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Bio-Surfactant Production And Its Application In Microbial Enhanced Oil Recovery On Laboratory Scale

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ABSTRACT

In current scenario, the demand for energy is considered to be the major target for industries. Among the various sources of energies, crude oil plays a vital role. Oil recovery is achieved using conventional primary and secondary recovery methods. In-order to recover the remaining residual oil, technologies like Enhanced Oil Recovery (EOR) are utilized which is also known as tertiary recovery. Among EOR, Microbial enhanced oil recovery (MEOR) is a technique which enables the improvement of oil recovery by injection of bio-surfactant produced by microorganisms. Bio-surfactant can retrieve unrecoverable oil from the cap rock which is held by high capillary force. Bio-surfactant is a surface active agent which can reduce the interfacial tension and reduce viscosity of oil and thereby can be recovered to the surface as the mobility of the oil is increased. Research in this area has shown promising results besides the method is echo-friendly and cost effective compared with other EOR techniques. In our research, on laboratory scale we produced bio-surfactant using the strain *Bacillus subtilis* (MTCC 1427) and comparative study was carried out at both 30 and 45 °C. Biosurfactant and analytical studies such as surface tension and interfacial tension have revealed that *Bacillus subtilis MTCC 1427* has the capability to grow and produce maximum biosurfactants leading to the reduction of surface tension from 71 mN/m to 34 mN/m at pH 8.0 and 30° C. However at temperature 45° C the strain did not produce biosurfactant. Also the strain failed to show growth below pH of 5.0 and above pH of 8.0. Produced bio-surfactant was characterized using FTIR analysis.

INTRODUCTION

Increased interest of biosurfactant in the recent years has stimulated attempts to enlarge the present range of microbial surfactants. Most of these biosurfactants are bio-degradable and less toxic than their chemically synthesized counterparts (Desai et al, 1994). Microbial enhanced oil recovery (MEOR) is considered a relatively cheap method to recover tertiary oil from reservoirs. MEOR improves macroscopic sweep efficiency through three mechanisms which include permeability profile modification (by microbial induction), reduction of interfacial tension between oil and water with microbial bio-surfactants (to lower capillary trapping forces) and stimulation of reservoir porosity and permeability with microbial products like acids. A combination of the above three mechanisms can also be used for improving macroscopic sweep efficiency to recover tertiary oil (Lazar et al, 2007).

An aqueous surfactant formulation when injected in to a mature oil reservoir contacts the small blobs of oil trapped in the pores of the reservoir rock and dramatically reduces the interfacial tension (IFT) and increases the capillary number, thus mobilizing trapped oil. (Sen, 2008). This surfactant MEOR represents one of the most promising methods to recover a substantial proportion of residual oil. *Bacillus subtilis* can reduce surface tension from 72 mN/m to 25 Mn/m (Hossein et al, 2010). *Bacillus subtilis* has been used to produce biosurfactants at both mesophilic and thermophilic conditions (Makkar and Cameotra, 1998). A strain of *Bacillus subtilis* has been reported to be able to grow and produce biosurfactant at 45° C (Makkar and Cameotra, 1997). In this paper we have studied the production of biosurfactants from *Bacillus subtilis (MTCC 1427)* and have compared biosurfactant production at both mesophilic and thermophilic conditions. In order to apply the biosurfactant in oil recovery, the stability of the surfactant at various pH, temperature, salinity and metal ions need to be studied. Hence the pH of the purified surfactant solution was adjusted to various pH ranging from 1.0 to 11.0, incubated for 2 h and the surface tension was measured. The surface tension decreased up to pH 5.0 suggesting that the biosurfactant was not stable below pH 5.0 (acidic conditions) and then the surface tension remained constant till pH 11.0 (Fig. 5a). This clearly suggests that biosurfactant was stable between pH 5.0 to 11.0. Similarly, the effect of temperature stability was studied by incubating the biosurfactant at various temperatures between 40 to 100^{o}C for 2 h and measured for surface tension. It has been found that the surface tension of the biosurfactant remained constant between 40 – 100^{o}C suggesting that biosurfactant produced by *P. putida* was highly thermostable (Kanna et al, 2014).

MATERIALS & METHODS

Microorganism and Maintenance

Bacillus subtilis (MTCC 1427) was procured from Microbial type culture collection center and gene bank (MTCC) has been used in this study. The culture was maintained in nutrient agar plates with the following composition (g/L): Peptone, 5.0; beef extract, 1.0; yeast extract, 2.0; NaCl, 5.0; agar, 15.0; pH 7.0 ± 0.2, storage temperature −2° C - −8° C.

Media and Cultivation conditions

Nutrient broth with the following composition (g/L) was used for inoculum preparation. Beef extract, 1.0; yeast extract, 2.0; peptone, 5.0; NaCl, 5.0. *Bacillus subtilis (MTCC 1427)* grown in Nutrient broth for 8 – 10 hours at 30 ^{o} C was used as inoculum. For production of biosurfactant mineral salt medium with the following composition (g/L) was used. KNO_3, 0.3; Na_2HPO_4, 0.2; KH_2PO_4, 0.014; NaCl, 0.001; $MgSO_4$, 0.06; $CaCl_2$, 0.004; $FeSO_4$, 0.002. 0.1 (g/L) of trace element solution containing $ZnSO_4.7H_2O$, 2.32; $MnSO_4.4H_2O$, 1.78; H_3BO_3, 0.56; $CuSO_4.5H_2O$, 1.0; $Na_2MoO_4.2H_2O$, 0.39; $CoCl_2.6H_2O$, 0.42; EDTA, 0.5; $NiCl_2.6H_2O$, 0.004; KI, 0.66; Sucrose, 20; K_2SO_4, 3. Growth studies were carried out separately for 30 ^{o}C and 45 ^{o}C in a rotatory shaker at 180 rpm. Growth analysis and biosurfactant production were done at pHs ranging from 5.0 – 8.0.

Biomass calculation

20mL samples were collected at different time interval of fermentation, centrifuged at 12352 x g for 25 minutes. The pellet was dried at 50^{o} C for overnight and the cell dry weight was determined.

Surface tension activity

The cell free broth obtained by centrifugation of the cultures at 12352 x g for 25 minutes was used for the determination of Surface tension and Interfacial tension. Tensiometer (Data Physics Scientific Company) was used for surface and interfacial tension measurements. 10mL of the sample were taken for analysis. Wilhelmy plate method was used to determine the surface tension. A thin plate (perimeter about 40 mm) is lowered to the surface of a liquid and the downward force to the plate is measured. Surface tension is the force divided by the perimeter of the plate. The plate must be completely wetted before the

measurement to ensure that the contact angle between the plate and the liquid is zero. The position of the plate must be maintained constant such that the lower end of the plate is exactly on the same level than the surface of the liquid.

Isolation & Purification of Biosurfactant

The culture was centrifuged at 12352 x g to remove bacterial cells. The supernatant was subjected to acid precipitation at a pH of 2.0 with 6 N HCl at 4 °C. The precipitate was pelleted out by centrifugation at 12352 x g for 25 minutes, re dissolved in DDH_2O, pH was adjusted to 7.0, freeze dried and weighed. The dried surfactant was extracted with Dichloromethane. The extract was dried using rotary evaporator under vacuum. This biosurfactant was further utilized for analysis purposes.

Bio-surfactant characterization using FT-IR analysis

Biosurfactant was extracted from the supernatant fluid (2 ml) with dichloromethane (2 ml), dried with Na_2SO_4 and evaporated on a rotary evaporator. The IR spectra of purified biosurfactant were recorded on the Bruker IFS113vFTIR-spectrometer, in the 4000 to 400 cm-1 spectral region at a resolution 2 cm-1, using a 0.23 mm KBr liquid cell.

RESULTS & DISCUSSION

Growth and biosurfactant production

The profiles of cell dry weight, biosurfactant production and sucrose concentration have been presented in Figs. 1 – 3. *Bacillus subtilis* (MTCC 1427) were able to grow in mineral salt medium both at 30° C and 45° C. Fig. 1A shows that *B.subtilis* of pH 8 at 84^{th} hour gave maximum of 1.98 g/l. Followed by pH 7 at 96^{th} hour, the dry cell weight was 1.8 g/l. As the pH had been reduced the maximum yield was only about 1.4 – 1.5 g/l. Fig. 1B shows that *B.subtilis* of pH 7 at 96^{th} hour gave maximum of 1.8 g/l followed by pH6 at 108^{th} hour gave maximum of 1.8 g/l. pH 8 and pH 5 gave only 1.6 and 1.7 g/l after 108^{th} hour of fermentation.

Fig. 2A and 2B indicates the relationship between sucrose consumption as time proceeds at varying pH with temperature maintained at 30 ^{o}C and 45 ^{o}C respectively. The figure shows the sucrose concentration has been decreased with cell growth with time. Fig. 2 A shows that at pH 7 the maximum reduction of sugar concentration was achieved at 120 hours. Fig. 2B shows that at pH 5 the maximum reduction of sugar concentration was achieved at 120 hours as 3 g/l. pH 8 gave the least amount of sugar reduction of 8 g/l at 120 hours of growth.

For the bacteria *Bacillus subtilis,* the production of biosurfactant was proportional to the cell growth, representing biosurfactant as a growth associated product. The results obtained by optimizing with varying pH at both 30 $^{\circ}$C and 45 $^{\circ}$C is shown in figure 3A and 3B respectively. Among all, pH 8 gave maximum biosurfactant yield of 1.3 g/l at 84^{th} hour of growth at 30° C.

Effect of biosurfactant on surface tension and interfacial tension

Surface tension and interfacial tension are the most important factors in oil recovery process. Biosurfactant produced by *B.subtilis* is known to reduce the surface tension of oil significantly. From figure 4A it can be noted that the surface tension has been reduced from 66, 67 and 68 mN/m to 45, 48 and 41 mN/m at pH5, 6 and 7 respectively. However growth at pH 8 showed a maximum reduction of surface tension from 71 to 34 mN/m. Figure 4B shows that maximun reduction of surface tension from 70 to 41 mN/m was observed at pH 7 and 45° C.

Interfacial tension is one of the key parameter since capillary number increases with decreases with interfacial tension which lowers the residual oil saturation in the core and increases the residual oil recovery rate. Figure 5A and 5B gives the profiles changes in interfacial tension versus time at both 30° C and 45° C. The maximum reduction in IFT at 30° C was 8 mN/m at pH 8. Similarly at 45° C IFT reduction was 11 mN/m at pH 6.

Characterization of bio-surfactant

The biosurfactant produced by *B.subtilis* was purified as mentioned in the materials and methods. Molecular composition present in biosurfactant evaluated using FT-IR analysis. Fig. 6 shows the spectra obtained from freeze dried biosurfactant sample produced by strain *B.subtilis..* It can be noted that biosurfactant obtained using bacteria mostly consist of absorption bands. The band 1724 cm^{-1} corresponds to ester group. Similarly the presence of amide I band at 1639 $cm^{-1}$was due to C=O stretch. The presence of these bands clearly represents that they belong to lipopeptide family. The band at 1724 cm^{-1} was due to C=O stretch of ester carbonyl and band at 1639 cm^{-1} denote the presence of -C=O stretch of 2° amide band I.The steep bend at 1532 cm^{-1} represents -C=O stretch of 2° amide band II and 1463 cm^{-1}represents asymmetric C-H bend of CH_3. The C-H bend of $CH_3$was due to the band at 1379 cm^{-1}. FT-IR spectrum obtained was compared with previous reports (Borgund *et al*., 2009, Pereira *et al*., 2013) and the spectrum with band N-H stretching at 3300 cm^{-1} and band at 3000 cm^{-1} was due to C-H stretching. Band at 1724 cm^{-1}corresponds to C=O in ester bond. The presence of band at 1639 and 1532 cm^{-1} are due to presence of amide group. It is clearly evident from FTIR results that biosurfactant produced by *Bacillus subtilis* is lipopeptide belonging to surfactin group

CONCLUSION

The study has revealed that *Bacillus subtilis* (MTCC 1427) can produce biosurfactant at both 30 °C and 45 °C. The bacteria showed a considerable reduction of surface tension from 71 mN/m to 34 mN/m and interfacial tension form 41 to 8 mN/m at 30^{o} C which is higher than growth at 45^{o} C. The potential use of this bacterium is highly suitable for MEOR applications at mesophilic condition than that of the thermophilic condition. Purified biosurfactant produced from *Bacillus subtilis* was characterized as lipopeptide which mainly consist of lipid and peptide belongs to surfactin molecule.

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FIGURES

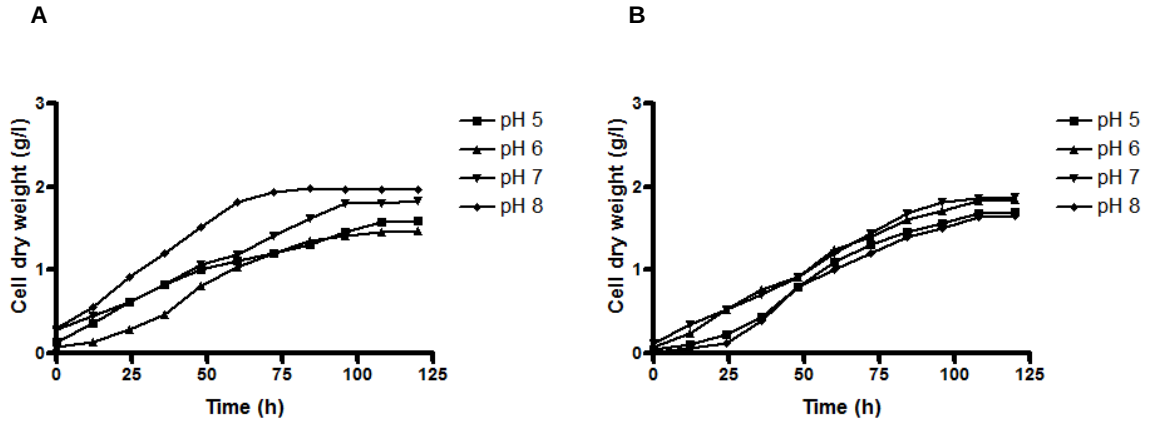

Figure 1: (A) Time vs Cell dry weight at 30° C with varying pH, (B) Time vs Cell dry weight at 45° C with varying pH

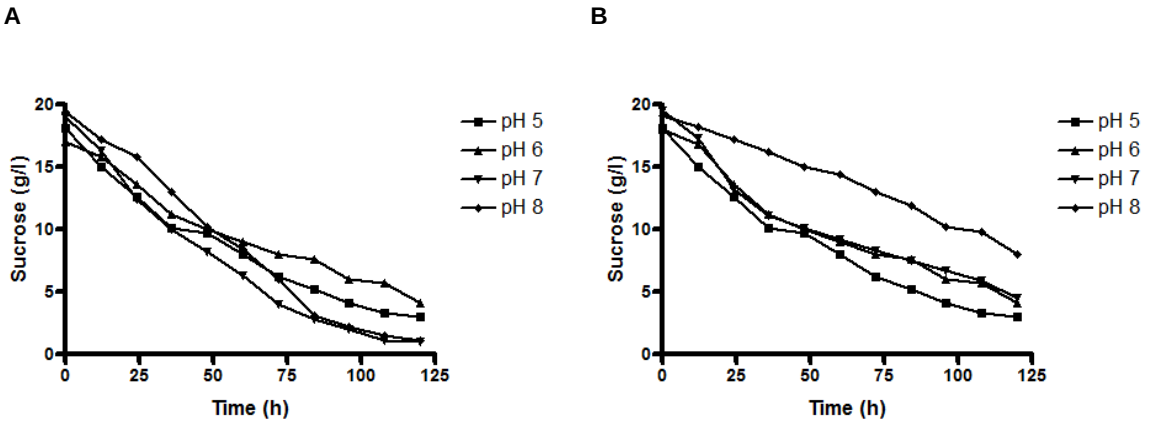

Figure 2: (A) Time vs reducing sugar for varying pH at 30° C, (B) Time vs reducing sugar for varying pH at 45° C

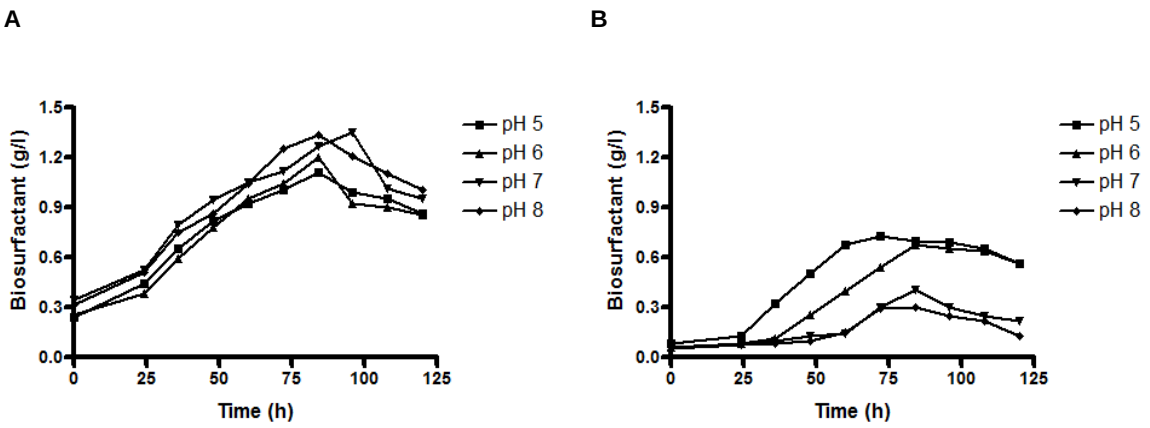

Figure 3: (A) Time vs Biosurfactant concentration for varying pH at 30° C, (B) Time vs Biosurfactant concentration for varying pH at 45° C

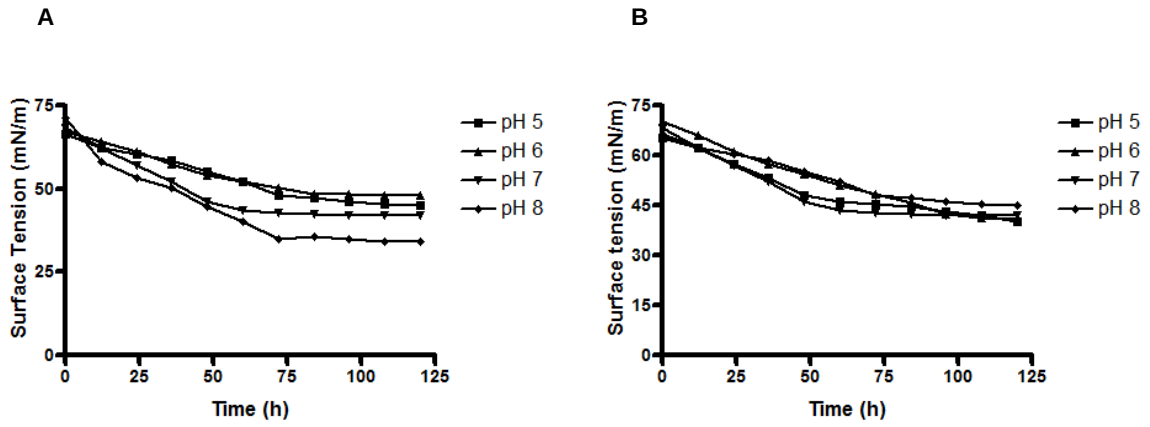

Figure 4: (A) Time vs Surface Tension for varying pH at 30° C, (B) Time vs Surface Tension for varying pH at 45° C

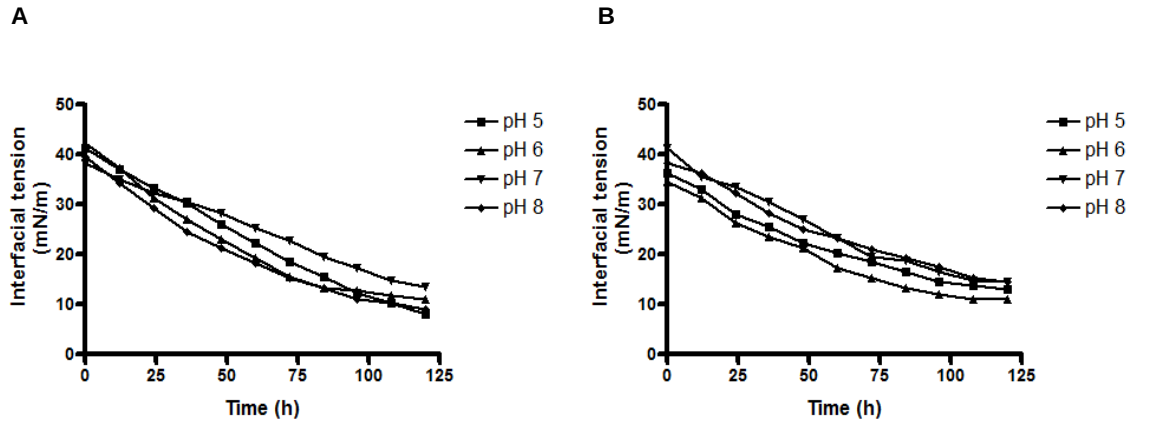

Figure 5: (A) Time vs Interfacial Tension for varying pH at 30° C, (B) Time vs Interfacial Tension for varying pH at 45°

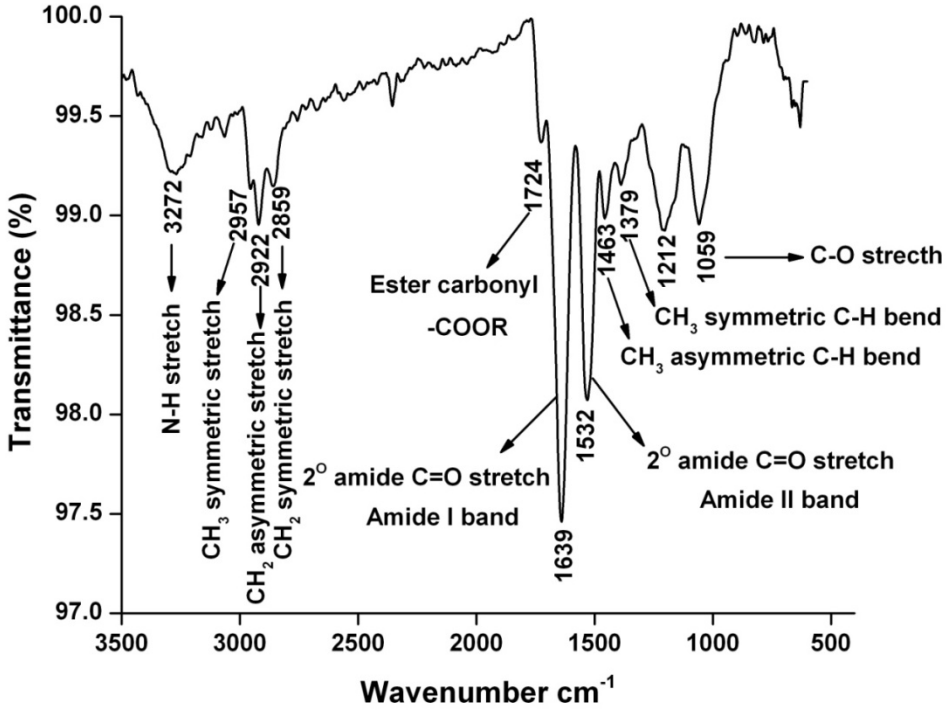

Figure 6: FT-IR spectra of bio-surfactant powder extract produced by *Bacillus subtilis* MTCC 1427