

#### **449b Recyclable Immobilized Cellulase Conjugates for Hydrolysis of Pretreated Lignocellulosic Biomass**

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Lignocellulosic biomass is considered to be a viable renewable resource for production of fuels, energy and chemicals. Most biomass conversion processes currently under investigation involve a pretreatment step to improve the accessibility of the cellulose, followed by enzymatic hydrolysis to produce fermentable sugars. However, one of the principal deterrents for economical conversion of cellulose to sugars via enzymatic hydrolysis is the high cost of the cellulase enzymes. Even with recent advances in the reduction of the cost of the cellulase enzymes, additional efforts are needed to improve the economics of the enzyme hydrolysis step. As part of a research effort at WRI to develop a commercial process for bioconversion of lignocellulosic biomass, we are addressing recycling of the cellulase enzymes with the objective of improving overall process economics.

Through our research efforts, we are developing a concept to immobilize the enzymes on reversibly soluble polymers (biocatalyst conjugates), which allows the enzymes to be recovered and recycled. The enzymes are immobilized on a reversibly soluble polymer to produce the biocatalyst conjugate, which in the soluble form is used to hydrolyze several fresh batches of cellulose. In the soluble form, the biocatalysts mediate the desired reaction, thus overcoming steric hindrance and mass transfer limitations encountered with insoluble matrices. After completion of the hydrolysis reactions, the biocatalyst conjugates are made reversibly insoluble by changing the pH of the solution, causing full precipitation and recovery of the enzymes for recycle.

We first focused on the preparation of the biocatalyst conjugates to determine the effect of pH on their solubility. The activities of the cellulase preparations on model cellulosic substrate (Avicel) were determined as a function of pH. We also evaluated the biocatalyst conjugate preparations for their ability to repeatedly hydrolyze the cellulose substrates prepared from corn stover and wheat straw by the WRI fractionation process. The results demonstrate the concept is a powerful approach that is scalable and can become a core technology in biorefineries.