Journal of Bioscience and Bioengineering
VOL. 114 No. 4, 440–445, 2012

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# Coreflood assay using extremophile microorganisms for recovery of heavy oil in Mexican oil fields

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Received 22 November 2011; accepted 11 May 2012
Available online 16 June 2012

**A considerable portion of oil reserves in Mexico corresponds to heavy oils. This feature makes it more difficult to recover the remaining oil in the reservoir after extraction with conventional techniques. Microbial enhanced oil recovery (MEOR) has been considered as a promising technique to further increase oil recovery, but its application has been developed mainly with light oils; therefore, more research is required for heavy oil. In this study, the recovery of Mexican heavy oil (11.1°API and viscosity 32,906 mPa s) in a coreflood experiment was evaluated using the extremophile mixed culture A7, which was isolated from a Mexican oil field. Culture A7 includes fermentative, thermophilic, and anaerobic microorganisms. The experiments included waterflooding and MEOR stages, and were carried out under reservoir conditions (70°C and 9.65 MPa). MEOR consisted of injections of nutrients and microorganisms followed by confinement periods. In the MEOR stages, the mixed culture A7 produced surface-active agents (surface tension reduction 27 mN $m^{-1}$), solvents (ethanol, 1738 mg $L^{-1}$), acids (693 mg $L^{-1}$), and gases, and also degraded heavy hydrocarbon fractions in an extreme environment. The interactions of these metabolites with the oil, as well as the bioconversion of heavy oil fractions to lighter fractions (increased alkanes in the $C_8$–$C_{30}$ range), were the mechanisms responsible for the mobility and recovery of heavy oil from the porous media. Oil recovery by MEOR was 19.48% of the residual oil in the core after waterflooding. These results show that MEOR is a potential alternative to heavy oil recovery in Mexican oil fields.**



[**Key words:** Microbial enhanced oil recovery (MEOR); Heavy oil; Extremophile; *Thermoanaerobacter*; Biodegradation; Coreflood]

Global energy demand requires a greater volume of crude oil. In the traditional oil recovery process, about 30–40% of the oil is extracted, but most of it stays entrapped in reservoirs (1,2). Competitive and innovative technologies for enhanced oil recovery (EOR) have been developed to target this (3). In Mexico, the characteristics of the oil increase the difficulty of oil recovery, because a high percentage of reserves (61.9%) corresponds to heavy (<20°API) and high viscosity oils (Anuario estadístico Petróleos Mexicanos, http://pemex.com/informes/pdfs/anuario_estadistico_2010.pdf). One of the EOR technologies for this problem is the application of microbial enhanced oil recovery (MEOR). In this technology, the addition of nutrients allows the development of microorganisms and the production of metabolites such as biosurfactants, biopolymers, biomass, acids, solvents, gases and enzymes (4).

Biomass can modify the permeability of porous media by selectively plugging highly permeable zones and redirecting the flow of fluids in the reservoir (5). The fermentation of carbohydrates produces gases such as $CO_2$, $CH_4$, and $H_2$, which contribute to repressurising the reservoir (3). Another benefit of fermentative activity is the production of acids, for example acetic and propionic acid, and the production of solvents such as ethanol, acetone and butanol. Acids can reduce the pH of the environment and can alter fluids and solid surfaces, which then affects the flow behaviour of oil (Ohno et al., 13th Oil Gas and Petrochemical Congress, www.ripi.ir/congress13/meor.pdf). Biosurfactants reduce oil viscosity and the interfacial tension between fluids, thus increasing oil mobility (6). Another important mechanism in MEOR is the bioconversion of heavy hydrocarbon fractions to light fractions and their redistribution in the oil (7).

A variety of microorganisms have been reported for the microbiological recovery process; some of them are aerobic and mesophilic (8). However, other studies have established that anaerobic, thermophilic, halotolerant, and indigenous microorganisms are most appropriate for the MEOR process (3,7).

Since the mechanisms of MEOR depend on the microorganisms and characteristics of the oil (7), additional studies must be carried out. The advantages of MEOR technology are that it is less expensive than thermal processes, it does not interfere with the waterflooding recovery process, and it is environmentally friendly (Brown et al., Sym. Improved Oil Recovery SPE, USA, SPE 59306, 2000). However, few studies have been carried out on MEOR with heavy oils; in addition, there are no reports of this technology with Mexican oils.

The aim of this research was to evaluate the recovery of heavy oil using extremophile microorganisms in coreflood experiments under oil field conditions.

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doi:10.1016/j.jbiosc.2012.05.011

## MATERIALS AND METHODS

**Oil and core samples** A crude oil sample was obtained from oil well A7 in the northern region of the Cordoba Platform, Veracruz, México. The downhole conditions were a temperature of 70°C and pressure of 9.65 MPa. The oil samples were collected in sterile bottles and transported and stored at 4°C until use. The core used in the oil recovery tests was made of Berea sandstone.

**Microorganisms** The mixed culture A7 was isolated from a Mexican oil reservoir as described by Castorena-Cortés et al. (9). Molecular analysis showed that the culture A7 was comprised mainly of *Thermoanaerobacter pseudethanolicus*, *Thermoanaerobacter ethanolicus*, and *Thermoanaerobacter* sp. The sequences were deposited in GenBank (NCBI) under accession numbers HQ686054 to HQ686059.

**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, 15.0 NaCl, 2.0 yeast extract, 2.0 tryptone peptone, 0.6 cysteine-HCl, 10.0 molasses as the carbon source, and 1 mL (0.1%) resazurin. The culture medium was sterilised with heat at 121°C, 15 psi, for 22 min. The pH of the culture medium was adjusted with a solution of sodium bicarbonate (10%) to 7.0. To maintain a reducing atmosphere, a sodium sulphide solution (2%) was added.

**Oil recovery test** The sandstone core was washed with solvents and vacuum dried at 80°C. The core porosity determination was carried out by calculating the weight difference between two states of saturation, and the permeability was calculated using Darcy's equation (10). These tests were conducted at 0.1 MPa and 25°C.

The experimental system consisted of a continuous injection pump, an oven for temperature control, transference cylinders, a pressure meter, a differential pressure transducer, a data acquisition system, and a stainless steel cell. The core fragment was placed inside a rubber sleeve in the cell and then placed in the oven for temperature control. Transference cylinders were used to displace fluids (brine, oil, and microorganisms plus nutrients) using an injection pump. A differential transducer was installed to monitor changes in pressure. A gas sampling trap at the outlet of the experimental system was adapted. The experiment was carried out at temperature, pressure, and salinity of the reservoir (70°C, 9.65 MPa, and 15 g $L^{-1}$ NaCl, respectively).

The characteristics of the core, oil, and conditions of the oil recovery system are shown in Table 1. Fig. 1 shows the stages of the coreflood assay; all fluids were injected at 10 mL $h^{-1}$. Brine (15 g $L^{-1}$ NaCl) was injected until core saturation. Subsequently, the core fragment was saturated with oil (75.5%). Before fluid injection, a vacuum was applied to eliminate air. Waterflooding was carried out by brine injection. Once this stage was concluded (no more oil was recovered), microbial enhanced oil recovery (MEOR) was initiated. BM with molasses and a 30 mg $L^{-1}$ inoculum of mixed culture A7 (the age was 48 h, and it was at the exponential phase of growth) was injected until 3.5 porous volumes were displaced. The system was held in confinement for 10 days (first stage). Over the period of confinement, a second injection of nutrients and microorganisms was carried out with the same concentration as the first injection, followed by a confinement period of 10 days (second stage). The third stage of MEOR consisted of brine injection for the displacement of oil released by the microorganisms. In each recovery stage, aqueous and oil phases were obtained, which were separated for subsequent analysis. In the aqueous phase, metabolites and biomass were determined. The recovered oil and gas phase were also quantified and analysed.

**TABLE 1.** Oil recovery test conditions, oil and core characteristics.

| Characteristic/conditions | Value |
|---|---|
| Crude oil | |
| Type of oil | Heavy |
| API gravity | 11.6 |
| Viscosity (mPa s) at 30°C | 32906 |
| Viscosity (mPa s) at 70°C | 841.94 |
| Saturated (%) | 10.81 |
| Aromatic (%) | 42.33 |
| Resins (%) | 23.16 |
| Asphaltenes (%) | 23.70 |
| Sulphur (%) | 4.81 |
| Core | |
| Type of rock | Sandstone berea |
| Length (cm) | 12.5 |
| Diameter (cm) | 10.6 |
| Area ($cm^2$) | 81.07 |
| Pore volume ($cm^3$) | 215.8 |
| Porosity (%) | 21.29 |
| Permeability (mD) | 100.13 |
| Oil saturation, So (%) | 75.5 |
| Residual water saturation, Swr (%) | 24.5 |
| Operation conditions | |
| Microbial culture | Mixed culture A7 |
| Inoculum concentration (mg $L^{-1}$) | 30 |
| Brine, NaCl (g $L^{-1}$) | 15 |
| Temperature (°C) | 70 |
| Pressure (MPa) | 9.65 |
| Flow (mL $h^{-1}$) | 10 |

**Analytical methods** Oil characterisation included measurements of API gravity and sulphur content, as well as the quantification of saturated and aromatic hydrocarbons, resins, and asphaltenes (SARA), conducted according to the standard methods ASTM D70-09, ASTM D4294-10, and ASTM D2007-03, respectively (11–13). Total hydrocarbon contents were analysed on a gas chromatograph (HP 6890N) with a flame ionisation detector, according to standard method EPA 8015 (14). The range of C-atom hydrocarbons present in the sample oil was determined by the boiling point distribution using the ASTM D7169-05 method (15).

Gas analysis was conducted on a gas chromatograph coupled to a thermal conductivity detector and a CTR 1 column. The injector and detector temperatures were 45°C and 100°C, respectively. The substrate (molasses) concentration was determined as glucose by the total sugar technique (16). The biomass concentration was determined as total protein by the Bradford method (17). The oil spreading technique was applied to evaluate the presence of biosurfactants or surface-active agents (18). Organic acid and solvent measurements were carried out on a gas chromatograph (HP 6890) coupled to a flame ionisation detector and an AT-Wax capillary column. The temperature of both the injector and detector was 250°C and the oven was maintained at 80°C.

## RESULTS AND DISCUSSION

**Oil and sandstone core characterisation** The crude oil used in this work was classified as heavy oil with 11.16°API according to Tiab and Donaldson (10), and contained a high percentage of resin-asphaltene (46.86%). The sulphur concentration was 4.81%. The viscosity evaluated at 30°C was 32,906 mPa s (Table 1).

The Berea core used in the oil recovery test presented the characteristics described in Table 1, with a porosity of 21.29% and a permeability of 100.13 mD.

**Oil recovery process** In the coreflooding assay, oil recovery was obtained at each stage. In the waterflooding stage, 2.1 pore volumes of brine were injected with a recovery of 76.8 mL oil, corresponding to 46.2% of the initial oil (IO) (Fig. 2A). At this phase, brine injection was carried out until no additional released oil was detected, and was extended for a further 4 h. After this stage, 53.8% of the residual oil (RO) remained in the Berea core. The microbial process (MEOR) began under these conditions and it was subdivided into three stages (Fig. 2). At the first MEOR stage, 3.5 pore volumes of the nutrient solution with microorganisms were injected (Fig. 2B). The quantity of oil recovered was 12.5 mL, corresponding to 7.5% IO and 13.98% RO after waterflooding. In this period, the microorganisms of mixed culture A7 were in the exponential phase of growth, which corresponds to the highest production of biomass and metabolites quantified in batch systems (9). After the first 10 days of confinement, the second stage of MEOR injection was performed (Fig. 2C), which yielded an additional recovery of 1.8% IO and 3.3% RO. After the second confinement, the third stage of MEOR (Fig. 2D) was carried out with brine injection where oil recovery of 1.2% IO and 2.2% RO were obtained.

A total recovery of 56.7% IO was obtained by waterflooding and MEOR. The recovery attributed to microbial activity was 10.5% IO and 19.5% RO. RO in tertiary recovery processes, including MEOR, is the most difficult to mobilise; in addition, the microbial recovery process depends on the degree of residual oil saturation of the system (Zekri, A. and El-Mehaideb, R., SPE Sym. Improved Oil Recovery, USA, SPE 75217, 2002).

The results of our work were similar to those obtained by other authors. Bao et al. (2) reported 9.4% additional oil recovery in the waterflooding process using indigenous bacteria from the Shengli oil field. Jinfeng et al. (19), in an oil displacement system employing the injection of bacteria, showed a 5.6% increase in oil recovery after 7 days of confinement. In the present work, the multiple injections and confinement periods increased oil recovery, which is consistent