Fed-batch fermentation for heterologous protein production by recombinant *Pichia pastoris*

M. S. O. Wanderley[2,3,4], C. Oliveira[1], S. I. Mussatto[1], D. Bruneska[2,3], J. L. Lima Filho[2,3,4], L. Domingues[1] and J. A. Teixeira[1]

[1]IBB – Institute of Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[2]Grupo de Prospecção Molecular e Bioinformática (ProspecMol), Universidade Federal de Pernambuco (UFPE), Av. Prof. Moraes Rego, n°1235, 50670-901, Recife-PE, Brazil.
[3]Lab. de Imunopatologia Keizo Asami (LIKA), UFPE, Av. Prof. Moraes Rego, n°1235, 50670-901, Recife-PE, Brazil.
[4]Programa de Pós-graduação em Ciências biológicas, UFPE, Av. Prof. Moraes Rego, s/n, 50670-901, Recife-PE, Brazil.

Pichia pastoris is a methylotrophic yeast widely used for heterologous recombinant protein production. This yeast has potential for high level expression, efficient secretion and growth to very high cell densities. Fed-batch fermentation has been widely used to enhance protein production by *P. pastoris*. Frutalin is a α-D-galactose-binding lectin isolated from *Artocarpus incisa* seeds, successfully used as a cancer diagnostic tool and thus its large-scale production is aimed. This lectin has been previously expressed and produced in *P. pastoris* using a batch process. Therefore, the present work aims at evaluating a fed-batch fermentation process as an alternative to improve the production of recombinant frutalin by *P. pastoris* KM71H.

Cultivations were carried out in a 1.6 L reactor, in three distinct phases: 1) initial batch fermentation for cells growth in BMGH medium; 2) a fed-batch phase with 50% glycerol and 12 mL/L of trace metal solution; 3) a fed-batch phase where cells were induced by 0.5% methanol and 12 mL/L of trace metal solution. During the fermentation, the dissolved oxygen was kept above 30% saturation, aeration ratio was fixed at 1.5 vvm, and pH values were controlled at 5.0. In the first and second fermentation phases, the temperature was maintained at 30 °C, being decreased to 21 ºC at the end of second stage. Total cell concentration was determined by measuring the absorbance of the broth at 600 nm, while glycerol consumption and methanol concentrations were detected by HPLC. The recombinant frutalin production was detected by denaturing SDS–PAGE, being the bands visualized by staining with Coomassie Brilliant Blue R250. The lectin activity was checked by hemagglutination assays toward rabbit red blood cells.

High cell density (98.8 g/L dry weight) was obtained during the fed-batch process (Fig. 1A), which is generally desirable since the concentration of secreted protein in the medium often increases proportionally to the cell density. Analysis by SDS–PAGE showed frutalin production at 120, 132 and 144 h. Native frutalin migrates in SDS–PAGE as a double band, where the upper band corresponds to the glycosylated isoforms and the lower band to the non-glycosylated isoforms. Recombinant frutalin migrated in gel as a single band (Fig. 1B) and exhibited hemagglutinating activity towards rabbit erythrocytes. Optimization of the induction phase is still on course. Nevertheless the results obtained so far show the feasibility of the fed-batch process for large-scale recombinant frutalin production by *P. pastoris* KM71H. Supported by: CNPq, ISAC- ERASMUS.

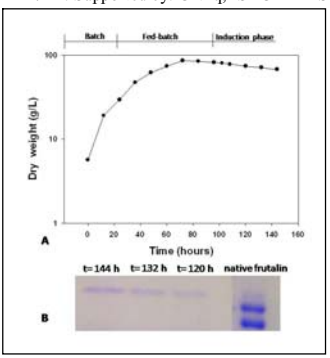

Fig. 1. Production of recombinant frutalin in fed-batch fermentation process by *P. pastoris* KM71H. A) Yeast growth profile and time course of batch, fed-batch and induction phases. B) SDS-PAGE analysis of the supernatant at induction phase.