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Production and Characterization of Biosurfactant by *Pseudomonas putida* MTCC 2467

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Abstract: Microbial compounds that exhibit pronounced surface and emulsifying activities are classified as biosurfactants. The effect of carbon and nitrogen sources and other physiological parameters affecting the biosurfactant production by *Pseudomonas putida* MTCC 2467 was studied. Different carbon and nitrogen sources were tested for biosurfactant production using minimal medium and it has been found that sucrose and ammonium sulphate were found to be the best carbon and nitrogen source. The effect of initial pH was studied by varying pH from 5.0-8.0 and maximum biosurfactant production, 2.8 g L⁻¹ was achieved at an optimum pH of 8.0. The biosurfactant production was also monitored by measuring the liquid surface tension and interfacial tension and the results showed that the medium surface tension reduced from 74-35 mN m⁻¹. The stability of biosurfactant was determined by varying pH, temperature, salinity and metal ions found that biosurfactant was stable at alkaline pH and higher temperature range suggesting that biosurfactant was highly stable, purified and checked for protein and lipid content and characterized by FTIR spectroscopy.

Key words: *Pseudomonas putida*, biosurfactant, interfacial tension, dissolved oxygen, stability, salinity, FTIR spectroscopy

INTRODUCTION

Biosurfactants are surface active compounds produced by variety of bacteria (Mukherjee *et al.*, 2008). Properties of biosurfactant include surface tension reduction, promoting foaming agents, stable and environment friendly (Singh, 2012). Microbial surfactants are considered to be interesting because of their biodegradable nature and effectiveness at extreme conditions of pH and temperature (Makkar and Cameotra, 1997). Biosurfactants can be divided into two groups; low-molecular-mass, lower surface and interfacial tension and high molecular-mass polymers mainly used as emulsion stabilizing agents. The class of low-mass surfactants includes phospholipids, glycolipids and lipopeptides and high-mass includes polymeric and particulate surfactants (Nitschke and Costa, 2007). Biosurfactants are either anionic or nonionic and the hydrophobic moiety is based on long chain fatty acids or their additives and in hydrophilic, it can be carbohydrate, amino acid, phosphate or cyclic peptide (Thenmozhi *et al.*, 2011).

Generally bacteria produce low molecular weight molecules that efficiently reduce surface and interfacial

tension such as glycolipids and lipopeptides. Rhamnolipid, trehalose lipids and surfactin are the most effective biosurfactants with the ability to reduce the water surface tension and interfacial tension of water/oil system significantly even at lower concentrations (Fracchia *et al.*, 2012). Majority of known biosurfactants are synthesized by microorganisms grown on water immiscible hydrocarbons whereas other biosurfactants are produced on water-soluble substrates such as glucose, glycerol and ethanol (Tabatabaee *et al.*, 2005).

Additionally, high product titers have been exclusively described with the vegetable oil as sole carbon source in combination with *Pseudomonas aeruginosa* strains (Muller *et al.*, 2012). In the present scenario, interest in biosurfactants has increased globally to expand the present range of microbial surfactants. These biosurfactants have potential use in oil industries such as cleaning oil sludge, mobilizing heavy crude oil and managing oil spillage. In addition to this, biosurfactants are being used in food industries as additives and emulsifiers which are applied in agriculture and cosmetics (Kosaric, 1992). Application of biosurfactants in microbial enhanced oil recovery depends on their stability at higher temperature and pH

conditions (Khopade *et al.*, 2012). *Bacillus subtilis* and *Pseudomonas aeruginosa* are well known bacteria which produce biosurfactant namely surfactin and rhamnolipid which are applied in microbial enhanced oil recovery (Fracchia *et al.*, 2012). Increasing demand for petroleum over recent years and to meet the gap, application of biosurfactant in oil recovery plays a major role in petroleum industries. However, stability of biosurfactant at extreme pH and temperature conditions is necessary for enhanced oil recovery. *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus thuringiensis* and *Staphylococcus aureus* are generally used for production of biosurfactants. *Bacillus subtilis* strains produced a broad spectrum of bioactive compounds with great potential for biotechnological and biopharmaceutical applications including surfactin, fengycin, iturin and bacillomycins which are amphiphilic membrane-active biosurfactants with potent antimicrobial activities (Sriram *et al.*, 2011).

The major problem faced by oil industries is to recover oil to the maximum possible extent using economical methods. In this regard, microbial enhanced oil recovery with the aid of biosurfactants is promising. Hence, extensive identification and characterization of new suitable strain for biosurfactant production and degradation during oil spills is necessary. In our study, biosurfactant is produced by *Pseudomonas putida* MTCC 2467 and potentially applied to reduce the surface and interfacial tension which influence the enhancement of oil. The strain producing biosurfactant can be suitably used in oil fields, biomedical and environmental applications. This is the first report describing biosurfactant production using strain *Pseudomonas putida* MTCC 2467.

MATERIALS AND METHODS

Microorganism and maintenance: *Pseudomonas putida* MTCC 2467 was procured from Microbial Type Culture Collection (MTCC) Institute of Microbial Technology, Chandigarh, India for this study. The culture was maintained in nutrient agar plates with the following composition (g L⁻¹): beef extract, 1.0; peptone, 5.0; yeast extract, 2.0; NaCl, 5.0; agar, 15.0; pH 7.0±0.2 and storage temperature, -2 to -8°C.

Media and cultivation conditions: Nutrient broth with the following composition (g L⁻¹) was used for inoculum preparation. Beef extract, 1.0; yeast extract, 2.0; peptone, 5.0 and NaCl, 5.0. *Pseudomonas putida* (MTCC 2467) was grown in nutrient broth for 8-10 h at 30°C (A_{600nm} 0.7-0.9) and 2% (v/v) of the inoculum was

used for production of biosurfactant using mineral salt medium with the following composition (g L⁻¹) KNO₃, 0.3; Na₂HPO₄, 0.2; KH₂PO₄, 0.014; NaCl, 0.001; MgSO₄, 0.06; CaCl₂, 0.004; FeSO₄, 0.002; 0.1 mL of trace element solution containing (g L⁻¹) ZnSO₄·7H₂O, 2.32; H₃BO₃, 0.56; CuSO₄·5H₂O, 1.0; MnSO₄·4H₂O, 1.78; Na₂MoO₄·2H₂O, 0.39; CoCl₂·6H₂O, 0.42; EDTA, 0.5; NiCl₂·6H₂O, 0.004; KI, 0.66; K₂SO₄, 3.0.

Effect of different fermentation parameters on biosurfactant production: To identify the best carbon source namely sucrose, glucose and starch were tested to evaluate best carbon source for biosurfactant production using the strain *Pseudomonas putida*. The 100 mL of production medium with 2% (w/v) of above mentioned carbon sources were grown separately at 40°C and 200 rpm for 5 days. Samples were collected for every 12 h and analyzed for biosurfactant production, growth and other parameters. To identify the best nitrogen source different nitrogen sources such as ammonium sulphate, ammonium nitrate and urea with 0.3 % (w/v) were added to the medium containing 2% (w/v) sucrose as carbon source. Fermentation was carried out for 5 days at 40°C and 200 rpm for 5 days. Samples were collected for every 12 h and analyzed for biosurfactant production, growth and other parameters. To investigate the effect of initial pH on biosurfactant production, the initial pH of the production medium was adjusted to 5.0, 6.0, 7.0 and 8.0 using 3 M HCl and 3 M NaOH and biosurfactant production experiments were carried out as mentioned earlier. Similarly, the effect of dissolved oxygen on biosurfactant production was studied by varying medium volume such as 25, 50, 75, 100, 125 and 150 in 250 mL flasks separately and fermentation was carried out as mentioned earlier.

Analysis of sucrose concentration: One millilitre of sample was centrifuged at 12325×g for 25 min and the supernatant was collected to determine sucrose concentration using dinitrosalicylic (DNS) method. One millilitre sample was mixed with 25 µL of 3 M HCl and heated at 100°C for 20 min. The 1 mL of DNS reagent was added to the hydrolyzed samples and heated for 100°C for 10 min. Sucrose concentration was determined by measuring absorbance at 540 nm (Miller, 1959).

Cell biomass determination: The 2 mL of sample was subjected to centrifugation for 25 min at 12325×g and the supernatant was decanted. The precipitate was washed twice with 0.8% NaCl and the pellet was dried in hot air oven at 50°C overnight. The pellet was cooled in desiccator and the dry weight of pellet was measured. The procedure was repeated till concurrent values were obtained.

Biosurfactant determination: The culture was centrifuged at $12325\times g$ to remove bacterial cells. Supernatant was subjected to acid precipitation at pH 2.0 by adding 6 N HCl at 4°C. The precipitate was pelleted out by centrifugation at $12325\times g$ for 25 min, re-suspended in double distilled water and pH was adjusted to 7.0, freeze dried and weighed. The dried surfactant was extracted with dichloromethane and the extract was dried using rotary evaporator under vacuum. The concentrated liquid obtained was the pure form of biosurfactant.

Surface and interfacial tension measurement: Surface tension measurement of the cell free broth was determined by K6 Tensiometer (Kruss GmbH, Hamburg, Germany), using plate method. Ten millilitre of sample was placed in the glass container. Measurements were carried out by automatic controller which smoothly pulls down the plate such that it gets into contact with the liquid placed. The force acting on the rectangular plate with known length were measured and converted into surface tension digitally. For interfacial tension measurement equal amount of oil is added to the sample and similar procedure is followed.

Effect of pH and temperature on stability of biosurfactant: The effect of pH on stability of biosurfactant, pH of the biosurfactant solution was adjusted to various pH ranging from 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 by adding 3 N NaOH and 3 N HCl. Surface tension was determined to check the stability of biosurfactant.

To investigate the effect of temperature on stability of biosurfactant, the samples were heated at 50, 60, 70, 80, 90 and 100°C for 2 h and analyzed for surface tension measurements before and after the heat treatment.

Effect of salinity and metal ions on stability of biosurfactant: To investigate the effect of salinity on stability of biosurfactant various amounts of NaCl 10, 20, 30, 40 and 50 g L⁻¹ were added to biosurfactant samples at CMC and retained for 48 h. Surface tension was measured using tensiometer.

To investigate the effect of metal ions on stability of biosurfactant, various metal ions K⁺, Ca²⁺, Mg²⁺, Fe²⁺ and Al³⁺ of 20 g L⁻¹ concentration were added to the biosurfactant samples at CMC. After 48 h, the samples were analyzed for surface tension.

Characterization of biosurfactant: Protein content in the extracted biosurfactant was estimated by using Lowry method (Lowry *et al.*, 1951). The samples were extracted with dichloromethane to determine the lipid content at room temperature. The extracted sample was lyophilized

and weighed to determine the amount of lipid present. Biosurfactant was extracted from the supernatant fluid (2 mL) with dichloromethane (2 mL), dried with Na₂SO₄ and evaporated in a rotary evaporator. The IR spectra of purified biosurfactant were recorded on the Bruker IFS113vFTIR-spectrometer in the 4000-400 cm⁻¹ spectral region at a resolution 2 cm⁻¹ using a 0.23 mm KBr liquid cell.

Statistical analysis: All the experiments were performed at least three times and the values reported are mean of three individual experiments with p<0.005.

RESULTS

Effect of different carbon sources on biosurfactant production: The effect of different carbon sources such as sucrose, glucose and starch on biosurfactant production by *Pseudomonas putida* MTCC 2467 was studied. Among the tested carbon sources, sucrose produced maximum of 2.3 and 1.3 g L⁻¹ of biomass and biosurfactant, respectively (Fig. 1a-b). In order to further confirm that biosurfactant was produced during fermentation, surface tension and interfacial tension of medium was measured at regular intervals of time. The surface tension and interfacial tension measurement showed that both these parameters decreased with time with increased biosurfactant concentration (Fig. 1c-d). The results also clearly showed that the decrease in surface and interfacial tension was maximum when *P. putida* was grown in medium containing sucrose (Fig. 1). This further confirmed that maximum biosurfactant production can be obtained using sucrose as the carbon source. Hence, sucrose of 2% (w/v) was used as carbon source for further studies.

Effect of different nitrogen sources on biosurfactant production: The effect of different nitrogen sources such as ammonium sulphate, ammonium nitrate and urea on biosurfactant production by *P. putida* MTCC 2467 was studied. The medium was supplemented with sucrose as carbon source and the concentration of nitrogen source was 0.3% (w/v). Ammonium sulphate produced 2.6 and 2.5 g L⁻¹ of biomass and biosurfactant, respectively (Fig. 2a-b) which was best among all nitrogen source tested. To ensure that biosurfactant was produced, the fermented sample at regular time interval was subjected to surface and interfacial tension measurements. Results show increased concentration of biosurfactant reduced the surface and interfacial tension of medium (Fig. 2c-d). Surface and interfacial tension reduction was maximum when *P. putida* was grown in medium containing

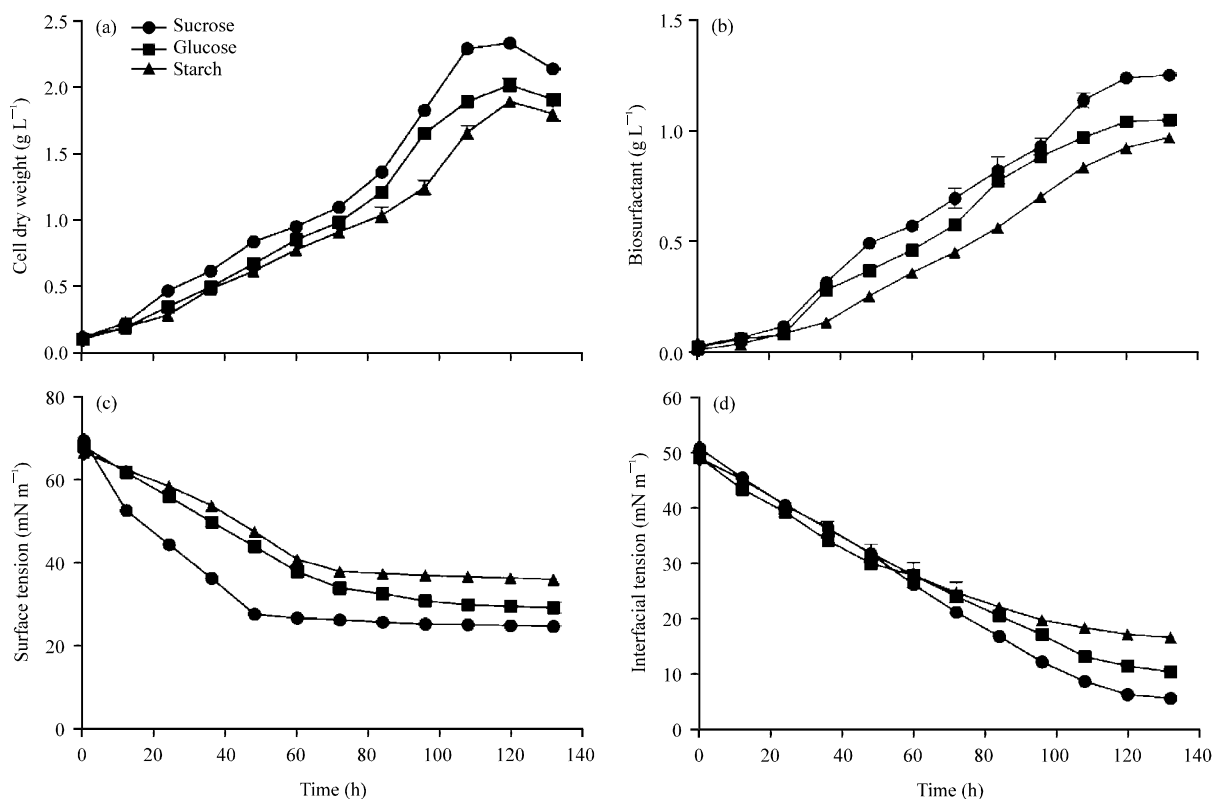


Fig. 1(a-d): Effect of different carbon sources on biosurfactant production by *Pseudomonas putida* MTCC 2467 (a) Cell dry weight profile, (b) Biosurfactant profile, (c) Surface tension and (d) Interfacial tension profiles. Each experiment was performed 3 independent times and error bars represent \pm SE ($p < 0.005$)

ammonium sulphate as nitrogen source which showed highest level of biosurfactant production. Hence, ammonium sulphate of 0.3% (w/v) was used as nitrogen source for further studies.

Effect of initial pH on biosurfactant production: To study the effect of initial pH on biosurfactant production by *P. putida* MTCC 2467, the initial pH of the medium was adjusted to 5.0, 6.0, 7.0 and 8.0. The medium was supplemented with sucrose and ammonium sulphate as carbon and nitrogen source, respectively. A maximum of 2.8 g L⁻¹ of biomass and 2.5 g L⁻¹ of biosurfactant was produced at pH 8.0 (Fig. 3a-b) which was best among all other pH variations tested. To ensure the biosurfactant production, samples at regular interval of time were subjected to surface and interfacial tension measurements. The results showed that decrease in surface and interfacial tension was maximum when *P. putida* was grown in medium containing pH 8.0 (Fig. 3c-d). This confirmed that maximum biosurfactant production can be obtained at pH 8.0 and therefore pH 8.0 was used for further studies.

Effect of dissolved oxygen on biosurfactant production:

The effect of DO on biosurfactant production by *P. putida* MTCC 2467 was studied by varying the medium volume (25, 50, 75, 100 and 125 mL) in 250 mL flasks. The medium was supplemented with sucrose, ammonium sulphate as carbon and nitrogen source and initial pH was adjusted to 8.0. Flasks containing 50 ml of culture medium produced a maximum of 3.2 and 2.8 g L⁻¹ of biomass and biosurfactant, respectively (Fig. 4a-b) which was best among all other medium volume variations tested. The surface and interfacial measurement results also clearly showed that decrease in surface and interfacial tension was maximum when *P. putida* was grown with 50 mL medium in 250 mL flask (Fig. 4c). The further increase in culture volume affects both the growth and biosurfactant production.

Stability of biosurfactant: 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 1.0-11.0,

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 surface tension remained constant till pH 11.0 (Fig. 5a). This clearly suggests that biosurfactant was stable between pH 5.0-11.0. Similarly, the effect of

temperature stability was studied by incubating the biosurfactant at various temperatures between 40-100°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°C suggesting that biosurfactant produced by *P. putida* was highly

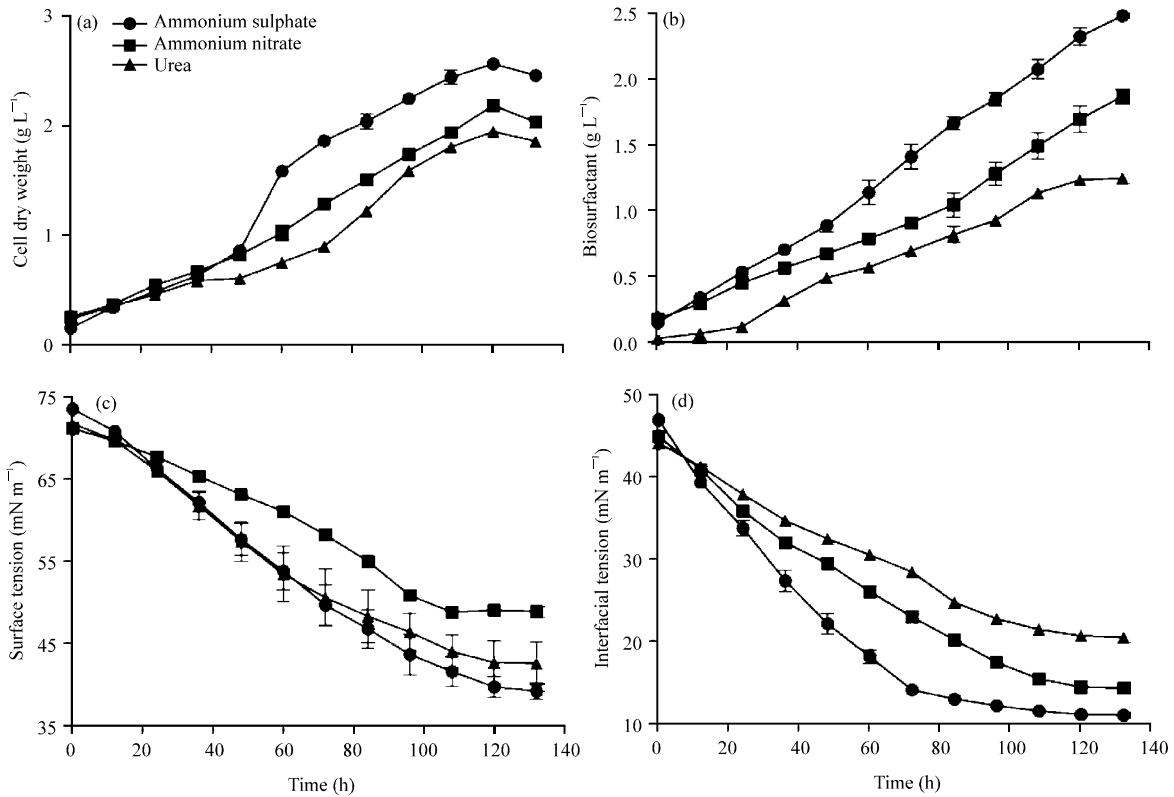


Fig. 2(a-d): Effect of different nitrogen source on biosurfactant production by *Pseudomonas putida* MTCC 2467 (a) Cell dry weight profile, (b) Biosurfactant profile, (c) Surface tension and (d) Interfacial tension profiles. Each experiment was performed 3 independent times and error bars represent \pm SE ($p < 0.005$)

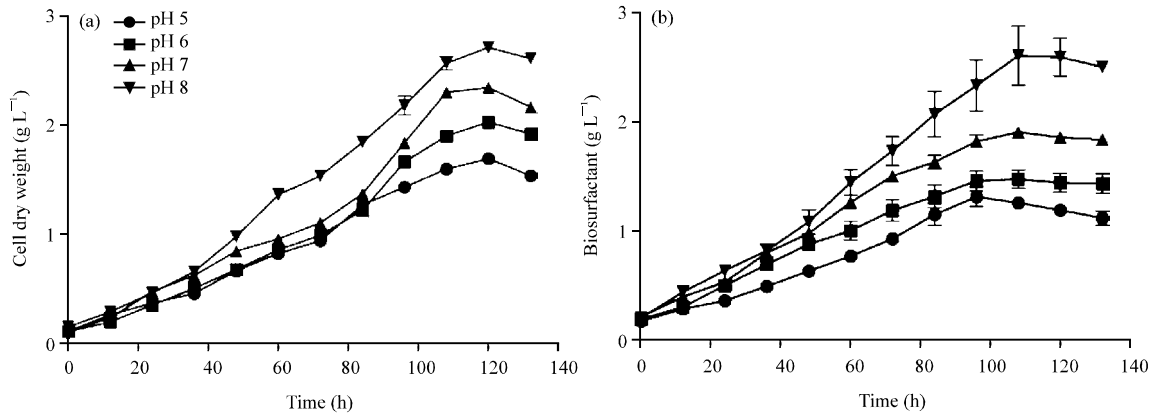


Fig. 3(a-d): Continue

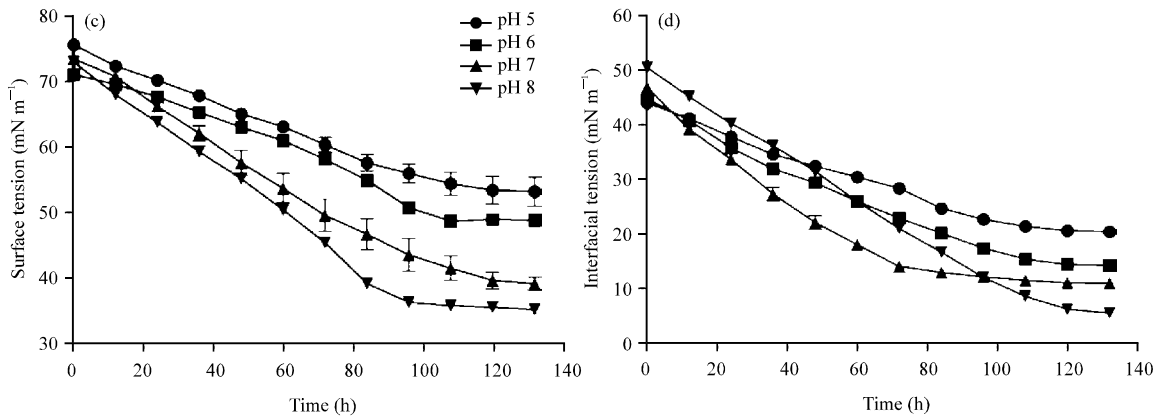


Fig. 3(a-d): Effect of initial pH on biosurfactant production by *Pseudomonas putida* MTCC 2467 (a) Cell dry weight profile, (b) Biosurfactant profile, (c) Surface tension and (d) Interfacial tension profiles. Each experiment was performed 3 independent times and error bars represent \pm SE ($p < 0.005$)

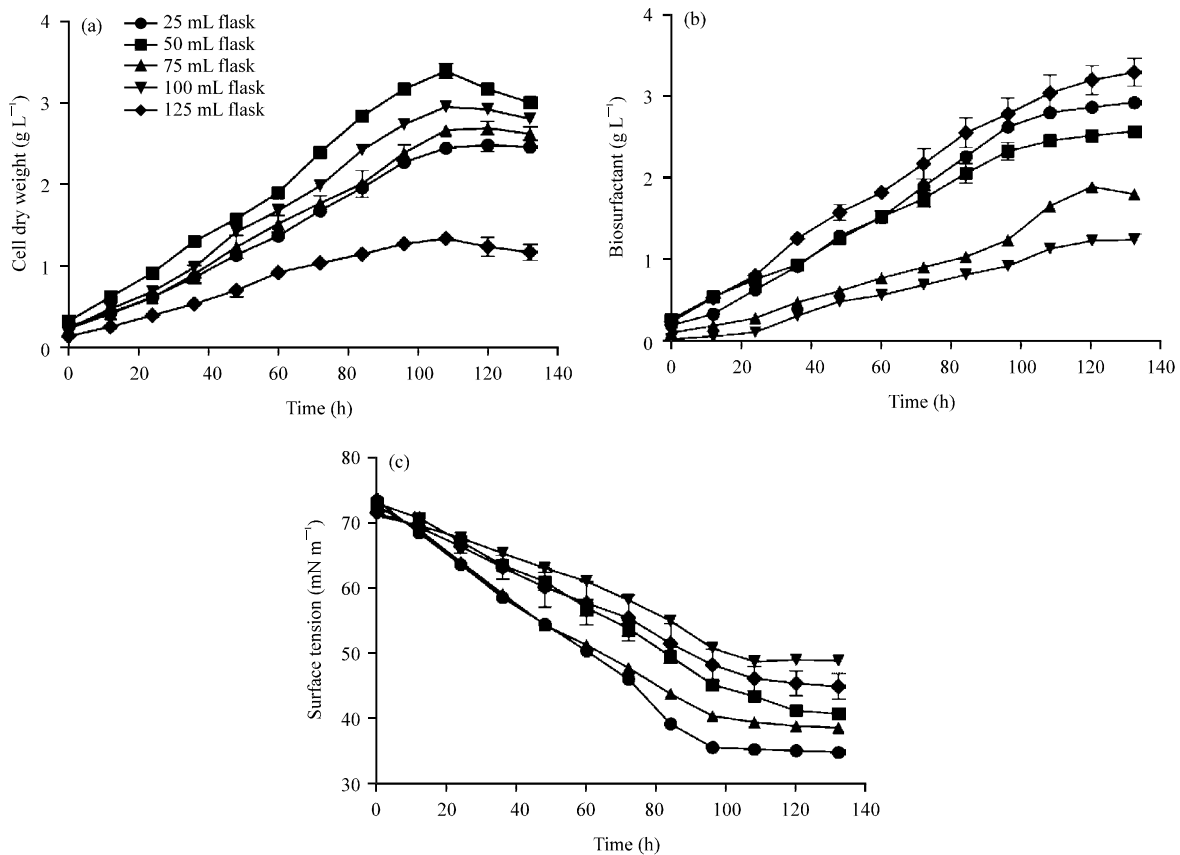


Fig. 4(a-c): Effect of dissolved oxygen biosurfactant production by *Pseudomonas putida* MTCC 2467 (a) Cell dry weight profile, (b) Biosurfactant profile, (c) Surface tension and (d) Interfacial tension profiles. Each experiment was performed 3 independent times and error bars represent \pm SE ($p < 0.005$)

thermostable (Fig. 5b). Effect of NaCl on biosurfactant samples produced by *Pseudomonas putida* was studied.

Pseudomonas putida exhibited good resistance to NaCl concentration upto 10 g L⁻¹ (Fig. 5c). Biosurfactant

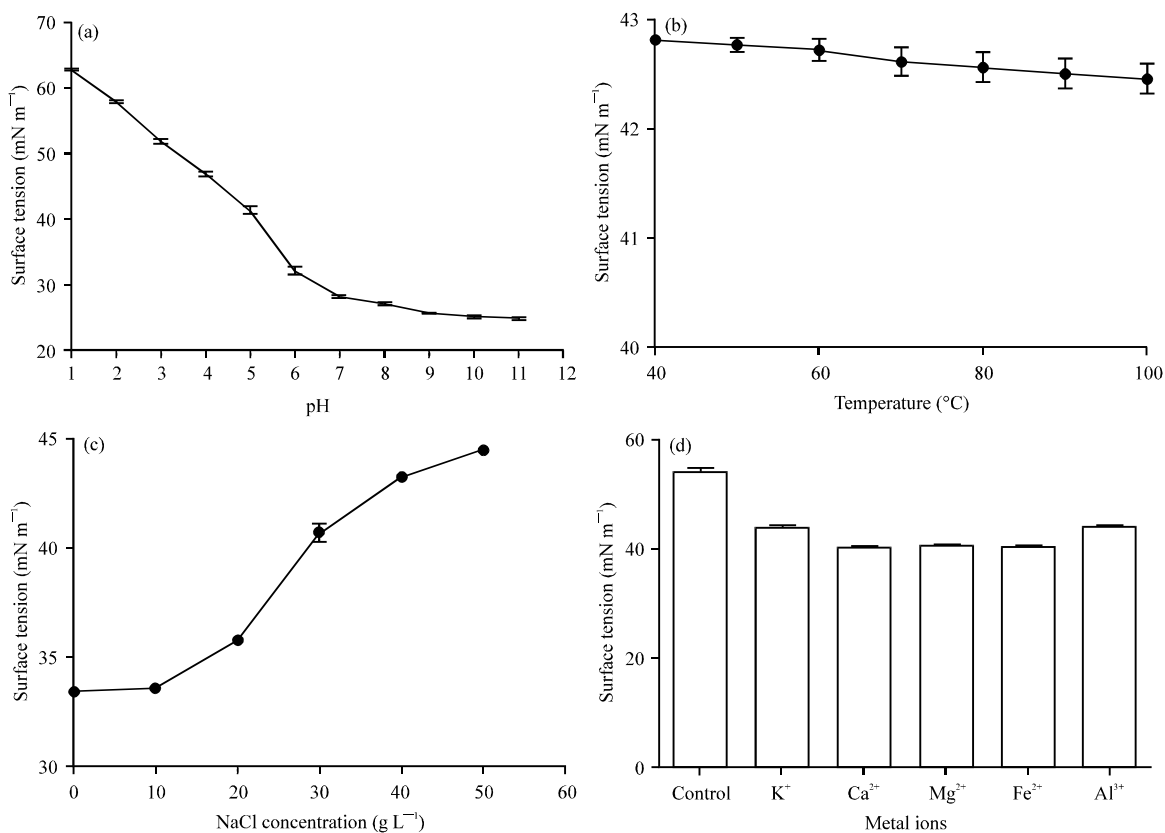


Fig. 5(a-d): Stability of biosurfactant, (a) pH stability; the pH of biosurfactant solution was adjusted to various pHs ranging from 1.0-11.0 and incubated for 2 h and analyzed for surface tension measurements, (b) Temperature stability: Biosurfactant solution was incubated at various temperatures between 40-100°C for 2 h and analyzed for surface tension measurements. Each experiment was performed 3 independent times and error bars represent \pm SE ($p < 0.005$), (c) Effect of salinity: Biosurfactant solution was added to various NaCl concentration between 10-50 g L⁻¹ and analyzed for surface tension after 48 h. Each experiment was performed 3 independent times and error bars represent \pm SE ($p < 0.005$) and (d) Effect of metal ions: biosurfactant solution was added to various metal ions at 20 g L⁻¹ concentration and analyzed for surface tension after 48 h. Each experiment was performed 3 independent times and error bars represent \pm SE ($p < 0.005$)

stability effect on different metal ions were performed by incubating the biosurfactant with various metal ions and measured for surface tension.

Characterization of biosurfactant: The biosurfactant produced by *P. putida* was purified as mentioned in the materials and methods and checked for protein and lipid content. The result showed that the protein and lipid level found in biosurfactant was 6.88 and 66.4%, respectively. Molecular composition present in biosurfactant was evaluated using FTIR analysis. Figure 6 shows the spectra obtained from freeze dried biosurfactant sample produced by strain *P. putida*. 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.

DISCUSSION

Biosurfactant are surface active compounds produced by variety of microorganisms belonging to the genus *Bacillus*, *Pseudomonas* and *Acinetobacter*. Biosurfactant producing microorganisms are generally known to grow on both water immiscible hydrocarbons and carbohydrate containing mineral salt medium. Various

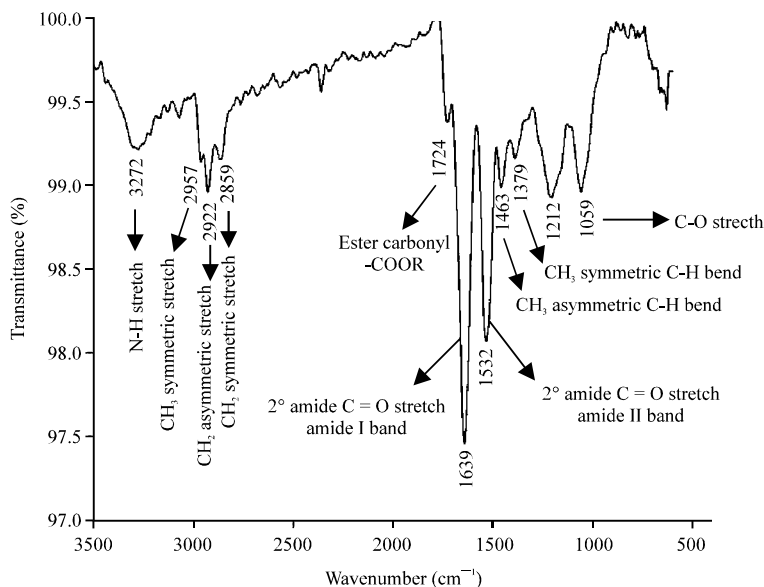


Fig. 6: FT-IR spectra of biosurfactant powder extract produced by *Pseudomonas putida* MTCC 2467

carbon sources such as glucose, starch, sucrose and hydrocarbons such as hexadecane and dodecane are routinely used for biosurfactant production (Makkar and Cameotra, 1997). In this study four carbon sources glucose, starch, sucrose and hexadecane was used in the mineral salt medium to study the ability of *P. putida* to produce biosurfactant. It was found that increase in cell biomass was obtained for all carbon sources except for hexadecane (data not shown) where no significant growth was observed. In the presence of 2% sucrose highest biomass (2.4 g L^{-1}) as well as biosurfactant (1.3 g L^{-1}) was obtained. Similar results were obtained using *Bacillus subtilis* MTCC 1427 where in presence of 2% sucrose, 3.3 g L^{-1} biomass and 1.1 g L^{-1} biosurfactant was produced (Makkar and Cameotra, 1997). At 40°C maximum biosurfactant (1.3 g L^{-1}) yield was achieved in 120 h of fermentation using sucrose as carbon source. Maximum reduction in surface tension (25 mN m^{-1}) and interfacial tension (8 mN m^{-1}) was achieved using sucrose as carbon source at 60 h of fermentation. Reports have shown that nitrogen source affects biosurfactant production and strains such as *Arthrobacter* sp. preferred ammonium salts and urea as nitrogen source (Tabatabaee *et al.*, 2005). Hence, in this study we used organic (urea) and inorganic nitrogen sources (ammonium sulphate and ammonium nitrate) to select the best nitrogen source. Our results show that ammonium sulphate was the best nitrogen source since maximum biomass (2.5 g L^{-1}) and biosurfactant (2.4 g L^{-1}) were obtained using it. Additionally the surface tension ($74\text{-}38 \text{ mN m}^{-1}$) and interfacial tension ($43\text{-}11 \text{ mN m}^{-1}$)

reduction was maximum when ammonium sulphate was used as a nitrogen source. These results are similar to earlier reported data on biosurfactant production by *Bacillus subtilis* MTCC 1427 where ammonium sulphate was found to be the best suited nitrogen source for the strain (Makkar and Cameotra, 1997).

Since, metabolite production by microbes is generally susceptible to changes in pH we optimized these variables to achieve maximum biosurfactant production. Our results show that maximum biosurfactant (2.5 g L^{-1}) was obtained at pH 8.0 at 108 h of fermentation. The yield of biosurfactant was found to be decreased at acidic pH as it is attributed to formation of precipitate. Dissolved oxygen is known to regulate carbon assimilation rate as well as metabolite synthesis rate (Nitschke and Costa, 2007). Hence, we evaluated the effect of oxygen on biosurfactant production by varying the medium volume. Our results show that flask with 50 mL medium gave maximum yield of 2.8 g L^{-1} of biosurfactant. Stability studies on biosurfactant produced was analyzed to ensure that biosurfactant is stable over a broad range of pH and temperature. The stability of biosurfactant decreased till pH 5.0 and then stabilized till pH 10.0. The results obtained were found to be similar with previous reports in which biosurfactant produced by *B. subtilis*, *P. aeruginosa*, *B. cereus* and *R. erythropolis* showed reduction in surface tension till pH 6 and thereafter stabilized (Amani *et al.*, 2010; Xia *et al.*, 2011). Additionally, temperature had no significant effect on surface tension reduction property over a temperature range of $40\text{-}100^\circ\text{C}$ which is consistent with previous

reports where strains showed stability from 40-120°C (Amani *et al.*, 2010; Xia *et al.*, 2011). These results confirmed that biosurfactant produced by *P. putida* exhibits both pH (alkaline) and thermal stability. Effect of NaCl on biosurfactant samples produced by *Pseudomonas putida* was studied. *P. putida* exhibited good resistance to NaCl concentration up to 10 g L⁻¹ (Fig. 5c). Similarly, biosurfactant produced by strains *P. aeruginosa*, *B. subtilis*, *R. erthropolis*, *P. auregonisa* and *B. cereus* showed good stability at NaCl concentration upto 20-25 g L⁻¹ (Amani *et al.*, 2010; Xia *et al.*, 2011). Studies on effect of metal ions on biosurfactant were examined and the results showed that surface tension was lower than control for all metal ions tested (Fig. 5d). However, there was no difference in the effect among the metal ions (K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Al³⁺) tested.

The biochemical analysis shows that purified biosurfactant contained 6.88% protein and 66.4% lipid content. The result compared with previous report and understood that the protein content and lipid were 7.21 and 89.7%, respectively (Sekhon *et al.*, 2011). The FT-IR spectra of the dried biosurfactant sample produced by *Pseudomonas putida* The following spectra were obtained with characteristic bands at; 3272 cm⁻¹ for N-H stretch, presence of aliphatic chains from bands at 2957 cm⁻¹ (symmetric C-H stretch of CH₃), 2922 and 2859 cm⁻¹ (asymmetric and symmetric C-H stretch of CH₂, respectively). 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 CH₃. The C-H bend of CH₃ was due to the band at 1379 cm⁻¹. The 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 *Pseudomonas putida* is a lipopeptide belonging to surfactin group as the spectra obtained is comparable with pure surfactin from sigma (Borgund *et al.*, 2009). To our knowledge, there was no previous attempt found in literature about production of biosurfactant and its stability using the strain *Pseudomonas putida* MTCC 2467. *Pseudomonas putida* which produces biosurfactant serves to be multipurpose not only in oil recovery but it can also be used for bioremediation and oil spillage.

CONCLUSION

In this study, *Pseudomonas putida* MTCC 2467 has been investigated for biosurfactant production under different process conditions. The strain produced highest amount of biosurfactant with sucrose and ammonium sulphate as carbon and nitrogen sources. Biosurfactant produced by *P. putida* exhibited higher surface activity (surface tension reduction from 72-34 mN m⁻¹, interfacial tension reduction from 51-8 mN m⁻¹). The produced biosurfactant was found to be thermo-stable (40-100°C). Biosurfactant pH was stable at alkaline pH (6.0-11.0). These results clearly suggest that the strain is suitable for possible applications in oil recovery and bioremediation applications. Purified biosurfactant produced from *Pseudomonas putida* was characterized as lipopeptide which mainly consist of lipid and peptide belongs to surfactin molecule.

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