**TITLE:** BIOEMULSIFIER IMMOBILIZED IN CHITOSAN BEADS FOR APPLICATION IN MICROBIAL ENHANCED OIL RECOVERY

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## ABSTRACT:

Chemical and biological surfactants are amphiphilic compounds, which can reduce surface and interfacial tension by accumulating at the interface of immiscible fluids and increase the solubility and mobility of hydrophobic or insoluble organic compounds. The majority of commercially available surfactants are derived from petroleum. However, the growth of environmental concern among consumers, combined with new environmental laws led to the demand for natural surfactants as alternatives to synthetic products. Thus, biosurfactants from microbial sources are becoming important products for industrial, environmental and medical applications. In this work, a bacterial strain isolated from a petroleum sample was identified by amplification and sequencing of 16S RNAr and evaluated for biosurfactant production. Initially, the whole culture and its supernatant were prospected for emulsify aqueous mixtures of hexadecane, hexane, isoctane, kerosene and toluene. Since the supernatant did not show activity, the study focused on the biomass. Therefore, we tested the properties of a new bioemulsifier based on bacterial biomass entrapped in chitosan beads. The culture was prepared in Mineral Medium containing 2% of glycerol as carbon source, under 160 rpm at 30 °C for 48 h. Biomass was obtained by centrifugation and washed three times with distilled water, lyophilized and autoclaved. For immobilization, chitosan powder was dissolved in 2 M sodium acetate buffer pH 3.8 at the proportion of 4% (w/v) and the dead cell biomass was added in the proportion of 1:1 (w/w) to the chitosan gel and mixed for 2 h to complete homogenization. This mixture was dropped in a solution of 8% NaOH (w/v) under agitation in order to coagulate the chitosan, forming the beads and entrapping the biomass. The strain was identified as Staphylococcus saprophyticus, a Gram-positive bacterium, whose cell wall presents peculiar tense active property. The entrapped biomass was the only responsible for the emulsification of different oily mixtures, since the chitosan alone did not show that activity. Besides the reduction of oil viscosity, the addition of S. saprophyticus biomass to chitosan enhanced the resistance and biodegradability of native chitosan. The bioemulsifier has potential to be applied in reservoir for microbial enhanced oil recovery using its ability to reduce the viscosity of the oil with the advantage to be biodegradable, resistant, and harmless to humans and to the environment.

Keywords: biosurfactants, immobilization, chitosan, beads.

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