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Bio-Surfactant Production by *Pseudomonasaeruginosa* ATCC 9027 and It's Application in Microbial Enhanced Oil Recovery

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Abstract:- Microbial enhanced oil recovery (MEOR) is a technique which enables the improvement of oil recovery by injection of microorganisms into depleted oil reservoirs. Bio-surfactants generated by these microorganisms play a significant role in enhancing oil recovery from the depleted reservoir after the primary and secondary recovery process. This work focuses on the production of Bio-surfactants from *Pseudomonas aeruginosa*(ATCC 9027)and the comparison of bio-surfactant production by experimental studies at both mesophilic and thermophilic conditions. The growth and bio-surfactant production was investigated at varying pH and temperature conditions resembling the actual reservoir conditions. A sensitivity analysis of the previously mentioned physical/biological parameters was carried out in order to deduce the optimal conditions required for enhanced growth and bio-surfactant production. Bio-surfactant and analytical studies such as surface tension and interfacial tension have revealed that *Pseudomonas aeruginosa*(ATCC 9027) has the capability to grow and produce maximum Bio-surfactants 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 bio-surfactant. Also the strain failed to show growth below pH of 5.0 and above pH of 8.0.

I. INTRODUCTION

Increased interest of bio-surfactant in the recent years has stimulated attempts to enlarge the present range of microbial surfactants. Most of these Bio-surfactants are bio-degradable and less toxic than their chemically synthesized counterparts [8]. 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 [3]. 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 [2]. 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 [1]. *Bacillus subtilis*had been used to produce bio-surfactants at both mesophilic and thermophilic conditions [6]. A strain of *Bacillus subtilis* has been reported to be able to grow and produce bio-surfactant at 45° C [7]. In our previous study we had producedbio-surfactant from *Bacillus subtilis* (MTCC 1427)and have compared bio-surfactant production at both mesophilic and thermophilic conditions.It is also found that the sucrose and bio-surfactant concentrations are highly sensitive to pH rather than reservoir microbial concentration, while at larger resident time and water saturation, the microbial and nutrient concentrations were lesser due to enhanced dispersion [11].

II. MATERIALS & METHODS

2.1 Microorganism and Maintenance

Pseudomonas aeruginosa(ATCC 9027) was procured from American Type Culture Collection Centre 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.

2.2 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. *Pseudomonas aeruginosa*(ATCC 9027) was grown in Nutrient broth for 8 – 10 hours at 30^{o} C (OD600nm 0.8 – 0.9). This was used as inoculum at 2% (v/v) level. For production of bio-surfactant 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 ^oC and 45 ^oC in a rotatory shaker at 180 rpm. Growth analysis and bio-surfactant production were done at pH ranging from 5.0 – 8.0.

2.3 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.

2.4 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.

2.5 Isolation & Purification of Bio-surfactant

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 bio-surfactant was further utilized for analysis purposes.

2.6 Effect of bio-surfactant on oil recovery using sand packed column

A simple sand packed column was designed to determine the efficiency of produced bio-surfactant in oil recovery rate. The column was made of plastic material with 10 cm in length. The diameter was 2.5 cm. The column was packed with fine sand material. Sand was saturated with brine initially followed by oil saturation to replicate petroleum reservoir condition. The sand packed column was flooded again with brine until no more oil received at the effluent. 0.5 PV (Pore Volume) of *Pseudomonas aeruginosa* ATCC 9027 (OD = 0.23) in mineral salt medium was injected into the column. The column was flooded with water (Secondary flooding) followed Tween separately (Chemical flooding). Similarly produced bio-surfactant (30 ^oC and 45 ^oC) was injected into the sand column to check how efficiently oil recovery can be improved. The effluent collected from the outlet of the column gives the amount of oil recovered.

III. RESULTS & DISCUSSION

3.1 Growth and bio-surfactant production

The profiles of cell dry weight and bio-surfactant production have been presented in Figs. 1 & 2. *Bacillus subtilis* (MTCC 1427) was able to grow in mineral salt medium both at 30° C and 45° C. Fig. 1A shows that *B.subtilis*of pH 8 at 84th hour gave maximum of 1.98 g/l, followed by pH 7 at 96th 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 96th hour gave maximum of 1.8 g/l followed by pH 6.0 at 108th hour gave maximum of 1.8 g/l. pH 8 and pH 5 gave only 1.6 and 1.7 g/l after 108th hour of fermentation.

Fig. 2A and 2B indicates the relationship between sucrose consumption as time proceeds at varying pH with temperature maintained at 30 ^oC and 45 ^oC 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 *Bacillus subtilis*, the production of bio-surfactant was proportional to the cell growth, representing bio-surfactant 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 bio-surfactant yield of 1.3 g/l at 84th hour of growth at 30° C.

3.2 Effect of bio-surfactant on surface tension and interfacial tension

Surface tension and interfacial tension are the most important factors in oil recovery process. Bio-surfactant 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 pH 5, 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 maximum reduction of surface tension from 70 to 41 mN/m was observed at pH 7 and 45° C. Our previous work on bio-surfactant produced by *Pseudomonas putida* MTCC 2467 exhibited higher surface activity of 72 – 34 mN/m which is in par with current result [9]

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

3.3 Effect of bio-surfactant on oil recovery using sand packed column

Oil recovered using bio-surfactant shown in Figure 6A and 6B at 30° C and 45° C respectively. With water injection, oil recovered was 32%. With tween injection the oil recovery was additional 3% and 4% Similarly, with bio-surfactant produced by maintaining temperature 30° C and 45° C the oil recovery was increased to 7% and 8.5% respectively. MEOR flooding was carried out by injecting pure bio-surfactantproduced by the metabolites produced by the strain *Pseudomonas putida*(MTCC 2467). The result was compared to our previous work where using water flooding % of oil was recovered. Tween injection gave additional oil recovery of 2.6% [10].

III. CONCLUSIONS

The study has revealed that *Pseudomonas aeruginosa*ATCC 9027 can produce bio-surfactant 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 where additional oil recovery of 7 – 8.5% after water injection.

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FIGURES

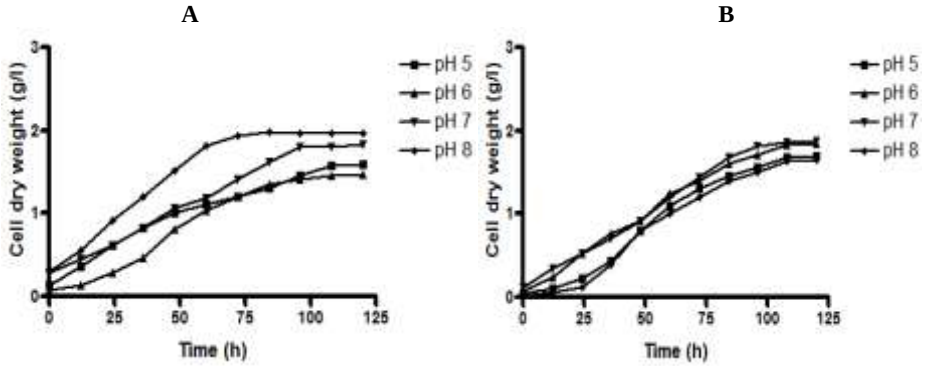

Figure 1: (A) Time vs Cell dry weight at 30° C with varying pH, (B) Time vs Cell dry weight at 45° C with varying pH

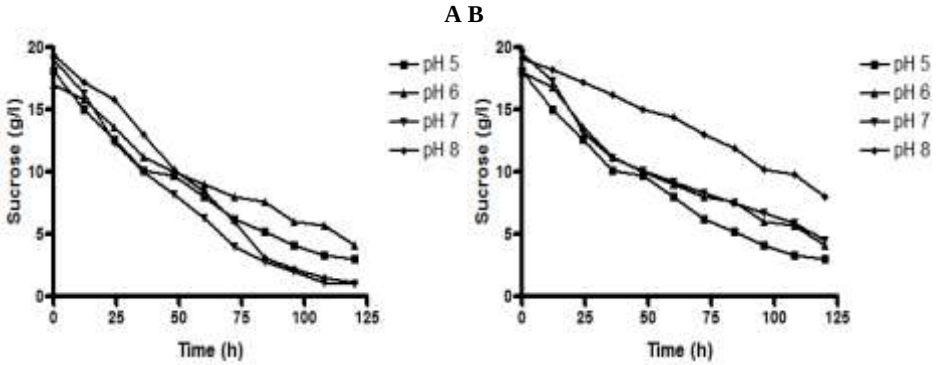

Figure 2: (A) Time vs reducing sugar for varying pH at 30° C, (B) Time vs reducing sugar for varying pH at 45° C

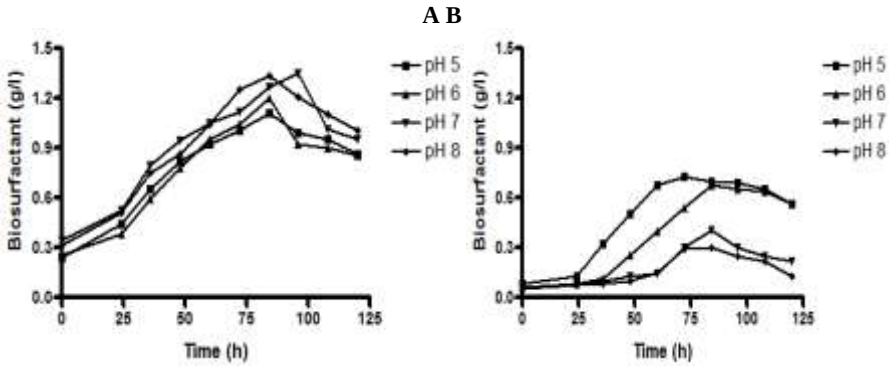

Figure 3: (A) Time vsBio-surfactant concentration for varying pH at 30° C, (B) Time vsBio-surfactant concentration for varying pH at 45° C

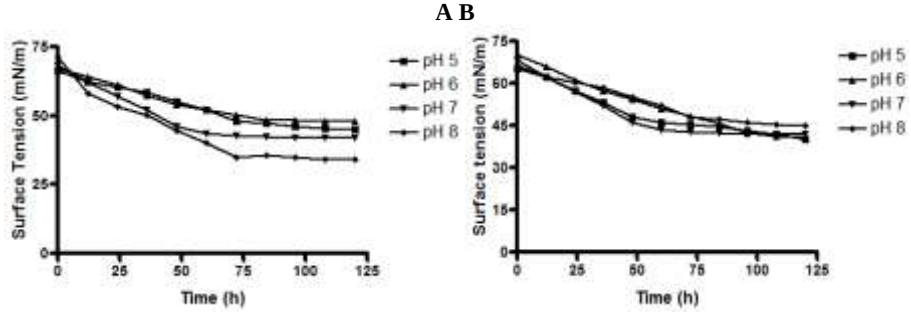

Figure 4: (A) Time vs Surface Tension for varying pH at 30° C, (B) Time vs Surface Tension for varying pH at 45°

A B

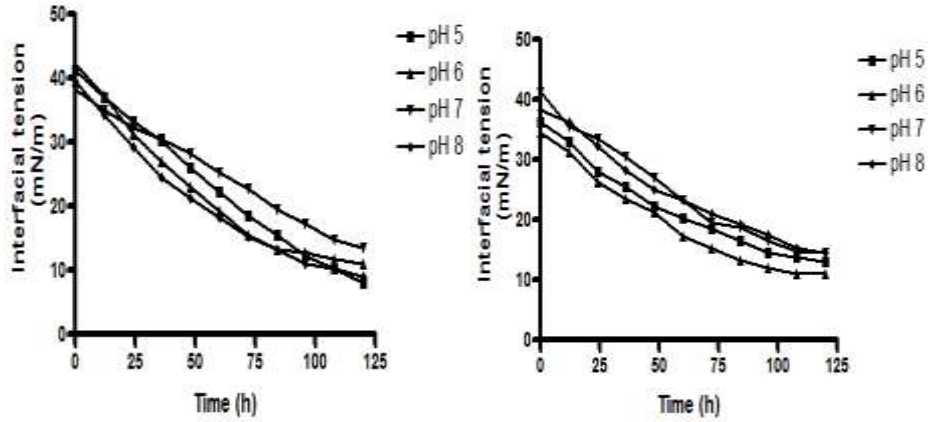

Figure 5: (A) Time vs Interfacial Tension for varying pH at 30° C, (B) Time vs Interfacial Tension for varying pH at 45° C

A B

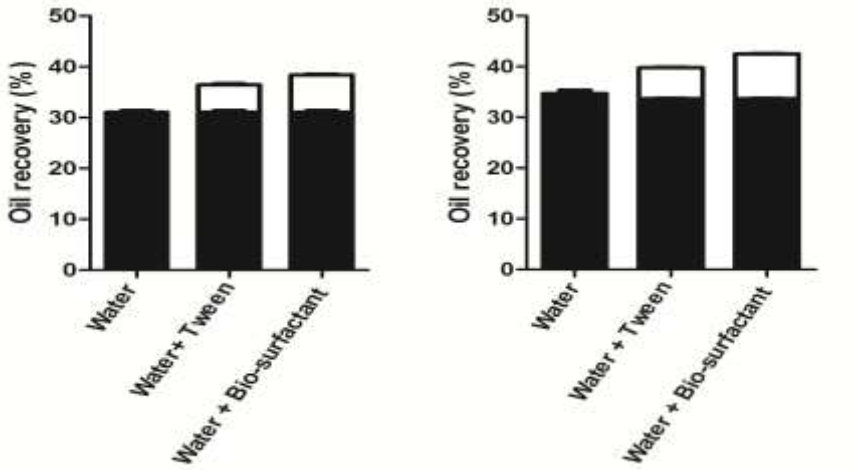

Figure 6: (A) % oil recovery using sand packed column at 30° C, (B) % oil recovery using sand packed column at 45° C