# Laboratory Research on Air-assisting Microbial Enhanced Oil Recovery

Cheng Mingming*[1], Gao Jianbo[2], Lei Guanglun[3], Geng Xiaoheng[1], Zhang Zaiwang[1], Ma Xiin[3]

*1. Binzhou University, Binzhou, Shandong, 256600, China*

*2. Safety evaluation center of Binzhou, Shandong, 256600, China*

*3. China University of Petroleum (East China), Qingdao, Shandong 266580, China*

*E-mail: Rachel19cheng@163.com*

**Abstract** The air-assisting microbial enhanced oil recovery is a new technology of tertiary recovery, which microbe can grow and metabolize better by help of oxygen. Experiments of microbe growing and oxygen consuming as well as oil-removing were performed under the different dissolved oxygen. Experiments showed that the time of microbial proliferation arriving the period of logarithmic phase was reduced in the oxygenated environment relative to the oxygen-deprived environments. The content of light hydrocarbons of biodegraded oil increased more than 5% in the oxygenated environment. What is more, the microbial de-oiling efficiency was obtained more than 80% after having added dissolved oxygen. Based on laboratory data, it is believed that the air-assisting microbial enhanced oil recovery is a promising new front of oil-displacement.

**Keywords**: Air-assisting; Microbial Enhanced Oil Recovery; Laboratory Experiment; Dissolved oxygen; Oxygen consuming; De-oiling efficiency

## 1. Introduction

Microbial enhanced oil recovery is one of the tertiary recovery methods, which using the bacteria and metabolites as oil- displacing agent [1−3]. Throughout the past several generations, MEOR has become highly developed [4-6]. However, an examination of the literature shows that MEOR generally does not recover as much remaining oil in place as other chemical enhanced oil recovery (EOR) processes [7-8]. A more likely explanation is that microbial growth and reproduction is subjected to the reservoir environment such as high temperature and lack of oxygen. Much more speculative is the possibility of fingering of bacterial suspension along the dominant flow channel because of low viscosity [9-10]. Air-assisting microbial enhanced oil recovery is a promising branch oil displacement technology, which could provide enough oxygen to maintain microbial growth and reproduction [11-12]. Meanwhile, because of the Jamin effect, the bubble produced by the injected gas is very conducive to form gas-resistance in high permeability strata to enhance the swept volume and conformance efficiency of microbial driving [13-15]. In this respect, it has important implications for the improvement of EOR technique to study on the air-assisting microbial enhanced oil recovery [16-18].

## 2 Materials and Methods

### 2.1 Experimental Cultures

A group of petroleum-degrading bacteria were obtained from the sludge samples that collected from the Triassic reservoir rock in 2009. Three bacteria were isolated by the plate methods and enrichment cultured at formation temperature 44.4℃. In addition, the isolated bacteria were inoculated to agar slant culture-medium, stored at -4℃ until use[19]. The identification result of the tested bacteria strains′16S rDNA was shown in table 1.

**Table 1 Blast result of the tested bacteria strains′16S rDNA**

| Tested bacteria Strain | | Reference strain | | Homology/% | Identification result |
|---|---|---|---|---|---|
| Strain name | Accession No. | Genus species | Accession No. | | |
| Cq 3-1 | KJ782614 | Pseudomonas veronii CIP 104663 | CIP 104663 | 99.93 | Pseudomonas |
| Cq 3-2 | KJ782615 | Enterobacter xiangfangenis 10-17 | 10-17 | 99.78 | Enterobacter xiangfangenis |
| Cq 3-3 | KJ782616 | Bacillus licheniformis ATCC 14580 | ATCC 14580 | 98.86 | Bacillus licheniformis |

The oil used in the experiment was obtained from an Oil production of China Petrochemical Corporation. The artificial brine used in the experiment was prepared according to the salinity of the oilfield brine, the physical property of which is shown in Table 2.

**Table 2 Physical property of oil and water**

| Physical property of oil | | | | Physical property of water | | |
|---|---|---|---|---|---|---|
| Viscosity(*mPa.S*) | | | freezing point(℃) | PH value | Salinity(*ppm*) | water type |
| Viscosity | underground | viscosity of gas-free | | | | |
| 1.91 | | 14.15 | 20.77 | 6.08 | 112920 | $CaCl_2$ |

## 2.2 Determination of microbial growth and oxygen consumption curve

Most of the oil displacement strains are facultative anaerobes in order to adapt the reservoir environment, that is, the microorganisms can survive in anoxic condition and rapidly multiply in oxygen-rich condition. The microbial growth and oxygen consumption curve was determined by incubation experiments of bacteria of different dissolved oxygen concentration.

The initial bacterial suspension was created by first put the artificial brine in a culture flask, then add the culture and stir until the culture have dissolved. Then the air was led continuous into the culture flask using accurate monitoring of detector until the dissolved oxygen concentration reaches a required level. After this 3% of bacterial cell was pour in the flask and incubated at 44.4℃. The bacterial counts and dissolved oxygen concentration were respectively measured by plate count and LDOTM at a particular point. The flowchart of bacterial culture experiments was shown in Fig.1.

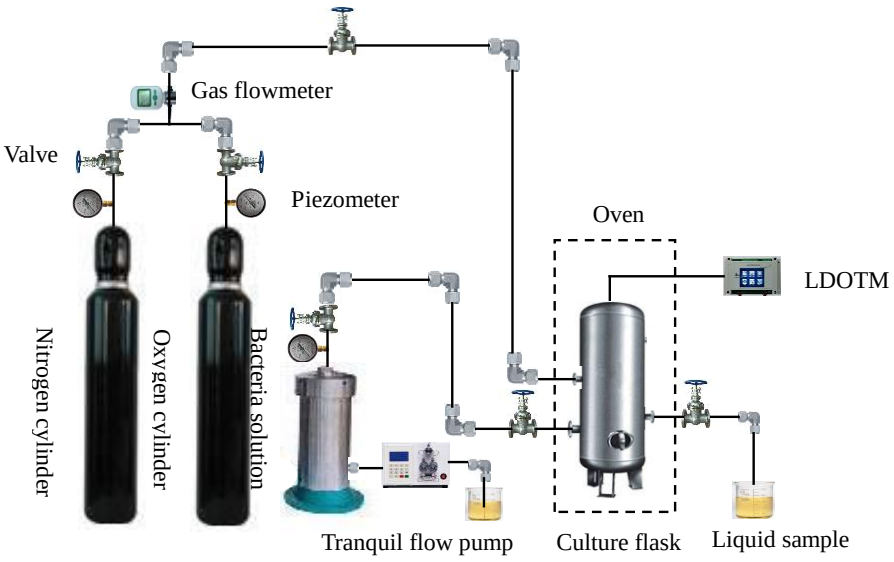



Fig.1 Flowchart of bacterial culture experiments

## 2.3 Gas chromatography of biodegraded oil of different dissolved oxygen concentration

Microorganisms utilize the hydrocarbon of the residual oil as a carbon source to maintain their reproduction [20-23]. Gas chromatography was conducted to measure the compositional change of biodegraded oil. The bacteria suspension was prepared by mixing appropriate concentration of bacteria and substrate as well as artificial brine with stirring. Then, the mixture was poured in erlenmeyer flasks, after this the air was led continuous into the erlenmeyer flask to create different dissolved oxygen conditions. Use another flask containing the same volume of the artificial brine as control. A certain amount of oil was added to the flasks, and then the erlenmeyer flasks were incubated at stratum temperature of 44.4℃ for 6days. To isolate and determine the constituents of oil using GC technique, quantitatively analyze their content by area normalization method [24-25].

$$P_i = \frac{f_i A_i}{\sum_{i=1}^{n} f_i A_i} \times 100\% \quad (1)$$

where $P_i$ is the relative contents of the components, $f_i$is the relative quality product factor of the components, $A_i$

is the peak area of the components.

The flowchart of gas chromatography of biodegraded oil was shown in Fig.2.

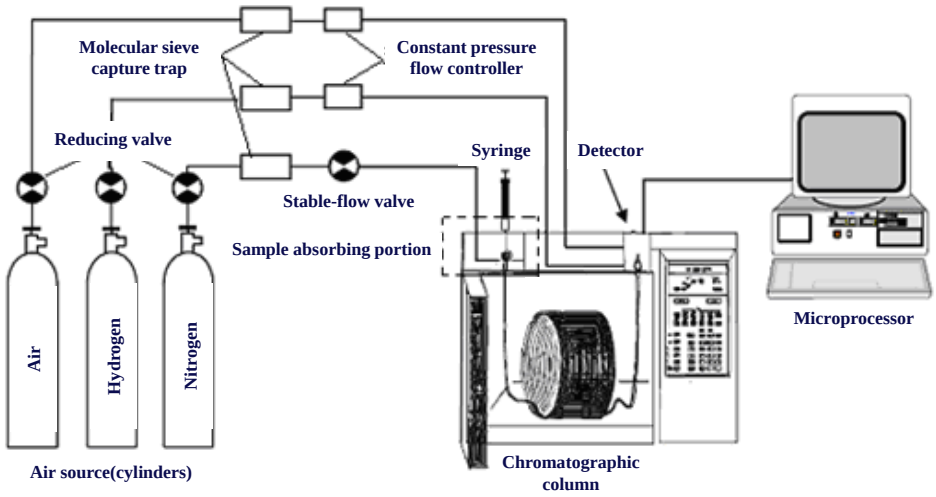



Fig.2 Flowchart of gas chromatography of biodegraded oil

### 2.4 Microbial de-oiling efficiency of different dissolved oxygen concentration

The formation sand was disposed by sifting, washing and drying, after this, the sands can be mixed with quantitive oil and then stirred thoroughly and let it sit on the element for 3days before using. The oil sands were put in water-absorbing and oil discharging equipment, in which, a certain amount of bacteria solution were poured in. Use another flask containing the same volume of the artificial brine as control. Then the equipment was cultivated in stratum temperature of 44.4℃ for 6days. The amount of expulsion oil was recorded every 12 hours.

## 3 Results and analysis

### 3.1 Relationship of microbial growth and oxygen consumption

The bacteria reproduction can be divided into 4 stages, that is, lag phase, ogarithmic growth phase, stationary phase and declining phase. The measured data was consistent with the typical microbial growth curve (Fig.3). When the dissolved oxygen concentration is 3.0mg/L, the biggest concentration of bacteria growth is $26\times10^8$ cells/mL; When the dissolved oxygen concentration is 5.5mg/L, the biggest concentration of bacteria growth is $33\times10^8$ cells/mL, indicating that the increase of dissolved oxygen had been exerting great impact on microbial multiplication.

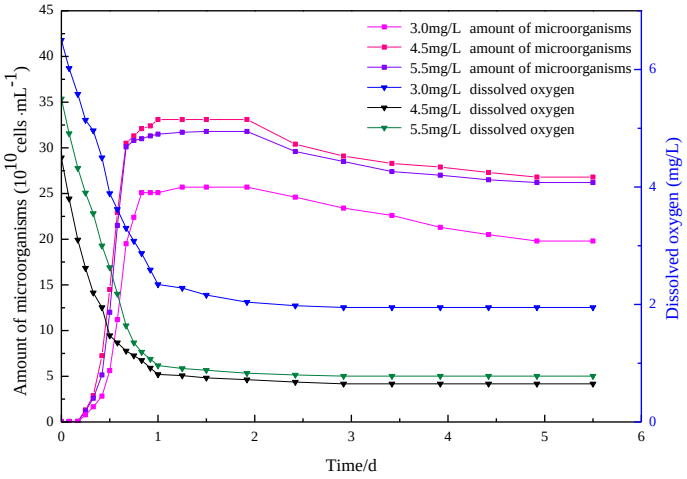



Fig.3 Microbial growth and oxygen consumption curve

### 3.2 Components change of biodegraded oil of different dissolved oxygen concentration

The components change of biodegraded oil of different dissolved oxygen concentration was shown in Fig.4and Fig.5.

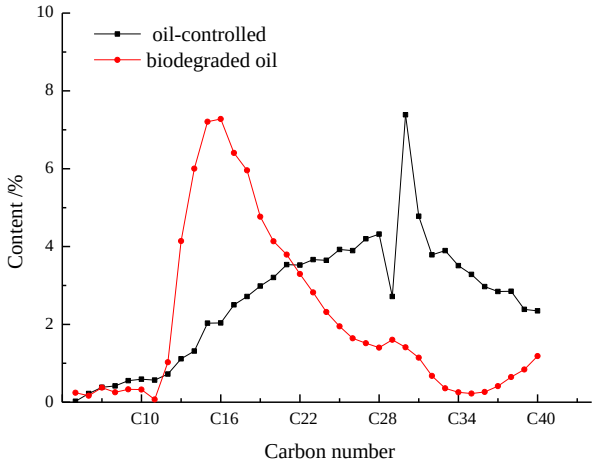



Fig.4 Carbon distribution curve of biodegraded oil with oxygen concentration of 0.5 mg/L

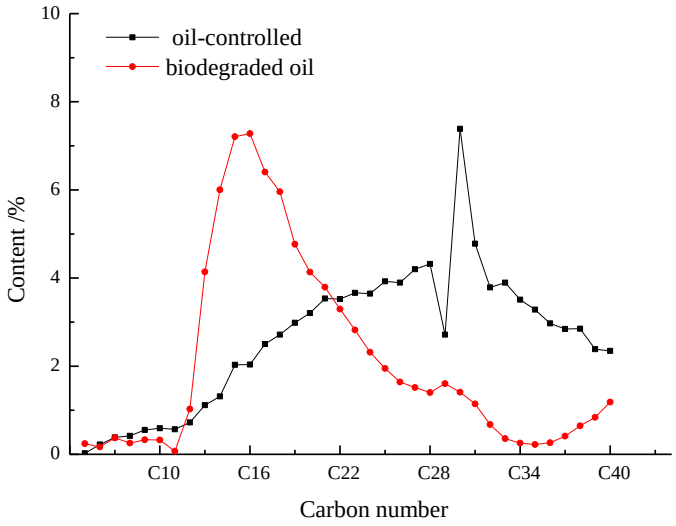
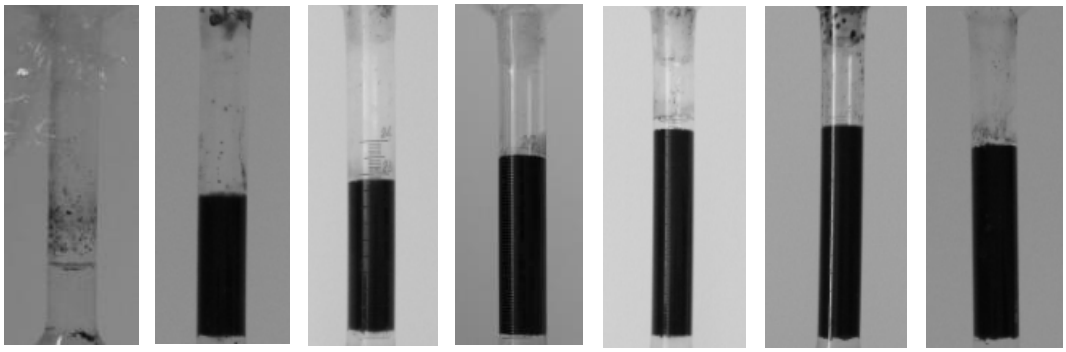



Fig.5 Carbon distribution curve of biodegraded oil with oxygen concentration of 5.5 mg/L

As illustrated in Fig.4and Fig.5, the microbial degrading oil efficiency positively correlates with dissolved oxygen condition. 5.18 percent of C15 of the oil that incubated with 5.5 mg/L dissolved oxygen increases, compared to a 1.71 percent increase that incubated with 0.5 mg/L dissolved oxygen. On the one hand, the C30 content of oil incubated with 5.5 mg/L dissolved oxygen reduce 5.98 percent relative to 4.77 percent of oil incubated with 0.5 mg/L. A more likely explanation is that aerobic and facultative bacteria utilize residual oil as a carbon source in an oxygenated environment. Oxygen atoms are introduced in the long-chain alkane by alkane oxygenases by means of terminal oxidation and penultimate oxidation. Then the long-chain alkane was oxidized to corresponding alcohol, aldehyde and fatty acids, which is very conducive to the decreases of oil viscosity.

### 3.3 Change of microbial de-oiling efficiency with different dissolved oxygen concentration

The photos of microbial de-oiling after incubating for 6 days were shown in Fig.4, the change of microbial de-oiling efficiency with different dissolved oxygen concentration was shown in Fig.6.



Artificial brine 0.5 mg/L 1.5 mg/L 3.0 mg/L 4.5 mg/L 5.5 mg/L 6.5 mg/L

Fig.6 Microbial de-oiling rendering after incubating for 6 days

As illustrated in Fig.6, there is hardly any oil released from the flask of artificial brine relative to bacterium solution, the amount of released oil is positive correlated basically with the dissolved oxygen concentration.

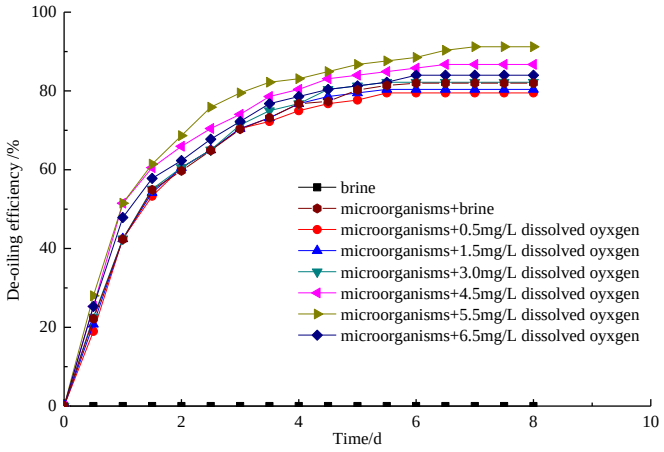



Fig.7 Change of microbial de-oiling efficiency with time under different dissolved oxygen conditions

The artificial brine is inefficiency in de-oiling at a percentage of only 2 percent of oil been released after 8 days. The oil sand prepared using artificial brine and microorganisms were found to have a fast speed of release oil in the initial stage, the upward trend slowed down on day 6, after which the efficiency reaches a maximum of 79%. From these data it could be concluded that microorganisms exhibited good abilities of changing crude properties, and as such, the oil adsorbs on the surface of sand was released to the liquid surface (Fig. 7).

The microbial de-oiling efficiency of different dissolved oxygen conditions has similar variation trend with the artificial brine and microorganisms. The difference was that the more the concentration of oxygen was, the higher the efficiency was. When the concentration was 6.5 mg/L, oxygen excessive amount adverse instead, because of the SOD theory proposed firstly by .M.McCord and I.Fridovich.

## 4. Conclusions

On the basis of the results, we came to following conclusions: the increase of dissolved oxygen is conducive to microbial reproduction to some extent. The maximum concentration of microorganisms in oxygenated environment is about 3 times as much as microorganisms in hypoxia environment. The mass reproductive microbe makes the oil degradation more efficient in oxygenated environment relative to hypoxia environment. Furthermore, the degradation rate of C15 in 5.5 mg/L dissolved oxygen environment was 1.21 percent higher than that in 0.5 mg/L dissolved oxygen environment. The de-oiling ability of microorganisms is far above that of artificial brine. Of these, it is noteworthy that, the microbial de-oiling efficiency in oxygenated environment was 3~12 percent higher than that in hypoxia environment, indicating that oxygen exhibited huge synergistic effect of de-oiling. These data support the hypothesis that air-assisting microbial enhanced oil recovery will be considered as a kind of new drive technology under good development.

## Acknowledgments

This study was supported by the natural science foundation of Shandong province (2016ZRB01A57)

## References


[1] Cheng M, Lei G, Gao J, et al. Laboratory experiment, production performance prediction model, and field application of multi-slug microbial enhanced oil recovery [J]. Energy & Fuels, 2014, 28(10): 6655-6665.

[2] Du S, Hu J. Evolution of Microstructure and Properties of AZ31 Magnesium Alloy during High Temperature Annealing[J]. Journal of Materials and Applications 2015,4(1) 27-31

[3] Wang S, Mulligan C N. Occurrence of arsenic contamination in Canada: sources, behavior and distribution[J]. Science of the Total Environment, 2006, 366(2): 701-721.

[4] Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications[J]. Biological psychiatry, 2008, 64(6): 527-532.

[5] Simpson D R, Natraj N R, McInerney M J, et al. Biosurfactant-producing Bacillus are present in produced brines from Oklahoma oil reservoirs with a wide range of salinities[J]. Applied microbiology and biotechnology, 2011, 91(4): 1083-1093.

[6] Bødtker G, Lysnes K, Torsvik T, et al. Microbial analysis of backflowed injection water from a nitrate-treated North Sea oil reservoir[J]. Journal of industrial microbiology & biotechnology, 2009, 36(3): 439-450.

[7] Banat I M, Franzetti A, Gandolfi I, et al. Microbial biosurfactants production, applications and future potential[J]. Applied Microbiology and Biotechnology, 2010, 87(2): 427-444.

[8] Nazina T N, Shestakova N M, Grigor'yan A A, et al. Phylogenetic diversity and activity of anaerobic microorganisms of high-temperature horizons of the Dagang oil field (PR China)[J]. Microbiology, 2006, 75(1): 55-65.

[9] Simpson D R, Natraj N R, McInerney M J, et al. Biosurfactant-producing Bacillus are present in produced brines from Oklahoma oil reservoirs with a wide range of salinities[J]. Applied microbiology and biotechnology, 2011, 91(4): 1083-1093.

[10] Sanjay M, Simanta B, Kulwant S. Paraffin problems in crude oil production and transportation: a review[J]. SPE Production & Facilities, 1995, 10(01): 50-54.

[11] Javaheri M, Jenneman G E, McInerney M J, et al. Anaerobic production of a biosurfactant by Bacillus licheniformis JF-2[J]. Applied and Environmental Microbiology, 1985, 50(3): 698-700.

[12] Gray M, Yeung A, Foght J, et al. Potential microbial enhanced oil recovery processes: a critical analysis[C]//SPE Annual Technical Conference and Exhibition. Society of Petroleum Engineers, 2008.

[13] Pathak K V, Keharia H. Application of extracellular lipopeptide biosurfactant produced by endophytic Bacillus subtilis K1 isolated from aerial roots of banyan (Ficus benghalensis) in microbially enhanced oil recovery (MEOR)[J]. 3 Biotech, 2014, 4(1): 41-48.

[14] Chatzis I, Morrow N R, Lim H T. Magnitude and detailed structure of residual oil saturation[J]. Society of Petroleum Engineers Journal, 1983, 23(02): 311-326.

[15] Pathak K V, Keharia H, Gupta K, et al. Lipopeptides from the banyan endophyte, Bacillus subtilis K1: mass spectrometric characterization of a library of fengycins[J]. Journal of the American Society for Mass Spectrometry, 2012, 23(10): 1716-1728.

[16] Tyson G W, Chapman J, Hugenholtz P, et al. Community structure and metabolism through reconstruction of microbial genomes from the environment[J]. Nature, 2004, 428(6978): 37-43.

[17] Frank D N, Amand A L S, Feldman R A, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases[J]. Proceedings of the National Academy of Sciences, 2007, 104(34): 13780-13785.

[18] Okpokwasili G C, Nweke C O. Microbial growth and substrate utilization kinetics [J]. 2006, 5, 453−456.

[19] Mingming C, Tian X, Guanglun, L, et al. Development index prediction of muti-slug microbial flooding in low permeability reservoir[J]. Journal of China University of Petroleum2014, 38(6): 113-120 (in Chinese)

[20] Bryant S L, Lockhart T P. Reservoir engineering analysis of microbial enhanced oil recovery[C]//SPE Annual Technical Conference and Exhibition. Society of Petroleum Engineers, 2000.

[21] Santisi S, Cappello S, Catalfamo M, et al. Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium[J]. Brazilian Journal of Microbiology, 2015, 46(2): 377-387.

[22] Deppe U, Richnow H H, Michaelis W, et al. Degradation of crude oil by an arctic microbial consortium[J]. Extremophiles, 2005, 9(6): 461-470.

[23] Wang F H L. Effects of reservoir anaerobic, reducing conditions on surfactant retention in chemical flooding[J]. SPE reservoir engineering, 1993, 8(02): 108-116.

[24] Mingtao Z，Shoude Z，Xudong H, et al. Experimental Study on Composition and Stability of Vegetation-growing Concrete[J]. Journal of Materials and Applications, 2015,4(1):1-6

[25] Kowalewski E, Ruesl åtten I, Steen K H, et al. Microbial improved oil recovery—bacterial induced wettability and interfacial tension effects on oil production[J]. Journal of Petroleum science and Engineering, 2006, 52(1): 275-286.