viscosity of heavy crude oil. Results demonstrated the potential of IMP-100 and IMP-200 consortia and LC-1, LAF-1 for utilization in MEOR.

**Evaluation and Characterization of Biosurfactants Produced by Microorganisms Isolated from Brazilian Oils**

**J. F. B. Pereira**¹, E. J. Gudiña², R. Costa¹, L. R. Rodrigues², J. A. Teixeira², J. A. P. Coutinho¹

¹ CICECO - Chemistry Department, University of Aveiro, Portugal  
² Institute for Biotechnology and Bioengineering (IBB), Centre of Biological Engineering, University of Minho, Braga, Portugal

Surface-active agents or surfactants are amphiphilic molecules that comprise both hydrophilic and hydrophobic moieties, allowing the reduction of the surface and interfacial tensions, as well as the formation of oil in water or water in oil emulsions. Due to their interesting properties, surfactants are widely used by petroleum industries to reduce the capillary forces that retain the oil inside the reservoir. However, since chemical surfactants present some limitations related to environmental issues and restrictive laws, the biosurfactants constitute a reliable alternative due to their lower toxicity, higher biodegradability and effectiveness at extreme temperature, salinity and pH conditions. In this work, different biosurfactant-producing microorganisms under reservoir conditions were isolated from Brazilian oils. Biosurfactant production was evaluated by measuring surface tensions, interfacial oil-water tensions and emulsification activities. Among the isolated microorganisms, two Pseudomonas and three *Bacillus* strains were able to grow and produce extracellular biosurfactants at 40°C. Furthermore, the biosurfactants were characterized using different spectroscopic techniques, namely FTIR, 1H NMR, ESI/MS and MS/MS. Structural characterization of these molecules is important to understand their surface-active properties, as well as the formation of molecular aggregates. Biosurfactants produced by *Pseudomonas* and *Bacillus* strains were found to be rhamnolipids and surfactins, respectively. The results obtained show that it is important to characterize the biosurfactants in order to optimize their application in bioremediation with crude oil, or in microbial enhanced oil recovery processes.

**A Biosurfactant-producing and Oil-degrading Bacillus Subtilis Strain Enhances Oil Recovery under Simulated Reservoir Conditions**

**E. J. Gudiña**¹, J. F. B. Pereira², R. Costa², L. R. Rodrigues¹, J. A. P. Coutinho², J. A. Teixeira¹

¹ Institute for Biotechnology and Bioengineering (IBB), Centre of Biological Engineering, University of Minho, Braga, Portugal.  
² Chemistry Department, University of Aveiro, Portugal.

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