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Numerical analysis of the impact of structural changes in cellulosic substrates on enzymatic saccharification

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HIGHLIGHTS

- ▶ The factors controlling cellulose conversion rate were verified.
- ▶ High dependency of initial reaction rate on cellulose surface area was verified.
- ▶ Increases in cellulose surface area reduce retardation of the cellulase reaction.

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ABSTRACT

Here, a simple cellulose conversion model that considers the cellulose surface area and surface density of adsorbed cellulase as substrate-derived and cellulase-derived factors controlling reaction rates is provided. Microcrystalline cellulose (Avicel) and delignifed softwood were used as controls, and structure-modified samples were prepared. It was shown that the initial cellulose conversion rate is largely controlled by the cellulose surface area. Moreover, the proposed model demonstrates that increases in cellulose surface area reduce retardation of the cellulase reaction.

The proposed model was used to estimate the impact of structural changes in a substrate (i.e., cellulose surface area) by pre-treatment on enzymatic saccharification. It was found that increasing the cellulose surface area is the most effective way to optimize enzymatic saccharification of cellulose substrates.

1. Introduction

Several studies have attempted to explain the reaction mechanisms of specific enzymes in the cellulase system. Since the reaction mechanisms of many enzymes have been clarified, the overall cellulase reaction can be roughly described as follows (Bansal et al., 2009): (1) adsorption of cellulase onto the substrate via the binding domain; (2) location of a bond susceptible to hydrolysis on the substrate's surface; (3) formation of an enzyme–substrate complex; (4) hydrolysis of the β-glycosidic bond and simultaneous forward sliding of the enzyme along the cellulose chain; (5) desorption of cellulases from the substrate or repetition of steps 2 and 3 or 4 if the catalytic domain detaches from the chain, and (6) hydrolysis of cellobiose by β-glucosidase to form glucose. Although the above steps are unique to the type of enzyme, and this process appears well explained, there remain problems in analyzing the cellulase reaction. In particular, all steps occur almost simultaneously and cannot be examined separately during enzymatic saccharification. Moreover, the heterogeneity of the substrates makes it difficult to interpret enzymatic saccharification. Therefore, various kinetic models of cellulase reaction have been developed for better quantitative analysis of enzymatic saccharification.

Michaelis–Menten (M–M) kinetics are based on a quasi-steady state approximation under conditions of excess substrate compared to available enzyme, and they are suitable for modeling the initial stage of homogenous liquid-phase enzymatic reactions (Schnell and Maini, 2003). However, the cellulase reaction with cellulosic substrates is a heterogeneous reaction that occurs on a solid surface, making the quasi-steady state approximation inapplicable. Nonetheless, some previous studies have shown that modified M–M and original M–M kinetics are applicable under certain conditions (Bezerra and Dias, 2004). Furthermore, empirical or semi-empirical models have been widely developed. Empirical models can be useful, but they do not provide insight into the mechanism of the reaction (Bansal et al., 2009). For reliable estimates of the cellulase reaction using a simple method, it is important to consider the rate-controlling factors, which provide insight into the mechanistic details of the cellulase reaction. Moreover, the factors should be easily expressed in numerical form. The reaction rate-controlling factors can be divided into enzyme-derived and substrate-derived factors.

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