APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR PRODUCTION OF XYLANASES AND LACCASES USING SPENT COFFEE BEANS AND SPENT GRAINS FROM THE BREWING INDUSTRY AS SUBSTRATES IN SOLID STATE FERMENTATION

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Introduction

Population growth has generated the need for more efficient industries and a sustainable approach that allows the collection of large amounts of high consumption products in less time. This has motivated the application of enzyme technology which in turn requires finding these enzymes producing organisms under different conditions, particularly fungi (1) The application of xylanases in the paper pulp industry is to confer the pulp brightness, its use decreasing the amount of organochlorine compounds traditionally used for the bleaching stage (1)

The laccase enzymes are widely distributed in white rot fungi and their function is the degradation of lignin, however not only are they used for the degradation of lignin in the paper bleaching process, but also promote the oxidation of a wide range of toxic organic compounds.(2)

The Response Surface Methodology (RSM) has been successfully utilized to optimize the improving solid state fermentation process (4)

The aim of the present study was to evaluate the influence of fermentation parameters (temperature and substrate concentration) on the production of Xylanases and Laccases enzymes by native white rot fungi (basidiomycetes) from the Northeastern region of Mexico under solid state fermentation conditions, using RSM.

Methods

Initially four different native strains identified as *Schizophyllum commune (Sc), RVAN2, CH5* and RVAN12 belonging to the laboratory L1 of the Institute of Biotechnology were grown in media made from spent coffee beans and spent grains from the brewing industry (5g/30ªC) and the xilanase and laccase activity was followed. Later, the quantitative effect of two independent variables, including temperature (X1) and the substrate concentration (X2), was evaluated to find the optimal concentrations of these two factors. Temperature ranges tested were 25-35 ° C and 3-7 g. To determine the xylanase activity, the method of Miller was used, 1959 (3). The laccase activity was determined in the filtrate by the oxidation of 2,2 'azino-bis-3-etilbenz-thiazoline-6-sulfonic acid (ABTS) (4). The RSM technique used in this study was a central composite design (CCD), which is a first-order equation (2^N) (4) Data was analyzed in the Design Expert software V8.

Results

The results obtained by the four strains showed that with spent coffee beans *Sc* reached xylanase activities of 237 (±19) U/g, whereas RVAN12 with grains from the brewing industry the laccase activity was of 1995 (±909) U/g.

The optimal point predictions in agreement with the contour plots and response surfaces for both enzymes and with both selected fungi (*Sc* and RVAN12) were: for the laccase activity in spent grains from the brewing industry 29.7 °C and 6.8g with activity of 2254 U/g (RVAN12), while for *Sc,* the xylanase activity obtained was 313 U/g at 31.1 °C and 6.73 g when the spent coffee beans were used.

Conclusions

The results show that when using Response Surface Methodology (RSM) it is possible to find the optimal values of specific variables such as temperature and substrate concentration for the design of production systems, upon selection of microorganisms with potential biotechnological application.

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