Research Journal of Chemical and Environmental Sciences
Res J. Chem. Environ. Sci. Vol 4 [4S] 2016: 84-91
Online ISSN 2321-1040
CODEN: RJCEA2 [USA]
©Academy for Environment and Life Sciences, INDIA
Website: www.aelsindia.com/rjces.htm

RJCES

FULL LENGTH ARTICLE

Studies on Production of Biosurfactant from *Pseudomonas Aeruginosa* (MTCC7815) & its Application in Microbial Enhanced Oil Recovery

Archana.S, Govind Singh Tomar, G. Srinikethan
Department of Chemical Engineering, National Institute of Technology, Surathkal, Mangalore,n India
Email: archana75biotech@gmail.com, govind07@outlook.com, srinikethan.g@gmail.com

ABSTRACT

Biosurfactants are generally microbial metabolites with the typical amphiphilic structure of a surfactant. This study investigated potential biosurfactants production from Pseudomonas aeruginosa *MTCC7815 and its application in oil recovery. Presence of glucose, urea and n-hexadecane in the medium majorly influenced the growth of the bacteria andyieldedf 3.937g/l of the biosurfactant. The kinetic studies revealed that the biosurfactant produced was growth-associated as its production coincides with the exponential growth phase of the P.aeruginosa. The biosurfactant producing ability of the strain was measured based on the reduction in surface tension and emulsification (E_{24}) index. The bacterial biosurfactants were found to be functionally stable at varying range of pH (2.5–11), temperature and salinity. The E_{24} and surface tension values were found to be 75.24% and 34.07 mN/m at pH 7.0, 74.6% and 35.15 mN/m at 80°C and 58.23% and 39.92mN/m at 5% salt concentration, respectively. Microbial enhanced oil recovery (MEOR) studies were carried out using a sand pack column to stimulate an oil reservoir. The use of cell free broth resulted in 36.84% additional oil recovery with 65.71% of total oil recovery, thus proving that the biosurfactant produced from P.aeruginosacould be a potential candidate in oil recovery thus replacing the hazardous chemical counterpart.*

Keywords—*Biosurfactant, Surface Tension, Emulsification (E_{24}) index, Microbial Enhanced Oil Recovery, Sand Pack Column*

INTRODUCTION

Biosurfactants are amphiphilic compounds consisting of hydrophobic and hydrophilic domain with the ability to reduce surface and interfacial tension, produced by microorganisms and have many advantages, such as low toxicity and high biodegradability, compared to synthetic counterparts.The minimum concentration of surfactant necessary to initiate micelle formation is defined as critical micelle concentration (CMC)[1].Surfactants are majorly used to remediate oil spills and in enhancing the oil recovery by increasing the solubility of the petroleum components or lower the interfacial tension to enhance the mobility of the petroleum[2].Environmental impact with use of synthetic surfactants has instigated the consideration of biosurfactants as an alternative. Biosurfactants are an important class of environment-friendlysurface-active products which are produced on microbial cell surfaces or secreted extracellularly [3, 4]. The biosurfactants produced is growth associated making use of hydrocarbon as the substrate for its growth. This ability of the organism enables it to degrade the oil, aid in bioremediation at oil spill sites and in microbial enhanced oil recovery [5]. About two third of oil explored is still unrecovered, and it is figured that only 30-40% oil recovered with conventional recovery techniques, while about 60-70% of total oil still remain trapped in the reservoir. Increased demand for crude oil and petroleum products has given strong threshold to the development of enhanced oil recovery (EOR) technologies[6]. Biosurfactants have been tested to enhance oil recovery and demonstrated to be effective in the reduction of the interfacial tension of oil and viscosity of the oil, increasing the mobility of fluid and in removal of water from the emulsions prior to processing [7]. Rhamnolipid is one class of biosurfactant produced by *Pseudomonas* specieshaving a prominent surface-activity as it can reduce surface tension of water from 72 to 25–30 mN/m with a critical micelle concentration of 5–65 mg/l and displays a high emulsifying activity of 74%–85%[8]. Given these features, rhamnolipid is well suited to apply in the petrochemical industry for enhanced oil recovery, hydrocarbon remediation, removal of heavy metals from soils, and decontamination of soil from oil[9, 10]. In this regard, there is a need for studies on biosurfactants production by optimizing various parameters to facilitate the microbial growth as the product is growth associated thereby improving the production of biosurfactant, followed by its kinetic

studies which could of use in developing a continuous process for a large scale production of biosurfactant. Also, it becomes crucial to study the stability of the biosurfactant produced at various temperature, pH and salinity for its utilization in the oil reservoir site for microbial enhanced oil recovery process. Therefore efforts have been made to improve the yield and to study its application in oil recovery.

MATERIALS AND METHODS

A. Inoculum

Pseudomonas aeruginosa (MTCC 7815) was purchased from IMTECH, Chandigarh received in lyophilised form, thus was revived and grown on.nutrient agar plates at 30°C for 48 h. The growth of inoculum was performed by the addition of a loopful of cells from nutrient agar plates to a 100-mL Erlenmeyer flask containing 50 mL of medium of the following composition: 1.0 g/L of $NaNO_3$, 3.0 g/L of KH_2PO_4, 7.0 g/L of K_2HPO_4,0.2 g/L of $MgSO_4 \cdot 7H_2O$, 5% yeast extract, 5% peptone, and 3% glycerol.The flask was incubated at 35^0C in an incubator at 150 rpm for 24 hours.

B. Biosurfactant production

1)Media and Growth condition: 250 ml Erlenmeyer flask containing 100 ml of the mineral salt medium (2.0 g of urea, 2.0 g of (NH_4) SO_4, 3.61 g of Na_2HPO_4 , 1.75 g of KH_2PO_4, 0.2 g of $MgSO_4.7H_2O$, 50 mg of $CaCl_2.2H_2O$, 1 mg of $FeSO_4.7H_2O$, 50 µg of $CuSO_4.7H_2O$, 10 µg of $MnSO_4.5H_2O$, 10 µg of H_3BO_3, 70 µg of $ZnSO_4.7H_2O$, 20 µg of $Na_2MoO_4.2H_2O$ and 20 g of glucose) were inoculated with cell cultures of *Pseudomonas aeruginosa* (MTCC 7815). All batch runs were performed in triplicate at 35^0C utilizing an incubator shaker at 150 rpm. Samples were collected every 12 hours, transferred to eppendorf tubes and then centrifuged at 12,000 rpm for 15 minutes at 4^0C for estimation of bacterial biomass. The total biomass, residual glucose concentration, surface tension measurement and biosurfactant production were analysed at regular time intervals. The residual glucose estimation was performed using DNS method.

2)Cell Biomass: The bacterial cell dry weight was determined as a function of bacterial growth [11]. 10ml of the broth was centrifuged at 12,000 rpm for 15 minutes at 4^0C. The supernatant was removed and the vial containing the biomass was dried in the oven at 50^0C and weighed.

3)Biosurfactant Extraction by Precipitation: Since the biosurfactant produced by *P. aeruginosa* was extracellular in nature, the biosurfactant was extracted from the whole cell-free culture broth. The cell-free broth was taken and the pH was set to 2 using 6N HCl and kept overnight at 4 ^{0}C for complete precipitation of biosurfactant. The precipitate was collected by centrifuged at 12000 rpm for 15 minutes at 4°C and maintaining pH 8.0 by using phosphate buffer [12].The biosurfactant quantity and surface tension value of were measured.

4)Surface Tension measurement and Emulsification (E_{24}) Index: The surface tension of cell-free broth was measured using an Attension Sigma702 Tensiometer (Biolin Scientific) by using Wilhelmy plate method according to the instructions of the manufacturer. The surface tension was measured in mN/m. All measured were taken as the mean of three replicates at room temperature. The emulsifying capacity of biosurfactant was analysed by emulsification index [13]. In this method 2 ml of oil was added to 2 ml of the cell-free broth in a test tube, vortex at high speed for 2 min and allowed to stand for 24h, the E_{24}index of the sample was calculated based on the height of the emulsion to total height of the solution.

C. Effect of various parameters on production of biosurfactant:

The bacterial strain *P. aeruginosa* MTCC 7815 was studied for the biosurfactant production under the influence of various physical and chemical parameters. All batchruns were performed in triplicate. Total biomass, surface tension, emulsification index (E_{24} %) and biosurfactant production were analysed.

1) Effect of carbon, nitrogen and hydrocarbon: 100 ml of MSM broth with four different carbon sources (glucose, glycerol, fructose and starch) were prepared in different 250 ml Erlenmeyer flask and sterilized in an autoclave and its pH was adjusted to 7. 1 ml of inoculum of *P. aeruginosa* MTCC 7815 was added and incubated at 150 rpm at 35 ^{0}C for 96 hr. Total biomass, surface tension, emulsification index (E_{24} %) and biosurfactant production were analysed. Similar studies were carried out for with five different nitrogen sources (urea, ammonium nitrate, yeast extract, peptone and ammonium chloride) and four hydrocarbons (n-hexadecane, kerosene, petrol and diesel) [14].

***2) Effect of pH and temperature on production*:** 100 ml of MSM broth of varying pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 and sterilized in an autoclave. 1 ml of inoculum of *P. aeruginosa* MTCC 7815 was added and incubated at 150 rpm at 35 ^{0}C for 96 h. Total biomass, surface tension, emulsification index (E_{24} %) and biosurfactant production were analysed. To study the effect of temperature MSM broth inoculated with the culture was incubated at varying temperature in the range of 25-50°C.

D.Growth Kinetics

Optimized medium components and operating conditions were considered for shake flask studies. Samples were collected every 12 hrs to estimate reduction in surface tension, substrate affinity, specific growth rate and yield coefficients[15].

 © 2016 AELS, INDIA

E.Stability Studies

The stability of biosurfactant at a different temperature range (4 ^{0}C to 80 ^{0}C), pH range (2-11), and alkaline conditions (NaCl conc. 10% to 25% w/v) was studied. The stability of biosurfactant was analysed by emulsification index and by measuring the surface tension of each sample after respective treatments [11].

F.Microbial Enhanced Oil Recovery (MEOR) studies

The potential application of the biosurfactant in MEOR was evaluated using the 'sand pack column' technique described by Qazi*et al.*[16]. A PVC column (50cm x 4.5cmdia) was packed with acid washed dry sand. The potential of the surfactant for oil recovery wasestimated by pouring 50 ml of aqueous solution of biosurfactant(extraction from 100 ml culture broth) in the column. Theamount of oil released was measuredto study the influence of biosurfactant in inducing oil recovery. Total oil and additional/enhanced oil recovery were determined by the following method [11].

G. Statistical analysis

Statistical analysis was done by Student's "*t*-test" and factorial two-way ANOVA.

RESULTS AND DISCUSSION

A Effect of various process parameters on biosurfactant production

1) ***Effect of Carbon source:*P.*aeruginosa*** MTCC 7815 was able to utilize glucose as a sole carbon source and produce higher amount of biosurfactant 3.876 g/l, biomass 5.672 g/l, E_{24} 76.77% and lowest surface tension 34.533 mN/m followed by glycerol 3.286 g/l biosurfactant, 4.937 g/l biomass, and 74.32% E_{24} and 35.192 mN/m surface tension (Fig. 1). The lowest production was observed with the starch (0.349 g/l).

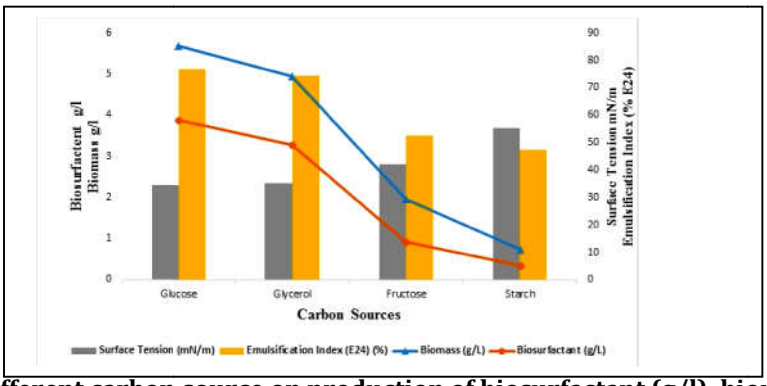

Fig.1. Effect of different carbon source on production of biosurfactant (g/l), biomass (g/l), surface tension (mN/m) and emulsification index (%).

Bordoloi and Konwar [11] obtained similar result during biosurfactant production by *Pseudomonas* sp. using different carbon sources but reported highest biosurfactant production (5.14 g/l) in the case of D-glucose as a carbon source, biomass 6.38 g/l , surface tension 30.25 mN/m and E_{24} 79.65%. Thompson *et al.* [17] reported glycerol as sole carbon source for biosurfactant production by *B. subtilis* ATCC 21332.

2) *Effect of Nitrogen source*: Urea was the best sources of nitrogen for biosurfactant production by *P.aeruginosa* MTCC 7815 (Fig.2). The biomass, biosurfactant,E_{24} was respectively 5.381 g/l, 3.663 g/l and 78.68% highest while the reduction in surface tension recorded as 34.871 mN/m.

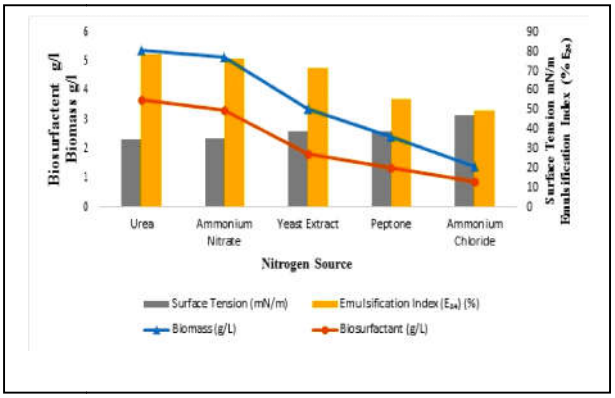

Fig.2. Effect of different nitrogen source on production of biosurfactant (g/l), biomass (g/l), surface tension (mN/m) and emulsification index (%).

 © 2016 AELS, INDIA

Bordoloi and Konwar[11]obtained urea as sole nitrogen source with 2.0% D-glucose. Rahman*et al.*[18] also obtained ammonium nitrate as nitrogen source with glycerol by*Candida lipolytica*.

3) Effect of hydrocarbons: In the case of *P. aeruginosa*MTCC 7815 n-hexadecane enhanced the biosurfactant production (3.821 g/l), the lowest surface tension was recorded as 33.74 mN/m and highest biomass 5.375 g/l and emulsification activity 75.77 % (Fig 3). Kerosene shows the second best results as the hydrocarbon source. Petrol and diesel also have a significant role in the production of biosurfactant and increase in yield parameter. Whang *et al.*[19] reported that n-hexadecane enhanced the biosurfactant production (4.99 g/l), biomass (5.23 g/l), surface tension (31.65 mN/m) and E_{24} (75.86%) in the case of *P. aeruginosa* PBSC1. Bordoloi and Konwar[11] also reported n-hexadecane as the main hydrocarbon in the case of *Pseudomonas* sp.

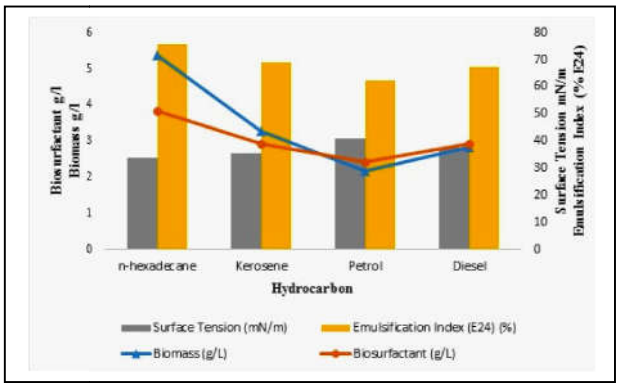

Fig.3. Effect of different hydrocarbon source on production of biosurfactant (g/l), biomass (g/l), surface tension (mN/m) and % E_{24}.

4) Effect of pH*:* In the case of P. aeruginosaMTCC 7815 maximum biomass (5.184 g/l), biosurfactant (3.962 g/l), surface tension reduction (33.857 mN/m) and E_{24} (77.53 %) were observed at pH 7.0 (Fig 4). pH 6.5 and 7.0 produced statistically par result for surface tension reduction and emulsification activity. At lower pH and higher pH values caused an appreciable drop in biosurfactant production. Bordoloi and Konwar[11]and Whanget al.[19]reported that pH 6.5 and 7.0 produced par result for the biosurfactant production. Decreased or increase in pH produced less biosurfactant. change.

5) Effect of temperature: The optimum condition of temperature observed was 35 ^{0}C and followed by 30 ^{0}C. The highest biosurfactant production was recorded as 3.882 g/l at 35^{0}C (Fig.5). The temperature 35 ^{0}C produced a highest result for the biosurfactant production, dry cell biomass weight (5.138 g/l), surface tension reduction (34.571mN/m) and emulsification activity (78.46 %). Temperature is one of the parameters that greatly affected the biosurfactant production. When temperature increased above 40^{0}C bacterial growth and biosurfactant production totally inhibited. A decrease and increase in the optimum temperature show the lower culture growth and biosurfactant production. As similar case as pH, Increased or decreased in optimum temperature reduce the biosurfactant production, biomass, higher surface tension and lower E_{24} [19].

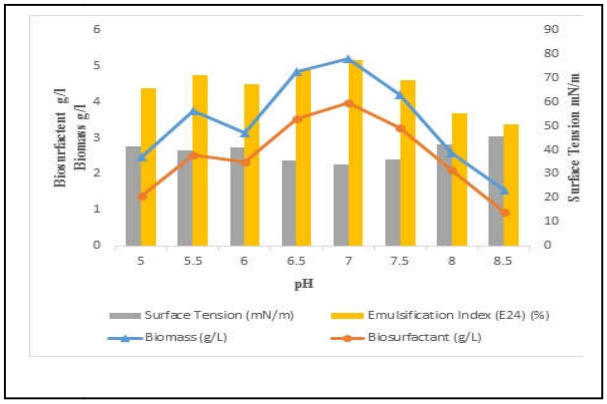

Fig.4. Effect of pH on production of biosurfactant (g/l), biomass (g/l), surface tension (mN/m) and emulsification index (%).

 © 2016 AELS, INDIA

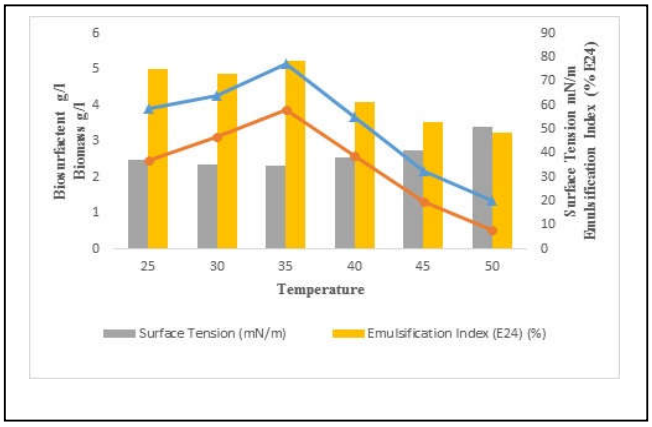

Fig.5. Effect of temperature on production of biosurfactant (g/l), biomass (g/l), surface tension (mN/m) and emulsification index (%).

B. Kinetics of biosurfactant production

Fig 6 shows the results of time course variation of biosurfactant production, cell growth, surface tension and glucose consumption by *Pseudomonas aeruginosa* MTCC 7815. The concentration of glucose was 20.0 g/l in growth media. The initiation of biosurfactant production was observed after 120 hours of incubation, the biosurfactant production increased in the exponential phase along with anincrease in bacterial biomass and continued till stationary phase of the bacterium. The organism utilized glucose slowly during the incubation time as indicated by adecrease in residual glucose concentration.

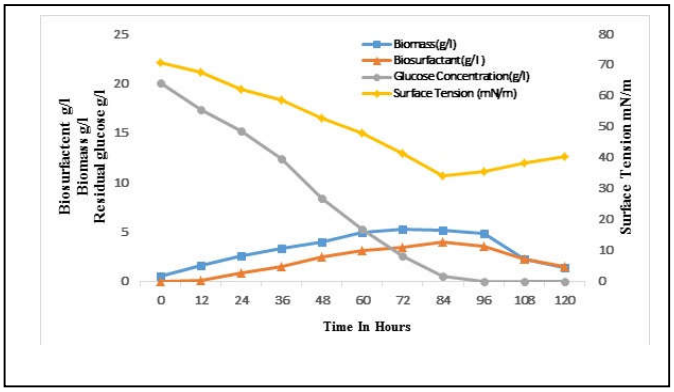

Fig.6. Time-course profile of biosurfactant synthesis.

The maximum growth of the bacterium was observed at 72 hours, where X_{max}= 5.273 g/l. However, maximum biosurfactant production (P_{max} = 3.937 g/l) occurred in the stationary phase (at 84 hours), the corresponding biomass was X = 5.158 g/l. A decline in the biosurfactant production was observed after 96 hours of incubation probably because of the exhaustion of the nutrients.

The kinetic parameters evaluated in terms of yield factors related to biosurfactant production by *P.aeruginosa*MTCC 7815 to substrate utilization ($Y_{P/S}$), dry cell biomass to substrate utilization ($Y_{X/S}$) and biosurfactant production to dry cell biomass ($Y_{P/X}$) are given in Table 1. $Y_{P/S}$ = 0.216 g/g, $Y_{P/X}$ = 0.847 g/g, and $Y_{X/S}$ = 0.606 g/g was obtained during surfactant production by *Pseudomonas aeruginosa*. Specific growth rate (μ) observed was 0.0324/hour whereas the maximum growth rate (μ_{max}) was found to be 0.09767/hour and $K_s$was 3.566g/l. Similar results were obtained by Gunther*et al.*and Soberon-Chavez *et al.*(20, 21) during biosurfactant production by *Pseudomonas aeruginosa* in minimal medium containing 3.5% glucose as carbon source, they observed that μ_{max} = 0.0935/hour, K_s = 2.18 g/l, $Y_{X/S}$ = 0.22 g/g, $Y_{P/S}$ = 0.43 g/g at 96 hours of incubation.

TABLE 1.KINETIC PARAMETERS FOR BIOSURFACTANT PRODUCTION BY P. AERUGINOSA MTCC 7815.

Parameter	***Values***
Specific growth rate (μ)	0.0324/hr
Maximum Specific growth rate (μ_{max})	0.09767/hr
$Y_{X/S}$	0.606 g/g

 © 2016 AELS, INDIA

$Y_{P/S}$	0.216 g/g
$Y_{P/X}$	0.847 g/g
X_{max}	5.273 g/l
P_{max}	3.937 g/l
K_s	3.566 g/l

C. Stability studies

Biosurfactant produced from *P. aeruginosa* MTCC 7815 was found to be stable over wide ranges of temperature, pH and alkaline conditions. Fig. 7 shows that maximum emulsification index and surface tension were 75.24% and 34.07 mN/m, respectively obtained at pH 7. The biosurfactant completely lost its activities when incubated at pH 2 and pH 9-11 as evident by lowest emulsification (15.51%) and higher surface tension (51.43 mN/m).

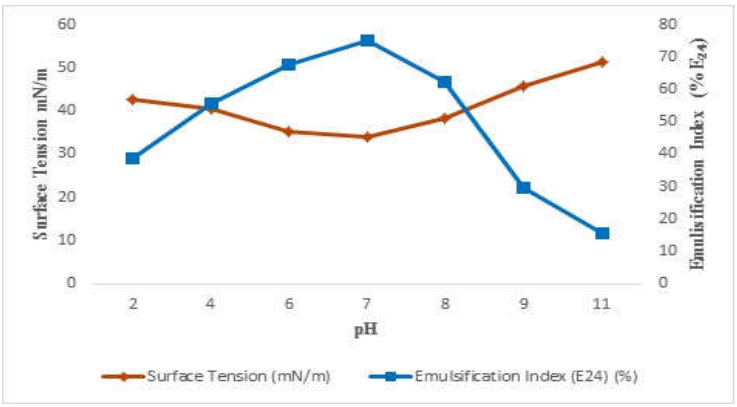

Fig.7. Stability of the biosurfactant at varying ranges of pH

The temperature stability of the biosurfactant is shown in Fig 8. The biosurfactant retained its activity over a wide range of temperature (20-80 ^{0}C) as indicated by stable emulsifying and surface tension values. The values were found to be 74.6% and 35.15 mN/m respectively. A gradual decline was observed at lower and higher temperature ranges.

Fig. 9 shows the stability of biosurfactant at different salt conditions. It was found that biosurfactant could tolerate only 1-10% salt while the continuously decline in emulsification observed but the surface tension of the broth at a high and low concentration of salt found lowered. The lowest surface tension (38.92 mN/m) and highest emulsification (58.23%) were achieved in the range of 5% salt concentration.

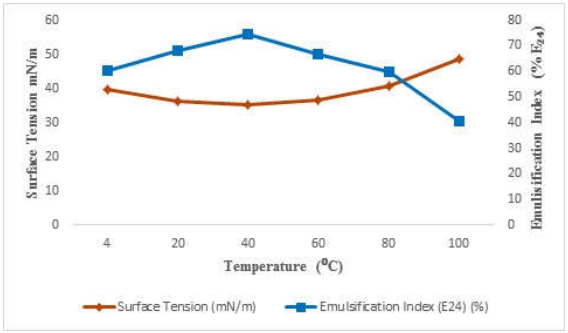

Fig.8. Stability of the biosurfactant at varying ranges of temperature (^{0}C).

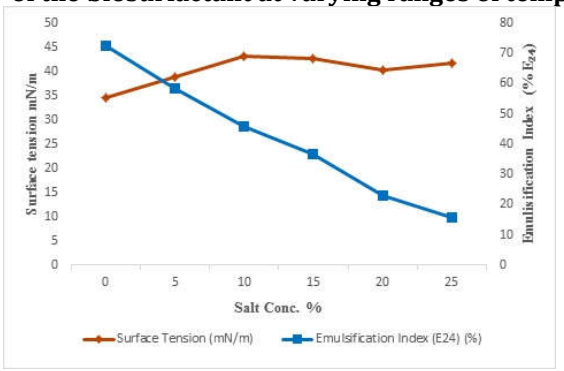

Fig.9. Stability of the biosurfactant at varying range salt concentration.

 © 2016 AELS, INDIA

Quazi*et al.[16]* obtained a similar result on the stability of biosurfactant. They reported biosurfactant show maximum activity at temperature (40-80^{0}C), pH (5-8) and salt concentration (0-15%) with maximum E_{24}=79% and ST=32.2 mN/m biosurfactant produced by *Pseudomonas* sp. The biosurfactant produced by *C.sphaerica* was found to be stable at temperature (5-120^{0}C), pH (2-12) and salinity (2%-20%) and reported 40% loss of biosurfactant activity at higher salinity (20%) [22, 23].

D.Microbial enhanced oil recovery by sand pack column

To investigate the efficiency and applicability of biosurfactant produced by *P. aeruginosa* MTCC 7815 in enhanced oil recovery, the cell-free broth was pour into the column at room temperature. This resulted in an additional oil recovery of 36.84%, whereas the total oil recovery was found to be 65.71% (Table 2). Quazi*et al.*[16] reported 46% enhanced oil recovery and 74% total oil recovery at room temperature. Bordoloi and Konwar [11] reported 15% more oil recovery at 90^{0}C and 10% more at 70^{0}C under laboratory condition. Zhao*et al.* and Amani *et al.*[24, 25] observed an additional oil recovery 43% from the biosurfactant obtain from *Pseudomonas* sp.

TABLE 2. ENHANCED OIL RECOVERY USING BIOSURFACTANT PRODUCED BY *P. AERUGINOSA* MTCC 7815.

Organism	*Pour Volume (ml)*	*S_0i (ml)*	*S_0rwf (ml)*	*S_0rbf (ml)*	*Tor (%)*	*Aor (%)*
P. aeruginosa MTCC 7815	200	70	38	24	65.71	36.84

S_0i= Initial oil saturation, **S_0rwf=** Residual oil after water flooding,
S_0rbf = oil saturation after biosurfactant flooding,
Tor= Total oil recovery, **Aor**= Additional oil recovery**]**

CONCLUSION

The production studies for biosurfactant from *P. aeruginosa* (MTCC 7815) by varying carbon sources, nitrogen sources, temperature and pH coincided with the optimal condition. The growth medium supplemented with simpler molecule glucose as carbon source and urea as the nitrogen source gave a better yield. The kinetic studies revealed that the biosurfactant produced was growth-associated asits production coincides with the exponential growth phase of the *P. aeruginosa*. The stability studies carried out revealed that the biosurfactant produced can be efficiently used over varying conditions of temperature, pH and salinity. The partial purification of biosurfactant was carried out by acidification thus improving its ability to reduce surface tension to greater extent. Microbial enhanced oil recovery (MEOR) studies were carried out using a sand pack column to simulate an oil reservoir. The use of cell-free broth resulted in 36.84% additional oil recovery with 65.71% of total oil recovery, thus indicating that biosurfactant produced from *P. aeruginosa* (MTCC 7815) could be a potential candidate in oil recovery thus replacing hazardous chemical surfactant. Further studies can be carried out on large scale of biosurfactant production along with its application studies in field of bioremediation and drug delivery.

REFERENCES

1. .I. M. Banat, A. Franzetti, I. Gandolfi, G. Bestetti, M. G. Martinotti, L. Fracchia*, et al.*, "Microbial biosurfactants production, applications and future potential," *Appl Microbiol Biotechnol,* vol. 87, pp. 427-44, Jun 2010.
2. C. N. Mulligan, "Environmental applications for biosurfactants," *Environ Pollut,* vol. 133, pp. 183-98, Jan 2005.
3. M. Sifour, M. H. Al-Jilawi, and G. M. Aziz, "Emulsification properties of biosurfactant produced from Pseudomonas aeruginosa RB 28," *Pak J Biol Sci,* vol. 10, pp. 1331-5, Apr 15 2007.
4. A. Dhasayan, G. S. Kiran, and J. Selvin, "Production and characterisation of glycolipid biosurfactant by Halomonas sp. MB-30 for potential application in enhanced oil recovery," *Appl Biochem Biotechnol,* vol. 174, pp. 2571-84, Dec 2014.
5. A. Fiechter, "Biosurfactants: moving towards industrial application," *Trends Biotechnol,* vol. 10, pp. 208-17, Jun 1992.
6. L. R. Brown, "Microbial enhanced oil recovery (MEOR)," *Curr Opin Microbiol,* vol. 13, pp. 316-20, Jun 2010.
7. C. Syldatk, S. Lang, U. Matulovic, and F. Wagner, "Production of four interfacial active rhamnolipids from n-alkanes or glycerol by resting cells of Pseudomonas species DSM 2874," *Z Naturforsch C,* vol. 40, pp. 61-7, Jan-Feb 1985.
8. M. S. Kuyukina, I. B. Ivshina, J. C. Philp, N. Christofi, S. A. Dunbar, and M. I. Ritchkova, "Recovery of Rhodococcus biosurfactants using methyl tertiary-butyl ether extraction," *J Microbiol Methods,* vol. 46, pp. 149-56, Aug 2001.
9. B. Bubela, C. L. Labone, and C. H. Dawson, "An apparatus for continuous growth of microorganisms under oil reservoir conditions," *Biotechnol Bioeng,* vol. 29, pp. 289-91, Feb 1987.

10. J. D. Desai and I. M. Banat, "Microbial production of surfactants and their commercial potential," *Microbiol Mol Biol Rev,* vol. 61, pp. 47-64, Mar 1997.
11. N. K. Bordoloi and B. K. Konwar, "Microbial surfactant-enhanced mineral oil recovery under laboratory conditions," *Colloids Surf B Biointerfaces,* vol. 63, pp. 73-82, May 1 2008.
12. A. Saimmai, O. Rukadee, T. Onlamool, V. Sobhon, and S. Maneerat, "Isolation and functional characterization of a biosurfactant produced by a new and promising strain of Oleomonas sagaranensis AT18," *World J Microbiol Biotechnol,* vol. 28, pp. 2973-86, Oct 2012.
13. D. G. Cooper and B. G. Goldenberg, "Surface-active agents from two bacillus species," *Appl Environ Microbiol,* vol. 53, pp. 224-9, Feb 1987.
14. A. S. Santos, A. P. Sampaio, G. S. Vasquez, L. M. Santa Anna, N. Pereira, Jr., and D. M. Freire, "Evaluation of different carbon and nitrogen sources in production of rhamnolipids by a strain of Pseudomonas aeruginosa," *Appl Biochem Biotechnol,* vol. 98-100, pp. 1025-35, Spring 2002.
15. S. S. Cameotra and R. S. Makkar, "Synthesis of biosurfactants in extreme conditions," *Appl Microbiol Biotechnol,* vol. 50, pp. 520-9, Nov 1998.
16. M. A. Qazi, T. Kanwal, M. Jadoon, S. Ahmed, and N. Fatima, "Isolation and characterization of a biosurfactant-producing Fusarium sp. BS-8 from oil contaminated soil," *Biotechnol Prog,* vol. 30, pp. 1065-75, Sep-Oct 2014.
17. D. N. Thompson, S. L. Fox, and G. A. Bala, "Biosurfactants from potato process effluents," *Appl Biochem Biotechnol,* vol. 84-86, pp. 917-30, Spring 2000.
18. P. K. Rahman, G. Pasirayi, V. Auger, and Z. Ali, "Production of rhamnolipid biosurfactants by Pseudomonas aeruginosa DS10-129 in a microfluidic bioreactor," *Biotechnol Appl Biochem,* vol. 55, pp. 45-52, Jan 2010.
19. L. M. Whang, P. W. Liu, C. C. Ma, and S. S. Cheng, "Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil," *J Hazard Mater,* vol. 151, pp. 155-63, Feb 28 2008.
20. G. Soberon-Chavez, F. Lepine, and E. Deziel, "Production of rhamnolipids by Pseudomonas aeruginosa," *Appl Microbiol Biotechnol,* vol. 68, pp. 718-25, Oct 2005.
21. N. W. t. Gunther, A. Nunez, W. Fett, and D. K. Solaiman, "Production of rhamnolipids by Pseudomonas chlororaphis, a nonpathogenic bacterium," *Appl Environ Microbiol,* vol. 71, pp. 2288-93, May 2005.
22. F. S. S. R. de Cassia, D. G. Almeida, R. D. Rufino, J. M. Luna, V. A. Santos, and L. A. Sarubbo, "Applications of biosurfactants in the petroleum industry and the remediation of oil spills," *Int J Mol Sci,* vol. 15, pp. 12523-42, 2014.
23. E. J. Silva, N. M. Rocha e Silva, R. D. Rufino, J. M. Luna, R. O. Silva, and L. A. Sarubbo, "Characterization of a biosurfactant produced by Pseudomonas cepacia CCT6659 in the presence of industrial wastes and its application in the biodegradation of hydrophobic compounds in soil," *Colloids Surf B Biointerfaces,* vol. 117, pp. 36-41, May 1 2014.
24. F. Zhao, F. Ma, R. Shi, J. Zhang, S. Han, and Y. Zhang, "Production of rhamnolipids by Pseudomonas aeruginosa is inhibited by H2S but resumes in a co-culture with P. stutzeri: applications for microbial enhanced oil recovery," *Biotechnol Lett,* vol. 37, pp. 1803-8, Sep 2015.
25. H. Amani, M. M. Muller, C. Syldatk, and R. Hausmann, "Production of microbial rhamnolipid by Pseudomonas aeruginosa MM1011 for ex situ enhanced oil recovery," *Appl Biochem Biotechnol,* vol. 170, pp. 1080-93, Jul 2013.

CITE THIS ARTICLE

Archana.S, Govind Singh Tomar, G. Srinikethan**.** Studies on Production of Biosurfactant from *Pseudomonas aeruginosa* (MTCC7815) & its Application in Microbial Enhanced Oil Recovery Res. J. Chem. Env. Sci. Vol 4 [4S] 2016. 84-91

 © 2016 AELS, INDIA