

# Methane Formation During the Degradation of N-alkanes and Total Crude Oils

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When oxygen, nitrate, ferric iron and sulphate are depleted as electron acceptors, the biological formation of methane is the terminal process in the degradation of organic substances. Biological methane contributes roughly 20% to the world wide natural gas reservoirs of economical interest and the physiological process of methane formation during the degradation of biomolecules such as proteins, carbohydrates and lipids is well understood in aquatic habitats and sediments. Recently, isotopic evidence was presented, that biological methane formation may also take part in older shales and coal bearing strata of significant maturity and form gas reservoirs of economic value (Scott et al., 1994; Martini et al., 1996). Under these geological setting easily degradable biomolecules are expected to be already diagenetically altered and may not serve a major substrate for biological methanogenesis. Thus, the biochemistry of methane formation in mature sediments and oil reservoirs remains unclear. Until Zengler et al. (1999) had enriched a methanogenic consortium capable to degrade n-alkanes during the formation of methane, major crude oil constituents such as alkanes were considered to be recalcitrant under methanogenic conditions. 16S ribosomal gene analysis elucidate the members of the microbial consortium. Bacterial clones belong to the delta subclass of proteobacteria closely related to *Synthrophus* spp, responsible for the fermentation of alkanes and concurrently production of acetate and hydrogen. The archaeal clones were related to acidoclastic (*Methanosaeta*) and CO<sub>2</sub>-reducing methanogens (*Methanospirillum* and *Methonoculleus*) consuming acetate and hydrogen in the consortium. The rapid consumption of these metabolite to very low concentration maintaining thermodynamic conditions feasible for the acidogenic degradation of hydrocarbons. The degradation of 1 mol hexadecane was calculated to require 7.5 mol H<sub>2</sub>O for the formation of 12.3 mol CH<sub>4</sub> and 3.8 mol CO<sub>2</sub> indicating that large amounts of CO<sub>2</sub> are released and significant quantities of water are consumed in this process. In this paper we discuss lines of

evidence for the geochemical significance of biological methanogenesis in geological settings such as oil reservoirs and coal strata. First degradation of n-alkanes in oil contaminated aquifer sediments accompanied by methane formation (Anderson and Lovley, 2000) show that this process is more abundant than previously thought. Second in addition to alkanes, the biodegradation of other major oil constituents such as alkenes and aromatic hydrocarbons can in principle result in the formation of methane (Schink, 1985; Heider et al., 1999). Finally, carbon and hydrogen isotopic composition of methane in our methanogenic consortium growing on n-hexadecane and total crude oil perfectly match the isotopic composition of methane observed in mature shales and coaliferous strata that are most likely formed by microbial methanogenesis. Depending on the carbon substrate, methane generated from n-alkanes and total crude oil has a carbon and hydrogen isotopic signature of -53 to -66 (PDB), -314 to -403 (SMOW); and -57 to -59 (PDB) -377 to -402 (SMOW), respectively. Moreover, results support the previous conception, that oil related compounds may serve as a substrate for microbial methane formation. The results further explain biodegradation in oil reservoirs in the absence of oxygen, nitrate, iron and sulphate as terminal electron acceptors.

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