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Development of a microbial process for the recovery of petroleum oil from depleted reservoirs at 91–96 °C

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HIGHLIGHTS

- Hyperthermophilic, alkalophilic, halophilic bacteria enriched from oil well.
- Bacterial consortium enhanced oil recovery at 96 °C in core flood studies.
- 96 °C is the highest temperature reported for Microbial Enhanced Oil Recovery.
- Microbial metabolites aiding oil recovery at 96 °C were identified.

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ABSTRACT

A consortium of bacteria growing at 91 °C and above (optimally at 96 °C) was developed for the recovery of crude oil from declining/depleted oil reservoirs having temperature of more than 91 °C. PCR-DGGE-Sequencing analysis of 16S rRNA gene fragments of NJS-4 consortium revealed the presence of four strains identified as members of the genus *Clostridium*. The metabolites produced by NJS-4 consortium included volatile fatty acids, organic acids, surfactants, exopolysaccarides and CO_2, which reduced viscosity, emulsified crude oil and increased the pressure that facilitated displacement of emulsified oil towards the surface. NJS-4 enhanced oil recovery by 26.7% and 10.1% in sand pack trials and core flood studies respectively in optimized nutrient medium comprised of sucrose and sodium acetate as carbon/energy source and urea as nitrogen source (pH 7–9, 96 °C, and 4% salinity). Nutrient medium for MEOR was constituted using commercial grade cheap nutrients to improve the economic viability of MEOR process.

1. Introduction

A petroleum reservoir is a subsurface pool of hydrocarbons contained in porous or fractured rock formations. There are three main stages by which oil is recovered from reservoir. The first stage is the primary recovery stage where oil is recovered due to natural energy inherent in the reservoir. Expansion of the dissolved gas following reduced pressure as a consequence of drilling operations is usually responsible for the driving energy that carries oil to the surface. When inherent pressure of reservoir tends to fall, secondary recovery methods are applied. In this method external fluid or gases are injected to maintain reservoir's pressure. Primary and secondary oil recovery processes can account for 30–40% oil productions leaving behind about 55% of oil as residual oil in the reservoirs (Sen, 2008). A third stage of oil recovery called tertiary recovery or enhanced oil recovery involves chemical flooding, thermal recovery and miscible displacement involving carbon dioxide (CO_2), hydrocarbon or nitrogen injection. Enhanced oil recovery methods are not cost effective (Al-Sulaimani et al., 2011). So there is need to develop alternate strategy for oil recovery.

Microbial Enhanced Oil Recovery (MEOR) is one among the most promising novel approaches that can be potentially implemented with an exceptionally low operating cost (Lazar et al., 2007). MEOR processes can be categorized into three main types. (i) In the first type of MEOR process, bacteria are grown *ex situ*. The culture broth, with or without bacterial cells, is injected in the oil wells in a process similar to chemical flooding. The microbial metabolites such as organic acids, biosurfactants and solvents, then remove adhered oil as well as paraffin deposits from well surfaces including tubing, rods, etc. Such type of MEOR process helps overcome clogging related problems which reduce oil production. This process is usually applicable to oil reservoirs rich in paraffin contents where paraffin clogs the well bores decreasing the efficiency of oil recovery. (ii) In the second type of

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MEOR process, a combination of anaerobic bacteria and nutrient media supplemented with cheap substrates (such as molasses) are injected in depleted oil wells. Bacterial fermentation of nutrient media occurs within oil wells producing bacterial metabolites which stimulate displacement of oil entrapped by capillaries and brine. (iii) The third MEOR process is a water flooding process boosted with microbial cultures but not supplemented with nutrient media. Microorganisms used in this type of MEOR process must be capable of using reservoir hydrocarbons as source of carbon and energy and produce desired metabolites. This type of MEOR process is practiced late in the course of a water flood and stimulates microbial activity throughout the reservoir.

MEOR methods were usually limited to oil reservoir having temperature up to 70 °C (Sen, 2008) but there are many oil reservoirs world over having temperature higher than 90 °C. So there is need to develop a microbial process which can be applied to such oil reservoirs characterized by extreme high temperature, pressure and salinity. In this study a unique combination of novel indigenous micro-organisms optimally growing at 96 °C and producing biosurfactant, exopolysaccharide, CO_2, organic acids and solvents was developed from formation water sample. The efficiency of consortium for MEOR was evaluated using simulated sand pack column and core flood assay.

2. Methods

2.1. Enrichment of the hyperthermophilic consortia at temperatures above 90 °C

Crude oil or formation water samples collected from oil wells in Ahmadabad and Mehsana oil fields in Western India were used as inoculum for the enrichment of hyperthermophilic bacteria. Enrichment for hyperthermophilic bacteria was set up in peptone–yeast extract base medium [in g/L: peptone, 10; yeast extract, 10; NaCl 10; Salt Solution SS30 (K_2HPO_4 1 g/L, KH_2PO_4 1 g/L, $NaHCO_3$ 10 g/L, NaCl 2 g/L, $CaCl_2$ anhydrous 0.2 g/L, $MgSO_4{\cdot}7H_2O$ 0.2 g/L), 40 ml] pH 7.0. Enrichments were incubated at 91 or 96 °C in hot air oven for 14–21 days.

2.2. Optimization of nutritional and growth parameters

Carbon and nitrogen source were optimized by using basal medium (K_2HPO_4 7 g/L, KH_2PO_4 3 g/L, yeast extract 10 g/L, NaCl 10 g/L) supplemented with carbon source (1% w/v) and nitrogen source (0.5% w/v). Actively growing culture (ca. 10^6 cells/ml) was inoculated at 1% v/v and incubated at 96 °C for 14 days. Temperature, pH and salinity were optimized using basal medium supplemented with 1% sucrose as carbon source and 0.5% urea as nitrogen source. Growth was evaluated at 80, 91 and 96 °C at pH 5–11 and at salinity 4, 7 and 11 g%.

2.3. Phylogenetic affiliation of microbial species present in NJS-4 consortium

Bacterial species present in NJS-4 consortium were identified by PCR-DGGE-Sequencing of 16S rRNA gene fragments of NJS-4 consortium as described previously by Sachdev et al. (2010) with following modifications. Genomic DNA extraction of NJS4 consortium was done by CTAB method (Zhou et al., 1996). PCR amplification of 16S rRNA gene was carried out using GC341F and 907R primers described by Muyzer et al. (1995). PCR amplicons were resolved by DGGE performed using DCode system (Bio-Rad, USA) over a denaturing gradient of 30–70% at 65 °C and 100 V for 14 h. Separated bands were excised, reamplified, purified and sequenced on an ABI 3100 sequencer as described earlier (Sachdev et al., 2010).

2.4. Sand-pack column assays

Vertically oriented jacketed glass columns (500 ml) were uniformly packed with sterilized dry sand. Temperature was maintained at 96 °C by circulating heated oil through the jacketed walls of the glass column using a circulating oil bath. Sand used to pack the glass column was washed with dilute acid (1×) then with deionized water (3×) and dried in hot air oven at 80 °C for 18 h. The column was then flooded with brine (2% NaCl) followed by oil under pressure till brine was washed out. The sand pack was subsequently flooded with nutrient medium to ensure maximum possible displacement of oil till saturation of residual oil was reached. The column was inoculated with 100 ml inoculums (10^6 cells/ml) in test and sterile distilled water in control column.

2.5. Core flood assay

Characteristics of Barea core used in the Core flood study are described in Table 3. The core fragment was placed inside a stainless steel core holder and placed in the oven at 96 °C. Fluids (brine, oil, and microorganisms plus nutrients) were injected using an injection pump. Core flood studies were performed at 96 °C, 700 psi and 40 g/L NaCl. Core was initially saturated with brine (2 g% NaCl) and then with oil. Brine was again injected to displace removable oil and allow only residual oil in place. Subsequently, nutrient medium with NJS4 consortium having a cell density of 8×10^6 cells/ml was injected. The sealed core was incubated at 96 °C for 14 days. Subsequently oil was recovered by displacement at the same temperature with brine and analyzed.

2.6. Metabolite analysis

Cell free supernatant was used for the metabolite analysis. Gas analysis for carbon dioxide, hydrogen, methane and nitrogen was performed using Gas chromatography (PerkinElmer) equipped with thermal conductivity detector (TCD), Porapak Q column (SS, 1/800 _ 80 mesh) with argon as carrier gas (flow rate: 40 ml/min). Temperature settings for TCD analysis were as follows: oven at 40 °C, injector and detector at 70 °C, injector at 100 °C. Biosurfactant production was analyzed in terms of emulsification index (Satpute et al., 2010). Exopolysaccharide was determined by precipitation by isopropanol (Pawar et al., 2013). Cell surface hydrophobicity of the consortium was determined by MATH measurement as per protocol described by Xiao et al. (2013). Organic acids and solvents were analyzed using high-performance liquid chromatography system (Dionex, USA) using Aminex HPX-87H, Biorad column, equipped with refractive index (RI) detector. The working conditions were: 5 mM H_2SO_4 as a mobile phase with a flow rate of 0.7 ml/min at 40 °C.

3. Results and discussion

Hyperthermophilic microbial consortia were enriched from the formation water samples collected from oil wells (*in situ* temperature −90 °C or more) in western India. Only those oil wells were selected where water injection was not practiced. This assured enrichment of only native flora and ruled out contamination of heterologously introduced microorganisms. Growth of hyperthermophiles was monitored in terms of cell density as well as production of CO_2 and desired metabolites which included biosurfactant, organic acids, solvents, exopolysaccharide and volatile fatty acids. One such consortium designated as NJS4 was selected for further studies as it showed maximum growth and production of desired metabolites (data not shown). PCR-DGGE analysis of 16S rRNA gene fragments performed to determine the composition of

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