In vivo biotinylation of recombinant beta-glucosidase enables simultaneous purification and immobilization on streptavidin coated magnetic particles

Beta-glucosidase from Bacillus licheniformis was in vivo biotinylated in Escherichia coli and subsequently immobilized directly from cell lysate on streptavidin coated magnetic particles. In vivo biotinylation was mediated by fusing the Biotin Acceptor Peptide to the C-terminal of beta-glucosidase and co-expressing the BirA biotin ligase. The approach enabled simultaneous purification and immobilization of the enzyme from crude cell lysate on magnetic particles because of the high affinity and strong interaction between biotin and streptavidin. After immobilization of the biotinylated beta-glucosidase the specific activity (using p-nitrophenyl-β-d-glucopyranoside as substrate) was increased 6.5 fold (compared to cell lysate). Immobilization of the enzyme resulted in improved thermal stability compared to free enzyme; after 2h of incubation (at 50°C) the residual enzyme activity of immobilized and free beta-glucosidase was 67 and 13%, respectively. The recyclability of immobilized beta-glucosidase was examined and it was observed that the enzyme could be recycled at least 9 times and retain 89% of its initial activity.
Semi-continuous in situ magnetic separation for enhanced extracellular protease production modeling and experimental validation

In modern biotechnology proteases play a major role as detergent ingredients. Especially the production of extracellular protease by Bacillus species facilitates downstream processing because the protease can be directly harvested from the bio-suspension. In situ magnetic separation (ISMS) constitutes an excellent adsorptive method for efficient extracellular protease removal during cultivation. In this work, the impact of semi-continuous ISMS on the overall protease yield has been investigated. Results reveal significant removal of the protease from Bacillus licheniformis cultivations. Bacitracin-functionalized magnetic particles were successfully applied, regenerated and reused up to 30 times. Immediate reproduction of the protease after ISMS proved the biocompatibility of this integrated approach. Six subsequent ISMS steps significantly increased the overall protease yield up to 98% because proteolytic degradation and potential inhibition of the protease in the medium could be minimized. Furthermore, integration of semi-continuous ISMS increased the overall process efficiency due to reduction of the medium consumption. Process simulation revealed a deeper insight into protease production, and was used to optimize ISMS steps to obtain the maximum overall protease yield. Biotechnol. Bioeng. 2013; 110: 2161–2172. © 2013 Wiley Periodicals, Inc.

Use of high-gradient magnetic fishing for reducing proteolysis during fermentation.

Proteolysis during fermentation may have a severe impact on the yield and quality of a secreted product. In the current study, we demonstrate the use of high-gradient magnetic fishing (HGMF) as an efficient alternative to the more conventional methods of preventing proteolytic degradation. Bacitracin-linked magnetic affinity adsorbents were employed directly in a fermenter during Bacillus licheniformis cultivation to remove trace amounts of unwanted proteases. The constructed magnetic adsorbents had excellent, highly specific binding characteristics in the fermentation broth (K(d) = 1.94 micromolar; Q(max) = 222.8 mg/g), which obeyed the Langmuir isotherm and had rapid binding kinetics (equilibrium in

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A magnetic adsorbent-based process for semi-continuous PEGylation of proteins

A semi-continuous magnetic particle-based process for the controlled attachment of PEG (PEGylation) to proteins is described for the first time. Trypsin and 2 kDa mono-activated PEG were used to systematically develop the steps in the process. Proof of concept was shown in a microfluidics system to minimize reagent consumption. Two streams containing (i) 1.2 g/L trypsin and (ii) 4 g/L magnetic adsorbents derivatized with the reversible affinity ligand benzamidine were pumped into a pipe reactor. At the exit, a third solution of activated PEG (0-40 g/L) was introduced and the solutions immediately fed into a second reactor. Upon exiting, the mixture was combined in a third reactor with a fourth stream of free amine groups to stop the reaction (50 mM lysine). The mixture continued into a high-gradient magnetic separator where magnetic supports, with PEGylated trypsin still attached, were captured and washing and elution steps were subsequently carried out. Analysis of the conjugates (with SDS-PAGE & LC-MS) showed that the extent of PEGylation could be controlled by varying the reaction time or PEG concentration. Furthermore, the PEG-conjugates had higher enzyme activity compared to PEGylation of non-immobilized trypsin.

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Scalable manipulation of proteins using magnetic adsorbents

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Contributors: Ottow, K. E.
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In Situ Magnetic Separation for Extracellular Protein Production

A new approach for in situ product removal from bioreactors is presented in which high-gradient magnetic separation is used. This separation process was used for the adsorptive removal of proteases secreted by Bacillus licheniformis. Small, non-porous bacitracin linked magnetic adsorbents were employed directly in the broth during the fermentation, followed by in situ magnetic separation. Proof of the concept was first demonstrated in shake flask culture, then scaled up and applied during a fed batch cultivation in a 3.7 L bioreactor. It could be demonstrated that growth of B. licheniformis was not influenced by the in situ product removal step. Protease production also remained the same after the separation step. Furthermore, degradation of the protease, which followed first order kinetics, was reduced by using the method. Using a theoretical modeling approach, we could show that protease yield in total was enhanced by using in situ magnetic separation. The process described here is a promising technique to improve overall yield in No production processes which are often limited due to weak downstream operations. Potential limitations encountered during a bioprocess can be overcome such as product inhibition or degradation. We also discuss the key points where research is needed to implement in situ magnetic separation in industrial production.

Avoiding proteolysis during fermentation by using high gradient magnetic fishing

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Integrated process for protein modification using smart magnetic handles

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Batch purification of the replication protein A complex using chelating M-PVA magnetic supports

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Projects:

Scaleable Assembly of Protein Machines using Magnetic Adsorbents
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