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# Effects of indigenous microbial consortia for enhanced oil recovery in a fragmented calcite rocks system

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ABSTRACT

Two indigenous bacterial consortia, IMP-100 and IMP-200, proved to have a functional effect on heavy crude oil recovery, indicating a potential implementation in Microbial Enhanced Oil Recovery (MEOR). Growth kinetics of the indigenous bacterial population was performed under anaerobic conditions at 70 °C and 33 g $L^{-1}$ of salinity. It was found that both extremophile consortia were able to grow under the latter conditions. Moreover, they synthesized metabolites that altered the surface properties of the supernatants derived from cell cultures, a useful property in oil recovery processes. *Ex situ* fermentations in the presence of crude oil-impregnated calcite rocks demonstrated that both bacterial consortia enhance crude oil recovery by 8.5% and 13%. In order to identify possible phenomena responsible for incremental oil recovery, emulsification index ($E_{24}$), surface tension, cell adhesion to hydrocarbons, and crude oil viscosity were characterized. The results demonstrate that IMP-(100, 200) consortia were able to recover heavy crude oil from calcite rocks, possibly due to a decrease in crude oil viscosity, induced by the presence of metabolites and/or the interaction between bacteria and oil hydrocarbons.



## 1. Introduction

Nowadays, crude oil is still a major energy source around the world. Additionally, it is used as raw material in many chemical processes and also for the manufacture of a wide variety of products at industrial level. The increased demand for crude oil has driven the implementation of enhanced recovery processes in order to maintain the crude oil production. However, the conventional technologies currently used for oil resource exploitation have become less efficient, since a large portion of the reserve oil is not fluid and remains trapped within the rocks. This heavy crude oil is especially important in several countries, including Mexico. Thus, the development of recovery processes of heavy-crude oil is sought in order to increase it by 3% and 8% regarding the original oil volume (México, PEMEX, 2014).

MEOR arises as a biological technology suitable to increase oil production. This technology is based on both microbial activities and the presence of metabolites within a reservoir. A previously employed strategy for MEOR is based on the stimulation of the indigenous microbial population (which is already capable of thriving under extreme conditions) by supplying suitable nutrients (Marshall, 2008; Youssef et al., 2007; Shibulal et al., 2014). Local microbial communities inside the oil wells can alter crude oil properties allowing it to display improved flow features and increased sweep efficiency, mediated by both stabilization of the interfacial tension and decreased viscosity (Lazar et al., 2007).

A bacterial consortium can be particularly effective for microbial oil recovery when compared to a single bacterial species, as it displays a broader range of activities and secretes several metabolic products (Brenner et al., 2008). Some of the products synthesized by these bacteria are potentially useful for oil recovery. These include biogas, bioacids, biosolvents, biomass, biosurfactants and biopolymers (Perfumo et al., 2010; Al-Sulaimani et al., 2011). Therefore, the stimulation of indigenous consortia *in situ* could enhance oil recovery. However, the actual impact of the *in situ* MEOR techniques is difficult to assess because of the lack of quantitative information regarding the microbial processes itself. Taking this into account, we analyzed the feasibility of growing indigenous microorganisms and obtaining consortia *ex situ* by supplying suitable nutrients to support bacterial metabolism. To investigate these aspects, viable bacteria were collected from a Mexican oil reservoir and their growth and metabolite production were evaluated in high salinity and high temperature conditions,

*Abbreviations:* BATH, bacterial adhesion to hydrocarbons; $E_{24}$, emulsification index; *I*, ionic strength; MEOR, Microbial Enhanced Oil Recovery; OD, optical density; SCR, specific consumption rate; TEM, transmission electron microscopy; $Y_{CO_2/x}$, $CO_2$ yield on biomass; $Y_{g\ oil/g\ biomass}$=, crude oil production yields; $\mu$, specific growth rate; $\mu_{ap}$, apparent viscosity

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according to the environmental conditions prevailing in their natural reservoir.

## 2. Materials and methods

### 2.1. Site description and sampling procedure

Samples were obtained from the Chicontepec oil reservoir, geographically located in the central eastern region of Mexico. Geologically it covers a 3875 $km^2$ area and the formation is located within a great depression. It is composed of carbonate rocks with sediments highly cemented by ferrous calcite and dolomite. The temperature of the oil well fluctuates from 70 to 100 °C and contains oil ranging from light to heavy (México, CNH, 2010). Crude oil samples were collected from different production wells. Production water samples were allowed to flow for several minutes before performing the collection. All samples were placed in sterile bottles filled with nitrogen to avoid oxygenation. They were transported and stored in a dark place at 4 °C.

### 2.2. Culture medium and growth conditions

The composition of the basal medium SS1-M was the following (g $L^{-1}$): $K_2HPO_4$ (0.28), $MgCl_2 \cdot 6H_2O$ (2), $CaCl_2$ (0.45), KCl (0.81), $NH_4Cl$ (0.5), peptone casein (4), yeast extract (4), cysteine-HCl (1), NaCl (33), BaCl (0.044), molasses (10) and 1 mL of a trace mineral mixture which composition was (g $L^{-1}$): $FeCl_2$ (0.025), $MnCl_2$ (0.45), $CuCl_2$ (0.005), $AlCl_3$ (0.105) and $ZnCl_2$ (0.005). In some cases oil-impregnated calcite rocks were added to the medium (SS1-MC). The pH of the culture medium was adjusted to 8.0 with $NaHCO_3$ (8.5%). The medium was mixed and dispensed into serum bottles under a stream of $O_2$-free $N_2$ gas. Serum bottles were subsequently autoclaved at 121 °C for 15 min. Prior to inoculation, a 0.2 mL aliquot of a sterile $Na_2S \cdot 9H_2O$ (2%) solution was added to maintain a reduced atmosphere. Cells were cultured in 160 mL serum bottles containing 35 mL of SS1-M medium under anaerobic conditions at 70 °C during 120 h. Each bottle was inoculated with 0.1 g $L^{-1}$ of cells pre-cultured for 12 h.

### 2.3. Microorganisms isolation

Fermentative microbial cultures were obtained after centrifugation in order to separate the oil phase from the brine. Serum bottles containing SS1-M medium were inoculated with oil (2 mL). Bottles were incubated at 70 °C for 10 days. After this time, fresh cultures were prepared by transferring 2 mL of this culture to fresh SS1-M medium. The most active cultures were selected and were named IMP-100 and IMP-200 consortium. Bacteria were cryopreserved at −70 °C in 14% glycerol solution and maintained through monthly subcultures at 4 °C.

### 2.4. Analytical determinations

#### 2.4.1. Biomass, reducing sugars, and bioproducts assessments

Biomass was quantified by the Lowry method (Lowry et al., 1951). Using samples pre-hydrolyzed with β-fructofuranosidase, reducing sugars were assessed by treatment with DNS reagent (Miller, 1959). Carbon dioxide was determined by thermal conductivity gas chromatography (Gow-Mac Instrument Co., NJ, USA), using a CTR-1 column (1.83 m × 0.64 cm) operated under the following conditions: carrier gas, He; flow rate, 65 mL $min^{-1}$; detector temperature, 100 °C; and injector temperature, 45 °C. Organic acids and solvents concentrations were quantified by gas chromatography (HP/Agilent Technologies, model 6890, CA, USA) coupled with a flame ionization detector (FID 356) using an At-Wax capillary column (Alltech). The temperature of both injector and oven was 200 °C and the FID detector was kept at 250 °C. Helium was used as a carrier gas at 4.3 mL $min^{-1}$.

#### 2.4.2. Supernatants surface properties

Emulsification capacity was determined by mixing 2 mL of cell-free supernatant and 3 mL of *n*-hexadecane in glass test tubes. The tubes were shaken vigorously using a vortex for 2 min and afterwards incubated at room temperature. The emulsion stability was investigated after 24 h and the $E_{24}$ was calculated as the percentage of the emulsified layer height divided by the overall liquid column height (Pirollo et al., 2008). The results were compared to SS1-M medium as a control. Surface tension was quantified at different culture time points using a digital tensiometer equipped with a Du Noüy ring (Biolin Scientific, Attention, model Sigma 703D, EPO, FI). The platinum–iridium ring was submerged below the supernatant surface and raised until the ring broke through the surface of the samples. The oil-spreading test was performed using a Petri dish (9 cm diameter) containing 30 mL of distilled water, followed by the placement of light crude oil (20 μL) over the aqueous surface. Afterwards, 10 μL of cell-free supernatants was placed on the oil surface. The diameter of the clear zone on this surface was measured after 1 min, as previously described (Morikawa et al., 2000; Qiao and Shao, 2010).

#### 2.4.3. Bacterial adhesion to hydrocarbons (BATH)

Cell hydrophobicity was measured using the BATH test, using a method similar to that reported by Dorobantu et al. (2004). Cells were washed twice and suspended in phosphate buffer (pH 7, 0.1 M, $I$=0.56 M) to obtain an optical density (OD) value of 0.6, as measured at 600 nm. A mixture consisting of 2 mL cell suspension and 3 mL *n*-hexadecane was shaken for 2 min using a vortex. Both, *n*-hexadecane and aqueous phases were allowed to separate for 30 min. OD at 600 nm was quantified in the former phase. Hydrophobicity is expressed as the percentage of adherence to hexadecane and it was calculated as follows: $100 \times [1-(OD_{600}$ of the aqueous phase$(OD_{600}$ of the initial cell suspension$)^{-1})]$.

#### 2.4.4. Viscosity measurements

Crude oil viscosity was measured in the samples from the oil recovery essay. These determinations were carried out using a cone/plate rheometer (Anton Paar, model MCR501, Ostfildern, GER) equipped with a stainless steel cone (1°, 25 mm) maintained at a constant temperature (25 °C). Measurements were performed employing a 100 $s^{-1}$ shear rate.

#### 2.4.5. Oil recovery test

A calcite core, possessing a porosity value of 15% and a 129 mD permeability, was fractionated. $20 \times 15 \times 7$ $mm^3$ fractions were washed with solvents, vacuum-dried for 24 h at 80 °C and finally sterilized at 121 °C for 2 h. These fractions were saturated with heavy crude oil (11.8 °API, 0.985 g/$cm^3$ of density, 5850 mP s of viscosity measured at 25 °C) at 110 °C for 24 h. Excess of oil was removed and oil saturation was estimated (25%). Eight grams of oil-impregnated rocks were packed into 160 mL serum bottles containing 32 mL SS1-MC medium. Rocks were used only once per each independent experiment. Serum bottles were inoculated with cells (0.1 g $L^{-1}$) pre-cultured for 24 h in SS1-MC medium at the established conditions (see Section 2.2). Fermentations were carried out at 70 °C. Serum bottles used as control contained only SSI-MC medium (without cells). Recovered oil was quantified gravimetrically. Each sample of the recovery test was filtered on Whatman paper to separate both cell-free aqueous and oil phases. They were subsequently dried at 80 °C for 24 h and weighted. For each sample of the kinetics, the weight differences were calculated between the pre-packed oil-impregnated rocks (8 g) and the dry oil-impregnated rocks; consistent results were obtained. All experiments were conducted in triplicate and the results