SPE 161458

Microbial Enhnaced Oil Recovery by *Bacillus subtilis* strains under simulated Reservoir conditionsE. J. Gudiña, L. R. Rodrigues, and J. A. Teixeira, University of Minho, J. F. Pereira, and J.A. Coutinho, University of Aveiro, L. P. Soares and M. T. Ribeiro, Partex Services Portugal

This paper was prepared for presentation at the Abu Dhabi International Petroleum Exhibition & Conference held in Abu Dhabi, UAE, 11–14 November 2012.

Abstract

Microbial Enhanced Oil Recovery (MEOR) is a tertiary oil recovery process in which microorganisms and their metabolites are used to retrieve unrecoverable oil from mature reservoirs. 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. In previous work, *Bacillus subtilis* strains that are useful for application in MEOR were isolated from crude oil samples. Those isolates were able to grow and produce extracellular biosurfactants at 40ºC under anaerobic conditions in mineral medium supplemented with hydrocarbons. In addition, some isolates degraded the long-chain *n*-alkanes of paraffinic mixtures. Mobilization of residual oil by those isolates was evaluated using sand-pack columns at 40ºC. Additional oil recoveries obtained with the different isolates using paraffin ranged from 16 to 31%. The recovered paraffin showed a decrease in the percentage of large alkyl chains and its viscosity was reduced. In the present work, the degradation of long-chain *n*-alkanes and the mobilization of residual oil by the same isolates were studied using heavy oil. The results obtained confirmed that the isolates were able of degrading the long chain *n*-alkanes of crude oil, and also additional oil recoveries between 8 and 10% were obtained. Furthermore, for a better simulation of the oil reservoir conditions, a core flooding equipment that can work at the oil reservoir pressure was designed and will be used to confirm the applicability of selected isolates to increase oil recovery. The sampled reservoir consists of sands with an average porosity of 25% and a permeability of 50mD. The oil is paraffinic, with low viscosity, high pour point and a gravity of 25º API, with very low solution gas. The reservoir pressure and temperature are respectively 398-440psi and 40ºC.

Introduction

The primary phase of oil recovery uses the natural stored energy of the reservoirs to produce oil and gas. As the reservoir pressure dissipates, the flow of oil to the well head can be improved by the injection of water into the wells. When the ratio of water to oil pumped out of the well becomes too great, the process is discontinued. However, after primary and secondary recovery operations, up to two-thirds of the original oil in place still remains in the reservoir. This is mainly due to the high viscosity of the residual oil, which limits its mobility, and the high interfacial tension between the hydrocarbon and the aqueous phases, which results in high capillary forces that retain the oil in small pores within the reservoir rock (Lazar et al. 2007; Sen 2008). A major challenge to the oil industry is extracting the maximum amount of oil from reservoirs. To recover entrapped oil, costly tertiary methods, known as Chemical Enhanced Oil Recovery (CEOR) are applied. Several compounds are used for CEOR. Surfactants reduce interfacial tension between oil/water and oil/rock interfaces. Polymers are used to increase the viscosity of water-flood. Acids, gases and solvents increase the permeability through the porous network and re-pressurize the oil reservoir. Since these compounds are petrochemicals, obtained from petroleum feedstock after refining and downstream processing, CEOR methods turned out to be economically unattractive as the finished products are utilized for the recovery of raw materials.

Microbial Enhanced Oil Recovery (MEOR) is an alternative tertiary oil recovery technology where microbial metabolites (biomass, biopolymers, gases, acids, solvents, enzymes and surface-active compounds) and activities (hydrocarbon metabolism, plugging) are used to improve the recovery of residual oil from depleted and marginal reservoirs, thereby extending their life (Brown 2010). This technology takes advantage of the ability of indigenous or injected microorganisms to synthesize useful products by fermenting inexpensive raw materials. MEOR processes offer major advantages over conventional EOR, namely they do not consume as large amounts of energy as the thermal processes, nor depend on the price of crude oil as many chemical processes. Furthermore, microbial products are biodegradable and have low toxicity (Lazar et al. 2007; Sen 2008; Youssef et al. 2009).

Biosurfactant production by microorganisms *in situ* constitutes an effective mechanism to recover large amounts of the residual oil from mature oil fields (Almeida et al. 2004; Banat et al. 2010; Bordoloi and Konwar 2008). Biosurfactants are a heterogeneous group of surface-active molecules synthesized by microorganisms with both hydrophilic and hydrophobic domains, which allow them to partition at the interface between fluid phases with different degrees of polarity, such as oil-water or air-water interfaces, reducing surface and interfacial tensions (Banat et al. 2010). Among them, lipopeptide biosurfactants produced by *Bacillus* species are capable of generating the low interfacial tension between the hydrocarbon and the aqueous phases required to mobilize entrapped oil (Simpson et al. 2011). These compounds are good candidates for application in MEOR processes and can efficiently replace synthetic surfactants due to their specific activity, low toxicity, high biodegradability and effectiveness at extreme conditions of temperature, pressure, pH and salinity (Bordoloi and Konwar 2008; Suthar et al. 2008). Biosurfactant production *in situ* at concentrations that allow the mobilization of significant amounts of residual oil has been demonstrated using selected microorganisms stimulated by the addition of nutrients into the wells (Youssef et al. 2007; 2012).

On the other hand, the degradation of heavy oil fractions by microorganisms can be an important process in MEOR, since it promotes the reduction of the oil viscosity, improving its mobility through the reservoir. In the last years, different species with the ability of degrading heavy hydrocarbons have been described (Wentzel et al. 2007). However, few studies presented *Bacillus* strains as potential *n*-alkanes degraders. Wang et al. (2006) isolated a thermophilic *Bacillus* strain that preferentially degraded the long-chain *n*-alkanes (C15-C36); and She et al. (2011) showed that the treatment of a reservoir in the Daqing Oilfield with *B. subtilis* allowed the degradation of higher paraffinic fractions of oil, thereby increasing the flow characteristics of the reservoir. Recently, the ability of different *Bacillus* strains to degrade different paraffinic mixtures, with preference for the higher paraffin fractions, was described by Gudiña et al. (2012). As widely known, to increase the MEOR potential it is important to combine multiple mechanisms, such as degradation of heavy oil fractions and biosurfactants production, which can be achieved by using consortia of microorganisms with different properties (Jinfeng et al. 2005).

Laboratory studies on MEOR have typically used sand-pack columns, which provide a convenient bench-scale approach to evaluate oil recovery for several reasons: it is an economic model; a battery of columns can be set up simultaneously; and they simulate oil recovery operations of oil reservoirs (Suthar et al. 2008). In this work, a sand-pack column model was used to study the effect of biosurfactant-producing and oil-degrading microorganisms isolated from crude oil samples on the mobilization of entrapped heavy oil.

Materials and Methods

Microorganisms

Three biosurfactant-producing and oil-degrading *Bacillus subtilis* strains (#309, #311 and #573) isolated from crude oil samples obtained from a Brazilian oil field (Gudiña et al. 2012) were used in this study. Isolates were stored at -80ºC in Luria-Bertani (LB) medium supplemented with 20% (v/v) glycerol. The composition of LB medium was (g/l): NaCl 10.0; tryptone 10.0; yeast extract 5.0. The pH was adjusted to 7.0.

Sand-pack column assays

Sand-pack columns were designed to simulate the oil reservoir and used to evaluate the effect of microorganisms in oil recovery. Vertically oriented acrylic columns with a volume of 250 ml were uniformly packed with acid washed dry sand. The columns were provided with a sieve and cap fixed at the bottom. After packing the sand tightly, a top sieve and cap were fixed. The caps on both the ends of the column were provided with holes for insertion of inlet and outlet tubes. Rubber 'O' rings surrounded the caps to hermetically seal the column.

The experiments were carried out at 40ºC using heavy oil. The column was first flooded with water at a constant flow rate of 3 ml/min; pore volume (PV, ml), defined as the empty volume of the model, was calculated by measuring the volume of water required to saturate the column. The porosity (%) of the column was calculated as the PV divided by the total volume of the column (250 ml). In the second step, the hydrocarbon was injected into the column in the same way to replace water, until there was no more water coming out from the effluent. Original oil in place (OOIP, ml) was calculated as the volume of hydrocarbon retained in the column. Initial oil saturation (S_{oi}, %) and initial water saturation (S_{wi}, %) were calculated as follows:

$$Soi\ (\%) = \frac{OOIP}{PV} \times 100 \quad (Eq.1)$$

$$Swi\ (\%) = \frac{PV - OOIP}{PV} \times 100 \quad (Eq.2)$$

The sand-pack column was incubated at 40ºC for 24 hours and afterwards flooded again with water to remove the excess of hydrocarbon, until no more oil was observed in the effluent. The amount of oil recovered, so called oil recovered after water flooding (S_{orwf}, ml) was determined volumetrically. Remaing oil saturation (S_{or}) was calculated as follows:

$$Sor\ (\%) = \frac{OOIP - Sorwf}{OOIP} \times 100 \quad (Eq.3)$$

The remaing oil was subjected to microbial recovery processes. The column was inoculated with 50 ml of the different isolates in MSS (OD_{600nm}= 0.2), sealed and incubated for 14 days at 40ºC. Control columns were inoculated only with MSS and incubated at the same conditions. The MSS consisted of (g/l): NaCl 10.0; Na_2HPO_4 5.0; KH_2PO_4 2.0; $MgSO_4$ $7H_2O$ 0.2; sucrose 10.0. After incubation, the column was flooded with water and the volume of hydrocarbon recovered (oil recovered after microbial flooding (S_{ormf}, ml)) was measured. Additional Oil Recovery (AOR, %) was calculated as follows:

$$AOR\ (\%) = \frac{Sormf}{OOIP - Sorwf} \times 100 \quad (Eq.4)$$

Hydrocarbon degradation determination

The degradation of heavy oil recovered after the sand-pack column assays performed with the three different *B. subtilis* strains was evaluated. The additional oil recovered at the end of the treatment in sand-pack columns was diluted (20 mg/ml) in dichloromethane for gas chromatography (GC) analysis. GC analysis of each sample were performed with CP 3800 Varian gas Chromatograph equipped with and on-column injection, FID detector, and DB-HT-SIMDIS capillary column (5 m × 0.53 mm i.d., 0.15 µm thickness) (Agilent J&W Scientific Inc., California, USA). Helium was used as gas carrier and a constant flow rate of 18 ml/min was set. Injector and detector temperatures were 350 and 370ºC, respectively. Oven temperature was set at 40ºC during 5 min, raised to 350ºC at the rate of 5ºC/min, and at last kept at 370ºC during 15 min. All the samples were analyzed in triplicate.

Results and Discussion

Sand-pack columns assays

B. subtilis #309, #311 and #573 were used to perform the oil recovery assays with heavy oil using sand-pack columns. These microorganisms, isolated in a previous work from crude oil samples, exhibit desirable properties for application in MEOR: they can grow under anaerobic conditions in presence of crude oil, in medium with NaCl concentrations up to 100 g/l and temperatures up to 50ºC. The biosurfactants produced by those isolates show thermo- and salt tolerance, reduce surface tension up to 30 mN/m, emulsify hydrocarbons, and their critical micelle concentrations are very low (0.02-0.03 g/l) (Gudiña et al 2012).

The results obtained in the current work with the sand-pack columns are shown in **Table 1**, (results represent the average of three independent experiments ± standard deviation). The pore volume of the columns ranged from 93.0 to 96.5 ml, and the porosity was between 37.2 and 38.6%. The OOIP values varied from 90 to 93 ml, meaning that most of the water present in the column was replaced by oil. After the water flooding process, more than 55% of the oil remained trapped into the columns.

When the different isolates were introduced into the columns with the appropriate nutrients (MSS) and incubated for 14 days at 40ºC, additional oil was recovered in all the cases. The lowest recovery was obtained with isolate #309 (15.4%), whereas isolates #311 and #573 gave similar results (17.1-17.7%). Taking into account the amount of oil recovered in the control columns (about 7%), it can be concluded that 8-10% of the oil was recovered due to the action of microorganisms.

Table 1 Summary of the results obtained in sand-pack columns for heavy oil recovery using *B. subtilis* isolates #309, #311 and #573.

	#309	#311	#573	Control
PV (ml)	94.5 ± 0.7	95.0 ± 1.4	93.0 ± 4.2	96.5 ± 0.7
Porosity (%)	37.5 ± 0.3	38.0 ± 0.6	37.2 ± 1.7	38.6 ± 0.3
OOIP (ml)	93.0 ± 1.4	91.0 ± 1.4	90.0 ± 2.8	89.5 ± 4.6
S_{oi} (%)	98.4 ± 2.2	95.8 ± 0.1	96.8 ± 1.4	92.8 ± 5.1
S_{wi} (%)	1.6 ± 2.2	4.3 ± 0.1	3.2 ± 1.4	7.2 ± 5.1
S_{orwf} (ml)	41.0 ± 1.4	35.5 ± 0.7	36.0 ± 1.4	36.5 ± 3.2
OOIP-S_{orwf} (ml)	52.0 ± 2.8	55.5 ± 0.7	54.0 ± 1.4	53.0 ± 1.4
S_{or} (%)	55.9 ± 2.2	61.0 ± 0.2	60.0 ± 0.3	59.4 ± 1.5
S_{ormf} (ml)	8.0 ± 0.0	9.5 ± 0.7	9.5 ± 0.7	4.0 ± 0.0
AOR (%)	15.4 ± 0.8	17.1 ± 1.0	17.7 ± 1.8	7.6 ± 0.2

Enhanced oil recovery has been demonstrated by several authors in sand-pack columns or cores after growing *in situ* different *Bacillus* strains which produce surface active compounds. The additional oil recoveries reported were between 5 and 22% (Almeida et al. 2004; Dastgheib et al. 2008; She et al. 2011; Yakimov et al. 1997). Therefore, the results obtained in this work with *B. subtilis* isolates show that these microorganisms are good candidates for application in MEOR processes.

The increase in oil recovery observed in sand-pack columns can be due to several factors. *B. subtilis* isolates used in this study produce biosurfactants which reduce surface tension and emulsify hydrocarbons (Gudiña et al. 2012), which can contribute to reduce the interfacial tension at the oil-water interface. Furthermore, the isolates used have the ability of degrading hydrocarbons, thus reducing oil viscosity (Gudiña et al. 2012), which can contribute to enhance the mobilization of the hydrocarbon into the column. Moreover, there are other factors which can contribute to the increase in oil recovery, like the production of gases by the isolates, increasing the pressure into the column, or the plugging of high permeability channels due to the accumulation of biomass, which redirects water to oil rich zones, increasing oil production.

For a better simulation of the oil reservoir conditions, a core flooding equipment that can work at the oil reservoir pressures and temperatures was designed. This model will be used to study the ability of these microorganisms in enhancing oil recovery at the reservoir conditions.

Bacillus strains have shown efficiency in enhancing oil recovery in field assays. Youssef et al. (2007; 2012) used biosurfactant-producing *Bacillus* strains to inoculate mature oil wells together with the addition of adequate nutrients. Analysis of the production water indicated the growth of injected microorganisms and biosurfactant production. Biosurfactant concentration in the produced fluids was 20-90 mg/liter, which is about 2 and 9 times the minimum concentration required to mobilize entrapped oil from sandstone cores. The results obtained showed an increase in oil production in the inoculated wells, as well as a decrease in the water to oil ratio, which was maintained for a period of 40-60 days following the treatment. Taking into account the cost of nutrients and equipment required, as well as the increase in oil production obtained, the process can be considered cost-effective.

Hydrocarbon degradation

In order to evaluate the degradation of the heavy oil recovered after the sand-pack column assays performed with *B. subtilis* #309, #311 and #573, the oils recovered were analyzed by GC and the relative degradation of each *n*-alkane was measured. The respective variations of relative weight fraction of each *n*-alkane present in the heavy oil are illustrated in **Fig.1**.

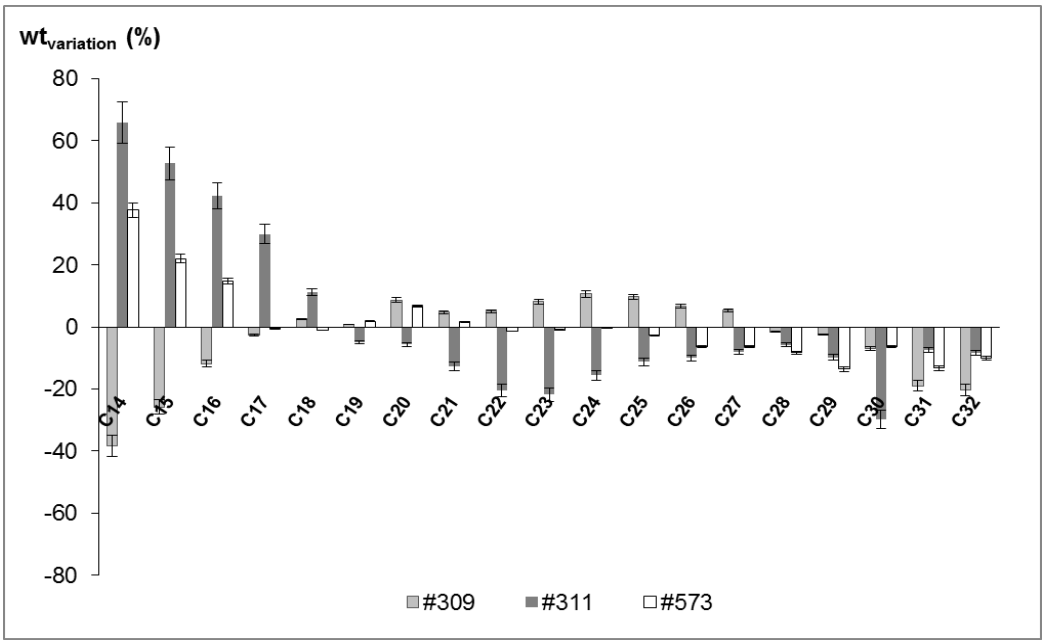

Fig.1 Variation of the relative weight fraction of *n*-alkanes present in the heavy oil after the treatment in sand-pack columns with isolates #309, #311 and #573, for 14 days at 40ºC as compared to the control (error bars represent the average error).

The GC analysis showed that the heavy oil recovered after the treatment with the strains #309, #311 and #573 were degraded. Among them, the isolate #311 presented the highest degradation rate with an increase in the relative weight fraction of *n*-alkanes lower than C19, and a decrease in the percentage of *n*-alkanes higher than C19. These results indicate that this isolate degraded the higher *n*-alkanes into lighter ones during the sand-pack column assays. Similarly, the strain #573 showed ability to degrade the higher *n*-alkanes (>C25), thus increasing the percentage of *n*-alkanes with chains containing 20 carbons

or less than 17 carbons, when compared with the control sample. On the other hand, the isolate #309 displayed a different oil degradation profile, degrading the higher *n*-alkanes (>C28) as well as the lower ones (chains lower than 17 carbons).

Other authors (Das and Mukherjee 2007; Etoumi 2007; Kato et al. 2001; She et al. 2011; Wang et al. 2006) reported the degradation of *n*-alkanes by different bacteria under aerobic conditions, However, degradation of hydrocarbons using *B. subtilis* under anaerobic conditions was only described by Gudiña et al. (2012). The authors found that *B. subtilis* #309 was able to degrade the higher *n*-alkanes (>C27) of a paraffinic mixture into *n*-alkanes with lower chains (between 20 to 26 carbons). The strain #573 was also studied using *n*-alkanes between C14 and C24, and similarly it was observed an increase of *n*-alkanes with chains lower than 18 carbons. The results obtained in the current study using crude heavy oil were similar to the ones obtained previously using paraffinic mixtures with *B. subtilis* isolates, thus suggesting that these isolates can be used to reduce the percentage of long chain *n*-alkanes of crude oil.

Bearing in mind the potential application of these *B. subtilis* strains to enhance the oil recovery in the reservoir, these results constitute important preliminary data. In summary, these strains were able i) to produce biosurfactants, reducing the oil-water interfacial tension and ii) to degrade the higher *n*-alkane fractions, decreasing the oil viscosity. In addition, the sand-pack column assays showed that the combination of both bioprocesses allows an increase in the mobilization of oil in the column, leading to an additional oil recovery after the treatment.

Conclusions

In the current work three different *B. subtilis* strains (#309, #311 and #573) isolated from a Brazilian reservoir were used to study their ability to degrade heavy oil fractions and to enhance oil recovery in sand-pack columns. Those isolates were able to recover between 8-10% of the heavy oil entrapped in the sand-pack column. Moreover, all the strains exhibited capacity to degrade the higher *n*-alkanes found in the heavy oil. These results highlight that the treatment of heavy oil with *B. subtilis* strains *in situ* can contribute to enhance the oil fluidity, increasing the additional oil recoveries. Those preliminary results are the plateau for future studies applying a core flooding equipment that can work at the oil reservoir pressure and temperature, thus validating them as good candidates for application in MEOR.

Acknowledgments

This work was supported by PARTEX OIL AND GAS. Jorge F. B. Pereira acknowledges also the financial support from Portuguese Foundation for Science and Tecnhology through doctoral research grant SFRH/BD/60228/2009.

References

Almeida, P.E., Moreira, R.S., Almeida, R.C.C., Guimaraes, A.K., Carvalho, A.S., Quintella, C., Esperidia, M.C.A. and Taft, C. 2004. Selection and application of microorganisms to improve oil recovery. *Engineering in Life Sciences* **4**(4): 319-325.

Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., Smyth, T.J. and Marchant, R. 2010. Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology* **87**(2): 427-444.

Bordoloi, N.K. and Konwar, B.K. 2008. Microbial surfactant-enhanced mineral oil recovery under laboratory conditions. *Colloids and Surfaces B-Biointerfaces* **63**(1): 73-82.

Brown, L.R. 2010. Microbial enhanced oil recovery (MEOR). *Current Opinion in Microbiology* **13**(3): 316-320.

Das, K. and Mukherjee, A.K. 2007. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresource Technology* **98**(7): 1339-1345.

Dastgheib, S.M.M., Amoozegar, M.A., Elahi, E., Asad, S. and Banat, I.M. 2008. Bioemulsifier production by a halothermophilic *Bacillus* strain with potential applications in microbially enhanced oil recovery. *Biotechnology Letters* **30**(2): 263-270.

Etoumi, A. 2007. Microbial treatment of waxy crude oils for mitigation of wax precipitation. *Journal of Petroleum Science and Engineering* **55**(1-2): 111-121.

Gudiña, E.J., Pereira, J.F.B., Rodrigues, L.R., Coutinho, J.A.P. and Teixeira, J.A. 2012. Isolation and study of microorganisms from oil samples for application in Microbial Enhanced Oil Recovery. *International Biodeterioration and Biodegradation* **68**:56-64.

Jinfeng, L., Lijun, M., Bozhong, M., Rulin, L., Fangtian, N. and Jiaxi, Z. 2005. The field pilot of microbial enhanced oil recovery in a high temperature petroleum reservoir. *Journal of Petroleum Science and Engineering* **48**(3-4): 265-271.

Kato, T., Haruki, M., Imanaka, T., Morikawa, M. and Kanaya, S. 2001. Isolation and characterization of long-chain-alkane degrading *Bacillus thermoleovorans* from deep subterranean petroleum reservoirs. *Journal of Bioscience and Bioengineering* **91**(1): 64-70.

Lazar, I., Petrisor, I.G. and Yen, T.E. 2007. Microbial enhanced oil recovery (MEOR). *Petroleum Science and Technology* **25**(11-12): 1353-1366.

Sen, R. 2008. Biotechnology in petroleum recovery: The microbial EOR. *Progress in Energy and Combustion Science* **34**(6): 714-724.

She, Y.H., Zhang, F., Xia, J.J., Kong, S.Q., Wang, Z.L., Shu, F.C. and Hu, J.M. 2011. Investigation of biosurfactant-producing Indigenous microorganisms that enhance residue oil recovery in an oil reservoir after polymer flooding. *Applied Biochemistry*

and Biotechnology **163**(2): 223-234.
Simpson, D.R., Natraj, N.R., McInerney, M.J. and Duncan, K.E. 2011. Biosurfactant-producing *Bacillus* are present in produced brines from Oklahoma oil reservoirs with a wide range of salinities. *Applied Microbiology and Biotechnology* **91**(4): 1083-1093.
Suthar, H., Hingurao, K., Desai, A. and Nerurkar, A. 2008. Evaluation of bioemulsifier mediated Microbial Enhanced Oil Recovery using sand pack column. *Journal of Microbiological Methods* **75**(2): 225-230.
Wang, L., Tang, Y., Wang, S., Liu, R.L., Liu, M.Z., Zhang, Y., Liang, F.L. and Feng, L. 2006. Isolation and characterization of a novel thermophilic *Bacillus* strain degrading long-chain *n*-alkanes. *Extremophiles* **10**(4): 347-356.
Wentzel, A., Ellingsen, T.E., Kotlar, H.K., Zotchev, S.B. and Throne-Holst, M. 2007. Bacterial metabolism of long-chain *n*-alkanes. *Applied Microbiology and Biotechnology* **76**(6): 1209-1221.
Yakimov, M.M., Amro, M.M., Bock, M., Boseker, K., Fredrickson, H.L., Kessel, D.G. and Timmis, K.N. 1997. The potential of *Bacillus licheniformis* strains for *in situ* enhanced oil recovery. *Journal of Petroleum Science and Engineering* **18**(1-2): 147-160.
Yakimov, M.M., Timmis, K.N., Wray, V. and Fredrickson, H.L. 1995. Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniformis* Bas50. *Applied and Environmental Microbiology* **61**(5): 1706-1713.
Youssef, N., Elshahed, M.S. and McInerney, M.J. 2009. Microbial processes in oil fields: culprits, problems, and opportunities. *Advances in Applied Microbiology*, **66**:141-251.
Youssef, N., Simpson, D.R., Duncan, K.E., McInerney, M.J., Folmsbee, M., Fincher, T. and Knapp, R.M. 2007. *In situ* biosurfactant production by *Bacillus* strains injected into a limestone petroleum reservoir. *Applied and Environmental Microbiology* **73**(4): 1239-1247.