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# A pilot study on large-scale microbial enhanced oil recovery (MEOR) in Baolige Oilfield

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ABSTRACT

Industrial application of microbial enhanced oil recovery (MEOR) has been hindered by a lack of large-scale data-based guidelines for process design and operation. In the present work MEOR was investigated in both laboratory and large-scale oilfield studies. Six microbial strains were initially isolated from Baolige Oilfield in China. Laboratory based investigation showed that all six strains were able to decrease the oil viscosity. Two mixtures of strains exhibited greater reduction effects, i.e., 35% and 56%, respectively. The optimal nutrient concentration was found to be 1.0%. The mixtures of strains tested in laboratory core flooding based MEOR also confirmed their greater MEOR performance, i.e., MEOR levels of 9.1% and 13.2%, respectively, compared to that of any single strain ranging from 7.0% % to 8.7%. Using the strain mixture that had been selected under the laboratory based conditions, the pilot field study achieved a significant MEOR: 210,000 tons of crude oil produced over 43 months from 169 production wells. The research results obtained in this work including both laboratory and field studies can be potentially applied in other oilfields with similar geological and physical conditions, for large-scale MEOR process design and operation.

## 1. Introduction

Water flooding is a commonly used method for secondary oil recovery. Water is injected into the oil field to physically displace and sweep the oil to the production wells (Taware et al., 2017). However, there remain challenges in using water flooding techniques. In particular, variable permeability, uncontrollability of fluidic conditions, and undesirable interface properties contribute to inefficient recovery (Taware et al., 2017). As a result, conventional, water-flood oil recovery operation often leaves one half to two-thirds of the oil in the reservoir within the complex capillary network (Brown, 2010; Gao and Zekri, 2011; Siegert et al., 2014; Song et al., 2015). Therefore, there has been tremendous effort in developing alternative and more efficient strategies to improve oil recovery. Among the alternative technologies in development, microbial enhanced oil recovery (MEOR) is regarded as an economic and environmentally friendly tertiary oil recovery, which has attracted much attention in recent years (Gao and Zekri, 2011; Lazar et al., 2007; Le et al., 2015; Nazar et al., 2011; Safdel et al., 2017; Sen, 2008). However, in spite of its potential benefits, MEOR is currently still not widely applied to industrial applications, that is largely attributed to a lack of sufficient field-test data, especially from large-scale tests to support design for industrial processes (Safdel et al., 2017), and regarded as a significant road blocker.

In principle, MEOR uses microorganisms (either indigenous or exogenous) and their metabolites to enhance oil recovery. It is generally understood that the MEOR process is facilitated either by metabolites or by biosurfactant production. Metabolites such as extracellular polymeric substance (EPS) selectively block high-permeability zones, leading to selective plugging and diverting the water into lower permeability zones. Biosurfactants, produced *in situ* reduce oil viscosity and interfacial tension between oil-water-rock interfaces. This reduced interfacial tension increases residual oil mobilisation (Armstrong et al., 2015; Kryachko et al., 2013; Le et al., 2015; Sen, 2008). It has been demonstrated that microbes and nutrients injected into a reservoir can stimulate the *in situ* production of MEOR agents such as biosurfactants, biopolymers, acid and gas (Dhanarajan et al., 2017; Joy et al., 2017; Saxena, 2015; Whitby and Skovhus, 2010).

At present, the majority of studies on MEOR have been conducted in laboratory-scale. Pilot studies in oilfields have mostly involved a small number of wells (ranging from two to dozens) (Amani, 2015; Armstrong et al., 2015; Bao et al., 2013; Fulazzaky et al., 2015; Huang et al., 2014; Kryachko and Voordouw, 2014; Sen, 2008; Youssef et al., 2013). Additionally, theoretical analysis and numerical modelling have been used to assist design and optimize MEOR operations (Liu et al., 2014; Nielsen

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et al., 2014; Sivasankar and Kumar, 2014; Spirov et al., 2014). However, it is challenging to use laboratory-scale models to closely simulate actual oilfield geophysicochemicals conditions. The dramatic difference in geometry scale and system complexity, that significantly limit the relevance of predictions based on laboratory microcosm studies (Brown, 2010). This unpredictability can become a huge risk factor for industrial operation failure, normally associated with significant cost. To date, there have been very few attempts to conduct large-scale applications in oilfields for MEOR.

Therefore, the aim of the present work was to address this research gap by conducting a large-scale, long term and systematic field test as a pilot to verify the feasibility of MEOR for industrial application, and ultimately provide data-based guidelines for further industrial design and operations.

Prior to the field test, six facultative anaerobes were isolated from the local production fluid. The performance of individual isolates and mixed cultures were first examined in laboratory. Parameters investigated included: growth rate, gas production rate and interfacial properties. The MEOR effect was then investigated with a laboratory-scale core flooding model.

The filed test was carried out in four fault-blocks (namely, B19, B38, B48 and B51) in Baolige Oilfield for this pilot study. The surface area of the four fault-block is approximately 20.8 $km^2$ with original oil in place of $35 \times 10^6$ tons. This investigation included results from the oilfield's 78 injection wells and 169 oil production wells.

## 2. Materials and methods

### 2.1. Materials

All chemicals and reagents were obtained commercially and used as received. The biochemical reagent kits were purchased from Beijing Leadman Biochemical Limited, China. Glucose, peptone, yeast extract, urea, ammonium sulphate, potassium dihydrogen phosphate, magnesium sulphate and sodium chloride were of analytical grade and purchased from Tianjin Tian Da Chemical Factory, China. Other reagents used in this study were also of analytical grade, and the water was deionized.

Instrument included multi-function core flow experimental device (LDY-III, Nantong Yi Chuang Experimental Instruments Co., Ltd.), Spectrophotometer (UV-2550, Shimadzu), Haake viscosimeter (RS-300, Thermo Scientific), Spinning Drop Interface Tensiometer (TX-500C, Kenuo of the United States), and Surface Tensiometer (A801S, Kenuo of the United States).

The strains used in this study were isolated from the production fluid of Baolige Oilfield. They were *Bacillus subtilis*, *Arthrobacter*, G. *subterraneus*, *Pseudomonas aeruginosa*, *Bacillus licheniformis* and *Rhodococcus* sp., and were named HB3, IV, H, Z-2, LC and JH, respectively.

### 2.2. Methods

#### 2.2.1. Isolation and characterization of bacterial strains

To obtain the high efficiency strains for MEOR, experiments were performed to enrich and isolate high efficient strains from the produced fluids of oil wells in Baolige oilfield. Because the average temperature of the target reservoir was 50 °C, all the experiments were carried out at this temperature.

The brine (20 mL) and crude oil (10 g) were transferred to a 500 mL conical flask containing 120 mL medium consisted of (wt.%): glucose 2.0%, peptone 0.05%, yeast extract 0.05%, urea 0.05%, ammonium sulphate 0.05%, potassium dihydrogen phosphate 0.5%, magnesium sulphate 0.02%, and sodium chloride 0.01%, and incubated at 50 °C, 180 rpm for 5 days. The choice of the concentration range was based on the previous feasibility investigation (data not shown). To isolate strains capable of biosurfactant production, the samples with good emulsifying effect on crude oil were selected for the further isolation. Taking 0.1 mL culture broth spread on LB agar plates and incubated at 50 °C for 48 h. The pure colonies were obtained by repetitive streaking on solid LB agar medium.

A loop of biomass was scraped off the agar plate, suspended in 20 mL distilled water to lyse by boiling for 10 min and freezing for 5 min. The supernatant was used as the template for PCR after centrifugation. Phylogenetic analysis based on 16S rRNA gene sequences were performed according to Wu et al. (2013).

#### 2.2.2. Batch growth conditions

Batch growth experiments were performed, following the published procedure (Xia et al., 2012), using 100 mL LB medium at different temperatures (20–60 °C) at 200 rpm. After 48 h incubation, the optical density at 600 nm ($OD_{600}$) was measured using the spectrophotometer. The results are averages of three independent experiments.

#### 2.2.3. Determination of surface tension and interfacial tension

The surface tension of the supernatant fluid was measured by the Wilhelmy plate method with a surface tensiometer DST-100 (SEO, Korea) at 25 °C. The interfacial tension of crude oil/water systems were determined using a Spinning Drop Video Tensiometer SVT20 (Dataphysics Instrument Gmbh, Germany). During measurement, the supernatant of the fermentation broth was filled in the capillary, then a drop of crude oil was added to the supernatant, and the interfacial tension was measured when the temperature was raised to 50 °C (Sakthivel et al., 2015). Each result was the average of three determinations.

#### 2.2.4. Emulsifying activity

The emulsifying activity of fermentation broth was determined at 25 °C, as follows: the supernatant liquid was mixed with equal volumes of crude oil for 2 min, and then it was settled at room temperature for 24 h. The emulsification index ($E_{24}$) was calculated as the ratio of the height of the emulsion layer to the total height of the mixture (Dastgheib et al., 2008).

#### 2.2.5. The determination of bacterial density

The samples of produced fluids from oil wells were diluted serially to desired concentrations. The bacterial density were then counted by the flat colony counting method (Baron et al., 2006). Each dilution was plated in triplicate on a nutrient agar plate and incubated at 37 °C for 24 h. The number of CFU at each dilution rate was counted after incubation and the average CFU/ml was determined.

#### 2.2.6. Core flooding tests

Prior to field applications, core flooding tests were conducted in laboratories, following a commonly used procedure (Gao et al., 2013).

**(i) Core tube filling and water saturation**. A 50 cm long core tube (inner diameter, 2.5 cm) was used. It was filled with silica sand (80, 150 and 200 mesh, mixed as desired) by mechanical loading. After sand loading the core tube was under vacuum conditions for 6 h in order to remove air. Saline solution was then pumped into the core tube. The total volume of saline solution pumped into the core tube and the pressure difference between the inlet and outlet of the core tube were recorded.

Based on the measurement, the porosity $\Phi$ and permeability $K$ of the core tube were calculated.

$$\Phi = \frac{PV}{V_T} \times 100\% \quad (1)$$

$$K = Q\frac{\mu_w L}{\Delta P A} \quad (2)$$

where $PV$ is pore volume and $V_T$ is the total volume of the core tube. Q is the displacement rate (mL/min), and $L$ and $A$ are the length and

cross-sectional area of core tube, respectively. $\mu_w$ is the fluid viscosity (mPa·s), and $\Delta p$ (atm) is the pressure difference between the inlet and outlet of the core tube.

**(ii) Oil saturation and water flooding.** Following water saturation was oil saturation with crude oil (a mixture of oil samples from the four blocks), and then aged at 50 °C for a week. The total amount of injected crude oil was recorded. The brine was then injected to conduct the secondary recovery stage (i.e., water flooding stage) until reaching a given water-cut level of 98%.

**(iii) Microbial flooding.** This was carried out by injecting a mixture of microbes (8 $\log_{10}$ cfu/mL) and nutrient (1.0%) in water into the core tube. The total volume of injection was equivalent to 1.0 PV. After injection the core tube was closed for 4 days. Then, the microbial flooding was started that was driven by water injected via the tube inlet. The produced fluid from the outlet of tube was collected for further analysis. Microbial flooding was completed when no detectable oil was produced from the outlet of the core tube. The experiments were carried out at 50 °C (the average temperature in Baolige Oilfield), with a displacement pressure ranging from 3 MPa to 5 MPa. The quantity of crude oil by microbial flooding was recorded. All tests were performed three times. The enhanced oil recovery by microbial flooding (MEOR%) was then calculated.

$$MEOR(\%) = \frac{m_{MEOR}}{m_{Tot}} \times 100\% \quad (3)$$

where $m_{Tot}$ is the total mass of crude oil injected and $m_{MEOR}$ is the amount of crude oil output by microbial flooding.

#### 2.2.7. Microbial injection and performance monitoring in field tests

Based on preliminary studies, the composition of the nutrient medium used in the field test was modified, consisting of (wt.%): glucose 0.4%, peptone 0.1%, yeast extract 0.08%, ammonium chloride 0.2%, and potassium dihydrogen phosphate 0.1%. The injected bacteria solution was prepared by diluting the fermentation liquid, which contained 8 $\log_{10}$ cfu/mL after 8 h fermentation, with 1 (wt.)% nutrient solution to reach a target concentration.

Fig. 1 shows a schematic diagram of the process for both MEOR and water flooding operations. In order to recycle the used microorganisms, the microbial-contained fluid following oil-water separation was re-injected into the reservoir.

The MEOR operation started in May 2012 when the mixture of exogenous bacteria and nutrient solution was injected through the 78 injection wells over a period of 40–60 days at a total volumetric flow rate of 2275 $m^3$/d. The concentrations of injected microbial solutions and nutrition liquids were controlled at 6.5 $\log_{10}$ cfu/mL and 1.0%, respectively. This was followed by a typical water flooding process that was monitored on a monthly basis for all the four blocks for the change of bacterial density. When bacterial density decreased to a given level (5 $\log_{10}$ cfu/mL), the second cycle of MEOR process (i.e., bacteria/nutrition injection followed by water flooding) was repeated.

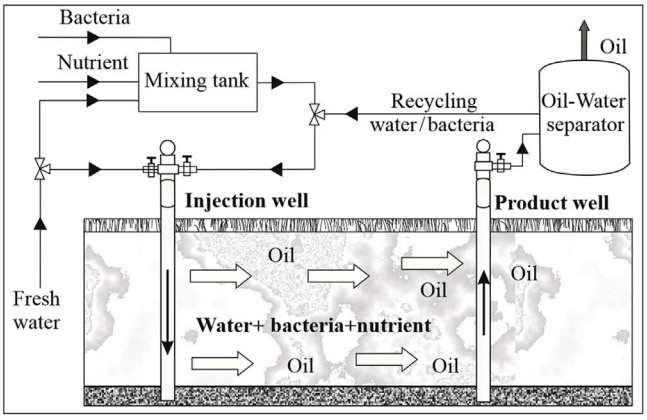


**Fig. 1.** Schematic diagram of the injection-production-recycling process.

## 3. Results and discussion

### 3.1. Characterisation of the activities of exogenous bacteria

To evaluate the performance and activities of the six strains, their growth was measured under both oxic and anoxic conditions in a temperature range of 20–60 °C. The results are presented in Fig. 2. All six strains grew well under both oxic and anoxic conditions where the overall anoxic growth rate was relatively lower which was about a half of the oxic growth rate at all temperature levels. This suggested that the six strains isolated were facultative anaerobes. Strain LC was found to be the most active at almost all the temperature levels.

Under both oxic and anoxic conditions, the growth rate as a function of temperature showed a similar trend where an optimal temperature around 40–50 °C was observed. It was interesting to note that this temperature was approximate to the overall average temperature of the blocks investigated.

To investigate the effect of the strains on crude oil properties, the interfacial tension and viscosity of crude oil from Baolige Oilfield were measured and compared before and after treatment by different strains or strain mixtures for 48 h. Fig. 3 shows the reduction in both interfacial tension and viscosity for six separate strains and also two mixtures. The two mixtures were prepared by mixing selected individual strains in media at same density with equal volumes. The selection of the strains to mix was based on their individual performance and similarity (data not shown). Fermentation liquids of these six strains significantly reduced the interfacial tension between oil and water (> 70%). The mixed strains resulted in higher reduction in interfacial tension than each individual strain with a reduction of 87.5% by Mixed strain 1 (IV:H:Z-2:HB3 = 1:1:1:1, v/v), and 90.8% by Mixed strain 2 (LC:JH = 1:1, v/v), respectively.

All of six strains and their mixtures reduced the viscosity ranging from 25% to 44%. Both Mixed strains 1 & 2 showed higher reduction than individual strains, with reductions of 38% and 56%, respectively (Fig. 3). This may be due to that the combination of metabolites produced by mixed strains were more effective than that by individual strain in lowering the surface tension and increasing the extent of degradation as suggested previously (Al-Hattali et al., 2013; Al-Sayegh et al., 2017; Darvishi et al., 2011; Dhanarajan et al., 2017; Jha et al., 2016; Nakamura et al., 2007; Satpute et al., 2010; Varjani and Upasani, 2017). To fully understand the mechanism, further studies are required. Nevertheless, this reduction in both interfacial tension and viscosity can be beneficial for improving fluidity of the crude oil, and also stripping oil from rock/sand surface where the oil film is attached (Wang et al., 2015).

### 3.2. Optimisation of nutrient concentration

To examine the effect of nutrient concentration on bacterial growth and their metabolism, bacterial density, gas production, surface tension of fermentation liquid and the emulsifying activity were measured after 48 h fermentation at 50 °C with nutrient concentrations ranging from 0.1% to 1.0% (wt.%).

As shown in Fig. 4, the bacterial density increased approximately linearly extending from 7.9 to 8.9 $\log_{10}$ cfu/mL when the nutrient concentration changed from 0.1% to 1.0%. The gas production increased significantly as well with increase in nutrient concentration, largely attributed to the rise of bacterial density. On the other hand, the surface tension of the fermentation liquid decreased from 54.5 to 35.2 mN/m when increasing nutrient concentration, that was also reflected by an increase in emulsification index $E_{24}$. These results clearly demonstrated the significant effect of nutrient concentration on the properties of oil-water system.

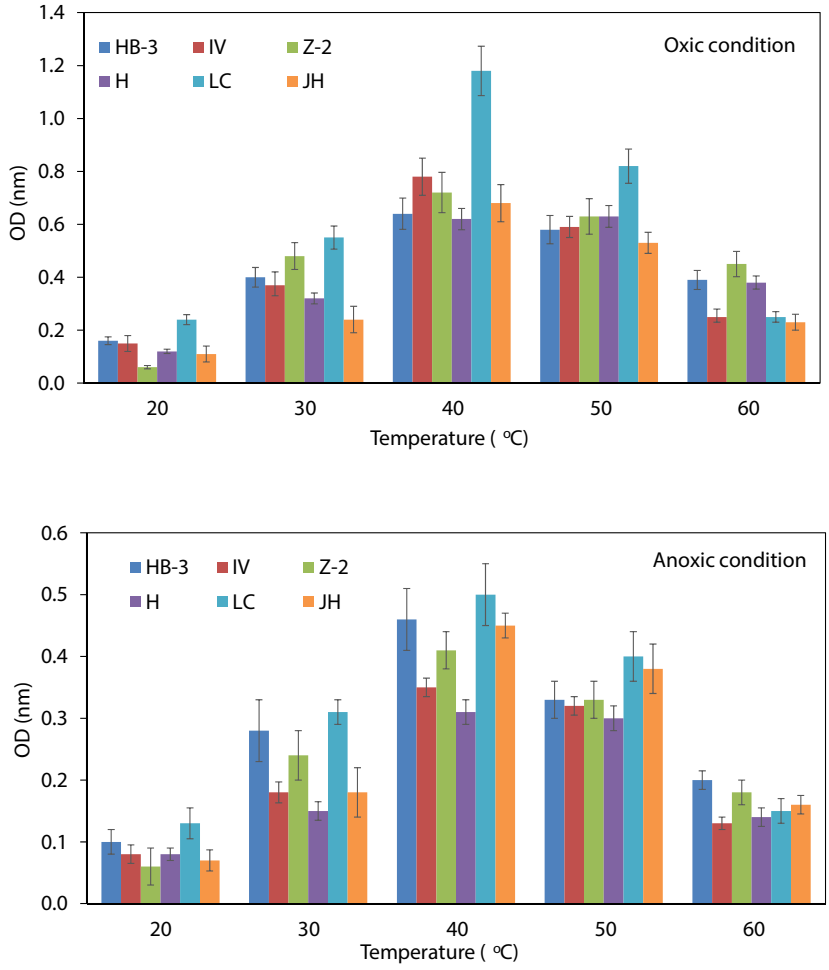


**Fig. 2.** The effect of temperature on the growth of six strains under oxic and anoxic conditions.

In view of the trend of the property changes as a function of nutrient concentration, significant variations were observed when nutrient concentration increased from 0.1 to 0.5%. Above 0.5%, all variations continued to extend in the same direction, however, the increase rate in $E_{24}$ and gas production rate tended to reach a plateau stage when nutrient concentration rose to 1.0%. This suggested the nutrient concentration level of 1.0% be appropriate for application. Compared with the commonly-used nutrient concentration range (typically 2.0%–4.0%) in other field tests (Patel et al., 2015), the concentration used in this study (i.e., 1.0%) was significantly lower which can help reduce the cost.

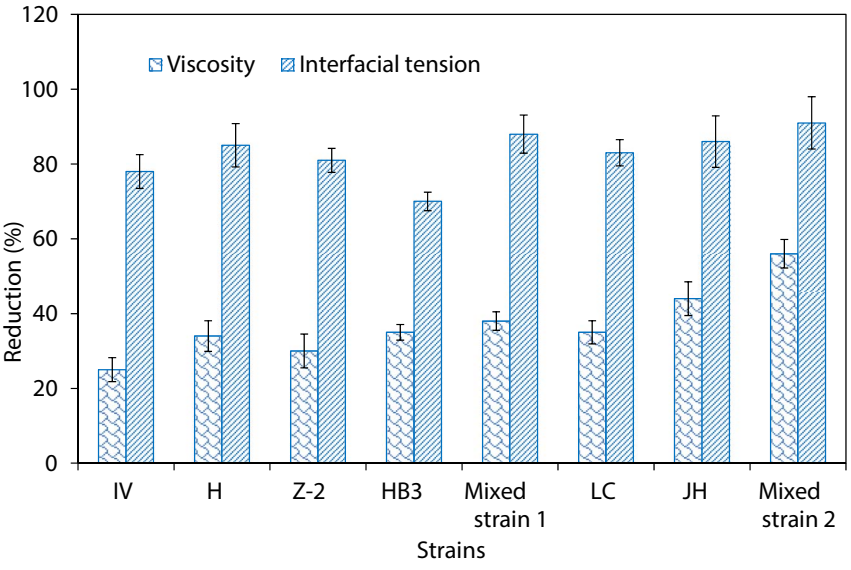


**Fig. 3.** Reduction of interfacial tension and viscosity by different strains (Mixed strain 1, IV:H:Z-2:HB3 = 1:1:1:1 v/v, Mixed strain 2, LC:JH = 1:1 v/v).

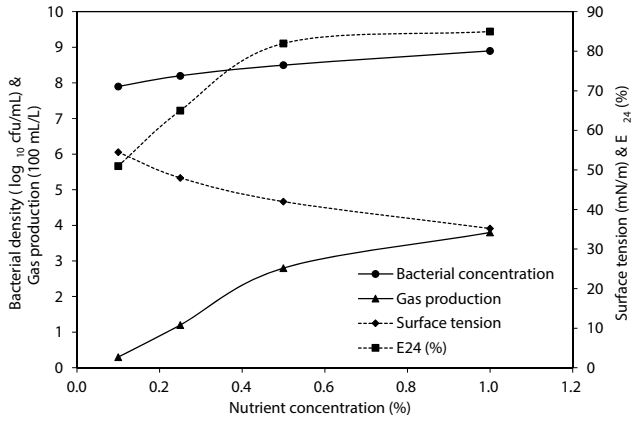


**Fig. 4.** Effects of nutrient concentration on bacterial density, gas production, surface tension and emulsifying activity. Mixed strain 2 (LC:JH = 1:1, v/v) was cultured at 50 °C for 48 h.

### 3.3. Core flooding based MEOR

The effect of the six separate strains and two mixtures on MEOR was investigated by using a laboratory-scale core flooding model, as summarised in Table 1. The properties of core columns used are also presented, in terms of porosity and permeability. The data shown are the average values of three repeats.

All six individual strains and two mixtures improved oil recovery, where the synergistic effect of mixed strains exhibited more significant effects. The two mixtures of strains showed higher MEOR levels of 9.1% and 13.2%, respectively, compared to that of any single strain ranging from 7.0% % to 8.7%.

It can be seen that the eight core columns used in the experiments had similar properties in terms of porosity (26.1%) and permeability $(226.4) \times 10^{-3}\,\mu m^2$. The insignificant variation in these properties suggested that the microbial activity was the key factor for MEOR (Kruger et al., 2016).

The results are in line with the previous observations with similar for MEOR (Aburuwaida et al., 1991; Al-Sayegh et al., 2017; Dhanarajan et al., 2017; Gao et al., 2017; Satpute et al., 2010; Zhao et al., 2017). In addition to producing different types of biosurfactants, the bacteria *Bacillus subtilis* HB3, *Pseudomonas aeruginosa* Z-2, *Bacillus licheniformis* LC and *rhodococcus* sp. JH were also able to degrade long chain hydrocarbons, thus reducing the viscosity of crude oil (Al-Sayegh et al., 2017; Gao et al., 2017; Liu et al., 2012; Zheng et al., 2012). In addition, *Arthrobacter* IV and *Bacillus licheniformis* LC can metabolize organic acids and produce biopolymer, respectively (Dhanarajan et al., 2017; Satpute et al., 2010). As these strains have different properties and can produce different metabolites, their effect on MEOR efficiency can vary. The results of core flooding experiments showed that the oil displacement efficiency of mixed bacteria was higher than that of single ones where Mixed strain 2 showed the highest.

**Table 1**
Effects on MEOR by different strains or strain mixtures.

| Core number | Strains | Porosity (%) | Permeability ($10^{-3}\,\mu m^2$) | MEOR (%) |
|---|---|---|---|---|
| 1 | IV | 26.65 | 224.8 | 7.0 |
| 2 | H | 27.00 | 231.5 | 8.5 |
| 3 | Z-2 | 25.38 | 215.1 | 8.7 |
| 4 | HB3 | 26.13 | 209.8 | 6.6 |
| 5 | Mixed strain 1 | 26.45 | 228.2 | 9.1 |
| 6 | LC | 24.91 | 215.4 | 8.3 |
| 7 | JH | 26.75 | 235.5 | 7.6 |
| 8 | Mixed strain 2 | 25.88 | 250.8 | 13.2 |

The data are the average of three repeated experiments.

### 3.4. Pilot study in the oilfield

After more than 10 years of operation in Baolige Oilfield (Inner Mongolia Autonomous Region, China), the production of crude oil has fallen sharply, and the water cut is rising. It is difficult to improve the recovery efficiency by conventional means. However, this field has unique characteristics suitable for MEOR based on water flooding. Firstly, it is a typical heavy oil reservoir with an average viscosity of 157 mPa s for crude oil. It is significantly higher than that of water, making conventional water flooding ineffective (Mai and Kantzas, 2009). Secondly, the formation has an average porosity and permeability of 18% and $144 \times 10^{-3}\,\mu m^2$, respectively. They are in the middle-high range of permeability, allowing introduction of bacterial cells (Bryant and Douglas, 1988). Thirdly, the average of formation temperature is 50 °C. That is favourable for the growth of the related bacteria. Finally, after decades of exploitation of the field, its water-cut has been increasing significantly in recent years exceeding 75%. That has resulted in a very low efficiency of conventional water flooding recovery.

The pilot study on MEOR was started on 1st May 2012. The oil production, water cut and bacterial density were monitored on a daily/monthly basis. The profiles of these variables are depicted in Fig. 5 over a period of 43 months for all the 169 oil wells studied. At the end of 2015, four cycles of microbial injection of Mixed strain 2 were carried out (indicated by the arrows in Fig. 5). With the first microbial injection for 60 days, the oil production increased noticeably from 820 t/d to 920 t/d over 95 days. The oil production remained at that high level with little fluctuation for about three months, which was followed by a gradual decline. When the bacterial density in produced water dropped close to 5 $\log_{10}$ cfu/mL, the second cycle of microbial injection was conducted. This pattern was repeated for four times.

The original profile with water flooding showed a clear decline trend (lower panel, Fig. 5). That indicated the oil production could have dropped to 750 t/d by the end of 2016 according to the natural decline curve (dashed line, Fig. 5). However, the production maintained at approximately 900 t/d with MEOR, though the four cycles of bacterial injections caused oscillations.

The profile of bacterial density showed a similar increasing trend while an additional lower plateau was observed in the first cycle operation, i.e. 5 $\log_{10}$ cfu/mL compared to the average of 6 $\log_{10}$ cfu/mL after the first cycle. The existence of the initial lower plateau was likely due to two reasons. Firstly, the bacterial was injected via the injection well while the bacterial density was measured using the fluid sample from the production well with a typical distance of 300 m, resulting in delays for detection. Secondly, the bacteria were inevitably adsorbed by the formation during the process of migration in reservoir until an apparent saturation was reached. This explanation can be further supported by the water cut profile which showed an opposite trend compared to the oil production profile, showing no initial lower plateau. In addition, water cut with MEOR (65.8%) was found to be significantly lower than that (78.5%) with conventional water flooding.

During the period of MOER application in Baolige Oilfield, to evaluate the effect of MEOR, no other recovery enhancing methods was employed at the same time. As indicated by the production decline curve (the oil production at different time was predicted according to the production decline law of oilfield) (Wachtmeister et al., 2017) (Fig. 5). Therefore, a significant increase in production was achieved that was $2.1 \times 10^5$ t of crude oil cumulatively over a period of 43 months. This may be attributed to the creation of a stable microbial field in the reservoir demonstrating significant MEOR effects. Compared with other MEOR applications reported in the literature (Behlulgil et al., 1992; Cheng et al., 2014; Li et al., 2002, 2015; Safdel et al., 2017; Sengupta, 2009; Le et al., 2015), the production increase in this pilot study was noticeably higher than that of more complex operation. For example, according to incomplete statistics (between 1998 and 2012), 10 MEOR projects (involving 45 injection wells and 144

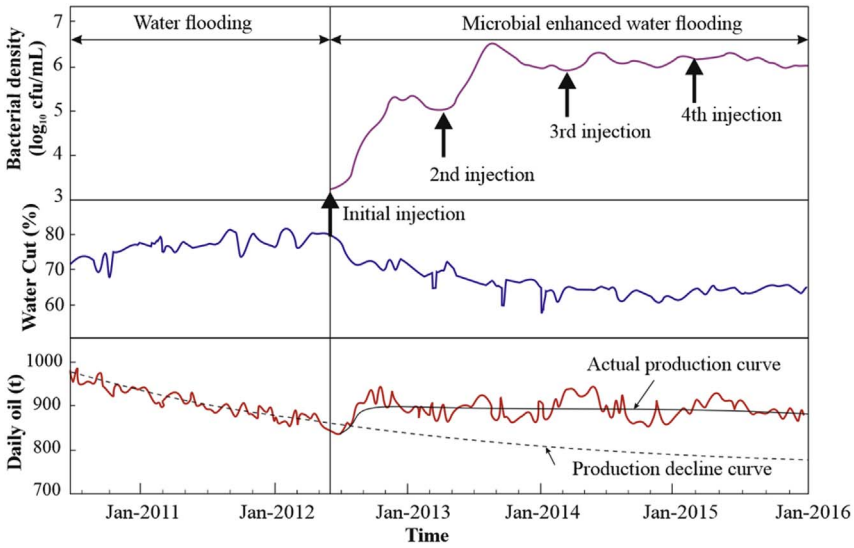


**Fig. 5.** Daily based oil production, water cut and bacterial density during MEOR, showing average values of 169 oil production wells.

production wells in total) in Daqing Oilfield had a cumulative incremental oil production of $5.7 \times 10^4$ t.

While the overall effect the MEOR pilot operation was significant, there were still some wells (about 15% of the wells investigated) which did not show notable increase in oil production. That may be attributed to a number of reasons. Firstly, some wells (about 6% of the wells investigated) were within formations which had poor homogeneity. If this type of formation contained large channels that could lead to rapid flow of injected bacterial towards production wells through short-cuts. This can be reflected by the reduction in the retention time in the reservoir, as observed in these wells during operation. To improve the sweep volume of bacteria in these formation, it is necessary to consider techniques for flow profile control, e.g., by selective plugging of these highly permeable zones (Nemati et al., 2005). Secondly, some wells were within formations which had poor connectivity in the underground network. This can affect the flow field of bacterial solution even trap bacteria in the network, resulting in lower bacterial densities detected in the production fluid. It was observed in this study that about 9% of the wells investigated showed significantly lower cell concentrations (i.e., $< 5$ $\log_{10}$ cfu/mL) in the production fluid. Thirdly, it was interesting to observe the increase in bacterial density of the production fluid in some wells (about 17% among the wells investigated) compared to the injected bacterial density. This was highly likely due to the existence of endogenous bacteria in the formation which were activated and driven out. Further studies are needed in order to identify the strains and understand their potential effect on the selected bacteria, e.g., in terms of growth and reproduction activities.

## 4. Conclusions

Laboratory based investigation showed that all six strains were able to reduce the oil viscosity, Two mixtures of strains exhibited higher reduction effects, i.e., 35% and 56%, respectively. The optimal nutrient concentration was found to be 1.0%. The mixtures of strains tested in laboratory core flooding based MEOR also confirmed their higher MEOR performance, i.e., MEOR levels of 9.1% and 13.2%, respectively, compared to that of any single strain ranging from 7.0% % to 8.7%.The pilot field study achieved a significant MEOR, that was 210,000 tons of crude oil cumulatively produced over 43 months in 169 production wells using the selected strain mixture under the laboratory based optimal conditions. The research results obtained in this work including both laboratory and field studies can be potentially applied in other oilfields with similar geological and physical conditions, for large-scale MEOR process design and operation. To further improve the technique, insights into the MEOR mechanism is needed especially about the interaction of exogenous with indigenous bacteria. Also, the importance of formation heterogeneity needs to be further addressed as it may result in microbial fluids short-cutting through high-permeability zones. In addition, the potential loss of microbial activity or stability over long-term operation requires further biological studies.

## Conflicts of interest

We declare that we have no conflict of interest.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2017.12.009.

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