Characterization and physicochemical properties of biosurfactant produced from an adaptive strain for microbial enhanced oil recovery (MEOR)

4.1. Introduction

World's demand for oil increased rapidly day by day due to industrialization and urbanization as it is an important source of energy for humans, but simultaneously it also plays a major role in environmental contaminant. It was figured that around 60-70% of total crude oil remain trapped in the reservoirs after primary and secondary oil recovery (Suthar et al. 2008). To recover this unrecovered crude oil various chemical process are used in which most commonly chemical surfactants were used this is known as Enhanced Oil Recovery (EOR), Surfactants having amphiphilic properties they interact with interfacial surface of oil and soil and reduce the viscosity of crude oil so it can easily mobilize and comes into the oil reservoir but chemical surfactants are hazardous, non-biodegradable and adversely affecting in food chain and environment.

Biosurfactants are the better alternative of chemical surfactants they have high surface activity, environmental friendly, lower toxicity, biodegradability, ecological acceptability and do not lose physico-chemical properties at extreme temperatures, pH and salinity levels (Kim et al. 2000; Silva et al. 2010; Thavasi et al. 2011).

They are widely used in microbial-enhanced oil recovery (MEOR), agriculture, food, cosmetics, and pharmaceuticals industries. Today crude oil and petroleum products are the major source of hydrocarbon pollutants for soil and marine environments. Due to the

insoluble nature of these pollutants in water, their removal from the environment is very difficult. Biodegradation of hydrocarbons by microorganisms is one of the promising ways to remove them from the soil and marine environments (Inakollu et al. 2004; Lawniczak et al. 2013; Moldes et al., 2011).

After primary and secondary methods in Oil recovery certain amount of oil remains in the oil wells, Biosurfactant is one of the efficient reagent to recover that percentage of oil from oil wells this is termed as Microbial enhanced oil recovery (MEOR). Biosurfactants have the ability to emulsify crude oil and to decrease the viscosity of crude oil that is one of the mechanisms for MEOR (Wang et al., 2011). Biosurfactant properties remain stable under extreme physico-chemical conditions this makes it feasible for enhanced oil recovery operations.(El-Sheshtawy et al. 2015)

According to their chemical nature biosurfactant are classified as glycolipids, lipopeptides, phospholipids, polymeric microbial surfactants and particulate biosurfactants (Varjani et al. 2017). Lipopeptides are most popular and efficient biosurfactantthey have high surface and interface activity, emulsion forming capacity and stabilization, wetting, anti-adhesive and the antimicrobial activities (Muthusamy et al. 2008). Surfactin, a lipopeptide biosurfactant is one of the most surface-active biosurfactants (Peypoux et al. 1999), in terms of critical micelle concentration (CMC) and minimum surface tensions. It is a versatile bioactive molecule having the ability to inhibit fibrin clot formation, enhanced oil recovery, bioremediation for cleaning soil and water. In addition, it has demonstrated antifungal, antiviral, antitumor, insecticidal, and antimycoplasma activities(Liu et al. 2015; Seydlová, et al. 2011). These properties of surfactin reflect its potential commercial applications.

In previous chapter *Candida tropicalis* MTCC230 get acclimatized in high hydrocarbon condition for biosurfactant production and optimization (Verma et. al., 2011). In this chapter, the produced biosurfactant was characterized by Fourier Transform Infrared Spectroscopy (FTIR), Near IR spectroscopy, Reversed-phase high-performance liquid chromatography (RP-HPLC) and Mass Spectroscopy (MS) analysis. The physicochemical properties of the biosurfactant including Critical Micelle Concentration (CMC), oil spreading activity and emulsification ability were also studied. Surface tension variation study was done at the different temperature, pH and salinity for determining the effectiveness of biosurfactant under extreme environmental condition. Furthermore, a soil column saturated with crude oil was washed with biosurfactant by batch type soil washing this revels its potential use in MEOR. Waste mobile oil contaminated soil was washed with biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC230. This study is very much helpful for evaluating the properties, effectiveness and characterizing the biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC230 and its potential commercial application in MEOR.

4.2. Material and Methods

Candida tropicalis MTCC230 was acclimatized in high hydrocarbon (petrol) condition for biosurfactant production and optimized fermentation conditions. Here, commercially known strain *Bacillus Subtilis* MTCC2423 for surfactin production was used as a standard and for comparative analysis.

Emulsification Activity

The production of biosurfactant was estimated in terms of the Emulsification index value i.e., $\%E_{24}$ at different time intervals. The $\%E_{24}$ is the height of the emulsion layer, divided by the total height of the liquid, multiplied by 100 in Eq. 1 (Donio et al., 2013; Saikia et al. 2012). Emulsification index (E_{24}) was measured after 24 h.

$$\%E_{24} = \frac{\text{Height of emulsified layer (cm)}}{\text{Height of total liquid (cm)}} \times 100 \qquad \text{……..(1)}$$

Effect of pH, temperature and salinity on biosurfactant stability

Stability of biosurfactant under stress Environmental conditions was done by determined surface tension changes and emulsification index of cell-free broth (Supernatant) under the wide range of temperatures, pH and different salt concentrations. The pH of cell-free broth was adjusted to 2, 4, 6, 7, 8, 10 and 12 with HCl or NaOH solutions; cell-free broth were incubated at 30, 40, 50, 60, 70, 80 and 90ºC for 30 min and then kept at room temperature; The salinity of cell-free broth was adjusted to 2%, 4%, 6%, 8% and 10% w/v (NaCl). The surface tension and emulsification index of these cell-free broths were measured at room temperature. All the experiments were carried out in triplicate.

Measurement of Surface Tension (ST) and critical micelle concentration (CMC)

Surface tension was measured on a ring tensiometer (Krüss tensiometer K6) according to the Du Noüy Ring method (Liu, et al. 2013) at room temperature. CMC value was estimated by measuring the surface tension of biosurfactant at different concentration. Dilutions of the biosurfactant in distillate water were prepared to determine CMC up to a

constant Surface Tension (ST) value. CMC was determined as mg/l by plotting the biosurfactant concentration against the ST value**.**

Measurement of Oil Spreading Activity

Morikawa et al. (2000) (Morikawa et al. 2000) described the Oil spreading experiment; In brief 20 ml of distilled water was added to a plastic Petri dish, followed by addition of 15ml of oil (Mobile oil discarded from a motorcycle) making a thin layer on the surface of the water. 10 µl of cell-free culture broth was then added to the oil surface. If biosurfactant is present in the cell-free culture broth, the oil will be displaced with an oil free clearing zone and diameter of this clearing zone indicates the surfactant activity.

Soil washing

In three conical flasks having 50g of garden soil was mixed with waste mobile oil discarded from four stroke bikes. First untreated flask act as a blank, second flask was flooded with water and third was with biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230. Shaking the flasks at 100rpm overnight at 37ºC after that observed the texture of soil and separation layers in flasks.

Thin Layer Chromatography

Preliminary characterization of the biosurfactant was done by thin layer chromatography (TLC) method which was separated on a silica gel plate using a mobile phase of $CHCl_3$:CH_3OH:H_2O (65:15:1) as developing solvent system with different color developing reagents. Isolated biosurfactant was characterized by spraying the plate with ninhydrin reagent and heated at 110 ºC for 5 min.

Near IR and FTIR analysis

Biosurfactants produced from *B. subtilis* MTCC2423 and from an acclimatized strain of *C. tropicalis* MTCC230 were characterized by using Aventes Spectrometer with wavelength range is 300-1100 nm and Avasoft 8 (software) was used to process data received.

To determine the functional groups and the chemical bonds, samples were prepared for infrared analysis by mixing approximately 1mg of crude biosurfactant with 100mg of KBr and pressing the mixture into the form of a pellet at 134MPa for 2–3 min to obtain transparent pellets. The IR spectrum of the pellet was collected from 500 to 4000 wavenumbers (cm^{-1}) by using a FTIR spectrometer (shimadzu) with sample dispersed in the pellets of KBr.

HPLC analysis

Crude biosurfactant was dissolved in methanol and was analyzed using Reverse Phase High Performance Liquid Chromatography (RP-HPLC) (Waters) on a reverse phase column [SunFire TM C18, 5 µm] using an Agilent 1100 series HPLC instrument and analyzed by PDA detector (waters 2998 Photodiode Array). The elution was done using a linear gradient of 90% (v/v) of methanol and 10% (v/v) water with 0.05% (v/v) trifluoro acetic acid (TFA) for 21 min at a flow rate of 1 ml/min. HPLC spectra were detected at 210 nm and was compared with authentic lipopeptide surfactin purchased from Sigma Chemicals (St. Louis, MO, USA).

Mass spectrometry analysis

LC/MS analysis was carried out using Agilent 6230 Time of Flight-Mass Spectrometer (TOF-MS) system equipped with dual electrospray ionization (Dual-ESI) source. Data was acquired in positive ion mode using mass range of 100-3200 *m/z*. The capillary gas temperature/ voltage (V_{cap}) was set to 275°C and 3500 V, respectively and the fragmentor voltage (V_{frag}) was 300V. Data acquisition and analysis of the MS data was carried out using the Agilent Acquisition Software and Agilent Qualitative Analysis Software along with Agilent MassHunterBioConfirm Software, respectively. Mass deconvolution was carried out over the mass range of 500-1500 Daltons (Da) with mass step of 5 Da and peak height filter of signal to noise ratio greater than 30:1. Maximum Entropy algorithm was used to deconvolute the multiply-charged ion envelope to zero charge mass spectrum. Intact mass was deduced from the deconvoluted spectrum based on the mass shift and mass error and was compared with mass of authentic lipopeptide surfactin purchased from Sigma Chemicals (St. Louis, MO, USA).

Sand Pack Studies for Enhanced Oil Recovery Applications

The potential application of biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 for Microbial Enhanced Oil Recovery (MEOR) was carried out by sand packed method described by Suthar et al., 2008. A jacketed glass column was packed with 35g of acid washed loam sand and completely dried at 100ºC in a hot air oven. The brine solution (5 % NaCl, w/v) was then passed through the column and pore volume (PV) was determined After saturating column with brine solution, Four stroke engine oil (Castrol ACTIV 4T) was passed until the column got saturated with oil, Initial oil saturation which will be original oil in place (OOIP) was calculated by replacing the brine solution present in

the column and measured initial oil saturation (Soi). Further, the oil saturated column was washed with brine solution until no further oil was discharged in the effluent; oil retained i.e. residual oil saturation (Sor). Cell-free fermentation broth containing biosurfactants produced from an acclimatized strain *C. tropicalis* MTCC230 was then loaded on to the oil saturated sand pack column and incubated for 24h. Discharged engine oil flowing out of the column was collected and the percentage of oil recovery was calculated as Additional Oil Recovery (AOR).

4.3. Results and Discussion

Effect of pH, salinity and temperature on biosurfactant stability

Physiochemical stability of biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 was done by estimating its surface tension and emulsification Index on a wide range of pH, temperature and salinity (Figure 4.1). It was observed that surface tension of biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 was not much fluctuating with the change of pH from 2 to 12 as shown in Figure 4.1 the highest surface tension was 42mN/m at pH 2 and the lowest surface tension was 38.5mN/m at pH10. Similarly, highest Emulsification Index (E_{24}) was 62% at pH 8 and the lowest was 47% at pH 2 as shown in Figure 4.1 (a). At different salinity concentration (2% to 10%), highest surface tension was 49mN/m at 8 % NaCl and the lowest was 42mN/m at 2% NaCl and the highest Emulsification Index (E_{24}) was 54% at 4% NaCl and the lowest was 48% at 2% NaCl as shown in Figure 4.1 (b). Similarly, at different temperature (30ºC to 90ºC) highest surface tension was 41mN/m at 80ºC and the lowest surface tension was 34 mN/m at 30ºC and the

highest Emulsification Index (E_{24}) was 62% at 60ºC and the lowest was 50% at 30ºC as shown in Figure 4.1 (c).

These results clearly suggest that biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 showed the minimal change in surface tension and Emulsification Index over the wide range of pH, temperature and salinity. So, it was suggested as a good candidate for enhanced oil recovery from the reservoir, as reservoirs usually possess such a harsh condition of temperature, pH and salinity.

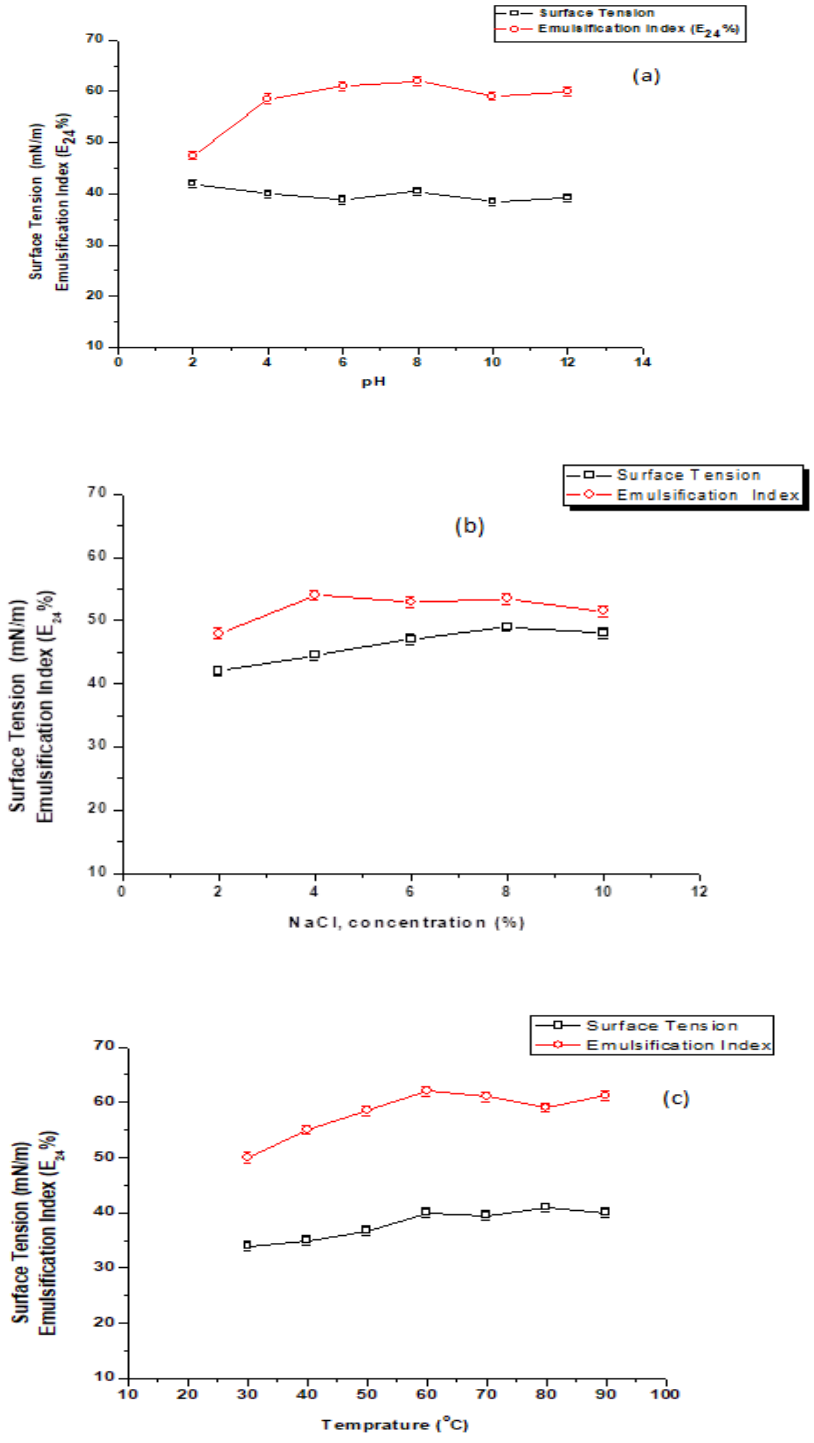

Figure 4.1 Effect of (a) pH, (b) salinity and (c) temperature on the stability of biosurfactant

Measurement of critical micelle concentration (CMC)

Critical micelle concentration (CMC) is an important parameter for the evaluation of biosurfactant activity. As we increase the concentration of biosurfactant the surface tension decreased, a minimum surface tension of biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 and from *B. Subtilis* MTCC2423 (standard surfactin producing strain) were 32mN/m at 32.5mg/l and 28.5mN/m at 33mg/l respectively, As shown in Figure 4.2. This reveals that CMC value of biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 and from *B. Subtilis* MTCC2423 was nearly the same. CMC value helps to determine the efficiency of biosurfactant, increase in the concentration of surfactant after CMC had no effect on the Interfacial Tension (IFT). Thus, the efficiency of the biosurfactant in reducing the interfacial tension between soil and oil makes it more attractive for use in microbial enhanced oil recovery (MEOR). Crude biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 having CMC value 32.5mg/l that could consider as good surfactant and efficiently reduce the IFT to enhance the oil recovery.

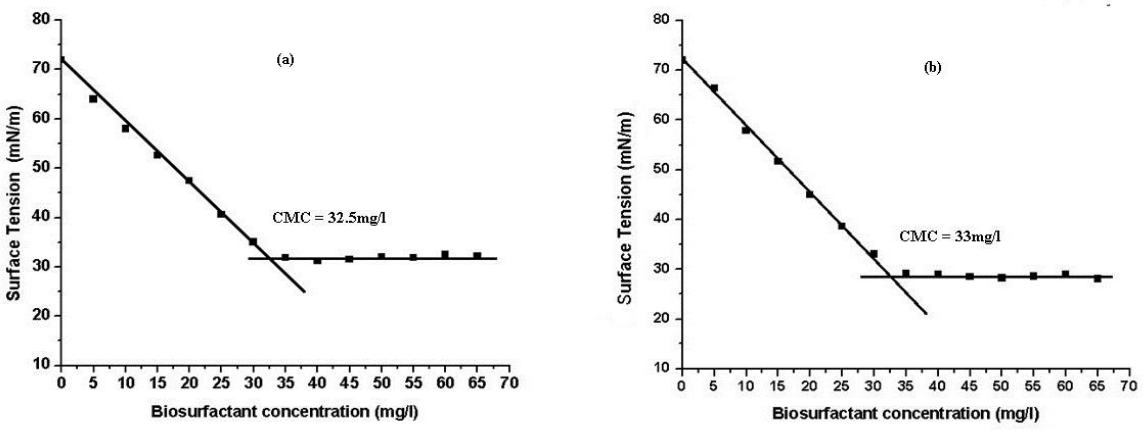

Figure 4.2 CMC value of biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 (a), and from *B.Subtilis*MTCC2423 (b).

Oil Spreading Activity

Oil spread or displacement activity is a qualitative method used to estimate the activity/efficacy of the surfactant to displace the oil. 10µl of cell-free culture (Supernatant) broth of biosurfactant, produced from an acclimatized strain *C. tropicalis* MTCC230 was dropped on the oil (Mobile oil discarded from a motorcycle) surface, a clear zone formed under which water appears as shown in Figure 4.3. This proves that supernatant having biosurfactant that have the efficiency to displace oil and could be used in bioremediation of water contaminated with hydrocarbon wastes.

Figure 4.3 Oil spreading analysis (a) control (b) oil displaced by biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230.

Soil washing

The performance of biosurfactant in soil washing was done on soil contaminated with four stroke engine oil (Figure 4.4). Figure 4.4(a) shows that soil contaminated with engine oil was in dark black color appearance, Figure 4.4(b) shows contaminated soil washed with water and Figure 4.4(c) shows contaminated soil washed with biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC 230 after 24hr of incubation at 37°C with 100rpm respectively, a clear layer formed where oil get flocculated on the top of the liquid and the texture of the soil changes from black to normal color after biosurfactant treatment as shown in Figure 4.4(c). These results reveal that biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 have potential to remediate the soil contaminated with crude oil or hydrocarbon wastes.

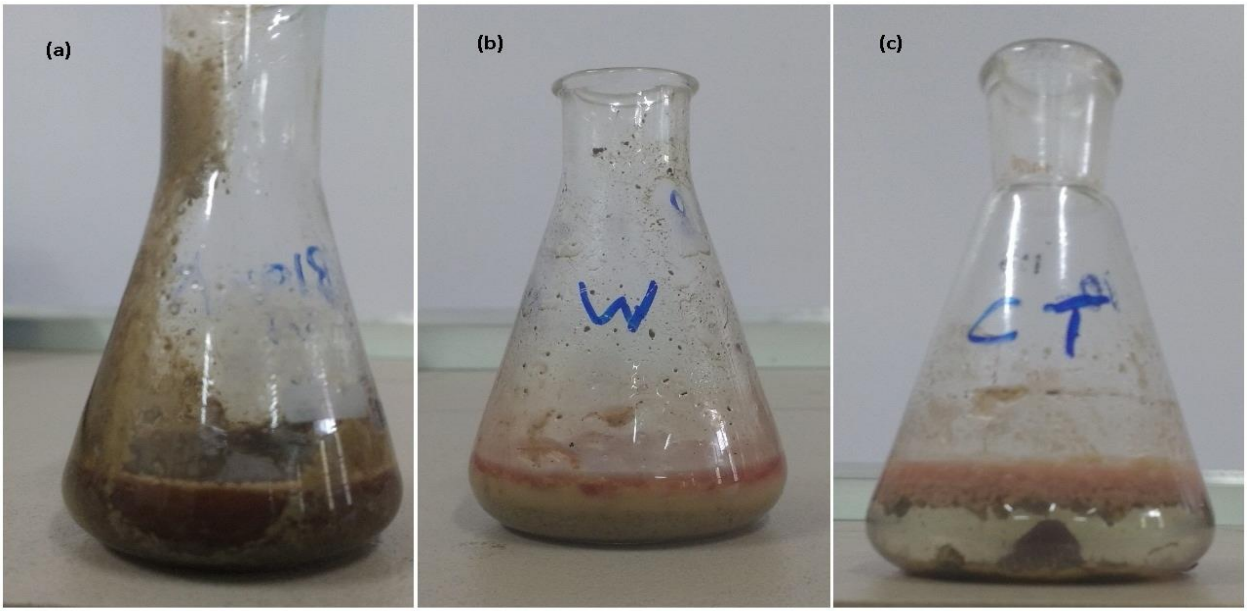

Figure 4.4 Soil washing analysis (a) control: soil contaminated with engine oil, (b) contaminated soil washed with water, (c) contaminated soil washed with biosurfactant produced *Candida tropicalis* MTCC 230.

Characterization of Biosurfactant

Thin Layer Chromatography (TLC)

The crude biosurfactant produced from an acclimatized *C. tropicalis* MTCC 230 was characterized as lipopeptide by the TLC method; a pink color spot was observed with an Rf value of 0.50 (Figure 4.5). From this study, a clear picture was received that the peptide is present in the partially purified biosurfactant produced from an acclimatized *C. tropicalis* MTCC 230, this is because peptide gives a pink color with ninhydrin test by TLC method.

Figure 4.5. Thin layer chromatography of extracted biosurfactant produced from *C. tropicalis* MTCC230.

Near Infrared (NIR) and Fourier transformed-infrared (FTIR) spectrum analysis

Comparative Studies of surfactin and Biosurfactant produced from a *B. Subtilis* MTCC2423 and from an acclimatized strain *C. tropicalis* MTCC230 respectively, were performed by using Near IR spectrum of wavelength range 300-1100nm as shown in Figure 4.6, result shows that the absorbance peak frequencies for surfactin and biosurfactant were fall in the same wavelength i.e., 396nm and 967nm, Absorbance units (AU) were different for each surfactant due to concentration difference.

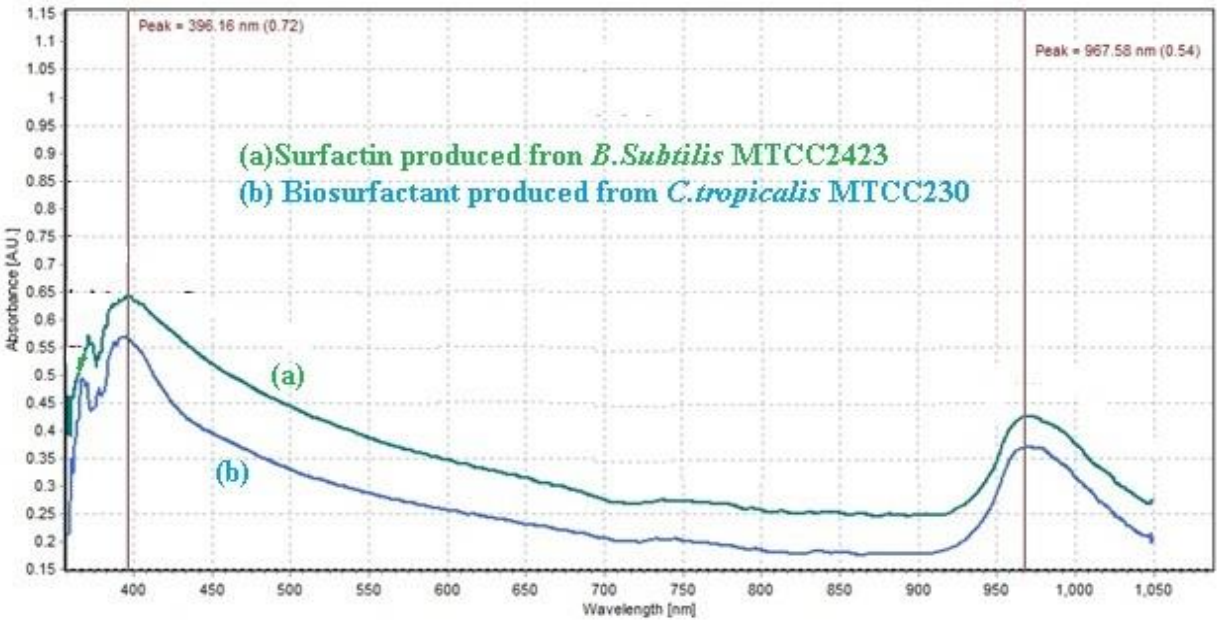

Figure 4.6 Near Infrared (NIR) spectrum (a) surfactin produced from a *B. Subtilis* MTCC2423 and (b) biosurfactant from an acclimatized strain *C. tropicalis* MTCC230.

Further, the biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 was confirmed and characterized by the FTIR spectra. As shown in Figure 4.7, the absorbance of N-H stretching bond at 3134 cm^{-1} indicated the presence of a peptide residue, 2773 cm^{-1}corresponding to the C–H (CH_3) and (CH_2) stretch (aliphatic chain), at 1730 cm^{-1}corresponds to C=O stretching bond (presence of lactone ring), CO–N at 1408 cm^{-1} corresponds to amide group and the presence of an aliphatic chain-CH at 1,066–946 cm^{-1} (Jung et. al., 2012; Das et. al., 2008). These results strongly indicate that the biosurfactant produced by *Candida tropicalis* MTCC230 contains aliphatic and peptide-like moieties i.e, cyclic lipopeptide and corresponds to surfactin and also showing same wave number positions as shown by surfactin produced from *Bacillus Subtilis* MTCC2423 (standard surfactin producing strain) shown in Figure 4.6 and Figure 4.7.

Functional characterization was confirmed by Near-IR and FTIR spectrometric analysis, crude biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 showed aliphatic and peptide-like moieties i.e, cyclic lipopeptide, that share the same wave number positions as shown by surfactin produced from a *B.Subtilis* MTCC2423 (standard surfactin producing strain), IR spectrometric analysis is one of the most promising technique to determine the functional characterization of an unknown compound (Das et al. 2008; Jung et al., 2012).

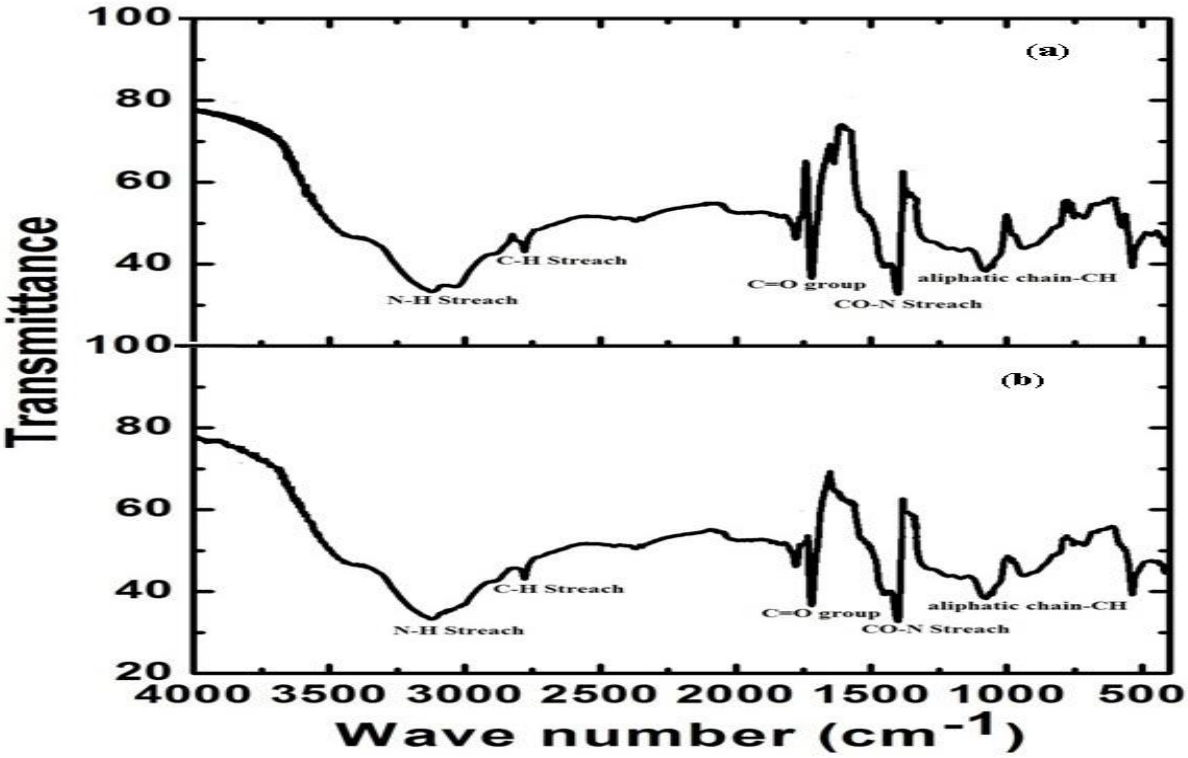

Figure 4.7. FTIR spectrum (a) Biosurfactant produced from *Candida tropicalis* MTCC230 and (b) surfactin produced from *Bacillus subtilis* MTCC 2423.

RP-HPLC analysis

HPLC chromatograms of standard surfactin (Sigma-Aldrich) and biosurfactant recovered from an acclimatized strain *Candida tropicalis* MTCC 230 was shown in Fig.24. RP-HPLC analysis revealed that the biosurfactant recovered from an acclimatized *Candida tropicalis* MTCC 230 and authenticated purchased standard surfactin (sigma-Aldrich) share similar chromatogram peak with similar retention time at approx. 4.3 to 6.8 minute during the 22 minutes of run time (Figure 4.8).

Comparative study with standard surfactin (sigma-Aldrich) and biosurfactant recovered from an acclimatized strain *Candida tropicalis* MTCC 230 by RP-HPLC, showed similar retention time. This result significantly confirms that the biosurfacatnt produced from an acclimatized strain *C. tropicalis* MTCC230 considered as a lipopeptide surfactin. Surfactin produced from an acclimatized strain was quantified using HPLC and it was found that approx. 398.88mg/l of surfactin was produced. This was quite good amount of surfactin when compare to other surfactin producing microorganisms, in most of the cases it was found that around 150 to 300mg/l is normally surfactin was produced (Jung et.al., 2012; Liu et. al., 2012; Sousa et. al., 2014; Hsieh et.al., 2004).

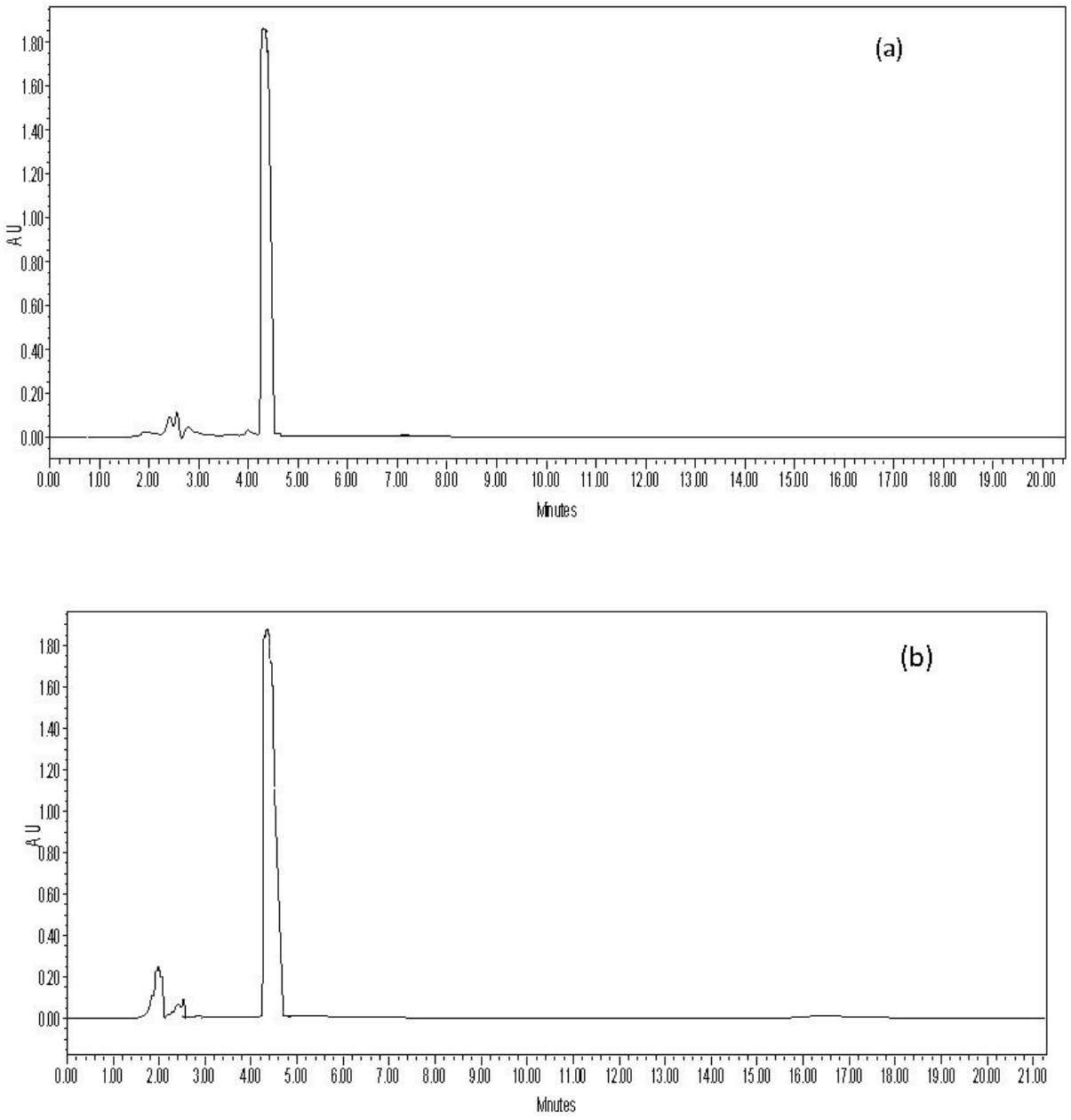

Figure 4.8. HPLC chromatogram of (a) standard surfactin (Sigma-Aldrich) and (b) biosurfactant produced from *Candida tropicalis* MTCC 230

Mass spectrometry analysis

Crude biosurfactant produced by acclimatized *C. tropicalis* MTCC230 were isolated using a two-step purification method. Firstly, biosurfactants were partially purified from the culture supernatant by ethyl acetate extraction and was further purified using RP-HPLC on a C18 column. Hereafter, active fractions were characterized by ESI-TOF-MS to confirm its identity with respect to the reference standard through intact mass analysis.ESI-TOF mass spectra was extracted from the total ion chromatogram (TIC) for the main peak corresponding to lipoprotein (surfactin) and revealed occurrence of the most abundant ion assigned to $[M+H]^+$. The two molecular ions of $[M + 2H]^{2+}$ at 518.8 m/z and $[M + H]^+$ at 1036.3 m/z were consistent with the presence of surfactin. Figure 4.9 shows the ESI-TOF-MS spectra of the lipoprotein (surfactin) with molecular mass 1036Da. Results confirmed that biosurfactant produced from the acclimatized *C. tropicalis* MTCC230 was found near identical to the standard surfactin (Sigma-Aldrich) sharing the same molecular mass i.e, 1036.34Da and confirming that biosurfactant produced from an acclimitized strain *Candida tropicalis* MTCC 230 was considered as surfactin class of lipopeptide biosurfactant.

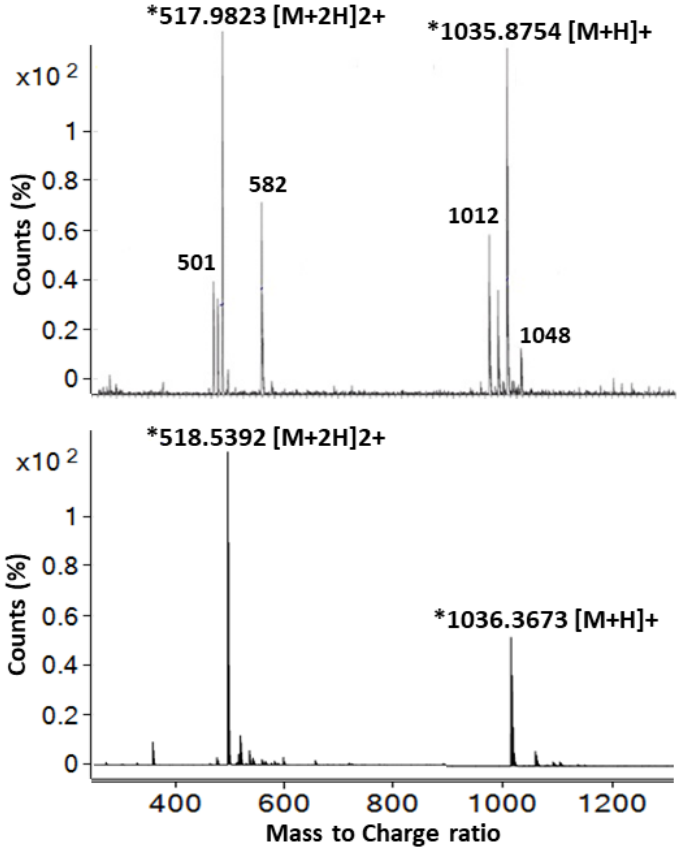

Figure 4.9. ESI-TOF-MS: Intact mass of singly charged and doubly charged of biosurfactant produced from an acclimitized strain *C. tropicalis* MTCC230 (a) and (b) mass of surfactin standard surfactin (Sigma-Aldrich).

Enhanced oil recovery using sand pack column

The applicability of biosurfactant produced from *C. tropicalis* MTCC230 in MEOR was done on sand pack column in laboratory scale (Figure 4.10) as it was found that its construction is easy, inexpensive, rapid and reliable. Table.1 show different parameters (PV, OOIP, Sorwf, Sorbf, Swi(%), Soi(%), Sor(%), AOR(%) that were helpful for determination of oil recovery experiment was done inthree replicates and mean value was considered. The

pore volume (PV) and OOIP (original oil in place) of the column are 31ml and 21.67ml (mean values), respectively. Brine solution flooding step in a column was similar to water flooding in oil wells during the secondary phase oil recovery (Suthar et al., 2008). After secondary phase oil recovery, still 60% - 70% of the oil was remained in the sand column/oil wells, to recover remaining oil from the column cell-free fermented broth (supernatant) containing biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC 230 was loaded onto the oil containing sand column for 24 hrs. The amount of oil recovered after biosurfactant flood i.e., S_{orbf} was 4.16ml (mean value). Oil displaced after 24 hrs from soil column was recovered and calculated as additional oil recovery (AOR) i.e., 39.80% (mean value) as shown in Table. 1. Many researchers have reported 20%-60% enhanced oil recovery from different biosurfactant producing microbial species such as *B. mojavensis* JF2, *B. licheniformis* TT42, *Fusarium sp*. BS-8, *B. licheniformis* K125, *B. subtilis* K1, *P. aeruginosa*SP4, *B. subtilis* MTCC 2423, *B. subtilis* MTCC 1427, *B. aureum*MSA13 (Makkar et al. 1997; Qazi et al. 2013; Seghal Kiran, et al. 2010; Suthar et al., 2008). Thus, the biosurfactant produced from an acclimatized strain *Candida tropicalis* MTCC 230 was efficient and seems to be a better agent for Microbial Enhanced Oil Recovery (MEOR) from oil reservoirs.

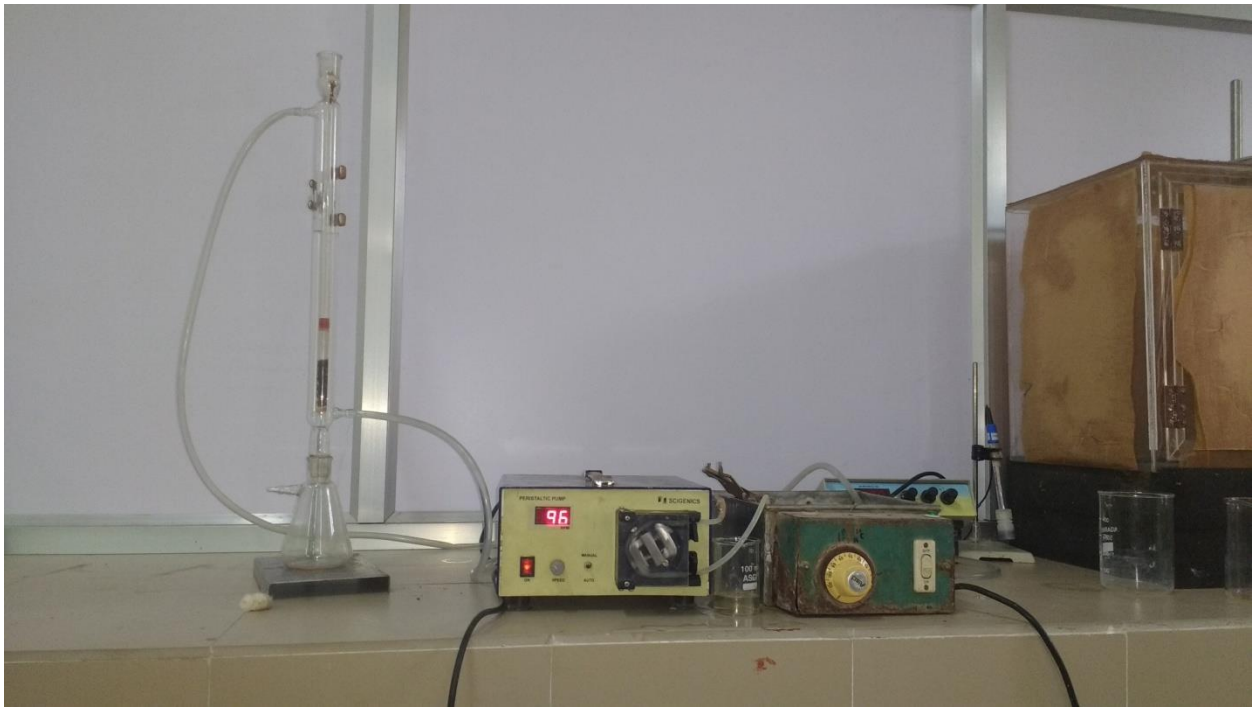

Figure 4.10. Lab scale setup of sand pack column

Result obtained from sand pack column was significantly acceptable as 39.80% (mean value) additional oil was recovered (Table 4.1). This result indicated that biosurfactant produced from *C. tropicalis* MTCC230 having ability to lowering the interfacial tension of oil that was trapped between the oil reservoir and facilitating the oil to mobilize. Stable physicochemical properties and effective surface and emulsification activity of biosurfactant produced from *C. tropicalis* MTCC230 makes it more effective agent for MEOR in oil industries.

Table 4.1 Parameters for oil recovery in sand pack column (SPC) using water flood and biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230

Parameters	Sand pack column (SPC)			
	SPC1	SPC2	SPC3	SPC mean
PV (mL)	30	32	31	31
OOIP (mL)	21	22	22	21.67
Sorwf (mL)	9.7	11	10.4	10.36
Sorbf (mL)	3.8	4.5	4.2	4.16
Swi (%)	30	31.2	29	30.06
Soi (%)	70	68.8	71.1	69.96
Sor (%)	53.8	50	52.7	52.10
AOR (%)	39.1	40	40.3	39.80

PV Pore volume, OOIP Original oil in place (Amount of brinesolution discharged upon displacement by oil sand pack column); Sowf—Oil retained after brine flooding; Sorbf—Oil released from sand pack column after treatment with biosurfactant; Soi %—Percent initial oil saturation; Swi %—Percent initial water saturation; Sor %—Percent residual oil saturation; AOR %—Percent additional oil recovery after biosurfactant flooding.

4.4. Conclusion

In this study biosurfactant produced from an acclimatized strain *C. tropicalis* MTCC230 considered as a lipopeptide surfactin that was confirmed by TLC, Near IR, FTIR spectrum, HPLC and MS analysis and its CMC value was found to be nearly the same of surfactin produced from *B. Subtilis* MTCC2423 (standard surfactin producing strain). It shows promising results in the field of bioremediation to remove the hydrocarbon contaminants from water and soil that was confirmed by oil spreading method and soil washing analysis. It was stable over wide range of pH, temperature and salinity that makes its

suitability in various industries and most importantly in MEOR. In sand-pack column experiment 39.80% additional oil was recovered (AOR) this shows its direct approach for MEOR in oil reservoirs.