



Original Research Article

An efficient thermotolerant and halophilic biosurfactant-producing bacterium isolated from Dagang oil field for MEOR application

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ABSTRACT

Keywords

Biosurfactant;
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core flood
study;
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degradation;
MEOR

An efficient biosurfactant-producing, thermotolerant and halophilic bacterium was isolated and cultured from petroleum reservoir of Dagang oil field, using haemolytic assay, and the qualitative oil-displacement test. On the basis of 16S rDNA analysis, the isolate was identified as *Bacillus subtilis* BS2. This bacterium was able to produce a type of biosurfactant which could reduce the surface tension of the culture broth from 70.87 to 28.97 mN m⁻¹ after 8 d of incubation at 37 °C and to 36.15 mN m⁻¹ after 20 d of incubation at 55 °C, respectively. The biosurfactant offering potential for biotechnology as it showed stability at high temperature, a wide range of pH and salt concentrations. The FT-IR spectrum of extracted biosurfactant tentatively characterized the biosurfactant as glycolipid derivative. Elemental analysis of the biosurfactant by EDS revealed that the biosurfactant was anionic in nature. Biodegradation experiments with crude oil suggested a preferential utilisation of n-alkanes as a carbon source by BS2 strain upon the synthesis of biosurfactant. Core flood studies for oil release indicated 9.6% of additional oil recovery during water flooding at 37 °C and 7.2% of additional oil recovery at 55 °C. The isolated *Bacillus subtilis* BS2 strain has the potential for application for MEOR through water flooding in China's oil fields.

Introduction

Biosurfactants are surface-active amphiphilic compounds produced by many microorganisms. Based on the chemical

composition and microbial origin, biosurfactants are classified as glycolipids, lipopeptides, fatty acids, and other

biological macromolecules and contain various functional groups including carboxyl, amino and phosphate groups (Kosaric, 1992, Desai and Banat, 1997). Most widely studied biosurfactants are surfactins of *Bacillus subtilis* (Cooper et al., 1981, Nitschke and Pastore, 2006), rhamnolipid of *Pseudomonas aeruginosa* (Maier and Soberon, 2000), glycolipid of *Rhodococcus erythropolis* and sophorolipid of *Candida rugosa* (Thanomsub et al., 2007). Their use is preferred over chemically synthesized surface-active agents with respect to lower toxicity, biodegradability, better environmental compatibility, higher foaming capabilities, high selectivity, specific activity at higher temperatures, wider range of pH and salinity (Georgiou et al., 1992; Desai and Banat, 1997). Such characteristics make these microbial products attractive for MEOR and environmentally acceptable.

Biosurfactant production is influenced by various physico-chemical factors including carbon–nitrogen ratio, divalent cations and specific substrate availability (Adamczak and Bednarski, 2000; Pruthi and Cameotra, 2003; Abouseoud et al., 2008). Hydrocarbons such as crude oil and gasoil and commonly various carbohydrates such as glucose, sucrose and glycerol have been used as carbon substrates for the production of biosurfactants. Since the biological function of biosurfactants is related to hydrocarbon uptake, a spontaneous release occurs with these substrates (Desai and Banat, 1997). Biosurfactants in particular have several benefits in enhancing oil displacement and movement through oil bearing rocks by means of reduction of interfacial tension between oil rocks and oil and formation water; and by means of modification of the wettability of porous media and emulsification of crude oil. In addition, the biosurfactant production contributes to the lowering of viscosity of oils, thereby increasing the flow.

Indigenous microorganisms inhabit aquatic as well as oil-bearing deep sub surface environments (Magot et al., 2000). In-situ biodegradation of crude oil in oil reservoirs have been reported by both the aerobic as well as anaerobic degradation (Aitken et al., 2004; Jones et al., 2008; Jimenez et al., 2012; Prince et al., 2013). Da Cruz et al. (da Cruz et al., 2008) advocated that anaerobic and aerobic processes can be synergistic and their microbiota coexist in the oil reservoir.

In petroleum industry, secondary recovery processes have been used as enhanced oil recovery strategies. Among these processes, water and polymer flooding are widely applied due to its operational simplicity and low-cost (Shedid, 2006; Pei et al., 2013). However, primary and secondary production recovery methods can produce only about 40% of the original in-place oil. Consequently, the importance of tertiary recovery technology has gained momentum. There is renewed worldwide interest in the use of microorganisms and their metabolic products to increase the oil production. These recovery processes are known as MEOR. The strategies investigated so far for MEOR involving biosurfactants include either injection of biosurfactants produced in bioreactors into the reservoirs or injection of biosurfactants producing microorganisms into the reservoirs to produce biosurfactants in situ through supplying suitable nutrients. Cultures suitable for MEOR have been studied by various authors (Castorena-Cortes et al., 2012; Gudina et al., 2012; Kobayashi et al., 2012).

In the present study, an efficient biosurfactant-producing bacterium *Bacillus sp.* strain BS2 have been isolated from Gang Xi and Kong Dian blocks of Dagang oil field. The properties of the strain regarding adaptability to reservoir conditions of temperature, pH and salinity

tolerance have been studied. The oil release property of this strain has been evaluated by the core flood studies for a MEOR approach.

Materials and Methods

Sampling

Water and crude oil samples were collected from production wells in Kongdian and Guangxi blocks, Dagang Oil field. This part of the oil reservoir is located in the west of BeiDagang structural belt and is an anticline structure complicated by faults. It comprises an area about 55 km². The main production layer is the lower member of the neogene Minghuazhen formation and the neogene Guantao formation (Cai et al., 2013). Samples of the crude oil along with formation water were collected directly from the well heads into pre-sterilized sample bottles. The temperature of the reservoir is about 55 °C in this section and the depth of the well screen ranges from 1002 to 1032 m. Samples were stored at 4 °C temperature in the laboratory until further use. All the chemicals used in the present study were of analytical grade and purity.

Enrichment and isolation of microorganisms

For isolation of microorganisms, the enrichment culture technique was applied. The water samples obtained from production wells were inoculated in enrichment medium (EM) containing 1% crude oil as a sole carbon source, and incubated at 30 °C on the rotary shaker at 150 rpm for 7 d. The composition of EM was (g L⁻¹): (NH₄)₂SO₄ 10; KCl 1.1; NaCl 1.1; FeSO₄·7H₂O 2.5×10⁻⁵; KH₂PO₄ 3.4; K₂HPO₄ 4.4; MgSO₄ 0.5; EDTA 1; yeast 0.5. The medium was adjusted to pH 7.0 and sterilized by autoclaving at 121 °C for 20 min. Enrichment of microflora was

carried out through four successive sub-culturing with EM medium. After four subsequent transfers of the enrichment, the bacteria were isolated and purified on Luria-Bertani (LB) agar plates.

Screening and identification of biosurfactant producing bacterial strains

The potential biosurfactant producers were screened among the isolated strains by haemolytic assay and the oil spreading technique. Initially, the isolated strains were tested for their potential ability of producing biosurfactant by haemolytic assay as reported by Mulligan et al. (Mulligan et al., 1984). The composition of blood agar medium (BAM) was (g L⁻¹): nutrient agar 23; sterile sheep blood 50 mL. Further, the tested bacterial strains were further screened for the biosurfactant production by the oil spreading technique with slight modification as reported elsewhere (Youssef et al., 2004). Briefly, 40 mL of distilled water was added to a Petri-dish (9 cm diameter) followed by addition of 40 µL of crude oil to the surface of the water. 10 µL of culture was then added to the surface of oil. The diameter of the clear zone on the oil surface was measured. Strains that produced a zone of diameters of above 4 cm were considered as efficient biosurfactant-producing bacteria and further studied. The diameters of triplicate samples from the same culture of each strain were determined. High biosurfactant producing strains were stored as frozen stock cultures at -20 °C in 25% (v/v) glycerol for further analysis.

For identification of bacteria, 16S rDNA fragments were amplified by PCR with the following set of primers: 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-GGTTACCTTGTTACGACTT-

3). The procedures of PCR amplification were described in details elsewhere (Yu et al., 2013). DNA sequencing was performed by BGI Company (Shenzhen, China). Sequence alignments were performed using the CLUTAL W program (Thompson et al., 1994). BLAST was used to analyse similarities (www.ncbi.nlm.nih.gov/BLAST). Phylogenetic trees were generated from alignments by the neighbour-joining method and the reliability of inferred trees was tested with bootstrap test using the MEGA4 program (www.megasoftware.net). The reference sequences from the GenBank were used for generating phylogenetic trees.

Growth of strain BS2 at different temperatures and salinities

Among several isolated bacterial strains, BS2 was identified as the most efficient biosurfactant-producing bacterial strain and used for further study. 5 mL LB broth medium was inoculated with the strain BS2 and incubated at 37 °C and 150 rpm for 12 h. The cells were collected by centrifugation at 8000 × *g* for 20 min and washed twice with 5 mL of sterile minimal salt medium (MSM, see below). 5 mL of bacterial suspension was transferred to a 500 mL flasks containing 300 mL of MSM and incubated at 55 °C, 150 rpm for 20 d. For comparison, the same culture were prepared and incubated at 37 °C, 150 rpm for 12 d. The samples of cultures were taken at appropriate time intervals and monitored for surface tension (ST) reduction and bacterial cell growth (OD₆₀₀). In order to examine the effect of salinities on the growth of strain BS2 and its biosurfactant production, sodium chloride was added to the MSM to achieve final concentrations of 2-40 g L⁻¹. Then the cultures were incubated at 37 °C and 150 rpm for 6 d.

The composition of MSM for the experiments was (g L⁻¹): NaNO₃ 2.0, KCl 0.5, Na₂HPO₄·H₂O 1.0, KH₂PO₄ 1.0, CaCl₂ 0.025; MgSO₄ 0.1, FeSO₄·7H₂O 0.001 and 2 mL L⁻¹ trace element solution. The pH was adjusted to 7.0. Crude-oil (1%, v/v) was used as a sole source of carbon. Trace element solution containing (mg L⁻¹ in distilled water): FeCl₃·6H₂O 60, ZnSO₄·7H₂O 600, MnSO₄·H₂O 200, CuSO₄·5H₂O 590, CoCl₂·6H₂O 60, H₃BO₃ 150 and Na₂MoO₄·H₂O 15.

Determination of Biomass and Surface tension (ST)

Biomass growth was determined at 600 nm wave length using ultraviolet spectrophotometer (UV1800, Shimadzu, Japan) and is indicated as OD₆₀₀. ST measurements were made by using a Du-Nouy tensiometer (Krüss, Germany) (Makkar and Cameotra, 1997). All measurements were made on the cell-free broth obtained by centrifuging the cultures at 8000 × *g* rpm for 30 min. ST values reported were the mean of three independent measurements.

Effects of temperature, salinity and pH on the biosurfactant stability

The surface tension was measured to determine the stability of the biosurfactant at different temperatures, pH and salinities. The cell-free culture supernatant was obtained by centrifugation at 8000 × *g* for 20 min at 4 °C. To determine the thermal stability of the biosurfactant, cell-free broth was maintained at a constant temperature range of 4–120 °C for 30 min, then cooled to room temperature and activity of the biosurfactant was investigated. To assess the effect of salinity on the surface activity of cell-free broth, various concentrations of sodium chloride (0–12%, w/v) were

employed. For analysing the effect of pH on the biosurfactant activity, the pH of the cell-free broth was adjusted in the range of 2.0–12.0 with HCl (6 N) and NaOH (6 N) and the surface tension were measured. All experiments were performed in triplicate.

Extraction and characterization of biosurfactant produced by strain BS2

Extraction and purification of the BS2 biosurfactant were carried out as described elsewhere (Chandankere et al., 2013). The functional groups and elemental composition of the dried biosurfactant was studied by fourier transform infrared spectroscopy (FT-IR) and energy dispersive X-ray spectroscopy (EDS), respectively. The sample for FT-IR analysis was prepared as mentioned elsewhere (Chandankere et al., 2013) and analysis was carried out by GX-FTIR system (Perkin Elmer, USA). Quantitative elemental analysis of the dried biosurfactant was carried out to determine the weight and atomic percentage of different elements present in the sample by using EDS (EDS, Oxford Instruments, UK).

Biodegradation of crude oil

The capability of the isolated BS2 strain to degrade crude oil at higher temperature was evaluated, aiming at its potential application in MEOR. Flasks containing 250 mL of MSM culture media supplemented with 1% (v/v) of the crude oil as sole carbon source were inoculated with strain BS2 culture and incubated at 55 °C, at 150 rpm for 15 d. Control incubation was kept under same conditions, without addition of bacterial culture. After the incubation period, the residual organic phase was extracted and analysed by gas chromatography (GC). The procedures of extraction and GC analysis as well as semi-quantitative analysis of oil biodegradation were reported elsewhere (Cai et al., 2013).

Oil recovery by the core flooding system

For core flood studies, a Berea sandstone core of 3.85 cm diameter and 20 cm length was used. The permeability of the core was about 170 md. The core pack was prepared with core by filling Serro metal around the core plug in a core holder. After determining the pore volume, the core was flooded with filtered (0.45 μ) formation water by displacement pump. Then the core was flooded with oil of an API gravity of 26 from the same sand till no further water comes out of core and has IOS (Initial Oil Saturation) condition. Further, the core was flooded with formation water until no oil is produced to obtain residual oil saturation (ROS). After this step, the isolated microbial culture with MSM was injected into the core. Changes in injection pressure and different pressure were monitored. A constant flooding rate of 20 mL h⁻¹ of liquid was applied. After that two different core pack were kept in incubator at 37 °C and 55 °C for 15 d so that bacteria can grow, multiply establish there and can produce metabolites. After completion of the incubation period, again formation water (MSM of same salinity) was injected to recover the additional residual oil from the core to simulate oil recovery by MEOR.

Results and Discussion

Dagang Oil field is located in Huanghua depression and belongs to the Dagang district of Tianjin. Dagang ranks on place 6 among 21 onshore oil and gas fields in China. The production covers twenty-five districts, cities and counties in Tianjin, Hebei and Shandong. Since the beginning of the oil and gas exploration, most of the blocks are under secondary recovery through water flooding and polymer flooding. MEOR, particularly in-situ MEOR technology, has previously been studied with limited success (Feng et al., 2006).

Isolation and identification of biosurfactant-producing strains

From several isolated bacterial strains with different types of colonies, 34 strains were tested to have haemolytic activity on blood agar plates. These isolates were able to produce clear displacement circles by the oil spreading technique. Cultures producing displacement circles with diameters of 4.0 cm and above were considered for further investigations. 9 out of 34 strains, producing circles within 4.0 - 5.7 cm were selected and considered as the most efficient biosurfactant-producing bacteria isolated from Dagang oil field.

The strains were subjected to 16S rDNA gene sequence analysis for identification of the isolates. The nucleotide sequences of the nearly complete 16S rDNA gene (1442 bp) were determined and compared to previously recorded sequences. The 9 most efficient biosurfactant-producing bacteria isolated from Dagang oil field were closely related to the species in genus *Pseudomonas*, *Staphylococcus* and *Bacillus* (Fig. 1). The 16S rDNA gene sequences of isolates BS1-BS9 have been deposited with the GenBank database, and can be obtained by accession number (Fig. 1). Out of the 9 efficient biosurfactant-producing bacteria, strain BS2 had the highest biosurfactant production and activity. This was selected for further study. Based on the 16S rDNA gene sequences and using the GenBank BLAST, isolate BS2 was found to be closely related to *Bacillus subtilis* strain Baws1, with 100% similarity. We thus tentatively classified strain BS2 as *Bacillus subtilis* BS2.

Growth of strain BS2 at different temperatures and salinities

In addition to the lack of oxygen,

temperature and salinity appear to be the most important environmental factors that influence bacterial growth in the reservoir. Therefore, the growth of the strain BS2 at different temperatures and salinities were studied. The surface tension dropped rapidly after inoculation at 37 °C, reaching its lowest value (28.97 mN m⁻¹) after 8 d of growth (Fig. 2a). The biosurfactant produced by strain BS2 at 55 °C, otherwise under the same conditions could reduce the surface tension from 70.87 to 43.31 mN m⁻¹ after 14 d of culture and drop to 36.15 mN m⁻¹ after 20 d of incubation (Fig. 2b). This suggests that strain BS2 is an efficient thermotolerant biosurfactant-producing bacterium which could be potentially used in microbial enhanced oil recovery processes, as the average temperature of the Dagang oil reservoir is about 55 °C.

Strain BS2 could grow under NaCl concentrations up to 40 g L⁻¹ with an OD value of 1.4 after 6 d (Fig. 2c). The surface tension remained relatively stable (ranging from 30.21 to 31.70 mN m⁻¹) at NaCl concentrations of 2 to 20 g L⁻¹. Even under the high concentration of 40 g L⁻¹ NaCl, the biosurfactant produced by strain BS2 could reduce the surface tension from 70.87 to 35.88 mN m⁻¹ after 6 d of incubation, which indicates that strain BS2 is a halo-tolerant biosurfactant-producing bacterium, and may also find potential application in bioremediation of contaminated marine environments (the average salinity of the sea water is 34.88 g L⁻¹). Growth in the marine environments and the capability to use oil as a carbon substrate offers strain BS2 biotechnological potential for its use in remediation of oil spills.

Stability of BS2 biosurfactant

The stability of the BS2 biosurfactant was studied at different temperature, salinity, and

pH. The thermal stability analysis of cell-free broth over a wide range of temperature (4–120 °C) gave a stable surface tension around 30 mN m⁻¹, revealing that the BS2 biosurfactant can operate at in a large temperature interval (Fig. 3a). Heating of the cell-free supernatant up to 120 °C caused no effect on the biosurfactant performance, suggesting thermo stability in the typical temperature range of oil reservoir. The mean value of six ST values obtained at different temperatures was 30.4 mN m⁻¹, with a standard deviation of 0.32‰. This suggests that the BS2 biosurfactant can be used in microbial enhanced oil recovery processes where high temperatures prevail. The surface tension reduction was stable even at temperature beyond the growth of this organism.

Fig. 3b shows the effect of salinity on the biosurfactant activity. Negligible changes were recorded in the biosurfactant activity with an increase in the NaCl concentration up to 12%. Steady surface tension of average 30.07 mN m⁻¹ ± 0.86‰ was observed.

Fig. 3c depicts the activity of the biosurfactant-containing broth a various pH. At acidic pH of 2 and 4, the ST values were 35.33 and 36.43 mN m⁻¹, respectively. This can be attributed to the decreased aqueous solubility of biosurfactant. However, the BS2 biosurfactant activity was retained over a pH range of 6–12 with minimal variation in surface tension.

The BS2 biosurfactant can be used under a large range of temperature, salinity and pH covering typical reservoir conditions of oil fields. Our findings further indicate that the strain BS2, not only could find the application in enhanced oil recovery operations, but may find application in bioremediation of soil as well as marine environments in a consortium.

Characterization of produced biosurfactant

The FT-IR spectrum was analysed to elucidate the functional groups of the produced biosurfactant. The broad peak located at 3410 cm⁻¹ is expected to be C-H stretching vibrations of hydrocarbon chain position (Fig. 4). Further two major adsorption bands at 1650 and 1540 cm⁻¹ attributed to the bonds formed between hydroxyl groups and carbons atoms, respectively. The adsorption peaks at 1210 cm⁻¹ could be ascribed due to the presence of sulphate group. From the result obtained, the produced biosurfactant was tentatively characterised as glycolipid derivative. These results were in concurrence with as mentioned elsewhere (Chandankere et al., 2013). To support this result, elemental analysis of the dried biosurfactant was carried out using EDS.

EDS analysis of BS2 biosurfactant revealed the composition of five elements (Na, C, S O and Cl) normalise to 100% of the biosurfactant (Table1). The distribution of cation (Na) element suggested its link with the negative charge of the sulphate or carboxylic groups. The anionic nature of the extracted biosurfactant was indicated by the presence of carboxyl and acetyl and functional groups in the biosurfactant. The result of EDX analysis supports those obtained by FT-IR analysis.

Crude oil biodegradation by Strain BS2

Relative distributions of n-alkanes and normalisation of concentration relative to 30-hopane, obtained from the control and degraded crude oil after 15 d of biodegradation by strain BS2 is depicted in Fig. 5. The results demonstrated that n-alkanes in the range of C14-C35 were degraded to varying extent.

Table.1 EDS analysis of BS2 biosurfactant.

Elements	Atomic ^a	Weight ^b
Na	35.12	30.48
C	30.32	19.62
S	7.09	9.12
O	4.12	7.33
Cl	23.35	33.45
Total	100	100

^{a, b}Data are normalized as weight and atomic percent.

Fig.1 The phylogenetic tree based on the 16S rDNA sequence demonstrating the phylogenetic affiliation of strains BS1-BS9 (bootstrap value 0.1)

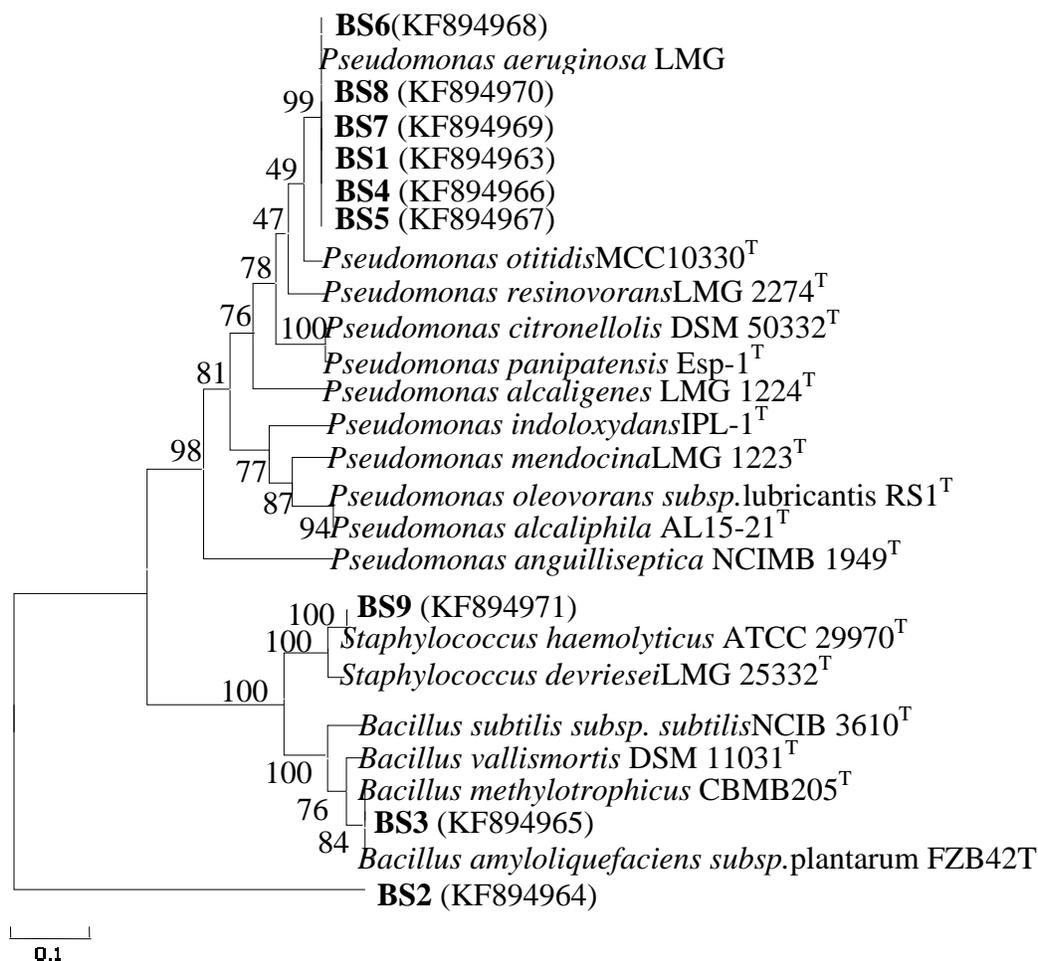


Table.2 Parameters of the oil recovery test by the core flooding system at 37 °C and 55 °C.

Parameters	55 °C	37 °C
Pore Volume	39 cc	41 cc
Porosity	20 %	21 %
Permeability	139 md	142 md
Oil Gravity	26° API	26° API
Water displaced in oil flooding	25 cc	27 cc
Oil left in Core	25 cc	27 cc
Water saturation	35.9 %	34.1 %
Initial oil saturation (IOS)	64.1 %	65.9 %
Oil recovery by water flooding	9.2 cc = 36.8%	12 cc = 44.4%
Oil remaining	15.8 cc	15 cc
ROS by water flood	40.5 %	36.58 %
Oil recovery by MEOR	1.8 cc = 7.2%	2.6 cc = 9.6%

Fig.2 Growth of strain BS2 in MSM at different temperatures and salinities. Kinetics of biosurfactant production by strain BS2 at 37 °C (a) and at 55 °C (b); effect of NaCl concentration on the growth of strain BS2 and surface activity (c).

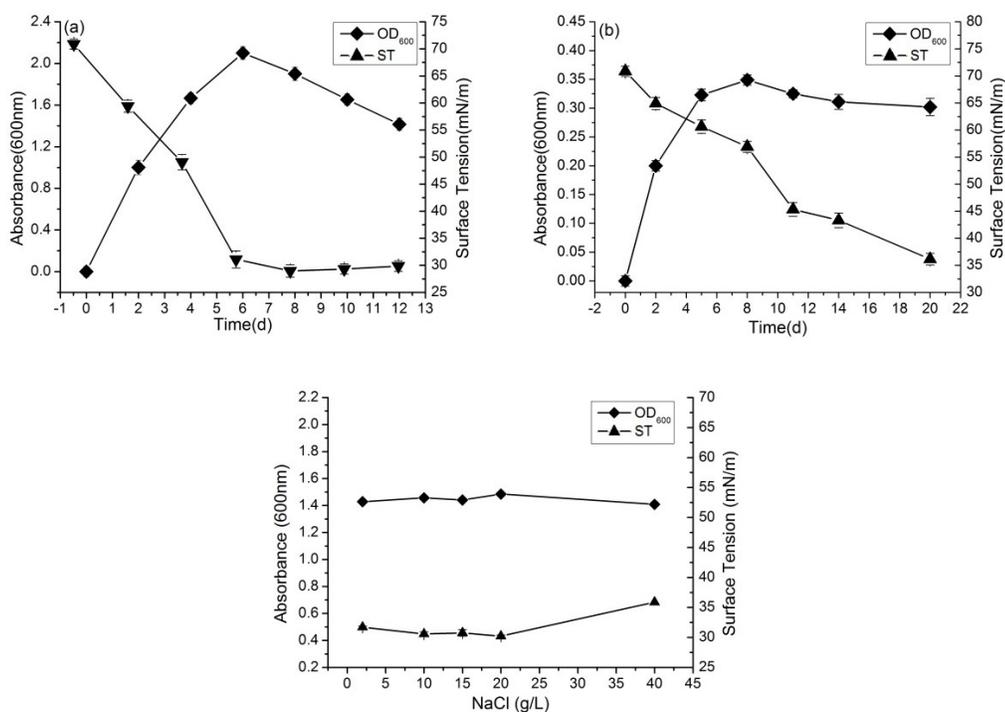


Fig.3 Effects of temperature (a), salinity (b) and pH (c) on the stability of BS2 biosurfactant.

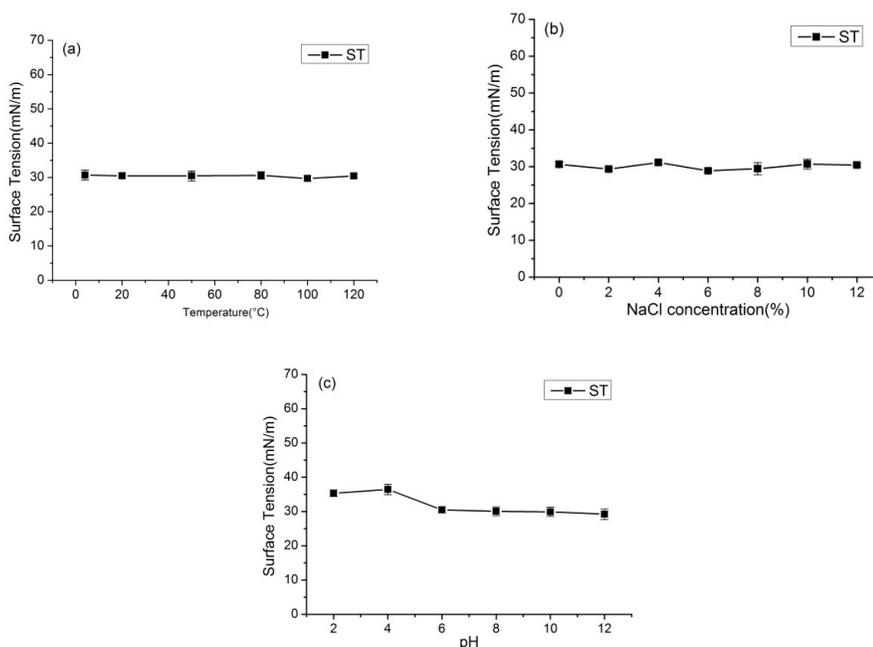


Fig.4 Characterization of biosurfactant produced by strain BS2 by Fourier Transform Infrared analysis (FT-IR)

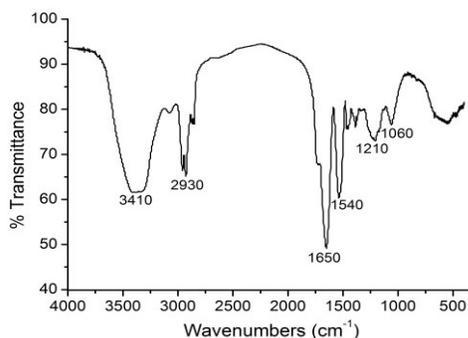
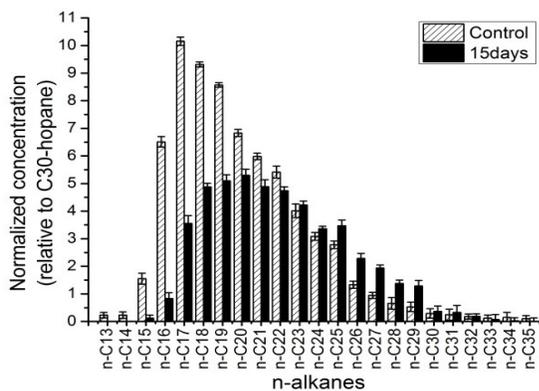


Fig.5 Relative concentration of n-alkanes with respect to 30-hopane. Each bar is the average of three independent replicates and the error bars represent the standard deviation.



The predominant utilization of C15-C19 ranging from 92.13 to 40.47%, suggest a preferential utilisation of n-alkanes upon microbial metabolism of strain BS2. Alkanes are the carbon substrate also used for the synthesis of biosurfactant. The biosurfactants probably increase the solubility of hydrophobic oil and make it more bioavailable for biodegradation. Partial biodegradation of n-alkanes will not increase the viscosity of the oil to a greater extent, however, the produced biosurfactant reduce the surface tension significantly, leading to mobilisation with the water flow in the reservoir.

The preferential removal of alkane is typical feature of reservoir degradation and also observed in the methanogenic Dagang reservoir (Jimenez et al., 2012). Reservoirs are mostly anoxic and the production of biosurfactants in an in-situ application will largely depend on O₂ concentration and thus can be controlled by the O₂ supply to avoid uncontrolled deterioration of the crude oil in the reservoir. Larter and colleagues (Larter et al., 2003) indicated very slow rate of biodegradation of crude oil in reservoir, limited to lighter alkanes and aromatic fractions.

Kumari and colleagues (Kumari et al., 2012) studied the degradation of aromatics and different carbon chain length alkanes and its relation to biosurfactant production and activity, also indicated the preferential degradation of C14-C18 alkanes, followed by degradation of aromatics, corroborating our findings. The oil degradation capacity of the isolated high biosurfactant producing *Bacillus subtilis* BS2 can also be used as a potent member of bacterial consortium for bioremediation of crude oil contaminated sites.

Oil recovery by the core flooding system

Core flood studies was carried out to evaluate the effects of the isolated BS2 bacteria, on oil release and recovery at nearly reservoir conditions at 55 °C, 10 g L⁻¹ salinity and under static growth condition for 15 d incubation period. After the desired period of incubation, some gas release was observed before oil release. The released gas was found to contain mainly CO₂ suggesting mineralisation of oil. The pH of the produced water decreased from 7.0 to 6.0, which is an indication of in situ bacterial propagation and metabolite production. The oil recovery was increased 7.2% over water flood (Table 2). Core flood studies for MEOR conducted by various authors corroborate our findings (Brown, 2010; Rabiei et al., 2013).

However, the core flood study carried out at 37 °C incubation by deploying this bacterial strain under the same set of conditions resulted in the oil recovery to the order of 2.6 cc or 9.6%, which is supported by the results of the higher surface tension reduction at 37 °C as compared to 55 °C and is as expected, The result further shows that the strain BS2 can be used in low as well as high temperature reservoir with good success.

Bacillus subtilis BS2 isolated from Dagang oil field was identified and characterized to be an efficient thermotolerant and halophilic biosurfactant-producing bacterium. Strain BS2 showed the ability to reduce the surface tension from 70.87 mN m⁻¹ to 36.15 mN m⁻¹ when the cells were grown on minimal salt medium containing 1% (v/v) crude-oil as the sole source of carbon at 55 °C and 150 rpm.

Additionally, strain BS2 could grow under NaCl concentrations up to 40g L⁻¹ and obtained the lowest surface tension of 35.88 mN m⁻¹ at 37 °C after 6 d. The produced biosurfactant also exhibited a robust tolerance for temperature, pH and salinity variations. The study of environmental factors on the produced biosurfactant stability increases its scope of application at conditions with varying pH, temperature and salinity. In core flood study, an increase of 9.6% and 7.2% of additional oil is recovered over water flooding at 37 °C and 55 °C respectively. This makes strain BS2 as a promising candidate for MEOR application. Its halotolerant capacity adds value for bioremediation of oil contaminated sites (oil spills), especially in the marine environment.

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