

## Who is who in anaerobic oil biodegradation?

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Anaerobic bioremediation is an important alternative for the common aerobic cleanup of subsurface petroleum-contaminated soil and water. Microbial communities involved in anaerobic oil biodegradation are scarcely studied, and only few mechanisms of anaerobic hydrocarbons (AHC) degradation are described. In this work, microbial degradation of aliphatic hydrocarbons (AHC) was studied by using culture-dependent and culture-independent approaches. Hexadecane and hexadecene-degrading microbial communities were enriched under sulfate-reducing and methanogenic conditions. The microorganisms present in the enriched cultures were identified by 16S rRNA gene sequencing.

From stable hexadecane-degrading sulfate-reducing enrichment, a co-culture composed of a rod-shaped bacterium closely related to *Desulfomonile limimaris* (94% identity) and an oval-shaped bacterium related to *Desulforhabdus amnigena* (99% identity) was obtained. Isolation of the new *Desulfomonile* is being performed, and the role of those microorganisms in AHC degradation is being investigated.

The methanogenic enrichment produced methane from hexadecene in an amount equivalent to 57% of the stoichiometric value. This culture was composed of bacteria belonging mainly to *Syntrophaceae* and *Synergistaceae* families, which include several syntrophic microorganisms. A *Syntrophus*-like microorganism was identified and it is most likely a key player in AHC degradation, since its presence in oil degrading environments was previously reported [1,2]. *Methanobacterium*, *Methanosaeta* and *Methanolinea*, were present with relative abundances of 31%, 10% and 3%, respectively, and were the ones converting hydrogen and acetate to methane in those enrichments.

The presence of the gene encoding for the alkyl-succinate synthase (*ass* gene), involved in the activation of alkanes, is being assessed in both methanogenic and sulfate-reducing enrichment cultures.

The results presented are important for understanding “who is doing what”, and at what rate in AHC biodegradation. The effects of the different electron acceptors on the composition and activity of AHC-degrading microbial communities were explored. This knowledge is highly relevant for the development of innovative strategies for in situ bioremediation of deep contaminated sites.

[1] N.D. Gray, A. Sherry, R.J. Grant et al., *Environmental Microbiology* 13(11), 2011, 2957-2975.

[2] T. Siddique, T. Penner, J. Klassen et al., *Environmental Science and Technology* 46, 2012, 9802–9810.