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Stimulation of methanogenic crude oil biodegradation in depleted oil reservoirs

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ABSTRACT

We examined the feasibility of stimulating methanogenic crude oil biodegradation through the combined supply of CO_2 and protein-rich matter in anaerobic microcosms, without the external supply of H_2, and using formation waters and crude oil collected from the Stillwater and Cushing oil fields of Oklahoma, USA. We found that, depending on the chemical and microbiological composition of the formation water, protein-rich matter can stimulate methanogenic crude oil biodegradation if supplied along with CO_2. For the low salinity (116, 714 mg/L) Stillwater oil field formation water, during the early stages of the stimulation process, CH_4 was produced by the microbiological reduction of the supplied CO_2 with H_2 produced from the syntrophic/fermentative biodegradation of the supplied protein-rich matter. As indicated by 16S rRNA gene community analysis, this resulted in a syntrophic enrichment of a methanogenic crude oil-degrading microbial community and led to > 42% crude oil biodegradation with the concurrent production of CH_4 after 100 days of incubation at 50 °C. For the high salinity (176,665 mg/L) Cushing oil field formation water, although H_2 was produced, enrichment of a methanogenic crude oil-degrading microbial community—and thus CH_4 production—was not possible. We tested two types of protein-rich matter (yeast extract and isolated soy protein) and found that isolated soy protein can be used as a substitute for yeast extract in field-scale applications. These findings have large implications toward the coupling of geological CO_2 storage and microbial enhanced oil recovery in depleted oil reservoirs.

1. Introduction

The biodegradation of crude oil via methanogenesis in oil reservoirs has been extensively studied [1–7]. Oil-degrading syntrophic microorganisms produce CO_2, H_2, and acetate, which are subsequently converted to CH_4 by hydrogenotrophic and acetoclastic methanogens. The sequence of syntrophic methanogenic reactions from alkanes ($C_{16}H_{34}$) is usually represented by the following global reactions [3,6]:

$$4C_{16}H_{34} + 64H_2O \rightarrow 32CH_3COO^- + 32H^+ + 68H_2 \quad (1)$$

$$68H_2 + 17CO_2 \rightarrow 17CH_4 + 34H_2O \quad (2)$$

$$32CH_3COO^- + 32H^+ + 64H_2O \rightarrow 128H_2 + 64CO_2 \quad (3)$$

$$32CH_3COO^- + 32H^+ \rightarrow 32CH_4 + 32CO_2 \quad (4)$$

where the concentration of H_2 and acetate typically remains low, proving their important role as key intermediates in methanogenic crude oil biodegradation [8–13].

On the evidences supporting the feasibility of methanogenic crude oil biodegradation at oil reservoir conditions, the possibility has been suggested of recovering crude oil from depleted oil reservoirs as CH_4 by stimulating methanogenic crude oil biodegradation *in situ* [3,7,14,15]. However, an appropriate approach to stimulate methanogenic crude oil biodegradation at the field-scale has not been developed.

The main factor impeding methanogenic crude oil biodegradation in oil reservoirs is the lack of nutritional resources available to the microorganisms [12,16]. In general, oil reservoirs contain ample carbon sources but usually contain low concentrations of nitrogen, phosphorus, and trace metals. A lack of these nutrients limits microbial growth in such habitats [17]. Due to the fact that nitrogen compounds are best supplied to microorganisms as a mixture of amino acids rather than inorganic nitrogen, they constitute the starting building blocks in the biosynthetic pathways for the majority of nitrogen-containing biomolecules. We recently proposed an approach to stimulate the biodegradation of crude oil and biogenic conversion of CO_2 to CH_4 in depleted oil reservoirs through the combined injection of CO_2 and a nutrient solution composed of protein-rich matter [18–21]. The combined injection of CO_2 and a nutrient solution composed of protein-rich matter would lead to the reduction of pH in the formation water (e.g.,

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6.8 to 5.0), which in addition to the availability of organic nitrogen compounds would create a suitable condition for the biogenic production of H_2 and acetate from the biodegradation of crude oil by indigenous fermentative microorganisms [22,23]. H_2 and acetate production constitutes the bottle neck in the biogenic conversion of CO_2 and acetate to CH_4 by hydrogenotrophic and aceticlastic methanogens in depleted oil reservoirs. Thus, stimulating the biogenic production of H_2 and acetate from the biodegradation of crude oil through the combined injection of CO_2 and a nutrient solution composed of protein-rich matter represents a promising and appealing approach to stimulate the biogenic conversion of both crude oil and CO_2 to CH_4. This new approach may reduce CO_2 emissions and also enhance the recovery of oil in the form of CH_4.

Broadly, crude oil biodegradation via methanogenesis has been extensively studied. The feasibility of crude oil biodegradation via methanogenesis using methanogenic microbial communities present in oil-contaminated subsurface sediments [1] and oil reservoir formation waters [24] has been proven using methanogenic microbial communities obtained by repeated enrichment transfers using freshwater supplied with nutrients (minerals, trace metals, vitamins, and reducer agents) and H_2/CO_2 gases. Here, we tested the effect of the combined supply of CO_2 and a nutrient solution composed of protein-rich matter on the biodegradation of crude oil by indigenous microbial communities present in depleted oil reservoirs, and we show that the combined supply of CO_2 and a nutrient solution composed of protein-rich matter can result in the stimulation of methanogenic crude oil biodegradation at salinity levels of oil reservoir formation waters, without the need for repeated enrichment transfers, and/or the external supply of H_2.

2. Materials and methods

2.1. Formation water and crude oil samples

Two distinct formation waters were examined in this effort: formation water collected from the Stillwater oil field (Separator tank of Duck #1 well: lat. 36.098189, log. −97.069614) and formation water collected from the Cushing oil field (Well head of Yahola # 17 well: lat. 35.7771504, log. −96.4850607) located in north-central Oklahoma, USA. Water and oil samples were collected aseptically in sterile Nalgene bottles flushed with produced natural gas. To prevent contact with air, prior to closing the bottles, the surface of collected formation waters were covered with produced crude oil. Collected samples were used within 2 days after collection.

2.2. Nutrient solution composition

Collected formation waters transferred to anaerobic media serum vials were supplemented with a nutrient solution containing resazurin as a redox indicator, $Na_2S{\cdot}9H_2O$ as a reducing agent in some cases, and a salt solution composed of the following salts (g/L): KH_2PO_4, 0.316; NH_4Cl, 0.54; $MgCl_2$, 0.2; Cysteine-HCl, 0.66 (Table 1). CO_2 was supplied as $NaHCO_3$.

Two types of protein-rich matter were tested in separate experiments: yeast extract (Fisher BioReagents™) and isolated soy protein (MP Biomedicals, LLC). Yeast extract was chosen because it has been shown to stimulate the biogenic production of H_2 from a wide variety of organic substrates including hydrocarbon compounds by pure and mixed microorganisms [25–28]. In addition, the less expensive and massively produced isolated soy protein was used to assess the feasibility of its use as a substitute for yeast extract in field-scale applications. Isolated soy protein is a protein-rich matter typically composed of 90% protein, 0.5% fat, 0.3% total hydrocarbons, and 4.5% ash, containing a number of essential trace metals [29].

Table 1
Composition of the nutrient solution.

Compound	Amount/120 mL	Role
Resazurin	100 µL	Anaerobic indicator
$Na_2S{\cdot}9H_2O$	0.1 g	ORP reducer
$NaHCO_3$	1.2 g	CO_2 source
Protein-rich matter	0.24 g	Amino acids source
$Na_2MoO_4{\cdot}H_2O$	0.01 g	Trace metal source
Salt solution	20 mL	Minerals source

2.3. Analytical methods

The composition of the collected Stillwater and Cushing oil field formation waters were determined using an inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis using the services of the Soil, Water, and Forage Analytical laboratory at Oklahoma State University.

The gas composition in the headspace of the microcosms was monitored using a gas chromatographer (GC) (Fisher Scientific Tracer 1310) equipped with two thermal conductivity detectors (TCD) and two columns (TG-Bond Msieve 5A, 30 m × 0.53 mm × 50 µm, and TG-Bond Q, 30 m × 0.32 mm × 10 µm). The gases that were analyzed included CH_4, CO_2, H_2, H_2S, N_2, and O_2.

The composition of the crude oil at the start and conclusion of the microcosm experiments was determined by using a GC (Perkin Elmer Clarus 500) equipped with a flame ionization detector (FID) and one column (Elite-5 capillary column, 30 m × 0.25 mm × 0.25 µm), where 1 mL of crude oil was dissolved in 9 mL of hexane (organic solvent), and the remaining of another 1 mL of crude oil supplied to the microcosm was extracted from the solution using 9 mL of hexane at the conclusion of the experiment. Both crude oil samples contained in the hexane were analyzed at the same time.

Major alkanes have been identified by analyzing and comparing a standard mixture of n-alkanes C_{10} – C_{40} (all even) in hexane, 50 µg/mL each (Cat. No. 68281, Sigma-Aldrich). A quantitative analysis of individual alkanes is beyond the scope of this research. However, the overall percentage of crude oil biodegradation was quantified according the following equation:

$$\text{Biodegradation}\,\% = \frac{A_i - A_f}{A_i} 100 \tag{5}$$

where A_i is the integration area of the chromatogram of the crude oil at the start of the experiments, and A_f is the integration area of the chromatogram of the remaining crude oil in microcosm at the conclusion of the experiment.

2.4. Experimental procedure

250-mL serum vials were supplied with 20 mL of the salt solution and then sealed with rubber stoppers and aluminum seals. To create an anoxic environment, the vials were autoclaved at 121 °C with needles inserted through the rubber stoppers to allow the salt solution to degas. To remove traces of O_2 from the headspace of the vials, autoclaved vials were stripped with pure N_2 gas in a biosafety cabinet for 15 min. The vials were then opened in an anaerobic chamber containing a pure N_2 gas atmosphere, and 100 mL of the formation water, $NaHCO_3$, and protein-rich matter were added (Table 1). To ensure zero initial concentrations of H_2 in the vials at the start of the incubation process, contrary to common practices the anaerobic chamber was operated without H_2 in its atmosphere. To compensate for the pH increase due to the addition of $NaHCO_3$, 1.0 mL of autoclaved HCl (0.2 N) was added to the solution. Crude oil was added last before the vials were resealed with new sterile rubber stoppers. Finally, microcosms prepared following the outlined procedure were stripped with pure N_2 gas to ensure anaerobic initial conditions, and they were incubated at 50 °C; this temperature was estimated based on the typical lithostatic temperature gradient of 25–30 °C/km and depths of the wells (800–1000 m) from