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Interfacing *Pichia pastoris* cultivation with expanded bed adsorption

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Background

For improved interfacing of the *Pichia pastoris* fed-batch cultivation process with expanded bed adsorption (EBA) technique, a modified cultivation technique was developed. The modification included the reduction of the medium salt concentration, which was then kept constant by regulating the medium conductivity at low value (about 8 mS cm^{-1}) by salt feeding. Before loading, the low conductivity culture broth was diluted only to reduce viscosity, caused by high cell density. The concept was applied to a one-step recovery and purification procedure for a fusion protein composed of a cellulose binding module (CBM) from *Neocallimastix patriciarum* cellulase 6A fused to lipase B from *Candida antarctica* (CALB).

Results

The modified cultivation technique resulted in lower cell death and consequently lower concentration of proteases and other contaminating proteins in the culture broth (see Figure 1). Flow cytometry analysis showed 1% dead (propidium stained) cells compared to 3.5% in the reference process. During the whole process of cultivation and recovery, no proteolysis was detected and in the end of the cultivation the product constituted 87% of the total supernatant protein. The lipase activity in the culture supernatant increased at an almost constant rate up to a value corresponding to 2.2 g L^{-1} of CBM-CALB. In the EBA process no cell-adsorbent interaction was detected but the cell density had to be reduced by a two-times dilution to keep a proper bed expansion. At flow velocity of 400 cm h^{-1}, the breakthrough capacity was 12.4 g L^{-1}, the product yield 98 %, the concentration factor 3.6 times, the purity about 90%, and the productivity 2.1 g L^{-1} h^{-1}.

Conclusion

Our achievements in the modified cultivation stage that increased the quality of the feedstock for the separation stages were: higher target protein to total protein concen-

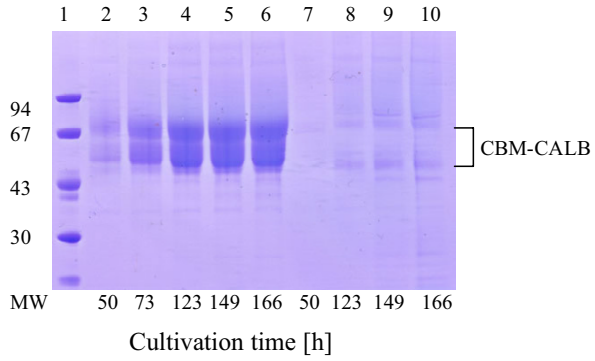

Figure 1
SDS-PAGE analysis of samples withdrawn from the bioreactor in two *P. pastoris* mTLFB cultivation processes. 20 μL of the culture supernatant was loaded in each lane. The two strong upper bands, in the lanes 2 to 6, represent protein of interest, which appears as two diffuse bands due to glycosylation. Lanes 7 to 10 correspond to samples from reference cultivation of the same *P. pastoris* strain, transformed with linear vector lacking DNA coding for the protein of interest (thus, the contaminating proteins).

tration ratio in the culture broth, low protease activities, and lower salt concentration and conductivity. The low salt and conductivity should also make the feedstock more suitable for loading on ion exchanger EBA media. These achievements made the expanded bed adsorption technique more efficient for initial recovery of CBM-CALB in particular but should also make the EBA technique more attractive for the recovery of other recombinant proteins from *P. pastoris* system.

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