## Abstract

## Title: Stability studies of a biosurfactant produced by a Bacillus strain isolated from a Brazilian oil reservoir rock sample.

Authors: ARGENTIN, M. N.<sup>1</sup>, MARTINS, L. F<sup>2</sup>, SOUSA, M. P<sup>2</sup>, BOSSOLAN, N. R. S.<sup>1</sup>

Afiliation: <sup>1</sup>IFSC-USP - Instituto de Física de São Carlos (campus 2 - Avenida João Dagnone, 1100 - Jardim Santa Angelina - 13563-120 - São Carlos, SP), <sup>2</sup>CENPES - Centro de Pesquisa e Desenvolvimento da Petrobrás, Cidade Universitária, Rio de Janeiro, Brasil.

Biosurfactants are low molecular weight compounds produced by a wide variety of microorganisms and, in the oil industry, can be employed in processes of microbially enhanced oil recovery (MEOR). The extreme conditions in oil reservoirs as temperature and salinity, for example, involve studies on the production of biosurfactants of indigenous bacterial strains, as well as its stability under these conditions. This study aimed to carry out stability tests with the biosurfactant produced by a Bacillus species, called Ar70C7-2, isolated from deep rock samples of an offshore oil reservoir located in southeastern Brazil. The supernatant obtained from strain Ar70C7-2, when cultured in LB medium (Luria-Bertani) medium with salinity of 70g/l and incubation at 55°C, showed high emulsifying index E<sub>24</sub>, tested in different organic substrates. For stability tests, biosurfactant was extracted from the culture medium by acid precipitation, yielding on average 0.24g of crude extract (semi-purified) per liter of culture. The stability of the emulsifying and reduction of surface tension properties of the biosurfactant was tested at different temperatures, pH and salinities, using 20ml of a 0.1% crude extract solution. In the thermal stability test, samples were kept for 24 hours at temperatures ranging from -18 to 121°C followed by stabilization at room temperature for 3h.  $E_{24}$  indices ranged from 62.2 (± 2.1)% at -18°Cto 69.8 (± 1.6)% at 100°C, being the lowest surface tension of 43.3 (± 0.3) mN/m at -18°C. For the assay at different pH conditions, samples pH were adjusted to values in the range of 2 to 12, with greatest  $E_{24}$  obtained at pH 12 - 70.4 (± 1.2)% - and minimum at pH 2 - 65, 2(± 3.4)% -, with lower surface tension of 44.3 (± 0.4) mN/m at pH 2. For testing the effect of ionic strength, salinities of the samples were adjusted to concentrations in the range of 0 to 21% NaCl. E<sub>24</sub> values ranged from 61.5 (± 3.7)%, obtained in concentration of 15% NaCl, to 70.3 (± 3.8)%, at 3% NaCl; lower surface tension 42.2 (± 0.2) mN/m was registered at 12% NaCl. The biosurfactant produced by strain Ar70C7-2 was very stable in wide ranges of temperature, pH, and salinity, and should be further studied for its chemical characterization and production conditions to validate its applicability in MEOR. An optimization of culture conditions for the biosurfactant production, with regard to the carbon-nitrogen ratio of the medium and salinity, is in progress.

Keywords: Bacillus, biosurfactant, oil reservoir, thermophilic.

Financial Support: PETROBRAS S/A