

TITLE: OPTIMIZATION AND CARACTERIZATION OF BIOSURFACTANT PRODUCED BY *Bacillus amyloliquefaciens* MO13 AS POTENTIAL BIOREMEDIATION STRATEGY

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ABSTRACT:

Microorganisms naturally metabolize numerous petroleum hydrocarbons, contributing to minimize oil pollutants on the oceans. Biosurfactants are non-toxic amphiphilic molecules produced by a multiplicity of these microorganisms. They are capable to make hydrophobic molecules available to local microbiota, allowing organic hydrocarbon to be used as carbon source and also can be used for oil recuperation in degraded areas. However, the major problem in biosurfactant production is its cost and application that could be minimized through bioprocess optimization. The objective of this research is to optimize the production of *Bacillus amyloliquefaciens* MO13 biosurfactants in bioreactors through surface responses. For this, different culture mediums (YPD, LB and LB glucose 2%) were tested as pre-inoculum of *Bacillus amyloliquefaciens* MO13 and incubated for 24 hours, at 30°C. After OD reached 2.0, 2% of the pre-inoculum were inoculated in the minimal saline medium (MSM) supplemented with different glucose or glycerin concentrations (1, 3 and 5%). After 40 hours the supernatant was collected and used to biosurfactant activity tests. Surface responses were developed through the following parameters: temperature (25, 30 and 35 °C), stirring (200, 250 and 300 rpm), aeration (0.1, 0.3 and 5 lpm) and pH (6.5, 7.0 and 7.5). In addition, CFU tests, gravimetric analysis and surface tension were performed. For the biosurfactant characterization, the culture supernatant was acidified and the biosurfactants were extracted and analyzed by mass spectrometry. The results demonstrate that YPD was the most appropriate pre-inoculum for biosurfactant production and 3% of glucose and glycerin was enough to produce high biosurfactant concentration. The equation obtained from the surface response reached an optimization of biosurfactant concentration 18g/L, CFU 2.6×10^{11} g/ml and surface tension 28.2 mN/m in the condition of 31°C, 252 rpm, 0.43 lpm and pH: 7 for glucose and 13.7 g/L, 2.2×10^{11} and 28.2 mN/m in 34°C, 239 rpm, 0.31 lpm and pH: 7.2 for glycerin. The mass spectrometry revealed a production of 5 surfactins and 2 iturins. The biosurfactants produced by *Bacillus amyloliquefaciens* MO13 represents a great advanced in bioremediation field which could be used to improve bioremediation process, microbial enhanced oil recovery or as antimicrobial. Therefore, bioprocess optimization is the first step to produce biosurfactants in large scale.

Keywords: *Bacillus*, bioremediation, biosurfactant, optimization

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