

THE ROLE OF BACTERIAL EXOPOLYMERIC SUBSTANCES (EPS) IN THE HYDROCARBONS BIODEGRADATION

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Understanding the mechanisms of microbiological petroleum degradation is of global interest. The limiting and crucial step of the biodegradation is the emulsification of hydrocarbons, a prerequisite to increase the bioavailability of this compound class (Churchill et al, 1995). The approach of this study was to evaluate the production of EPS by aerobic bacterial strains from a Brazilian oil reservoir. The EPS production was compared to the biodegradation potential and the microbial growth in the presence of hydrocarbons, evaluating if good hydrocarbons biodegraders will be efficient EPS producers.

The oils and petroleum formation water samples were obtained in May 2002 from Campos Basin (Brazil). The oil samples used in this study were chosen from 73 producing wells within the field and are representative examples of a non-biodegraded oil produced from the Coquina sequence of the Lagoa Feia Formation ($\approx 3,070$ - $3,240$ m, reservoir temperature ≈ 85 °C) and of a biodegraded oil (biodegradation level of 5-6 according to Peters & Moldowan, 1993) produced from sandstones of the Carapebus formation ($\approx 2,066$ m, reservoir temperature ≈ 51 °C). The Campos Basin covers an area of around $100,000$ km², mostly offshore to the $3,400$ m isobath, and is the most prolific oil basin in Brazil holding approximately 85 % of the total Brazilian oil reserves and 40% of total gas reserves (Nascimento et al, 1999). The aerobic microbial enrichments from the samples permitted the recuperation of 29 strains of bacteria. All bacterial strains were identified by molecular taxonomy based on the partial sequential and phylogenetic analysis of 16S ribosomal DNA. From these bacteria, 10 strains were analyzed for their potential of EPS production, microbial growth and biodegradation of the hydrocarbons phenanthrene and n-octadecane.: *Microbacterium* sp., *Dietzia* sp., *Bacillus subtilis*, *Micrococcus* sp., *Halomonas* sp., *Brevibacillus* sp., *Bacillus pumilus*, *Bacillus megaterium*, *Pseudomonas* sp. and *Bacillus* sp.

Bacillus subtilis showed the best EPS production rate when phenanthrene (36.5 mg of EPS per gram of biomass) and n-octadecane (27.33 mg EPS / g of biomass), alone, were

applied as carbon sources. When these hydrocarbons were used as mixtures, *Bacillus megaterium* presented high rates of EPS production, 69.33 mg / g of biomass. The cellular density increase of the bacteria during the hydrocarbon degradation experiments was monitored by spectrophotometric analysis. Through the correlation of EPS production values and the obtained growth curves of the bacterial strains it was possible to verify that the cellular density increase does not correspond to the major production of EPS. The best producers of EPS (*Bacillus subtilis* and *Bacillus megaterium*) were not the same strains that presented the highest rates of microbial growth, e.c. *Dietzia* sp and *Pseudomonas* sp. The growth of microorganisms using hydrocarbons as substrate is frequently associated to the production of biosurfactants. Cultures that presented excellent results in the EPS production (*Bacillus subtilis*, *Bacillus megaterium*, *Dietzia* sp. and *Pseudomonas* sp.) or that showed the major indexes of microbial growth with the applied hydrocarbons (*Pseudomonas* sp. and *Dietzia* sp.) were tested for their biodegradation activity. Our results suggest that EPS production is playing a key role in the biodegradation processes of aliphatic and aromatic hydrocarbons.

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