Role of Biosurfactant Produced By *Fusarium sp.* BS-8 in Enhanced Oil Recovery (EOR) Through Sand Pack Column

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***Abstract*—Microbial enhanced oil recovery (MEOR) represents the use of microorganisms to extract the remaining oil trapped up in reservoirs. Recently, the biosurfactant application in MEOR has been preferably encouraged over microorganisms due to their potential role in oil mobility and miscibility. The present study was conducted to develop practical microbial solutions and approaches for improving oil production. A newly isolated fungal strain *Fusarium sp.* BS-8 (JQ860113) produced an effective biosurfactant. The crude biosurfactant was able to reduce the surface tension of water from 72 mNm^{-1} to 32 mNm^{-1}, with 71% hydrocarbon emulsifying index and an oil displacement activity (ODA) in the range of 7-13 cm. Under optimized conditions, up to 5.25 g L^{-1} crude biosurfactant was recovered from fermentation broth with ethyl acetate/methanol (5:1) with an ODA of 13 cm. A sand pack column was constructed to simulate an oil reservoir. The use of crude biosurfactant resulted in the additional oil recovery of 46% over untreated column. The biosurfactant was found to be stable at wide range of temperature (0-90 ℃), pH (5-9) and salt concentration (1-15%), suggesting it to be suitable candidate for biosurfactant mediated enhanced oil recovery.**

***Index Terms*—Biosurfactant, MEOR, sand pack column, *Fusarium* sp. BS-8.**

I. Introduction

The rapid urbanization and industrialization has lead multifold increase in the consumption of the crude oil [1]. About two third of oil explored is still unrecovered, and it is figured that only 30-40% oil is recovered with conventional recovery techniques,while about 60-70% of total oil still remain trapped in the reservoir [2]-[5]. The world's demand for oil increased sharply for several years, peaking at 86 million barrels per day in 2007 and expected to accelerate over the next years reaching 89 million barrels per day in 2012 and nearly 91 million barrels per day in 2013. This shortage of oil is leading to major energy crisis as well as gradual increase in oil price hence affecting the economy. Increased demand for crude oil and petroleum products has given strong threshold to the development of Enhanced Oil Recovery (EOR) technologies [6]. Hapless oil recovery from the oil wells is due to low permeability of the rocks forming the reservoir, oil globules that are formed are trapped in pores in reservoirs due to high capillary pressure. High viscosity of the crude oil is another major factor reducing the efficiency of recovery of oil from wells [5], [6]. Enhanced oil recovery (EOR) alleviates oil production by modifying the interfacial tension, changing the fluid properties, mobility of fluid, altering permeability of zones, thus oil moves towards production well in controlled manner [7].

Microbial enhanced oil recovery (MEOR) is economical alternative to conventional enhanced oil recovery techniques involving chemical and industrial methods. Microbial enhanced oil recovery is a biological process that involves the application of microorganisms or their metabolic product to stimulate the reservoir condition to release the trapped oil [4]. Microorganisms play very important role in oil production, especially the trapped residual oil. These produce different metabolites like biosurfactants, biopolymers, acids gases and solvents that can remove heavy and light crude oil. In either case, the mechanism behind oil recovery is always dependent on such microbial metabolites[8]-[10].

The biosurfactant mediated MEOR strategy is based on production of biosurfactants by specific microorganisms from inexpensive raw materials and its injection in oil reservoir [11]. During the process of oil recovery biosurfactants act mainly in two ways; first one is reduction in the interfacial tension between oil and water, resulting in decrease of the capillary effect and increase in capillary number [12]. Second one is the formation of micelles which provides a physical mechanism whereby oil can be mobilized by a moving aqueous phase. Both of these effects result from the presence of hydrophilic and hydrophobic structural elements, with affinities for the water phase and for the oil phase, respectively [3]-[13].

The microbial surface active agents are super seated over conventional ones as they offer several features like reduction in surface and interfacial tension, by being biocompatible [14]. They are biodegradable, non-toxic and eco-friendly in comparison to commercially synthesized surfactants .They show low irritability [15] and maintain their properties even at high temperatures [16]. These are very specific in their actions due to functional groups present on them [17]. They even require low capital investment in comparison to chemically synthesized surfactants [18]. Biosurfactants produced from microorganisms result in decreasing the interfacial tension between oil and water, and reduction in viscosity of oil, thereby releasing the trapped residual oil from reservoirs [19], [20]. This is a cost effective method resulting in increased oil production that can result in self-sufficiency and decreasing oil imports from other

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countries and hence generating revenue.

Therefore, the main objectives of the study were to investigate the oil recovery potential of biosurfactants produced by a newly isolated fungus *Fusarium sp.* BS-8 from oil contaminated soil using sand pack column. The current study may form a basis for a more elaborated in situ application of this biosurfactant for enhanced oil recovery.

II. Materials and Methods

A. Microorganism and Culture Conditions

Fungal isolate *Fusarium sp.* BS-8 (NCBI GeneBank: JQ860113), previously isolated from crude oil contaminated soil from MissaKeswal oil field, Gujar Khan, Pakistan (unpublished data) was used in this study. This strain was maintained on Sabourauddextrose agar (SDA) plates for routine use and was activated usingSabouraud dextrose broth (SDB)at 30 ℃ and 150 rpm for 5-7 days before using it as inoculum for biosurfactant production in mineral salts medium (MSM). The production medium, i.e. MSM contained (g L^{-1}): Sucrose 40; Yeast extract 18.8; Sodium acetate 0.5; Sodium benzoate 0.1; $MgSO_4.7H_2O$ 0.5; $(NH_4)_2SO_4$ 3; KH_2PO_4 2; NaCl 0.9; and 2% vegetable oil used as an additional carbon source. The production medium was inoculated with 9% v/v 48-72 h seed culture of BS-8 in MSM. The cultivation was performed in 2L Erlenmeyer flask at 30 ℃ and 150 rpm in a shaker incubator. The cell free supernatant was collected after centrifugation at 14000 ×g for 20 min and filtered through Whatman filter paper No.1, and was analyzed for biosurfactant production.

B. Recovery of the Biosurfactant

The biosurfactant was recovered from fermentation broth by using the method of Bagheri *et al.*, [21]. Centrifugation of culture broth was done at 14000 ×g for 20 min to obtain cell-free supernatant (CFS) which is acidified to pH 2 using 6N HCl. The acidified cell free supernatant is kept overnight at 4 ℃ for complete precipitation of the biosurfactant. The precipitated biosurfactant was centrifuged at 15000 ×g at 4 ℃ for 15 min and dissolved in phosphate buffer having pH 8. Different solvents systems were used for efficient product recovery by solvent extraction. The precipitate obtained is re-suspended in desired solvent system. Biosurfactantwas extracted three times by organic solvents and evaporatedusing rotary evaporator. The solvents systems used for recovery optimization were: methanol/chloroform (1:1, 1:2, 1:5); methanol/ethylacetate (1:1, 1:2, 1:5); andchloroform, ethylaccetate, butanoland diethylether individually.

C. Analytical Methods

Quantitative and qualitative analysis of biosurfactant production is done by analytical methods including oil displacement activity ODA test, Drop collapse test, Emulsification activity, Surface tension and interfacial tension measurement.

1) Oil displacement area(ODA) test

This method indicates the production of biosurfactant by displacing the oil film forming the clear zone [22]. The method is performed by using 50 ml water in a petri plate having a diameter of 15cm, followed by addition of 40 µl of crude oil to surface of water. A thin film of oil was formed when 20 µl of CFS was added in center of plate. Clear zone displacing the oil was measured in cm.

2) Emulsification index (E24)

The emulsifying capacity of biosurfactant was analyzed by emulsification index according to Cooper and Goldberg [23]. In this method 2 ml of kerosene was added to 2 ml of the cell-free broth in a graduated tube, vortex at high speed for 2 min and allowed to stand for 24h, afterwards,the E_{24} index of sample was calculated (1).

$$E_{24}=\frac{height\ of\ emulsion}{total\ height\ of\ solution}\times 100 \quad (1)$$

3) Surface tension (SFT) measurement

Surface tension (SFT) of CFS was measured using a KRUSS K20digitalTensiometer (Kruss GmbH, Hamburg, Germany) by using Wilhelmy plate method according to the instructions of the manufacturer. The SFT was measured in mNm^{-1}. All the measurements were taken as mean of five replicates at room temperature.

4) Dry biomass estimation

Dry biomass was determined by centrifugation of fungal cultures. Centrifugation is done at 12000 ×g for 20 min, the supernatant is decanted and pellet remains behind. Centrifugation is repeated twice under similar conditions to achieve complete separation of fungal biomass. The pellet obtained is washed twice with distilled water. The pellet is dried on pre-weighed filter paper in hot air oven at 80 ℃ for 60 min. The dry biomass is calculated by measuring the total weight of filter paper again.

D. Characterization of the Biosurfactant

1) Stability testing of biosurfactant

The stability of crude biosurfactant at different temperature range (4 ℃ to 120 ℃), pH range (3-11), and at alkaline conditions (NaCl conc. 10% to 25%, w⁄v) was investigated. The stability of biosurfactant was analyzed by emulsification index, oil displacement assay and by measuring the surface tension of each sample after respective treatments.

2) Toxicity testing of biosurfactant

E. Cytotoxicity Assay

Cytotoxic effect of biosurfactant was determined by Brine Shrimp Assay [24]. 100 ppm, 1000 ppm and 10,000 ppm solutions of partially purified biosurfactant dissolved in distilled water were made. For preparation of artificial seawater 34 g of commercial sea salt (Harvest Co. K. H.) was dissolved in 1 litre of distilled water. It was aerated with vigorous shaking and continuous stirring on magnetic stirrer for two hours. A shallow rectangular dish (20 ×30cm) was filled with artificially prepared seawater. The dish was divided into two unequal compartments by fitting a plastic divider of 5mm containing several holes. Brine shrimp (*Artemiasalina*) eggs (Sera, Heidelberg, Germany) were sprinkled in the larger portion of the dish. The larger portion was covered with aluminium foil in order to provide darkness and allow hatching of brine shrimps while smaller portion was kept in the light. Brine shrimp eggs began to hatch within

24-36 h. Pasteur pipette was used to collect the phototropic nauplii (brine shrimp larvae) from the illuminated side. For this assay two-dram vials were used. 2 ml of seawater was taken in the vial and then 0.5 ml of each solution (100 ppm, 1000 ppm and 10,000 ppm) was added to it. By using Pasteur pipette ten shrimps were transferred to each vial and volume in the vial was raised up to 5 ml. The cytotoxicity assay was performed in triplicate. The vials were placed in the light at 25-30 ℃. The number of survivors and dead shrimps were counted with the aid of magnifying glass in the stem of Pasteur pipette after 24 and 48 h.

F. Phytotoxicity Assay

Phytotoxic effect of cell free supernatant and different concentration, i.e. 30, 40, 50, 60 (mg ml^{-1}) of partially purified biosurfactantwas analyzed on wild reddish seeds. Seeds were surface sterilized by rinsing in 0.1% mercuric chloride for 4-5 min and again thoroughly washed twice with the sterile distilled water. Seeds were allowed to dry on filter paper. Circle shaped autoclaved filter paper was placed inside a petri plate.5 ml of each sample was added into a single plate to wet the filter paper. Total 20 seeds were placed in a single plate on a wetted filter paper and incubated at 25 ℃ under light bench.The total number of seeds germinated was counted at 3rdday of incubation. Mean value of root length and shoot length was determined at 5th day of incubation. Distilled water was used as control. Experiment was performed in triplicates to achieve accurate results. The percentage of seed germination inhibition was calculated by formula (2):

$$A\text{-}B = \frac{C}{\text{total no. of seeds}} \times 100 \quad (2)$$

where *A*=total number of seeds germinated in control; *B*=total number of seed germinated in sample.

G. Construction of Sand Pack Column

A vertically oriented PVC column with an internal diameter of 7.62 cm and length of 97.5 cm with a rubber cork (2.54 cm) at the end was used to demonstrate microbial enhanced oil recovery by biosurfactant produced from *Fusariumsp.*BS-8. The column was packed with the sand having size of 1.5 mm collected from construction site near Department of Bioinformatics, Quaid-i-Azam University, Islamabad. To ensure homogenous packing of sand, the sand was poured into the column with gentle tapping in small amounts. After packing the sand top cork was fixed. Both corks had a hole for insertion of rubber pipes. A peristaltic pump was fixed and connected to the column to maintain the constant steady flow rate.

H. Operation of Sand Pack Column

The column packed was evacuated using nitrogen gas. Nitrogen gas was passed through its one end for 7 min to remove excess of oxygen. The column was than flooded with the brine (5% NaCl) at a flow rate of 36 ml min^{-1}. Six pore volumes (PV) of brine were flooded to ensure complete saturation of column. Next the column was saturated with the motor oil of (20W-50 Shell Helix HX3) shell Pakistan Ltd (density at 35 ℃ 878 kg m^{-3}). As the oil entered the column it displaced the brine from the column. Oil was introduced in column with the same flow rate of 36 ml min^{-1} with the contact time of 18 min. Initial oil saturation which will be original oil in place (OOIP) was calculated. Initial oil recovery was done by water flooding with brine. 10-11 PV of brine were flooded across the column to achieve residual oil saturation. The amount of oil left in column was determined volumetrically. Residual oil was removed by biosurfactant flooding. Crude biosurfactant produced from *Fusariumsp.* BS-8, able to reduce the surface tension of water from 72 mNm^{-1} to 33.26 mNm^{-1}, was added to the column at same flow rate. Column was again flooded with brine after the incubation of 48 h. Liquid was collected in fractions of 100 ml, discharged from the column. Each of the samples was extracted with 50 ml n-hexane, which was further evaporated to collecting flask in rotary evaporator and oil left was measured. Total oil recovery and additional/enhanced oil recovery was determined by the following method [25]:

$$Tor\% = \frac{S_o rwf - S_o rbf}{S_o rwf} \times 100 \quad (3)$$

$$Aor\% = \frac{S_o i - S_o rbf}{S_o i} \times 100 \quad (4)$$

where, *PV* is the volume of brine required for saturatingcolumn; OOIP was the volume of brine displaced by oil; S_oI was the initial oil saturation prior water flooding; S_orwf was residual oil after water flooding; S_orbf was oil saturation after biosurfactants flooding; Tor% denotes total oil recovery; and *Aor*% describes additional oil recovery

III. Results

A. Production of Biosurfactant

Biosurfactant production by *Fusariumsp.*BS-8 was best achieved at optimized conditions having 9% of inoculums size, using sucrose as main carbon source and yeast extract as nitrogen source in corresponding ratio of 9:1 at pH of 7, temperature 30 ℃ and 150 rpm after 15 days of incubation. The biosurfactant produced was able to reduce the surface tension of water from 72 $mNm^{-1}$to 32 mNm^{-1}. In ODA test,biosurfactant produced displaced the crude oil and gave a zone of 16mm. Emulsification index was found to be ~71% (Fig. 1).

B. Optimization of Solvent for Extraction of Product

Fig. 2 shows the results for optimization ofbiosurfactantrecovery using different solvent systems. The results were expressed in terms of weight of crude extract (mg 100 ml^{-1}) and ODA (cm).Up to 172 mg 100ml^{-1} was recovered withan ODA of 4.3 cm when chloroform/methanol at a ratio of 5:1 was used, while~90 mg 100$ml^{-1}$with chloroform only was showing the ODA of 28cm. Biosurfactant recovery was promising when ethyl acetate (with or without methanol) was used as the solvent system. Maximum product recovery of 525 mg 100 ml^{-1} was achieved with ethylacetate/methanol ratio of 5:1 which showed the ODA of 13 cm, however,only 80 mg 100ml^{-1} product was recovered with a significant (ODA of 9 cm) activity whenonlyethylacetatewas used. Therefore, methanol/ethylaccetae (1:5) was selected as the solvent

system of choicefor product recovery from CFS throughout the study.

C. Stability Analysis of Biosurfactant

Biosurfactant produced from *Fusarium*sp. BS-8 was found to be stable over wide range of temperatures, pH and alkaline conditions. Fig. 3 a) shows that maximum ODA (33 mm) was attained at pH 7, while emulsification index and surface tension were 63% and 32.3 mN m^{-1}, respectively. The biosurfactant completely lost its activities when incubated at pH 1 and pH 9-11 as evident by lowest emulsification (10.3%), ODA (12 mm) and higher SFT (47.1 mN m^{-1}).The temperature stability of the crude biosurfactant is shown in Fig. 3 b). The biosurfactant retained its activity over wide ranges of temperature (40-80 ℃) as indicated by stable emulsifying, ODA and surface tension values. The values were found to be 66%, 33.2mN$m^{-1}$and 31 mm,respectively (Fig. 3 b)). A gradual decline was observed afterwards at higher temperature ranges. Fig. 3 c) shows the stability of biosurfactant at different salt concentrations.

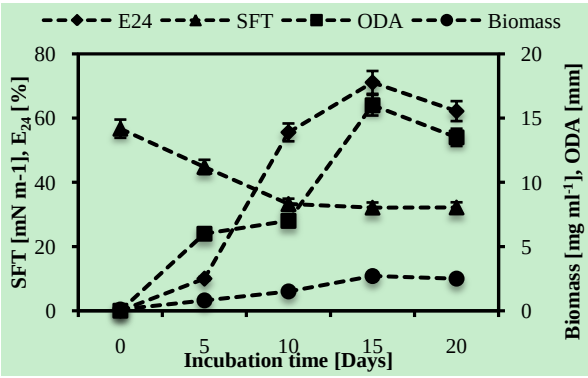

Fig. 1. Growth and biosurfactant production trends of BS-8 isolate in shake-flask settings.

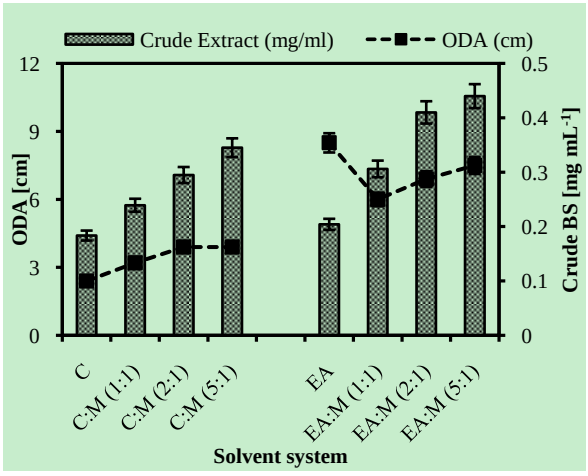

Fig. 2. Counter-current extraction of the crude biosurfactant from cell-free fermentation broth [M= methanol; C=chloroform; EA= ethylacetate].

It was found that biosurfactant could tolerate only 1-10% salt while continuously decline in emulsification and ODA with increasing concentration of salt was observed. Luckily, the surface tension of the broth at high and low concentration of salt was found lowered. The lowest SFT (31.8 mNm^{-1})and highest emulsification (56.5%) and ODA(23mm) were achieved in the range of 1-15% salt concentration.

D. Phytotoxicity Testing

To determine the pertinence of biosurfactant as bio-control agent, phytotoxic assay was performed against wild radish seeds.

Table I shows that there was a significant inhibition of root and shoots' length at higher biosurfactantconcentration (60 mg ml^{-1}) after 3rdand 5thday. It showed slight phytotoxic effect at the lowest concentration (40 mg/ml) tested.

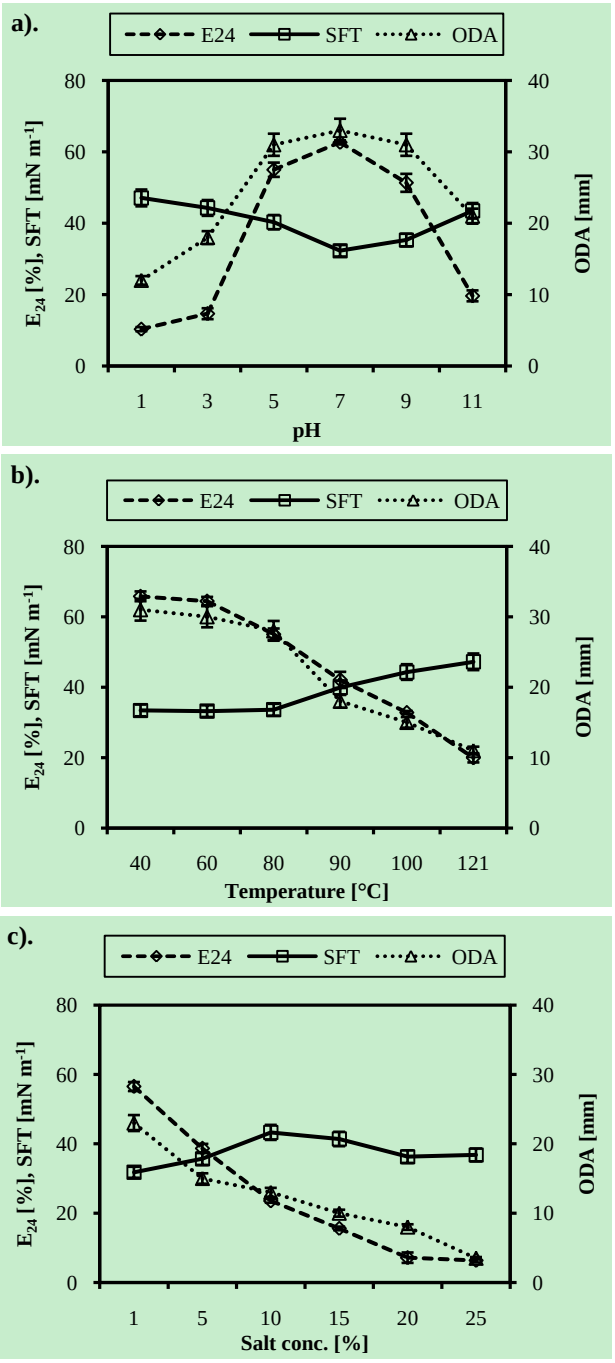

Fig. 3. Biosurfactant stability at varying ranges of (a) pH, (b) temperature, and (c) percent salt concentration.

The biosurfactant had significant effect on seed germination and showed 5%,10% and 15% of germination inhibition at 40, 50 and 60 mg ml^{-1}, respectively.Cell free supernatant also showed the significant effect on root and shoot length, with 25% of seed germination inhibition. Distilled water was used as control.

E. Cytotoxicity Test

Cytotoxic effect of biosurfactant was analyzed by using brine shrimps assay is shown (Table II). The ED_{50} for this

sample was too high showing biosurfactant to be inactive in this assayand results were insignificant.

F. Operation of Sand Pack Column

To investigate the efficiency and applicability of crude biosurfactantrecovered from the fermentation broth of *Fusarium sp.* BS-8 in enhanced oil recovery, the biosurfactant solution was injected into the column at room temperature. This resulted in an additional oil recovery of 46%, whereas the total oil recovery was found to be ~74% (Table III).

IV. Discussion

Biosurfactants are amphiphilic molecules possessing characteristics like low toxicity, biocompatibility, high biodegradability, ecological acceptability and stability at extreme levels of pH, salinity and temperatures [26], [27]. Biosurfactant gained tremendous importance nowadays because of wide array of applications in bioremediation especially in enhanced oil recovery [28], [29].

TABLE I: Phytotoxic Activity of Cell-Free Supernatant, and Different Conc. of Crude Biosurfactant against Wild Reddish Seeds

Sample	Seed Germination		Seed Germination Inhibition (%)	Root Length (cm)	Shoot Length (cm)
	3rd Day	5th Day			
Cell free supernatant	2	2	25%	2.1	1.5
60 mg ml-1	4	4	15%	1.38	0.76
50 mg ml-1	5	5	10%	2.83	3.66
40 mg ml-1	6	6	5%	4.36	4.23
Distilled water	7	7	0%	8	6

TABLE II:Cytotoxic Activity of Crude Biosurfactant against Brine Shrimps Larvae

No of shrimps	Crude BS Conc. (ppm)			EC50 µg ml-1
	100	1000	10,000	
Total shrimps	30	30	30	
Alive after 24hrs	27	24	15	10,000
Alive after 48hrs	24	18	9	1,848

TABLE III: Total and Enhanced Oil Recovery by *Fusarium sp.* BS-8

Organism	Pore Volume (PV)	S_oi (ml)	$S_o$rwf (ml)	$S_o$rbf (ml)	Tor (%)	Aor (%)
Fusarium sp. BS-8	400	263	126	68	74.1	46

Biosurfactants also have medicinal importance and can also be used in various other areas such as cosmetics, textile, food industry, oil, agriculture, mining etc. [30].

In present study, a newly isolated fungal strain *Fusarium sp*. BS-8 was used for production of biosurfactant at previously optimized conditions with MSM. This fungal strain was evaluated for biosurfactant production by using up to 2% oil and without oil because it is reported that fungus use vegetable oil as an additional source of carbon which effect biomass and induce the production of biosurfactant as it triggers the gene activation responsible for biosurfactant biosynthesis and also provides the fatty acid to elongate the lipid chain in hydrophobic domain of biosurfactant [31]. Maximum biomass and high activities were observed with 2 % vegetable oil the results were correlated with previous studies of with Konishi *et al.* [32] and Sarubo *et al.* [33], who used olive oil and canola oil for production of biosurfactant from *Candida batistae* CBS8550 and *C.lipolytica* UCP 0988 respectively. However Fontes *et al.* [34] reported that there is no increase in biosurfactant production by using olive oil from *Yarowialipolytica* which may be due to adhesion of surfactant to *Y.lipolytica* to cell wall.

Maximum biosurfactant from cell free supernatant was recovered by using most appropriate organic solvent. Single solvent as well as combination of solvents were used to achieve maximum recovery of biosurfactant. Maximum recovery as well as maximum activity was achieved using solvents ethyl acetate and methanol in ratio of 5:1 (Fig. 4), as reported by Luna *et al.* [35] during production from *C.sphaerica* UCP 0995.

Fig. 4. Set-up for sand-pack column.

Stability of biosurfactant was analyzed over a broad range of temperature (40℃ to 121 ℃), pH (1-11) and salinity (1-25%) by different analytical methods (Fig. 3 a)-3 c)). The biosurfactant showed maximum activity at 40-80 ℃ with maximum E_{24}=66%, SFT=33.2 mNm^{-1} and ODA=31 mm. A thermo-tolerant biosurfactant was obtainedand was found stableeven at higher temperature up to 121 ℃ (Fig. 3 b)). Our studies were in full agreement with studies on*C.lipolytica* [26], *C.glabrata* [36] and *Bacillus subtilis* C9 [37].The activity of biosurfactant was sustained at all pH levels with minimum deviation in surface tension values, showing greater pH stability (Fig. 3 a)). However, surface tension value increased at lower pH, which is due to the biosurfactant precipitation in CFS [38].Sarubbo *et al.* [33] reported that biosurfactant produced by *C.lipolytica* was stable at different pH values giving best activities at pH 12.

The varying salt concentration (1-25%) had a significant effect on the activity of the biosurfactant (Fig. 3 c)).Similar results were observedfor biosurfactant production by *C.tropicalis* which tolerated 5% salinity, while at 10% salinity lost 20% of its activity [39]. Luna *et al.*, [40] also investigated stability of biosurfactant produced by *C.sphaerica* at temperature (5-120 ℃), pH (2-12) and salinity (2%-10 %). The stability of biosurfactant in present work suggested it to be thermo-tolerant and halo-tolerant anda good candidate for enhanced oil recovery from the reservoir, as reservoirs usuallypossess such a harsh condition of temperature, pH and salinity.

In present study, the biosurfactant product was investigated for cytotoxic and phytotoxic potential because of environmental concerns. The results werenon-significant in brine shrimp cytotoxicity assay (Table II), which showed that the biosurfactant would not be cytotoxic to the indigenous microflora and found safe for environmental applications. However biosurfactantshowed phytotoxic effects only when applied at high concentration levels (Table I). These results weresimilar with the previous reports on rhamnolipids contributing the inhibition of plantgrowth with gradual increase in concentration [41], [42]. Seed germination inhibition in Lactuca sativa (lettuce) was also observedwith gradual increase in concentration of surfactants [43], [44]. Thesefinding showed that the product can be produce pesticidal effect and can playimportant role in agriculture field to control weeds.

The biosurfactant produced by *Fusarium sp*. BS-8 was tested for its application in *ex-situ* microbial enhanced oil recovery by using sand pack column, which resulted in enhanced oil recovery of 46% (Table III), these findings are correlated with work done for oil recovery with microbial by-products [3]-[47]. Many researchers have reported oil recovery up to 20%-60% from different species ofBacillus spp. and Pseudomonas spp. such as like *P.aeruginosa* SP4, *B.subtilis* PT2, *B.mojavenis* JF-2by biosurfactant using sand packed column [4]-[48].

V. Conclusion

To the best of our knowledge, this is very first report on biosurfactant production by Fusarium spp. It appears to be a novel candidate for the production of possibly a new class glycolipid biosurfactant and its application in microbial enhanced oil recovery (MEOR).

It was concluded that under previously optimized conditions (unpublished data) *Fusarium sp*. BS-8 was able to produce biosurfactant, while its optimum recovery was achieved by ethylaccetate/methanol in 5:1 indicated lipid containing moieties attached with some hydrophilic components like glycolipids. The stable activities of biosurfactant at extreme environmental conditions (pH, salinity and temperature) suggested it to be thermo-tolerant and halo-tolerant, which is the most needed and important property of any biosurfactant for application in microbial enhanced oil recovery (MEOR). *Fusarium* sp. BS-8 is found to be halo-tolerant and thermo-tolerant so these properties can be exploited and used in the treatment of marine oil spills as well as recovery of oil from deep sea reservoirs having these extreme conditions.

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