

## OPTIMIZATION OF THE BIOPRODUCTION OF ENZYME XYLANASE IN *ESCHERICHIA COLI* BY FED BATCH FOR BLEACHING PROCESS

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Enzymes have been used in pulp and paper industries as an attractive approach to reduce the amount of toxic chemicals in bleaching process. The company VERDARTIS develops customized enzymes and xylanase is one of the most important enzymes of the company which was adapted to cellulose bleaching process. The production process developed by Verdartis is constitutive and has ability to secrete enzymes and its production occurs through the fed-batch process. The present work was aimed to optimize the bioproduction of recombinant xylanase in *Escherichia coli* grown in shake flasks by fed-batch with glucose pulses in order to improve production of recombinant xylanase. *E. coli* DH5 $\alpha$  cells using the pT7BsXA plasmid containing the xylanase native, developed in VERDARTIS in partnership with USP Ribeirão Preto. The cultivation was carried out in three steps (LB medium; synthetic and fed-batch using synthetic supplementary medium). After ~12h, glucose was depleted from media indicating this was the right time to start feeding and samples were taken for analysis of enzyme activity and cell density. We compared different conditions, e.g. glucose concentration in the medium, control of pH, addition of yeast extract and MgSO<sub>4</sub>. The results showed that the fed-batch method was influenced by pH, yeast extract, cell density, glucose and MgSO<sub>4</sub>. The highest production levels were related to lower cell-density cultures, pH controlled between 6.5 and 7.5, adequate availability of glucose in the culture medium, absence of yeast extract and glucose pulses without MgSO<sub>4</sub>. The negative control (grown in batch mode) had higher production in all experiments, yielding the maximum production of 50,000 U/L, while 35,000 U/L was the best enzyme production in fed-batch. This study demonstrated that using glucose as sole source of feeding to produce xylanase was unsuitable and another feeding strategy is required for optimization of the bioproduction process.

Keywords: Xylanases, Bleachings, *E. coli*, Fed-batch cultivation.

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