World J Microbiol Biotechnol (2013) 29:103–108
DOI 10.1007/s11274-012-1162-7

ORIGINAL PAPER

Enhancement of bunker oil biodesulfurization by adding surfactant

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Received: 13 July 2012 / Accepted: 24 August 2012 / Published online: 1 September 2012

Abstract Biodesulfurization (BDS) is a promising method to remove sulfur compounds from diesel and gasoline. However, the information on BDS of heavy oil is scanty, which might be due to their "undesirable" physical properties and more complicated sulfur diversities. In this study, the BDS of one kind of heavy oil, bunker oil MFO380 was investigated. The biocatalyst was obtained by the enrichment with oil sludge as the seed and using dibenzothiophene (DBT) as the sole sulfur source. The enriched biocatalyst (microbial mixed culture) could selectively remove sulfur from DBT and DBT was transformed into 2-hydroxybiphenyl, which indicates that the BDS process is beneficial to non-destructive carbon bonds and thus can maintain the calorific value. The bunker oil BDS results showed that after 7 days of incubation, the removal efficiency of sulfur in MFO380 was only 2.88 %, but this could be significantly improved by adding surfactants Triton X-100 or Tween 20. This effect could be attributed to greatly reduced viscosity of heavy oil and increased mass transfer of sulfur compounds in heavy oil into water. Adding Triton X-100 achieved the highest removal efficiency of sulfur, up to 51.7 % after 7 days of incubation. The optimal amount of Triton X-100 was 0.5 g/50 ml medium. When toluene was added as an organic solvent for MFO380, the BDS activity was improved, while lower than the effect of adding surfactants.

Keywords Biodesulfurization · Bunker oil · Surfactant · Viscosity

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Introduction

As the conventional oil reservoirs mature and their production declines, the world will increasingly depend on heavy oil. The International Energy Agency (IEA) estimates that there are 6 trillion barrels of heavy oil in place globally, which is more than twice as much as conventional oil resources (Alboudwarej et al. 2006). Hence, heavy oil has the potential to be a major energy source for the twenty-first century. Bunker oil is one common type of heavy fuel oil, used mainly for marine applications, such as shipping. However, bunker oil contains elevated levels of sulfur, which generate serious air pollution during combustion, e.g. SO_x. The high content of sulfur in heavy oil is one of the most important issues that have seriously restricted the application of heavy oil (Shennan 1996). Therefore, there is an urgent need for removing sulfur contaminants from heavy oil.

Hydrodesulfurization (HDS) is commonly used in oil refineries to remove sulfur from fuel oils under elevated pressure and temperature. However, further application of HDS is hindered by its high cost and ineffectiveness on polycyclic thiophenes (Rana et al. 2007). The diesel and heavy oil contain significant amounts of benzothiophenes, dibenzothiophenes (DBTs) and its derivatives (CxDBT) (Choudhary et al. 2006), which are considerably more

difficult to remove by HDS processes (Grossman et al. 1999; Papizadeh et al. 2010). Biodesulfurization (BDS) is one of the alternative methods to remove sulfur compounds from diesel and gasoline, and this has attracted worldwide attention (Luo et al. 2003; Kilbane 2006). The BDS process can be carried out under ambient temperature and pressure. Moreover, it is environmentally cleaner and less energy-intensive.

Sulfur compounds can be aerobically biodesulfurized through a sulfur-specific pathway, which is preferred as it leaves the carbon structure of the target compounds intact. The specific pathway of removal of sulfur from DBT was named as "4S" and implied consecutive oxidation of DBT sulfur to sulfoxide (DBTO), sulfone (DBTO2), sulfinate (HPBS) and hydroxybiphenyl (HBP) (Gallagher et al. 1993). The sulfur atom is oxidized into sulfite and sulfate, which will be released to the aqueous phase (Kilbane 2006). Many studies have used DBT-degrading microorganisms to investigate the BDS capability of crude oil, gasoline and biodiesel (Oldfield et al. 1997; Zhang et al. 2011; Wang et al. 2011). It was reported that 26–71 % of sulfur could be removed biologically from different petroleum oils, with the highest removal efficiency of sulfur from light crude oil (Bhatia and Sharma 2010).

So far, very few studies have been found on BDS of heavy oils. This might be due to their "undesirable" physical properties and more complicated sulfur diversities. Moreover, the viscosity of heavy oil is much higher, compared with gasoline and diesel. Our preliminary study found that heavy oil forms big particles or balls in the aqueous phase, which decreases the mass transfer sharply and limits the effectiveness of heavy oil biosulfurization process significantly. Thus, it is very challenging to remove sulfur from heavy oil by a biological process.

The low solubility of heavy oil in aqueous phase might be one of major limitations to BDS of heavy oil. To reduce the viscosity of bunker oil and increase the contact of bunker oil with biocatalyst is the critical step in BDS. Heating is a common method utilized to overcome the high viscosity for chemical reaction. However, it is not suitable for bioreaction because high temperature will do harm to biocatalysts (Chang et al. 1999). The use of surfactants to form an emulsion is one of the effective methods to reduce the viscosity of heavy oil. Heavy oil is suspended as micro-spheres stabilized in a water continuous phase by the use of surfactants forming an O/W emulsion (Langevin et al. 2004) and the emulsion formed can promote the mass transfer between heavy oil and water phase (Van Hamme and Ward 2001; Feng et al. 2006). Thus, heavy oil BDS process might be improved by adding surfactants. However, the role of surfactants in heavy oil BDS has not yet been investigated.

In this study, the objective is to explore the feasibility of sulfur removal from heavy oil using suitable microorganisms. The BDS process was investigated by using bunker oil MFO 380 as the representative heavy oil, and the biocatalyst was enriched by using DBT as sulfur source. The BDS of MFO 380 with adding surfactants was investigated to explore the potential improvement of BDS efficiency of heavy oil through adding surfactants.

Materials and methods

Chemicals

DBT was purchased from Sigma–Aldrich, USA. Bunker oil MFO380 was kindly provided by the Maritime and Port Authority (MPA) of Singapore. MFO 380 represents the medium fuel oil with the maximum viscosity of 380 cSt. Other chemicals were of analytical grade, commercially available and used without further purification.

Culture medium and enrichment

The culture medium was a sulfur-free basal salt medium (BSM), consisting of (in g/L) KH_2PO_4 2.44, Na_2HPO_4 5.57, NH_4Cl 2.00, $MgCl_2{\cdot}6H_2O$ 0.36, $FeCl_3{\cdot}6H_2O$ 0.001, and $MnCl_2{\cdot}4H_2O$ 0.004. The final pH was 7.0. Glycerol was added as carbon source with a final concentration of 10.0 g/l. DBT was used as model compound and the sole sulfur source, and ethanol was used to dissolve the model sulfur species.

The mixed culture was enriched from an oil sludge which was collected in Pulau Sebarok Island, Singapore. The oil sludge was mixed with BSM at a volume ratio of 1:5 and shaken reciprocally at 200 rev/min and room temperature for 4 days to digest the remaining oil and detach the microbial seed. Fifty mL of the mixture was transferred to a 1–L flask containing 250 mL of BSM supplemented with 0.5 mmol/L of DBT and the mixture was reciprocally shaken at 200 rev/min and room temperature for 4 days. The above step of enrichment was repeated four times before the enriched culture was used as the seed in the BDS test.

Biodesulfurization experiment

Bunker oil MFO 380 was used as feedstock in this study. Five mL of microbial seed was mixed with 45 mL BSM and 1 g bunker oil in 250-mL flasks. The ratio of oil to water was 1:50 (w/w). Glycerol was added as carbon source with a final concentration of 10.0 g/l. BDS experiment was carried out at 30 °C on a rotary shaker operated at

200 rev/min. Control experiment was also conducted under the same conditions but without adding microbial inoculums.

Biodesulfurization by adding surfactants

According to the hydrophobicity and the solubility of bunker oil, three kinds of surfactants Tween 20, Triton X-100 and PEG 4000 were used to test the influence of surfactants on the BDS of MFO380. The ratio of surfactant to water was 1:50 (w/w). The ratio of oil phase to aqueous phase was 1: 50 (w/w). The experiment was carried out at 30 °C on a rotary shaker operated at 200 rev/min for 7 days.

Analytical methods

Oil–water emulsion was extracted with dichloromethane (DCM). 50 g of emulsion was transferred to a 250 ml separation funnel and 50 ml of DCM was added. The funnel was shaken for 3 min, and the sample-solvent mixture was placed still for 24 h. The lower layer was released from the separation funnel, and the solvent was evaporated using a Buchi evaporator at 50 °C. The oil extract obtained was further heated in a Kilm muffle oven at 50 °C for 1 h, subsequently cooled in a dessicator at room temperature for 40 min, and weighed by an analytical balance. The heating–cooling cycle was repeated until two consecutive weighing showed weight losses lower than 5 mg.

High-performance liquid chromatography (HPLC) was used for the quantitative analysis of DBT in the n-hexane phase. HPLC was performed on a Waters 2695 liquid chromatograph equipped with an auto-sampler, a reversed-phase Zorbax SB-C18 column (4.6 mm × 150 mm), and a diode array detector. The mobile phase was 90 % of methanol in water (v/v, %) with a flow rate of 0.5 ml/min. For the quantification of DBT, the external standard method was used at 280 nm.

The elements in heavy oil were determined concurrently using a Leco CHN elemental analyser. Sulfur analysis of oil fraction was performed with a Leco SC-432 instrument (furnace temperature 1,371 °C). Three analyses for each sample were carried out for consistency. The results were expressed in terms of weight percent of each element in the samples.

DBT and its metabolites were monitored by GC (Agilent 6890N, USA) equipped with a MS detector (Agilent 5973N). The conditions of the GC–MS were as follows: injector, 300 °C splitless; carrier gas, helium 1 ml/min; column, DB-5 ms; oven temperature program, 50 °C for 2 min, 45 °C/min to 140 °C, 10 °C/min to 300 °C, hold for 6 min, total run time per sample 26 min.

Results and discussion

DBT biodesulfurization

DBT was used as a model compound to study the BDS process in the aqueous phase by the enriched biocatalyst. The concentration of DBT was about 0.5 mM and the concentration of the biocatalyst was 6.5 g/l. As shown in Fig. 1, the concentration of DBT decreased significantly within the first 10 h of incubation process, and then decreased slowly. The DBT concentration changed from 0.576 to 0.062 mM after 30 h of incubation. The degradation products of DBT, 2-HBP and sulfate increased accordingly.

The chromatogram of GC–MS analysis for DBT BDS by enriched biocatalyst after 20 h is shown in Fig. 2. The results of GC–MS analysis show that the final desulfurization product of DBT is 2-HBP. As shown in Fig. 1, the sulfur removed was oxidized to sulfate. This indicates that DBT can be aerobically biodesulfurized through a sulfur-specific pathway. Thus, the enriched culture can be used as biocatalyst for BDS of bunker oil, and the calorific value of heavy oil will not be lost after the BDS.

Generally, it is believed that the aerobic conversion of DBT–2-HBP is catalyzed via a multi-enzyme pathway consisting of two mono-oxygenases and a desulfinase, which is also known as the "4S" pathway (Monticello 2000). The key enzymes in the pathway are dibenzothiophene monooxygenase (DBT–MO), a tetramer encoded by the *dszC* gene; dibenzothiophene sulfone monooxygenase (DBTO2–MO), a dimer encoded by the *dszA* gene; 2-(2′-hydroxyphenyl) benzenesulfinic acid (HPBS) desulfinase, encoded by the *dszB* gene, and the NADH: FMN oxidoreductase encoded by the *dszD* gene. The final reaction catalysed by the desulfinase appears to be the rate-limiting

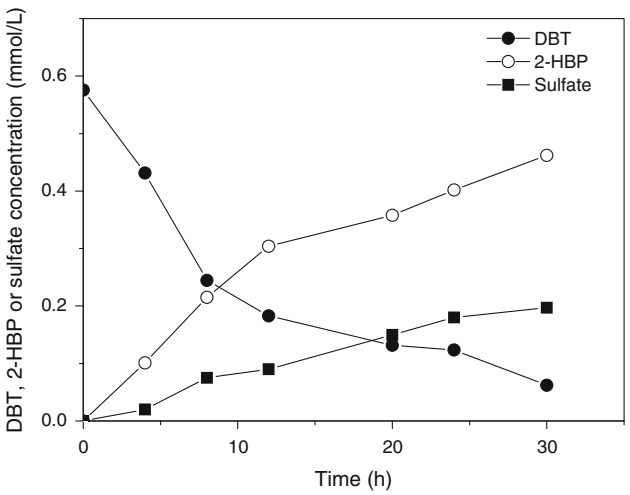

Fig. 1 Biodesulfurization of DBT by mixed culture in aqueous phase

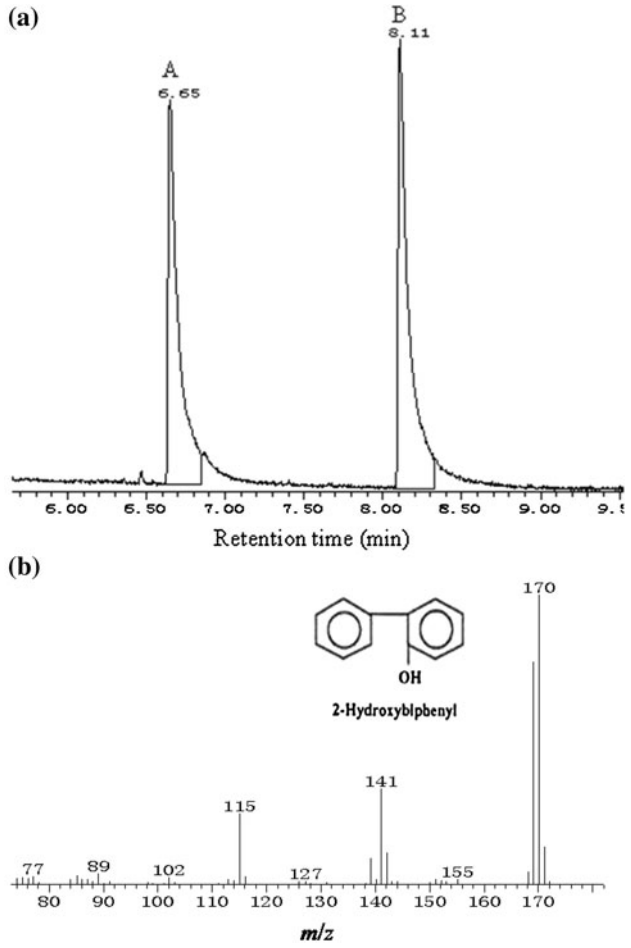

Fig. 2 GC–MS chromatogram **a** of the DBT metabolites and mass spectrum **b** of the final metabolite by mixed culture after 20 h of incubation (peak *A* 2-HBP, molecular mass 170; *B* DBT, molecular mass 184)

step in the pathway (Gray et al. 1996). Through the well recognized "4S" pathway, DBT is biodegraded to dibenzothiophene sulfoxide, dibenzothiophene sulfone, hydroxyphenyl benzene sulfonate and finally 2-HBP.

Biodesulfurization of bunker oil

Firstly, received oil-contaminated soil and oil sludge were used directly for the BDS process of MFO 380. The elemental compositions of MFO 380 were analysed before and after 10 days of the biotreatment. The control was carried out with adding MFO 380 into medium without biocatalysts. As shown in Table 1, after the biotreatment the carbon content in MFO 380 did not change and the hydrogen content decreased slightly. The ratio of hydrogen to carbon decreased slightly, which indicates that the compounds with shorter carbon chains might be consumed by the biocatalysts. The nitrogen content in MFO380 remained rather constant and the sulfur content increased slightly after the biotreatment process. The results show that the sulfur in MFO 380 has not been utilized during the biotreatment process when received soil or oil sludge was used directly. The possible reason is that the specific microorganism for BDS did not exist in the received seed. It is recommended that the specific microbial strains should be concentrated by enrichment of a mixed culture system by model compounds first. This will help in establishing the effectiveness of BDS of heavy oil.

Table 1 Elemental composition of bunker oil after BDS by soil and oil sludge (%)

Samples	C	H	N	S	H/C
MFO380	85.4	10.8	0.27	2.68	12.65
Control	85.3	10.6	0.28	2.75	12.43
BDS by soil	85.4	10.2	0.27	3.04	11.94
BDS by oil sludge	85.5	9.9	0.29	3.11	11.60

BDS biodesulfurization

On the other hand, the low solubility of MFO 380 in the aqueous phase might be another major limitation to poor BDS of heavy oil. When MFO 380 was added into the aqueous phase, large particles or irregular balls were formed (Fig. 3a). A large amount of heavy oil was tightly pasted on the wall of flasks. Thus, sulfur compounds in heavy oil cannot contact and react with biocatalysts efficiently. Reducing the viscosity of bunker oil and increasing the contact of bunker oil with biocatalyst are the critical steps in the BDS process.

Enhanced solubility of bunker oil in water by adding surfactants

The surfactants were added to the aqueous phase to improve the solubility of heavy oil in water. Surfactants Triton X-100, PEG 2000 and Tween 20 were selected. After adding surfactant Triton X-100 or Tween 20, bunker oil MFO 380 could easily form an emulsion with the aqueous phase, while the solubility of heavy oil by adding PEG 2000 was not good.

Figure 3b shows the picture of MFO 380 in water after adding 2 % surfactant Triton X-100. It can be seen that the bunker oil could be fully mixed with aqueous phase after adding surfactant Triton X-100, and the mixing effect was better than the condition without adding surfactant (Fig. 3a). This indicates that adding surfactant Triton X-100 can increase greatly the mass transfer of heavy oil in water, which may have a significant influence on the BDS of heavy oil.

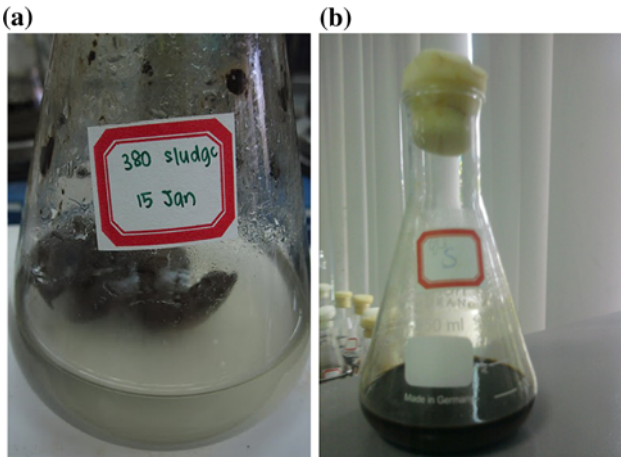

Fig. 3 Visual observation of oil–water mixing condition before **a** and after **b** adding surfactant Triton X-100

Enhanced biodesulfurization of bunker oil by adding surfactants

The surfactants Tween 20, Triton X-100 and PEG 2000 were used to investigate the influence of surfactants on the BDS of bunker oil MFO 380. The biocatalyst was enriched from oil sludge by using DBT as sulfur source. As shown in Fig. 4 and Table 2, the BDS efficiency of bunker oil was very low without adding surfactants (BDS). Only 2.88 % of sulfur in MFO380 was removed after 7 days of incubation. When the surfactants were added into the reaction system, removal efficiencies were significantly improved. The highest removal of sulfur was achieved by adding Triton X-100. When Triton X-100 was added, the sulfur content decreased from 2.73 to 1.32 % (wt/wt) and the removal efficiency of sulfur in MFO380 was 51.7 % after 7 days of incubation. The improvement of BDS could be attributed to greatly reduced viscosity of heavy oil and increased mass transfer of sulfur compounds in heavy oil into water by adding surfactants. A previous study reported that adding surfactants could improve the mass transfer of DBT between organic and aqueous phases in biphasic (O/W) systems, so that the BDS rate of DBT was improved (Wang et al. 2006).

From Table 2, it can be seen that the content of nitrogen, carbon and hydrogen also decreased slightly after the biotreatment. The ratio of H/C increased after the biotreatment when adding Triton X-100 or Tween 20. The possible reason is that the enriched biocatalyst is mixed bacteria, which have the ability of BDS, biodenitrogenation and biocracking simultaneously. In the process of BDS, polycyclic aromatics could be biodegraded which can decrease the carbon content. As a result, the ratio of H/C increased after the biotreatment.

In addition, the effect of adding toluene on BDS of bunker oil was also studied. The addition of toluene can greatly reduce the viscosity of bunker oil and can improve the separation of bunker oil in medium. The ratio of toluene to bunker oil was 5 ml/g. Figure 5 shows that the sulfur content decreases from 2.67 to 2.32 % by adding toluene, to 2.01 % by adding surfactant Triton X-100 and to 1.89 % by adding both toluene and Triton X-100 after 7 days of incubation. This indicates that adding toluene can improve

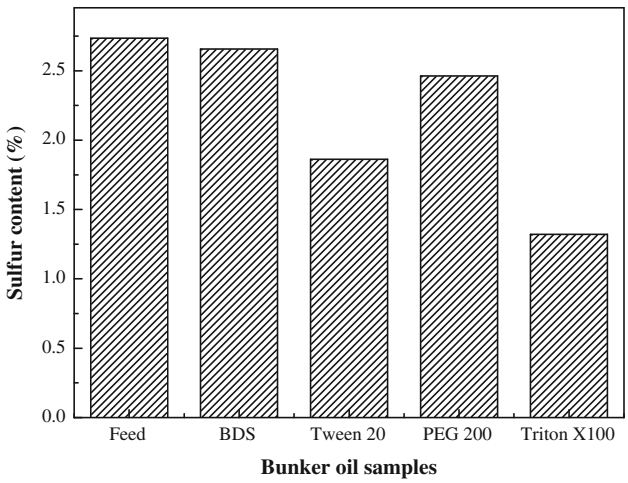

Fig. 4 Biodesulfurization of bunker oil by adding surfactants

Table 2 Elemental composition of bunker oil after BDS by adding surfactants (%)

Samples	N	S	C	H	H/C
MFO380	0.33	2.73	85.3	10.10	11.84
BDS	0.57	2.65	83.4	9.50	11.40
BDS + Tween 20	0.27	1.86	79.5	9.63	12.11
BDS + PEG 2000	0.30	2.46	83.4	9.73	11.67
BDS + Triton X-100	0.19	1.32	77.2	9.48	12.28

BDS biodesulfurization

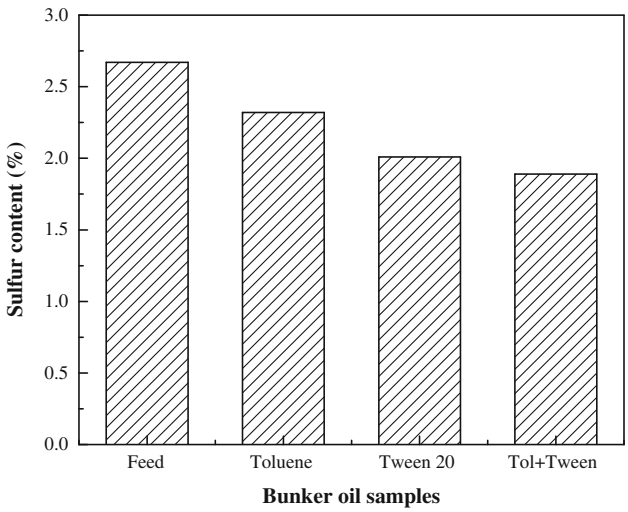

Fig. 5 Biodesulfurization of bunker oil by adding toluene

the BDS process. However, the improvement of bunker oil BDS by only adding toluene is lower than those by adding surfactants. This might be attributed to the toxicity of toluene to cells.

Effect of surfactant amounts on biodesulfurization of bunker oil

The amount of surfactants can influence both the cost of BDS process and the separation of heavy oil from the biotreatment system. The influence of the amount of Triton X-100 and Tween 20 on BDS efficiency of MFO 380 was investigated. The results (data not shown here) show that the optimal amount of surfactant Triton X-100 was 0.5 g per 50 ml medium while that of Tween 20 was 0.3 g per 50 ml medium. Too small amount of surfactant cannot provide enough emulsion for the BDS process, and thus, the mass transfer is limited. However, too much surfactant will cause damage to the biocatalyst and decrease the biological activity. Further investigation is needed to understand it better.

Conclusions

The feasibility of heavy oil BDS was studied with bunker oil MFO380. The biocatalyst was enriched with oil sludge using DBT as the sole sulfur source. The enriched culture could selectively degrade DBT into 2-HBP, which means that the BDS process follows the "4S" pathway. The heavy oil BDS results show that, without adding surfactants, the removal efficiency of sulfur in bunker oil MFO380 was only 2.88 % within 7 days. Adding surfactants was an efficient method to reduce the viscosity of heavy oil. When the surfactants were added into the reaction system, the BDS efficiency of heavy oil was significantly improved. Tween 20 and Triton X-100 were better surfactants than PEG 4000. Adding Triton X-100 achieved the highest sulfur removal efficiency 51.7 %. The optimal amount of surfactant Triton X-100 is 0.5 g per 50 ml medium while the optimal of Tween 20 is 0.3 g per 50 ml medium. Further investigation is needed to understand the role of surfactants in enhancing heavy oil BDS.

Acknowledgments The authors would like to express the deep appreciation to the Maritime and Ports Authority (MPA) of Singapore for the financial support.

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