TITLE: AHEST: A NOVEL THERMOSTABLE ESTERASE WITH POTENTIAL USE IN MEOR AND BIOTECHNOLOGICAL APPLICATIONS

AUTHORS: GUTIERREZ, R.F.[1]; MARTINS, L.F.[2]; CASTRO, A. M.[2]; BOSSOLAN, N. R. S.[1], ARAÚJO, A.P.U.[1]

INSTITUTION: [1]IFSC-USP - SÃO CARLOS INSTITUTE OF PHYSICS, UNIVERSITY OF SÃO PAULO, SÃO CARLOS, BRASIL; [2]CENPES - PETROBRAS RESEARCH & DEVELOPMENT CENTER, RIO DE JANEIRO, BRAZIL.

ABSTRACT:

Surface-active compounds (SACs) are widely used in many sectors of modern industry. At present biosurfactants are attracting much attention due its environmental advantages over the synthetics compounds, mainly derived from petroleum. Oil industry is one of the main areas for biosurfactant studies and application. This study aims to investigate the surfactant properties and the activity in high salinity conditions of the AhEst protein, a thermostable esterase from *Acetomicrobium hydrogeniformans* present in an oil-water separation unit, for potential use in MEOR. AhEst was previously identified by our group in an *in silico* search for potential esterases produced by thermophiles. AhEst was produced as a recombinant protein in *E. coli*, purified and used in experiments for the reduction of surface tension and emulsification capacity (E24 index). A test to evaluate the AhEst ability to change the wettability of the calcite powder from oil-wet to water-wet was done. AhEst activity was measured by the release of p-nitrophenol hydrolyzed from the p-nitrophenyl acetate at increasing salt concentrations (35 to 180g/L). Circular dichroism measurements (CD) were used to evaluate the structural stability of AhEst in the same salinity range. AhEst showed a high E24 index for the tested concentrations (58% to 67%). The reduction of surface tension was not so low (54mN/m for 500ppm - high concentration tested) as expected for a good biosurfactant like ones described in the literature (30mN/m). The qualitative wettability test showed that AhEst was able to change the wettability of the oil-wet calcite powder to water-wet condition when compared to the positive control. The CD measurements showed that AhEst is structurally stable until 70 g/L of salt and then begins to lose secondary structure. The melting temperature could not be determined due AhEst high thermostability. Nonetheless, AhEst exhibited an increasing esterase activity as salt were added. Our results show AhEst as a good emulsifier, but not a good surface tension reducing agent. Moreover, Ahest exhibited an increased activity even showing secondary structure changes at high salt concentrations. These preliminary results present AhEst as a potential enzyme for use in MEOR and others biotechnological processes due to its emulsifier properties and its stability in extremely conditions of temperature and salinity.

Keywords: *Acetomicrobium hydrogeniformans*, thermoestable esterase, AhEst, MEOR

Financial support: Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP); PETROBRAS S/A.