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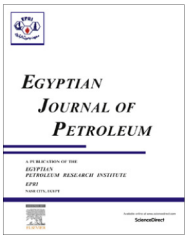

FULL LENGTH ARTICLE

Production of biosurfactant from *Bacillus licheniformis* for microbial enhanced oil recovery and inhibition the growth of sulfate reducing bacteria

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Abstract In this study, the bacterium Bacillus licheniformis has been isolated from oil reservoir; the ability of this bacterium to produce a biosurfactant was detected. Surface properties of the produced biosurfactant were confirmed by determining the emulsification power as well as surface and interfacial tension. The crude biosurfactant has been extracted from supernatant culture growth, and the yield of crude biosurfactant was about 1 g/l. Also, chemical structure of the produced biosurfactant was confirmed using FTIR analysis. Results revealed that, the emulsification power has been increased up to 96% and the surface tension decreased from 72 of distilled water to 36 mN/m after 72 h of incubation. The potential application of this bacterial species in microbial-enhanced oil recovery (MEOR) was investigated. The percent of oil recovery was 16.6% upon application in a sand pack column designed to stimulate an oil recovery. It also showed antimicrobial activity against the growth of different strains of SRB (sulfate reducing bacteria). Results revealed that a complete inhibition of SRB growth using 1.0% crude biosurfactant is achieved after 3 h.

1. Introduction

Surface active agents produced by different groups of microorganisms are known as biosurfactants. Biosurfactants reduce surface tension in both aqueous and hydrocarbon mixtures. Biosurfactants can aggregate at interfaces between fluids having different polarities, such as water and oil, leading to the reduction in interfacial tension. Because of their efficiency in lowering interfacial tension, biosurfactants have been employed for the enhancement of oil production especially in tertiary oil recovery. Low toxicity, high biodegradability and ecological acceptability are among the main characteristics of these surface active materials [1–6]. These favorable features make biosurfactants potential as one of the good alternatives of chemically synthesized surfactants in a variety of applications [7,8].

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Biosurfactants can be categorized into four main groups: lipopeptides and lipoproteins, glycolipids, phospholipids, and polymeric surfactants [9].

Biosurfactants are widely used in different industries, such as cosmetics, special chemicals, food, pharmaceutics, agriculture, cleaners and microbial enhanced oil recovery (MEOR) [10–13]. The last mentioned application has attracted more attention because only 30% of oil present in reservoir can generally be recovered using primary and secondary recovery techniques [1]. MEOR is considered as a tertiary recovery technique that could recover the residual oil using microorganisms or their products (biosurfactants). However, the application of biosurfactants in microbial enhanced oil recovery depends on their stability at extreme conditions of temperature, salinity and pH, or surface activities [12]. Stimulation of microorganisms that produce biosurfactants and degrade heavy oil fractions in situ reduces the capillary forces that retain the oil into the reservoir and decreases oil viscosity, thus promoting its flow. As a result, oil production can be increased [14].

Another interesting application of biosurfactants is the use of biosurfactant as an antimicrobial agent. In this study sulfate reducing bacteria were chosen as target organisms. Sulfate reducing bacteria (SRB) were detrimental bacterial species that used sulfur-based compounds present in formation waters as a feedstock. SRB could produce hydrogen sulfides which are poisonous to humans [15], and corrosive to oil-extracting equipments.

These bacteria could be responsible for H_2S production, which causes "souring" of crude oil, microbiologically influenced corrosion (MIC), and increased solid loading in water injection systems (iron sulfides, etc.), and can cause plugging of producer and injection wells with sulfide scale or biofilm proliferation.

In addition, souring lowers the economic value of produced oil and induces safety hazards [16,17]. A number of methods for controlling sulfate reducing bacteria in different oil production facilities have been proposed to reduce microbial activity, including use of oxidizing (such as halogen and ozone) or nonoxidizing biocides (such as formaldehyde, glutaraldehyde, isothiazolones, and quaternary ammonia compounds) [18]. Also, SRB would play a very negative role in MEOR [19].

The main objective of this study:

The present study aimed to isolate and identify bacteria from oil reservoir and detect their ability in biosurfactant production. The potential application of these isolated bacteria in microbial-enhanced oil recovery (MEOR) and also their antimicrobial activity against sulfate reducing bacteria was investigated.

2. Experimental

2.1. Sampling procedure

Water samples were collected from a well (Fadl 9) located in the Niage field, Badr El-din Petroleum Company, west desert, Egypt. The samples were collected in sterile bottles by 50 ml sterile syringe from sample points, preserved in refrigerator at 4 °C and transported to the laboratory and bacteriological analyses were conducted within 24 h [20].

2.2. Reservoir brine characterization

The production of Fadl 9 well is 10% oil and 90% water; the salinity (as NaCl) of the accompanying (formation) water is 9% and the temperature of the well head is 45 °C.

2.3. Isolation of crude oil degrading bacteria

Bushnell Hass Mineral Salts (BHMS) medium was used for the isolation of hydrocarbon-degrading bacteria, BHMS medium composed of the following (g/l): KH_2PO_4, 1; K_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.02; NH_4NO_3, 1; NaCl, 2 and 2 droplets of 60% $FeCl_3$. The pH was adjusted to 7. The BHMS medium was supplemented with 1% (v/v) crude oil as the sole carbon source [14].

Formation water (1 ml) was added to Erlenmeyer flasks containing 100 ml of the medium, and the flasks were incubated for 10 days at 30 °C on a rotary shaker (150 rpm). Then 5-ml aliquots were removed and placed in a fresh medium. After a series of three further subcultures, inoculums from the flask were streaked out, and phenotypically different colonies on the BHMS agar were purified. The pure isolates were stored in stock media with glycerol at −20 °C for further characterization [21,22].

2.4. Identification of the bacterial isolate

The selected bacterial isolate was identified at the Natural Care for Scientific Consultation and Research (NCSCR), Cairo, Egypt.

- Isolation of template DNA

The genomic DNA was isolated using QIAamp DNA Mini kit.

- PCR amplification

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) (GeneAmp® PCR System 9700 in 9600 emulation mode) using the following primers:

Forward primer (5′ to 3′): AAGCAACGCGAAGAACC TTA
Reverse primer (3′ to 5′): AGAGTGCCCAACTGAAT GCT

The following PCR program set to thermal-cycling contains: initial step at 10 min, 95 °C: this heating step was required to activate the AmpliTag Gold® DNA Polymerase, Melt step at 95 °C, 30 s, Anneal step at 60 °C, 30 s and Extend step at 72 °C, 45 s. (each of 30 cycles), final extension at 72 °C, 10 min and final step at 4 °C, ∞.

- DNA sequencing

The 16S rDNA was sequenced by electrophoresis and data gathering was done automatically by ABI prism® 310 Genetic Analyzer.

- Blast analysis of 16S rRNA

Blast program (basic local alignment search tool) is a web search tool to show the identity and similarity between different microorganisms (www.plast.ncbi.nlm.nih.gov/plast.cgi).

Then this sequence was compared with the 16S rDNA sequence in the MicroSeq® ID16S rDNA 500 Library (v 1.0). Based on the comparison, the software provided a potential ID for the unknown bacterial species. The MicroSeq® ID16S rDNA 500 Library (v 1.0) included over 1435 validated 16S rDNA sequences. All sequences and strains were carefully checked and quality controlled to achieve maximum reliability. Polymorphic positions were taken into account to ensure the highest degree of accuracy. The MicroSeq® 500 ID (Applied biosystems) Analysis software provides the phylogenetic tree which includes a list of all the top matches.

2.5. Growth kinetics and production of biosurfactant by the bacterial isolate

The bacterial strain was streaked on a nutrient agar slant and incubated for 24 h at 30 °C. Two loops of culture were inoculated in 40 ml of nutrient broth in a 100 ml Erlenmeyer flask and incubated in a rotary shaker at 150 rpm for 30 °C for 8–12 h until cell numbers reached 10^8 CFU/ml. This was used as inoculum at the 5% (w/v) level. For biosurfactant production, a mineral salt medium with the following composition was utilized: 2.5 g/l of $NaNO_3$, 0.1 g/l of KCl, 3.0 g/l of KH_2PO_4, 7.0 g/l of K_2HPO_4, 0.01 g/l of $CaCl_2$, 0.5 g/l of $MgSO_4{\cdot}7H_2O$, and 5 ml of a trace element solution. Trace element solution contained 0.116 g/l of $FeSO_4{\cdot}7H_2O$, 0.232 g/l of H_3BO_3, 0.41 g/l of $CoCl_2{\cdot}6H_2O$, 0.008 g/l of $CuSO_4{\cdot}5H_2O$, 0.008 g/l of $MnSO_4{\cdot}H_2O$, 0.022 g/l of $[NH_4]_6Mo_7O_{24}$ and 0.174 g/l of $ZnSO_4$ [23]. The respective carbohydrate (glucose) was added to make a final concentration of 2%. The concentration of the yeast extract was 3%. Cultivation studies have been done in 500 ml flasks containing 150 ml of the medium at 30 °C for 48 hs. Experiments were conducted in independent triplicates [24].

2.6. Biosurfactant recovery

The bacterial broth (10 ml) was inoculated into the medium MSM (1000 ml) using glucose as a sole carbon source and the pH value was adjusted to 7.5. Incubation was carried out at 30 °C, 150 rpm, for 72 h. The extraction technique is a combination of acid precipitation and solvent extraction [25]. The broth culture sample was centrifuged (at 4 °C using 130,000×*g* for 15 min). The obtained supernatant was treated by acidification to pH 2.0 using 6 M HCl, and the acidified supernatant was left overnight at 4 °C for complete precipitation of the biosurfactants. Supernatant was removed to obtain the pellet and the pellet was extracted with methanol for 2 h while stirring continuously. Methanol was filtered to remove the remaining material and evaporated to dryness using rotary evaporation.

2.7. Chemical structure of the crude biosurfactant

Infrared (IR) spectra of the biosurfactant (a film of each sample on KBr pellet) were obtained using a Nicolet IS-10FTIR spectrometer. IR spectra were conducted between 4000 and 500 cm^{-1} with a resolution of 1 cm^{-1} [20].

2.8. Surface properties

Surface properties including surface tension, critical micelle concentration (CMC), emulsification index (E_{24}) and foaming were determined as indicator of biosurfactant production and these measurements were done in triplicate. The surface properties of the crude biosurfactant were evaluated by measurement of the interfacial tension and the Critical micelle concentration (CMC).

2.8.1. Surface tension

Surface tension was measured on a ring tensiometer (Krüss-tensiometer K6) using the bacterial supernatant solution (50 ml) at 30 °C, while a solution of 0.1%, was tested at 30 °C when evaluating the crude biosurfactant [4].

2.8.2. Critical micelle concentration (CMC)

Critical micelle concentration is the concentration of an amphiphilic component in a solution in which the formation of micelles is initiated. Concentrations ranging from 0.1 to 1×10^{-6}% of the crude biosurfactant recovered from the isolate of *Bacillus licheniformis* were prepared. The critical micelle concentration values of the biosurfactant were determined using surface tension method. The CMC was determined from a semilog plot of surface tension versus surfactin concentrations. All measurements were done in duplicate [26].

2.8.3. Interfacial tension

Interfacial tension of surfactin biosurfactant was estimated using a surface tensiometer. Interfacial tension at different concentrations ranging from 100 to 1000 ppm of surfactin solutions was carried out and measured against paraffin oil at 25 and 45 °C [27].

2.8.4. Emulsification index (E_{24})

Emulsification power of the produced biosurfactant in the culture supernatant was measured by adding kerosene (6 ml) to the aqueous phase (of culture supernatant) and by vortexing for 2 min. After 24 h the emulsion index (E_{24}) was calculated according to the following equation [28]:

$$(E_{24}) = 100 \text{ (height of the emulsion layer/the total height)}$$

2.8.5. Foaming

Foaming of the biosurfactant in the culture medium was determined by shaking the supernatant (10 ml) for 2 min, and then foaming was calculated according to the following equation [29]:

$$\text{Foaming} = (\text{height of foaming/total height}) \times 100$$

2.9. Effect of some different environmental factors on the production of biosurfactant

2.9.1. Salinity

The effect of salinity on surface tension was determined by adding NaCl at different concentrations (1, 5, 10, 15 and 20%) of Nacl to 0.1% surfactin solution and the surface tension was measured at 25 °C [30].

2.9.2. pH

In order to investigate the stability of the surfactin at different pH values, the crude surfactin solution (0.1%) was adjusted to pH values of 3, 5, 7, 10 and 12. The surface tension of the resultant solutions was measured at room temperature [26].

2.10. Application of the produced biosurfactant in enhanced oil recovery

The potential application of the indigenous bacteria and biosurfactant-producing bacterial strain for MEOR was evaluated using the sand pack column technique designed to stimulate an oil recovery, described elsewhere [31]. The operation of the sand pack column was as follows:

(1) Saturation of the sand pack with brine: The column was flooded with brine under pressure to ensure its 100% saturation with brine. Pore volume of the column was calculated by measuring the volume of brine required to saturate the column (PV).
(2) Saturation of the sand pack with oil: the oil was collected from Niage 1 oilfield Badr El-din Petroleum Company. The oil filled in a tank was passed under pressure into the sand pack column, in the same way as the brine, until residual brine saturation was reached. As the oil entered into the column, the brine was displaced and discharged from the pack through a tubing inserted at the bottom end of the column. Initial oil saturation (S_{oi}) was calculated by measuring the volume of brine displaced by oil saturation, also called original oil in place (OOIP).
(3) Brine flooding: the sand pack was again flooded with brine until there was no oil coming in the effluent, i.e. residual oil saturation (S_{or}) was reached. The amount of crude oil retained in the sand pack was determined volumetrically. S_{or} was calculated by measuring the volume of the displaced oil.
(4) Biosurfactant flooding: this was done in a manner similar to oil and brine floods. 0.6 pore volume of the crude biosurfactant was passed through the column at a flow rate of approximately 2.5 ml/min and incubated for 24 h; then the column was again flooded with brine. Discharges from the column were collected in 25 ml quantities to measure the amount of oil recovered using crude biosurfactant.

2.11. Antimicrobial test against sulfate reducing bacteria (SRB)

This test has been conducted using NACE Standard test method TM 0194-94 (Field Monitoring of Bacterial Growth in Oilfield Systems) and ASTM [32]. The tested water collected from the Niage 1 oilfield Badr El-din Petroleum Company has been subjected to a growth of about 10^8 bacterial cells/ml. One type of biosurfactant (surfactin) produced from *Bacillus licheniformis* was tested as biocide at different concentrations (0.1, 0.3, 0.5, 0.75 and 1%) and the system was incubated for a contact time of 3 h. Each system was recultured in SRB specific media.

3. Results and discussion

3.1. Isolation and identification of biosurfactant producing bacteria

Bacteria isolated from the formation water sample taken aseptically from Fadl 9 oil reservoir as described earlier were enumerated at 3.1×10^7 CFU/ml using Bushnell Hass Mineral Salts (BHMS) medium. Only one bacterial strain has been found to be predominant in the culture medium using crude oil as carbon source. The bacterial isolate was rod shaped and gram positive, bacilli.

The identification of the bacterial isolate was carried out using 16S rRNA technique, where the isolated genomic DNA of the strain was amplified using PCR reaction. The PCR product was purified and sequenced. The bacterial isolate was identified as *B. licheniformis* DSM = 13 strain ATCC 14580 applying the 16S rDNA sequence with 99% identity.

3.2. Growth kinetics and evaluation the production of biosurfactant

The stationary phase of the *B. licheniformis* that appeared after 24 up to 72 h is shown in Table 1 and Fig. 1. On the other hand, the surface tension and emulsification power of the supernatant obtained from the corresponding broth culture of the *B. licheniformis* were taken as an indication of the ability of the bacteria to produce biosurfactants (Table 1). The maximum biosurfactant production has been achieved in 72 h of incubation during the stationary phase of the growth curve, so that the production of biosurfactants is considered as secondary metabolites. Lin [33] reported that, most biosurfactants are considered as secondary metabolites and some may play essential roles in the survival of the producing microorganisms either through facilitating nutrient transport or microbe–host interactions, or as biocides. It has been suggested that the production of biosurfactants can enhance emulsification and solubilization of hydrocarbon substrates, and therefore facilitate the growth of microorganisms on hydrocarbons. By secreting biosurfactants into the growth medium, microorganisms relying on non-polar substrates as sole carbon sources, ensure

Table 1 Evaluation of growth kinetics and biosurfactant production of the bacterial isolate.

Incubation period h	Log count	Emulsification power (E_{24}) %	Surface tension (S.T.) mN/m
Control*	0	0	59
0**	8.2	0	59
3	8.2	0	56
6	8.8	25	50
12	9.0	62	50
24	9.3	75	46
48	9.3	85	42
72	9.7	96	36
96	9.1	80	45

Control*: Sample without inoculum.
0**: Inoculation time.

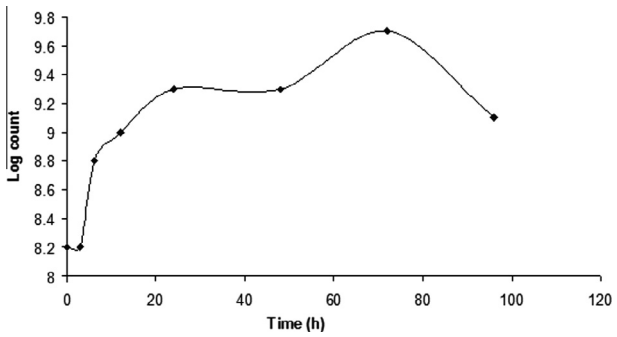

Figure 1 Growth curve of *Bacillus licheniformis*.

the timely supply of carbon source to maintain their survival and growth. One of the most important properties of biosurfactants should be foaming power. The observed foaming due to the biosurfactant obtained in the supernatant of *B. licheniformis* was found to be 51%. Stable foaming coupled with reduction in surface tension and an increase in the emulsification power of the medium is considered as a qualitative indication of biosurfactant production [34]. It is worth noting that the formation of foam during enrichment of a culture in a mineral medium with glucose as a carbon source was a potential application of biosurfactants in microbial enhanced oil recovery [35].

3.3. Biosurfactant recovery

After the bacterial strain *B. licheniformis* was grown under optimum conditions, the recovery of biosurfactant from cell free culture was done by the classical technique. This technique is well suited for batch recovery as a partial purification process. They include solvent extraction, precipitation and crystallization. The yield of the biosurfactant was relatively low (1 g/l). In fact, the modification of the succeeding fermentation process is expected to raise the production rate. This is supported by the results of Rodrigues et al. [36] where they reported that the potential use of alternative fermentative medium instead of the synthetic medium for biosurfactant production by *Lactococcus lactis* 53 and *Streptococcus thermophilus* effectively proceeded with high yields and productivities of biosurfactant. An increase about 1.2–1.5 times the mass of the produced biosurfactant per gram cell dry weight was achieved. About 1.8 g/l dry weight of the crude bioemulsifier was obtained after the partial purification process by *B. licheniformis* K125 [31]. Rhamnolipids were produced at a concentration of 1.3 and 0.709 g/l by *Pseudomonas aeruginosa* J4 using diesel and kerosene as sole carbon source has been reported by Wei et al. [37].

3.4. Structural characterization

The infrared spectrum of the *B. licheniformis* biosurfactant and the spectrum of a standard sample of surfactin from *B. subtilis* produced from de Oliveira et al. [38] are shown in Fig. 2. In both spectra it is possible to observe bands characteristic of peptides (wavelength 3430 NH, wavelength 1655 CO, and wavelength 1534 CN) and aliphatic chains (wavelength 3000–2800), indicating that this compound is a lipopeptide. Similar results were obtained by other authors [38] with *B. subtilis* and [39] when determining the chemical structure of the surfactant produced by *B. licheniformis*. No significant difference in IR spectra of the biosurfactant produced in this work and the standard sample is observed.

3.5. Surface properties

3.5.1. Critical micelle concentration (CMC)

One of the most important properties of a surfactant is their spontaneous aggregation in water and formation of well-

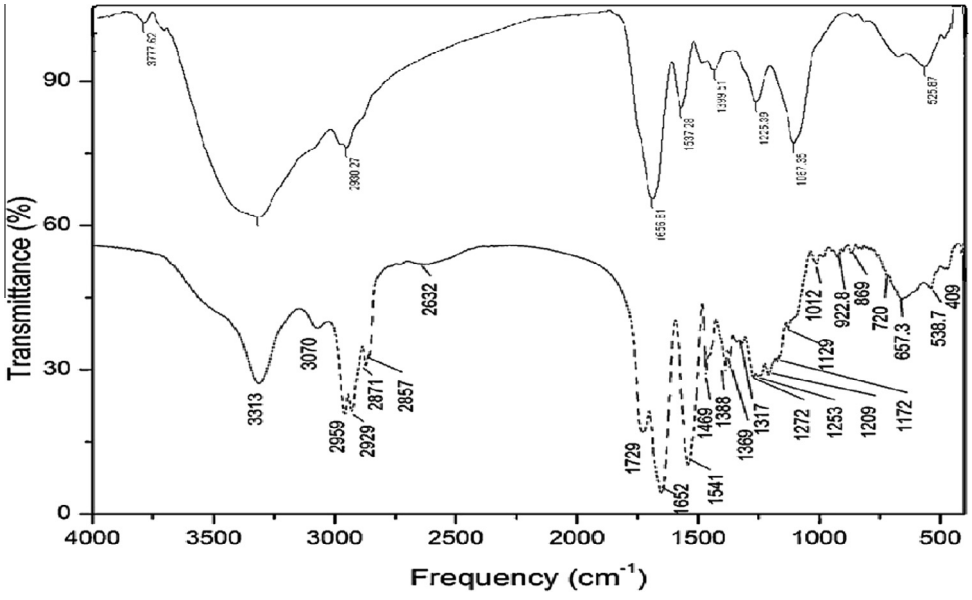

Figure 2 A comparison of FTIR spectroscopy of standard surfactin (bottom) and crude extracellular biosurfactant (top) produced by *Bacillus licheniformis*.

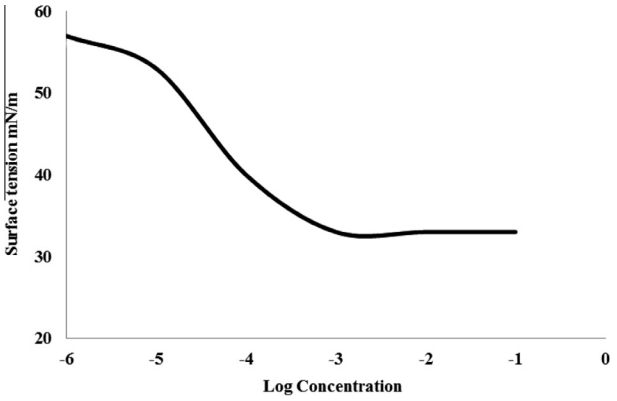

Figure 3 CMC and minimum surface tension reduction by crude biosurfactant produced by *Bacillus licheniformis*.

known structures such as spherical micelles, cylinders, etc. The surface tension decreases gradually with increasing surfactant concentration. At a certain concentration called critical micelle concentration (CMC), this decrease stops. Above the CMC, the surface tension remains almost constant [40]. The lowest CMC value was obtained at a concentration of 10^{-3}% with a minimum surface tension value of 32 mN/m (Fig. 3). As surfactant concentration increases the surface tension of the surfactant solution decreases up to a certain value and then becomes almost constant due to the interface saturation with the surfactant molecules. The results are in agreement with those of [41,4]. For practical purposes, it is important to distinguish between an effective biosurfactant and an efficient biosurfactant. Effectiveness is measured by the minimum value to which the surface tension can be reduced, whereas efficiency is measured by the biosurfactant concentration required to produce a significant reduction in the surface tension of water. The latter can be determined from the CMC of the biosurfactant [42].

3.5.2. Interfacial tension

The capacity of surfactin biosurfactant to reduce the interfacial tension between water and tested hydrophobic substance (paraffin oil) is shown in Table 2. The minimum reduction in interfacial tension was obtained at 1000 ppm concentration of the biosurfactant at 25 and 45 °C. Results suggested that the biosurfactant is very much effective. Thus, the efficiency of the biosurfactant in reducing the interfacial tension between sea water and hydrophobic substance makes it more attractive for use in microbial enhanced oil recovery (MEOR) [27].

Table 2 Measurement of interfacial tension at different biosurfactant concentrations in sea water at different temperatures.

Biosurfactant concentration (ppm)		Control*	1000	500	400	300	200	100
Interfacial tension of sea water (mN/m)	25 °C	13	5	8	9	8	10	12
	45 °C	10	4	8	7	7	10	11

Control*: Sample sea water without biosurfactant.

Table 3 Effect of salt concentration on surface tension reduction by the crude biosurfactant produced in the study.

Salinity% (NaCl)	Control*	1	5	10	15	20
Surface tension of biosurfactant (mN/m)	35	38	37	38	40	41
Surface tension of dist. water (mN/m)	71	71	72	74	75	76

Control: Sample without NaCl.

3.6. Environmental factors

3.6.1. Salinity

The surface activity of the surfactin solution was slightly affected by increasing sodium chloride concentrations up to 10% (Table 3). On the other hand high concentrations of sodium chloride (15–20%) revealed a slight increase in surface tension. Furthermore, sodium chloride alone had only a slight effect on the surface tension of the used distilled water (Table 3). Therefore, it can be concluded that the activity of the surfactin is not affected at high salt concentrations and exhibited high stability. Furthermore, it was necessary to study the effect of different concentrations of salinity on the activity of the test surfactin to investigate its applicability in the petroleum industry (MEOR), so that the surfactin is an excellent candidate, if compared with chemical surfactants, which are deactivated by 2–3% salt concentrations and cause environmental toxicity [43].

3.6.2. pH

The surface activity of the surfactin was greatly affected by changes in pH values. It was found that the surfactin solution has a low surface activity at pH 3 and the surface activity was detected starting with the increase in pH to 5 with its maximum value at pH 7 (Table 4). So, it could be concluded that, the surfactin biosurfactant is generally active at pHs around the neutral value. However, highly acidic conditions cause more reduction in the surface activity than highly alkaline ones. These findings are in accordance with those obtained by Wei and Chu [44]. They reported that the surfactin started to precipitate out of the production medium at pH 5 and was dissolved completely when the pH returned to 6.1.

As a result, physical characterization revealed that the surfactin has excellent surface and emulsifying activities and it showed high stabilities over a wide pH range (5–10). These properties make the surfactin biosurfactant potential candidate to be used in the bioremediation of contaminated sites and in the petroleum industry (MEOR) where drastic conditions commonly prevail.

3.7. Oil recovery using sand-pack column

B. licheniformis was used to perform the oil recovery technique with crude oil using sand-pack column. This microorganism

Table 4 Effect of different pH values on surface activity of surfactin biosurfactant.

pH	3	5	7	10	12
Surface tension (mN/m)	38	35	34	39	40

Table 5 Summary of results obtained in sand-pack column for crude oil recovery using *Bacillus licheniformis*.

Parameter	*Bacillus licheniformis*	Control
PV (ml)	43	45
OOIP (ml)	37	38
S_{oi} (%)	86	84.4
S_{wi} (%)	14	15.6
S_{orwf} (ml)	25	20
OOIP-S_{orwf}(ml)	12	18
Sor (%)	32.4	47.4
Sorbf (ml)	2	1
AOR (%)	16.6	5.6

OOIP, Original oil in place; S_{oi}, Initial oil saturation; S_{wi}, Initial water saturation; S_{or}, Residual oil saturation; S_{orbf}, Oil recovered after biosurfactant flooding; S_{orwf}, Oil recovered after water flooding; AOR, Additional Oil Recovery.
Soi (%) = OOIP/PV * 100 (Eq. 1), Swi (%) = PV – OOIP/PV * 100 (Eq. 2), Sor (%) = OOIP – S_{orwf}/OOIP * 100 (Eq. 3), AOR (%) = Sorbf/OOIP – Sorwf * 100 (Eq. 4).

can be reduced of the surface tension value up to 36 mN/m, and emulsify hydrocarbon to about 96%. So, it exhibits desirable properties for application in MEOR. Table 5 shows that, *B. licheniformis* has the ability to enhance oil recovery with the sand-pack column. The pore volume (PV) of the column is about 43 ml, OOIP (original oil in place) of the column is 37 ml. After the water flooding process, 32.4% of the oil remained trapped in the column. When the biosurfactant of *B. licheniformis* was introduced into the column and incubated for 24 h at 35 °C, the amount of oil recovered after biosurfactant flood was 2 ml. This means that additional 16.6% crude oil was recovered due to the action of the biosurfactant from *B. licheniformis*. This result is in agreement with that of [45,31]. It was also found that the construction of a sand pack column is easy, rapid and inexpensive and the problems associated with core flood studies like preservation of live cores. This makes the sand pack column a suitable bench-scale technique for screening microorganisms showing potential for oil recovery.

3.8. Antimicrobial activity of crude surfactin against SRB

Sulfate reducing bacteria (SRB), which have relatively simple growth requirements in sulfate and carbon as energy sources, play a very negative role in MEOR [19,46]. Also it is well known that the cationic surfactants were commonly used to control the SRB growth [47,48]. Table 6 shows the effect of different concentrations of crude biosurfactant produced by *B. licheniformis* as antimicrobial activity on the growth of sulfate reducing bacteria (SRB). Biosurfactants can gradually reduce the growth of bacteria at different concentrations until a complete decline of growth at 1% concentration. Results from Tables 1–4 suggested that a microbial metabolite possessing a combination of surface and emulsification activity could be effective in oil recovery and can be applied as a biocontrolling agent against SRB. So it can control the production of H_2S, which causes "souring" of crude oil.

Table 6 Effect of crude biosurfactant on the growth of sulfate reducing bacteria (SRB).

	Different concentration (%)					
Growth cell/ml	Control 10^8	0.10 10^5	0.30 10^5	0.50 10^3	0.75 10	1.00 Nil

4. Conclusion

In the present work *B.* licheniformis was the dominant bacteria isolated from oil reservoir. It was grown on the MSM medium to produce biosurfactants.

The produced biosurfactant was able to decrease the surface tension, interfacial tension and increase emulsification capacity. Also it enhanced oil recovery in the sand-pack column technique. It is found that the isolated biosurfactants have the ability to recover about 16.6% of the crude oil entrapped in the sand-pack column.

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