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Research Article

Application of bacterial and yeast biosurfactants for enhanced removal and biodegradation of motor oil from contaminated sand

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ABSTRACT

Background: This study investigated the potential application of two biosurfactants for enhanced removal capability and biodegradation of motor oil contaminated sand under laboratory conditions. The biosurfactants were produced by the yeast *Candida sphaerica* and by the bacterium *Bacillus* sp. cultivated in low-cost substrates. The ability of removing motor oil from soil by the two biosurfactants was identified and compared with that of the synthetic surfactants Tween 80 and Triton X-100.

Results: Both crude and isolated biosurfactants showed excellent effectiveness on motor oil removal from contaminated sand under kinetic conditions (70–90%), while the synthetic surfactants removed between 55 and 80% of the oil. A contact time of 5–10 min under agitation seemed to be enough for oil removal with the biosurfactants and synthetic surfactants tested. The crude and the isolated biosurfactant from *C. sphaerica* were able to remove high percentages of motor oil from packed columns (around 90%) when compared to the biosurfactant from *Bacillus* sp. (40%). For the degradation experiments conducted in motor oil contaminated sand enriched with sugar cane molasses, however, oil degradation reached almost 100% after 90 d in the presence of *Bacillus* sp. cells, while the percentage of oil degradation did not exceed 50% in the presence of *C. sphaerica*. The presence of the biosurfactants increased the degradation rate in 10–20%, especially during the first 45 d, indicating that biosurfactants acted as efficient enhancers for hydrocarbon biodegradation.

Conclusions: The results indicated the biosurfactants enhancing capability on both removal and rate of motor oil biodegradation in soil systems.

1. Introduction

In recent years, much attention has been directed towards biosurfactants owing to their different advantages such as lower toxicity, higher biodegradability, better environmental capability, higher foaming, high selectivity, specific activity at extreme temperatures, pH and salinity, and the ability to be synthesized from renewable feed stocks [1]. Some disadvantages can be mentioned for the use of biosurfactants: at the time, a small amount of biomolecules is produced at industrial level. Many biosurfactants are yet in a laboratory scale level and some of them are quite expensive. The discovery of new biosurfactants, development of new fermentation and recovery processes and the use of cheap raw materials (specifically the use of agro-industry wastes as carbon sources) will allow that more inexpensive biosurfactants can be available for remediation process [2].

The major difficulty in bioremediation of oil-contaminated soil is the bioavailability or mass transfer limitation of the oil pollutants in the soil, causing poor food-microorganism contact and thus poor biodegradation efficiency [3]. Oil penetration through soil is an extremely complex process related to physical, chemical, and biological factors [4]. Petroleum hydrocarbons are highly hydrophobic material with low water solubility and those components attach to soil particles, reducing the bioavailability of oil compounds to microorganisms, thereby limiting the rate of mass transfer for biodegradation. The possible physical forms for oil contaminants in soil can be dissolved in pore water, adsorbed onto soil particles, absorbed into soil particles, or be present as a separate phase, which can be a liquid or a solid phase [3]. The key process to enhance the bioavailability of the oil contaminant is to transport the pollutant to the aqueous bulk phase [5]. One of the effective ways to increase the bioavailability (or solubility) of hydrophobic pollutants in soil is using surfactants to enhance the desorption and solubilization of petroleum

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hydrocarbons, thereby facilitating their assimilation by microorganisms [5,6,7].

Enhanced soil washing generally has been performed with synthetic surfactants, including anionic, nonionic, cationic and mixed surfactants, and some of them have shown great washing capabilities for hydrophobic organic compounds (HOCs) from contaminated soils and groundwater [8]. Some synthetic surfactants, such as Triton X-100, Tween 80, Afonic 1412-7, are shown to be able to enhance the concentration of nonpolar compounds in the aqueous phase [5,6]. However, the residual synthetic surfactants in soils and groundwater have the potential toxicity risk or hazard to environment and human health. So, an improved strategy for soil washing technology is to use biosurfactants [9]. Therefore, biosurfactants seem to be better candidates for using in soil washing technology. The literature data indicated that most of previous studies have focused on few biosurfactants [5,10,11]. More other biosurfactants should be investigated for their properties in enhancing soil washing because they may have more promising properties [9].

At low concentrations, biosurfactants are soluble in water, and with increasing concentrations, they form micelle in solution. The concentration at which micelle begins to form is called the critical micelle concentration (CMC); above the CMC, biosurfactants can solubilize petroleum hydrocarbons in soil-water systems, but some biosurfactants may increase the water solubility of hydrocarbon molecules below the CMC. Therefore, biosurfactants may be useful in degradation of soil contaminating hydrocarbons [12].

The aims of this work were to use two biosurfactants, *i.e.*, a glycolipid produced by *Candida sphaerica* [13] and another new biosurfactant produced by *Bacillus* sp. to remove motor oil from a laboratory oil-contaminated sand and to compare their efficiency with two commonly used synthetic surfactants (Tween 80, and Triton X-100) in agitated (flasks) and static assays (packed columns). Additionally, potential application of the two biosurfactants for enhanced biodegradation of motor oil contaminated sand with a series of bench-scale experiments was evaluated.

2. Materials and methods

2.1. Materials

All chemicals were of reagent grade. Growth media were purchased from Difco Laboratories (USA).

Three types of industrial waste were used as substrates to produce the biosurfactants. Ground nut oil refinery residue was obtained from ASA LTDA in the city of Recife, in Pernambuco state, Brazil. Corn steep liquor was obtained from Corn Products of Brazil in the city of Cabo de Santo Agostinho, Pernambuco, Brazil and sugar cane molasses was obtained from a local plant cane sugar in the city of Igarassu, Pernambuco, Brazil.

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

Samples of 100/50 mesh (0.15–0.3 mm) of Brazilian standard sand NBR 7214 [14] were used in the experiments. Laboratory impregnated sand samples with motor oil were prepared and left to stand at room temperature for 24 h until subsequent use.

2.3. Synthetic surfactants used

Two chemically synthesized surfactants (namely, Tween 80 and Triton X-100) were also used for motor oil removal from contaminated soil to compare their performance with that from biosurfactants. Tween 80 (purchased from Sigma Chemical Co. St. Louis, MO, USA) is a nonionic surfactant and an oil-in-water emulsifier. The CMC of Tween 80 is about 0.0124% (w/v) (120 mg/L) and the surface tension is able to be reduced to 43.7 mN/m. Triton X-100, also obtained from Sigma Chemical Co. (St. Louis, MO, USA), is a nonionic surfactant possessing a hydrophilic polyethylene oxide group and a hydrocarbon lipophilic or hydrophobic group. The CMC of Triton X-100 is about 0.0183% (w/v) (183 mg/L) and the surface tension is able to be reduced to 32.7 mN/m.

2.4. Microorganisms and preparation of seed cultures

C. sphaerica UCP 0995 was obtained from the culture collection of the Universidade Católica de Pernambuco, Brazil. The microorganism was maintained at 5°C on yeast mold agar slants. The *C. sphaerica* inoculum was prepared by transferring cells grown on a slant to 50 mL of yeast mold broth. The seed culture was incubated at 28°C and 150 rpm for 24 h.

The *Bacillus* sp., an indigenous bacterium, was isolated from a petroleum contaminated soil site located in Recife city, Brazil. The bacterium culture was maintained on nutrient agar slants at 4°C. For pre-culture, the strain from a 24 h culture on nutrient agar was transferred to 50 mL of nutrient broth to prepare the seed culture. The cultivation conditions for the seed culture were 28°C, 150 rpm and 10 to 14 h of incubation.

2.5. Production of biosurfactant

The microorganisms were cultivated in a submerged culture in a Marconi MA832 shaker (Marconi LTDA, Brazil).

The yeast biosurfactant was produced in a medium composed of 9% ground nut oil refinery residue and 9% corn steep liquor dissolved in distilled water. The final pH of the medium was 5.3 and the surface tension prior to inoculation was 50 mN/m. The inoculum (1%, v/v) was added to the cooled medium at the amount of 10^4 cells/mL. Fermentation was carried out in 500 mL Erlenmeyer flasks at 28°C and 150 rpm for 144 h [13].

The bacterium biosurfactant was produced in Bushnell-Hass medium (Difco) composed by 0.1% of KH_2PO_4, 0.1% of K_2HPO_4, 0.02% of $MgSO_4 \cdot 7H_2O$, 0.02% of $CaCl_2 \cdot H_2O$ and 0.005% of $FeCl_3 \cdot 6H_2O$. The pH was adjusted to 7.0 by 1.0 M of HCl. The surface tension prior to inoculation was 56 mN/m. Three percent sugar cane molasses and 3% corn steep liquor were added. Two percent aliquots (v/v) of the cell suspension (0.7 optical density at 600 nm), corresponding to an inoculum of 10^7 CFU/mL, were used to inoculate 500 mL Erlenmeyer flasks containing 100 mL of sterile production medium. Cultivation was carried out at 27°C with agitation at 200 rpm for 120 h.

2.6. Determination of surface tension

The CMC of *C. sphaerica* biosurfactant is about 0.025% (w/v) (250 mg/L) and the surface tension is about 25.0 mN/m [13] while the CMC of *Bacillus* sp. biosurfactant was determined as 0.5% (w/v) (5000 mg/L) and the surface tension as 29 mN/m (data not shown).

Since the biosurfactant from *C. sphaerica* was previously produced, measurements of the surface tension were conducted to assess the quality of the biosurfactant obtained. Changes in surface tension were determined in the cell-free broth obtained by centrifuging the cultures at 5000 × g for 30 min. Surface tension was determined using a Sigma 700 Tensiometer (KSV Instruments LTD, Finland) at room temperature. Tensiometers determine the surface tension with the aid of an optimally wettable ring suspended from a precision scale. With the ring method, the liquid is raised until contact with the surface is registered. The sample is then lowered again so that the film produced beneath the liquid is stretched for the determination of maximum force, which is used to calculate the surface tension. The instrument was calibrated against Mill-Q-4 ultrapure distilled water (Millipore, Illinois, USA). Prior to use, the platinum plate and all glassware were