



Enhanced methanogenesis from hexadecane and ethylbenzene under non-methanogenic conditions

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Microbially enhanced oil recovery (MEOR) may provide access to remaining, but yet inaccessible petroleum in reservoirs. The microbial conversion of heavy hydrocarbon remnants into gaseous methane could at least provide access to energy which would otherwise be lost. On the other hand, methanogenesis could remove toxic hydrocarbons from contaminated aquifers and sediments. Therefore, sediment samples from a contaminated sea port basin were investigated to assess the in situ potential for methanogenic hydrocarbon degradation. Since this process is believed to be a sequential syntrophic procedure, non-methanogenic conditions were created in sediment microcosms to facilitate the first hydrocarbon attacking step. To achieve this, a high electron potential was created by the addition of ferrihydrite, manganese oxide, nitrate or sulfate as electron acceptors. Hexadecane, ethylbenzene or naphthalene were used as model carbon substrates. Methanogenesis evolved rapidly from set ups treated with iron and manganese, but not nitrate, reflecting the in situ conditions at the site. Surprisingly, on sulfate methanogenesis was neither inhibited nor significantly supported. Methane formation rates were the highest with hexadecane as substrate, followed by ethylbenzene and naphthalene. Methane was removed in high rates at the same time by anaerobic methanotrophs. The microbial community in situ and in vitro was dominated by members of the Geobacteraceae. Their methanogenic partners were quantified, targeting the genes encoding for the methyl coenzyme M reductase (*mcrA*). Methane consumption in the microcosms and the presence of methanotrophic anaerobes belonging to the ANME-1 and ANME-2 clusters suggest anaerobic methanotrophy as an accompanying process. *mcrA* genes belonging to the ANME-1 & -2 clusters were detected in lower copy numbers than the methanogenic *mcrA*, which is in good agreement with the activity measurements. These results indicate that the in situ stimulation of hydrocarbon dependent methanogenesis is possible with relatively simple methods.