Journal of Petroleum Science and Engineering 81 (2012) 86–93

Contents lists available at SciVerse ScienceDirect

Journal of Petroleum Science and Engineering

journal homepage: www.elsevier.com/locate/petrol

Evaluation of indigenous anaerobic microorganisms from Mexican carbonate reservoirs with potential MEOR application

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ARTICLE INFO

Article history:
Received 9 February 2011
Accepted 9 December 2011
Available online 21 December 2011

Keywords:
carbonate reservoir
extremophile
fermentative
Thermoanaerobacter
Microbial Enhanced Oil Recovery (MEOR)

ABSTRACT

The success of biotechnological processes for oil recovery depends on adequate understanding of the system components: microorganisms, oil and porous media.
Nine oil samples were collected from a carbonate oil reservoir in Cordoba Platform, Veracruz, Mexico. Geochemical characterisation demonstrated heavy oils with: API gravity of 10.7 to 14.5°, 3 to 5% sulphur, 36.58 to 54.06% aromatic hydrocarbons and −23.98 to −24.24 (‰) $\delta^{13}C$ isotopic values in total oil. Additionally, the pristane/phytane ratio (Pr/Ph)<1 indicated an anoxic depositional environment. Results showed that native microbiota could have contributed to the biogeochemical transformation of the oil in the reservoir. Anaerobic, thermophilic, halotolerant and fermentative enrichment cultures were obtained from the nine oil samples (A1–A9). Metabolites such as CO_2, CH_4, ethanol, acetone, acetate and biosurfactants were detected. Mixed culture from the A7 sample showed the highest activity among all the mixed cultures screened, growing under 50 to 80 °C, 5 to 35 g L^{-1} NaCl and 0.8 to 14.2 MPa pressure. Its kinetic parameters were $\mu_{max} = 0.321\ h^{-1}$ and $Ks = 0.54\ g\ L^{-1}$. These results were used to determine the conditions for oil recovery assays. A7 culture enhanced recovery of up to 12% of heavy oil (11.16 °API) in oil impregnated carbonate granular porous media. Analysis of the V3 region of the 16S rRNA gene of the A7 culture showed that the predominant taxon was *Thermoanaerobacter*. Phylogenetic approximations demonstrated that similitude percentage of 99.9% corresponded to *Thermoanaerobacter ethanolicus*, 99.6% to *Thermoanaerobacter pseudethanolicus* and 98.9% to *Thermoanaerobacter brockii* and *Thermoanaerobacter finii*.

1. Introduction

Mexico is one of the most important oil producers in the world and several reserves are found in the continental platform and the Gulf of Mexico. The main Mexican oil reserves are in carbonate reservoirs (PEMEX, 2009; Rodríguez and Christopher, 2004). Some of these oil fields are located in the Cordoba Platform in Veracruz, Mexico, formed by a sedimentary sequence of Tertiary carbonate rocks (Román and Holguin, 2001).

Oil from the fields in the north region was generated in carbonate-evaporite rock platform during the middle–late Cretaceous period. Oil from the south region fields were originated in siliciclastic-carbonate facies during the late Jurassic and Turonian. These oils are heavy with less than 20 °API (Guzmán et al., 2001).

Geological, structural and physicochemical characteristics of the carbonate reservoir turn oil extraction into a complex process. Enhanced recovery processes, such as microbial enhanced oil recovery (MEOR), could be useful for oil extraction from this type of reservoir. MEOR technology consists of injecting or stimulating microorganisms present in the reservoir that are able to produce CO_2, CH_4, H_2, acids, solvents and biosurfactants. Gas production increases reservoir pressure and reduces oil viscosity (Youssef et al., 2009). Solvents alter rock wettability at the oil–rock interface, releasing the oil from the porous matrix. Solvents can also be dissolved in oil, decreasing its viscosity (McInerney et al., 2005). Acids, such as acetic acid, can modify rock permeability and facilitates oil mobility. Furthermore, biosurfactants reduce the superficial and interfacial tension between oil and water (Sayyouh, 2002; Youssef et al., 2009). All these mechanisms can enhance the recovery of oil.

Anaerobic, thermophilic and fermentative bacteria are some of the physiological groups found in oil reservoirs (Takahata et al., 2000; Magot, 2005; Dahle et al., 2008). It is possible to stimulate microbial growth and the production of useful metabolites for oil recovery (Ollivier and Cayol, 2005). However, there are few studies of native microorganisms and their physiological characterisation from the oil of Mexican carbonate reservoirs. Furthermore, molecular descriptions of microbial communities have not been sufficiently investigated. In these analyses, molecular tools can be included, such as denaturing

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doi:10.1016/j.petrol.2011.12.010

gradient gel electrophoresis (DGGE) and gene amplification and sequencing to assemble a phylogenetic description of the involved species (Marzorati et al., 2008).

The aim of this research was to evaluate and characterise indigenous microorganisms obtained from the oil of a Mexican carbonate reservoir in order to be applied in heavy oil recovery processes.

2. Materials and methods

2.1. Sampling site

The sampling site was the north region of the Cordoba Platform in San Felipe Formation, Veracruz (Fig. 1), constituted mainly of carbonate rocks (limestone and breccia) that produce heavy oil.

The oil producing interval is located at a depth of 1250 m in an estimated reservoir of 20 m thickness. The downhole conditions are a temperature of 70 °C and a pressure of 14.2 MPa. Nine oil samples were collected in sterile bottles from different wells and identified as A1, A2, A3, A4, A5, A6, A7, A8 and A9. Samples were transported and stored at 4 °C until use. They were used as inocula for enrichment cultures.

2.2. Culture medium

The composition of the culture base medium (BM) was (g L^{-1}) 0.25 NH_4Cl; 0.14 K_2HPO_4; 1.0 $MgCl_2 \cdot 6H_2O$; 0.14 $CaCl_2 \cdot 2H_2O$; 0.34 KCl; 2.0 yeast extract; 2.0 tryptone peptone; 0.6 cysteine-HCl; 1 mL (0.1%) resazurin and molasses as carbon source. The pH of the culture medium was adjusted to 7.0.

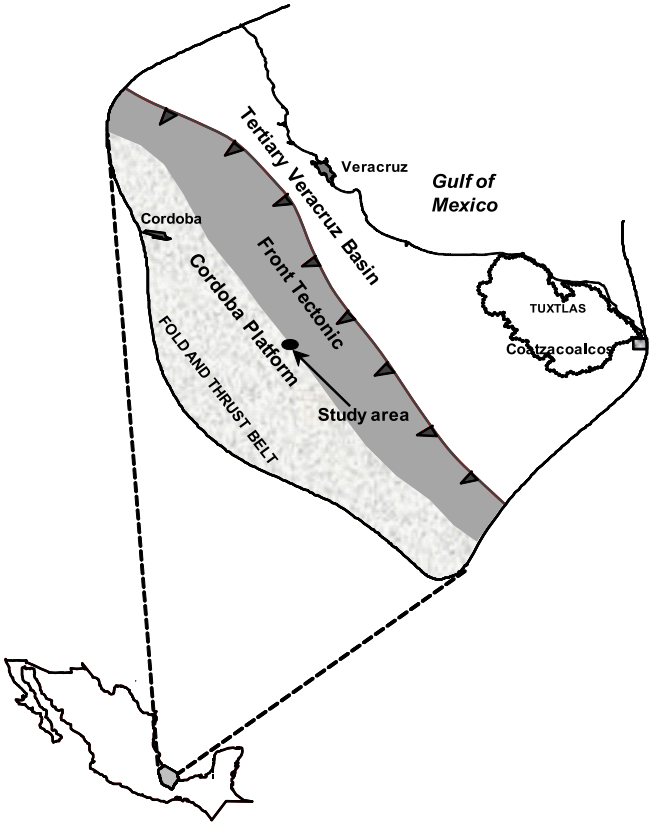

Fig. 1. Localisation map of the oil sampling area in the Cordoba Platform.

2.3. Enrichment cultures

Fermentative enrichment cultures were performed in 125 mL serum bottles containing 50 mL of BM, 5 g L^{-1} NaCl and 10 g L^{-1} molasses (5 g L^{-1} glucose) and were inoculated with 1 mL oil. The cultures were incubated at 70 °C and 100 rpm for 9 days under anaerobic conditions; microbial growth and CO_2 production were evaluated. The most active culture was selected.

2.4. Temperature and salinity effect

The temperature and salinity effect on CO_2 production by the selected mixed culture was evaluated. The systems contained 50 mL BM, 10 g L^{-1} molasses and 1 mL oil. Temperatures tested were 50, 60, 70 and 80 °C and NaCl concentrations were adjusted to 5, 15, 25 and 35 g L^{-1}. Systems were incubated for 9 days.

2.5. Evaluation of kinetic parameters

For kinetic characterisation, 125 mL serum bottles were used containing 70 mL of BM medium, 15 g L^{-1} NaCl, 1 mL of the selected microbial inoculum (OD 0.520 at 620 nm), 1 mL of oil and molasses at different concentrations (0.5 to 11.3 g L^{-1}). The bottles were incubated at 70 °C for 15 days and substrate consumption, CO_2, biomass, acid and solvent productions were monitored. Growth kinetic parameters were estimated with the Lineweaver–Burk model (Zhou et al., 2009). Experimental data for CO_2 production were adjusted with the modified Gompertz equation (Shi and Yu, 2004). All the experiments were carried out in triplicate.

2.6. Oil recovery test

This assay was conducted to test the oil recovery ability of the A7 culture in granular carbonate as porous media. 125 mL bottles with 30 g washed granular carbonate (0.3 to 0.4 mm size) were used. Porous media were saturated with 12 mL of heavy oil (11 °API, viscosity 32,906 mPa.s at 30 °C) in a vacuum condition for 2 h. 50 mL BM with 10 g L^{-1} molasses and 1 mL selected culture inoculum (OD 0.520 at 620 nm) were added to the systems. Control systems were composed of oil-impregnated granular porous media with brine (NaCl 15 g L^{-1}) and without inoculum. The bottles were incubated at 70 °C for 7 days. Released oil volume was measured.

2.7. Analytical methods

The geochemical characterisation of the oil samples included measurements of API gravity, sulphur content, quantification of saturated and aromatic hydrocarbons, resins, asphaltenes (SARA) and $\delta^{13}C$ isotopes were conducted following ASTM D 70–03; ASTM D 4294–03; ASTM D, 2007–03 procedures.

Total hydrocarbon contents were analysed by using gas chromatography (HP 6890N) with a flame ionisation detector, according to the EPA 8015 method, under the following conditions: DB-1 capillary column, column temperature 45 °C for 3 min, 45–100 °C at 20 °C min^{-1}, 100–200 °C at 10 °C min^{-1}, 200–255 °C at 10 °C min^{-1}, 255–290 °C at 10 °C min^{-1} and 290 °C for 5 min. Helium was used as the carrier gas at a flow rate of 1.4 mL min^{-1}. Injector and detector temperatures were set at 250 °C.

Sterane and terpane biomarkers were analysed in the saturated hydrocarbon fraction with a gas chromatograph coupled to a mass selective detector, according to the EPA 8270 method, under the following conditions: DB-5 capillary column, column temperature 40 °C for 1 min, 40–290 °C at 6 °C min^{-1} and hold for 8 min, 290–320 °C at 6 °C min^{-1} and hold for 15.33 min. Helium was used as the carrier gas at a flow rate of 1.4 mL min^{-1}. Injector and detector temperatures were set at 250 °C.