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Original Research Article

The role of sulphate-reducing bacteria in oil recovery

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A B S T R A C T

Keywords

SRB, Surface tension, IFT, EOR, TPH, SO_4

Sulphate-reducing bacterial (SRB) metabolites could reduce both surface and interfacial tensions between air/liquid and oil/liquid respectively, and also able to form a stable emulsion system with crude oil. Oil recovery rate of SRB from saturated oil sand was determined as 39.2%. Three fraction ratios (alkane, asphaltene and NSO) were significantly reduced after 3-month bio-degradation. Also, significant decreases of three *n*-alkanes (C_{11}-C_{13}) were determined. However, there was no remarkable change in composition of aromatics during 3-month experiment. Both SRB metabolite/oil emulsion and bio-degradation partially contributed to a reduction of oil viscosity. The amount of reduced SO_4^- was about 77.4 mg in 20 days by SRB using TPH as the unique carbon sources.

Introduction

Sulfate-reducing bacteria (SRB) are a group of microbes that use sulfate (SO_4^-) as a final electron acceptor instead of oxygen for respiration (Al-Zuhair, El-Naas et al. 2008). SRB are able to use a very wide spectrum of different low molecular organic compounds (e.g. lactate, acetate, proprionate, succinate, pyruvate, ethanol, sugars, etc.) for growth, with SO_4^- being reduced to hydrogen sulfide (H_2S) (Benaroudj, Lee et al. 2001; Al-Zuhair, El-Naas et al. 2008; Brioukhanov, Pieulle et al. 2010; Sherry, Gray et al. 2013). SRB is known as harmful bacteria in the productive process of oilfields. They might cause serious problems in oilfield water systems, including corrosion of iron in anaerobic conditions and reduction of the injectivity of water injection wells by precipitation of amorphous ferrous sulfide (Castro, M. et al. 1997).

Recently, some studies reported that SRB might play a role in microbial enhancing oil recovery (MEOR) (Champagne, Mondou et al. 1996; Callbeck, Agrawal et al. 2013). For example, SRB could diminish oil viscosity, replenish the declining pressure of reservoir, and change heavy oil to light oil through yields of bio-generated acids, gas (H_2S) and degradation of hydrocarbons (Aliphatic and Aromatic). Moreover, different types of SRB are widely distributed in global oil

reservoirs. Therefore, SRB could be a great target that used for enhancing oil recovery (EOR).

This study was mainly focused on the mechanism of SRB in EOR. To do this, the whole research was divided into four parts. In the first part, surface activity of SRB metabolites including interfacial and surface tensions, oil recovery rate from saturated oil sand, and effectiveness of emulsification were studied. Many previous studies demonstrated that metabolites produced by variety of microbes could act as bio-surfactants to reduce interfacial tension (IFT) between oil and water, and thus improve the solubility and mobility of oil (Singer and Finnerty 1988; Banat 1993; Singh, Van Hamme et al. 2007).

In the second part, 3-month bio-degradation of total petroleum hydrocarbons (TPH) was investigated. Fraction changes of TPH were estimated by comparing between before and after the SRB bio-degradation. It has been reported that SRB could directly use alkanes, aromatics and other oil components as electron donors for SO_4^- reduction (Callaghan, Morris et al. 2012; Wohlbrand, Kube et al. 2013), thereby changing oil compositions. Therefore, composition changes of two fractions (alkane and aromatic) were studied subsequently by gas chromatography-mass spectrometry (GC-MS).

Oil viscosity is well known to play an important role in EOR (Ghosh and Al Shalabi 2011; Homayuni, Hamidi et al. 2011). In the third part, the role of SRB in oil viscosity was studied. To do this, viscosities of three different SRB pre-treated oil samples (metabolites/oil emulsion system, bio-degraded oil and metabolites/biodegraded oil emulsion system) were measured and compared to that of crude oil. Both aerobically and anaerobically stored oil samples under experimental conditions (as same as bio-degradation experiment) were studied as controls.

Bio-gas has been reported to reduce oil viscosity and replenish the pressure of oil reservoir (Al-Sulaimani, Joshi et al. 2011). However, the effect of H_2S on oil viscosity was not directly tested in this study. Our laboratory lacks of such equipment to measure oil viscosity under the actual reservoir temperatures and pressures. Moreover, H_2S could easily escape from contaminated liquids and is extremely toxic if inhaled. Handling (produce, conduction and storage) of H_2S should be strictly restricted and minimized in laboratory condition. Therefore, the productivity of H_2S was estimated indirectly by using bio-consumption of SO_4^- as an index. In the final part, SRB bio-reduction rate of SO_4^- was tested with TPH acting as the unique carbon source. Sodium lactate (in SRB medium ATCC 1249 type III) was also used as an electron donor in SRB anaerobic respiration and acted as a control.

Materials and Methods

Chemicals

All reagents (media and buffers) used were prepared gravimetrically using a Sartorius A200S analytical balance, and made up to volume with room temperature sterile distilled water (dH_2O). All chemicals used in this paper were reached the analytical standard, and have been autoclaved at 121 °C for 20 min for sterilizing before use.

Isolation

The SRB was isolated from pipe line of water injection well in Daqing Oilfield, China. The isolation was carried out in Postgate medium C (sPGC) (Postgate 1984).

The medium consists of the following: NaCl (0.12 M), $MgCl_2 \cdot 6H_2O$ (5.9×10^{-3} M), KH_2PO_4 (3.6×10^{-3} M), NH_4Cl (0.019 M), Na_2SO_4 (0.032 M), $CaCl_2 \cdot 2H_2O$ (2.8×10^{-4} M), $MgSO_4 \cdot 7H_2O$ (1.2×10^{-4} M), $FeSO_4 \cdot 7H_2O$ (1.4×10^{-5} M), trisodium citrate (1.1×10^{-3} M), sodium lactate (70% w/v, 0.077 M), yeast extract (1 g L^{-1}) and agar (20 g L^{-1}). The pH was finally adjusted to 7.2.

The plates were incubated at 45 ºC for 20 days under anaerobic conditions in a 3.5 L anaerobic jar (Traditional system; Oxoid Company) filled with carbon dioxide and hydrogen which was produced by using anaerogen sachets according to the instructions of the manufacturer. Preparation and inoculation of plates were carried out inside an environmental chamber which contained a mixture of gases (Nitrogen 87%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment. After incubating for a week, several colonies of SRB were observed. The different bacteria were isolated and allowed to grow on separate plates and were found to be of the same type belonging to *Desulfobacteriaceae* family.

Microbial culture

The cell culture was carried out in ATCC medium 1249 type III (Shao, Kang et al. 2012). Cell culture was prepared by inoculating a single colony from the agar plate into 80 mL of broth in a 120 mL headspace vial. The headspace vial was covered by septa, and subsequently sealed with aluminum cap by capping clamp. Preparation and inoculation were carried out inside an environmental chamber which contained a mixture of gases (Nitrogen 87%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment. Headspace vials were then grown anaerobically on an orbital shaker (150 rpm) at 45 °C for 20 days (The cell concentration was grown to A600 nm to 1.5 (stationary phase) and stored at 4 °C until required, but no longer than 8 hours.

Surface property of SRB metabolites

Cells were removed by centrifugation (Eppendorf 5810R centrifuge) at 10,000×g for 5 min at room temperature. The supernatant was restored in a 500 mL clean flasks and stored at 4 °C until required, but no longer than 24 hours.

Surface tension assay: surface tension between liquid samples and air was measured by using a Dataphysics DCAT21 Tensiometer (Germany) at 45 °C (target reservoir temperature) based on its operation manual.

IFT assay: IFT between liquid samples and oil was measured by the TEXAS-500 Spinning Drop interfacial Tensiometer (CNG, USA) at 45 °C (target reservoir temperature). The oil sample was degased and dehydrated in an electronic oven at 110 °C for 48 hours. The interfacial tension was then recorded repeatedly in every 15 min. The equilibrium interfacial tension value stable in two hours was recognized as the ultimate value in analytical process.

Amott spontaneous imbibition test (Yu, Evje et al. 2009): oil sand was prepared by saturation of sand with crude oil (with density was 0.8 g cm^{-3}) as mass ratio of 1 (oil):7 (sand). Oil sand was placed in an electronic oven for dehydration and aging at 45 °C for two weeks. Each component (Amott sample holder, supernatant, and measuring cylinder) was heated to 45 °C before use.

16 g of oil sand was weighted and placed into an Amott sample holder, and filled with SRB supernatant (metabolites). The holder was sealed with parafilm, and placed into an orbital shaking hot-water bath, and then inoculated at 45 °C, 150 rpm for two hours.

After inoculation, parafilm was removed, and the Amott sample holder was covered by a measuring cylinder (with gradations from 0 to10 mL). The measuring cylinder was then filled with supernatant up to 5 mL (or any other integral gradations might facilitate further observation). The Amott system was placed into an air circulation oven at 45 °C for 8 hours. The recovered oil was measured with one hour interval during the test by visual inspection at the top of the imbibition cell (measuring cylinder). Afterwards, final volume of recovered oil was extracted and determined after dehydration, and the oil recovery rate could be calculated by Eq. 1.

$$\% \text{ Recovery rate} = \frac{\text{Dehydrated recovery volume}}{\text{Total oil volume in 16 g saturated oil sand}} \times 100 \quad (1)$$

Microbial emulsion: 20 mL of SRB supernatant was mixed with 20 mL of oil sample into a clean tube (with gradations from 0 to 50 mL). The mixture was pre-heated to 65 °C in a hot water bath, and then emulsified by using a 2.5 L digital ultrasonic cleaner (CE-7200A, Jeken, China), at 80 watt (W) for 15 min. Three-phase oil/emulsion/supernatant sample was then allowed to equilibrate. A sample was considered fully equilibrated once the interface was distinct and the phase behavior did not change for at least several days (generally last for 100 hours).

Bio-degradation

50 mL of freshly cultured SRB (A600 nm was 1.5) was well mixed with 50 mL of crude oil (dehydrated and degased) in a 120 mL headspace vial by gently shaking up and down for 5 min. The headspace vial was covered by septa, and subsequently sealed with aluminum cap by capping clamp. Preparation and inoculation were carried out inside an environmental chamber which contained a mixture of gases (Nitrogen 87%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment. Headspace vials were then placed on an orbital shaker (150 rpm) and incubated at 45 °C for three months. During the incubation, headspace vials were frequently opened for releasing the produced H_2S every half-month.

Estimation and analysis of residual oil

Fractions of TPH: 30 mg of crude oil (before/after biodegradation) were consecutively extracted with hexane, dichloromethane, and chloroform (100 mL each). All three extracts were pooled and dried at room temperature by evaporation of solvents under a gentle nitrogen stream in a fume hood. After solvent evaporation, the amount of residual TPH was then determined gravimetrically. After gravimetric quantification, the residual TPH was fractionated into alkane, aromatic, asphaltene, and NSO (nitrogen, sulfur, and oxygen-containing compounds) on a silica gel column. To do this, samples were dissolved in hexane and separated into soluble and insoluble fractions (asphaltene). The soluble fraction was located on a silica gel column and eluted with different solvents. The alkane fraction was eluted with 100 mL of toluene. Finally, the NSO fraction was eluted with methanol and chloroform (100 mL each) (Hadibarata and Tachibana 2009). The alkane and aromatic fractions were then analyzed by GC-MS.

A Petro column (60 m × 0.25 mm × 0.25mm) is used as the chromatographic column for GC-MS analysis (Agilent 7890A GC, US). The temperature program of saturated hydrocarbon was set as: started from 80 °C (held for 0.2 min), then heated to 220°C at the rate of 4°C/min, then to 320°C at the rate of 2°C/min (held for 20 min). The temperature program of aromatic

hydrocarbon was set as: started from 100°C (held for 0.2 min), then heated to 300°C at the rate of 3 °C/min (held for 20 min). The samples were injected into a heated (300°C) splitless injector, and the injection volume was 0.5 mL. Helium was used as the carrier gas, with the constant flow rate of 1.5 mL min^{-1}. The MS transfer line and ion-source temperature were 280°C and 240°C, respectively.

Oil viscosity study

Viscosity of different oil samples (e.g. crude oil, bio-degraded oil and SRB metabolites/oil emulsion system, etc.) was measured by using a NDJ-8S digital viscometer (Nirun Intelligent Technology, China) at 45°C.

Bio-consumption of SO_4^-

SO_4^- background measurement: 100 mL of oil samples (dehydrated and degased) were washed by using 100 mL of 5% isopropanol and 20 mL of pentane for at least five times to extract the residual SO_4^-. The extracted solution was then precipitate with 10 mL of 0.1 M $BaCl_2$.

100 µL of SRB (A600nm was adjusted to 1.5) was inoculated into 100 mL of oil (TPH assay) and ATCC medium 1249 type III (medium assay) in two separated 120 mL headspace vials. 1.75 mL of 2 M $NaSO_4$ (final concentration was 35 mM) was added into both assays for maintaining 20 days anaerobic SO_4^- respiration. The headspace vials were covered by septa, and subsequently sealed with aluminum cap by capping clamp. Preparation and inoculation were carried out inside an environmental chamber which contained a mixture of gases (Nitrogen 87%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment. Headspace vials were then placed on an orbital shaker (150 rpm) and incubated at 45 °C for 20 days. During the incubation, headspace vials were frequently opened for releasing the produced H_2S every week.

After incubation, oil was removed for further extraction with 100 mL of 5% isopropanol and 20 mL of pentane for at least five times, while the SRB was removed by centrifugation at 10,000×g for 5 min at room temperature. Extracted solutions (two assays) were restored into a 1000 mL clean flask, separately. 1.75 mL of 2 M $BaCl_2$ was added for each assay to precipitate the residual SO_4^-. The precipitates were boiled at 100 °C for 15 min (eliminate BaS_2O_3 precipitate), and then dried in an oven at 50 °C for overnight to remove moisture. The dry stuffs were weighted gravimetrically using a Sartorius A200S analytical balance, and the consumed SO_4^- was subsequently calculated by Eq. 2.

$$\text{Reduced sulphate (mg)} = 336 - \frac{96 \times \text{dry weight}}{233}$$

Results and Discussion

Surface activity study

To evaluate the surface property of SRB metabolites, both surface (between air and metabolites) and IFT (between oil and metabolites) were tested in this study. dH_2O (used to make SRB culture media) and SRB culture media were conducted as negative controls.

Surface tension of SRB metabolite assay was about 25.88 mN m^{-1} (Figure 1a), which was significantly lower than that of dH_2O (around 72 mN m^{-1}) (data was not shown) and culture medium assays (Figure 1b). Moreover, IFT between oil and SRB was around 0.13 mN m^{-1}, which was nearly one order of magnitude lower than that of control assays (Figure 2).

The significant difference was determined in paired test. n=4, error bars are standard error.

In order to assess the efficiency of oil recovery by SRB metabolites, Amott spontaneous imbibition assay was conducted. Both dH_2O and culture medium were used as controls. As results shown below (Figure 3), SRB metabolites could recover oil remarkably from saturated oil sand. In contrast, there was no oil extracted in both control assays during 120 inoculation hours. Oil recovery profile of metabolite assay could be described as (Figure 3): oil was recovered rapidly before the first 24 hours; afterwards, oil recovery volume increased slightly with a significantly decreased rate as incubation time from 24 to 120 hours; finally, oil recovery becomes to a saturation level after 120 inoculation hours. The recovered oil was then extracted and dehydrated. The recovery volume of dehydrated oil was about 0.98 mL. Therefore, oil recovery rate was finally determined as 39.2% (Eq. 1).

To evaluate the efficiency of emulsion by SRB metabolites, volume changes of emulsion phase were measured over 120 hours. Both dH_2O and culture medium were used as negative controls. Emulsion phase gradually decreased to about 5.4 mL as incubation time increased up to 80 hours in SRB metabolite assay (Figure 4). Afterwards, the emulsion system reached to the equilibrium after 80 hours incubation. In contrast, emulsion phases of both dH_2O and medium assays were decreased rapidly since the experiment was initiated, and completely separated into two individual phases (oil and dH_2O/medium) before 10 hours. The equilibrated Oil/metabolite emulsion phase was then extracted for later viscosity measuring.

Fraction ratios in TPH after 3-month bio-degradation

To find out the role of SRB bio-degradation in variations of TPH fraction ratios, oil was inoculated with SRB cells by 1:1 (volume) ratio for 3-month period. Four fractions (alkane, aromatic, asphaltene and NSO) of TPH were then tested in this study. Oils (no microbial) stored at both aerobic and anaerobic conditions (exactly same condition with bio-degradation assay) were also conducted as controls. Fresh oil used for comparison was zero-time stored crude oil (dehydrated and degased).

During 3-month anaerobic storage, fraction ratios of TPH were slightly different compared to their fresh counterparts. However, those variations were remained in estimated errors (Table 1). However, ratios of fractions, including aromatic, asphaltene and NSO, of aerobic oil were slightly increased probably due to its significantly decreased alkane. After 3-month bio-degradation, ratios of alkane, asphaltene and NSO were significantly decreased, while aromatic was remarkably increased.

To deeply understand SRB degradation, composition of alkane and aromatic of different oil samples were studied then by GC-MS. In alkane assay, SRB degraded oil showed significantly lower contents of Undecane, *n*-Dodecane and *n*-Tridecane, compared to their fresh counterparts (Figure 5). Significant decreases in Undecane and *n*-Dodecane were also found in aerobically stored oil samples. There was no significant difference of composition change between fresh and anaerobically stored oil samples. Moreover, no remarkable composition changes were found in all four different samples in aromatic assay (data not shown).

The significant difference was determined in paired test. n=6, error bars are standard error.

Oil viscosity study

To estimate the effects of both SRB metabolites and bio-degradation on oil viscosity, viscosity of different oil samples were measured by using a NDJ-8S digital viscometer (Nirun Intelligent Technology, China) at 45 °C (reservoir temperature). There were totally six oil samples, including fresh oil (dehydrated and degased), aerobically and anaerobically stored (3-month storage) oils, biodegraded oil, oil/metabolite emulsion system and bio-degraded emulsion system (metabolite/ bio-degraded emulsion system). Of the six, fresh oil, aerobically and anaerobically stored oils were used as controls.

In contrast to fresh oil assay, three assays (degraded oil assay, metabolite/oil emulsion assay and bio-degraded emulsion assay) showed significant decreases in oil viscosities (Figure 6). Of the three, bio-degraded emulsion assay exhibited the best viscosity reduction level. Furthermore, aerobic oil assay showed a significantly higher oil viscosity after 3-month storage, compared to its fresh counterpart.

SO_4^- bio-reduction study

To evaluate the yield of bio-gas by using TPH as an unique carbon source, SRB was inoculated into crude oil and incubated under experimental condition for 20 days. SO_4^- reduction of SRB was then studied as an index to indirectly indicate the bio-productivity of H_2S. SO_4^- reduction by cells grown in medium (medium assay) was conducted as a control. The amounts of reduced SO_4^- in TPH and medium assays were about 193.9 mg and 77.4 mg, respectively (Figure 7). Moreover, SO_4^- reduction of TPH assay was significantly lower than that of media assay (Figure 7).

SRB is generally recognized as a hazard in the productive process of oilfields (Castro, M. et al. 1997). However, it has been reported that SRB could contribute to improving oil recovery more recently (Champagne, Mondou et al. 1996; Callbeck, Agrawal et al. 2013). To better understand the role of SRB played in EOR, oil recovery mechanism of SBR was studied in detail in this research.

SRB metabolites could significantly reduce surface tension and IFT in contrast to both control assays (Figure 1 and 2), and were also able to recover oil from saturated oil sand with recovery rate of 39.2% (Figure 3). Moreover, SRB metabolites showed remarkable emulsion effectiveness with crude oil. The metabolite/oil emulsion phase equilibrated and retained 5.4 mL last for more than 40 hours (Figure 4). Metabolites produced by microbes contain amphipatic molecules (with both hydrophilic and hydrophobic parts) which are able to reduce both of ITF and surface tension (Al-Sulaimani, Joshi et al. 2011). Amphipatic molecules could accumulate at the interface of immiscible fluids thereby increasing the solubility and mobility of hydrophobic or insoluble organic compounds (Singh, Van Hamme et al. 2007). Those results indicated that SRB metabolites exhibited a significant surface activity. It also has been reported that microbes could produce variety of bio-surfactants (Rosenberg, Zuckerberg et al. 1979; Kim, Powalla et al. 1990; Yakimov, Amro et al. 1997; Lang and Wullbrandt 1999; Ghojavand, Vahabzadeh et al. 2008). However, type of bio-surfactants produced by SRB was not determined in this research. Undoubtedly, SRB could directly react with the fractions of TPH. After 3-month bio-

degradation, aromatic ratio was significantly increased due to the remarkable decreases in the other three fractions (alkane, asphaltene and NSO), in contrast to fraction ratios of fresh and anaerobic oils (Table 1). Composition changes of both alkane and aromatic in bio-degraded oil were then analyzed by GC-MS. Results indicated that ratios of three *n*-alkanes (C_{11}-C_{13}) including Undecane, *n*-Dodecane and *n*-Tridecane were significantly reduced during 3-month degradation (Figure 5). This result was similar with a previous study (Cravo-Laureau, Matheron et al. 2004). Also it has been reported that SRB could degrade alkanes, aromatics, toluene and other oil components (Callaghan, Morris et al. 2012; Wohlbrand, Kube et al. 2013). The alkane degradation mechanism has been demonstrated that the carbon chain of the alkane is altered at the end by terminal addition of a C_1-unit (Aeckersberg, Rainey et al. 1998). Decreases in both Undecane and *n*-Dodecane of aerobic assay might be due to volatilization during 3-month incubation.

However, there were no significant changes in composition of bio-degraded aromatics in our study. This might be caused by two reasons. Firstly, the degradation period in this research maybe not long enough for exhibiting the sufficient bio-degradation activity of SRB. Microbes generally degrade hydrocarbons in order of *n*-alkanes, branched-chain alkanes, branched alkanes, low molecular weight *n*-alkyl aromatics, polycyclic aromatics (Van Hamme, Singh et al. 2004). Secondly, degradation of aromatic compounds was based on types of SRB (Harms, Zengler et al. 1999).

The effects of SRB on oil viscosity were estimated in this study. Both of SRB metabolites/oil emulsion and bio-degradation contribute to a significant reduction of oil viscosity (Figure 6), due to its surface activity (Figure 1-4) and consumption of asphaltene and NSO (Table 1). By combination of emulsion and bio-degradation, oil viscosity was apparently further decreased (Figure 6). Our results demonstrated that SRB could partially reduce oil viscosity through either form of emulsion system and/or bio-degradation.

In the final part of this study, SO_4^- reduction of SRB was tested as an index to indicate the productivity of H_2S. SRB could directly use TPH for their anaerobic respirations (Figure 7). This result is also agreed with previous studies (Callbeck, Agrawal et al. 2013; Sherry, Gray et al. 2013). The total reduced SO_4^- of TPH assay was about 77.4 mg in 20 days.

In contrast, SRB showed a significantly higher SO_4^- reduction rate by using sodium lactate as an electron donor. The reason might be that sodium lactate is more readily to be utilized than carbon sources in TPH. Even that SRB were still seemed to produce significant amount of H_2S by using TPH as their unique carbon sources (Figure 7). The produced H_2S might play a role in further improving oil recovery through both of oil viscosity reduction and replenishment of reservoir pressure (Al-Sulaimani, Joshi et al. 2011). However, the actual effects of H_2S on EOR were not studied in this research.

Figure.1 Surface tension (Mean ± SD) between air and two different liquids
a: SRB medium assay; b: SRB metabolite assay

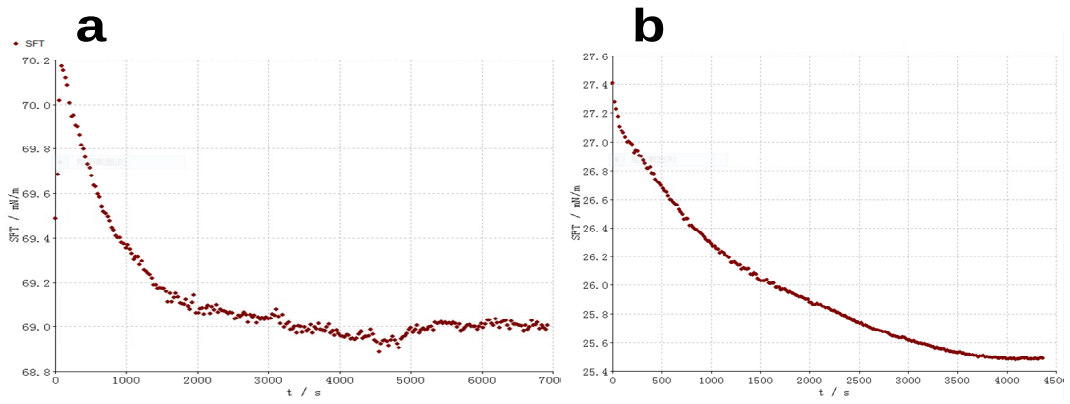


Figure.2 Comparison of IFT between dH_2O, SRB medium and SRB metabolite assays. *: significantly different from dH_2O assay. The number of stars (*) indicates the significance level. *: P<0.05; **: P<0.01; ***: P<0.005; ****: P<0.001

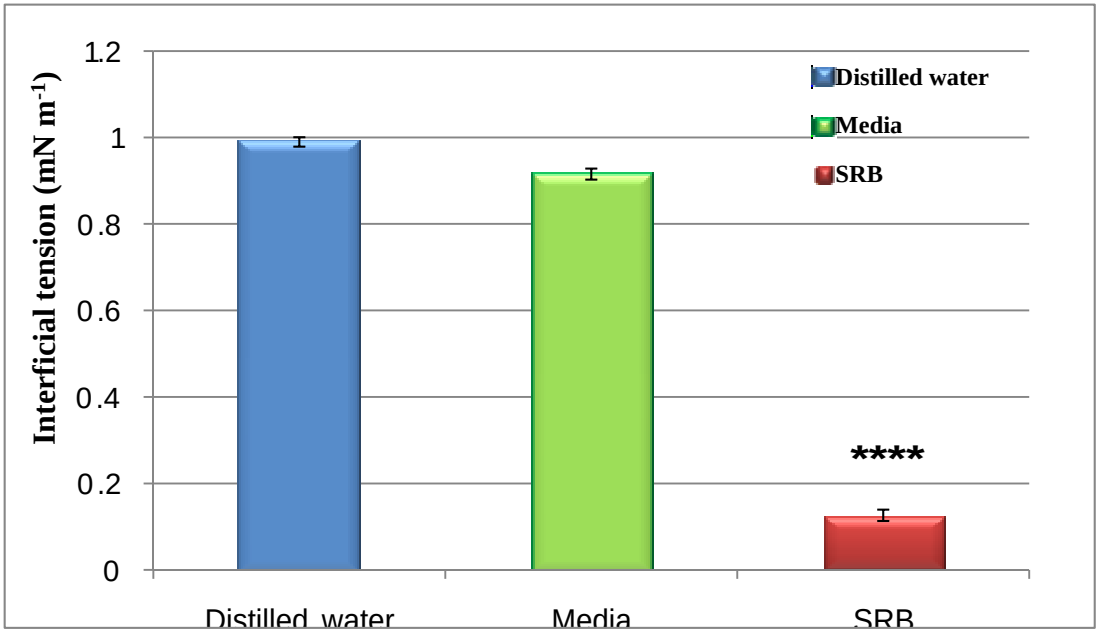


Figure.3 Comparison of oil recovery volumes (Mean ± SD) between three different assays during 240 incubation hours.

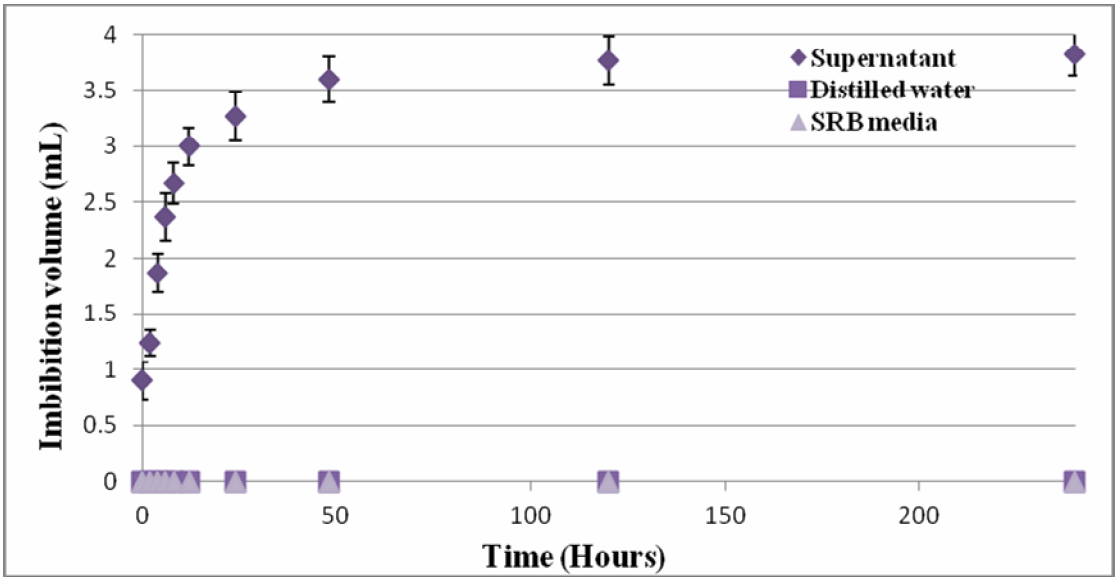


Figure.4 Comparison of emulsion volume (Mean ± SD) changes between three different solutions

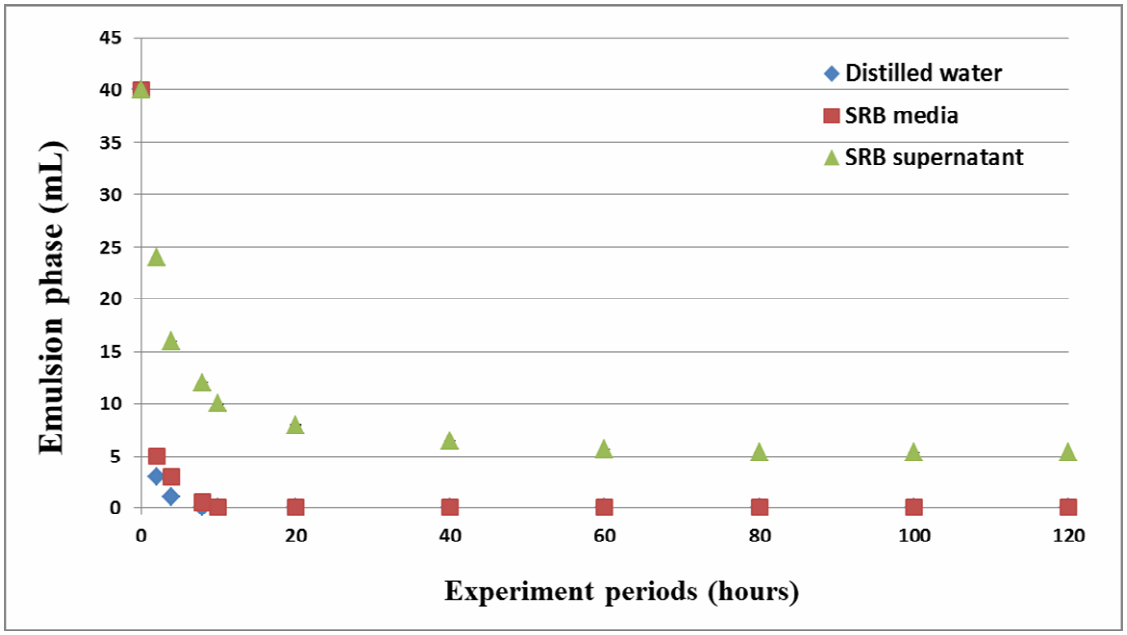


Table.1 Fraction changes in TPH between different oil samples

Fraction	Contents (Mean ± SD) (%)			
	Fresh oil	Anaerobic oil	Aerobic oil	SRB degraded oil
Alkane	55.71 ± 0.24	55.22 ± 0.23	54.25 ± 0.27*	52.77 ± 0.41*
Aromatic	11.25 ± 0.28	12.05 ± 0.24	12.12 ± 0.19	19.66 ± 0.06*
Asphaltene	1.96 ± 0.03	1.98 ± 0.02	2.16 ± 0.12	1.76 ± 0.04*
NSO	30.97 ± 0.26	30.65 ± 0.49	31.47 ± 0.18	25.81 ± 0.12*

Figure.5 Comparing the composition changes of three alkanes between four oil samples. *: significantly different from fresh oil assay. The number of stars (*) indicates the significance level. *: P<0.05; **: P<0.01; ***: P<0.005; ****: P<0.001

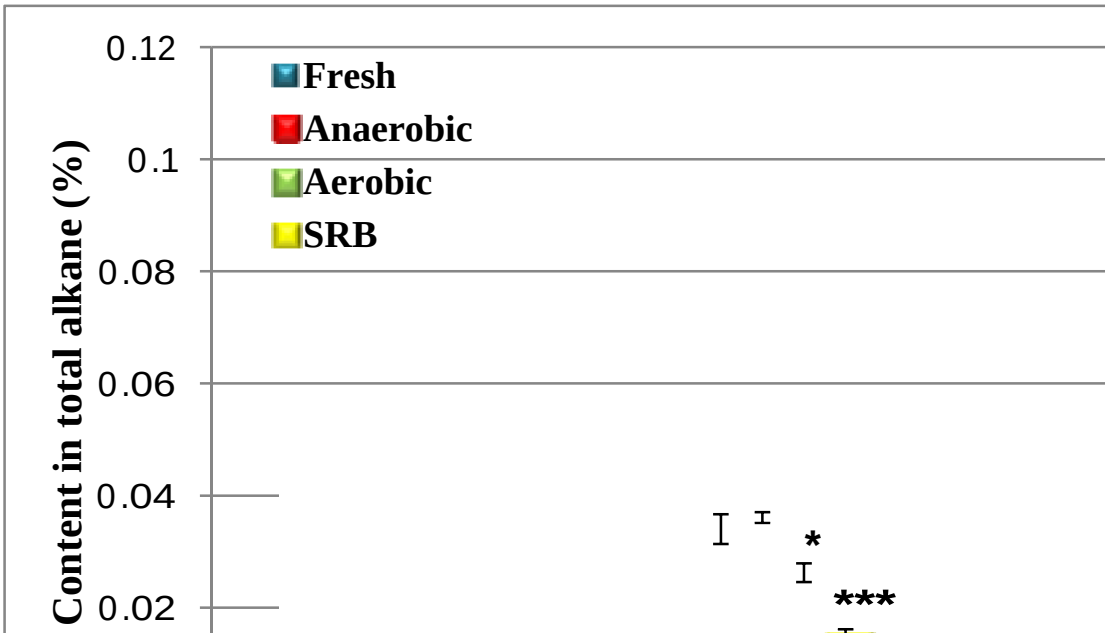


Figure.6 Comparison of viscosity between different oil samples. *: significantly different from fresh oil assay. The number of stars (*) indicates the significance level. *: P<0.05; **: P<0.01; ***: P<0.005.

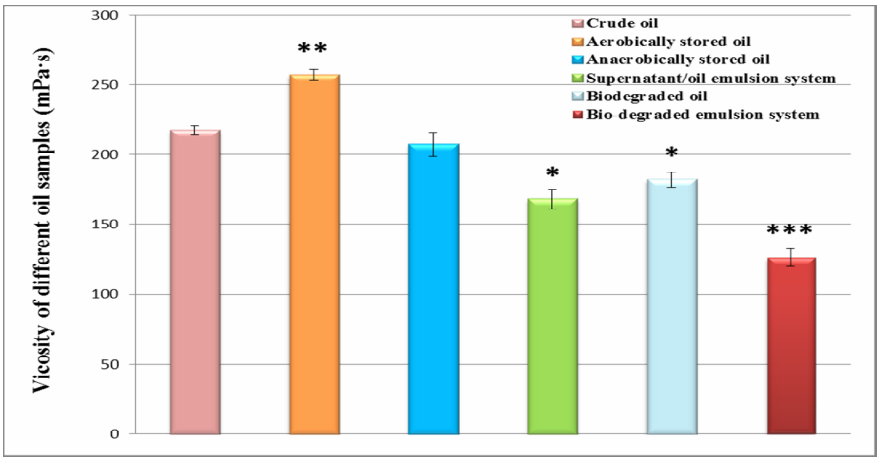
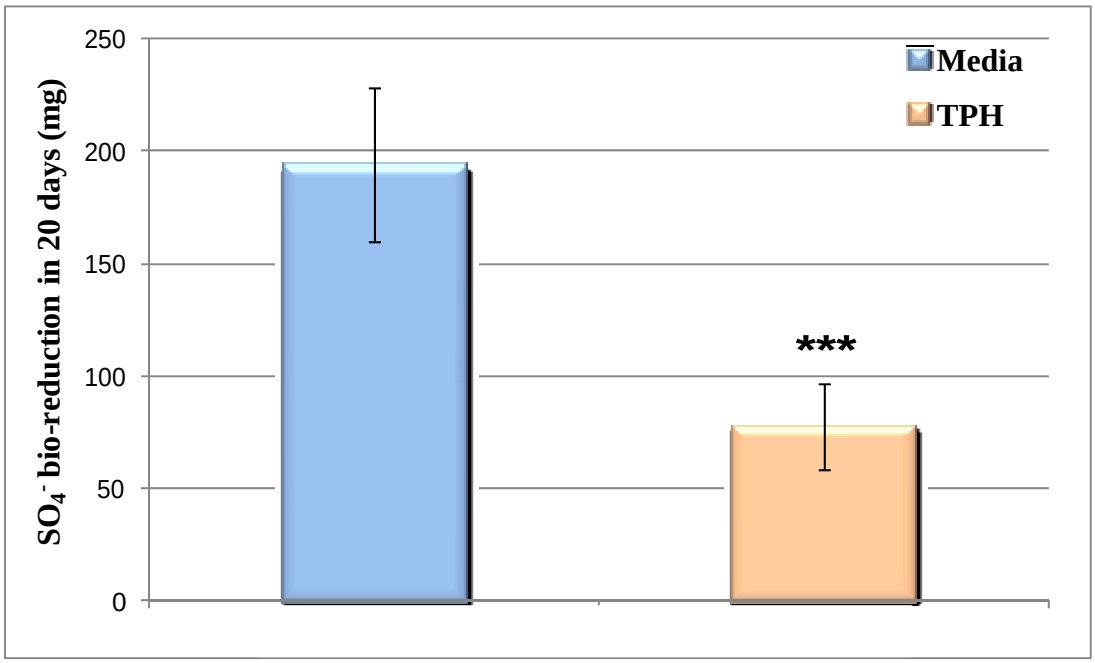


Figure.7 Comparison of SO_4^- reduction between two different carbon-sources assays (Medium assay and TPH assay). *: significantly different from medium assay. The number of stars (*) indicates the significance level. *: P<0.05; **: P<0.01; ***: P<0.005.



SRB metabolites exhibited a surface activity to reduce surface and interfacial tensions and also able to form stable metabolites/oil emulsion. The metabolites could recover around 39.2% of total oil from two-week aged saturated oil sand. Moreover, SRB could directly use TPH for their anaerobic respiration, resulting in bio-degradation of oil components (C_{11}-$C_{13\ n}$-alkanes) and bio-reduction of SO_4^-. Oil viscosity was also significantly reduced through either metabolites/oil emulsion and/or bio-degradation (asphaltene and NSO).

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