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Microbial Enhanced Oil Recovery (MEOR) is potentially useful to increment oil recovery from reservoirs beyond primary and secondary recovery operations using microorganisms and their metabolites. *In situ* stimulation of microorganisms that produce biosurfactants and degrade heavy oil fractions reduces the capillary forces that retain the oil inside the reservoir and decreases oil viscosity, thus promoting its flow and increasing oil production. *Bacillus subtilis* #573, isolated from crude oil samples obtained from a Brazilian oil field with a moderate temperature (40°C), was selected for further use in MEOR. This isolate can grow at temperatures up to 55°C and salinities up to 100 g/l, and produces extracellular biosurfactants under both aerobic and anaerobic conditions in the presence of hydrocarbons. The biosurfactants produced reduced the surface tension to 30 mN/m, decrease the interfacial tension oil-water and exhibited a high emulsifying activity, as well as thermo- and salt-tolerance. The microbial isolate also showed the ability of degrading long-chain n-alkanes under aerobic and anaerobic conditions. Mobilization of heavy crude oil by this isolate was evaluated using sand-pack columns at 40°C. Growing *in situ* *B. subtilis* #573 for 14 days allowed a 17% recovery of the entrapped crude oil. The recovered crude oil showed a decrease in the

percentage of *n*-alkanes higher than C₂₅ and its viscosity was reduced about 32%, which contributed to enhance its mobility. A core flooding equipment was used for a better simulation of the oil reservoir conditions (40°C and 32.4 bars). Preliminary results showed an additional oil recovery of 4%. The results obtained demonstrated that the selected isolate can be useful to recover residual oil from mature reservoirs.