Exploration of enzymatic strategies for synergistic degradation of lignocellulosic substrates

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論 文 名　：Exploration of enzymatic strategies for synergistic degradation of lignocellulosic substrates
（リグノセルロースバイオマスの高効率分解における協奏的酵素触媒系の探索）

区　　分　：甲

論 文 内 容 の 要 旨

Owing to the prior abundance and appreciable polysaccharides percentage, lignocellulosic biomass is considered an ideal renewable resource for substitution of fossil fuels. However, because of the complex matrix of polysaccharides within the rigid structure of the plant cell wall, the complete degradation via enzymatic hydrolysis needs a variety of enzymes working synergistically in addition to various pretreatment techniques of lignocellulosic biomass. Considering the fact that cost of enzymes is still the main obstacle that impeding the biofuel production, it is vital to develop the strategies that can improve the enzyme performance and reusability to achieve high biofuel yields at low enzyme loadings. Thus, in the present thesis, the enzymatic strategies that focus on improving reducing sugars production are stated.

This thesis is composed of five Chapters, which are the general introduction in Chapter1, the research achievements in Chapters 2 to 4, the general conclusion and future perspectives in Chapter 5.

In Chapter 2, the effect of pretreatment with peracetic acid (PAA) or an ionic liquid (1-ethyl-3-methyl imidazolium acetate, [Emim][OAc]) on the synergism between three purified recombinant cellulase and xylanases was investigated. The physical structure and chemical components of bagasse before and after two individual pretreatment methods were systematically investigated. Under the same pretreatment conditions, PAA pretreatment was more efficient at removing the hemicellulose from the substrate, while [Emim][OAc] pretreatment was more capable at decreasing the crystallinity of cellulose. Afterwards, two endoxylanase, XynZ and Xyn11A, were selected and mixed with an endoglucanase Cel6A for demonstrating the relationship between substrate property and enzyme synergism. The results showed that the hemicellulose content, especially arabinan, and the cellulose crystallinity of bagasse, as well as the molecular architecture of enzymes were found to affect the cellulase-xylanase synergism.

In Chapter 3, five endoxylanases from different glycoside hydrolase families and microorganisms were tested with an arabinofuranosidase, Araf51A, for the hydrolysis of insoluble wheat arabinoxylan, which is a structural component of hemicellulose. The optimized combination was XynZ/Xyn11A/Araf51A with a loading ratio of 2:2:1, and the value of degree of synergy increased with the increase in the Araf51A

proportion in the enzyme mixture. Afterwards, selected enzymes were immobilized on commercial magnetic nanoparticles through covalent bonding. Both free and immobilized enzymes showed a similar conversion to reducing sugars after hydrolysis for 48 h. After 10 cycles, approximately 20% of the initial enzymatic activity of both the individual or mixture of immobilized enzymes was retained. A 5.5-fold increase in the production of sugars was obtained with a mixture of enzymes immobilized after 10 cycles in total compared with free enzymes. Importantly, a sustainable synergism between immobilized arabinofuranosidase and immobilized endoxylanases in the hydrolysis of arabinoxylan was demonstrated.

In Chapter 4, a novel polymeric proteinaceous scaffold was successfully constructed by crosslinking two tyrosine residues at N- and C-termini of SpyCatcher protein via horseradish peroxidase-catalyzed reaction. Subsequently, the characteristics of the SpyCatcher polymer were systematically investigated. Taking the advantage of SpyCatcher-SpyTag interaction, two SpyTagged hemicellulases, XynZ and Araf51A, with specific ratios, were site-specifically assembled on to the SpyCatcher polymer, yielding a binary enzyme complex. The ratio-controllable binary artificial hemicellulosome exhibited higher sugar conversion than free enzymes in the case of high Araf51A proportion sample, possibly due to the spatial proximity between the conjugated enzymes.

In Chapter 5, the main contents of this thesis were summarized and future perspectives regarding the sustainable development by biocatalytic degradation of lignocellulosic substrate was discussed.