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Recombinant L-asparaginase induction process for pharmaceutical application

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L-asparaginase (ASNase) is an important biopharmaceutical used in the treatment of lymphatic system oncological diseases, mainly acute lymphoblastic leukemia (LLA). LLA is the most common type of cancer in childhood, but it can also occur in adults, and expected to afflict over 53,000 people worldwide by 2020. The treatment with ASNase presents several side effects, mainly allergic reactions and inactivation. Aiming to overcome these problems, a recombinant E. coli BL21(DE3) able to overexpress protease resistant ASNase was constructed. ASNase protease resistance is promising since higher immune response occurs when the enzyme is hydrolyzed and protease activity leads to its inactivation and the half-life decrease. To improve this ASNase production, induction conditions studies were performed. E. coli was cultivated in defined medium with 5g/L of glucose and the induction was carried using IPTG in different stages of exponential growth phase (0.2, 0.5 and 0.8 gcell/L). Also, IPTG concentration per cell mass was evaluated (1.3, and 3.0 mMol/gcell). Enzyme activity (U/mL and U/gcell) was used as response. Results showed that the inducion in advanced stage of exponential phase (0.8 gcell/L) was more advantageous, resulting in a maximum of 1.04 U/mL (527 U/gcell) and a higher cell concentration (1.97 gcell/L) in the end of the cultivation (18 h). When the induction was performed with 0.2 gcell/L (beginning of exponential phase) the glucose was not totally consumed. No statistic difference was observed among the IPTG concentrations, indicating that lower IPTG concentration could be used. The study of the induction process is necessary not only to obtain a high protein expression, but also to try to reduce production costs.

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