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Improving Bitumen Recovery from Poor Processing Oil Sands Using Microbial Pretreatment

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ABSTRACT: Low bitumen recovery and poor froth quality are always encountered when processing poor oil sands using water-based extraction processes. Application of microbial treatment of the ore prior to bitumen extraction was proposed to resolve the challenges and develop a more versatile and effective extraction process. Microbial treatment was carried out by placing a poor processing ore in the culture solution with strain of *Bacillus subtilis* for a restricted period of time. Flotation tests showed that an improved bitumen recovery of 97% was obtained after the poor ore was microbially treated. The wettability of solids was found to be changed from hydrophobic to hydrophilic, leading to a great decrease of the long-range attractive force and adhesion between bitumen and solids. Saturate, aromatic, resin, and asphaltene (SARA) fraction analysis indicated that the content of heavy components of asphaltenes and resins was decreased with an increase in saturate and aromatic fractions after the microbial treatment of the ore. This was well-correlated with the rheological characterization of bitumen. The improved processability was attributed to the biosurfactant production in the culture solutions, alteration of solids wettability, and decrease in bitumen viscosity, which collectively promoted the liberation of bitumen from the solids surface, and gives a better oil quality.

1. INTRODUCTION

Water-based extraction processes (WBEPs) have been successfully used to recover bitumen from Canadian oil sands. However, a low bitumen recovery and poor froth quality are always obtained when extracting bitumen from poor processing ores containing high electrolytes, high fines, and weathered ores.[1,2] It has been reported that bitumen recovery of the poor processing ores could be improved by increasing the operating temperature and solution pH or adding kerosene and/or short-chain amines in the slurry.[1−5] Increasing the operating temperature decreases bitumen viscosity and enhances its fluidity, leading to the improvement of bitumen liberation from the solids surface.[4−6] The addition of kerosene or short-chain amines could increase the hydrophobicity of the air bubble and, thus, decrease the induction time for the attachment of bubbles with bitumen droplets.[3,7] When using WBEPs to process oil sands, liberation of bitumen from sand grains is a key step for bitumen recovery from oil sands.[8] Efficient bitumen liberation is a prerequisite for obtaining a high bitumen recovery and good froth quality. It has been recognized that the wettability of solids in oil sands plays a significant role in bitumen liberation.[9,10] For example, the oil sands processability is often seriously deteriorated by weathering, which causes connate water evaporation and mineral oxidization, leading to a distinct hydrophobic feature of the solids and resulting in a poor bitumen liberation from the sand grains.[10,11]

Bitumen liberation can be accelerated by altering the solids wettability from hydrophobic to hydrophilic to increase the long-range repulsive force and decrease the adhesion force between bitumen and solids.[10] The solids surface wettability could be well-controlled in solutions with proper pH value, metal ions, biodegradable polymers, and/or surfactants.[12−14] Among them, biosurfactants are particularly interesting because of their high surface activity and biodegradability.[15−18] Biosurfactants are referred to as microbial compounds exhibiting good surface activity,[15] which include glycolipids, lipopeptides, polysaccharide−protein complexes, phospholipids, fatty acids, and neutral lipids.[16] Lipopeptides produced by *Bacillus subtilis* are well-known for their strong surface-active ability to reduce the surface tension of aqueous solution from 72 to 27 mN/m and oil/water interfacial tension less than 1.0 mN/m.[19,20] The strain of *B. subtilis* shows potential application in various fields such as tertiary recovery of oil, food, and health processing. It is believed that the produced surface-active compounds by the strain play a significant role on enhancing oil recovery.[20−23] The bacterium was injected into the sand-packed columns, contributing to a release of 35% residual oil, which was considerably larger than that of 21% using the nutrient solution.[20] The improved oil recovery was mainly attributed to the wettability alteration of the reservoir rock and the oil viscosity reduction by *B. subtilis*, which enabled the water flood to sweep out more oil from the capillary network.

In our previous study, a strain of *Pseudomonas aeruginosa* was applied to pretreat a weathered ore to improve bitumen recovery.[24] It was found that produced biosurfactants in the culture solution decreased solids hydrophobicity and bitumen viscosity collectively facilitated bitumen separation from the solids surface, leading to an improved bitumen recovery. However, the collected bitumen froth quality was still poor, which might be attributed to limited alteration of the solids surface wettability. Because the strain of *B. subtilis* has been

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successfully applied in tertiary oil recovery, it might find its application in processing surface-mined poor ores. In this paper, the effect of microbial pretreatment of a poor processing ore by a strain of *B. subtilis* to improve bitumen recovery was studied. The wettability of the solids separated from the microbial-treated ore was characterized. Changes in bitumen composition and viscosity were also determined to understand how the microbial pretreatment improves the processability of the poor processing ore. The findings show that the microbial treatment is an effective option to improve bitumen recovery from processing ores and might have its potential for industrial field application.

2. EXPERIMENTAL SECTION

2.1. Materials and Medium. Strain of *B. subtilis* (32811) purchased from the Gansu Microbiology Save Center was used to pretreat a poor processing oil sand without further acclimation. *B. subtilis* was preserved on nutrient agar slants at 4 °C to avoid biomutation and subcultured every 3 months. A poor processing ore with a bitumen content of 10.0% used in this work was sampled from Zhalaiteqi, Inner Mongolia of China. The preliminary oil sands extraction tests using a conventional water-based extraction method showed almost zero bitumen recovery from these ores. The composition of the ore was determined by the Dean−Stark apparatus. The mineral salts medium was selected according to the literature containing 4 g L^{-1} NH_4NO_3, 4.1 g L^{-1} KH_2PO_4, 14.3 g L^{-1} $Na_2HPO_4 \cdot 12H_2O$, 0.096 g L^{-1} $MgSO_4$, 0.0008 g L^{-1} $CaCl_2$, 0.0011 g L^{-1} $FeSO_4$, and 0.0015 g L^{-1} Na_2 ethylenediaminetetraacetic acid.[25] The C/N and C/P ratios in the feed solutions were kept constant at an optimum value, which resulted in maximum biosurfactant production.[26] The final pH of the medium was maintained at 6.8. All chemicals used were of analytical grade without further purification. Deionized water was used throughout the work to eliminate the possible influence of trace elements on the microbial growth and production.

2.2. Microbial Pretreatment of the Poor Ore. Microbial pretreatment of the poor ore was performed in an Erlenmeyer flask with a total volume of 500 mL and a working volume of 300 mL. Before pretreatment of the ore, *B. subtilis* was cultivated in mineral medium with glucose as a carbon source. Then, the culture was incubated in a shaking incubator at 30 °C and 150 rpm. After 48 h, 0.5 mL of the culture was injected into the sterile Erlenmeyer flask under sterile conditions, in which a mixture of 300 g of oil sands, 200 mL of mineral medium, and 1 g of glucose had been added. The presence of glucose was to stimulate the microorganism growth. The pretreatment of the poor ore was conducted in an incubator at 30 °C and 120 rpm. To obtain an appropriate incubation time, different incubation periods of 7, 10, 13, 17, 21, 27, 33, and 51 days were chosen.

2.3. Measurements on Surface Tension of the Culture Solution. Prior to evaluating the effect of microbial treatment of the ore on bitumen recovery, surface tension of the culture solution was measured to examine the biosurfactant production during incubation. The culture solutions for different incubation times were centrifuged at 15000*g* for 15 min to remove the bacterial cells and various particles. Measurement of the surface tension was then performed with a pendent drop method at room temperature using a contact angle analyzer (JC2000 D3, Shanghai, China). During the measurement, a water droplet was extruded and kept for a while to attain equilibrium conditions. To calibrate the instrument, the surface tension of deionized water was first measured. After the measurement, the pH of the culture solution was also analyzed. All of the measurements were performed 3 times, and the average values were reported.

2.4. Flotation Tests. After measurements of the surface tension and pH of the culture solution, the oil sands mixtures were transferred to a Denver flotation cell to carry out the flotation test. Extra deionized water was added to the cell to meet the requirement of 900 mL. The pH of the slurry for the flotation test was maintained at 8.0−8.5. The slurry was conditioned under mechanical agitation at 1500 rpm and 60 °C for 5 min. After conditioning, a flow rate of 150 mL/min air was then introduced and bitumen froth was collected as a function of time for a total of 15 min. The composition of collected bitumen froth was then determined using a Dean−Stark method with toluene as the reflux solvent.[27]

2.5. Extraction of Solids from Oil Sands and Wettability Characterization. It is well-known that oil sands processability is closely related to the solids wettability. The wettability of solids isolated from the microbial-treated ore was examined. Two methods, i.e., water drop penetration time (WDPT) measurement and particles partition test, were performed to examine the wettability of the fines from the microbial-treated ore. The solids extraction, disc preparation, WDPT measurement, and particles partition test were conducted according to procedures proposed by Dang-Vu et al.[28] The solids were isolated from various ores using a toluene washing method. In brief, the ores were first washed with toluene repetitively until the upper supernatant became colorless and then washed exhaustively with ethanol and deionized water in turn. After being washed, the solids were collected and dried under vacuum at room temperature. For wettability measurements, fine solids with a size of less than 50 μm were screen-separated and used.

The WDPT measurement has been applied in determining water repellency of soil in soil science.[29] The solids wettability was determined according to the penetration time for a deionized water droplet completely penetrating into a solids disc. It is believed that, the longer the penetration time, the more hydrophobic the solids. To prepare a disc, 2 g of the fines was compressed in a manual hydraulic press apparatus using a 20 mm diameter die. The disc was left undisturbed for 2 min under a pressure of 20 MPa. The deionized water droplet was placed on the disc to determine the solids wettability via a contact angle analyzer (JC2000 D3, Shanghai, China) with a real-time video. Five discs of each sample were used for the measurements, and the average value was reported.

For fine solids in the oil/water partitioning test, 0.3 g of fines was placed in a glass bottle. A total of 4 mL of deionized water and 4 mL of mineral oil were added to the bottle. After the bottle was sufficiently shaken, it was left undisturbed for 5 min to allow for phase separation. The bottle was then photographed. For a quantitative analysis, the upper oil phase was removed from the bottle and the fines remaining in the water phase was collected, dried, and weighed.

2.6. Saturate, Aromatic, Resin, and Asphaltene (SARA) Fraction Analysis. As a complex mixture of molecules ranging from nonpolar saturated hydrocarbons to highly polar polynuclear aromatics, crude oils, including heavy oil and bitumen, are usually reported on a weight percentage of saturates, aromatics, resins, and asphaltenes (known as SARA).[30−32] Several techniques have been proposed to fractionate the bitumen, all of which provide either rapid or bulk fractionation. However, no agreement is reached currently because of the complexity of the available methodologies and the lack of understanding on their correlation.[33] A classical chromatography separation method based on ASTM D2007 allows for obtaining sizable quantities and, hence, was employed in this work. The detailed procedure was described in our previous study.[24] Briefly, asphaltenes need to be precipitated from the bitumen prior to chromatographic separation analysis. The precipitation test was carried out with *n*-heptane as the solvent at room temperature. The remnant components of the bitumen were then obtained and referred to as maltenes. The maltenes were then separated in an open glass column (2 × 60 cm), where neutral alumina (200−300 mesh) and silica gel (200−300 mesh) were placed successively. Prior to using, the silica gel was purified by washing with a toluene/dichloromethane/methanol (1:1:3, v/v/v) mixture and then dried. The neutral alumina and silica gel were activated for 12 h at 450 and 160 °C, respectively, and then left overnight before use. A total of 0.2 g of maltenes was dissolved in 10 mL of *n*-heptane and mixed with 1 g of silica gel to load the sample for separation. After evaporation of *n*-heptane, the sample was then transferred into the open column for SARA fraction analysis. A series of mobile phases, including 180 mL of *n*-heptane, 140 mL of a solvent mixture of *n*-heptane/toluene (2:1), and 80 mL of a solvent mixture of toluene/dichloromethane/methanol (1:1:1), was successively used to elute the saturates, aromatics, and resins. Each fraction was dried using

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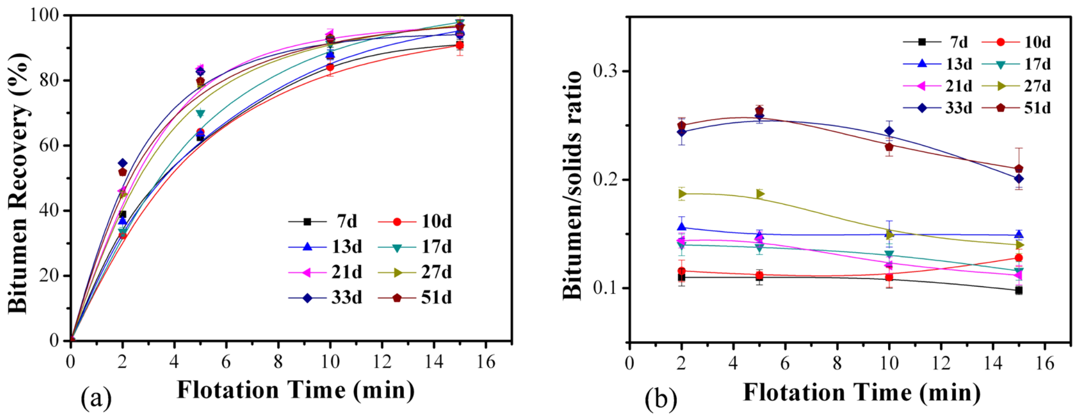

Figure 1. Effect of the incubation time on the (a) bitumen recovery and (b) froth quality when processing the microbial-treated oil sands at pH 8.2 and temperature of 60 °C.

a rotary evaporator and then weighed to calculate the final percentage composition.

2.7. Analysis on Degradation of Asphaltenes. For an analysis on the degradation of the asphaltenes, the asphaltenes were first separated from the bitumen and then treated by the strain. Precisely weighed 1 g asphaltenes were dissolved in 3 mL of toluene. Clean sand grains with a size of 50−250 μm were then added to the asphaltene solution to adsorb the asphaltenes. After volatilization of toluene, the imitated "oil sand" was added to a 250 mL Erlenmeyer flask, followed by the addition of 100 mL of mineral media, 0.2 mL of trace element solution, and 1 g of glucose. After sterilization, the strain of *B. subtilis* was implanted and the mixtures were incubated for 33 days at 30 °C and 150 rpm. After the water solution was filtered, microbial-treated "oil sand" was obtained, from which the microbial-treated asphaltenes were extracted with toluene. The recovered asphaltenes were first weighed to determine the mass loss, which was attributed to the degradation by the strain. SARA analysis on the microbial-treated asphaltene composition was then conducted according to the method mentioned before.

2.8. Rheological Measurements. Rheological characterization of bitumen was carried out with a controlled strain and stress rheometer (Ares G2, TA Instruments) at 60 °C, using plate−plate geometry and an axial force controlled gap. The measurement procedures were described in detail previously.[5] Briefly, before the rheological measurements, transducer calibration and standard oil calibration were first performed in turn. The transducer calibration included a torque procedure and a normal procedure, both of which were normal. The experimental conditions and operating parameters for the rheological characterization were validated by measuring the viscosity of the polydimethylsiloxane (PDMS) standard oil. The agreement of the result with the recommended data was within ±3%. The operating temperature was controlled at 60 °C with stability better than ±0.5 °C.

2.9. Force Measurements. Surface force measurements were carried out with an atomic force microscope (AFM, Multimode 8, Bruker, Billerica, MA). The AFM probes were prepared by gluing a fine solid particle (∼8 μm) onto a standard silicon nitride AFM cantilever (Bruker, Billerica, MA) using a two-component epoxy (EP120, 3M). The fine solids isolated from the untreated poor ore and the microbial-treated ore with an incubation time for 33 days were used and referred to as fines 1 and 2. Cantilever with a nominal spring constant of 0.35 mN/m was used and calculated using a thermal tune method during the force measurements. The glued probe particle was kept in a particle-free environment for more than 24 h before being used. Single-crystal silicon pieces with a size of 1.2 × 1.2 cm^2 were used as the substrate for preparing the bitumen surface. Prior to coating the bitumen, the silicon pieces were cleaned by immersing them in a piranha solution [a mixture of 7:3 (v/v) 98% H_2SO_4 and H_2O_2] at 90 °C for 30 min. The cleaned substrates were rinsed with enough ultrapure water and then blow-dried with ultrapure nitrogen. The silicon substrates processed as such possess a strong hydrophilic surface with a water contact angle of almost 0°. To have a stable bitumen coating, the hydrophilic substrate was first hydrophobized according to the literature.[34] The bitumen layer was then spin-coated on the hydrophobic silicon substrate with a spin coater. About 10 drops of the prepared bitumen/toluene solution (2.5 mg/mL) were dropped slowly within 20 s onto the substrate spinning at 4000 rpm and then kept for 30 s. The prepared bitumen surface was then used for the colloidal force measurement immediately.

The interaction forces between a fine particle and the bitumen surface were carried out in 1 mM KCl solution at pH 8.0−8.5. In the experiment, the solution was injected into a fluid cell slowly to avoid trapping air bubbles. Both the probe and bitumen surface were immersed in the solution for about 30 min to equilibrate the system. The detailed process of the colloidal force measurement can be found elsewhere.[10,35−37] Briefly, the long-range interaction force was recorded when the piezo stage brought the bitumen surface to approach the probe particle. A pull-off force was generated as the bitumen surface departed from the probe particle. The measured pull-off force that the probe particle needed to detach from the bitumen surface was regarded as the adhesion force. In each test condition, four bitumen−fine pairs were repeated and the measurements were carried out at different locations on the bitumen surface to ensure that the measured force profiles were representative. Adhesion forces for each test condition were reported from the statistical value calculated using more than 100 retraction profiles. For quantitative comparison, both the measured interaction forces and adhesion forces were normalized with the mean radius R of the probe particle. All of the experiments were conducted at room temperature of 22 ± 1 °C.

3. RESULTS AND DISCUSSION

3.1. Flotation Test. To study the effect of microbial treatment on bitumen extraction from the poor ore, the microbial-treated ore with different culture periods was processed using a Denver flotation cell. As shown in Figure 1a, bitumen recovery from the microbial-treated ore with an incubation period of 7 days could reach 90%, which increased to 94% when the incubation period was prolonged to 21 days or more. In contrast, no bitumen froth was obtained when processing the untreated poor ore. In other words, the bitumen recovery was zero for the original poor ore processed using WBEPs, and the result was not shown in Figure 1. Those findings suggest that microbial pretreatment of the ore was an effective way to recover bitumen from poor processing oil sands. The bitumen froth quality was also analyzed. The results shown in Figure 1b indicated that the bitumen froth quality was relative poor, especially for those of the incubation period less than 33 days.

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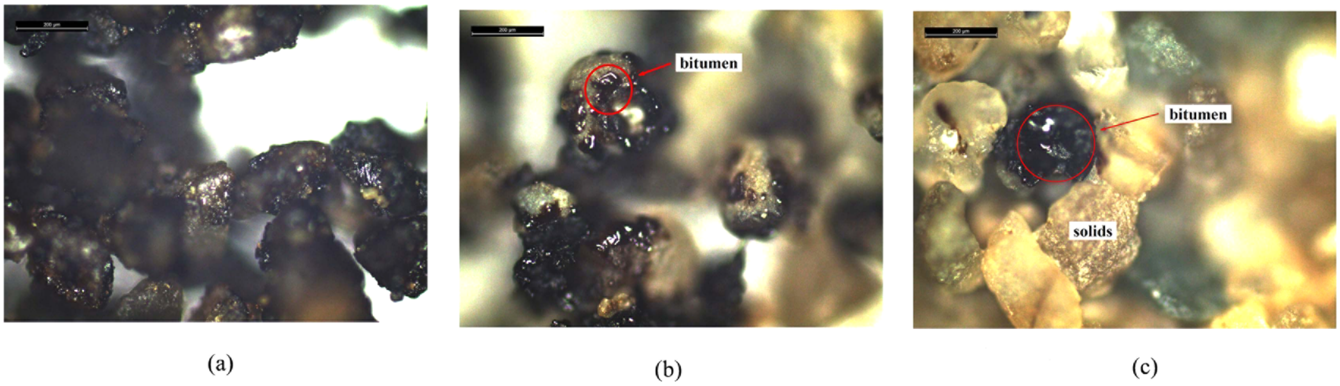

Figure 2. Surface morphologies of the (a) microbial-treated oil sands, (b) bitumen froth collected before aeration, and (c) bitumen froth collected after aeration for 5 min, when processing the microbial-treated ore at pH 8.0–8.5 and a temperature of 60 °C.

To confirm whether the bitumen has been successfully released from the sand grains during the flotation, the bitumen froth was examined with a microscope (Leica, DM2500P). The results in Figure 2 showed that the sand grains were covered with a bitumen layer for the oil sand ore (Figure 2a). When the ore was conditioned under mechanical stirring in the water solution, ablation and recession of bitumen occurred and resulted in bitumen droplets on the sand surfaces (Figure 2b). After the air bubbles were introduced into the slurry, the bitumen droplets were released from the sand surface, leaving most of the solids exposed to the outside (Figure 2c). These findings indicated that the bitumen could be well-separated with the sand solids during the flotation. However, there were still lots of solids brought into the bitumen froth, which possibly resulted from hydrodynamic cavitation.[38] The existence and formation of the tiny bubbles generated by hydrodynamic cavitation were found to increase the contact angle of the solids and, hence, attachment force.[38] In addition, a large quantity of foams was produced during the flotation, which promoted the transfer of solids from the slurry to the bitumen froth. Generation of the large quantity of the foams was attributed to the biosurfactants produced through microbial metabolism during the pretreatment of the oils sands by the strain. Another possible reason was from the device equipment, which is a modified product from a mineral flotation cell. Some process parameters, such as the agitation mode and bubble size, may not be exactly suitable for the oil sands separation.

3.2. Examination of the Solids Wettability. It is well-known that the solids wettability plays a significant role in both bitumen liberation and aeration.[1,2] High bitumen recovery can be obtained even at a lower temperature of 35 °C when processing a good ore, in which the solids are hydrophilic.[2] Low bitumen recovery and poor froth quality are always encountered when processing poor ores, such as the weathered ore.[1,2] Therefore, investigation on changes of the solids wettability is of great importance to understand how the microbial pretreatment improves the bitumen recovery from poor processing ores. The solids wettability was thereby examined by measuring the water droplet penetration time and partitioning of the fine solids between mineral oil and water phases. Figure 3 shows the time that a water drop needed to penetrate into a fines disc. It was found that the solids extracted from the original poor ore gave a penetration time of 205 s, which was comparable to that of a weathered ore reported in the literature.[9] The penetration time greatly decreased when the poor ore was microbially treated for a given incubation period. Especially, it decreased to less than 10 s when the incubation time was extended to over 21 days. It was believed that a shorter penetration time represented a more hydrophilic surface of the solids. Therefore, the great decrease of the penetration time with the incubation period over 21 days indicated that the solids wettability had been changed from hydrophobic to hydrophilic.

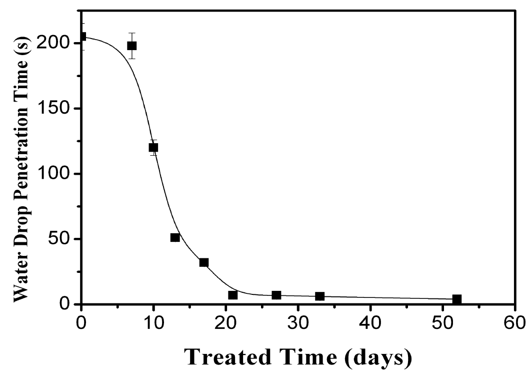

Figure 3. Water drop penetration time for fines extracted from the microbial-treated ore with different incubation periods.

Visual observation about the partition of fine solids between mineral oil and water phases is shown in the inset of Figure 4. Obviously, the fine solids extracted from the untreated poor ore mainly resided in the mineral oil, while they were mainly in the water phase for the microbial-treated ore. The amount of solids

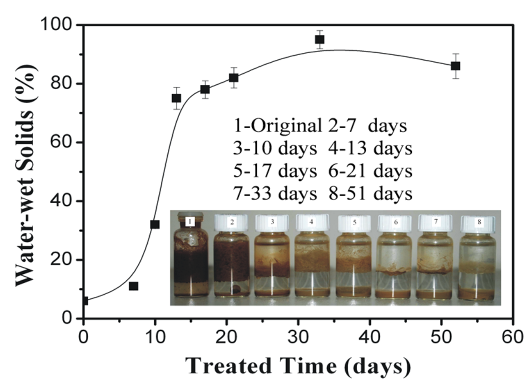

Figure 4. Partition of the fine solids between the mineral oil and water phases. The solids were extracted from the untreated poor ore or the microbial-treated ores with different incubation periods. (Inset) Observation of the fine solids distributed in the water and oil phases.

in the aqueous phase was increased with increasing the incubation time. For a quantitative analysis (Figure 4), the fine solids from the untreated poor ore that resided in the aqueous phase were less than 10%, while they were over 80% for solids extracted from the microbial-treated ore with an incubation time over 21 days. With the incubation time less than 10 days, the percentage of fines in the aqueous phase was comparable to that of reported weathered ores.[9] With increasing the incubation time over 21 days, the percentage of fines in the aqueous phase had been comparable to that of the reported good processing ore.[28] These results were consistent with that of the water penetration time measurement, indicating that microbial treatment of the ore is an effective technique to alter the solids surface wettability and then improve the processability of poor ores. It should be pointed out that bitumen recovery could also be improved by adding hydrophobic particles in the slurry.[39] On the premise that the bitumen had been liberated from the sand grains, the added hydrophobic particles could facilitate coagulation of the liberated bitumen and increase the attachment probability of bitumen aggregates with air bubbles. As a result, the added carriers were found useful to improve the aeration efficiency. However, for oil sands used in this work, it is not easy for bitumen to liberate from the hydrophobic solids surface. Therefore, alteration of the solids wettability from hydrophobic to hydrophilic accelerated the bitumen liberation and, hence, a high bitumen recovery.

It has been reported that the organics on the solids surface could be well-washed away by surfactants.[13] In comparison to the chemical surfactants, the biosurfactants were found more effectively to reduce the solids hydrophobicity.[40] To confirm the generation of the biosurfactants, the surface tension and pH of the culture solution were determined and the results are shown in Figure 5. It is found that the initial surface tension of

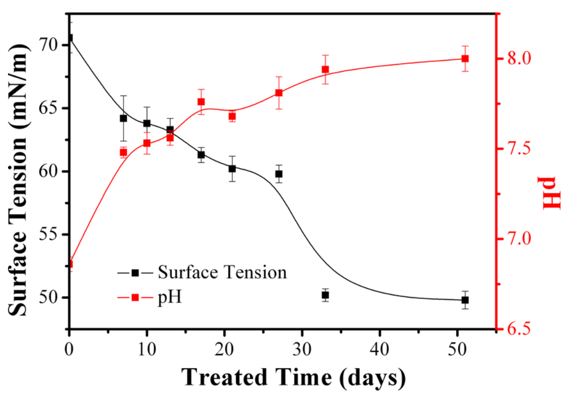

Figure 5. Measurements of the surface tension and pH value of the culture solution from the microbial-treated oil sands mixture.

the culture solution is about 71 mN/m, which is not significantly different from that of the deionized water, indicating that surface-active compounds were not produced at the beginning of the cultivation process. The surface tension gradually decreased with extending the incubation time and attained about 50 mN/m at the incubation time over 33 days. It is also noticed that the pH of the culture solution increased from about 6.9 to about 8.0, with the incubation time extending to over 33 days, which was related to the biosurfactant production. These findings clearly showed that bacterial growth could be well-accomplished in the culture medium and surface-active compounds were produced during the microbial treatment of the oil sands. Therefore, the variation of the solids wettability was believed to result from the biosurfactant production during the microbial treatment of the oil sands.

In addition to the effect on the solids wettability, the biosurfactants could also greatly affect the interfacial tension of bitumen with the aqueous phase.[18] The produced biosurfactants might adsorb on the bitumen and mineral solids surfaces, resulting in a decrease of the interfacial tension. It has been reported that bitumen release from the sand grains strongly depends upon the bitumen–water interfacial tension. The bitumen recovery could be enhanced by decreasing the bitumen–water interfacial tension.[41,42]

3.3. SARA Fraction Analysis.

During the cultivation of the oil sands, the microorganisms grew and reproduced using the bitumen as the sole carbon source. Therefore, the bitumen loss after cultivation was analyzed. It is found that the bitumen content in the oil sands decreased from 10.0 to 9.0% after the ore was treated for an incubation time of 33 days. In other words, 10% of the bitumen was consumed by the microorganisms during the cultivation of the ore. The SARA fraction of various bitumens was further analyzed. The asphaltene content in the bitumen was first determined using the *n*-heptane precipitation method. The result of Figure 6 shows

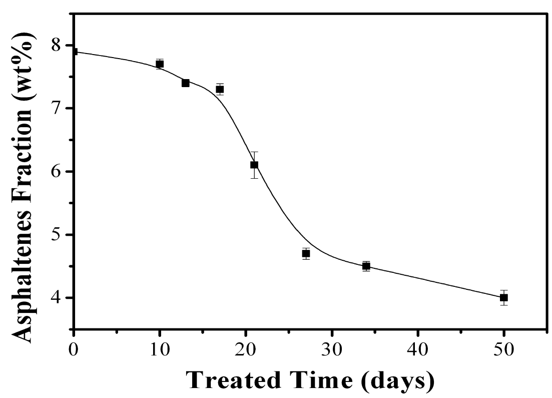

Figure 6. Asphaltene content in various bitumen from the untreated poor ore and microbial-treated ores with different incubation periods.

that the asphaltene content gradually decreased from about 7.9 to about 4.1% with extending the incubation time to 51 days. Such a result indicated that the asphaltene component was biodegraded during the treatment of the oil sands by the strain, leading to the improvement of the bitumen quality. After the asphaltene was removed, the left components of maltenes were separated into saturates, aromatics, and resins to have a more detailed view on the variation of the bitumen. The contents of various components in bitumen are shown in Table 1. It is observed that the saturate content was about 37% for bitumen from the untreated ore, which gradually increased after the ore was treated by the strain and attained about 46.4% for ore with an incubation time of 33 days. The aromatic content was also correspondingly increased from 9.6 to 15–19%. With regard to the content of resins, it increased first from 35.5 to 46.6 or 45.2% for a relatively short incubation time of 10 or 21 days and then decreased to 34.8% for a long incubation time of 33 days. It is also interesting to find that part of maltenes for the untreated bitumen was left in the chromatographic column. This might be attributed to the high polar molecules, such as the resins, which strongly adsorbed on the neutral alumina or

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Table 1. Various Fractions of the Maltenes in Various Bitumen from the Untreated Poor Ore and Microbial-Treated Ores with Different Incubation Periods

various fractions	original	10 days	21 days	33 days
saturates (%)	37.0 ± 1.5	38.1 ± 1.3	39.4 ± 3.1	46.4 ± 2.7
aromatics (%)	9.6 ± 0.1	15.3 ± 1.8	15.4 ± 1.6	18.8 ± 1.1
resins (%)	35.5 ± 0.5	46.6 ± 1.3	45.2 ± 3.8	34.8 ± 2.9
total recovery (%)[a]	82.6	100	100	100

[a]Total recovery: total percentage of maltenes that could be eluted by the mobile phase.

the silica gel and could not be eluted by the solvents. In contrast, the microbial-treated maltenes could be fully recovered, indicating that the molecules with high polarity were degraded by the strain during the cultivation of the ore. It is believed that resins and asphaltenes possess a similar chemical structure composed of the aliphatic chain and aromatics, but the resins has relatively smaller molecules.[43] Therefore, the increase of the resin content in the microbial-treated bitumen with a relatively short incubation time of 10 or 21 days was attributed to the degradation of asphaltenes to resins. With extension of the incubation time to 33 days, the resins were further degraded, resulting in the decrease of its content. It is also well-understood that the increase of the saturates and aromatics resulted from the degradation of the resins and asphaltenes.

To have an in-depth understanding on degradation of the heavy fraction in bitumen, asphaltenes were separated from the bitumen and treated with the strain. The result shows that only 94.4% of asphaltene was recovered after being cultivated by the strain, which was attributed to the biodegradation or loss during the collection. The components of the microbial-treated asphaltenes were analyzed. It was found that 88% of the recovered asphaltenes was *n*-heptane-soluble, indicating that most of the asphaltenes were biodegraded to light molecules.

The obtained *n*-heptane-soluble fraction was further fractionated into saturates, aromatics, and resins. The data in Table 2 show that the saturates, aromatics, and resins were about 12.8, 28.6, and 58.6%, respectively. Such a finding is well-consistent with the SARA analysis of the bitumen, in which the content of saturates and aromatics was increased because of the microbial treatment of the ore. It is also noticed that more than half of the degradation products was resins. In other words, the asphaltenes could be well-degraded to resins because they have a similar chemical composition and structure.

Table 2. Various Components of the *n*-Heptane-Soluble Fraction Obtained from the Asphaltene Degradation

	saturates (%)	aromatics (%)	resins (%)	total recovery (%)
microbially treated	12.8 ± 0.3	28.6 ± 0.7	58.6 ± 1.2	100

Degradation on aromatic compounds using a strain of *B. subtilis* has been considerably investigated in the literature. For example, degradation of pyrene and benz[*a*]pyrene with a strain of *B. subtilis* was investigated by Rochelle et al.[44] They found that about 40% of pyrene and 50% of benz[*a*]pyrene were degraded, respectively. Naphthalene and phenanthrene, two kinds of polycyclic aromatic hydrocarbons (PAHs), were also used to examine the capability of degradation of the strain.[45] The results showed that the losses of the two PAHs are about 71 and 62%, respectively. A strain of *B. subtilis* isolated from wastewater was used to degrade acenaphthene, anthracene, and benzo[*b*]fluoranthene. The results showed that the above PAHs were removed completely.[46] In this work, the results showed that most of the heavy molecules of asphaltenes in bitumen were degraded into lighter production. According to the above studies, it is believed that *B. subtilis* is a good strain with good selectivity of degrading PAHs, such as the asphaltenes and resins in bitumen, rather than the saturated hydrocarbons and aromatics. The degradation of asphaltenes and resins might occur via attacking the aromatic ring and/or locations contacting the *n*-alkanes and aromatics.

3.4. Rheological Characterization of Bitumen. It is well-known that the bitumen viscosity has a significant role on the oil sands processability.[6,8] The rheological properties of various bitumens were presented in Figure 7. It is found that the viscosity of the untreated bitumen was extremely high at about 10^5–10^6 Pa s, which considerably decreased to less than 10^5 Pa s for the microbial-treated bitumen. Especially, it decreased 2 orders of magnitude for the microbial-treated bitumen with an incubation time of 33 days. It has been reported that, to have a satisfactory bitumen recovery, the bitumen viscosity should be reduced below 1.5 Pa s.[6] An investigation on diluted bitumen liberation from a silicon surface also confirmed the importance of bitumen viscosity on the release of bitumen from the solids surface.[5] Although the viscosity of the microbial-treated bitumen was not reduced to the required value of 1.5 Pa s, it was believed that the drastic decrease of the bitumen viscosity could still enhance its fluidity and contribute to an efficient bitumen recovery. The bitumen viscosity is mainly dependent upon its composition, especially the content of the resins and asphaltenes. The decrease in bitumen viscosity was attributed to the degradation of the resins and asphaltenes. It is further observed that the viscosity of various bitumens varied little at a low shear rate from 10^{-3} to 10^{-2} s^{-1}, which behaved like Newtonian fluids. Because the shear rate was over 10^{-2} s^{-1}, the viscosity gradually decreased with increasing the shear rate. In other words, the bitumen behaved like a non-Newtonian fluid at such a shear-thinning region. However, it is noticed that the shear-thinning region for the microbial-treated bitumen

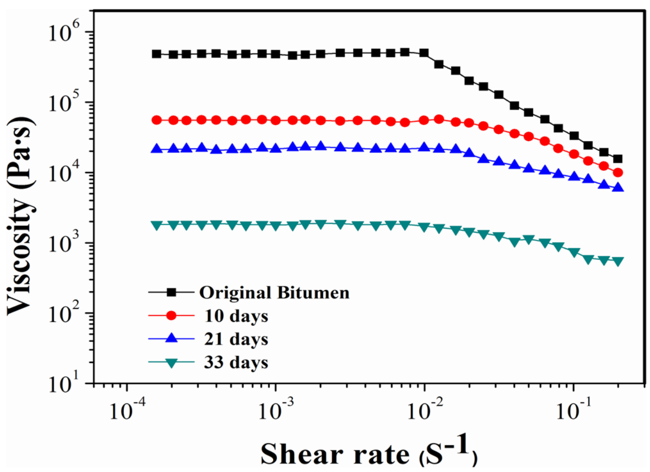

Figure 7. Effect of the microbial-treated time on the bitumen viscosity at 60 °C.

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obviously became weak, especially for the bitumen being treated for 33 days. Such a phenomenon was believed to be related to the chemical structure of the resins and asphaltenes in bitumen.[5,47] Asphaltene is an important component of the bitumen, which could be easily self-aggregated to form nanoparticle structures surrounded with the resins. Because of the strong attractive interaction, including hydrogen bonds or charge-transfer $\pi-\pi$ bonds between asphaltenes and resins, the gel-type microstructure and network particles are always produced,[48,49] which greatly affect the bitumen viscosity. At higher shear stress, the gel-type microstructure and network particles could be destructed or fractured, leading to the generation of the shear-thinning region.[50] After the bitumen was treated by the strain, the associated asphaltene aggregates in the microbial-treated bitumen became much less because of the degradation of the asphaltenes and resins. As a result, the shear-thinning region was reduced for the microbial-treated bitumen. It has been reported that reduction of viscosity via the addition of diluents in bitumen could well-facilitate the bitumen liberation from the solids surface.[4,5] Therefore, a drastic decrease of the bitumen viscosity is another important factor contributing to the bitumen liberation from the solids surface. Refinery is a process to improve the crude oil quality through catalytic cracking of the highly polar compounds or the acidic and alkaline compounds.[51] Therefore, the degradation of the heavy molecules in bitumen by a strain of *B. subtilis* improved not only the oil sands processability but also the bitumen quality.

3.5. Interactions between Bitumen and Fine Solids. The AFM colloidal probe technique has been widely used to improve our understanding on the process of water-based bitumen extraction from oil sands.[10,52−55] In oil sands processing, the interaction forces between bitumen and solids play an important role in determining the bitumen recovery and froth quality. A recent study reported that the interaction forces between bitumen and fine solids could be controlled through decreasing the hydrophobicity by removing organics from the solids surfaces, thereby improving the processability of the weathered ore.[2,10] To have a better understanding on how the microbial treatment affects the bitumen recovery, the long-range interactions and adhesion forces between bitumen and fines were measured using an AFM. The results are shown in Figure 8. It is found that the long-range interaction between bitumen and fines from the untreated poor ore (fines 1) showed a strong attraction starting from a separation distance of about 20−25 nm (fines 1). Correspondingly, a strong adhesion force of 9.6 mN/m was obtained when fines 1 were retracted from the bitumen surface. In contrast, both the long-range attractive force and adhesion were greatly decreased when the bitumen surface interacted with the fines from the microbial-treated ore (fines 2). Such reductions in long-range attraction and adhesion forces resulted from the alteration of the solids wettability, leading to the much easier liberation of bitumen from the sand grains. These findings were consistent with the results of flotation tests, where improved oil sands processability was obtained after microbial treatment of the poor ore.

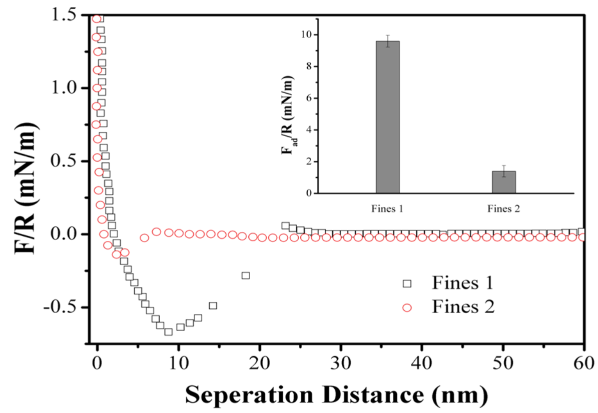

Figure 8. Normalized long-range interaction forces (F/R) between bitumen and fine solids extracted from the untreated poor ore (fines 1) and the microbial-treated ore (fines 2) as a function of the separation distance in 1 mM KCl solutions at pH 8.0−8.5. (Inset) Corresponding nominalized adhesion force.

4. CONCLUSION

Microbial treatment of a poor oil sands prior to the water-based bitumen extraction was carried out to improve bitumen recovery. Flotation tests showed that an improved bitumen recovery of 97% was obtained after the poor ore was microbially treated. The wettability of solids was found to be changed from hydrophobic to hydrophilic, leading to a great decrease of the long-range attractive force and adhesion between bitumen and solids. SARA fraction analysis indicated that the content of the heavy components of the bitumen, i.e., asphaltenes and resins, was decreased corresponding to an increase of the saturate and aromatic fractions after the microbial treatment, which was correlated well with the rheological characterization of bitumen. The altered solids wettability, biosurfactant production in the culture solutions, and decrease in the bitumen viscosity resulted from the degradation of asphaltene and resin components being considered as the major causes for the improved oil sands processability. Moreover, the decrease in bitumen viscosity not only contributes to the bitumen liberation from the solids surface but also gives a better oil quality, which is significant for its subsequent industrial refining. Therefore, microbial treatment is an effective option to improve bitumen recovery from poor processing ores and might have potential application in an industrial field.

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Notes

The authors declare no competing financial interest.

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