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A mathematical model for the inhibitory effects of lignin in enzymatic hydrolysis of lignocellulosics

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HIGHLIGHTS

- Cellulose conversion was calculated at cessation of cellulase activity.
- Steam-exploded pine feedstock provided data for a worked example.
- Conversion at relatively low enzyme loading characterized enzyme deactivation.
- Conversion at relatively high enzyme loading characterized cellulose occlusion.
- Enzyme requirements doubled when the temperature was raised from 30 °C to 50 °C.

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ABSTRACT

A new model for enzymatic hydrolysis of lignocellulosic biomass distinguishes causal influences from enzyme deactivation and restrictions on the accessibility of cellulose. It focuses on calculating the amount of unreacted cellulose at cessation of enzyme activity, unlike existing models that were constructed for calculating the time dependence of conversion. There are three adjustable parameters: (1) 'occluded cellulose' is defined as cellulose that cannot be hydrolysed regardless of enzyme loading or incubation time, (2) a 'characteristic enzyme loading' is sufficient to hydrolyse half of the non-occluded cellulose, (3) a 'mechanism index' measures deviations from first-order kinetics. This model was used to predict that the optimal incubation temperature is lower for lignocellulosics than for pure cellulose. For steam-exploded pine wood after 96 h incubation, occluded cellulose was 24% and 26% at 30 °C and 50 °C, and the characteristic enzyme loadings were 10 and 18 FPU/g substrate, respectively.

1. Introduction

The lignin in lignocellulosic biomass inhibits enzymatic hydrolysis, and the unique chemistry of softwood lignin makes softwoods particularly challenging for bioconversion (Mabee et al., 2006). Mansfield et al. (1999) suggested that two distinct mechanisms are involved: lignin binds cellulases in non-productive complexes, while also blocking cellulose from being accessible to cellulases. This paper describes a mathematical model developed to assist in distinguishing between the two mechanisms, and uses softwood biomass to illustrate use of the model.

Lignin and other phenolic substances can inhibit enzymatic hydrolysis through non-productive binding (Pan, 2008; Ximenes et al., 2010) or permanent deactivation (Ximenes et al., 2011). The distinction between the two mechanisms is important. Non-productive binding to the substrate or hydrolysis products slows the hydrolysis of cellulose to glucose, but the enzymes are eventually released to continue the hydrolysis process. On the other hand, permanent deactivation, e.g. through denaturation or chemical deactivation reactions with the substrate, can lead to cessation of hydrolysis before all of the cellulose has been converted to glucose. Ximenes et al. (2011) studied the effects of phenolic substances formed by degradation of lignin, e.g. vanillin, cinnamic acid and 4-hydroxycinnamic acid, and reported considerable enzyme deactivation for substances that showed only traces of non-productive binding. Sinitsyn et al. (1982) washed steam-exploded hardwood and found that the wash water contained cellulase inhibitors. They also found that adding the wash water to the washed wood resulted in markedly inferior glucose yields, with hydrolysis halted after incubation for 84 h. The latter observation indicated enzyme deactivation by water-soluble components of pretreated wood. Sewalt et al. (1996) suggested that deactivation of cellulase by lignin involves chemical reactions with quinone methide intermediates.

Permanent deactivation can also occur in the absence of lignin, e.g. as a result of shear forces generated by agitation (Taneda et al.,

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