Purification of lipase CalB with parallely operated membrane adsorber

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Introduction

Lipases (EC 3.1.1.3) are enzymes that catalyze the hydrolysis of long chain triacylglycerides into glycerol and free fatty acids. Their structure displays an α/β-hydrolase fold, forming the catalytic triad that binds the substrate (Figure 1). Due to their high stereo- and regioselectivity, but also to the large range of substrates, they open new pathways in the synthesis of structured lipids. The lipase *Candida antarctica* lipase B (CalB) is one of the most used biocatalysts (e.g. manufacturing of pharmaceuticals, bulk chemicals and food applications). CalB has a pI of 6.0, but it shows an unusual pH profile with a broad isoelectric region from pH 4.0 to 8.0 [2]. This special feature makes the purification of CalB challenging.

Disposable membrane adsorbers (MA) are replacing traditional flow-through chromatography more and more, because protein transport is fulfilled by convection while pore diffusion is minimal. Higher flow rates, reduced buffer consumption, shorter process times are some more benefits of this technique. One main topic in modern biotechnology are continuous processes.

Several advantages, e.g. significant increase in productivity, greater flexibility, cost reduction and improved product quality, can be named. A continuous set-up with several MAs was build to purify the recombinantly produced CalB (*E. coli*).

PCC can be used with conventional bead resins or with membrane adsorbers. Nevertheless, MAs have some main advantages. Mass transfer is realized by convection while pore diffusion is minimal. Higher flow rates, reduced buffer consumption, shorter process times are some more benefits of this technique. One main topic in modern biotechnology are continuous processes. Several advantages, e.g. significant increase in productivity, greater flexibility, cost reduction and improved product quality, can be named. A continuous set-up with several MAs was build to purify the recombinantly produced CalB (*E. coli*).

Results and Conclusion

✓ Recombinant production of CalB in *E. coli*
✓ First approach: Purification of CalB with cationic membrane adsorber
✓ Development of a flexible PCC device
• Optimization of CalB purification
• Determination of breakthrough curves
• Column switching strategy
• Long-term continuous operation

References