

**TITLE:** OPTIMIZATION OF CULTURE MEDIA USING GLYCEROL AS CARBON SOURCE FOR RHAMNOLIPIDS PRODUCTION

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**ABSTRACT**

Cheap and readily available microbial biosurfactants are an important issue in achieving sustainable and greener alternatives to petrochemical surfactants. Rhamnolipids are glycolipid biosurfactants naturally produced by *Pseudomonas aeruginosa* and can be used in diverse fields since they present elevated surface activity and their production yield is high in relation to the culture time when hydrophobic carbon sources are used in the production. However, the use hydrophilic renewable carbon sources, as glycerol, can diminish production and extraction costs adding value to such remarkable biomolecules. The evaluation of glycerol as substrate for rhamnolipids production is a promising strategy for reducing costs in this bioprocess. In this study we proposed a culture media optimization using a response surface methodology through central composite design to evaluate the influence of different C/N (3.27; 6; 10; 14; 16.72), C/P (16; 50; 100; 150; 184) ratios and grams per liter of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (0.04; 0.12; 0.24; 0.36; 0.45), using  $\text{NaNO}_3$  and  $\text{K}_2\text{PHO}_4$  as sources of N and P, respectively. The experiments were performed at two different temperatures (30 °C and 37 °C), to evaluate the effect of temperature, the carbon source concentration was maintained in 63 g/L (5% v/v) and were varied N, P and Fe concentrations to achieve the established ratios. From the response surface methodology was concluded that the C/N ratio was the most significant factor for rhamnolipid production at 30 °C, as the response surface model did not show a significant raise for C/P and Fe variations. Experiments conducted at 37 °C did not show the same success on the response as obtained in 30 °C, after 72 h of fermentation were obtained 1.168 g/L and 4.395 g/L, respectively. Finally, for optimum culture media were defined the following conditions C/N ratio of 16.72, C/P of 100 and 0.24 g/L of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ . Experimental design used in this study led to the optimization of a culture media containing glycerol as carbon source for scaling up the production of rhamnolipid.

**Keywords:** Biosurfactants, Experimental design, Glycerol, *Pseudomonas aeruginosa*.

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