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Investigation of a hydrocarbon-degrading strain, *Rhodococcus ruber* Z25, for the potential of microbial enhanced oil recovery

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ABSTRACT

A hydrocarbon-degrading strain, *Rhodococcus ruber* Z25, was isolated from the formation brine in Daqing Oilfield, China. The strain Z25 was able to grow under facultative anaerobic condition and produce biosurfactant while hydrocarbon was used as sole carbon source. The biosurfactant of *R. ruber* Z25 showed a perfect emulsification activity and was able to lower the interfacial tension to approximately 1.0 mN/m and achieved a CMC value of 57 mg/L. The biodegradation experiments of the crude oil by the strain Z25 under aerobic and anaerobic conditions exhibited positive effects in improving the physical properties of the crude oil, including mobility enhancement, cloud point reduction and wax degradation. Waterflooding experiments were done to investigate the MEOR potential of the strain and varied oil recovery efficiencies from 8.88% to 25.78% were achieved. The main MEOR mechanisms of the strain Z25 included hydrocarbon degradation, improvement of oil mobility, wettability alteration and selective plugging. These results revealed that strain Z25 exhibited a tremendous potential for MEOR application.

1. Introduction

Microbial enhanced oil recovery (MEOR) has been recognized as a potentially cost-effective method for enhancing oil recovery. The technology is flexible, relatively inexpensive, and can be applied by independent producers. MEOR formulations can be used in a variety of methods including microbial wax removal, single-well treatment (Bryant and Burchfield, 1989), permeability modification treatment (Gullapalli et al., 1998), and microbial enhanced water flooding process (Bryant and Douglas, 1988). Hydrocarbon degradation is the main mechanism for microbial wax removal (Barker et al., 2003; Becker, 2001). Selected inocula and nutrients were injected together to degrade the paraffin and other hydrocarbons that have accumulated on the production equipment, within the well, or within the reservoir (Etoumi, 2007; Ford et al., 2000). The microbial single-well treatment (also called well stimulation) may be localized to the well-bore region or occur several meters to ten or more meters in the reservoir. The objective of well stimulation technologies is to stimulate the production of large amounts of acids, gases, solvents, biosurfactants and/or emulsifiers in the near well region of the reservoir to improve oil rates. In addition to removing scale, wax, asphaltenes, and other debris, well stimulations may change wettability and flow patterns to allow more oil to flow to the well (Grula et al., 1985). Microbial enhanced water flooding differs from the above methods in that the nutrients with or without inocula are injected into the injectors in order to stimulate microbial activity in a large portion of the reservoir and the oil is recovered in producers (Hitzman, 1983; Lazar, 1991). The goal of microbial enhanced water flooding is to increase the ultimate oil recovery of the whole oil reservoir. This is done by improving the microscopic displacement efficiency through a reduction in the capillary forces that entrapped oil or by improving the volumetric sweep efficiency of the recovery fluid by blocking water channels and high permeability zones to push bypassed oil to production wells.

The *in situ* stimulation of hydrocarbon degrading bacteria by injection of oxygen and inorganic nutrients has long been studied to recover additional oil (Andreevskii, 1961; Nazina et al., 2007b). The method has been widely used in Russia and China that very strong evidence links microbial activity with oil recovery (Belyaev et al., 2004; Ivanov et al., 1993; Nazina et al., 2007a). In this approach, the stimulation of aerobic hydrocarbon metabolism in the vicinity of the injection well results in the production of acetate, other organic acids, alcohols and biosurfactants. High concentrations of aerobic hydrocarbon degraders are also detected in fluids close to the injection well that are able to produce biosurfactant while growing on hydrocarbon.

A hydrocarbon-degrading strain, *Rhodococcus ruber* Z25, was isolated from the formation brine in Daqing Oilfield, China (Zheng et al., 2009). The strain exhibits tremendous potential for MEOR application, including hydrocarbon mechanism, facultative anaerobic

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respiration and biosurfactant producing ability. Therefore, the mechanisms for MEOR of the strain Z25 were investigated and the treatment strategies were also discussed in the present work.

2. Materials and methods

2.1. Chemicals, media and growth conditions

Luria-Bertani (LB) medium was used for preparing the inoculum of the strain. The early log phase microbial culture broth of the bacteria prepared in LB medium was transferred to mineral salts medium (MS medium) at the concentration of 2.0% as the inoculum. The compositions of LB medium, MS medium, the trace elements solution and the vitamin solution were listed in Table 1 (Bao et al., 2009). Liquid paraffin (or crude oil) was supplied as carbon source and added at a concentration of 5.0% (v/v). In anaerobic cultivation, 8.0 g/L of NH_4NO_3 was added to the MS medium instead of 5.4 g/L NH_4Cl to provide the nitrate as alternative electron acceptor for facultative anaerobic respiration.

Anaerobic cultivation was conducted as follows (Hao et al., 2008): the water for media preparation was boiled for 20 min in order to dispel all the dissolved oxygen prior to use. L-Cysteine and resazurin as oxygen indicators were added to the medium to final concentrations of 500 mg/L and 100 mg/L, respectively. Pure nitrogen gas was poured into the anaerobic culture bottles until the oxygen indicators in the medium became achromatic. The anaerobic culture bottles were then sealed with rubber caps, sterilized at 121 °C for 20 min before use. The medium was then inoculated via injection. Aerobic and anaerobic cultivations were carried out at 37 °C on a rotary shaker at 150 rpm for 7 days.

The formation brine and crude oil were collected from the oil production station of Unit ZX-201 in Daqing Oilfield, China, and were employed for bacterial isolation, oil biodegradation and water flooding experiment. The physical and chemical parameters of MEOR potential reservoir in Daqing Oilfield were listed in Table 2. All the other chemicals in the study were of analytical grade.

2.2. Microorganism

Several hydrocarbon-degrading strains were isolated from the production brine of Daqing Oilfield. The strain Z25 was able to degrade hydrocarbon under facultative anaerobic condition and selected for further study. The strain Z25 was identified as *R. ruber* by 16S rDNA sequencing.

2.3. Biomass and biosurfactant production

Members of the genus *Rhodococcus* are known to produce surface-active trehalose-lipids associated with cell growth (Philp et al., 2002). In the present work, the biosurfactant production of *R. ruber* Z25 was determined via emulsification activity test (data not shown). Liquid paraffin was supplied as carbon source for biomass and biosurfactant production and the culture was centrifuged at 12,000 rpm and 4 °C for 30 min. The biomass was collected and washed twice with methyl *tert*-butyl ether (MTBE) to remove residual carbon source and biosurfactant. Then the biomass was dried in an oven at 110 °C to constant weight and the biomass was calculated.

The hydrophobic layer located at the surface of the culture was extracted using methyl *tert*-butyl ether (MTBE) method (Kuyukina et al., 2001). The solvent layer was separated from the aqueous phase and combined with the cell-washing MTBE for biosurfactant isolation. The solvent was removed by rotary evaporation at 50 °C under reduced pressure. The extract was then thoroughly washed thrice with petroleum ether to remove residual carbon source to obtain crude biosurfactant. The crude biosurfactant was then freeze-dried and stored under nitrogen.

Table 2
The physical and chemical parameters of MEOR potential reservoir in Daqing Oilfield.

Reservoir parameters		Formation brine parameters		Crude oil parameters	
Depth, m	800–1000	pH	8.0–8.5	Density, g/cm^3	0.86–0.89
Pressure, MPa	8.3–11.3	Salinity, g/L	6300–7000	Viscosity (*in situ*), mPa s	9.3–15.5
Temperature, °C	35–45	Water type	$NaHCO_3$	Natural gas/crude oil ratio	38–50
Pay thickness, m	45.1–73.4	Oxygen, mg/L	<0.3		

2.4. SFT/IFT and contact angle measurements

Surface tension (SFT) and interfacial tension (IFT) were measured by ring Du Nuoy method using a Kruss K100 Tensionmeter (Hamberg, Germany) at room temperature. As crude biosurfactant was sparingly soluble in water, its SFT and IFT measurements were performed immediately after emulsification by ultrasonic treatment in the formation brine (20 kHz, 1 min). Interfacial tension was determined against *n*-hexadecane. Critical micelle concentration (CMC) was calculated as the lowest concentration at which the SFT and IFT values were minimal.

The contact angle was measured using a Thermo Cahn Automated dynamic contact angle (DCA) analyzer (Cahn, USA) (Serro et al., 1997). The analyzer was operated by holding a quartz plate in a fixed vertical position, attaching it to a microbalance and moving a probe liquid (the formation brine) at constant rate up and down past the plate (Fig. 1). A unique contact angle hysteresis curve was produced by the microbalance as it measured the force exerted by the moving contact angle in advancing and receding directions. The dynamic contact angle was then calculated from the modified Young's Equation (Wilhelmy Equation). The DCA measurements were conducted in duplicate.

Table 1
The compositions of media in the present study.

Luria-Bertani (LB) medium		Mineral salts (MS) medium		Trace elements solution		Vitamin solution	
Peptone, g/L	10.0	KH_2PO_4, g/L	1.0	H_3BO_3, mg/L	280.0	Bioton, mg/L	2.0
Yeast extract, g/L	5.0	K_2HPO_4, g/L	1.0	$ZnSO_4 \cdot H_2O$, mg/L	178.0	Thiamine HCl, mg/L	5.0
NaCl, g/L	10.0	NH_4Cl, g/L	5.4	$MnCl_2 \cdot 4H_2O$, mg/L	8.0	Thioctic acid, mg/L	5.0
pH	7.0–7.2	$MgSO_4 \cdot 7H_2O$, g/L	0.	$CuSO_4 \cdot 5H_2O$, mg/L	8.0	Nicotinic acid, mg/L	5.0
		$CaCl_2 \cdot 2H_2O$, g/L	0.01	$NiCl_2 \cdot H_2O$, mg/L	80.0	Riboflavin, mg/L	5.0
		$FeSO_4$, g/L	0.01	$Na_2MoO_4 \cdot H_2O$, mg/L	50.0	Pyripoxin HCl, mg/L	10.0
		Trace elements solution, ml/L	1.0			D-Calcium Pantothenate, mg/L	5.0
		Vitamin solution, ml/L	1.0			Folic acid, mg/L	2.0
		pH	7.5			Cyanocobalamin, mg/L	0.1
						P-aminobenzic acid, mg/L	5.0