



SHIKIMIC ACID PRODUCTION OPTIMIZATION FOR A LABORATORY-EVOLVED *Escherichia coli* STRAIN BY DOEHLERT MATRICES

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Introduction. Compounds derived from the aromatic amino acid pathway play an important role in the pharmaceutical and alimentary industries as synthesis precursors for high valued chemicals, additives and even as final high value products (1). Shikimic acid (SA) is an aromatic amino acid pathway intermediate used as synthesis precursor of oseltamivir phosphate, an antiviral for influenza A and B treatment (2). This compound is usually obtained by non cost efficient chemical synthesis or plant extraction processes (3). Metabolic pathway engineering of *Escherichia coli* lacking the phosphotransferase system (PTS) has rendered new SA overproduction strains which have become an alternative to extractive processes (4). Nevertheless, for these strains to be utilized on an industrial level, study and optimization of fermentation process parameters should be done.

Methods. Multivariate methods are used for optimization of many variables along with their interactions. Doehlert matrices use a hexagonal homogeneous response surface capable of studying multidimensional spaces. This optimization method also allows new variables to be included in the experimental design reducing the number of experiments needed to be performed for acquiring new insights on the system (5). In this work a Doehlert optimization matrix has been used for glucose (Glu) and yeast extract (YE) concentrations to find the optimal batch operation conditions for SA production with a laboratory-evolved *E. coli* strain.

Results. The response surface for growth rate shows higher rates as glucose concentration diminish and revealing a minimum near 60 g/L of Glu and 30 g/L YE. SA production grid showed a maximum near 100 g/L Glu and 30 g/L YE which correlates with the maximum found on the surface constructed for the yield as presented in Figure 1. The use of these response surface experimental designs allowed us to calculate different fermentation parameters grids as

substrate consumption and product formation rates. The information retrieved from these results allowed us to identify the optimum condition for SA batch production as well as an optimum condition for fed-batch fermentations.

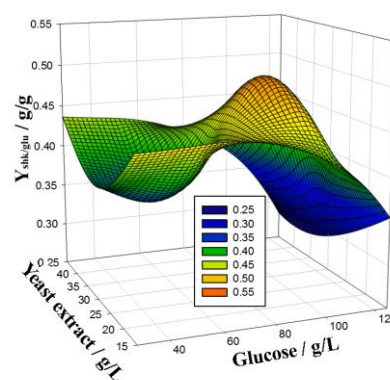


Fig.1 Response surface for shikimic acid yield on glucose derived from a Doehlert matrix experimental design.

Conclusions. Doehlert Matrices were successfully used for the study and optimization for the SA production process with a laboratory evolved *Escherichia coli* strain. The parameters for batch fermentation were optimized to 100 g/L glucose and 30 g/L of yeast extract. The use of this experimental design will allow further studies with other fermentation parameters over this experimental range.

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References.

- 1 Gosset G. (2009). *CURR OPIN BIOTECH.* vol.(20.): 651-658.
- 2 Escalante A, Calderón R, Valdivia A, de Anda R, Hernandez G, Ramírez O, Gosset G, Bolívar F. (2010). *MICROB CELL FACT.* vol(9):21-33.
- 3 Draths K.M, Knop D, Frost J.W. (1999). *J. Am. Chem. Soc.* vol(121):1603-1604.
- 4 Frost J.W, Frost K.M, Knop D. (2002). Biocatalytic synthesis of shikimic acid. United States. Patent US 6,472,168 B1.
- 5 Ferreira S, dos Santos W, Quintella C, Neto B, Bosque-Sendra J. (2004), *TALANTA.* vol(63):1061-1067.