



Insights into the Anaerobic Biodegradation Pathway of Hydrocarbons in Oil Reservoirs by Detection of Signature Metabolites and functional genes

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Microbial degradation of petroleum hydrocarbons in situ oil reservoirs plays an essential role in geo-bio-chemical process, and it has attracted an increasing attention from both scientific and industrial communities due to the promising application of energy recovery via bio-conversion of residue crude oil trapped in porous media of depleted oil reservoirs into methane, a clean energy of natural gas. Several pathways involved in hydrocarbon anaerobic biodegradation and methane generation by methanogenic microbial consortia were proposed in past years, yet the knowledge about the mechanism of hydrocarbon anaerobic biodegradation in situ oil reservoirs is still limited. This presentation will discuss hydrocarbon anaerobic biodegradation pathways by focusing on the initial activation by addition of fumarate to alkanes. Five representative biomarker molecules in hydrocarbon anaerobic biodegradation were synthesized and were applied to detect the intermediates of hydrocarbon anaerobic biodegradation in 32 production water samples from 3 different oilfields. Alkylsuccinates of parent alkanes from C₁ to C₈, 2-benzylsuccinate and naphthoic acid were detected, and the downstream metabolites such as alkylmalonates and 5,6,7,8-tetrahydro-naphthoate was also detected in the samples using GC-MS, which suggested a primary pathway framework of hydrocarbon anaerobic degradation via fumarate addition. Meanwhile, the key functional genes for encoding alkylsuccinate synthase (*assA*) and benzylsuccinate synthase (*bssA*) were detected in the samples, which confirmed the present of an anaerobic biodegradation pathway of the initial activation by addition of fumarate to hydrocarbons. In addition, alkane-degrading enrichment cultures from production water amended with C₁₅-C₂₀alkanes and incubated at 55° under methanogenic condition were established, and the methane generation pathways were analyzed. Phylogenetic analysis of 16S rRNA gene sequences retrieved from the enrichment cultures showed that *Firmicutes* (*Gelria* and *Moorella*) and CO₂reducing methanogens were dominant after incubation at 55° under methanogenic condition; and *assA* and *mcrA* were also detected in the enrichment cultures. In summary, our studies revealed that there inhabited abundant microbial consortia for hydrocarbon degradation and methane production in oil reservoirs and the fumarate addition and methanogenesis was involved in the process with a dominant methanogenesis of CO₂reducing route, which is fundamental for the in-situ bio-conversion of hydrocarbons into natural gas (methane) in petroleum reservoirs.

Keywords: alkylsuccinate; alkylsuccinate synthase; anaerobic degradation of hydrocarbon; fumarate addition; oil reservoir; signature metabolites