Bioethanol from lignocellulose - pretreatment, enzyme immobilization and hydrolysis kinetics - DTU Orbit (02/12/2018)

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Pretreatment and enzymatic hydrolysis are two of the processes involved in the production of cellulosic ethanol. Several pretreatment methods were proposed, however new pretreatment strategies to increase enzymatic hydrolysis efficiency are still under investigation. For enzymatic hydrolysis, the cost of enzyme is still the bottleneck, re-using the enzyme is possible way to reduce the input of enzyme in the process. In the point view of engineering, the prediction of enzymatic hydrolysis kinetics under different substrate loading, enzyme combination is useful for process design. Therefore, several kinetic models were proposed previously. In view of the connections between pretreatment and enzymatic hydrolysis. The hypotheses and objective of this PhD study consists of three parts:

(1) Pretreatment of barley straw by 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac), which was done during 2009. Ionic liquid had been reported to be able to dissolve lignocellulose. However, as our knowledge, in all published researches, the concentration of lignocellulose in ionic liquid were low (5~10%). Besides, pretreatment time were long (from 1 hr to 1 day). Based on the hypothesis that the amount of ionic liquid and pretreatment time can be reduced, the influence of substrate concentration, pretreatment time and temperature were investigated and optimized. Pretreatment of barley straw by [EMIM]Ac, correlative models were constructed using 3 different pretreatment parameters (temperature, time, concentration of barley straw substrate) and sugar recoveries obtained following enzymatic hydrolysis. Elevated pretreatment temperature and longer pretreatment time favoured hydrolysis. However intensive pretreatment at high temperature also causes degradation of cellulose. In addition, [EMIM]Ac pretreated lignocellulose was found to stabilize and protect the enzymes at elevated temperatures. Therefore lower levels of enzymes were required to obtain similar hydrolytic efficiencies. Optimal pretreatment condition was found with the aid of models based on multiple linear regression. Consider the balanced against economic considerations, barley straw can be pretreated under 150°C for 50 min with dry matter of 20% (w/w). Glucose yield can be up to 70% after enzymatic hydrolysis.

(2) Immobilization of ß-glucosidase (BG), which was done during 2010. One of the major bottlenecks in production of ethanol from lignocellulose is the required high cellulase enzyme dosages that increase the processing costs. One method to decrease the enzyme dosage is to re-use BG, which hydrolyze the soluble substrate cellobiose. Based on the hypothesis that immobilized BG can be re-used, how many times the enzyme could be recycled and how coupling with glutaraldehyde affected enzyme recovery after immobilization were investigated. Glutaraldehyde cross-linked BG aggregates were entrapped in 3.75% calcium alginate. Glutaraldehyde inactivate enzyme activity but also reduce the leakage of enzyme from calcium alginate. Findings showed that more than 60% of enzymatic activity could be maintained under optimized immobilization condition. In order to evaluate stability, the immobilized enzymes were re-used for the hydrolysis of Avicel. No significant loss of activity was observed up to 20th round. Similar glucose yields were obtained following enzymatic hydrolysis of hot water pretreated barley straw by immobilized and free BG. Finally, this is the first time that BG aggregates in a calcium alginate were visualized by confocal laser scanning microscope. The images prove that more BG aggregates were entrapped in the matrix when the enzyme was cross-linked by glutaraldehyde.

(3) Validation and modification of a semimechanistic model, which was done during 2010 ~ 2012. A number of cellulosic hydrolysis kinetic models were proposed. Among the models, a simple and useful mathematical model proposed by Kadam et al. (2004) has potential for supporting process design. However, like the other models, it was not validated intensively, especially under high glucose concentration background and high substrate loading. Thus, the role of transglycosylation was not considered in previous reports. Based on the hypothesis that transglycosylation plays an important role under these conditions, the influence of transglycosylation was introduced into the model and evaluated. The semimechanistic multi-reaction kinetic model consists of homogeneous and heterogeneous reaction proposed by Kadam et al. (2004) was systematically validated and modified under a step by step analysis. The objective is to perform a comprehensive analysis in view of validating and further consolidating the model. A number of dedicated experiments were carried out under a wide range of initial conditions (Avicel versus pretreated barley as substrate, different enzyme loadings, and different product inhibitors such as glucose, cellobiose and xylose) to test the hydrolysis and product inhibition mechanism of the model. Nonlinear least squares method was used to identify the model and estimate kinetic parameters based on the experimental data. The analysis showed that transglycosylation reaction at high glucose level play a key role in the model. Thereforwith the introduction of transglycosylation into the model, prediction of cellulose hydrolysis behavior over a broad range of substrate loading is possible. It also revealed that the experimental data used for parameters estimation or different estimation strategies influence the values of parameters and performance of the model. The revised model structure can now be used to support process design and technology improvement efforts at pilot and full-scale studies especially under high cellulose loading.

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