ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 10 (2): 183-189, 2011
ISSN 1682-296X / DOI: 10.3923/biotech.2011.183.189

Influence of Nitrogen and Phosphorus on Rhamnolipid Biosurfactant Production by *Pseudomonas aeruginosa* DS10-129 using Glycerol as Carbon Source

[2]Saher Mehdi, [1]Jesse S. Dondapati and [1]Pattanathu K.S.M. Rahman
[1]School of Science and Engineering, Chemical and Bioprocess Engineering Group, Teesside University, Middlesbrough-TS1 3BA, Tees Valley, UK
[2]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford-OX1 3RB, UK

Abstract: Biosurfactants are extracellular, amphipathic surface-active agents produced by many types of microorganisms which are capable of reducing the surface and interfacial tensions. The present study aims to optimize the biosurfactant production in *Pseudomonas aeruginosa* DS10-129 using (2^4) factorial design. The best experimental designs were selected on the basis of response variables such as: surface tension and bacterial growth (optical density). Two types of phosphate and nitrogen sources at different concentrations were used in the experiment. Further, main effect plots and interaction plots of the selected four important factors for the study has revealed the most significant medium that influence the production of biosurfactants. It was clearly indicated that the nitrogen source is the most significant factor with greater effect on the production of rhamnolipids by *P. aeruginosa* among the four selected factors. Hence, it is apparent that the combination of peptone (organic nitrogen source) and high phosphate concentration with glycerol is the best for maximum production of rhamnolipid.

Key words: Optimisation, factorial design, biosurfactants, dirhamnolipids, critical micelle concentration

INTRODUCTION

Most of the industrially produced surfactants in the market today are exclusively produced by synthetic methods and it has evolved into a multi-billion dollar industry worldwide with its demand rising above 300% in the last decade alone (Wei *et al*., 2008). However, the biosurfactants that are extracellular, surface active, amphipathic molecules produced by several species of bacteria were known to be a better alternative due to their low toxicity, biodegradability and specific activity even at extreme conditions (Benincasa *et al*., 2002; Mukherjee *et al*., 2006). Lately the rhamnolipids, a class of biosurfactants, have received great attention and were extensively reported to possess applications in commercial, therapeutic, environmental and even biomedical quarters (Dusane *et al*., 2010). Despite the fact that rhamnolipids are commercially applicable in various fields, their production at industrial scale was not yet exploited particularly because of the constraints, such as: low yields, high production costs, expensive raw materials and inefficient product recovery methods (Dos Santos *et al*., 2010).

An indigenous oil-degrading strain, *P. aeruginosa* DS10-129, which was isolated from the diesel contaminated sites was reported previously at Teesside University's research to produce rhamnolipids (Rahman *et al*., 2002; Rahman and Gakpe, 2008). Rhamnolipids naturally occur mainly in two forms: mono and di-rhamnolipids also referred as RL-F1 (rhamnoacyl-β-hydroxydecanoyl-β-hydroxydecanoate) and RL-F2 (rhamnoacyl-rhamnoacyl-β-hydroxydecanoyl-β-hydroxydecanoate), respectively. The RL-F1 and RL-F2 differ in their properties, such as: hydrophobic and hydrophilic nature based on their structures (Pornsunthorntawee *et al*., 2008). The *P. aeruginosa* DS10-129 produces a mixture of RL-F1 and RL-F2, similar to other strains; however, the di-rhamnolipids were predominantly found in its production levels (Bondarenko *et al*., 2010; Rahman *et al*., 2009, 2010).

The optimization of rhamnolipid production by regulating bacterial growth conditions and media compositions in various species of *Pseudomonas* utilizing low cost raw materials, have been immensely relied upon by the researchers for the last few years (Maneerat, 2005; Wei *et al*., 2008). In our previous study, the rhamnolipid production was reported to be 4.3 to 2.9 g L^{-1} utilizing soybean and sunflower oil as carbon sources (Rahman *et al*., 2002). Few others have successfully reported enhanced production of rhamnolipids by

Corresponding Author: Dr. Pattanathu K.S.M. Rahman, School of Science and Engineering, Chemical and Bioprocess Engineering Group, Teesside University, Middlesbrough-TS1 3BA, Tees Valley, United Kingdom Tel: 0044-1642-384669 Fax: 0044-1642-384418

optimization procedures; however, to our knowledge, a study on optimization of indigenous strain *Pseudomonas aeruginosa* DS10-129 using factorial design techniques and cost effective carbon source like water soluble glycerol have never been reported.

Optimizing single factor at a time while other factors being held constant is the most common method of experimentation; however, this strategy is time consuming as it involves large number of trials which increases the complexity exponential with an increase in the number of factors (Charyulu and Gnanamani, 2010). Unlike the conventional single factor at a time methods, the analysis of the factorial designed experiments helps to investigate the interrelationship of the factors as well as the effect of most significant variable on the response variable accurately. Since, this technique helps to organise the possible least number of experiments that will involve all the selected factors at given levels (Oliveira *et al*., 2009). In the present study, four prominent medium factors: N (nitrogen), P (phosphate), V (vitamins) and T (trace elements) that influence the production of rhamnolipids in *Pseudomonas* species and related strains were selected from the literature (Maneerat, 2005; Rahman and Gakpe, 2008; Dos Santos *et al*., 2010). The aim of the study is to investigate the interdependent influences of the selected four important factors and their resultant effect on surface tension and optical density, thereby to propose optimized values of each factor to enhance biosurfactant production in *Pseudomonas aeruginosa* DS10-129.

MATERIALS AND METHODS

Experimental design: A two level, full factorial design for four selective test factors (2^4) and three replicates of each was generated using an inbuilt DOE (Design of experiment) package of MINITAB 14 statistical software. The maximum and minimum values of phosphate sources are arbitrarily designated as high and low while, nitrogen is designated as organic and inorganic to reduce experiments to two levels respectively. Besides, their original values are shown in Table 1. The outcomes of the designed experiment, such as: values of Optical Density (OD) and Surface Tension (ST) values were considered as response variables for further analysis. The main effect plots and interaction plots were generated by utilizing the 'analyze factorial design' function of DOE (Design of Experiments) package of Minitab 14.

Micro-organism and medium conditions: *Pseudomonas aeruginosa* DS10-129 bacterial strain (EBI accession No: AM419153) was used in this study. One milliter of the cultures grown in nutrient broth was inoculated into the experimental shake flasks with oxoid 100 mL of mineral salts medium (Table 1) and incubated at 30°C, 160 rpm for 96 h in a rotary shaker. Water-soluble glycerol was used as the sole carbon source for the entire experiment. The samples of 10 mL size were drawn at every 24 h from the start of the experiment such as: 0, 24, 48, 72 and 96 h.

Table 1: Composition of medium with different nitrogen and phosphate sources

	Source			
	Nitrogen		Phosphate	
Composition	Organic	Inorganic	High	Low
Peptone	1.38 g	-	-	-
$(NH_4)_2$. SO_4	-	2 g	-	-
NaH_2PO_4. H_2O	-	-	4 g	0.8 g
Na_2H PO_4. H_2O	-	-	1 g	0.2
Mg SO_4.7 H_2O-	1 g	1 g	1 g	1 g
$CaCl_2$. 2 H_2O-	0.005 g	0.005 g	0.005 g	0.005 g
Glycerol	25 mL	25 mL	25 mL	25 mL

A total of 2^4 = 16 combinations of four most prominent media compositions according to the basic experimental factorial design were prepared (Table 1). Each of the sixteen media combinations were distributed into 250 mL shake flasks labeled accordingly (C_1 to C_{16}) in order to contain distinct media of either organic or inorganic nitrogen sources along with high or low phosphate sources in given proportions according to the factorial design (Table 2). Further, the pH of the medium for all the sixteen combinations was calibrated using 1 M NaOH, which were then autoclaved for 15 min at 121°C. Later, 1 mL of trace element and vitamin working solutions were added into the shake flasks as per the basic design of experimental combinations of factorial design. Further, 1 mL of inoculum was transferred into each shake flask containing 100 mL of the medium in each of the experimental replicates and incubated for 240 h.

Characterization of rhamnolipids by IR spectroscopy: FTIR (Fourier transform infrared) spectrometer (PerkinElmer 100 series) was used to characterize the presence of rhamnolipids in the cell-free extract (Mukherjee *et al*., 2008). The 2 M Sulphuric acid is used to acidify (pH: 2) the cell-free supernatant in order to precipitate rhamnolipids. An equal volume of 2:1 dichloromethane/methanol solution is utilized to extract the precipitated rhamnolipids. Further, the organic phase was dried and evaporated by anhydrous Sodium sulphate (Na_2SO_4) using rotary evaporator (Buchi, rota vapour R-200 Germany) at 60-70°C. Around 2-5 mg of the concentrated rhamnolipids was examined on the FTIR spectrophotometer.

Table 2: Sixteen basic combinations of the four factors at two levels: Nitrogen (N), Phosphate (P), Vitamine (V) and Trace elements (T), obtained from the 2^4 factorial designing, MINITAB v14

Combinations	N[α]	P[β]	V[ε]	T[ϖ]
C_1	$(NH_4)_2.SO_4$	High	Present	Present
C_2	$(NH_4)_2.SO_4$	Low	Present	Present
C_3#	Peptone	Low	Present	Present
C_4	$(NH_4)_2.SO_4$	High	Absent	Present
C_5#	Peptone	Low	Present	Absent
C_6*	Peptone	High	Absent	Present
C_7	$(NH_4)_2.SO_4$	High	Present	Absent
C_8	$(NH_4)_2.SO_4$	Low	Absent	Absent
C_9#	Peptone	Low	Absent	Present
C_{10}#	Peptone	Low	Absent	Absent
C_{11}*	Peptone	High	Present	Present
C_{12}	$(NH_4)_2.SO_4$	Low	Absent	Present
C_{13}	$(NH_4)_2.SO_4$	High	Absent	Absent
C_{14}	$(NH_4)_2.SO_4$	Low	Present	Absent
C_{15}*	Peptone	High	Present	Absent
C_{16}*	Peptone	High	Absent	Absent

*The combinations (C_3, C_5, C_9, C_{10}) that comprises of peptone+high phosphate exclusively, #The combinations (C_6, C_{11}, C_{15}, C_{16}) that comprises of peptone+low phosphate exclusively, αNitrogen source (organic or inorganic), βPhosphate source (high or low), εConcentration of vitamins: 0.5 mL per 100 mL (present or absent), ϖConcentration of trace elements: 1 mL/100 mL (present or absent)

Surface tension and optical density: Samples of 5 mL culture were centrifuged at 10,000 rpm, 4°C for 10 min and the supernatant was used for measuring the surface tension using the Kruss Digital Tensiometer (model k9, Germany). Growth of bacteria was measured as Optical density using Spectrophotometer (Pharmacia LKB, UltrospecIII) at 600 nm (Rahman *et al*., 2002).

CMC and glycerol concentration: In the present study, the CMC was determined from a graph plotted between Surface Tension (mN m^{-1}) versus concentration (mg L^{-1}) of the rhamnolipid surfactant (Yin *et al*., 2009). The intercept of the base line drawn from the minimum ST and tangential line of immediate ST decline in the graph indicates the critical point approximately (Rahman *et al*., 2010). The CMC was measured selectively for the significant combinations that have shown surface tension below 30 mN m^{-1}. The CMC values for each combination were determined by measuring ST initially at room temperature for a series of rhamnolipid solutions prepared with deionised water and by plotting those values against different concentrations (Table 3).

Further, the utilization of glycerol by the *Pseudomonas aeruginosa* DS10-129 culture throughout the experiment was estimated by a micro assay at various stages of experiment. The 166 µL of samples were collected at regular intervals into an eppendorf containing equal amounts of 21% sodium hydroxide and 1 mL of absolute alcohol. After the centrifugation, 100 µL of cupric chloride reagent and 233 µL of alcohol were added to the supernatant and centrifuged again. Glycerol depletion in the medium was estimated from the

Table 3: The values of various parameters of the best eight designs in terms of surface tension and OD plots after 48 h

Combinations	Surface tension (mN m^{-1})	OD (600 nm)	CMC (mg L^{-1})	Glycerol reduction (%)
C_3#	30.7	0.857	14	90.7
C_5#	28.2	0.833	12	80.0
C_6*	29.9	0.805	20	89.0
C_9#	29.8	0.798	22	88.0
C_{10}#	30.1	0.959	14	89.8
C_{11}*	28.0	0.895	14	87.4
C_{15}*	27.7	0.827	13	82.0
C_{16}*	27.5	0.776	10	80.0

*The combinations (C_3, C_5, C_9, C_{10}) that comprises of peptone+high phosphate exclusively, #the combinations (C_6, C_{11}, C_{15}, C_{16}) that comprises of peptone+low phosphate exclusively

calibration curve developed using the absorbance of the supernatants measured at 635 nm (Bondioli and Bella, 2005).

RESULTS AND DISCUSSION

Benefited by a plethora of information available in the literature on rhamnolipids' chronology of enhanced production in various bacteria; four prominent medium components at two levels were selected for further medium optimization studies. The 2^4 experimental factorial designs with three replicates for corner points and zero blocks were selected which resulted in a complete set of 48 runs. The set of basic experimental designs consisted of sixteen permutations of four factors that include their combinations at two levels as shown in the Table 2.

Identification and characterization of rhamnolipids: Based on the observations made from the resultant FTIR graph (Fig. 1), the broad band at region A (3367.89 cm^{-1}) indicates the presence of Hydroxyl group (-OH) free stretch due to hydrogen bonding. The broad band observed each in the region B and D (2925-2856 and 14551380 cm^{-1}) infers the presence of aliphatic CH_3, CH_2 and C-H bond stretching. Further, bands observed at 1737.20 and 1647.31 cm^{-1} of region C points to the occurrence of carbonyl (C = O) stretching and the other two peaks observed between 1121-1033 cm^{-1} (region E) indicates the presence of C-O-C bond stretching that are characteristic to ester functional group. Moreover, pyranyl isorption band observed in the region F at 918 cm^{-1} and β-pyranyl II sorption band observed in the region G at 838 cm^{-1} points out to the presence of dirhamnolipid in the mixture (Guo *et al*., 2009).

Effect of Nitrate (N), Phosphate (P), Vitamins (V) and Trace metals (T): In the present study, eight experimental combinations (C* and C# of Table 2): C_3, C_5, C_6, C_9, C_{10}, C_{11}, C_{15} and C_{16}, revealed a remarkable decreasing patterns of Surface Tension (ST) with time between 24 to 48 h (Fig. 2c, d) in comparison to the rest of the eight

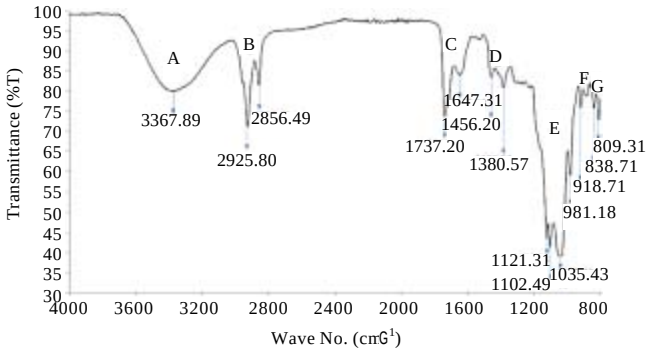

Fig. 1: IR spectra of rhamnolipids produced by *Ps. aeruginosa* DS10-129 (Region A: free (-OH) group, Region B and D: CH3, CH2 and C-H bond stretching, Region C: Carbonyl bond stretching, Region E: C-O-C bond stretching, Region F and G: Pyranyl I and II sorption bands, respectively)

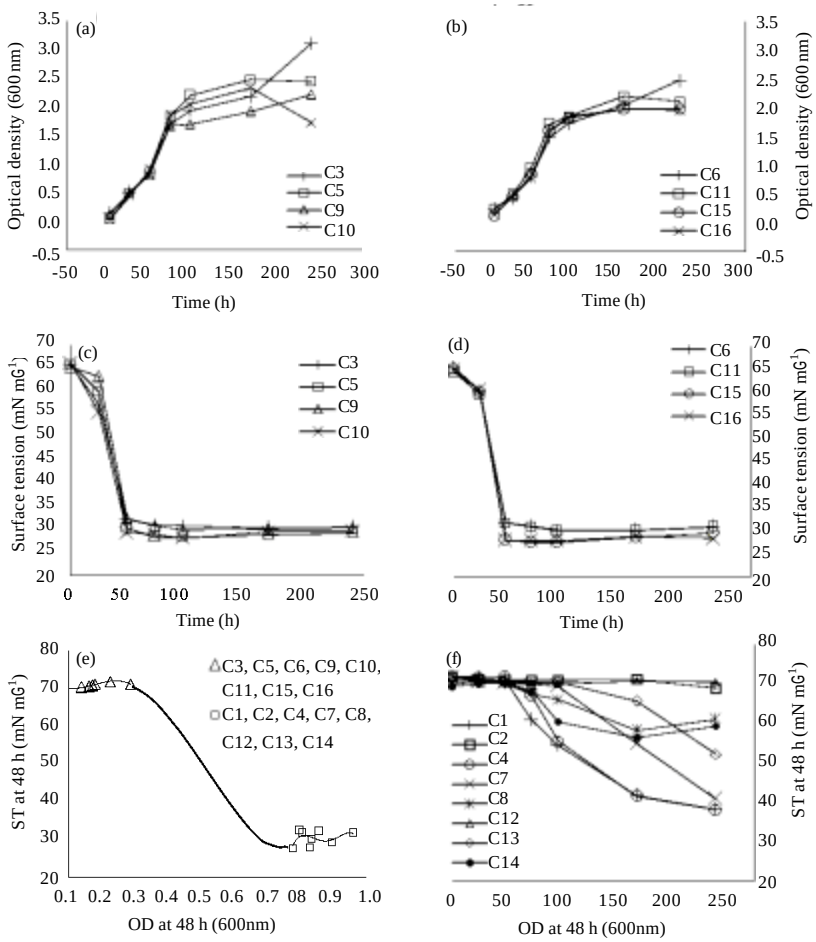

Fig. 2: (a, b) Optical density versus time, (c, d) Surface tension versus time for the eight best experimental combinations, (e) Surface tension versus optical density at 48 h for the complete set of combinations (C_1 to C_{16}) and (f) Surface tension versus time for the rest of the experimental combinations

experimental combinations: C_1, C_2, C_4, C_7, C_8, C_{12}, C_{13} and C_{14} (Fig. 2f). In fact, the graph plotted between the ST and OD (600 nm) of all the sixteen combinations in the experiment at 48 h, clearly revealed that the eight best combinations (C* and C# of Table 2) being characterized by low ST and high OD, had distinctly separated them from the rest of the other eight combinations as shown in the Fig. 2e.

Notably the entire C* and C# combinations are comprised of peptone as their only source for nitrogen (Table 2): which explains the significance of peptone in rhamnolipid production. However, the C*, combinations have shown better uniformity in bacterial growth (Fig. 2b) and slightly enhanced ST values (Table 3) than 'C#' combinations as evident from the Fig. 2a-d which points out the significance of the peptone with high concentration of phosphate (4 g NaH_2PO_4. H_2O + 1 g Na_2HPO_4. H_2O) influencing the bacterial growth and Surface Tension (ST) values. A reduction of the ST values from 72 to 27.5 mN m^{-1} was observed in the present study which can be considered as a better results compared to many of the previous studies; for instance, Liang *et al.* (2005) reported a ST reduction from 72 to 28.6 mN m^{-1}, Costa *et al.* (2006) reported 72 to 29.8 and Yin *et al.* (2009) reported 72 to 33.9 mN m^{-1}. Further, a recent report of Rahman *et al.* (2010) from Teesside University has reported ST value of 27.9 mN m^{-1} by the rhamnolipids produced from *Pseudomonas aeruginosa* DS10-129 supplemented with glycerol and yeast extract in a microfluidic bioreactor. However, in the present study the combinations, C_{15} and C_{16} have resulted in ST values as low as 27.7 and 27.5 mN m^{-1}, respectively.

The individual effects of either the presence or absence of vitamins and trace elements on the ST and OD values was not found noteworthy. If the eight experimental combinations that revealed minimum ST values and steady increase in OD values are observed, they are comprised of all the possible combinations of 'V' and 'T' without showing favor to any particular combination as in the case of 'N' and 'P' (C*and C# of Table 2). In the present study, the main effect plots (Fig. 3a-b) deduced for every 24 h has confirmed the positive effect of peptone as nitrogen source at significant levels. The main effect plots also called as one

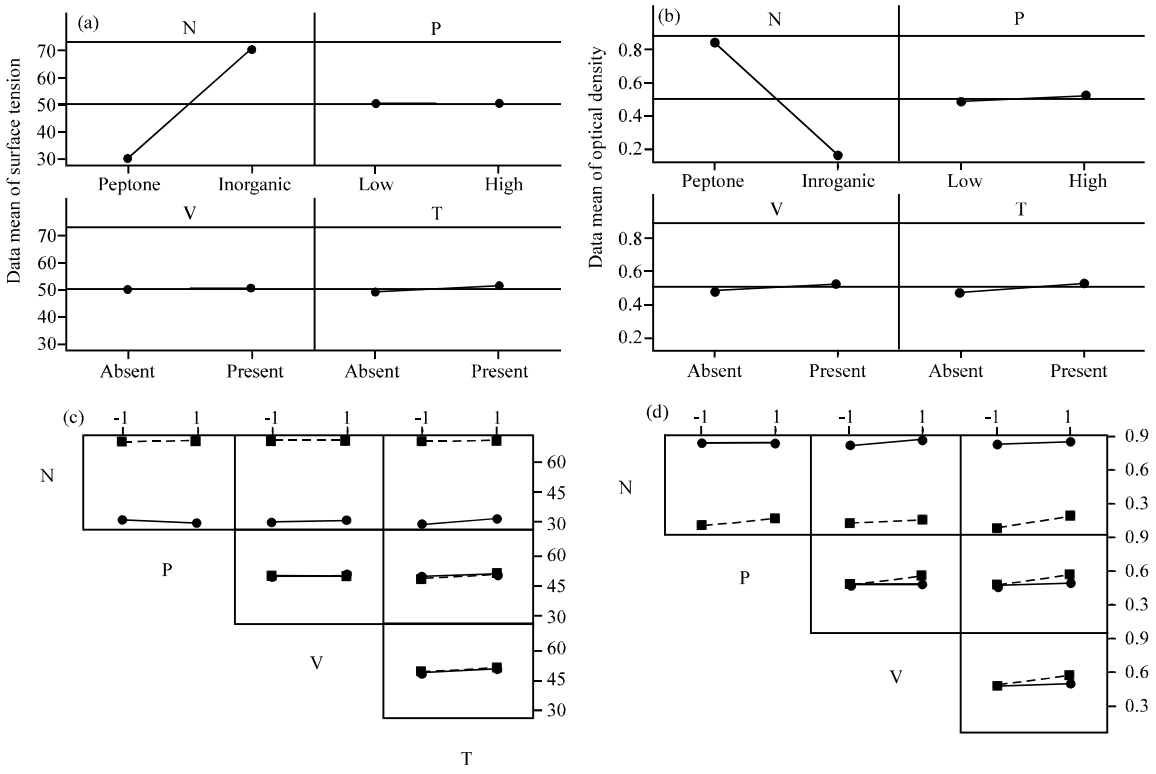

Fig. 3: Plots confirmating the individual and interaction effects over ST and OD values: (a and b) Main effect plot (data means) for ST and OD at 48 h, (c and d) Interaction plot (data means) for ST and OD at 48 h (Solid line indicates either peptone/low phosphate/absent and dotted line indicates either inorganic nitrogen/high phosphate/present correspondingly; -1 and 1 represent no and yes, respectively)

factor effects usually points out the difference that would result for changing a single factor in a process (Masson and Loftus, 2003). Further, the slope of the line connecting the two means values of ST corresponding to N were observed to be far greater than the other three factors throughout the experiment in case of nitrogen. Besides, the individual effect of phosphate was clearly evident in the graphs plotted only after 72 h.

Effect of interactions between N, P, V and T over response variables (ST and OD): The combined effect of all the interactions within the four factors: N, P, V and T over the ST and OD response values were further determined by plotting a series of interaction plots, for every sample retrieved after regular intervals (24 h). The interaction plots show the interaction or effect of one factor on the other in a given design (Masson and Loftus, 2003). In this study, the interaction plots have confirmed that the effect of organic nitrogen is significant in the presence of high phosphate source over both the ST and OD values (Fig. 2c-d). Moreover, the interactions between P, V and T were completely ruled out based on the interaction plots as evident from the Fig. 2c-d.

Critical Micelle Concentration (CMC) and glycerol utilization: Ideally CMC reveals the lowest concentration of surfactants required to attain the minimum surface tension for an emulsion; however, one should be aware that a CMC determined from a plot of ST versus concentration, could have an error rate of 3 to 4 mg L^{-1} or even higher depending on the rate at which the sampling was carried out. The typical range of CMC for the rhamnolipids produced by various bacteria was previously reported as 10 to 230 mg L^{-1} in several instances (Maier and Chavez, 2000; Nitschke *et al*., 2005; Rahman *et al*., 2010). Previously, some of the lowest CMC values were reported for the rhamnolipids, such as: 30 mg L^{-1} (Clifford *et al*., 2007), 22 mg L^{-1} (Rahman *et al*., 2010). In the present study, the C_{16} combination has shown a minimum value (10 mg L^{-1}) which was identified to fall into a category of minimum CMC values ever reported by a *Pseudomonas aeruginosa* sp. (Fig. 4).

The bacterial growth and rhamnolipid production by *Pseudomonas aeruginosa* DS10-129 strain were found maximum during 24 and 48 h with 2.5% glycerol supplemented as the sole carbon source. The percentage reductions of glycerol concentrations were monitored at regular intervals and the glycerol reductions at 48 h for the eight best combinations C* and C# were reported in the Table 3. The lowest glycerol utilization was observed in both C_5 and C_{16} combinations; however, the robust and

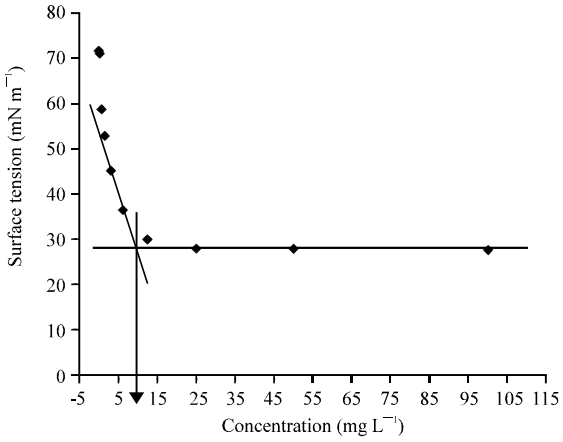

Fig. 4: The graph showing the determination of CMC for the combination C_{16} by plotting the surface tension values versus the concentration of rhamnolipds

optimized experimental combination resulted was C_{16} based on the minimum CMC, OD, ST and glycerol utilization values.

CONCLUSION

In conclusion, addition of peptone and phosphate in mineral salts medium with glycerol substrate has shown a minimum CMC value of 10 mg L^{-1} along with the lowest surface tension of 27.5 mN m^{-1}. This study revealed peptone as a better nitrogen source over inorganic sources for rhamnolipid production. It will be useful in scaling up of biosurfactant production using glycerol as a major substrate.

ACKNOWLEDGMENTS

Authors wish to thank the UK- Bioscience for Business KTN for the award of FROPTOP fund to further explore the bio-catalytic study of biosurfactant production from renewable resources.

REFERENCES

Benincasa, M., J. Contiero, M.A. Manresa and I.O. Moraes, 2002. Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soapstock as the sole carbon source. J. Food Eng., 54: 283-288.

Bondarenko, O., P.K. Rahman, T.J. Rahman, A. Kahru and A. Ivask, 2010. Effects of rhamnolipids from *Pseudomonas aeruginosa* DS10-129 on luminescent bacteria: Toxicity and modulation of cadmium bioavailability. Microb. Ecol., 59: 588-600.

Bondioli, P. and L.D. Bella, 2005. An alternative spectrophotometric method for the determination of free glycerol in biodiesel. Eur. J. Lipid Sci. Technol., 107: 153-157.

Charyulu, E.M. and A. Gnanamani, 2010. Condition stabilization for *Pseudomonas aeruginosa* MTCC 5210 to yield high titers of extra cellular antimicrobial secondary metabolite using response surface methodology. Curr. Res. Bacteriol., 3: 197-213.

Clifford, J.S., M.A. Ioannidis and R.L. Legge, 2007. Enhanced aqueous solubilization of tetrachloroethylene by a rhamnolipid biosurfactant. J. Colloid. Interface Sci., 305: 361-365.

Costa, S.G.V.A.O., M. Nitschke, R. Haddad, M.N. Eberlin and J. Contiero, 2006. Production of *Pseudomonas aeruginosa* LBI rhamnolipids following growth on Brazilian native oils. Process Biochem., 41: 483-488.

Dos Santos, S. C., L. G. Fernandez, J. C. Rossi-Alva and M. R. de Abreu Roque, 2010. Evaluation of substrates from renewable-resources in biosurfactants production by Pseudomonas strains. Afr. J. Biotechnol., 9: 5704-5711.

Dusane, D.H., S.S. Zinjarde, V.P. Venugopalan, R.J. Mclean, M.M. Weber and P.K.S.M. Rahman, 2010. Quorum sensing: Implications on Rhamnolipid biosurfactant production. Biotechnol. Genet. Eng. Rev., 27: 159-184.

Guo, Y.P., Y.Y. Hu, R.R. Gu and H. Lin, 2009. Characterization and micellization of rhamnolipidic fractions and crude extracts produced by *Pseudomonas aeruginosa* mutant MIG-N146. J. Colloid Interface Sci., 331: 356-363.

Liang, S.K., X.L. Wang, J.R. Lu, Q.Q. Zhang, 2005. Structure characterization of physicochemical properties of rhamnolips produced by *Pseudomonas* O-2-2. Fine Chem., 22: 499-502.

Maier, R.M. and G.S. Chavez, 2000. *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. Applied Microbiol. Biotechnol., 54: 625-633.

Maneerat, S., 2005. Production of biosurfactants using substrates from renewable-resources. Songklanakarin J. Sci. Technol., 27: 675-683.

Masson, M.E.J. and G.R. Loftus, 2003. Using confidence intervals for graphically based data interpretation. Can. J. Exp. Psychol., 57: 203-220.

Mukherjee, S., P. Das and R. Sen, 2006. Towards commercial production of microbial surfactants. Trends Biotechnol., 24: 509-515.

Mukherjee, S., P. Das, C. Sivapathasekaran and R. Sen, 2008. Enhanced production of biosurfactant by a marine bacterium on statistical screening of nutritional parameters. Biochem. Eng. J., 42: 254-260.

Nitschke, M., S.G.V.A.O. Costa, R. Haddad, L.A.G. Goncalves, M.N. Eberlin and J. Contiero, 2005. Oil wastes as unconventional substrates for rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* LBI. Biotechnol. Progress, 21: 1562-1566.

Oliveira, F.J.S., L. Vazquez, N.P. de Campos and F.P. de Franca, 2009. Production of rhamnolipids by a *Pseudomonas alcaligenes* strain. Process Biochem., 44: 383-389.

Pornsunthorntawee, O., P. Wongpanit, S. Chavadej, M. Abe and R. Rujiravanit, 2008. Structural and physicochemical characterization of crude biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from petroleum-contaminated soil. Bioresour. Technol., 99: 1589-1595.

Rahman, K.S., T.J. Rahman, S. McClean, R. Marchant and I.M. Banat, 2002. Rhamnolipid biosurfactants production by strains of *Pseudomonas aeruginosa* using low cost raw materials. Biotechnol. Prog., 18: 277-281.

Rahman, K.S.M. and E. Gakpe, 2008. Production, characterisation and applications of biosurfactants-review. Biotechnology, 7: 360-370.

Rahman, P.K.S.M., G. Pasirayi, V. Auger and Z. Ali, 2009. Development of a simple and low cost microbioreactor for high-throughput bioprocessing. Biotechnol. Lett., 31: 209-214.

Rahman, P.K., G. Pasirayi, V. Auger and Z. Ali, 2010. Production of rhamnolipid biosurfactants by *Pseudomonas aeruginosa* DS10-129 in a microfluidic bioreactor. Biotechnol. Applied Biochem., 55: 45-52.

Wei, Y.H., C.L. Cheng, C.C. Chien and H.M. Wan, 2008. Enhanced di-rhamnolipid production with an indigenous isolate *Pseudomonas aeruginosa* J16. Process Biochem., 43: 769-774.

Yin, H., J. Qiang, Y. Jia, J. Ye, H. Peng, H. Qin, N. Zhang and B. He, 2009. Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater. Process Biochem., 44: 302-308.