

THERMAL KINETIC ANALYSIS OF ALIVIIBRIO FISCHERI RECOMBINANT L- ASPARAGINASE

Environmental and industrial biotechnology (Bioenergy, bioremediation)

OP - (689) - THERMAL KINETIC ANALYSIS OF ALIVIIBRIO FISCHERI RECOMBINANT L- ASPARAGINASE

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Body

Thermal kinetic analysis is one of the main factors to establish a biopharmaceutical application. L-Asparaginase is an important enzyme for the pharmaceutical industry due to its application in the treatment of some lymphoid malignancies as acute lymphoblastic leukemia. Novel recombinant enzymes may be the solution in order to reduce or eliminate the side effects showed by the commercial preparations. The objective of the present study is to evaluate the thermal kinetic of a novel recombinant *Aliivibrio fischeri* L-Asparaginase produced by engineered *Bacillus subtilis*. Cultivations were carried out in orbital shaker using 500 mL Erlenmeyer flasks containing 100 mL of Luria-Bertani medium, at 30 °C, 200 rpm for 24 h. Cells were recovered by centrifugation and submitted to sonication for cell lysis. Enzymatic extract was evaluated at 25 °C, 37 °C, 45 °C and 60 °C considering the enzymatic activity over times. L- Asparaginase activity was measured according the ammonium release in L-Asparagine hydrolysis. Thermal parameters were calculated considering a single and a consecutive reactions model. Models analysis indicated that enzyme thermal inactivation follow a single reaction model, where k values of 0.128 h⁻¹, 0.148 h⁻¹, 0.262 h⁻¹, 0.701 h⁻¹ and 1.187 h⁻¹ were obtained for 25 °C, 37°, 45 °C and 60 °C, respectively. The enzymatic extract showed a half life time of 13 h at 25 °C and 5 h at 37 °C, indicating the product have satisfactory properties at room temperature and at human body temperature. The results herein showed the analyzed recombinant L-Asparaginase have potential to be used as a biopharmaceutical. **Acknowledgements** Funding: FAPESP (2018/06908-8 and 2020/15513-7); FCT (POCI-01-0145-FEDER-031268)

Palavras-chave : Thermal kinetic, Biopharmaceutical, L-Asparaginase