

PETROLEUM DEGRADATION STUDIES USING AEROBIC AND ANAEROBIC MICROBIOTA ISOLATED FROM PAMPO FIELD, CAMPOS BASIN, BRAZIL

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The biodegradation of hydrocarbons in petroleum reservoirs leads to substantial petroleum composition changes, decreasing the crude oil quality and economic value. The strongest change of petroleum quality occurs during the biodegradation initial phase as consequence of the crude oil hydrocarbons microbial biotransformation which is a sequential process. The biodegrading microbes have a pronounced preference for *n*-alkanes, followed by branched and cyclic hydrocarbons (Peters *et al.*, 2005). At advances stages of biodegradation, certain biomarkers, such as 25-norhopanes and secohopanes, are either created or enhanced by depletion of more abundant biomarkers (Bost *et al.*, 2001).

Petroleum biodegradation processes have been investigation for several decades and there are two major hypothesis: aerobic microbiota is responsible for the predominant biodegradation process in deep water reservoirs and caused by infiltration of fresh water carrying oxygen; anaerobic microorganisms are responsible for the oil biodegradation in deep water reservoirs and in shallow reservoirs (< 500 m) (Roling *et al.*, 2003). From our point of view these two apparently conflicting hypotheses can be merged into one in which the aerobic and anaerobic microbiota life cycles coexist but do not overlap, each are really active at different geological times both using petroleum and each other metabolites. Our hypothesis, based on recent bacterial research (among others Xu J *et al.*, 2004), goes further by quoting that the anaerobic bacteria present in water droplets inside the crude oil produce oxygen by reducing nitrates and perchlorates during the anaerobic life cycle The oxygen might be trapped into the bacterial biofilms at the interface oil water of the droplet and acting like an oxygen sponge. When the oxygen content is high the anaerobic consortium suffers from these conditions and ceases activity and the aerobic consortium takes over consuming the stored oxygen in the biofilm “sponge”, biodegrading the petroleum and some of the anaerobic bacteria byproducts. As soon as the oxygen is depleted the anaerobic microbiota takes over again. We cannot estimate the life cycle but we are talking about geological times and our experiments are optimized to be evaluated in few months. To evaluate this hypothesis, our group investigated the biodegradation potential of both aerobic and anaerobic microbiota

present in biodegraded Brazilian reservoirs oils and formation waters from Campos Basin, Brazil. Biodegradation potential was assessed by using either isolated strain or consortia and crude oil or mixtures of five biomarkers. Presently the samples were submitted to enrichment techniques producing aerobic and anaerobic consortia from which 29 strains of aerobic bacteria were isolated and identified (Consortium 1, Co1) and 63 not yet identified (Consortia 2, Co2). The biodegradation potentials of these consortia were assessed using 10 mg of crude oil, incubated at 30 °C and 200 rpm, during 60 days with 2g of wet cells in 50 mL of Zinder (Zinder *et al.*, 1984). Here we present preliminary results of the aerobic consortia with two sample of crude oil (P1 and P2), at different stages of biodegradation. Internal standards were used to estimate crude oil biodegradation monitoring *n*-alkanes (m/z 71) and biomarkers more resistant to biodegradation (i.e., steranes, m/z 217 and triterpanes, m/z 191). After 60 days of monitoring, the relative amounts of *n*-alkanes decreased from 75 to 33% to P1 and 61 to 28% to P2, both using Co1, while decrease from 75 to 60% to P1 and 61 to 45% to P2, using Co2.

In relation to the steranes, biodegradation was more efficient to P2Co1 with 78%. This consortium also degraded hopanes in both samples, depleting the C₃₀ 17 α (H), 21 β (H)-hopane and the C₃₁₋₃₄ homohopanes with preferential degradation of the lower molecular weight homologues (C₃₁ > C₃₂ > C₃₃ > C₃₄ > C₃₅). The homohopane index (C₃₁-C₃₄ homohopanes (22R+22S)/C₃₅ homohopanes (22R+22S), in m/z 191) decreased in the two consortium when compared to the control experiment (P1Co1, 10.09 to 9.91 and P2Co1, 10.14 to 7.40). Biodegradation of hopanes has been associated with the C-10 demethylation of the hopane A/B rings generating the corresponding 25-norhopanes (Peters *et al.*, 2005). In the present study, 25-norhopanes were detected after 20 days of incubation.

Assays with a mixed anaerobic and aerobic microbiota are in progress in a controlled experiment with alternate aerobic and anaerobic cycles.

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