was calculated as follows:

$$\text{Motor oil degradation (\%)} = (Od - Os) / Od \times 100\% \qquad (1)$$

Where Od is the amount of motor oil degraded (g) Os is the amount of motor oil added in the sand or seawater (g).

3. Results and Discussion

3.1 Application of the biosurfactant in hydrophobic contaminant cleaning test

After stirring for 10 minutes and 24 h of rest, it was observed removals of 40%, 50% and 80% of the oil from the beaker walls by the biosurfactant at concentrations of 1/2xCMC, CMC and 2XCMC respectively.

3.2 Application of the biosurfactant in hydrophobic contaminant spreading

The results obtained showed values of 100%, dispersion of the oily compound in seawater after the addition of the isolated biosurfactant at 2X CMC (Figure 1).

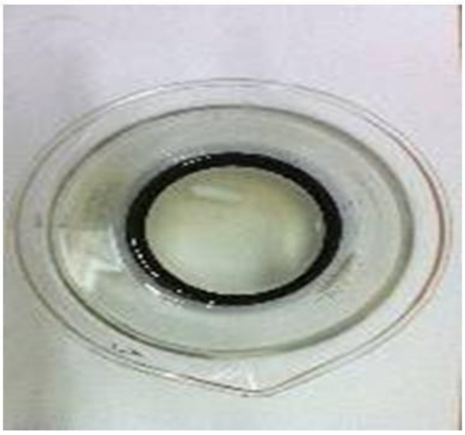

Figure 1: Illustration of dispersion due to the action of biosurfactant produced by C. sphaerica.

3.3 Motor oil biodegradation

Five different sets were used to study motor oil biodegradation. The results were analyzed during 5, 30, 45, 60, 75 e 90 days for each set as shown in Figure 2.
The addition of molasses provided required nutrients for enhanced growth of the microorganisms and the biodegradation of the petroleum derivate. Molasses is a co-product of sugar production, both from sugar cane as well as from sugar beet industry in Brazil. Molasses is rich in carbon, organic nitrogen and mineral compounds required for growth of microorganisms. Therefore, molasses was added to the mixtures of contaminated sand along the experiments.
Degradation was not observed in the control (contaminated sand + sugar cane molasses). In the first set of experiment (Contaminated sand + sugar cane molasses + *C. sphaerica*), the oil degradation reached 20% after 90 days.
The performance of biosurfactant was excellent, removing 95% of oil at concentration of twice the CMC value. The increase of biosurfactant concentration produced also accelerated the degradation process in the first 45 days of the experiment (Set 5), i.e., the biosurfactant increased the degradation rate in 10%, indicating that biosurfactant acted as an efficient enhancer for hydrocarbon biodegradation. It may be due to i) increase in the surface area of hydrophobic water-insoluble substrates and ii) increase in the bioavailability of hydrophobic compounds (Jadhav et al., 2013). Variable results have been shown concerning the utility of using biosurfactants in hydrocarbon solubilization and biodegradation (Chang et al., 2004). According to Zheng et al. (2012), the solubilizing capacity of a specific surfactant is determined only by its intrinsic micelles property and thus enhancing its solubilizing capacity is usually very difficult. Therefore, continuing efforts have been made to search for new surfactants or biosurfactants with much higher solubilizing efficacy, lower cost and low microbial toxicity.

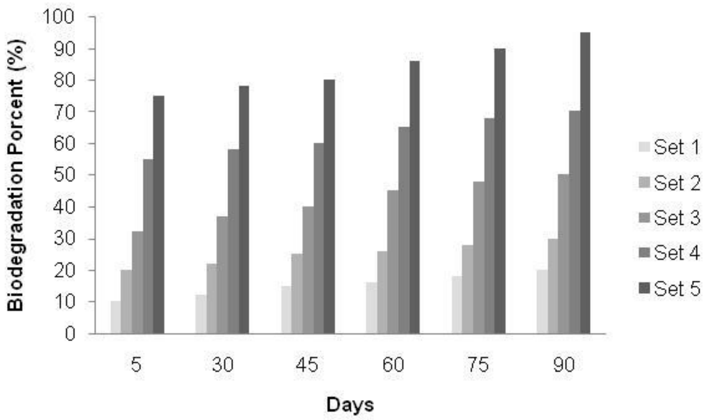

Figure 2: Biodegradation of motor oil. Set 1— contaminated sand + sugar cane molasses; Set 2 — contaminated sand + sugar cane molasses + C. sphaerica.; Set 3 — contaminated sand + sugar cane molasses + C. sphaerica biosurfactant (0.0125%) + C. sphaerica ; Set 4 — contaminated sand + sugar cane molasses + C. sphaerica biosurfactant (0.025%)+ C. sphaerica; Set 5 — contaminated sand + sugar cane molasses + C. sphaerica biosurfactant (0.05%)+ C.sphaerica.

3.4 Application of biosurfactant in removal of motor oil from seawater

The dispersive capacity of a biosurfactant is of extreme importance when it is intended to be used in the treatment of marine environments contaminated with hydrocarbons, since this characteristic will be responsible for facilitating the access of autochthonous microorganisms to the pollutant, providing the occurrence of the bioremediation process (Luna et al., 2015).

The results were analyzed during 10, 20 e 30 days for each set as shown in Figure 3. It was possible to observe that the time (days) and the increase of the biosurfactant concentration were favorable for the increase of the percentage of degradation of the oil. The best result was during the day 30 of experiment, obtaining 85% oil removal when the biosurfactant was added in 2xCMC (Set 5), but it can be observed that in experiments with lower concentrations of biosurfactant, also obtained good results, where the percentage of removal has increased over time.

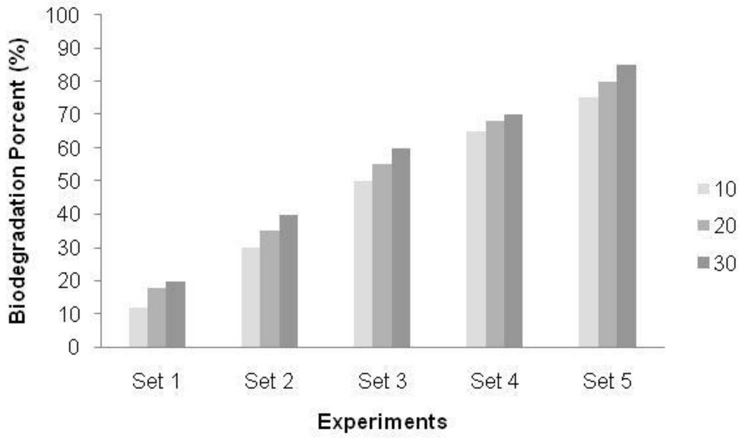

Figure 3: Biodegradation of motor oil. Set 1— Seawater +oil motor; Set 2 — Seawater +oil motor + C. sphaerica.; Set 3 — Seawater +oil motor+ C. sphaerica biosurfactant (0.0125%) + C. sphaerica ; Set 4 — Seawater +oil motor+ C. sphaerica biosurfactant + C. sphaerica biosurfactant (0.025%)+ C. sphaerica; Set 5 — Seawater +oil motor + C. sphaerica biosurfactant (0.05%)+ C.sphaerica.

4. Conclusions

The results of the removal of oily stains experiments under different contamination conditions clearly demonstrate the viability of application of this biomolecule as an additive for remediation processes that consider the preservation and the reduction of environmental impacts on ecosystems water, essential aspects for maintaining quality of life. It is mandatory to have conclusions in the manuscript**.** This ensures completeness of the presentation as well as provides the readers with an idea about the significance of the achievements in the presented work.

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