

## ETHANOL PRODUCTION BY *ZYMOMONAS MOBILIS* DURING SUCROSE FERMENTATION: OPTIMIZATION OF CULTURE CONDITIONS USING FACTORIAL DESIGN

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Current interest in ethanol as a potential fuel has stimulated research on various aspects of the fermentation process. Different techniques for increasing productivity, such as continuous culture, cell recycle and vacuum distillation, have been evaluated, but another important consideration is the improvement of the fermenting organism to give maximum productivity. One of the most promising ethanol-producing organisms is the bacterium *Zymomonas mobilis*. *Zymomonas mobilis* is a unique bacteria among the microbial world, with peculiar growth, energy production and responde to culture conditions, causing a great interest in scientific, biotechnological and industrial fields. The bacteria's ability to make possible energy production in favor of product formation, respond to physical and chemical environmental manipulation as well as its limited product formation make it an ideal microorganism for the study and development of microbial processes for ethanol production. The aim of this work was analyse the optimum operational conditions for the ethanol production by *Zymomonas mobilis* CCT 4494. Inoculum was prepared from activated culture using Erlenmeyer flasks containing 50 mL of fermentation medium. The flasks were placed on orbital shaker (model MA 830) under controlled temperature (30°C), 200 rpm, during a period of 24 hours. The pre-fermentation medium was composed in gL<sup>-1</sup> by: yeast extract 5.0, KH<sub>2</sub>PO<sub>4</sub> 1.0, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0, MgSO<sub>4</sub>.7 H<sub>2</sub>O 1.0 and sucrose 1.0. The medium for ethanol production had the same composition of pre-fermentation medium, differing only in concentration of sucrose and nutrientes. The fermentations were carried out batchwise in Erlenmeyer flasks containing 50 mL of fermentation medium, placed on orbital shaker (model MA 830) under controlled temperature, 200 rpm, during a period of 24 hours. The *cellular biomass* was determined in a spectrophotometer (model Cintra 5 UV-VIS "DoubleBeam") based on 660 nm an calibration curve (Calazans *et al.*, 1997). *Ethanol* was determined by gas chromatography using Chromatograph - HP-5890 Series II - detector FID (Flame Ionization Detector). The experiments were performed under the principles of statistical methodology of response surfaces (Box; Hunter, 1978). For the experimental design, we used the software *Statistica 6.0*, from a factorial design 2<sup>7-2</sup>. The independent variables were: pH; temperature, KCl, K<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub>, CaCl<sub>2</sub> and Sucrose. The bacterium *Zymomonas mobilis* CCT 4494 well adapted in fermentation medium containing high concentrations of sucrose and tolerated pH and temperature variations. The optimum conditions were pH 8; 40°C; KCl (18 g.L<sup>-1</sup>); K<sub>2</sub>SO<sub>4</sub> (5 g.L<sup>-1</sup>); MgSO<sub>4</sub> (5 g.L<sup>-1</sup>); CaCl<sub>2</sub> (1 g.L<sup>-1</sup>) and sucrose (250 g.L<sup>-1</sup>), resulting in a maximum ethanol concentration of 76.6 g.L<sup>-1</sup>. Observed in the analysis of variance (ANOVA) that the independent variables significant (*p*<0.05) were: temperature, KCl and sucrose, with a positive (coefficient >0) and K<sub>2</sub>SO<sub>4</sub> and CaCl<sub>2</sub>, with negative influence (coefficient <0) on the ethanol production.

**Keywords:** ethanol, *Zymomonas mobilis*, factorial design, sucrose, fermentation

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