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The Effects of culture Conditions on biosurfactant activity of *Pseudomonas aeruginosa* 181 using response surface methodology

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Abstract

Response surface methodology (RSM) was used to study interactive effects of the parameters (pH, stirring rate, casamino acid concentration and incubation period) on the biosurfactants activity. It was implied that the effect of any one parameter could not, on its own, explain the outcome of the reaction without considering the input of the other parameters. For this reason, experiments in this following were performed to gain some insight into their combined interactive effects. Within the experimental range studied, optimal conditions for the biosurfactant activity were predicted using the optimization function of the Design Expert software. The actual biosurfactant activities by *Pseudomonas aeruginosa* 181 were 28.43 dyne / cm for surface tension and 85% Emulsification Index (E24).

Introduction

Biosurfactants have tremendous potential for applications in petroleum recovery and the pharmaceutical, cosmetics and food industries as emulsifiers and deemulsifiers due to their easy biodegradability and mild toxicity as compared with the synthetic surfactants (Sen, 1997). Biosurfactants can improve the bioavailability of hydrocarbons to the microbial cells by increasing the area at the aqueous-hydrocarbon interface. This increases the rate of hydrocarbon dissolution and their utilization by microorganisms (Tuleva et al., 2002). Among the best studied biosurfactants are rhamnolipids that belong to the glycolipids class. Rhamnolipids have been identified predominantly from *Pseudomonas aeruginosa* (Beal & Betts, 2000). The aim of this work includes statistical optimization of the medium concentrations and physical factors which play a very significant role in enhancing the biosurfactant activity. These critical media components are casamino concentrations. Although pH and stirring rate are a very important and critical component of both the growth and the production media for *Pseudomonas aeruginosa*, monitoring the concentration as an independent variable within a fixed ran and at the desired levels as required in the statistical experimental design seems to be very difficult in shake flask experiments. The classical method of media optimization involves changing one variable at a time, keeping the others at fixed levels. Being single dimensional, this laborious and time-consuming method often does not guarantee determination of optimal conditions. On the other hand carrying out experiments with every possible factorial combination of the test variables is impractical because of the large number of experiments required. Thus a 2 full factorial central composite design and response surface modeling method was used in this study.

Material and Methods

Microorganism
Pseudomonas aeruginosa 181, was isolated from contaminated soils of a motor workshop in Serdang, Selangor, Malaysia (Ali, 1998). The strain was grown at 37°C for 18h and maintained at 4°C on TSA plates and alternatively at -80°C.

Media preparation
The experiments were prepared by various pH, casamino acid concentration, stirring rates and the incubation period were varied according to the experiments design. The seed culture at 5% (v/v) was used as inoculums into 1L of the production medium in a Bioreactor which was incubated at 37°C.

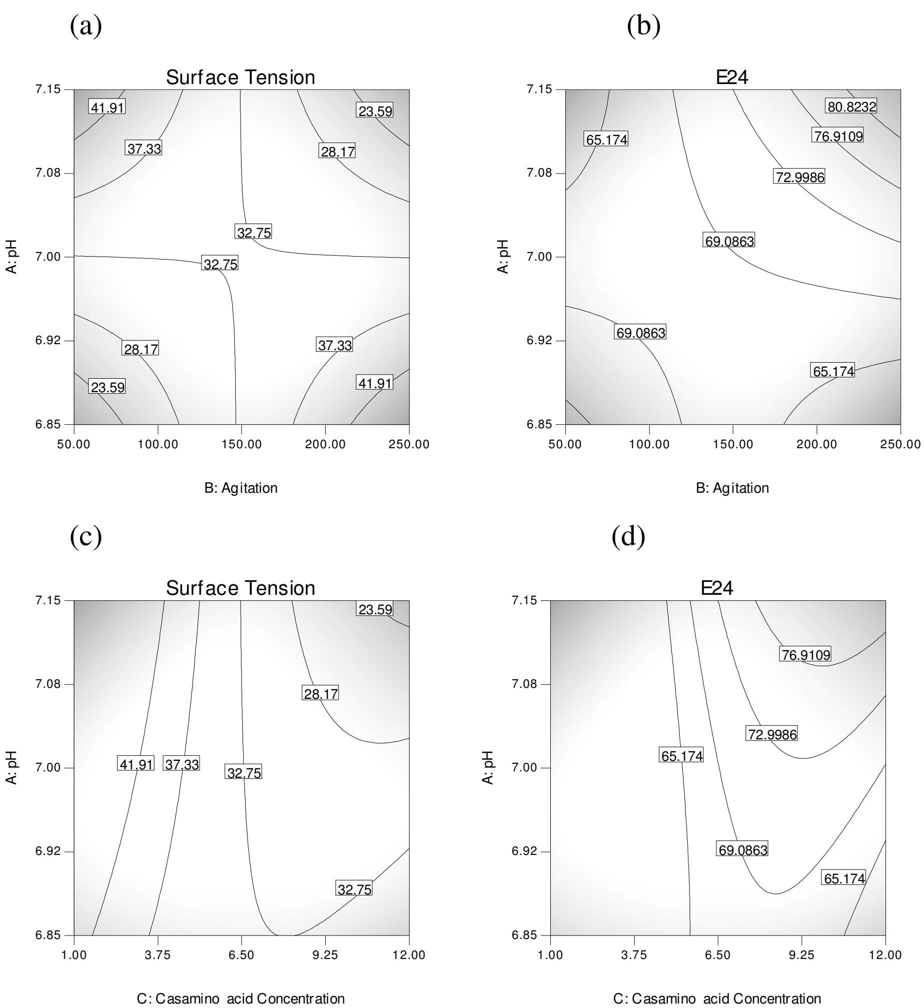
Analytical methods
Biosurfactants activity was determined by measuring the reduction of surface tension of the culture supernatant using a Fisher surface tensiomat model 21. The E24 was determined by vortexing equal volume of culture supernatant with hexadecane at high speed for 4 min. and left for 24h. The E24 was calculated as the height of emulsion layer/ total height X 100 (Patel & Desai, 1997).

Experimental Design and Optimization by RSM
Response surface methodology (RSM) consists of a group of empirical techniques devoted to the evaluation of relations existing between a cluster of controlled experimental factors and the measured responses, according to one or more selected criteria. A prior knowledge and understanding of the process and the process variables under investigation are necessary for achieving a more realistic model.

Results

Figure 1 show the response surface plots as functions of pH versus stirring rate, casamino acid concentration, and incubation period, respectively, for the surface tension, and emulsification index (E24). Slight or marked positive effects of pH were seen in most cases, negative or cradle (a minimum point between two peaks) effects were observed in the following interaction regions: In the surface tension, negative effects of pH were indicated with the increase of the casamino concentration (Figure 1c) while cradle effects were indicated with the incubation period (Figure 1e).

In the emulsification index (E24), negative effects were indicated with the increase of stirring rate (Figure 1b). Several trends were observed for the predicted combined effects of pH and stirring rate (Figure 1) at fixed casamino acid concentration (6.5 g / L) and incubation period (3day). When pH was acidic (6.85 - 7) high reduction of surface tension was expected at low rate of stirring rate (50-150 rpm) in which casamino acid concentration is 6.5 g / L (Figure 1a).



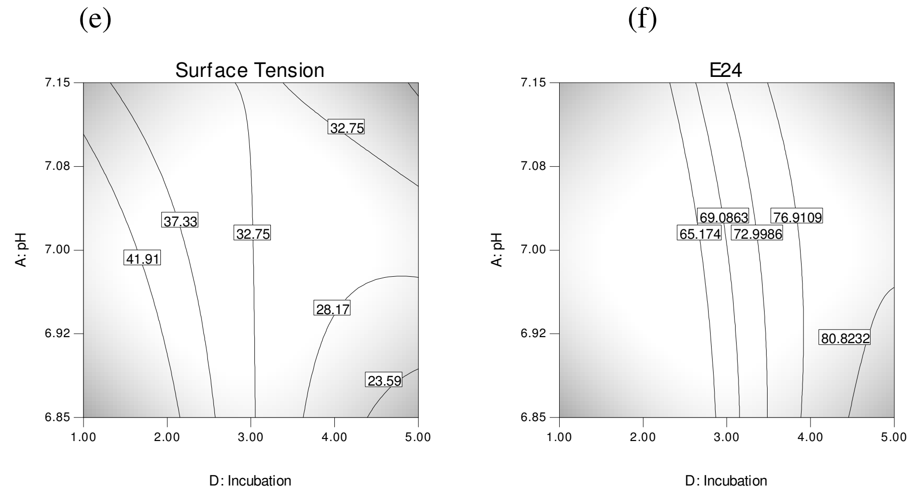


Fig. 1: Response Surface Plots of Surface Tension and E24, (a, b) pH versus Stirring rate (AB) (c, d) pH versus Casamino Acid Concentration (AC) (e, f) pH versus Incubation Period (AD)

Table 1: Optimal Conditions Derived by RSM for Biosurfactant Activity by *Pseudomonas aeruginosa* 181

Optimal Condition				Predicted*		Actual**		Relative Deviation %***
X_1	X_2 rpm	X_3 g/L	X_4 day	Surface Tension dyne/cm	(E24) %	Surface Tension dyne/cm	(E24) %	
7.15	250	4.06	1.93	29.18		28.43		2.57
7.11	250	5.78	2.18		85.13		85	0.15

X_1=pH, X_2= Stirring rate, X_3=Casamino acid Concentration, X_4=Incubation Period
*Predicted based on respective models developed
**Average value of triplicate runs

***Refer to this equation: Relative Deviation % = $$\frac{\text{Predicted Yield - Actual Yield}}{\text{Predicted Yield}} \text{X } 100$$

Discussion and Conclusion

The effects of pH can be apportioned to its effects on substrate as well as its direct influences on the bacteria growth. On increasing pH the whole cell protein was improved increased (Cooper & Goldenberg, 1987) and making the substrate more available to be utilized (Makkar & Cameotra, 1997). The nature of some bacteria also renders them more effective at acidic medium; however, its action is also simultaneously influenced by other factors such as agitation rate and medium composition (Swaminathan, 1997). In the high rate of stirring, on the other hand, surface tension was expected to increase markedly with decreasing pH which is in agreement with the significant and negative effect predicted by its statistical data (-13.45). This may be due to the *Pseudomonas aeruginosa* 181 utilized casamino acids at acidic and alkaline pH. However, effect of increasing stirring rate at acidic medium appears to be less significant than that at alkaline medium. This suggests that the negative effects of pH medium predominated over the positive effects of stirring rate with the use of acidic medium. When carrying out the medium at alkaline pH, the positive effect of stirring rate was clearly evident. Together, then alkaline pH and high rate of stirring rate promote the reduction of surface tension.

Within the experimental range studied, optimal conditions for the biosurfactant production by *Pseudomonas aeruginosa* 181 were predicted using the optimization function of the Design Expert software. These are presented in Table 1 along with their predicted and actual values. Comparison of predicted and experimental values revealed good correspondence between them, implying that empirical models derived from RSM can be used to adequately describe the relationship between the factors and response in biosurfactant activity by *Pseudomonas aeruginosa* 181. The relative deviation values obtained 2.57% (surface tension) and 0.15% (E24) are comparable to those reported in RSM studies on enzymatic synthesis 2.1% (Hari Krishna et al*.*, 1999), and

0.7% (Hamsaveni et al., 2001). These models can therefore be used to predict biosurfactant yields under any given conditions within the experimental range.

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