

EVALUATION OF THE PRE-INOCULUM UTILIZATION IN BIOSURFACTANT PRODUCTION BY SOLID STATE FERMENTATION

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Biosurfactants are one of the mainly class of natural surfactants and are classified according to their chemical composition and microbial origin. The production of biosurfactants by microorganisms is intimately linked to the environmental and nutritional conditions provided and the factors that influence microbial growth also affect their production. Currently, most of the compounds are synthetic surfactants and the main factor restricting the widespread use of biosurfactants is their production cost when compared to the ones with synthetic origin. The solid-state and submerged fermentation can be used for the production of biosurfactants, but the solid-state fermentation is considered a simple technology for production of compounds with interest and an alternative to avoid foaming, a limiting factor in obtaining these compounds by submerged fermentation. This study aimed to evaluate the influence from the pre-inoculum use in biosurfactant production by *Bacillus subtilis* in solid state fermentation. The fermentation was carried out in polyethylene bags containing 10 g of wheat bran and phosphate buffer solution and glycerol concentration to obtain 60% moisture and incubated in a chamber at 37°C for 96 hours. The fermentation was conducted in three ways: without pre-inoculum, with liquid pre-inoculum and solid pre-inoculum. *Bacillus subtilis* was grown in tubes with nutrient agar and incubated in a chamber at 37°C for 12 hours. After this period a cellular suspension was prepared from the tube where 2 ml of this suspension served as inoculum in the bags of wheat bran without pre-inoculation. The same cellular suspension were used in 1 ml to prepare the pre-liquid inoculum, which consisted of 50 ml of nutrient broth, kept under stirring at 37°C for 12 hours. This cellular suspension was also applied at the solid pre-inoculum containing 10g of wheat bran with buffer and glycerol, incubated at 37°C for 12 hours in an erlenmeyer flask. 2 ml of liquid and solid pre-inoculum were removed after 12 hours and inoculated in the bags. The surface tension and emulsifying activity were analyzed, the surface tension was performed in free-cell extracts, using a tensiometer and the emulsifying activity of the medium was analyzed using 3.5 mL of free-cell extract and 2 mL of toluene. This mixture was stirred in vortex for 2 minutes and the absorbance was read in spectrophotometer at 620 nm. The results showed that there was a decrease in surface tension in the three fermentations, with similar results, around 55 mN/m⁻¹, and the emulsifying activity values were also close, although the fermentation used as liquid pre-inoculum showed better results. The use of pre-inoculum, both the liquid and solid, didn't influence the surface tension and emulsifying activity, compared to the fermentation without pre-inoculum.

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