TITLE: ETHANOL PRODUCTION FROM GLYCEROL BY *WICKERHAMOMYCES ANOMALUS* CCC32.

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ABSTRACT

In 2016, Brazil produced about 3.8 billion liters of biodiesel, which corresponds to an approximate production of 3.8 million liters of crude glycerol as a by-product. As a source of carbon used by some microorganisms, glycerol can be converted into high added value products such as 1,3-propanediol and ethanol. The objective of this study was to evaluate the production of ethanol from glycerol by *Wicherhamomyces anomalus* CCC32, a yeast that has several biotechnological applications, such as fruit preservation and ethanol production. Fractional factorial design (2^{4-1}) and Box-Behnken matrix were used for three variables, whose planning matrix was generated by Statistica 7.0 software. The fermentations were conducted in 500 mL vials containing 190 mL of culture medium (gL^{-1}): $(NH_4)_2SO_4$ (2.0), K_2HPO_4 (1.7), KH_2PO_4 (0.65), $MgSO_4$ (0.2), NaCl (1.0), Yeast extract (1.0) and varied concentrations of pure glycerol, according to the planning matrix. To this medium was added 1.0 mL of micronutrient solution, in addition to the vitamins thiamine and nicotinic acid (5 mgL^{-1}) and 5% (v/v) inoculum. Growth was determined by spectrophotometry (DO_{600}) and the residual substrate and fermentation products by high performance liquid chromatography, using the Rezex ROA column and 0.005 M H_2SO_4, with a flow of 0.6 $mL.min^{-1}$ and 60ºC. According to the fractional factorial design, of the analyzed variables, pH was not significant at 95% confidence, being fixed at the lowest level, 4.0. According to the Box-Behnken design, the optimal conditions for glycerol bioconversion in ethanol were 33.64 ° C, 33.83 g glycerol L^{-1} and 0.98 g $NaNO_3L^{-1}$. According to Statistica, the R was 0.926, indicating that the model is adequate. The ethanol production by *W. anomalus* CCC32 under optimal conditions was 5.78 gL^{-1}, yielding 0.22 g g^{-1}, biomass reaching 6.8 g L^{-1} and there were still 7.52 g of residual glycerol L^{-1}. These results are superior to those obtined with genetically modified yeasts, such as *Hansenula polymorpha*. Therefore, *W. anomalus* CCC32 presents the potential for the production of ethanol from glycerol in a mineral medium and with low formation of co-products, besides, being classified in level 1 of biosafety, it becomes an attractive factor for future experiments.

Keywords: biodiesel, agroindustrial wastes, biofuels.

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