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Biodegradation of crude oil by *Chelatococcus daeguensis* HB-4 and its potential for microbial enhanced oil recovery (MEOR) in heavy oil reservoirs

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ABSTRACT

Biodegradation of crude heavy oil was investigated with *Chelatococcus daeguensis* HB-4 that was isolated from the produced fluid of Baolige Oilfield in China. Batch growth characterization and crude oil degradation tests confirmed HB-4 to be facultative anaerobic and able to degrade heavy oil. The oil degradation was found to occur through degrading long hydrocarbons chains to shorter ones, resulting in oil viscosity reduction. By mixing crude oil with glucose, or using sole crude oil as carbon source, the content of light fractions (C_8-C_{22}) increased by 4.97% while heavy fractions (C_{23}-C_{37}) decreased by 7.98%. It was also found that bioemulsifiers were produced rather than commonly observed biosurfactants in the fermentation process, which was attributed to the extracellular degradation of hydrocarbons. Core flooding tests demonstrated 20.5% oil recovery by microbial enhancement, and 59.8% viscosity reduction, showing potential of strain HB-4 for application in the oil industry, especially in enhanced heavy oil recovery.

1. Introduction

Heavy oil (typically with viscosity > 100 mPa·s and density > 0.92) reservoirs are widely distributed across the world and the total reserves are estimated to be around 1000×10^8 t (Wang et al., 2012). Taking China as an example, the proven heavy oil reserves are claimed to be 20.6×10^8 t, of which about 7.0×10^8 t remains unexplored (Zhao et al., 2013). In recent years, due to the declining of conventional light oil reservoirs, the exploitation of heavy oil reservoirs has attracted increasing attention (Al-Sayegh et al., 2015).

The characteristic high viscosity and density of heavy oil are associated with poor fluidity, that makes it challenging to exploit and recover (Mai and Kantzas, 2010; Nazina et al., 2017a,b; Sun et al., 2017). At present, the commonly used technologies for heavy oil recovery include steam soak (Ning et al., 2007; Zheng et al., 2011), steam huff and puff (Li et al., 2011), in-situ combustion (Ahmadi et al., 2014; Bagci, 2006) and chemical viscosity-reduction (Li et al., 2017; Tian et al., 2017). These technologies mostly aim to improve the crude oil fluidity through the reduction of its viscosity in order to improve oil recovery. However, they have limitations in different aspects, such as high cost, complicated operation and limited scope of applicability (Wang et al., 2012).

Microbial enhanced oil recovery (MEOR) operates, in principle, through the introduction of biological activities and metabolic products of microorganism into oil reservoirs. It has been increasingly applied in oilfields mainly owing to its wide compatibility, simple process, low cost and environmental friendliness, compared to conventional technologies (Ke et al., 2018a; Ke et al., 2018b; Patel et al., 2015; Safdel et al., 2017). In MEOR, microbial degradation of crude oil is believed to be one of the two key contributors, along with the production of metabolites such as biosurfactant, fatty acids and biogas. Therefore, it is crucially important to understand and, ultimately, employ suitable microorganism in specific reservoirs for MEOR, normally through screening and selecting bacteria with capability of high degradation (Gao et al., 2017; Souayeh et al., 2014; Shreve et al., 1995; Varjani 2017; Zhao et al., 2016).

Recently, there have been a range of oil-degrading bacteria being studied, either aerobic or anaerobic (Al-Sayegh et al., 2015; Kowalewski et al., 2006; Lan et al., 2015; Zheng et al., 2012). However, their MEOR performance has been limited (Xia et al. 2016). This may be attributed to the selection of unsuitable oil degrading strains, non-optimal operational conditions, or a combination of both. Therefore, much effort has been placed in understanding the mechanism, and selecting highly efficient oil degrading bacteria for MEOR (Gao et al.,

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2017; Cai et al., 2013). *Chelatococcus daeguensis* has been regarded as aerobic, when initially isolated from textile dye wastewater and biofilm of an on-site biotrickling filter for nitrate removal (Yoon et al., 2008; Liang et al., 2012). *Chelatococcus daeguensis* TAD1 has been studied, particularly, on its denitrifying performance under aerobic and thermophilic conditions (Li et al., 2016; Wei et al., 2017; Yang et al., 2017). However, there has been limited information in the literature about such strains associated with oilfield applications under facultative or anaerobic conditions.

In our previous studies, we isolated from Baolige Oilfield in China strains of *Luteimonas huabeiensis* sp. nov. HB-2 with capabilities for biosurfactant production, *Bacillus subtilis* HB-3 being able to reduce the oil viscosity (Ke et al., 2018a,b), and HB-4 from stratum water in Huabei Oilfield (Wu et al., 2013b). In the present study, we further isolated from the produced fluid within Baolige Oilfield and confirmed the strain as *Chelatococcus daeguensis*, capable of degrading hydrocarbons especially heavy oils. The performance of HB-4 was then systematically characterized, in terms of growth rate with different carbon sources, degradation of heavy oil, reduction in oil viscosity, and chemical composition. The effect of HB-4 on MEOR of heavy oil was then quantified with a core-flooding test.

2. Experimental

2.1. Materials

All chemicals and reagents used in this study were of analytical grade (Purity > 98%) and obtained commercially. The biochemical reagents were purchased from Beijing Leadman Biochemical Limited, China. Chemicals, including glucose, sucrose, molasses, starch, peptone, yeast extract, urea, ammonium sulphate, potassium dihydrogen phosphate, magnesium sulphate and sodium chloride (AR), were supplied by Tianjin Tian Da Chemical Factory, China. The samples of crude oil (saturates 43.2%, aromatic13.3%, Colloid 39.7%, asphaltene 3.8%) and brine were collected from Baolige Oilfield in Inner Mongolia Autonomous Region, China. The depth of the reservoir is approximately 1100–1150 m with a temperature of 38 °C and oil viscosity of 293.8 mPa·s (after dehydration). The samples were stored in 500 mL plastic bottles and immediately transported to the laboratory at 4 °C for further research.

Instrument and apparatus used in this study included a multifunction core displacement 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), Surface Tensiometer (A801S, Kenuo of the United States), and Gas Chromatography with FID and TCD detector (7890B, Agilent).

2.2. Methods

2.2.1. Bacterial isolation and screening

10.0 g of each collected sample was weighed into 500 mL flasks containing 100 mL enrichment medium (glucose, 2 g; NaCl, 1.0 g; water, 100 mL; cruid oil, 2 g; autoclaved at 121 °C for 30 min before use). After 48 h enrichment culture at 38 °C and 120 rpm , a series of 10^{-2}–10^{-6} diluents were prepared by gradient dilution method, and 150 μL diluents were coated on NA medium respectively. The inoculated plates were incubated at 38 °C and 180 rpm under aerobic conditions for 14 days. Bacterial colonies with rapid growth and large diameter were picked and purified (Gao et al., 2017). After activation, the purified strains were inoculated into 500 mL flasks containing 200 mL screening medium (glucose, 4g; peptone, 0.1g; yeast extract, 0.1g; urea, 0.1 g; ammonium sulphate, 0.1g; potassium dihydrogen phosphate, 1g; magnesium sulphate, 0.04g; sodium chloride, 0.02g; crude oil, 20 g; water 200 mL, pH adjusted to 7.0 and autoclaved at 121 °C for 30 min) before use) and incubated at 38 °C and 180 rpm under aerobic conditions for 7 days. Bacterial growth and emulsification of crude oil was observed and recorded during incubation.

The viscosity of crude oil was determined after 7 days incubation, and the strains with good performance for emulsification and viscosity reduction of oil were selected for further studies. Finally, a strain which had the highest activity for emulsification and viscosity reduction was isolated and named as HB-4. The strain was preserved in 20%, v/v sterile glycerol solution at −70 °C (Varjani and Upasani, 2016).

2.2.2. Bacterial identification

The strains were identified by16S rDNA sequence (Wu et al., 2013a,b). Phylogenetic tree of the 16S rRNA was constructed by the neighbour-joining method and their close relatives were retrieved from the GenBank database. Alignments to related sequences (shown with accession number) were performed with MEGA 4.1 software.

2.2.3. Batch growth with different carbon sources

HB-4 was activated by transferring culture from nutrient agar slants into BH medium (0.1% NH_4NO_3; 0.02% $MgSO_4{\cdot}7H_2O$; 0.1% K_2HPO_4; 0.01% $CaCl_2{\cdot}2H_2O$; 0.015% KCl), after 24 h incubation. 100 mL of the above fermentation broth was transferred to 500 mL flasks containing 2 g different carbon sources (including glucose, sucrose, molasses, starch and crude oil). Aerobic cultures were conducted by bubbling sterile air through the culture solution at a flow rate of 50 mL/min at 38 °C. During incubation, the bacterial density were counted, on a daily basis for 2 weeks, by the flat colony counting method (Baron et al., 2006). Each dilution was plated in triplicate on a nutrient agar plate and incubated at 38 °C for 24 h. The number of colony-forming units (CFU) at each dilution rate was counted after incubation and the average CFU/mL was determined.

2.2.4. Crude oil degradation test

20 g crude oil was added as the sole carbon source into 500 mL conical flasks containing 200 mL BH medium. It was then autoclaved at 121 °C for 30 min and cooled before use. Strain HB-4 was activated by transferring culture from nutrient agar slants into BH medium and incubated at 38 °C and 180 rpm for 24 h. Inoculum (2%, v/v) of HB-4 (optical density 1.0 at AU_{600}) was added into the test flask and incubated at 38 °C and 180 rpm for 0–35 days. Another flask was kept as control without inoculum. Aerobic cultures were conducted by bubbling sterile air through the culture solution at a flow rate of 50 mL/min at 38 °C. The anaerobic conditions were performed in an anaerobic incubator where nitrogen was used to displace oxygen. After incubation, the collected samples were extracted and analysed to check total petroleum hydrocarbon (TPH) degradation by gas chromatography (Varjani and Upasani, 2016). The extraction and analysis of crude oil were carried out according to the method described by (Varjani et al., 2015). The residual oil left after biodegradation was measured by weighing the quantity of oil. It was run in triplicate and the results were averages of three independent experiments. Degradation rate was defined as:

$$\text{Degradation}(\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (1)$$

where m_1 is the mass of crude oil before degradation, and m_2, the mass of crude oil after microbial degradation.

2.2.5. Viscosity and surface/interface properties

The extracted crude oil was analysed using viscosity measurement (Zhang et al., 2016). The viscosity of crude oil samples recovered from the degradation test was measured using a Haake viscosimeter (MARS III, Thermo Scientific). The emulsification activity (E24) was measured 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 (Lai et al., 2009). The emulsification index (E24) was calculated as the ratio of the height of the emulsion layer to the total

height of the mixture.

$$E_{24}(\%) = (\text{total height of the emulsified layer})/(\text{total height of the liquid layer}) \quad (2)$$

The surface tension (SFT) of the HB-4 broth was measured using the Wilhelmy plate method on a surface tensiometer (DCAT25, dataphysics Co. Ltd., Germany) at room temperature (Varjani and Upasani, 2016). The interfacial tension of crude oil/water systems were determined using a Spinning Drop Video Tensiometer SVT20 (Dataphysics Instrument Gmbh, Germany) (Ke et al., 2018b). Each result was the average of three determinations.

2.2.6. Chemical analysis by gas chromatography (GC)

Both control and biodegraded crude oil samples were further analysed by gas chromatography (GC) (Varjani et al., 2015). GC analysis was carried out with an Agilent 7890B, equipped with flame ionization detector (FID). The components of biodegraded samples were separated by a polymethylsiloxane capillary column (length, 30 m; internal diameter, 0.25 mm and thickness, 0.1 mm). Oven initial and final temperatures were set at 40 °C (10 min) and 330 °C, respectively. The oven temperature program was 10 °C/min. Vaporizer and detector temperature were 330 °C. Helium was used as carrier gas with a flow rate of 20 mL/min. Hydrogen gas flow rate and air flow rate were 40 and 400 mL/min, respectively. After the instrument reached stable, 0.5 μL samples was injected. Meanwhile, temperature program was started, and chromatographic charts and raw data were recorded.

2.2.7. Core flooding test

Core flooding tests were conducted according to the published procedure with minor modification (Gao et al., 2013). Briefly, in order to simulate the reservoir environment, the test temperature was set to be 38 °C, and the pressure, at 8 MPa. The length and the diameter of the core columns were 50 cm and 2.5 cm, respectively. The columns were packed with mixed silica sands (80–200 mesh) by mechanical loading. The permeability of cores were controlled at 150–200 $\times 10^{-3}$ μm^2. The process started with saturating the core column with crude oil (500 mPa·s), which was followed by ageing for 7 days at 50 °C. Then, water flooding was conduct with brine (the produced water from oilfield after sterilization) until reaching a given water-cut level of 98%. Subsequently, a 0.5 pore volume (PV) of the fermentation broth of HB-4 (with bacterial density 1×10^8 cfu/mL and 2.35%, v/v, nutrient) was injected into the core, followed by a 14-day "shut-in" period at 38 °C. The nutrients contained (w/v) 2% glucose, 0.12% NH_4NO_3, 0.02% $MgSO_4{\cdot}7H_2O$, 0.1% K_2HPO_4, 0.01% $CaCl_2{\cdot}2H_2O$ and 0.01% KCl). Finally, water flooding was repeated until no further oil was collected, and the oil recovery, oil composition and viscosity were determined. The control experiment was conduct under identical conditions without HB-4.

3. Results and discussion

3.1. Morphological characterization and bacterial identification

The isolated strain was a gram-negative, rod-shape bacterium. Cell measured 1.0–1.7 μm long and 0.5 μm wide. Colonies on TSA medium appeared to be white, round (diameter: about 1.0 mm), convex, smooth, reflective, and translucent, with regular edges.

The results of 16S rDNA sequence analysis showed that the isolated strain HB-4 had 100% sequence similarity with *Chelatococcus daeguensis*. Phylogenetic tree of the 16S rRNA was constructed by the neighbour-joining method and their close relatives were retrieved from the GenBank database (Fig. 1). Based on morphological observation and 16S rDNA sequence analysis, HB-4 was confirmed to be *Chelatococcus daeguensis* (CGMCC 6458 2012.08.17).

3.2. Growth under aerobic and anaerobic conditions

Batch growth experiments under aerobic and anaerobic conditions were performed. Strain HB-4 was found to be facultative anaerobe which grew well in the temperature range of 20–50 °C, while the optimum growth temperature was 37 °C. It grew well under both conditions, however, its growth under aerobic conditions was clearly more active than that under anaerobic conditions.

3.3. Utilization of different carbon sources

Under aerobic conditions, the effects of five commonly used carbon sources on the growth of HB-4 were investigated, including glucose, sucrose, molasses, starch and crude oil. The results are presented in Fig. 2. The strain grew best when glucose was used as carbon source, followed by starch and crude oil, but slower in sucrose and molasses. With glucose, the bacterial density was 8.6 log10 (cfu/mL) after one day fermentation, and reached a maximum of 9.5 log10 (cfu/mL) at day 5, which was followed by a slight decline to 9.2 log10 (cfu/mL) at day 7. The growth trend of bacteria in sucrose or molasses was similar with that of glucose, however, the overall bacterial density was significantly lower than that of glucose. When crude oil was used as the sole carbon source, the growth rate of bacteria was much lower than that of glucose and starch, where the bacterial density increased with the extension of time and reached 7.1 log10 (cfu/mL) over seven days.

In the process of microbial flooding, the carbon source in microbial nutrient solution not only directly affects the growth, reproduction and metabolism of microbes, but also determines the cost of MEOR. Traditional carbohydrates and starch as carbon sources can maintain the rapid growth of microbes, but the metabolism of these carbon sources in the fermentation process can be too fast; the carbon source may be depleted before reaching the desired depth of the reservoir, resulting in unsustainable growth and metabolism of microbes in the deep reservoir. In this respect, by using crude oil as the sole carbon source following the injection of sugar as carbon source, it not only allows the growth of microbes to be more controllable, but also reduces the cost of nutrient solution injected. The growth characteristics of HB-4 (Fig. 2) demonstrated that HB-4 is able to utilize crude oil as the sole carbon source, demonstrating potential for MEOR application.

3.4. Biodegradation of crude oil under aerobic and anaerobic conditions

To quantify the effects of strain HB-4 on crude oil degradation and viscosity reduction, the degradation rate and viscosity of heavy crude oil under either aerobic or anaerobic conditions, were measured over five weeks. The results are illustrated in Fig. 3.

Strain HB-4 was able to degrade crude oil under both conditions, while the crude oil degradation efficiency was clearly higher under aerobic conditions than that under anaerobic conditions. When the crude oil was incubated with HB-4 for 5 weeks, the degradation rate of crude oil under these two conditions reached 48.9% and 27.2%, respectively. Correspondingly, the reduction in oil viscosity under aerobic conditions (by 58.6%) was more significant than that under anaerobic conditions that decreased by 28.7%.

It was also observed that the trends of both parameters examined under aerobic conditions were different from that under anaerobic conditions. Aerobic degradation and viscosity reduction both had a rapid change at the first stage (for about 3 weeks), followed by a stage having a lower rate of change. Under anaerobic conditions, in contrast, the initial change rates were lower, also over the first 3 weeks, that increased at the second stage. Under aerobic conditions, the degradation rate of crude oil was faster because of the rapid increment of microbial density, while the metabolites effectively reduced the viscosity of crude oil. Following that, the second stage tended to have a slower rates of change due to the consumption of substrates and the production of metabolites. As a result, the degradation rate of crude oil showed a

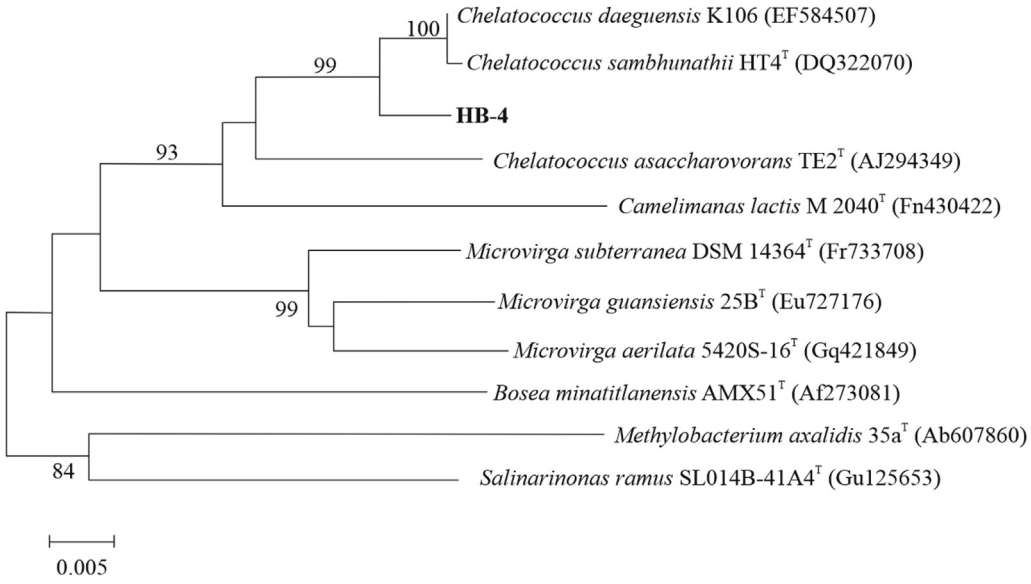

Fig. 1. Phylogenetic tree of the 16S rRNA constructed by the neighbour-joining method and their close relatives retrieved from the GenBank database. Alignments to related sequences (shown with accession number) were performed with MEGA 4.1 software. Bootstrap values shown at nodes for frequencies above a 50% threshold (n = 1000 replicates). Superscript "T" represents the type strain.

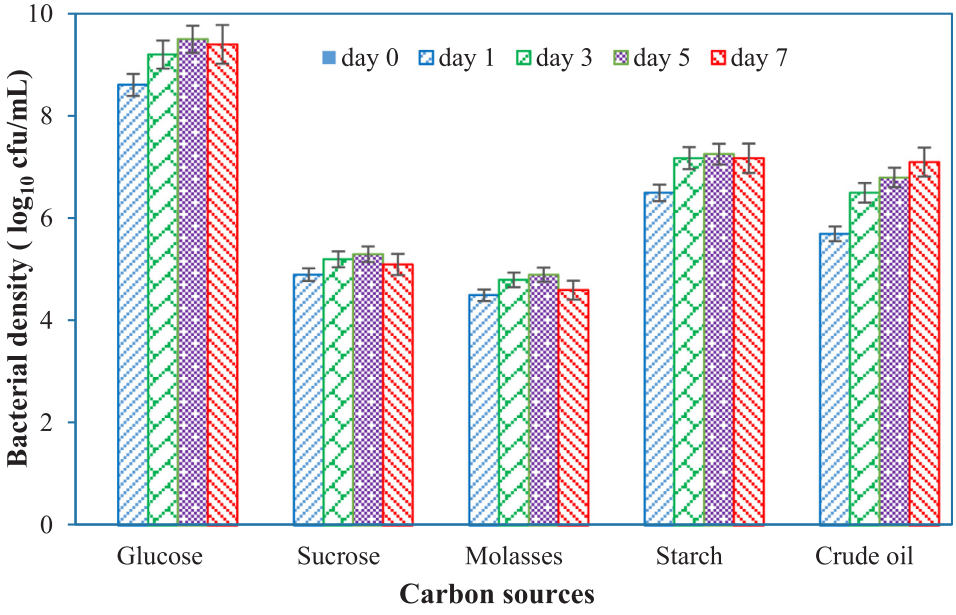

Fig. 2. The effect of different carbon sources on the growth of HB-4 under aerobic conditions.

trend of first stage of fast change and then a slower one, which was largely consistent with the change rate of crude oil viscosity.

However, under anaerobic conditions, although the degradation rate of crude oil increased with the decrease in viscosity during incubation, the change was much slower than that under aerobic conditions, mainly due to the slower growth of strain HB4 under anaerobic conditions. In the absence of sugar as a carbon source, the metabolites in the first 20 days were limited. As time went on, the amount of metabolites gradually increased, which in turn further promoted the degradation of crude oil by bacteria, thus accelerating the degradation rate.

At present, the majority of studies on MEOR including filed pilots have been focused on aerobic bacteria, which did demonstrate good performance, while little effort has been directed to the application of anaerobic bacteria on MEOR (Varjani and Upasani, 2016; Xia et al., 2011). However, during the industrial process of MEOR in oilfields, the injected bacteria can undergo aerobic, anoxic, or anaerobic fermentation, and more likely a combination of these processes in the reservoir. It is generally understood that in the near wellbore zone aerobic fermentation likely takes place where more oxygen is presented in nutrient solution carried in from wellhead. However, in regions being distant from the wellbore or in strata where microbes migrate towards, the fermentation tends to be anaerobic due to the gradual consumption of oxygen. Therefore, it is crucially important to understand and preferably quantify the performance of facultative anaerobes in order to effectively achieve MEOR.

Based on the growth characterization under either aerobic or anaerobic conditions that clearly showed strain HB-4 to be facultative, further characterization on its performance related to MEOR was carried out, in terms of emulsification index (E_{24}), surface tension and interfacial tension (IFT). The measurement results are depicted in Fig. 4. When the incubation period increased, the effect on the emulsification of crude oil was clearly pronounced. Emulsification index E_{24} increased from 8% (Day 0) to 78% (Day 3), and continued to increase though at a slower rate, reaching 89% after 2 weeks incubation when crude oil and broth appeared to be emulsified completely. The trend is in line with the observation made with measurement of oil degradation (Fig. 3). It should be noted that the effect on the emulsification could

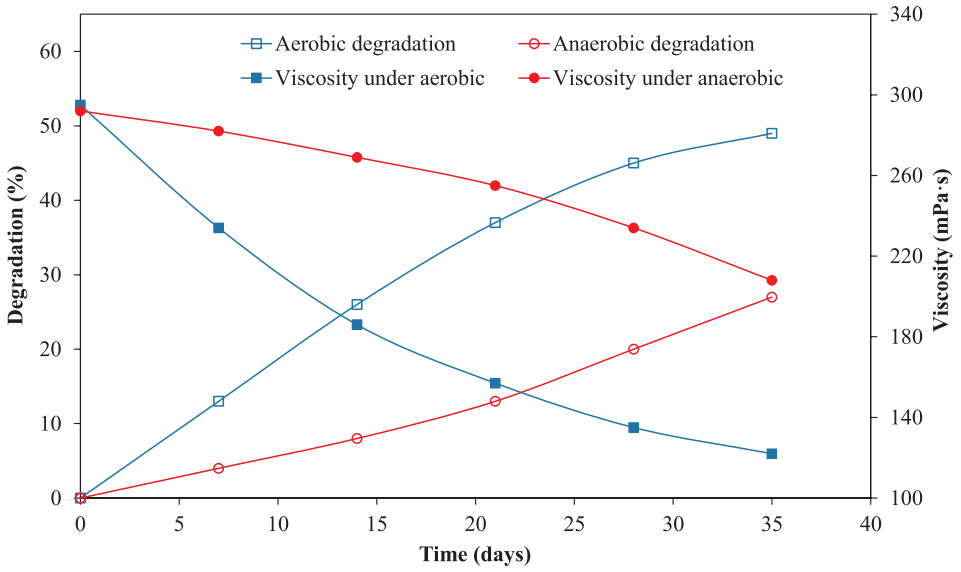

Fig. 3. Degradation and oil viscosity of heavy crude oil treated by HB-4 under aerobic and anaerobic condition.

also be potentially attributed to the production of biosurfacants by bacteria through metabolism, as observed in our previous studies (Ke et al., 2018a).

It was interesting to observe that, whilst the emulsification index increased significantly, there was only a slight decrease in surface tension, from 72.1 mN/m to 66.2 mN/m over the 4-week incubation, while interfacial tension remained approximately unchanged. The observation was clearly indicative of the contribution of potential metabolite biosurfactants to be insignificant, where the effect of biosurfactants was often markedly measureable (with surface tension reductions typically > 60%) in previous studies (Souayeh et al., 2014; Liang et al., 2017; Varjani and Upasani, 2017). This further suggested that the emulsification activity was likely associated with other types of bioemulsifiers produced through HB-4 metabolism. Unlike commonly observed biosurfactants produced by bacteria, such metabolite bioemulsifiers were able to emulsify crude oil, but cannot significantly reduce the surface tension of broth or interfacial tension of oil and water water (Shreve et al., 1995; Martinez-Checa et al., 2002; Rahman et al., 2003). More recent studies attributed the emulsification activity to the extracellular degradation of hydrocarbons (or oil) into shorter chain molecules (Ke et al., 2018a; Varjani, 2017; Zhao et al., 2016). In addition, it indicated oil biodegradation to be a crucial and even controlling process for oil (or other hydrocarbons) emulsification towards MEOR (Churchill et al., 1995).

3.5. Chemical analysis of biodegraded crude oil

In order to gain insights into the biodegradation process, chemical analysis with gas chromatography (GC) was carried out to identify and quantify the changes in different hydrocarbons including crude oil, in order to quantify the degree of the degradation progress (Cai et al., 2013; Varjani and Upasani, 2016). Fig. 5 and Table 1 illustrate the variation in contents of main components in degraded crude oil samples with either crude oil only, or oil mixed with glucose (mass ratio = 5:2) as carbon source. The analytical results of the crude oil control sample are also presented for comparison.

After incubation with HB-4 for 2 weeks under aerobic conditions with glucose and crude oil as carbon sources, the relative content of

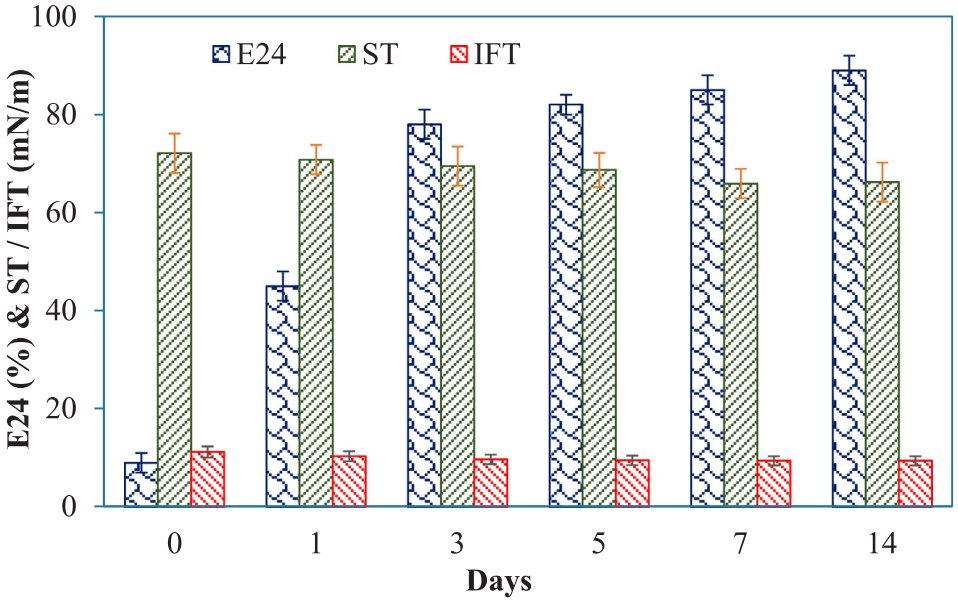

Fig. 4. Emulsification index (E_{24}), surface tension (mN/m) and interfacial tension (IFT, mN/m) of HB-4 broth as a function of incubation time.

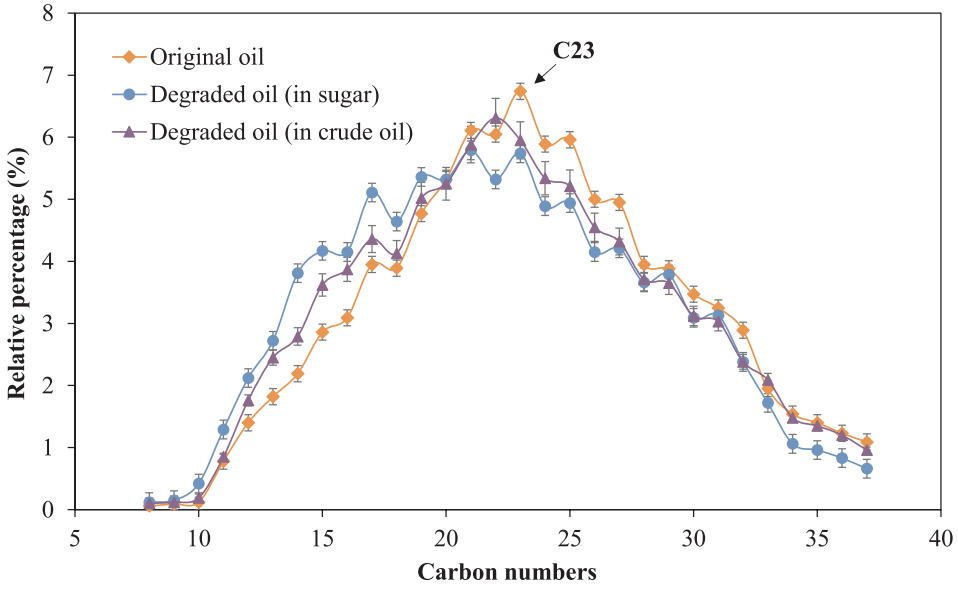

Fig. 5. Variation in degraded oil composition with different carbon sources.

Table 1
Variation in crude oil properties after HB-4 degradation with different carbon sources.

Characterization	Before treatment	Biodegradation in Sugar and crude oil	Biodegradation in Crude oil
Max peak	C_{23}	C_{22}	C_{21}
(C_8-C_{22})%	42.52	50.49	46.70
(C_{23}-C_{37})%	53.19	45.21	48.34
Viscosity (50 °C), mPa·s	298.9	132.5	185.6
Wax content, %	20.12	16.91	16.53
Asphaltenes content, %	27.74	25.29	25.70

degraded crude oil components changed most significantly; the relative content of lighter fractions (C_8-C_{22}) increased from 42.52% to 50.49%, whilst the heavier fractions (C_{23}-C_{37}) decreased from 53.19% to 45.21%. The carbon number (which typically indicates the length of hydrocarbon chains) associated with the maximum content shifted to lower carbon number, i.e., from C_{23} to C_{22}. The changes in Wax and Asphaltenes contents together with viscosity reduction (Table 1) further confirmed the effect of HB-4 on the degradation of long chain hydrocarbon of heavy crude oil. A similar trend of change in content of components was observed when crude oil only was the carbon source, while maximum content peak shifted from C_{23} to C_{21} though the positions of the two peaks were insignificantly distinguishable. The enhanced effect of adding glucose on biodegrading of crude oil was believed due to the favourable, more efficient bacterial metabolism with glucose which can facilitate the rapid growth of bacteria, resulting in higher efficiency of crude oil degradation (Atlas, 1981; Cooper et al., 1981).

3.6. Core flooding tests

Following the characterization of microbes HB-4 on the performance related to MEOR, it was further evaluated with commonly used core flooding tests in terms of oil recovery and water cut, by using a laboratory scale core column set-up (Xia et al., 2011). The profiles of oil recovery and water cut curves during the core flooding test are depicted in Fig. 6.

At the water flooding stage, the water cut of the outlet liquid increased rapidly with water flooding. When the 0.6 PV of water was injected, the water cut of the output liquid increased to a maximum and stable level of 98%, and the oil recovery reached a plateau at 42.3%. As water flooding became ineffective, a 0.4 PV of microbes HB-4 and nutrient were injected into the core, which was then shut in for incubation at a constant temperature of 38 °C for 14 days. Following the "shut-in" period was the post water flooding stage. During the first phase of post water flooding (1.0–1.5 PV injection), oil recovery increased significantly, from 42.3% to 56.5%, whilst water cut decreased correspondingly from 98.5% to 82.3%. Then, the increase rate of oil recovery became slower gradually reaching 62.5%, that was likely due to the consumption of microorganism and nutrient, while water cut raised correspondingly.

At the second phase of post water flooding (after 2.0 PV injection), oil recovery remained unchanged with no oil production in the outlet of core, indicating the ineffectiveness of microorganisms on oil recovery. At this point, a total amount of 62.8% of oil was recovered, where the microbial enhanced oil recovery was 20.5%, and MEOR level was much higher than that (typically, below 10%) reported in the literature (Wu et al., 2013a,b).

At the same time, the viscosity of the crude oil before and after the MEOR were measured to be 362.3 mPa·s and 145.7 mPa·s, respectively, showing a viscosity reduction of 59.8%. As discussed above, the significant reduction in oil viscosity was mainly attributed to microbial degradation where oil hydrocarbons were degraded into smaller molecules. To confirm this, GC analysis was conducted for samples taken during the first water flooding stage and microbial incubation. GC results showed that the composition of crude oil changed noticeably during the core flooding MEOR test. Compared to the components of crude oil from water flooding, after microbial treatment the heavy components (C_{23}-C_{37}) relatively reduced while the light components relatively (C_8-C_{22}) increased, that was consistent with the change in oil viscosity. The results demonstrated that the degradation effect of bacteria HB-4 on crude oil effectively reduced the viscosity of crude oil, resulting in significant improvement for oil recovery.

4. Conclusions

Biodegradation of crude heavy oil was investigated with facultative anaerobic strain *Chelatococcus daeguensis* HB-4 and its grew was also characterized at 37 °C with glucose, starch and crude oil as carbon sources. It was found that HB-4 degraded crude oil through breaing long-chain hydrocarbons (C_{23}-C_{37}) to shorter chains (C_8-C_{22}) that also

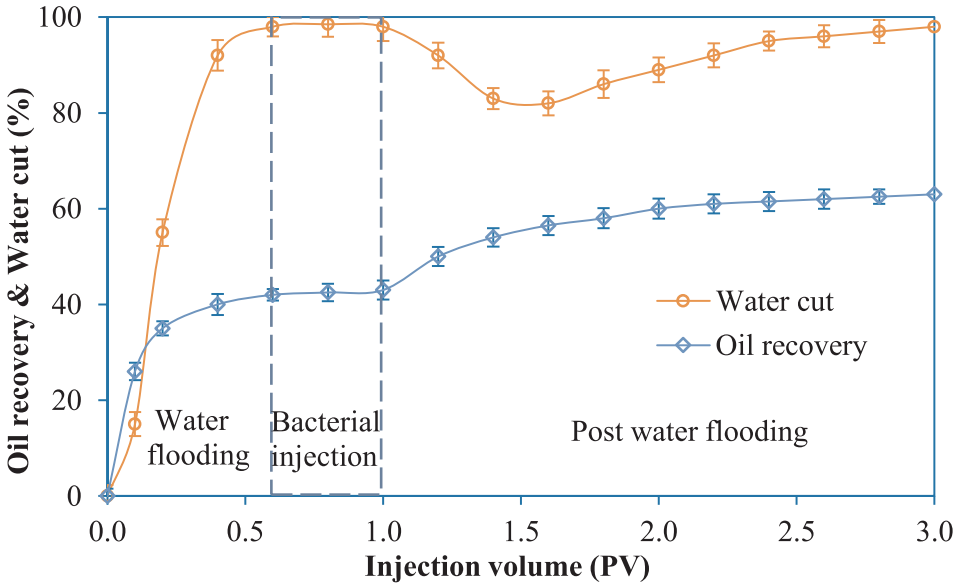

Fig. 6. Oil recovery and water cut curves of core flooding tests.

acted as bioemulsifiers. Along with degradation, the oil viscosity was reduced under aerobic and anaerobic conditions by 58.6% and 28.7%, respectively. Core flooding tests demonstrated that the fermentation broth of strain HB-4 increased the oil recovery efficiency by 20.5%, showing potential for applications in heavy oil reservoirs exploration.

5. Notes

The authors declare no competing financial interest.

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