



## Hydrocarbon activation under sulfate-reducing and methanogenic conditions proceeds by different mechanisms.

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Microbial degradation of alkanes typically involves their conversion to fatty acids which are then catabolised by beta-oxidation. The critical step in this process is activation of the hydrocarbon. Under oxic conditions this is catalyzed by monooxygenase enzymes with the formation of long chain alcohols. In the absence of oxygen alternative alkane activation mechanisms have been observed or proposed. Fumarate addition to alkanes to form alkyl succinates is considered a central process in anaerobic hydrocarbon degradation. Comparative studies of crude oil degradation under sulphate-reducing and methanogenic conditions revealed distinctive patterns of compound class removal and metabolite formation. Alkyl succinates derived from C7 to C26 n-alkanes and branched chain alkanes were found in abundance in sulfate-reducing systems but these were not detected during methanogenic crude oil degradation. Only one other mechanism of alkane activation has been elucidated to date. This involves addition of carbon derived from bicarbonate/CO<sub>2</sub> to C-3 of an alkane chain to form a 2-ethylalkane with subsequent removal of the ethyl group leading to the formation of a fatty acid 1 carbon shorter than the original alkane. 2-ethylalkanes have never been detected as metabolites of anaerobic alkane degradation and were not detected in crude oil-degrading methanogenic systems. Due to the range of alkanes present in crude oil it was not possible to infer the generation of C-odd acids from C-even alkanes which is characteristic of the C-3 carboxylation mechanism.

Furthermore genes homologous to alkysuccinate synthetases were not detected in the methanogenic hydrocarbon degrading community by pyrosequencing of total DNA extracted from methanogenic enrichments cultures. beta-oxidation genes were detected and intriguingly, alcohol and aldehyde dehydrogenase genes were present. This offers the possibility that alkane activation in the methanogenic system does not proceed via acid metabolites, but may be initiated by an anaerobic hydroxylation reaction. This is not unprecedented and hydroxylation of ethylbenzene has been demonstrated. However the C-H bond dissociation energy of alkanes is typically considered too high to readily permit alkane hydroxylation. It is however clear that alkane activation in these methanogenic crude oil-degrading systems involves mechanisms other than the well-known fumarate-addition reactions.