Analysis of Biosurfactant Production by a New Thermophilic and Halotolerant Bacterium, Geobacillus sp. QT

Wenjie Xia¹,², Hao Dong³, Li Yu², Yongqiang Bi² and Weichu Yu⁴

¹Power Environmental Energy Research Institute, California 91722, USA; ²Institute of Porous Flow & Fluid Mechanics, Chinese Academy of Sciences, Langfang 065007, P.R. China; ³State Key Laboratory of Heavy Oil Processing, China University of Petroleum, Beijing 102249, P.R. China and ⁴College of Chemistry and Environmental Engineering, Yangtze University, P.R. China

Abstract: Geobacillus sp. QT was isolated from the brines of oil reservoir in Daqing oilfield, China, and could produce biosurfactants with different hydrophobic or hydrophilic hydrocarbons. As a result of biosurfactant production, the surface tension of the culture was reduced from 71.59 mN/m to 25.9 mN/m. Two types of biosurfactants of this strain by using different carbon source were obtained. GPC analysis showed that the number-average molecular weights of the biosurfactants were 466-652 Da for type I and 269,485-406,158 Da for type II, with PDI values of 1.079-1.161 and 1.155-1.22, respectively. Chemical composition studies exhibited that two types of biosurfactant consisted of carbohydrates, lipids and proteins with different composition ratio. Emulsification assays approved the effectiveness of biosurfactants over a wide range of temperature, pH, salinity and with different metal ions. Sand packs tests indicated the potential value of the biosurfactants of this strain in enhanced oil recovery.

Keywords: Geobacillus sp., Biosurfactants, Surface activity, Emulsifying activity, Oil recovery.

1. INTRODUCTION

Microbial surface-active agents or biosurfactants are amphiphilic molecules consisting of hydrophobic and hydrophilic moieties and tend to interact with interfaces of various polarities and reduce the surface and interfacial tension of solutions [1,2]. Many microorganisms produce biosurfactants during growth on variety of substrates, and the biochemical and structural characteristics of these compounds are very diverse [3,4]. Biosurfactants can be conventionally subdivided into two groups. The first group comprises biosurfactants with low molecular weight, such as glycolipids and lipopeptides; the second group includes high molecular weight polymeric surfactants like polysaccharides, lipoproteins, lipopolysaccharides and complexes of these molecules [5,6]. For instance, the biosurfactant obtained from Streptococcus thermophilus A was a multi-component biosurfactant, consisting of protein and polysaccharides [7].

Practically all the usable surfactants are chemically synthesized at present. The utilization of conventional chemical surfactants and their derivatives are yet costly and of serious environmental concern, since they are detrimental to environment and the need to the enormous chemicals lead to the gradual environmental pollutions. Therefore, attentions have been paid to the alternative environmental friendly manners for the production of these surface-active compounds especially microbiological production of surfactants. The main reason is that biosurfactants take advantages over synthetic counterparts like lower toxicity, biodegradability, better environmental compatibility, higher foaming, high selectivity, specific activity at extreme temperatures, pH and salinity [2,8,9].

Like chemically synthesized surfactants, biosurfactants have potential applications in several fields of industrial processes including: agriculture, cosmetics, pharmaceuticals, detergents, food processing, textile manufacture and paint industries [8,9]. Several reports on successful applications of biosurfactants for oil spill remediation, oil tank clean up and microbial enhanced oil recovery (MEOR), revealed the special importance of these biological products in up and downstream processes of oil industry [3,8]. Enhanced oil recovery methods were devised to recover oil remaining in reservoirs after primary and secondary recovery procedures. MEOR is an important tertiary recovery technology that utilizes microbial cells or their metabolites for residual oil recovery [3,10]. In classic MEOR methods, microorganisms in reservoir are stimulated to produce polymers and surfactants that lower interfacial tension at the oil–rock interface and aid oil recovery [11]. To be suitable and useful in the MEOR processes, microorganisms must be able to grow under the severe environmental conditions encountered in oil reservoirs including high temperature, pressure, salinity and low oxygen levels [8]. Only a small number of bacteria were
identified with the capability of growing and producing surface-active compounds under these extreme conditions, and there will be even fewer if we consider more severe conditions such as what dominate in very deep oil reservoirs. Application of microbial surfactants instead of live microorganisms could be of great advantage and value in harsh growth-limiting conditions prevailing in deep reservoirs [11].

The present study reports the isolation of a potent biosurfactants-producing bacterium, *Geobacillus* sp. QT is with potential value in microbial enhanced oil recovery. The effects of carbon sources on biosurfactants production and chemical characterization of the biosurfactants were also investigated. MEOR suitability tests and biosurfactants stability to environmental stresses were presented. Moreover, oil recovery experiments of biosurfactants were carried out in sandpack column.

2. MATERIALS AND METHODS

2.1. Sampling Procedure

The Daqing oilfield is located in the Northwest of China. The reservoir is located at depths of 1300 to 1600 meters subterranean, with an in situ fluid temperature of about 55-68°C. The parameters of the Daqing oil reservoir is as follows: air permeability \(575 \times 10^{-3}\) \(\mu\)m^2; porosity - 34.7%; crude oil viscosity - 79.1 mPa·s; crude oil density - 0.898 g/cm^3; total salinity - 1852.26 mg/L. Samples of brine and crude oil were collected from an operating LHP wells in the DaQing oil reservoir in March 2010. The samples were taken in sterile bottles and transported to the laboratory at 4°C, and bacteriological analyses were conducted as soon as possible.

2.2. Media and Cultivation Conditions

In order to enrich and cultivate biosurfactant-producing bacteria, a basic salts medium (BSM) was used. The composition of the liquid medium was as following (g/L of distilled water): \(Na_2HPO_4\) (3.5), \(KH_2PO_4\) (1.5), \(MgSO_4\cdot7H_2O\) (0.6), \(NH_4NO_3\) (4.0), \(CaCl_2\) (0.02), \(FeSO_4\cdot7H_2O\) (0.01), and yeast extract (0.3) supplement with 1ml trace element solution containing (mg/L of distilled water): \(MnCl_2\cdot4H_2O\) (0.1), \(CoCl_2\cdot6H_2O\) (0.17), \(H_3BO_3\) (0.019), \(ZnCl_2\) (0.1), \(NaMoO_4\cdot2H_2O\) (0.1), vitamin B_12 (0.50), D(+)-biotin (10.0), folic acid (10.0), and 5% of carbon source. The pH was adjusted to 6.8-7.2. Carbon source was sterilized by using 25 mm diameter and 0.25 \(\mu\)m pore size filter, and was added to the heat-sterilized medium. The effects of different carbon sources including molasses, n-octane, n-hexadecane, n-paraffin, phenanthrene and crude oil on growth and biosurfactant production were investigated. Cultivation were carried out at 55°C and 200 rpm in 500 ml Erlenmeyer flasks with a working volume of 200 ml. Solid LB medium was made by adding 1.8% agar.

2.3. Screening and Isolation of Biosurfactant-Producing Bacteria

To enrich biosurfactant producing bacteria, 20 ml of the brine and 5 g of crude oil were transferred to the 500 ml Erlenmeyer flask containing 180 ml of BSM and incubated on a shaker at 200 rpm at 55°C. After 14 days, 0.1 ml of the enrichment culture were transferred and spread onto LB agar plates. There appeared several colonies and pure cultures of each morphologically distinct colonies were obtained by repetitive streaking onto solid LB medium. To isolate the strains capable of biosurfactant production, oil spreading method [2,12] and hemolytic activity [13] were applied. Among several bacterial strains isolated, a potent biosurfactant-producing bacterial strain was identified and examined as creation of significant clear zone on the oil surface and maintained on LB agar medium at 4°C for further experiments.

2.4. Characterization and Identification of Bacterial Strain

The selected bacterial isolate was examined by using standard biochemical tests by the Berge’s manual of systematic bacteriology [14] and identified by 16S rRNA gene sequencing. The chromosomal DNA of selected bacterial isolate was extracted using soil DNA Kit (Omega Biotek, USA). Polymerase chain reaction (PCR) was carried out and two sets of universal primers (27F, \(5’-AGAGTTGTATCCTGGCTCAG-3’\)) (1492R, \(5’-TACCCTGTTTATCTGAGCTC-3’\)) and \(V9(1055F, 5’-ATGGCTGTCGTCAGCT-3’)/V9(1406R-GC, 5’-ACGGGCGGTGTGTAC-3’)\) were used to amplify the V9 hypervariable region of the bacterial 16S rRNA gene [15]. The reaction was carried out in a 25 µl volume containing 1 x PCR buffer, 1.5 mmol/l MgCl2, 2 mmol/l dNTP mixture, 1 µmol/l each primers, 1 µl of pfu enzyme and 1 ng of template DNA. The PCR product was purified using a High Pure PCR product Purification Kit (Roche Applied Science, Germany) and PCR again, then sequenced with an ABI Prism 377 automatic sequencer (Applied Biosystems, USA) using the V9 primers. Sequence homologies were examined using BLAST version 2.2.12 of the National Center for
Biotechnology Information [16]. Multiple sequence alignments were carried out using Clustal X and a consensus neighbor-joining tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0 [17].

2.5. Surface Activity Measurement

Surface tension, interfacial tension and critical micelle concentration (CMC) were measured by Du Nouy ring method using a KRÜSS-K 8600 E interfacial tensiometer (Germany) with a 6 cm diameter platinum-iridium ring. CMC is defined as the surfactant concentration necessary to initiate micelle formation and the surface tension does not continue to decrease if more of the surfactant is present. CMC was measured following the references [13]. The reciprocal of CMC (CMC−1) is proportional to the total amount of surface-active compound present in the solution, and can be used as an approximate measure for biosurfactant concentration.

2.6. Emulsification Activity Determination

The emulsification activity was determined by addition of 6 ml hydrocarbon to 4ml of the test broth in a 15 ml graduated tube. The mixture was mixed vigorously for 10 min and allowed to stand for 24 h at room temperature. The emulsification index (EI) was calculated by dividing the measured height of the emulsion layer by the total height of the mixture and multiplying it by 100 [18,19].

2.7. Measurement of Dry Cell Weight

Biomass dry weight was determined by centrifugation of 100 ml sample of culture broths at 8,000 rpm for 15 min. The pellet was washed with distilled water, re-suspended in 10 ml of distilled water, filtered through prewashed 0.25µm filter paper, placed on a pre-weighed plate and dried at 105 °C to obtain constant weight.

2.8. Extraction and Characterization of Biosurfactant

After the incubation, the culture was centrifuged at 8,000 rpm for 20 min at 4°C. The residual hydrocarbon was carefully removed by extraction. The hydrocarbon-free culture broth was used for product extraction. 200 ml samples were extracted twice with an equal volume of chloroform-ethanol (2:1, v/v) after adjusting pH to 2.0 using 6N HCl. The solvent was removed by reduced-pressure distillation and the dried product was washed with distilled water [13,18].

The number-average molecular weight (Mn), weight-average molecular weight (Mw) and polydispersity index (PDI) of the biosurfactant were measured by gel permeation chromatography (GPC) using pullulan standards as described previously [20]. Total protein content of the bioemulsifier was determined according to the method described by Lowry [21]. Total carbohydrate content of the biosurfactant was determined according to the phenol-sulfuric acid method [22]. Lipid was analyzed by diethyl ether extraction [23].

2.10. Biosurfactant Stability Studies

The effect of several environmental parameters on the surface activity of the biosurfactant was determined. NaCl at different concentrations (0–40% (w/v)) was mixed with the solution of biosurfactant (0.1%, w/v) and the emulsification index was measured. To determine the temperature stability of the surface active compounds, the samples were incubated at different temperature (4°C to 120°C) for 120 min and allowed to room temperature and the emulsification index was measured. To study the pH stability of the solution of biosurfactant, the pH of the samples was adjusted to different pH values (1–12) with HCl (6 N) and NaOH (6 N) and subsequently the emulsification activity were measured. In order to investigate the effects of the metallic ion on emulsification, several metallic ions (e.g. K+, Ca2+, Mg2+, Fe2+, Zn2+, Mn2+, Cr3+ and Al3+) were added into the solution of biosurfactant.

2.11. Sandpack Test

To evaluate the potential application of biosurfactants in enhanced oil recovery (EOR), the core flooding system was employed. A standard core flooding equipment used was similar to that described before [24]. The acid-washed sandstone with particle size distribution of 380 µm (35%), 120–109 µm (30%), 80–75 µm (35%) was packed in proportion in a cylinder-shape stainless steel tube to obtain 35% porosity. The column was then saturated with crude oil (Daqing) and flooded with pore volumes of brines until the water flooding released oil became less than 2%. The remaining residual oil was flooded with biosurfactant solution and released residual oil percentage was measured.

3. RESULTS AND DISCUSSION

3.1. Isolation and Selection of Biosurfactant-Producing Bacteria

After a multi-step isolation program, 8 strains had hemolytic activity among 26 bacterial strains that iso-
lated and tested for hemolytic activity. Surface tension measurement was employed for the surface activities of hemolytic strains. 3 strains was confirmed when the surface tension below 40 mN/m was used as a selection criterion. By combining two screening criteria of hydrocarbon utilization and hemolytic activity, we obtained the best frequency of biosurfactant-producing strains (more than 10%). Bosh et al. reported the frequency of 3.2% by employing direct surface tension measurement as a screening standard [2,25] and Carrillo et al. observed only 1% frequency by using hemolytic activity as a screening criterion [13]. Possibly the higher frequency of biosurfactant producers is the result of primary enrichment of biosurfactant producers by growing on water immiscible substrate, which has shown to have stimulatory effects on biosurfactant production [5].

Temperature and salinity are important parameters in MEOR. For oil reservoirs with high temperature and salinity, further investigation was performed with respect to cell growth and biosurfactant stability at high temperature and salinity. The isolate QT was selected for further studies since it lowered culture broth surface tension from initial values of approximately 71.54 mN/m to below 25.9 mN/m following 100 h incubation at 55°C and displayed the highest biosurfactant production.

QT cells is a facultative anaerobic, gram-positive, non-flagellum, non-motile, rod-shaped bacterium with a length of 1.9 to 3.6 mm and a width of 0.6 to 0.9 mm. Colonies were yellow round with translucent halo and flattened out with longer incubation times. The sporangium was not swollen, whereas the spores were oval and subterminal positioned. QT used a variety of carbon sources for growth, including L-arabinose, fructose, galactose, glucose, inulin, D-xylose, D-mannool, salicin, and lactose but not adonitol, melibiose. This strain showed catalase and H₂S test are positive, and could utilize citrate, propionate, and gluconate; Reduce nitrate and did not produce indole or hydrogen sulfide. Arginine dihydrolase and b-galactosidase activities were observed, but no activities of oxidase, ornithine decarboxylase, lysine decarboxylase, phenylalanine desaminase, or trypothan desaminase were detected. The strain was identified as a member of the Geobacillus genus by the keys given in Bergey’s manual of systematic bacteriology [14]. It could grow at temperature of 4°C-80°C and at salinity of 0%-15% (w/v). The sequencing of 16s rRNA gene also confirmed the assignment of the strain to Geobacillus sp. and submitted to GenBank with an accession number FJ788895.1.

### 3.2. Biosurfactant Production

Biosurfactant production was studied using molasses as the sole source of carbon. Figure 1 illustrates biosurfactant production and growth characteristics of QT strain. Maximum biomass concentration (18.95 g/l) was achieved after 44–50 h of growth. The minimum surface tension of the culture broth was 25.9 mN/m and was observed 36 h after incubation, during the exponential growth phase. Comparison of these values with previously reported biosurfactants [2,13] indicates that QT biosurfactant is a potent biological surface-active agent. The emulsification capacity of the culture broth was not maximal at this point and would continue to increase with further growth and biosurfactant pro-

![Figure 1: Cell growth, Biosurfactant production, surface activity emulsification index of the Geobacillus sp. QT during growth on mineral salt medium supplemented with 5% molasses at 37°C.](image-url)
duction. This reveals that the biosurfactant concentration became sufficient for micelle formation after 36 h of fermentation, beyond which constant surface tension is observed. In fact, emulsification capacity was increased with more biosurfactant concentration. But after 44h, when turned in stable growth phase, biosurfactant production start increasing and then reach the maximum point at 70 h, which indicates that the biosurfactant production is no-associated with growth and biosurfactant is second metabolite. Similar observations have been made for other biosurfactant-producing microorganisms. Wu et al. have reported that growth and biosurfactant production by Pseudomonas aeruginosa EM1 using glucose as a carbon source follow the same pattern [7].

3.3. The Effect of Carbon Source on Growth and Biosurfactant Production

The effect of different carbon sources including hydrophilic and hydrophobic substrates on growth and biosurfactant production by the Geobacillus sp. QT was examined. The strain was grown in 200 ml BSM containing 5% of carbon source for 72 h and dry cell weight, surface tension, critical micelle concentration (CMC) and emulsification index (EI) with kerosene were determined. The strain was able to grow and produce biosurfactant using different types of carbon sources including molasses and hydrocarbons (n-octane, n-dodecane, n-hexadecane phenanthrene, paraffin and crude oil), which indicates that hydrophobic and hydrophilic carbon source may be used as substrate for biosurfactant production. The highest biomass production, as dry cell weight, was obtained when the strain grown on molasses as a sole carbon source. The maximum emulsification capacity was observed in the culture grown on crude oil whereas the cultures prepared on crude oil and paraffin showed the lowest surface tension and CMC (Figure 2). From these results, we may conclude that the optimum carbon source for QT growth is different from the best carbon source for biosurfactant production. These findings are in accordance with those of Wu et al. who observed that Pseudomonas aeruginosa strains had the highest yield of biomass when grown on glucose whereas the highest yield of rhamnolipid was seen with the glycerol as a carbon source [7]. Prieto et al. have also shown that the initiation of rhamnolipid production by P. aeruginosa is induced by the presence of poorly soluble hydrophobic substrates [26]. The surface tension of the cultures grown on molasses (25.9 mN/m) and crude oil (24.6 mN/m) was comparable, whereas the biosurfactant yield was higher in the culture grown on crude oil. The higher biosurfactant yield of the culture grown on crude oil (30.5 g/L) in comparison to the culture grown on molasses (14 g/L) maybe is the result of more hydrophobic nature of the cells prepared on hydrophobic substrates. The effect of hydrophobic substrates on increasing the biosurfactant was also confirmed by high biosurfactant yield of the culture grown on paraffin (26.5 g/L) in spite of its low biomass yield and surface activity.

3.4. Biosurfactant Characteristics

It was reported that low-molecular weight biosurfactants are able to reduce the surface tension below 40 mN/m [27] while the high molecular biosurfactant (bio-emulsifiers) can form and stabilize emulsions without remarkable surface tension reduction [10]. Therefore,
According to the above results, it can be inferred that the bio-surface active compounds synthesized by *Geobacillus* sp. QT belonged to low-molecular weight biosurfactants when using octane, dodecane, hexadecane and phenanthrene; or the mixture of low-molecular biosurfactants and bioemulsifiers when using paraffin, crude oil and molasses.

As shown in Table 1, according to the number-average molecular weights ($M_n$), the seven biosurfactants were classified into two types. Type I was below 700 Da when using octane, dodecane, hexadecane and phenanthrene with PDI values of 1.111; Type II was between 260,000 and 310,000 Da with PDI average values of 1.212, respectively. The result again confirmed that type I is the low-molecular biosurfactants and type II is the bioemulsifiers [10]. Further studies on chemical composition of the biosurfactants were conducted and showed that the biosurfactant of type I consisted of carbohydrates (from 52.18 to 68.23%), lipids (from 31.77 to 46.81%) and proteins (less than 1%, even null when using octane, dodecane and hexadecane) while Type II (bioemulsifier) was composed by carbohydrates (from 51.48 to 75.45%), lipids (11.47 to 30.12%) and proteins (from 7.02 to 18.4%). Obviously, the protein portion of Type II is higher than type I. The hydrophobic portion (lipid) always attached to the polysaccharide or protein backbone (hydrophilic portion) and provided the amphiphilic structure common to surface-active agents [28]. Biosynthesis of glycolipids, as surface-active agents, has been reported for a variety of bacteria. They are carbohydrates in combination with long-chain aliphatic or hydroxyl aliphatic acids [29]. Marchant et al. has reported the isolation of glycolipid biosurfactants from *Geobacillus* sp. strain [30]. Kuyukina et al. also described the determination of polar lipids and mostly glycolipids as *Geobacillus* biosurfactant extraction [23]. The bioemulsifiers with similar composition were broadly reported previously [31]. Based on these findings we may assume that *Geobacillus* sp. QT biosurfactant of type I is glycolipids and type II is the mixture of glycoproteins and glycolipids when using different carbon source.

### 3.5. Emulsification Activity

In addition to surface activity, good emulsification property is critical for biosurfactants to be promising in different environmental and industrial applications [3]. The interface tension (IFT) and emulsification capacity of QT biosurfactant against various hydrocarbons was investigated. Most microbial surface-active compounds are substrate-specific and emulsify different hydrocarbons at different rates. The type I (solution of biosurfactant produced with n-dodecane, 0.1% w/v) and type II (solution of biosurfactant produced with crude oil, 0.1% w/v) were employed. The emulsions were stable for 2 days and interface tension was measured at room temperature. Results as shown in Figure 3 indicated that emulsification indexes of type II were higher than that of type I whereas interface tension of type I were lower than that of type II with nine hydrocarbon and oils. This phenomenon could further confirm the above assumption about the characterization of the *Geobacillus* sp. QT biosurfactant produced when using different carbon sources. The EI value of two types were found to decrease with the increase of carbon numbers for the short n-alkane and the mixture of long-chain hydrocarbons such as paraffin, kerosene and crude oil, exhibited a positive emulsifying activity. The equivalent alkane carbon number (EACN) for type I was eight when interface tension reached at the lowest point whereas twelve for type II. These results suggest that the emulsification activity of surfactant solution is not simply determined by its surface tension, but it also depends on the direct interaction of the hydrophobic moiety of biosurfactant with hydrocarbon substrates.

### Table 1: Characterization of Biosurfactants Synthesized by *Geobacillus* sp. QT Using Different Carbon Sources

<table>
<thead>
<tr>
<th></th>
<th>Octane</th>
<th>Dodecane</th>
<th>Hexadecane</th>
<th>Phenanthrene</th>
<th>Paraffin</th>
<th>Crude Oil</th>
<th>Molasses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biosurfactant yield, g/L</strong></td>
<td>3.88</td>
<td>1.25</td>
<td>10.06</td>
<td>8.57</td>
<td>26.57</td>
<td>30.05</td>
<td>14.88</td>
</tr>
<tr>
<td>$M_n$, Da</td>
<td>652</td>
<td>591</td>
<td>466</td>
<td>492</td>
<td>304,782</td>
<td>406,158</td>
<td>269,485</td>
</tr>
<tr>
<td>$M_w$, Da</td>
<td>704</td>
<td>647</td>
<td>541</td>
<td>562</td>
<td>369,458</td>
<td>479,258</td>
<td>329,854</td>
</tr>
<tr>
<td>PDI</td>
<td>1.079</td>
<td>1.094</td>
<td>1.161</td>
<td>1.142</td>
<td>1.216</td>
<td>1.155</td>
<td>1.221</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>68.23</td>
<td>62.17</td>
<td>58.94</td>
<td>52.18</td>
<td>65.18</td>
<td>51.48</td>
<td>75.45</td>
</tr>
<tr>
<td>Lipid, %</td>
<td>31.77</td>
<td>37.83</td>
<td>41.06</td>
<td>46.81</td>
<td>22.48</td>
<td>17.47</td>
<td>18.4</td>
</tr>
<tr>
<td>Protein, %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.01</td>
<td>12.44</td>
<td>7.02</td>
<td></td>
</tr>
</tbody>
</table>
An important property of biosurfactants is their effectiveness over a wide range of temperature, pH and salinity [3]. Previous literature reported that the emulsifying activity was significantly inhibited at a NaCl concentration greater than 5 % [32], whereas the two types of biosurfactants by *Geobacillus* sp. QT showed a better halotolerance, remaining active under the salinity up to 20% (Figure 4). The activity of the two biosurfactants was significantly inhibited under the acid conditions and showed the relative stable at pH from 7 to 12 (Figure 5). On the contrary, the bioemulsifiers produced by fungi were stable under acidic condition. Figure 6 showed the effect of the temperature on the emulsification ability.

The type II exhibited the highest activity stably over a wide temperature range, whereas the temperatures after 100 °C did affect on emulsifying activity for type I biosurfactant. A slight decrease was detected with the treatment higher than 80°C. Previous studies have proved that the high molecular biosurfactants were thermostable [33]. Figure 7 indicated that the biosurfactant activity was not sensitive to the metallic ions.

Many species from the genus *Geobacillus*, as recently described, have come from oil-rich environments and their capability has been well documented. However, the different hydrocarbons (especially crude oil, kerosene and phenanthrene) degrading activity by *Geobacillus* sp. had not been reported until 2006 [34].
Previous review reported no biosurfactant-producing species of Geobacillus and none of the strains showed any evidence of surface-active components [30]. This is the first report of two-biosurfactant production by Geobacillus sp. by using different carbon sources. The production of surface-active compounds by bacteria is often viewed as aiding hydrocarbon degradation and enhancing the solubility of hydrophobic substrates, which can be used broadly in biotechnology applications. Based on the findings in the present study, including the hydrocarbon-degrading and biosurfactant-producing properties, the thermophilic bacteria exhibited a promising potential for applications within extreme environmental conditions, such as MEOR [3,10].

3.6. Oil Recovery from Sandpack Column

The potential application of the product in microbial enhanced oil recovery (MEOR) and trapped oil immobilization was evaluated using the sand pack technique described by Kuyukina et al. [24]. The biosurfactant solution of two types by Geobacillus sp. QT was able to recover 35% for type I and 55% for type II of the trapped oil. The reduction in interfacial tension caused by low molecule weight surfactant and the plugging of these high permeability zones by bioemulsifier increases microscopic displacement efficiency in porous medium and reduces the residual oil saturation [35]. So the efficiency of oil recovery for type II was high than that for type I.

4. CONCLUSIONS

A newly thermophilic strain of bacterium isolated from oil reservoir samples in Daqing oilfield of China and identified as Geobacillus sp. The bacteria could grow on various hydrocarbons and produce the low-molecular weight biosurfactant and bioemulsifiers. The two types of biosurfactants were isolated and identified as a complex of carbohydrates, lipids and proteins with different composition ratio. The emulsifying properties and interface tension of two biosurfactant approved the effectiveness over a wide range of temperature, pH, salinity and with different metal ions, which were considered to be ideal candidates for industrial applications.

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