Title

Methane generation accompanied by an unusual degradation of long-chain alkyl substituted moieties in heavy oil as evidenced by anoxic biodegradation experiments

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Abstract

Methanogenic crude oil degradation is a common process in subsurface oil reservoirs. Biodegradation of the light fraction of crude oils (e.g *n*-alkanes) has been well documented. However, little is known about the biodegradability of the resulting heavy oil. In this study, a methanogenic enrichment culture obtained from the Shengli oilfield could efficiently degrade heavy oil and generate 1.3 – 1.9 mmol CH_4/g of oil with a rate of 3.0 – 8.8 μmol CH_4/g of oil/day. Surprisingly, longer-chain-hydrocarbons (including *n*-alkylcyclohexanes, methyl-*n*-alkylcyclohexane, *n*-alkyldecalin, *n*-alkylbenzenes, *n*-alkyltoluenes, n-alkylxylenes and *n*-alkylnaphthalenes) were preferentially depleted relative to shorter-chain ones. Correspondingly, *n*-fatty

acids and naphthenic acids with 1 – 3 naphthenic rings accumulated over time. Two bacterial phylotypes affiliated to *Soehngenia* and *Dehalococcoidia* (GIF 9) were consistently among the most numerous bacteria in the successive oil-degrading cultures, indicating that these presently uncultured bacteria may account for this unusual heavy oil degradation process, most likely through a currently unknown degradation mechanism, and probably in cooperation with aceticlastic and hydrogenotrophic methanogens. The observation of the existence of methanogenic communities that preferentially utilize long-chain alkyl substituted moieties of heavy oil, has important implications for our understanding of heavy oil degradation in subsurface oil reservoirs and oil-contaminated environments.