P342. Effect of high pressure on surfactin production by *Bacillus subtilis*: implications for its application by the oil industry

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Surfactin, a lipopeptide biosurfactant produced by Bacillus subtilis strains, exhibits extraordinary surface active properties, as well as stability at a wide range of temperatures and salinities, making it useful to replace the chemical surfactants in many industrial applications. The oil industry can take advantage of its application to increase the productivity of oil reservoirs, through a technology known as microbial enhanced oil recovery (MEOR). However, in order to make this technology advantageous from an economic point of view, the surfactin-producing strains must be able of growing and producing the biosurfactant inside the oil reservoirs. In this work, *B. subtilis* #573, isolated from an oil reservoir, was evaluated regarding its ability of producing surfactin under oxygen limited conditions at high pressure. A central composite design (CCD) was used to model the effect of pressure (3.8-46.2 bar) and temperature (35.3-46.7°C) on surfactin production. The results obtained demonstrated that pressure (in the range studied) did not exhibit a negative effect on surfactin production by this isolate, whereas temperatures higher than 45°C reduced its production. For most of the different combinations of pressure and temperature assayed, surfactin production was observed after 24 h, and the surface tension was reduced to values bellow 26.5 mN/m. At 41°C and 47 bar, B. subtilis #573 produced 31 \pm 2 mg of surfactin per liter after 24 h, reducing the surface tension to 25.6 \pm 0.6 mN/m. These results were similar to those achieved at the same temperature at atmospheric pressure ($26.0 \pm 0.3 \text{ mN/m}$ and 27 ± 3 mg surfactin/L). The surfactin produced in both cases exhibited a critical micelle concentration value around 15 mg/L, and the chemical characterization (through UHPLC-MS) demonstrated the production of similar percentages of the different surfactin isoforms (C12-, C13-, C14-, C15- and C16-surfactin) in both conditions. Finally, the applicability of *B. subtilis* #573 in MEOR was studied in sand-pack columns. In assays performed at 41°C and 47 bar, additional oil recoveries around 14% were obtained after 14 days in in situ assays. These results demonstrate the applicability of *B. subtilis* #573 in in situ oil recovery processes.