

## **Biogenic Methane Production from Crude Oil by Enrichment from a Low-Temperature Western Canadian Oil Reservoir**

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### **Introduction**

Anaerobic conversion of crude oil to methane could be a cost-effective and environmentally-friendly enhanced recovery technology in mature or spent-oil reservoirs. In-reservoir methanogenic conversion of light-oil to heavy-oil with resultant production of methane is a dominant anaerobic biodegradative process since a majority of the world's oil reservoirs are already biodegraded (Dolfing et al. 2008; Roadifer, 1987). Though before now, anaerobic biodegradation in petroleum reservoirs was believed to be driven by oxygen-containing meteoric water. However, studies combining field evidence with laboratory studies has unequivocally demonstrated that methane is the end-product of anaerobic biodegradation of crude oil in petroleum reservoirs (Jones et al. 2008). In this respect, in-situ methanogenic conversion of non-conventionally recoverable oil to natural gas may be a future enhanced oil recovery technology. It is thus encouraging that a much recent study demonstrates conversion of residual oil, in marginal sandstone reservoir core, to methane using inoculum obtained from gas-condensate contaminated subsurface sediments (Gieg et al., 2008).

Studies on biodegraded oil has shown that petroleum hydrocarbons are degraded step-wise beginning with n-alkanes, monocyclic alkanes, alkylbenzenes, isoprenoid alkanes, alkyl-naphthalenes, bicyclic alkanes, steranes and hopanes (Connan, 1984). However a majority of the studies on crude oil or hydrocarbon methanogenesis were done using microbial enrichments from oil contaminated sediments or anaerobic sediments (Gieg et al., 2008; Bekins et al., 2005; Warren et al., 2004; Anderson and Lovely, 2000; Zengler, 1999) and the few studies (Morikawa et al., 1996; Stetter and Huber, 2000) using enrichment cultures from petroleum reservoirs were not thought to be sufficiently convincing (Roling et al., 2003).

In view of the above, a study was initiated using a methanogenic enrichment that was obtained directly from an oil reservoir production fluid. The objective of the ongoing study was to demonstrate methane production by oil reservoir microorganisms under different culture conditions that may inhibit or enhance methanogenesis, as well as demonstrate the removal of crude oil components from light oil or heavy oil. The source of the enrichment was a low temperature reservoir, producing crude oil with an API gravity of

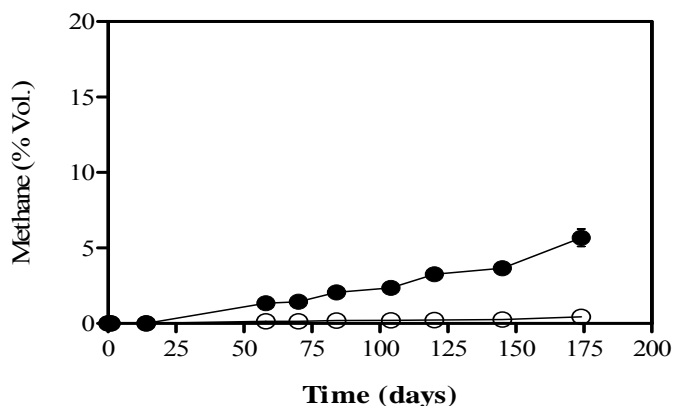
16. The same production fluid was previously the source of a nitrate-reducing oil-degrading enrichment (Lambo et al., 2008). Results obtained so far show that methane production from oil does not require amendment with minimal salt media; however the addition of phosphate into produced water inoculated with the methanogenic enrichment significantly improved methane production.

## Methods

A primary methanogenic enrichment culture was initially obtained using a mixture of three produced water samples as inocula (25%) in sterile minimal salts media that was amended with (1ml/L) EDTA-chelated trace elements, tungstate-selenate solution, vitamin B12, thiamine solution, Wolfe's vitamin solution, and 30 ml of 1M NaHCO<sub>3</sub>. The medium was prepared under an atmosphere of N<sub>2</sub>/CO<sub>2</sub> (90/10%, v/v) and made anaerobic by amendment with Na<sub>2</sub>S to a final concentration of 1mM. The final pH was 7.0 – 7.3. Sterile crude oil (4 – 20%, v/v) was added into the primary and subsequent enrichments after degassing the oil under vacuum. Incubations initiated without minimal salts media were carried out using phosphate (0.5 – 1 mM) and sulfide (1 mM) containing mixtures of produced water that was amended with or without the following: 10% methanogenic inoculum, added light oil or heavy oil (5%, v/v), and vitamins solutions (1 ml/L). The production of methane from different classes of hydrocarbons (10 mM toluene plus a non-degradable carrier, hexadecane, or naphthalene) and the effect of different concentrations of sulfate (0 – 10 mM) on methane production were also investigated. Methane production from unamended injection water and produced water was also investigated. Long-term incubation with oil (2.5%, v/v) was carried using minimal salts media that was inoculated with 10% oil-grown methanogenic enrichments. Methane production was measured at regular intervals by injection of 0.2 or 1 ml of culture headspace into a Hewlett-Packard 5890 gas chromatograph equipped with a stainless steel column (0.049 cm x 5.49 m) packed with Porapak R (Injector and detector temperature, 40 and 70 °C, respectively). Accumulation of acetate was measured by HPLC in secondary enrichments incubated with 20% (v/v) light oil. Archaeal community composition in the oilfield samples was analyzed by denaturing gradient gel electrophoresis (DGGE).

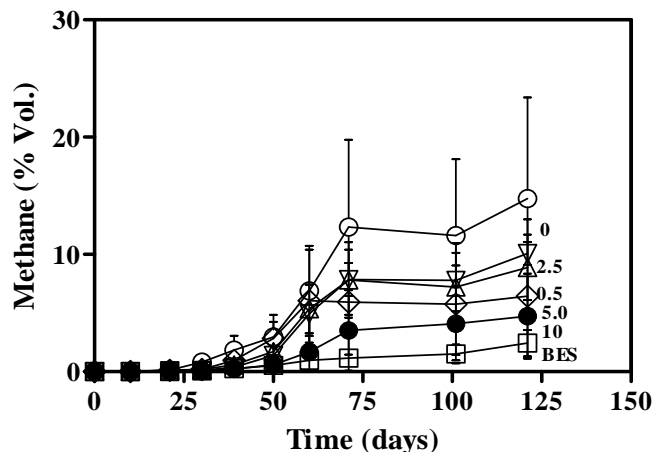
## Results

Primary enrichment incubated with light oil initially produced methane at a rate that was similar to control incubations without oil. However, the level of methane in experimental bottle increased to 15% (v/v) of the culture headspace after 280 days of incubation. Secondary enrichment cultures amended with 20% light oil produced methane at rates that were much higher than control cultures without oil (Fig. 1). Acetate was only detected in this culture during the early stages of incubation (data not shown).



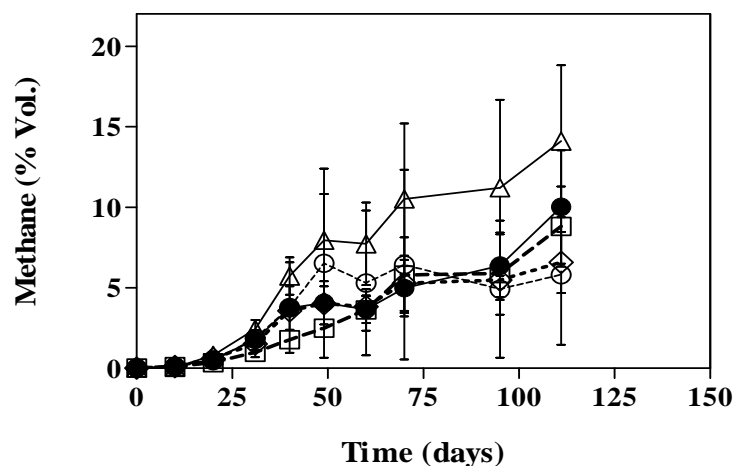
**Fig.1.** Methane production in secondary enrichment cultures incubated with 20% (v/v) light oil (●), as compared to a control (O). Data are mean of duplicates trials. Error bars are completely covered by the symbols.

Methane production was inhibited in the presence of 0.5 mM 2-bromoethanesulfonic acid (BES) or higher concentration (10 mM) of sulfate compared to low or moderate concentrations (0.5 – 2.5 mM) of sulfate (Fig. 2). Sulfate was not consumed in any of the sulfate containing cultures (data not shown).



**Fig. 2.** Methane production in cultures incubated with light oil and varying concentrations of sulfate. Inset values are indicative of the respective sulfate concentration in each culture. BES indicates cultures with 0.5 mM 2-bromoethanesulfonic acid, a physiological inhibitor of methanogenesis. Data are mean of three replicates.

Methane production from light oil-amended produced water inoculated with 10% methanogenic enrichment was higher (Fig. 3) than that observed for produced water amended with light oil only, or for unamended produced water alone, while methane production from produced water amended with inoculum and heavy-oil was comparable to that from produced water amended with light-oil only. A moderate level of methane was produced in cultures incubated with naphthalene or hexadecane after 140 days. In contrast, cultures incubated with toluene performed poorly (data not shown). Unlike unamended produced water, methane was not formed by injection water after 55 days of incubation. A total of 7 different genera, including *Methanobus*, *Methanoculleus*, *Methansaeta*, *Methanocalculus*, *Methanomicrobium*, and *Methanoplanus*, were identified in the oilfield samples.



**Fig. 3.** Methane production from amended and unamended produced water. Symbols indicate unamended produced water (O); light-oil amended produced water (□); produced water amended with light-oil and methanogenic inoculum (Δ); produced water amended with light-oil, methanogenic inoculum, and vitamin solutions (◇); and produced water amended with heavy-oil and methanogenic inoculum (●).

## Conclusion

Methane production could be observed in real time from light and heavy oil. The production was stimulated by appropriate nutrients (e.g. phosphate) and inhibited by a methanogen-specific inhibitor (BES), as well as by sulfate.

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