Application of Microbial Enhanced Oil Recovery Technology in Water-Based Bitumen Extraction from Weathered Oil Sands

Mingshan Ding, Yan Zhang, and Juan Liu
School of Chemistry and Chemical Engineering, University of Chinese Academy of Sciences, Beijing 100039, P.R. China

State Key Laboratory for Oxo Synthesis and Selective Oxidation, Lanzhou Institute of Chemical Physic, Chinese Academy of Sciences, Lanzhou 730000, P.R. China

Weihong Jia, Bin Hu, and Sili Ren
State Key Laboratory for Oxo Synthesis and Selective Oxidation, Lanzhou Institute of Chemical Physic, Chinese Academy of Sciences, Lanzhou 730000, P.R. China

DOI 10.1002/aic.14483
Published online May 8, 2014 in Wiley Online Library (wileyonlinelibrary.com)

When using the water-based extraction processes (WBEPs) to recover bitumen from the weathered oil sands, very low bitumen recovery arisen from the poor liberation of bitumen from sand grains is always obtained. Application of microbial enhanced oil recovery (MEOR) technology in WBEPs to solve the poor processability of the weathered ore was proposed. It was found that processability of the microbial-treated weathered ore was greatly improved. The improved processability was attributed to the biosurfactants production in the culture solution, alteration of the solids wettability, degradation of the asphaltene component, and the decrease of the bitumen viscosity, which collectively contributed to the bitumen liberation from the surface of sand grains. Although it still has many issues to be solved for an industrial application of the MEOR technology in oil sands separation, it is believed that the findings in this work promote the solution to the poor processability of the weathered ore. © 2014 American Institute of Chemical Engineers *AIChE J*, 60: 2985–2993, 2014
Keywords: oil sands, microbial enhanced oil recovery, water-based extraction processes, wettability, viscosity, asphaltenes

Introduction

Oil sands, the so-called tar sands widely distributed throughout the world, are unconsolidated sand deposits impregnated with high-boiling viscous crude oil which is normally referred to as bitumen.[1] With the excessive consumption of conventional crude oil and the burgeoning worldwide demand for petroleum, exploiting bitumen from oil sand ores has attracted much attention from both the industry and researchers.[2–5] Currently, the major technology used to process oil sands is the water-based extraction processes (WBEPs), which is traced back to the Clark hot water processes.[6] In water-based bitumen extraction processes, two key steps are involved[7,8]: the bitumen is first liberated from the sand grains and then the liberated bitumen is aerated and floated to the top of slurry to form a bitumen-rich froth. Any factors, such as the solids wettability, bitumen viscosity, and the water chemistry affecting the bitumen liberation and aeration will have a significant influence on the bitumen final recovery. It has been extensively documented that the bitumen recovery and froth quality are closely related to the oil sands properties. For example, Liu et al[9] investigated the processability of one good processing ore and three poor processing ores using a Denver flotation cell. The results indicated that the good processing ore with hydrophilic sand grains has good bitumen recovery and froth quality, while the weathered ore containing strong hydrophobic solids exhibited an extremely poor processability with very low bitumen recovery and poor froth quality. Because of the high fines content (e.g., 40%) or high divalent cation concentration (e.g., the calcium ions content in the formation water is larger than 40 ppm.[10]), poor processability was also obtained for the high fines ore and high electrolyte ore, which was attributed to the poor bitumen liberation and serious slime-coating of the fine solids on the bitumen droplets or bubbles. These findings reflected that the oil sands processability was closely related to the ore types.

Among different types of oil sands, the weathered ore's reserve is abundant. Weathered oil sands is referred to as ore that has been exposed to external environment for an extended period e of time and/or is not deeply buried under the overburden.[11] The effect of weathering on oil sands processability has been reported recently.[9,12–16] By studying a laboratory weathered ore and a naturally weathered ore, a mechanism on the deteriorated processability of oil sands due to weathering was proposed by Masliyah and coworkers.[11,13] It was suggested that the formation water loss because of weathering led to a close contact of bitumen with the solids surface and hence resulted in the adsorption of organic matter. The increased hydrophobicity of the solids

Correspondence concerning this article should be addressed to S. Ren at slren@licp.cas.cn.

greatly enhanced the adhesion force between bitumen and solids and thereby made it hard for the bitumen to liberate from the sand grains. As a result, lower bitumen recovery and poor froth quality are always obtained on processing the weathered ores. Therefore, it is highly desirable to find an effective way to improve the processability of weathered ore.

Microbial enhanced oil recovery (MEOR) technology has been successfully applied in crude oil recovery. MEOR[17] is a way to utilize microorganisms or their metabolic products to help the recovery of crude oil from reservoirs,[18,19] which can be traced back to 1926. Systematic laboratory studies were not carried out until 1940s by Zobell.[18] Subsequently, the first field test was conducted in 1954.[19] From that time on, numerous engineers and scientists have left their track on the progress of MEOR technique. Today, in addition to conventional technologies for crude oil production,[20,21] MEOR technique has been an alternative way used in classical or modern tertiary enhanced oil recovery.[22–24] The MEOR technique takes many advantages, such as cost efficiency and environmental friendliness. The main mechanisms on how microorganisms enhance oil release from reservoir rock involve: (1) the bacterial metabolic products of biosurfactants could reduce the interfacial tension between liquid and solids and alter the surface wettability of solids[25,26]; (2) the microorganisms could degrade the heavy component of crude oil and thus improve the oil quality[27]; and (3) gas production could reduce the viscosity of the crude oil and thereby facilitating its flow. In consideration of the successful applications of MEOR technique in the crude oil fields and oil reservoirs, its potential applications in oil sands separation could be anticipated.

As pointed out from the reports that the low bitumen recovery and poor froth quality of the weathered ores are mainly attributed to the poor bitumen liberation from the sands and the slime coating of the bitumen droplets with fines.[9,11,13] The important reason leading to these problems is that the hydrophobic characteristic of the solid sands or the high bitumen viscosity.[11,28] However, as far as we know, there is no one effective method yet to solve the poor processability of the weathered oil sands. Considering that the MEOR processes could produce biosurfactants to alter the solids wettabiltiy and degrade the heavy component of oil to reduce the oil viscosity, the MEOR technique was first proposed to be applied in the water-based bitumen extraction from weathered ores. In this work, a weathered ore was microbial treated by placing it in the culture solution with strain for a period of time. It was found that the microbial treatment of the oil sands greatly decreased the hydrophobicity of the solids and reduced the bitumen viscosity by degrading the asphaltene component. As a result, the processability of the weathered ore was greatly improved. The findings indicated that the MEOR technology might find its application in the water-base bitumen extraction from the weathered oil sands.

Experimental

Materials

A weathered oil sand ore with a bitumen content of 10.4% used in this work was sampled from Zhalaiteqi, Inner Mongolia of China. A strain of *Pseudomonas aeruginosa* (GSICC 31614) used as the microorganism was purchased from Gansu Microbiology Save Center (Gansu, China). The strain was first acclimated in laboratory to adapt to the mineral culture. The objective strain was preserved on nutrient agar slants at 0°C and was subcultured every 3 months. The mineral salts medium used in this study was amended from that of reported,[29] and contained (g L^{-1}): $Na_2HPO_4 \cdot 12H_2O$, 11; KH_2PO_4, 3; $NaNO_3$, 5; NaCl, 1; $CaCl_2$, 0.1; $MgSO_4$, 0.25. The trace elements necessary for the microorganism growth consisted of the following (g L^{-1}): $FeCl_3 \cdot 6H_2O$, 0.08; $ZnSO_4 \cdot 7H_2O$, 0.75; $CuSO_4 \cdot 5H_2O$, 0.75; $MnSO_4 \cdot H_2O$, 0.75; H_3BO_3, 0.15. The C:N and C:P ratios in the feed solutions were kept constant at an optimum value to generate maximum biosurfactants production.[30] The pH of the culture solution was maintained at 6.8. All chemicals used are analytical reagent. Deionized water was used throughout the experiments. The capability of *P. aeruginosa* growing in oil sands using bitumen as sole source of carbon and energy was evaluated as described later.

Microorganism growth evaluation

The growth of microorganism in mineral medium using oil sands as the sole carbon source was studied and compared with that of using glucose at 30°C for 6 days. Determination of the biomass was conducted according to the describes elsewhere.[31,32] The optical density of diluted cultures was evaluated with a UV-Vis spectrophotometer by measuring absorbance of the strain at 650 nm. The measured absorbance of the sample was then converted to dry cell weight using a standard curve reported in the literature.[33] Briefly, 2 mL of the culture solution was drawn using a sterile syringe in a 50-mL volumetric flask followed by the addition of deionized water to reach the capacity. After shaking the bottle, the absorbance of the sample was determined.

Microbial treatment of the oil sands

Treatment of the weathered ore by the *P. aeruginosa* was performed in an Erlenmeyer flask with a total volume of 500 mL and a working volume of 300 mL. For each test, 300 g of oil sand ores was added to the 200 mL mineral salts medium in the flask, following the trace elements solvent and 1 g glucose were added in turn. The presence of glucose in the tests was to stimulate the microorganism growth and eliminate the short dormancy stage. The cultures were sterilized using the intermittent sterilization method and cooled to room temperature. Thereafter, 0.5 mL of the *P. aeruginosa* incubated 2 days with glucose was injected into the cultures under sterile conditions. The cultures were incubated on a rotary shaker at 30°C and 200 rpm.[34] To obtain appropriate incubation time, different incubation period of 7 days, 14 days, and 20 days was chosen.

Flotation tests

Bitumen flotation was carried out using a Denver flotation cell with a water jacket for temperature control. After cultivation, the mixtures including the 300 g microbial-treated oil sands and the upper solution were transferred to the 1-L cell. Extra deionized water was added to the cell to meet the requirement of 900 mL.[11] Then, the extraction processes was conducted at 60°C under mechanical agitation at 1500 rpm for 5 min. After conditioning, air was introduced at a flow rate of 150 mL/min and the bitumen froth was collected as a function of time for a total of 15 min. The gathered froth was then transferred to Dean-Stark for further bitumen, solids, and water content analysis. Moreover, to study the effect of the culture solution on bitumen recovery, the culture solution was separated from the microbial-treated oil sands by using filter after the cultivation. With sufficient deionized water added, the treated oil sands were floated according to the procedure described earlier.

Extraction of solids from oil sands and wettability characterization

Solids extracted from the oil sands were carried out by toluene washing according to the method reported in the literature.[11,35] Briefly, 50-mL toluene was added to 50 g ores and kept shaking for hours. The obtained mixtures were then centrifuged at 15 000 g for 30 min. After decanting the upper bitumen solution, fresh toluene was added to the solids and repeating the washing processes until the supernatant become colorless. The solids were then washed with ethanol and deionized water in turn. Finally, the solids were collected and dried under vacuum at room temperature.

Water drop penetration time (WDPT) measurements has been widely used to determine the water repellency of soil,[36] which is an effective way to evaluate the wettability of solids by examining the time of a water drop penetrating into a solids disk. It is believed that the longer the penetration time is, the more hydrophobic the solids will be.[35] In details, 2 g of fines ($<$50 μm) was compressed into a disk in a manual hydraulic press using a 20 mm diameter die. After reaching a value of 20 MPa, the disk was left undisturbed for 2 min. The solids disk sample was immediately used after being drawn out from the die. The wettability characterization was conducted on a contact angle analyzer (JC2000D3, Shanghai, China). A deionized water drop was placed on the pressed solids disk and a real-time video of the drop was recorded. Five measurements at different locations for each disk were conducted and average values were reported.

The solids wettability was also examined by partitioning the solids in the mineral oil phase and water phase.[11,35] Dried fines of 0.3 g was placed in a glass bottle followed by adding the mineral oil and deionized water with a volume ratio of 1:1. After shaking the bottle, the sample was left undisturbed for 5 min to allow phase separation. Then, the bottle was photographed and the water-wet fines were collected, dried, and weighed.

Measurements on surface tension of the culture solution

To examine the biosurfactants production during the microbial treatment of the oil sands, the surface tension of the culture solution was measured. The culture solutions for an incubation time of 7, 14, and 20 days were centrifuged at 15,000 rpm for 15 min, respectively, to eliminate the fine particles. The surface tension measurement was performed with pendent drop method using a surface tension analyzer (JC2000D3, Shanghai, China). Pure water was first used to calibrate the instrument at room temperature. After the surface tension measurement, the pH of the culture solution was also determined. All the measurements were performed three times and the average values were reported.

SARA fraction analysis

Crude oils including the heavy oil and bitumen generally contain four fractions of saturates, aromatics, resins, and asphaltenes, which were known as SARA. Great effort has been made on SARA separation and characterization of the structure and a number of ways were developed.[37–40] Among those approaches, a chromatography separation method based on ASTM D 2007[41] was used in this work. Prior to chromatographic separation analysis, asphaltenes need to be precipitated from other components. The procedure was described elsewhere.[42] Briefly, bitumen was dissolved in toluene and centrifuged at 15,000 rpm for 30 min to remove residual solids. Then, the toluene was left to volatilize gradually from the diluted bitumen by natural evaporation in a fume hood for 1 week. Two grams of the air dried bitumen was dissolved in *n*-heptane (40 cm^3/g). The *n*-heptane-diluted bitumen was stirred on a laboratory shaker for 2 h at room temperature and left 24 h for asphaltenes precipitation. Then, the supernatant was carefully decanted with a syringe. Repeating the washing processes at least 17 times until the supernatant appeared colorless. Then, the asphaltenes precipitates were put in a fume hood for a week to remove *n*-heptane under ambient conditions.

After removing the asphaltenes, the remanent components of the bitumen is normally defined as maltenes. Fractionation of maltenes was carried out on a silica/alumina column chromatography.[43–46] An open glass column of 2×60 cm^2 were packed with neutral alumina (200–300 mesh) in its lower half and silica gel (200–300 mesh) in its upper half. Silica gel was washed with toluene/dichloromethane/methanol (1:1:3, v/v) mixture and then dried. The neutral alumina and silica gel were activated for 12 h at 450 and 160°C, respectively, and left overnight before utilization. The sample of maltenes (0.2 g) was dissolved in 10 mL of *n*-heptane, which was mixed with 1 g silica gels and left to remove the *n*-heptane by natural evaporation. The sample was then introduced into the column chromatography apparatus and separated into saturated hydrocarbons, aromatics, and resins by a successive elution with series of mobile phase according to the modified chromatographic methods.[44] A total of 180 mL *n*-heptane was used to elute the saturate fraction, and a 140 mL solvent mixture of *n*-heptane/toluene (2:1) to elute the aromatics fraction. Finally, a total of 80 mL solvent mixture of toluene/dichloromethane/methanol (1:1:1) was used to desorb the resins fraction. After chromatography separation, each fraction was dried using a rotary evaporator to remove the solvent and weighed to determine the content.

Rheological characterization of the bitumen

The bitumen viscosity was evaluated with a controlled strain and stress rheometer (Ares G2, TA Instruments) at 50°C, using plate–plate geometry and an axial force controlled gap. Details on the measurement procedure were described elsewhere.[28] Briefly, the operation temperature was kept constant with an accuracy of ±0.5°C. Both transducer calibration and standard oil calibration were performed before the rheological characterization. No fluctuation was obtained, when conducting the transducer calibration which includes a torque procedure and normal procedure. The experimental conditions for the rheological experiments were validated using PDMS standard oil with viscosity of 24,990.0 Pa s at 30°C under the shear rate of 5.090 rad/s.

All the experiments including the microbial treatment of the oil sands, floatation tests, and various characterizations were repeated 3–4 times.

Results

Microorganism growth evaluation

The time course of the strain growth on different carbon source was shown in Figure 1. It was observed that the growth of strain with glucose as carbon source started

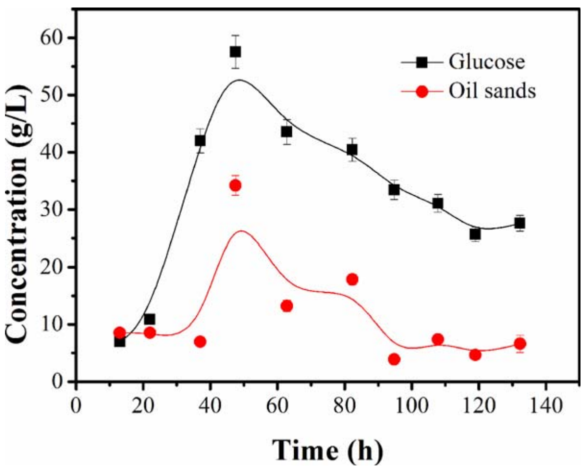


Figure 1. Growth curves of *P. aeruginosa* in the mineral medium containing glucose or oil sands as sole carbon source.

The strain growth was monitored by the optical density method. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

exponentially and reached a yield of 57.5 g/L after 50 h. While the strain growth with oil sands started with a short dormancy stage and reached a yield of 35 g/L. The results indicate that the glucose is more favorable to the strain growth comparing with the bitumen in oil sands. In other words, the glucose is more degradable by the microorganism than the bitumen, attributing to the fact that the aliphatic saturated hydrocarbon could be degraded more easily than the aromatics. The later is an important component of the bitumen. The decrease of both growth curves after 50 h indicates that the strain growth in both glucose and oil sands started to slow down, which might attribute to the excessive consumption of nutrition without extra supplement. The results clearly showed that the strain could use the bitumen as the carbon source for growth, although it had a lower growth in oil sands comparing with that in glucose. To stimulate the growth of strain in the oil sands and short the dormancy stage, a small amount of glucose was added during the cultivation of oil sands with the microorganism.

Processability of the microbial-treated oil sands

It is well-known that the processability of oil sands significantly depend on the characteristics of the ores.[9,11] The effect of MEOR technique on oil sands processability was evaluated using a Denver flotation cell. The bitumen recovery and froth quality (bitumen/solids ratio) as a function of flotation time are shown in Figure 2. It can be seen that the bitumen recovery increased with increasing the incubation time and good bitumen recovery was obtained for the microbial-treated ores. In contrast, the original weathered oil sands without treatment by the microorganism could not be processed at all using the WBEPs. In other words, no bitumen froth could be collected under the same processing conditions. Therefore, the floatation curve for the untreated ore was not offered in Figure 2. These results obviously indicate that the microorganism play significant role on the oil sands and could greatly improve the processability of the weathered ore. However, it is also noticed from inset of Figure 2 that the bitumen froth quality is still lower, indicating a lot of solids or fines were brought into the bitumen froth.

To better understand the effect of incubation time on the flotation efficiency, a first-order kinetics model was used to describe the flotation process.[47]

$$R = R_\infty(1 - e^{-kt})$$

The above equation shows the flotation recovery (R, %) as a function of flotation time (t, min). Where R_∞ (%) is the ultimate bitumen recovery and k is the flotation rate constant (min^{-1}). By best fitting the experimental data in Figure 2 to above equation, the flotation rate k was obtained. The results in Figure 2 show that a relative lower bitumen recovery of about 70% was observed for the microbial-treated ores with an incubation period of 7 days. Especially, the corresponding flotation rate constant is much lower and only about 0.09 min^{-1}. While for the ores with an incubation period of 14 and 20 days, the bitumen recovery could attain 93 and 98% with flotation rate constants of 0.18, and 0.19 min^{-1}, respectively. The flotation rate constant in the later two cases are more than 2 times than that for the ore treated with the incubation time of 7 days. Moreover, the bitumen froth quality was also slightly improved as increasing the incubation period (inset of Figure 2). These results indicated that appropriate extension of the incubation time of the ores could contribute to get a better oil sands processability. Therefore, if no special instructions, the microbial-treated oil sands with an incubation time of 20 days was used for the following studies.

To examine the function of the culture solution on bitumen recovery in the oil sands flotation, flotation tests of the microbial-treated ore and the original weathered ore were conducted using the culture solution or deionized water, respectively. The results in Figure 3 show that both the bitumen recovery and froth quality were considerably decreased when processing the microbial-treated ore without using the culture solution. Such result indicates that the culture solution play significant role on the oil sands processability. It is believed that biosurfactants produced by the microorganism

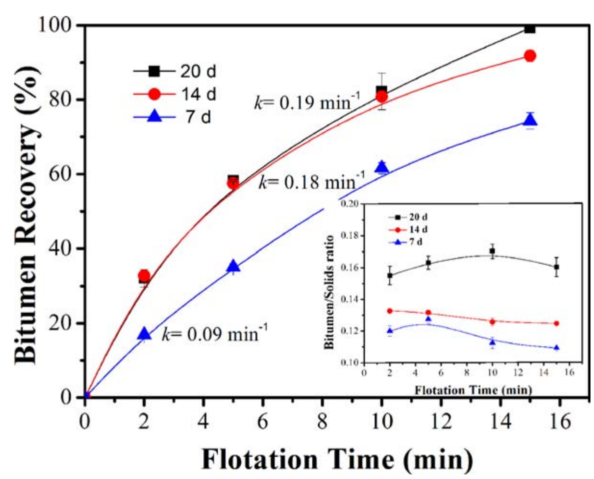
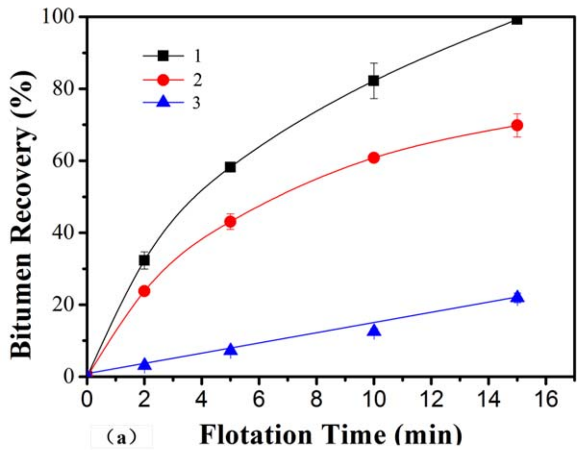


Figure 2. Effect of incubation time on bitumen recovery from the microbial-treated oil sands at pH = 8.2 and a temperature of 60°C (Solid lines represent the fitting profiles by a first-order kinetic model. In the Figure, *k* is the flotation rate constant).

Inset: the bitumen froth quality. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



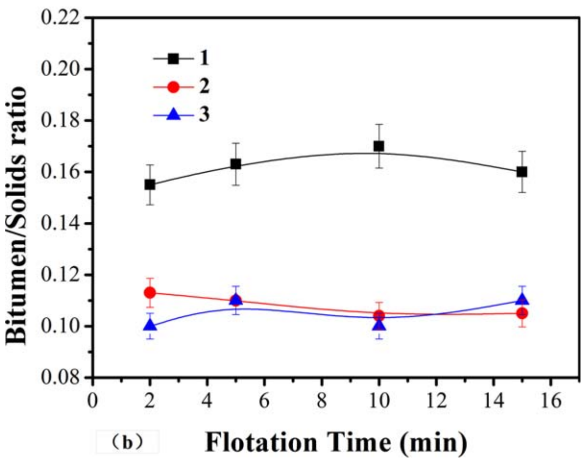


Figure 3. Effect of the culture solution on the bitumen recovery (a) and froth quality (b) when processing various ores at pH = 8.2 and temperature of 60°C. [(1) The solid square represents the microbial-treated ore processed with the culture solution; (2). the solid circle represents the microbial-treated ore processed with deionized water; and (3) the solid up triangle represents the original weathered ore processed with the culture solution].

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in the culture solution facilitated the bitumen liberation from sand grains. To confirm the biosurfactants production, the surface tension of the culture solution with different incubation times was measured and the results were shown in Figure 4. It was found that the surface tension of the culture solution decreased from 68.5 to 57.9 mN/m as the incubation time increased from 0 to 7 days. It further slightly decreased to a value of 53.0 mN/m as the incubation time extended to 20 days. The decrease of the surface tension indicates that biosurfactants was produced during the microbial treatment of the weathered oil sands. Variation of the solution pH was also examined. It was seen in Figure 3 that the pH of the culture solution gradually increased from 6.6 to 8.2 as increasing the incubation time. The increase of the pH might be related to the biosurfactants production, which was also in favor of the bitumen liberation from the solids.

However, it was also noted that the function of the culture solution on oil sands flotation was limited. For example, the bitumen recovery was still very low and only about 22% at 15 min when using the culture solution to process the original weathered ore (Figure 3). These findings indicated that besides of the function of biosurfactants in the culture solution, the microorganism might also have a significant role on the physicochemical properties of the solids and/or bitumen to improve the oil sands processability.

Examination of the solids wettability

It is well-known that the surface wettability of the oil sand solids play a significant role on the processability of the ores.[9,13] Therefore, the solids from various oil sands were toluene extracted and their surface wettability was analyzed. WDPT measurements were performed on various fine solids disks. It was found that the WDPT for the fine solids from the original weathered ore and microbial-treated ore were 204.8 and 62.8 s, respectively. It is believed that a longer water penetration time indicates a more hydrophobic surface of solids. Therefore, the large decrease of the WDPT reflected that the hydrophobicity strength of the solids was greatly reduced.

The solids wettability was also evaluated by partitioning the solids in the mineral oil phase and water phase and the results are shown in the inset of Figure 5. For a visual observation of the fine solids isolated from the original weathered ore, the oil phase was very cloudy and light-tight while the water phase was relative clear. For the fine solids isolated from the microbial-treated ore, both the oil and water phases were muddy. However, in a detail comparison, the water phase was a little bit cloudier than the oil phase. It was also observed that oil droplets coated with solids resided at the water–oil interface for both cases. Especially for the original ore solids, much of them were encapsulated in the oil phase forming solids-oil lump, which almost entered in the water phase because of the gravity. For a quantitative analysis of the solids partition, the solids resided in the water phase (water-wet solids) were collected and weighted. The results in Figure 5 showed that the content of the fines from the original weathered ore was 5.5%, while it was 55.2% for the fines from the microbial-treated ore. These findings indicated that the microbial treatment of the ore greatly reduced the solids amount in the oil phase reflecting the improvement of

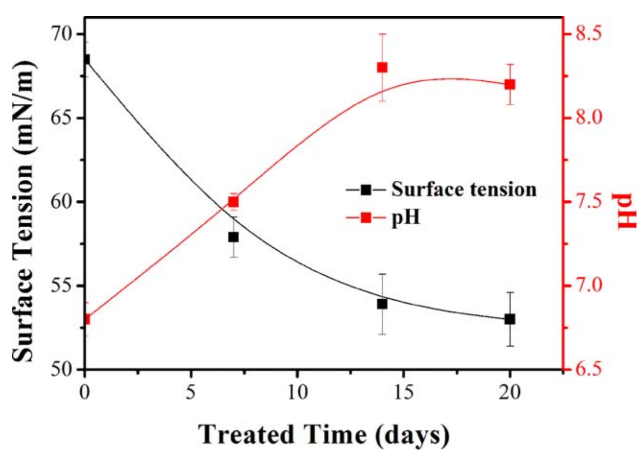


Figure 4. Measurements of the surface tension and pH value of the culture solution from the microbial-treated oil sands mixture.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

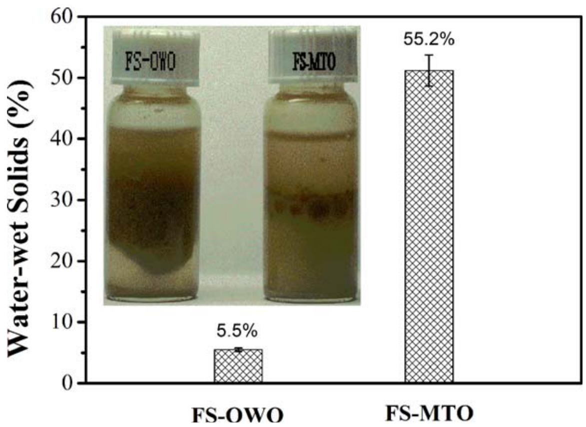


Figure 5. Qualitative and quantitative analysis for the partitioning test in the mineral oil and water phases of the fine solids isolated from the original weathered ore (FS-OWO) and the microbial-treated ore (FS-MTO).

Inset: photograph of the fine solids distribution in the water and mineral oil phases. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the solids wettability, which was in a good agreement with the results of the WDPT measurements.

It is believed that the alternation of the solids wettability was attributed to the washing effect of biosurfactants on the organics adsorbed on the solids surface. It has been reported that surfactants could be used for surface wettability alternation.[48] For example, the petroleum sulfonates were found effectively to clean the solids surface and then obtain a hydrophilic surface. Strong attractive force is always generated as the surfactants interacting with the adsorbed organic materials, which is responsible for the surfactants washing effect of removing the oil stain from the solids surface. Especially, the biosurfactants were found more effectively to reduce the surface/interfacial tension and the solids surface hydrophobicity compared with chemical surfactants.[15,18]

SARA fraction analysis

The content of asphaltenes in the bitumen was first determined using the *n*-heptane precipitation method. The result shows that the content of asphaltenes in the bitumen extracted from the original weathered ore is about $6.9 \pm 0.3\%$, while it decreases to $1.6 \pm 0.03\%$ for the bitumen extracted from the microbial-treated ore. Such result clearly indicates that most of the asphaltenes was degraded by the microorganism during the cultivation. Thereafter, the left maltenes were separated into Saturates, aromatics, and resins using chromatographic separation method. The results are shown in Table 1. It is interesting to find that the content of aromatics was not changed and maintained at about 11%, while it decreased from 46.4 to 40% for the saturates and increased from 31.9 to 39.9% for the resins after cultivation of the weathered ore. Decrease of the saturates fraction was attributed to its consumption as carbon source by the strains. As to the increase of the resins fraction, it might be mainly resulted from the degradation products of the asphaltenes. Such speculation is possible considering that resins and asphaltenes possess similar chemical structure and composition but only the resins have relative smaller molecules.[49–51]

Table 1. Various Fractions of the Maltenes in Bitumen from the Original Weathered Ore (MB-OWO) and Microbial-Treated Ore (MB-MTO), Respectively

Various fractions	MB-OWO	MB-MTO
Saturates (%)	46.4 ± 1.2	40.0 ± 2.6
Aromatics (%)	11.3 ± 1.0	11.2 ± 0.6
Resins(%)	31.9 ± 0.2	39.9 ± 1.6
[a]*Total recovery(%)*	89.6 ± 0.1	91.1 ± 0.8

[a]Total recovery: Total percentage of the maltenes that could be eluted by the mobile phase. The data indicated that there was about 10% of maltenes left in the chromatographic column.

Rheological characterization of bitumen

It is well-known that the viscosity of bitumen is an important factor affecting the bitumen liberation from the sand grains.[28,52] Effect of microbial treatment of the ore on bitumen viscosity was, therefore, examined. The rheological behavior of the original and treated bitumen at temperature of 50°C is shown in Figure 6. It was found that both the bitumen displayed a Newtonian rheological behavior at lower shear rate ($10^{-4}-10^{-1}$ s^{-1}) and a shear-thinning region at higher shear rate of over 10^{-1} s^{-1}. A similar phenomenon has been reported in the previous study, which was attributed to the reduce of the gel-type microstructure or the break-up of the continuous network of the asphaltene particles in bitumen at higher shear rate.[28] It was worthy to note that the viscosity of the microbial-treated bitumen decreased one order of magnitude comparing with that of the original bitumen. It is believed that the asphaltene molecules in bitumen are easy to be aggregated and surrounded with the resins. Because of their similar chemical structure, asphaltenes have strong interaction with resins through the hydrogen bonds or charge-transfer π—π bonds,[53] which is an important factor affecting the bitumen viscosity. Therefore, the great decrease of the bitumen viscosity might be attributed to the degradation of the asphaltenes. With decrease of the asphaltenes content in bitumen, the associated asphaltenes aggregation and asphaltenes-resins complex will be

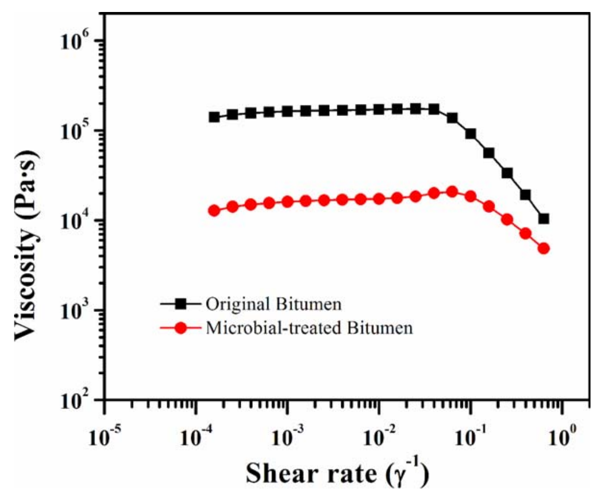


Figure 6. Rheological characterization of bitumen extracted from the original ore and microbial-treated ore.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

considerably reduced. As a result, the bitumen viscosity was greatly decreased.

Discussion

The WBEPs has been successfully used to recover bitumen from oil sands. However, very low bitumen recovery and poor froth quality are always obtained on dealing with the poor processing ore. For example, the weathered ore used in this work cannot be processed by using WBEPs at all. Application of MEOR technique in water-based bitumen extraction processes showed that the bitumen recovery could attain about 98% although the froth quality was still poor (Figure 2).

The flotation results in Figure 3 show that the culture solution plays important role on the oil sands processability. Surface tension measurement of the culture solution in Figure 4 clearly shows that the biosurfactants were produced by microorganism through secondary metabolism, which could reduce the oil–water interfacial tension and thereby help to the liberation of bitumen from the sand grains. In addition, the alteration of the solids wettability might play a more significant role on the processability of the weathered ore. It is well-known that the oil sands processability is closely related to the surface wettability of the solids. Colloidal force measurements showed that long-range attractive force and strong adhesion were always generated as bitumen interacting with hydrophobic solids, which resulted in a poor bitumen liberation from the sand grains and severe slime coating of the fine solids on the liberated bitumen or air bubble surfaces.[13,14] Therefore, with increasing the hydrophobicity of the solids, low bitumen recovery and poor froth quality are always obtained. In this work, the alteration of solids wettability from strong hydrophobic to weak hydrophobic or even to hydrophilic would greatly decrease the attractive force and adhesion between bitumen and sand grains, which is contributed to the bitumen liberation and then a good bitumen recovery. It has been extensively reported that the solids surface properties could be well controlled in solutions with proper pH value, metal ions, surfactants, or biodegradable polymers.[48,54–57] The wettability alteration of the oil sand solids might be attributed to the fact that most of the organic matters were removed from the solids surface through strong interaction between the biosurfactants and adsorbed hydrocarbons.

Decrease of the bitumen viscosity is another important factor contributing to the bitumen liberation from the sand grains. In hot-water process, the separation efficiency and the bitumen recovery are closely related to the bitumen viscosity.[52] Reduction of the bitumen viscosity to a recommended region by increasing the operating temperature and/or adding diluent could well facilitate the bitumen disengagement and displacement from the solids surface and result in a good bitumen liberation. It was also reported in our previous study that the bitumen liberation from a glass surface was greatly improved as the bitumen viscosity decreased by addition of diluents in the bitumen.[28] In this study, the great decrease of the bitumen viscosity enhanced the flowability of bitumen and then facilitated the bitumen liberation and recovery. The SARA fraction analysis showed that the decrease of the bitumen viscosity was attributed to the microbial degradation of the asphaltenes. It is well-known that the asphaltenes is a special fraction in crude oil or oil residues, which often poses numerous challenges during oil production, transport, and refining.[58–60] Therefore, the degradation of the asphaltenes improved not only the oil sands processability but also the bitumen quality.

The above discussion clearly shows that the MEOR technique is an effective way to increase the bitumen recovery, when using WBEPs to process the weathered ore. More importantly, the strain of *P. aeruginosa* used in this study did not consume too much bitumen as it greatly improved the processability of the weathered ore. It was found that the bitumen content only decreased 0.4% from 10.4 to 10.0% after the weathered ore was treated in the culture solution for 20 days. The loss of the bitumen was attributed to a trivial degradation of the saturates by the microorganism (Table 1). In contrast, the strain could well selectively degrade most of the asphaltenes. As the heaviest and least reactive compounds, asphaltenes exhibit complex bridged structures of naphthenic and aromatics comprising heteroatoms of sulfur and nitrogen.[61–64]

The strain of *P. aeruginosa* has been reported to degrade *n*-alkanes and polycyclic aromatic hydrocarbons (PAHs).[65–68] It was proposed that the *n*-alkanes was degraded via a terminal oxidation pathway, while the degradation of PAHs with three or four aromatic rings was through monooxygenation or dioxygenation of the carbon at certain position of the PAHs molecules.[65] It was also found that the strain could grow on 2-chlorobenzoate and a wide range of monohalogenated or dihalogenated benzoic acids. Chlorocatechols were believed the main intermediates for all chlorobenzoate catabolic pathways. It was suggested that the initial attack on chlorobenzoates was oxygen dependent and most likely mediated by dioxygenases.[66] Afferden et al.[68] isolated a strain of *P. aeruginosa* to desulfurize dibenzothiophene (DBT) and benzyl methyl sulfide (BMS). They believed that the biochemical mechanism on desulfurization of DBT and BMS were similar. The sulfur atom was first oxidized to sulfone and then the C═S bond was cleavaged via the formation of a chemically unstable hemimercaptal (S═O) by oxidation of the carbon linked to the sulfur atom. According to the above studies, we proposed that the degradation of saturates was performed via attacking C—C bond and the degradation of asphaltenes might occur via attacking various bonds of C—C, C═C, C—S, and C═S. As a result, cleavage of the long-chain alkanes and macromolecules of aromatic hydrocarbons were occurred leading to the decrease of molecular weight and then the reduction of bitumen viscosity.

Conclusions

MEOR technology was first applied in the WBEPs to recover bitumen from weathered oil sands. It was found that the oil sands processability was greatly improved and the bitumen recovery could attain about 98% after the weathered ore was treated in the culture solution with strain for 20 days. The improved oil sands processability was attributed to biosurfactants production in the culture solution, alteration of the solids wettability, degradation of the asphaltene component of the bitumen, and the decrease of the bitumen viscosity, which collectively contributed to the bitumen liberation from the surface of sand grains. At present, the poor processability of weathered oil sands is generally improved by increasing operating temperature or adding chemicals. Considering the massive energy consumption, greenhouse gas emission, and environmental pollution, microbial pretreatment of the ore is a more promising way to improve the oil sands processability as using WBEPs to recover bitumen from weathered ore. However, it should be pointed out that, for an industrial application of the MEOR technology in oil sands separation, it still has many issues to be solved. For example, the microorganism has strong selectivity and should be properly chosen and used according to the ore type and origin. Another issue is how to treat the oil sand ores using the strain on a field scale. Whether the oil sand ores are *in situ* treated by injecting the culture solution underground or treated after open mining ground should be well considered. Nevertheless, it is believed that the findings in this work promote the solution to the poor processability of the weathered ore. Therefore, MEOR technique might find its application in oil sands separation especially for the poor processing ore.

Acknowledgments

The authors acknowledge the financial support from the National Science Foundation of China (Grant No.51374195) and the "Top Hundred Talents Program" held by the Chinese Academy of Science.

Literature Cited

1. Masliyah JH, Zhou ZJ, Xu ZH, Czarnecki J, Hamza H. Understanding water-based bitumen extraction from athabasca oil sands. *Can J Chem Eng.* 2004;82(4):628–654.
2. Hirsch RL, Bezdek R, Wendling R. Peaking of world oil production and its mitigation. *AIChE J.* 2006;52(1):2–8.
3. Charpentier AD, Bergerson JA, MacLean HL. Understanding the Canadian oil sands industry's greenhouse gas emissions. *Environ Res Lett.* 2009;4(1):1–11.
4. Shah A, Fishwick R, Wood J, Leeke G, Rigby S, Greaves M. A review of novel techniques for heavy oil and bitumen extraction and upgrading. *Energy Environ Sci.* 2010;3(6):700–714.
5. Betancourt-Torcat A, Elkamel A, Ricardez-Sandoval L. Optimal integration of nuclear energy and water management into the oil sands operations. *AIChE J.* 2012;58(11):3433–3453.
6. Clark K, Pasternack D. Hot water seperation of bitumen from Alberta bituminous sand. *Ind Eng Chem.* 1932;24(12):1410–1416.
7. Hepler LG, Hsi C. *AOSTRA Technical Handbook on Oil Sands, Bitumen and Heavy Oils.* Edmonton, AB: Alberta Oil Sands Technology and Research Authority, 1989.
8. Hepler LG, Smith R. *The Alberta Oil Sands: Industrial Procedures for Extraction and Some Recent Fundamental Research.* Edmonton, AB: Alberta Oil Sands Technology and Research Authority, 1994.
9. Liu JJ, Xu ZH, Masliyah JH. Processability of oil sand ores in Alberta. *Energy Fuels.* 2005;19(5):2056–2063.
10. Kasongo T, Zhou ZA, Xu ZH, Masliyah JH. Effect of clays and calcium ions on bitumen extraction from Athabasca oil sands using flotation. *Can J Chem Eng.* 2000;78(4):674–681.
11. Ren SL, Dang-Vu T, Zhao HY, Long J, Xu ZH, Masliyah JH. Effect of weathering on surface characteristics of solids and bitumen from oil sands. *Energy Fuels.* 2009;23(1):334–341.
12. Su L, Xu ZH, Masliyah JH. Role of oily bubbles in enhancing bitumen flotation. *Miner Eng.* 2006;19(6):641–650.
13. Ren SL, Zhao HY, Dang-Vu T, Xu ZH, Masliyah JH. Effect of weathering on oil sands processability. *Can J Chem Eng.* 2009; 87(6):879–886.
14. Ren SL, Zhao HY, Long J, Xu ZH, Masliyah JH. Understanding weathering of oil sands ores by atomic force microscopy. *AIChE J.* 2009;55(12):3277–3285.
15. Wang LX, Dang-Vu T, Xu ZH, Masliyah JH. Use of short-chain amine in processing of weathered/oxidized oil sands ores. *Energy Fuels.* 2010;24(6):3581–3588.
16. Yang HQ, Wang YH, Ding MS, Hu B, Ren SL. Water-assisted solvent extraction of bitumen from oil sands. *Ind Eng Chem Res.* 2012; 51(7):3032–3038.
17. Lazar I, Petrisor IG, Yen TF. Microbial enhanced oil recovery (MEOR). *Pet Sci Technol.* 2007;25(11):1353–1366.
18. ZoBell CE. Bacterial release of oil from oil-bearing materials (Part 1). *World Oil.* 1947;126:36–47.
19. Yarbrough HF, Coty VF. Microbially enhanced oil recovery in the upper cretaceous nacatoch formation, Union County, AR. In: Donaldson EC, Clark JB, editors. *Proceedings of 1982 International Conference on MEOR.* OK: US Department of Energy, 1983;149–153.
20. Reddy PC, Karimi IA, Srinivasan R. Novel solution approach for optimizing crude oil operations. *AIChE J.* 2004;50(6):1177–1197.
21. Economides MJ, Nikolaou M. Technologies for oil and gas production: present and future. *AIChE J.* 2011;57(8):1974–1982.
22. Li QX, Kang CB, Wang H, Liu CD, Zhang CK. Application of microbial enhanced oil recovery technique to Daqing Oilfield. *Biochem Eng J.* 2002;11(2–3):197–199.
23. Soudmand-asli A, Ayatollahi SS, Mohabatkar H, Zareie M, Shariatpanahi SF. The in situ microbial enhanced oil recovery in fractured porous media. *J Pet Sci Eng.* 2007;58(1–2):161–172.
24. Thomas S. Enhanced oil recovery-an overview. *Oil Gas Sci Technol.* 2008;63(1):9–19.
25. Makkar R, Cameotra S. Biosurfactant production by a thermophilic Bacillus subtilis strain. *J Ind Microbio Biotechnol.* 1997;18(1):37–42.
26. Urum K, Pekdemir T. Evaluation of biosurfactants for crude oil contaminated soil washing. *Chemosphere.* 2004;57(9):1139–1150.
27. Widdel F, Rabus R. Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Curr Opin Biotechnol.* 2001;12(3):259–276.
28. Wang YH, Jia WH, Ding MS, Yang HQ, Hu B, Ren SL. Effect of diluent addition on bitumen liberation from a glass surface. *Energy Fuels.* 2012;26(2):1019–1027.
29. Sim L, Ward OP, Li ZY. Production and characterisation of a biosurfactant isolated from Pseudomonas aeruginosa UW-1. *J Ind Microbiol Biotechnol.* 1997;19(4):232–238.
30. Guerra-Santos L, Kappeli O, Fiechter A. Pseudomonas aeruginosa biosurfactant production in continuous culture with glucose as carbon source. *Appl Environ Microbiol.* 1984;48(2):301–305.
31. Rehmann K, Noll HP, Steinberg CEW, Kettrup AA. Pyrene degradation by Mycobacterium sp. *strain KR2. Chemosphere.* 1998;36(14): 2977–2992.
32. Rehmann K, Hertkorn N, Kettrup AA. Fluoranthene metabolism in Mycobacterium sp. strain KR20: identity of pathway intermediates during degradation and growth. *Microbiology.* 2001;147(10):2783–2794.
33. Moon HJ, Lim YK, Kim HS, Kwon DY, Chung WJ. Glycolipid biosurfactants produced by Pseudomonas aeruginosa D2D2 from diesel-contaminated soil. *J Microbiol Biotechnol.* 2002;12(3):371–376.
34. Robert M, Mercade ME, Bosch MP, Parra JL, Espuny MJ, Manresa MA, Guinea J. Effect of the carbon source on biosurfactant production byPseudomonas aeruginosa 44T1. *Biotechnol Lett.* 1989;11(12): 871–874.
35. Dang-Vu T, Jha R, Wu S-Y, Tannant DD, Masliyah J, Xu Z. Wettability determination of solids isolated from oil sands. *Colloids Surf A.* 2009;337(1):80–90.
36. Buczko U, Bens O. Assessing soil hydrophobicity and its variability through the soil profile using two different methods. *Soil Sci Soc Am J.* 2006;70(3):718–727.
37. Jewell DM, Weber JH, Bunger JW, Plancher H, Latham DR. Ion-exchange, coordination, and adsorption chromatographic separation of heavy-end petroleum distillates. *Anal Chem.* 1972;44(8):1391–1395.
38. Selucky ML. Quantitative analysis of coal-derived liquids by thin-layer chromatography with flame ionization detection. *Anal Chem.* 1983;55(1):141–143.
39. Aske N, Kallevik H, Sjöblom J. Determination of saturate, aromatic, resin, and asphaltenic (SARA) components in crude oils by means of infrared and near-infrared spectroscopy. *Energy Fuels.* 2001; 15(5):1304–1312.
40. Fan T, Buckley JS. Rapid and accurate SARA analysis of medium gravity crude oils. *Energy Fuels.* 2002;16(6):1571–1575.
41. ASTM D 2007. Standard test method for characteristic groups in rubber extender and processing oils by clay-gel adsorption chromatography method. 1993.
42. Zhang LY, Xu ZH, Masliyah JH. Langmuir and Langmuir-Blodgett films of mixed asphaltene and a demulsifier. *Langmuir.* 2003;19(23): 9730–9741.
43. Hirsch D, Hopkins RL, Coleman HJ, Cotton FO, Thompson CJ. Separation of high-boiling petroleum distillates using gradient elution through dual-packed (silica gel-alumina gel) adsorption columns. *Anal Chem.* 1972;44(6):915–919.

44. Šebor G, Blažek J, Nemer MF. Optimization of the preparative separation of petroleum maltenes by liquid adsorption chromatography. *J Chromatogr A*. 1999;847(1):323–330.
45. Kharrat AM, Zacharia J, Cherian VJ, Anyatonwu A. Issues with comparing SARA methodologies. *Energy Fuels*. 2007;21(6):3618–3621.
46. Shi Q, Hou DJ, Chung KH, Xu CM, Zhao SQ, Zhang YH. Characterization of heteroatom compounds in a crude oil and its saturates, aromatics, resins, and asphaltenes (SARA) and non-basic nitrogen fractions analyzed by negative-ion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Energy Fuels*. 2010;24(4):2545–2553.
47. Zhou ZA, Kasongo T, Xu ZH, Masliyah JH. Assessment of bitumen recovery from the Athabasca oil sands using a laboratory denver flotation cell. *Can J Chem Eng*. 2004;82(4):696–703.
48. Liu W, Jin Y, Tan X, Yeung A. Altering the wettability of bitumen-treated glass surfaces with ionic surfactants. *Fuel*. 2011;90(9):2858–2862.
49. Ortega-Rodríguez A, Cruz SA, Gil-Villegas A, Guevara-Rodriguez F, Lira-Galeana C. Molecular view of the asphaltene aggregation behavior in asphaltene-resin mixtures. *Energy Fuels*. 2003;17(4):1100–1108.
50. Speight J. Petroleum asphaltenes-Part 1: asphaltenes, resins and the structure of petroleum. *Oil Gas Sci Technol*. 2004;59(5):467–477.
51. Marques LC, Pereira JO, Bueno AD, Marques VS, Lucas EF, Mansur CE, Machado LC, Gonzalez G. A study of asphaltene-resin interactions. *J Braz Chem Soc*. 2012;23(10):1880–1888.
52. Hupka J, Miller JD, Drelich J. Water-based bitumen recovery from diluent-conditioned oil sands. *Can J Chem Eng*. 2004;82(5):978–985.
53. Luo P, Gu YG. Effects of asphaltene content on the heavy oil viscosity at different temperatures. *Fuel*. 2007;86(7):1069–1078.
54. Liu Q, Dong MZ, Asghari K, Tu Y. Wettability alteration by magnesium ion binding in heavy oil/brine/chemical/sand systems—analysis of electrostatic forces. *J Pet Sci Eng*. 2007;59(1):147–156.
55. Jiang TM, Hirasaki GJ, Miller CA, Ng S. Wettability alteration of clay in solid-stabilized emulsions. *Energy Fuels*. 2011;25(6):2551–2558.
56. Wang SQ, Segin N, Wang K, Masliyah JH, Xu ZH. Wettability control mechanism of highly contaminated hydrophilic silica/alumina surfaces by ethyl cellulose. *J Phys Chem C*. 2011;115(21):10576–10587.
57. Angle CW, Hamza HA. Effects of sand and process water pH on toluene diluted heavy oil in water emulsions in turbulent flow. *AIChE J*. 2009;55(1):232–242.
58. Dickakian G, Seay S. Asphaltene precipitation primary crude exchanger fouling mechanism. *Oil Gas J.(United States)*. 1988; 86(10):47–50.
59. Sheu EY, De Tar MM, Storm DA, DeCanio SJ. Aggregation and kinetics of asphaltenes in organic solvents. *Fuel*. 1992;71(3): 299–302.
60. Speight JG. *The Chemistry and Technology of Petroleum, Vol 114*. Boca Raton, FL: The Chemical Rubber Company Press, 2010.
61. Sheremata JM, Gray MR, Dettman HD, McCaffrey WC. Quantitative molecular representation and sequential optimization of Athabasca asphaltenes. *Energy Fuels*. 2004;18(5):1377–1384.
62. Ali FA, Ghaloum N, Hauser A. Structure representation of asphaltene GPC fractions derived from Kuwaiti residual oils. *Energy Fuels*. 2006;20(1):231–238.
63. Boek ES, Yakovlev DS, Headen TF. Quantitative mlecular representation of asphaltenes and molecular dynamics simulation of their aggregation. *Energy Fuels*. 2009;23(3):1209–1219.
64. Gray MR, Tykwinski RR, Stryker JM, Tan X. Supramolecular assembly model for aggregation of petroleum asphaltenes. *Energy Fuels*. 2011;25(7):3125–3134.
65. Zhang ZZ, Hou ZW, Yang CY, Ma CQ, Tao F, Xu P. Degradation of *n*-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated Pseudomonas aeruginosa DQ8. *Bioresour Technol*. 2011;102(5):4111–4116.
66. Hickey Wj, Focht Dd. Degradation of mono-, di-, and trihalogenated benzoic acids by Pseudomonas aeruginosa JB2. *Appl Environ Microbiol*. 1990;56(12):3842–3850.
67. Romero M, Cazau M, Giorgieri S, Arambarri A. Phenanthrene degradation by microorganisms isolated from a contaminated stream. *Environ Pollut*. 1998;101(3):355–359.
68. Afferden MV, Tappe D, Beyer M, Trüper HG, Klein J. Biochemical mechanisms for the desulfurization of coal-relevant organic sulfur compounds. *Fuel*. 1993;72(12):1635–1643.

Manuscript received Nov. 21, 2013, and revision received Jan. 26, 2014.