Immobilization of cellulases on magnetic particles to enable enzyme recycling during hydrolysis of lignocellulose - DTU Orbit (29/12/2018)

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There is an urgent need to replace petroleum-based fuels and chemicals with more environmentally sustainable options since oil contributes to a net production of greenhouse gases and is a limited resource. Lignocellulosic biomass is currently one of the most extensively studied feedstocks for biochemicals and biofuels production because of the great abundance of the feedstock and the fact that it is a waste material and does not directly compete with food production. Lignocellulose consists of cellulose (the most prevalent component), hemicellulose and lignin and the polymeric sugars (in cellulose and hemicellulose) can be enzymatically hydrolyzed into monomers and subsequently fermented to bioethanol or another desirable biochemical. One of the main costs and obstacles in making the bioprocess economically viable is the costs of cellulases which catalyze the hydrolysis of cellulose into glucose. One approach to decrease the costs of the cellulases could be to immobilize the enzymes on particles and thereby enable enzyme re-use. However, recycling of immobilized cellulases using common separation unit operations such as centrifugation or filtration may be difficult when dealing with lignocellulosic feedstocks containing insolubles. This could potentially be overcome by immobilizing the cellulases on magnetically susceptible particles. Consequently, the immobilized cellulases could be magnetically recovered and recycled for a new cycle of enzymatic hydrolysis of cellulose. The main objective of this thesis was to examine the possibility of immobilizing cellulases on magnetic particles in order to enable enzyme re-use. Studies at lab and pilot scale (20 L) were conducted using model and real substrates. In paper I and III beta-glucosidase or a whole cellulase mixture was covalently immobilized on commercial, but expensive, magnetic particles activated with different chemistries. It was observed that the highest immobilized enzyme activities were obtained using magnetic particles activated with cyanuric chloride. In paper II biotinylated recombinant beta-glucosidase was produced and immobilized on commercial magnetic particles coated with streptavidin. The procedure enabled simultaneous purification and immobilization from crude cell lysate because of the very strong interaction and high affinity between biotin and streptavidin. A third method of immobilizing enzymes was employed in paper IV where two types of magnetic anion exchange particles were used to immobilize beta-glucosidase through electrostatic interactions. For both covalent coupling and adsorption (anion exchange binding) between enzyme and support the specific activity (U/mg protein) of immobilized enzyme was lower compared to the free form, while for enzyme immobilization using the biotin-streptavidin system the specific activity increased by 6.5-fold upon immobilization compared to the crude cell lysate. Following enzyme immobilization the possibility of recycling the enzyme was examined using both synthetic substrates (soluble) and real lignocellulosic biomass (containing insolubles such as lignin, hemicellulose and non-hydrolyzed cellulose). The most promising particles for recycling were the anion exchange magnetic particles from Orica Watercare (MIEX® particles) since they promoted rapid magnetic separation and very low interaction with residual insolubles. In addition to these features, they are extremely cheap. It was also possible to strip adsorbed enzyme under special conditions and re-charge the particles with new fresh enzyme (which could decrease the cost associated with purchase of base particles). For these reasons, 400 g of the magnetic MIEX® particles were used for enzyme immobilization and enzyme recycling during the 20 L pilot scale study conducted in paper V. A new type of high gradient magnetic separator, a magnetically enhanced centrifuge, was employed and it was possible to recover the immobilized enzyme and separate the magnetic particles from residual cellulose in pilot scale, before using them in 3 subsequent 20 L hydrolysis cycles. The results in this thesis thus demonstrate that cheap magnetic immobilized cellulases can be used for repeated hydrolysis cycles at pilot scale and demonstrate the potential for use in large scale applications such as in the production of lignocellulosic derived biochemicals.

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