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FULL LENGTH ARTICLE

Production of biosurfactants by *Bacillus licheniformis* and *Candida albicans* for application in microbial enhanced oil recovery

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Abstract In this study, the bacterium *Bacillus licheniformis* DSM = 13 strain ATCC 14580 and the yeast *Candida albicans* IMRU 3669 were used for biosurfactant production. Surface properties of the produced biosurfactants were confirmed by determining the emulsification power as well as surface tension. The crude biosurfactants have been extracted from supernatant culture growth. FTIR analysis confirmed the chemical structure of the produced biosurfactants. The yields of crude biosurfactants were about 1 and 12 g/l for *B. licheniformis* and *C. albicans* respectively. Also, the results revealed that the emulsification power has been increased up to 96% and 65% with kerosene for bacterial and yeast strain respectively. Surface tension decreased from 72 to 36 mN/m after 72 h of incubation with *B. licheniformis* and 45 mN/m after 4 days of incubation with *C. albicans*. The potential application of this bacterial species in microbial enhanced oil recovery (MEOR) was investigated. The percent of oil recovery were 16.6 and 8.6 wt% for the bacterial and yeast strains respectively, upon application in sand pack column designed to stimulate an oil recovery.

1. Introduction

Surface active agents which are produced by different groups of microorganisms are known as biosurfactants. Biosurfactants reduce surface tension in both aqueous and hydrocarbon mixtures. Biosurfactants can aggregate at interfaces between fluids having different polarities, such as water and oil, leading to the reduction of interfacial tension. Because of their efficiency in lowering interfacial tension, biosurfactants have been employed for the enhancement of oil production especially in tertiary oil recovery. Low toxicity, high biodegradability and ecological acceptability are among the main characteristics of these surface active materials [1–6]. These favorable features make biosurfactants potential as one of the best alternatives of chemically synthesized surfactants in a variety of applications [7,8]. Biosurfactants can be categorized into four main

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groups: lipopeptides and lipoproteins, glycolipids, phospholipids, and polymeric surfactants [9].

Biosurfactants are widely used in different industries, such as cosmetics, special chemicals, food, pharmaceutics, agriculture, cleaners and microbial enhanced oil recovery (MEOR) [10–13]. The last mentioned application has attracted more attention because only 30% of oil present in a reservoir can generally be recovered using primary and secondary recovery techniques [1]. MEOR is considered as a tertiary recovery technique that could recover the residual oil using microorganisms or their products (biosurfactants). However, the application of biosurfactants in microbial enhanced oil recovery depends on their stability at extreme conditions of temperature, salinity and pH, or surface activities [12]. Stimulation of microorganisms that produce biosurfactants and degrade heavy oil fractions in situ reduces the capillary forces that retain the oil into the reservoir and decreases oil viscosity, thus promoting its flow. As a result, oil production can be increased [14].

The present study aimed to investigate the potential of *Bacillus licheniformis* and *Candida albicans* in biosurfactant production and the ability of these microbes to enhance the microbial oil recovery.

2. Experimental

2.1. Microorganisms

The bacterium strain *B. licheniformis* ATCC 10716 and yeast strain *C. albicans* IMRU 3669 used in the present study were purchased from Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Cairo, Egypt.

2.2. The growth kinetic and screening for the production of biosurfactant

The bacterial strain *B. licheniformis* was streaked on a nutrient agar slant and incubated for 24 h at 30 °C. Two loops of culture inoculated in 40 ml of nutrient broth in a 100 ml Erlenmeyer flask. The flask was incubated in a rotary shaker 150 rpm at 30 °C for 8–12 h until cell numbers reached 10^8 CFU/ml. This was used as inoculum at the 5% (w/v) level. For biosurfactant production, a mineral salt medium with the following composition was utilized (g/l): 2.5 of $NaNO_3$, 0.1 of KCl, 3.0 of KH_2PO_4, 7.0 of K_2HPO_4, 0.01 of $CaCl_2$, 0.5 of $MgSO_4{\cdot}7H_2O$, and 5 ml of a trace element solution [15]. The carbohydrate (glucose) added to make a final concentration 2%. The concentration of yeast extract at 3%. Cultivation studies have been done in 500 ml flasks containing 150 ml medium at 30 °C for 48 h [16].

C. albicans was cultivated on Yeast malt broth medium [17] used for developing the seed culture contained (g/l) glucose, 10; yeast extract, 3; malt extract, 3; peptone, 5; and pH adjusted to 6.0. Erlenmeyer flasks (250 ml) containing 50 ml of the seed culture medium were autoclaved at 121 °C for 20 min. The flasks were inoculated with a loop full of the microorganism freshly grown on yeast malt agar slant. The culture was then incubated for 24 h at 30 °C and 150 rpm in a rotary shaker. The final biomass weight after 24 h was estimated to be 10 g l^{-1}.

For sophorolipids (SL) production by *C. albicans* the medium composed of (g/l), glucose, 100; sunflower oil, 100; yeast extract, 1; urea 1, was inoculated with 5% (v/v) seed culture. The cultivation studies have been done in 2000 ml flasks containing 1000 ml medium at 26 °C for 5 days and the pH was adjusted to 6.0. Samples were taken periodically for carrying out the surface properties of the medium in different time intervals [18].

2.3. Extraction of the crude biosurfactants

The bacterial broth (10 ml) was inoculated into the medium MSM (1000 ml) using glucose as a sole carbon source and the pH value adjusted to 7.5. Incubation was carried out at 30 °C, 150 rpm, for 72 h. The extraction technique is a combination of acid precipitation and solvent extraction [19]. The broth culture sample was centrifuged (at 4 °C using 13,000 × *g* for 15 min). The obtained supernatant was treated by acidification to pH 2.0 using 6 M HCl, and the acidified supernatant was left overnight at 4 °C for complete precipitation of the biosurfactants. Remove supernatant to obtain pellet then extracted with methanol for 2 h while stirring continuously. Filter methanol to remove remaining material and evaporate to dryness using a rotary evaporator.

Extraction of sophorolipids (SL) is carried out by solvent extraction method, but without acidification after centrifugation at 4 °C using 13,000 × *g* for 15 min [20]. The obtained supernatant was extracted three times with an equal volume of ethyl acetate, shaking vigorously each time and allowing the two layers to separate in a separating funnel. Transfer bottom aqueous layer and the top ethyl acetate layer to separate flasks. Re-extract the aqueous portion twice more or until no further color persists in the ethyl acetate layer. Add 0.5 g of magnesium sulfate per 100 ml of ethyl acetate portion, to remove the traces of water present. The filter to remove materials other than biosurfactant then evaporates using rotary evaporator to yield a brown gum extract.

2.4. Chemical structure of the produced biosurfactants

Infrared (IR) spectra of the biosurfactant (a film of each sample on KBr pellet) were obtained using a Nicolet IS-10 FTIR spectrometer. IR spectra were conducted between 4000 and 500 cm^{-1} with a resolution of 1 cm^{-1} [21].

2.5. Surface properties

Surface properties including surface tension, emulsification index (E_{24}) and foaming were determined as indicators of biosurfactant production.

2.5.1. Surface tension

Surface tension was measured on a ring tensiometer (Krüss-tensiometer K6) using the broth supernatant solution (20 ml) at 30 °C, samples were taken and tested periodically in different time intervals [4].

2.5.2. Emulsification index (E_{24})

Emulsification power of the produced biosurfactant in the culture supernatant was measured by adding kerosene (6 ml) to the aqueous phase (of culture supernatant) and severe shaking

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